SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF CAMEL BRUCELLOSIS IN SELECTED DISTRICTS OF FAFAN ZONE, SOMALI REGION. EASTERN ETHIOPIA

MSc Thesis

By

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Department of Clinical Studies

JUNE 2017

BISHOFTU, ETHIOPIA

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A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Epidemiology

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STATEMENT OF THE AUTHOR

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ABSTRACT

A cross-sectional study was conducted on 450 camels in 35 herds, from Oct, 2016 to April, 2017 with the aim of determining sero-prevalence and assessing the associated risk factors for camel Brucellosis in purposively selected three districts (Jigjiga, Babile and Gursum) of Fafan zone, Somali Regional state, Ethiopia. Among the districts, a total of 10 settlements or pastoral associations (Kebeles) (5 Kebeles from Babile, 3 Kebeles from Gursum and 2 Kebeles from Jigjiga 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. The overall sero-prevalence of Brucella in Fafan zone was 4.8% (95%, CI: 2.8−6.8). The seroprevalence with respect to district level was 10% (95%, CI: 1.7 − 18.7), 5.7% (95%, CI: 0.9 -10.5) and 1.9% (95%, CI: 0.007−0.0522) in Jigjiga, Gursum and Babile district, respectively. Univariate logistic regression analysis on potentially assumed associated risk factors against seroprevalence of brucellosis, showed a statistically significant difference in sex, age, districts, parity, herd sizes, camels that co-exist with other ruminants and reproductive disorder (abortion) ($p<0.05$). Furthermore, multivariable logistic regression analysis of the risk factors, revealed that the age, herd size and camels that are kept closely together with other ruminants with adjusted odds ratio (OR) of 3.3 (95%, CI: 1.58- 6.74), 4.6 (95%, CI: 2.66 - 8.10) and 11.4 (95%, CI: 1.39 - 85.46), respectively were the major risk factors for the occurrence of seropositivity to Brucella infection in camels. Moreover the questionnaire survey revealed that most respondents in the study area (67%) did not know about the transmission of Brucellosis. Therefore, this study provided the sero-prevalence status and associated risk factors for camel Brucellosis and also the local practices of pastoral communities that can potentially contribute to the spread of the disease to humans.

Keywords: *Brucellosis, Camels, CFT, Ethiopia, Fafan Zone, RBPT, Risk factors, Seroprevalence.*

1. INTRODUCTION

1.1. Background and Justifications of the Study

Eastern Africa is known to be the heartland for camel production as 80% and 63% of the Africa and world population, respectively produced in the region. Camels are subset of huge livestock resources in Ethiopia with the population estimated to be 2.3 million. This number ranks the country third in Africa after Somalia and Sudan and fourth in the world (CSA, 2007). Since camels (*Camelus dromedaries*) are species that are well adapted to a hot and arid environment of Ethiopian pastoralists (Tefera and Gebreah, 2001) this makes camel rearing, the most sustainable livestock production system. All camels in Ethiopia are owned by pastoralists (MoARD, 2008).

Although camels are hardy and undemanding in their maintenance, they are not resistant to diseases affecting other livestock, as frequently assumed in the past. Camels are infected with different diseases including brucellosis, particularly when they are in contact with other infected ruminants. Brucellosis which caused by Brucella species is an important zoonotic disease and has become a major worldwide human concern (Neta *et al.*, 2010). It is an infectious disease of domestic and wild animals. According to the Office International des Epizooties (OIE), it is the second most important zoonotic disease in the world, accounting for the annual occurrence of more than 500,000 human cases (Pappas *et al.*, 2006).

Brucellosis is considered by the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) and Office International des Epizooties (OIE) as one of the most widespread diseases that has still of veterinarian, public health and economic concern in many developing countries including Ethiopia (Hadush and Pal, 2013). The disease in dromedary camels can be caused by *B. abortus*, *B. mellitensis* and *B. ovis* (Seifert, 1996). The organism can enter the body through the lungs, the digestive tract, mucous membranes and intact skin, where it is transmitted among animals from infected to susceptible individual animals. The disease spreads from camels to human through milk and/ or other infected animal products (Greenfield *et al.*, 2002).

Clinical signs of the disease in breeding camelids are the same as those in bovines and small ruminants. In humans, the disease may occur acutely with mild flu like symptoms. While successful isolation of the agent can be achieved by sampling different body tissues, some classical serological tests for detecting antibodies against *Brucella* species, like Rose Bengal plate test (RBPT) and complement fixation test (CFT) are applicable (Musa *et al.,* 2008). Treatment of brucellosis in animals is not as such effective when undertaken, while in humans the administration of effective antibiotics for an adequate length of time results successful (Corbel, 2006).

Brucellosis has great losses in livestock of developing countries by causing tremendous economic losses due to abortion, premature birth, decreased milk production, reduced fertility, mortality and cost of treatments and cross transmission to other animal species. On the other hand it has an obvious impact on human health and environment. Since camels suffer from lack of attention and negligence in numerous countries, the control of Brucellosis in camels is severely hampered. In Ethiopia, brucellosis in camels has not been extensively studied as compared to other species of animals.

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 man. 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 Ethiopian 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 had poor knowledge about brucellosis . Most of the camel owners believed that camel milk to possess superior storage life, medicinal properties (against dropsy, jaundice, diabetes, glycaemia) and has an aphrodisiac effect. However, most of them did not have any knowledge about the transmission of brucellosis from consumption of raw milk. Hence the isolation of *B.*

abortus and *B. melitensi*s (Radwan *et al.*, 1992; Gameel *et al.*, 1993; Abou-Eisha, 2000) 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 Ethiopia. Thus, there is a need for further study on the epidemiology of camel brucellosis and associated risk factors for zoonotic transmission and to design and implement control measures aiming at preventing further spread of the disease both in animal and pastoral communities.

1.2. Objectives

1.2.1. General objectives

- ✓ To estimate the sero-prevalence and associated risk factors for the occurrence of camel Brucellosis in the selected districts (JigJiga, Babile and Gursum) of Fafan Zone, Somali Region, Ethiopia.

1.2.2. Specific objectives

- ✓ To estimate the sero-prevalence of camel brucellosis in the three selected districts of Fafan zone, Ethiopian Somali Region.
- ✓ To assess potential risk factors that are associated with the occurrence of camel Brucellosis in the study areas.
- ✓ To investigate the public health significance, level of awareness and risk factors for zoonotic transmission of camel brucellosis in the study areas.

2. LITERATURE REVIEW

2.1.Camel Brucellosis

2.1.1. Aetiology

Brucellae are Gram-negative, facultative, non-spore-forming, intracellular coccobacilli or rods; that lack capsules, flagellae, and endospores. Brucella organisms grow slowly, but can be enhanced by using enriched media, such as Ferrell's media supplemented with 5% horse serum and six added antibiotics. The growth of *B. ovis* and *B. abortus*, biotype 2, always requires media enriched with serum or blood incubated in an atmosphere of 5% to 10% carbon dioxide. The genus presently consists of 10 classified (*B. abortus, B. melitensis, B. ovis, B. canis, B. suis, B. neotomae, B. ceti, B. innopinata, B. microti* and *B. pinnipedials*) and 3 unclassified potential species (Baboon isolate, Bo2 and Australian rodent strains) of which seven are pathogenic for humans. Each Brucella species has a preferred host species but can be transmitted to other species, including man (Sprague *et al.*, 2012).

Although Brucella species are genetically highly related to each other, they are described by the following parameters: colony morphology, agglutination with anti-S sera, and lysis by different phages, oxidase and urease activity, and host preference. Within the species, the biovars are defined by their requirement of CO_2, H_2S production, growth on dyes (thionin, basic fuchsine) and the agglutination with the monospecific sera A, M, and R. Recent approaches include genomic information e.g.*OMP*2 sequence, 16S rRNA gene sequences, and grouping systems based on Multilocus Sequence Typing (MLST) profiles (Banai, 2010).

Brucellae are generally susceptible to heat, direct sun light, acidic conditions and common disinfectant. 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. 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).

Camels are most frequently infected by various biovars of the two species *B. abortus* and *B. mellitensis.* Also they may contract infection from *B. ovis. Brucella abortus* has seven recognized biovars. The distribution of biovars could be important in ascertaining the source of some infections. *Brucella mellitensis* is the most virulent species of the genus Brucella and has three biovars, with biovars 1 and 3 being those isolated most frequently in small ruminants (Blasco and Molina, 2011).

2.1.2. Epidemiology

2.1.2.1.Occurrence

Brucellosis has a worldwide distribution and affects cattle, pigs, sheep, goats, camelids, dogs and, occasionally, horses. Brucella infections have also been documented worldwide in a great variety of wild life species and, more recently, in marine mammals. A spillover of infection from domestic animals to bisons, elks or African buffalos may also be possible. Theoretically, the three *Brucella* species known to cause brucellosis in camels (*B. abortus*, *B. melitensis*, *and B. ovis*) can cause infection anywhere. However, it is surmised that B. melitensis is widespread in Africa and the Middle East and *B. abortus* is widespread in the former Union of Soviet Socialist Republics (USSR) (Saegermann *et al.,* 2010).

The incidence appears to be closely related to breeding and husbandry practices. 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. High animal and herd prevalences have been reported from different countries, which not only pose a severe risk to humans but also to other livestock. A seroprevalence 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).

Also, introduction of camels into cattle, sheep and goat areas in the Darfur region of Sudan led to high incidence levels, as shown by Musa and Shigidi (2001). In another study in Sudan,

conducted by the same authors, in 3,413 dromedaries that were intermingled with cattle and small ruminants, the herd infection rate was 45.5%, with prevalence rates of between 1.4% and 90%. Since no camels had been culled due to brucellosis, it is believed that the reduction in camel Brucellosis was caused by the reduction in brucellosis in sheep and goats.

2.1.2.2.Sources of infection and Transmission

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 brucellas in their uterine exudates and placenta. 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. 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 . 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. 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 bacteria may be spread from animals to people in three main ways as:-Raw dairy products through infected raw or undercooked meat, unpasteurized milk, ice cream, butter and cheeses. Inhalation**,** via air especially farmers, laboratory technicians and slaughterhouse workers can inhale the bacteria and/or direct contact with living or dead infected animals and their carcasses

or secretions (including their tissues, blood, urine, vaginal discharges, aborted fetuses, and especially, placentas) through a cut or other wound (MDPH, 2006).

Even though people rarely get Brucellosis from their pets, normal contact with animals, touching, brushing or playing does not cause infection. But, people with weakened immune systems should avoid handling dogs known to have the disease. 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).

2.1.2.3.Risk factors

2.1.2.3.1. Host and pathogen risk factors

Susceptibility of camels to *Brucella* infection is influenced by the Age, Sex and reproductive status of individual animals. 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. 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 (Ghanem *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. 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 prevalences between 21 and 70% (Al-Majali and Al-Shorman 2009).

The male predominance is most likely related to occupational exposure, whereas children usually have a history of raw milk ingestion, consumption of unpasteurized milk products or in very rare cases animal contact (Al-Shamahy *et al.*, 2000).

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. The organism is susceptible to heat, sunlight and standard disinfectants, but freezing permits almost indefinite survival. 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).

2.1.2.3.2. Environmental and 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. In humans, prevalence of the disease is high (39.5%) in summer season (Salari *et al.*, 2003). Camel brucellosis caused by *B. abortus* or *B. melitensis* biovars can be encountered in all camel rearing countries with the exception of Australia. High individual animal and herd prevalences 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: habitat, herd size, cohabitation with other ruminants, and contact with other camels, the latter indicating an inter-camel cycle. At herd level, the risk factors are herd size and cohabitation with other ruminants (Ghanem *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. 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. This observation is supported by the fact that all *Brucella* spp. and biovars infecting other ruminants have also been isolated from camels. 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 rates of the disease were higher than those herded separately (Isam, 2016).

2.1.3. Pathogenesis

Almost all domestic species can be affected with brucellosis. It is essentially a disease of the sexually mature animals, the predilection site being the reproductive tract, especially the gravid uterus. Allantoic factors including, erythritol, possibly steroid hormones and other substances stimulate the growth of most of the Brucellae (Musa *et al.*, 2008).

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 reticuloendothelial 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 tropism of *Brucella* to the male or female reproductive tract is thought to be by erythritol, which stimulates the growth of the organism, but Brucella has also been found in the reproductive tract of animals with no detectable levels of erythritol. Erythritol, a sugar alcohol synthesized in the ungulate placenta and stimulates the growth of virulent strains of *B. abortus* (Anonymous, 2007).

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).

2.1.4. Clinical Signs

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. 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. Infections may cause stillborn calves, retained placenta, fetal death and mummification and reduced milk yield. Also, delayed service age and fertility have been reported. A retained placenta is rare in Camelidae. This may be a result of the difference in the placental attachment as they possess a placenta diffuse like the horse and not a cotyledonary placenta (Fowler, 2010).

According to the study done by Gwida *et al.*,(2011) 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, $\times 10^6$ 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.

No clinical signs were observed in the four camels inoculated with S19, whereas slight non-specific 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 (Gwida *et al.,* 2011).

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. Complications affecting the bones

and joints are common (they occur in 20–60% of cases) with Sacroilitis occurring most frequently. Other symptoms that may occur with this disease include muscle pain and swollen glands. Ultimately, the illness may become chronic and last for years (Franco *et al.,* 2007).

2.1.5. 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. 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. 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. 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 Manual of Diagnostic Test and Vaccines for Terrestrial Animals (OIE, 2000).

2.1.5.1.Serology

Chromatographic kits available for diagnosis of brucellosis in cattle can be used for camels with high precision (Godfroid *et al.,* 2010). The most commonly used serological tests are based on the detection of antibodies against the smooth surface lipopolysaccharide (LPS), since they are immunodominant antigens of Brucella. The indirect serological tests of Brucellosis diagnosis include agglutination tests, Rose Bengal plate test, complement fixation tests, milk ring test

(MRT) (This test is prescribed by the OIE for use only with cow's milk), precipitation tests and primary binding immunoassays (Poester *et al.,* 2010).

Rose Bengal Plate Test (RBPT) is used as a screening test for diagnosis of the acute and chronic forms of Brucellosis. It is a simple spot agglutination test where drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a positive reaction. The results are received in several minutes. The test is an excellent screening test but may be oversensitive for diagnosis in individual animals, particularly vaccinated animals (Poester *et al.,* 2010).

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 min. Due to high specificity of CFT than any other conventional tests, it has been recognized as a confirmatory serological test for brucellosis. This test is a "prescribed test for trade" by the OIE (OIE, 2000). Since this test is difficult to standardize, it is progressively being replaced by primary enzyme-linked Immiunosorbent assays (ELISA). The ELISA has not been widely evaluated for camel species, but is potentially useful subject to adequate standardization (Poester *et al.,* 2010).

Cross-reactivity exists to different other bacterias including, *Yersinia enterocolitica* O: 9; *Escherichia hermannii* and *E. coli* O: 157; .On the other hand, abortion and reduced fertility in the camel frequently have other causes, such as Salmonellosis, Trypanosomosis, or infections with Campylobacter or Trichomonas fetus, Therefore, difficulties may arise in the diagnosis of brucellosis and making laboratory examinations are essential. Usually an incorrect diagnosis of brucellosis particularly in camels may occur when based on serology alone; hence confirmation is made through prescribed bacteriological tests to isolate and identify the bacteria, (Yohannes *et al.,* 2012).

2.1.5.2.Skin test

The skin test is an allergic test that detects the specific cellular immune response induced by *Brucella spp.* infection. The injection of brucellergene, a protein extract of a rough strain of *Brucella spp,* is followed by a local inflammatory response in a sensitized animal. This delayed

type hypersensitivity reaction is measured by the increase in skin thickness at the site of inoculation. This test is highly efficient in discriminating between true brucellosis cases and false positive serological reactions (FPSR). It cannot discriminate between infection and vaccination. This test is prescribed as an alternative test by the OIE (Godfroid *et al.,* 2004).

2.1.5.3.Bacteriological Culture

The "**gold standard**" of the brucellosis diagnosis is the direct bacteriological testing that is, cultivation of Brucella, isolated from body fluids (blood, cerebrospinal fluid, milk, urine and others) or tissues (vaginal mucus, placenta and fetal stomach contents). Identification of the bacteria is based on their morphology, staining and metabolic profile (tests for catalase, oxidase and urease activities). This method of diagnosis is severely limited by the fact that Brucella is a hazardous bacterium and its isolation has to be done in specially equipped level three laboratories. Moreover, it is a very labor-intensive and time-consuming procedure. However, the isolation and cultivation of bacteria are also necessary preliminary steps for staining and biotyping of Brucella species (Godfroid *et al.,* 2010).

Classical biotyping of Brucella species is made on the base of phenotypic differences of surface lipopolysaccharide (LPS) antigens, sensitivity to staining, CO_2 dependence, H_2S production and other metabolic properties .Smooth (S-LPS) and rough (R-LPS) phenotypes are differentiated. The S-LPS phenotype is found in most Brucella species, only *B. canis* and *B. ovis* possess the R-LPS. Some proteins of Brucella are responsible for serological cross-reactions between Brucella spp. and other bacterial species (Emmerzaal *et al.,* 2002).

Laboratory diagnostic techniques for brucellosis mainly rely on serological tests that detect antibodies against Brucella and cultivation of blood or tissue cultures. To overcome the limitations posed by these techniques, research developed significant DNA diagnostic techniques for brucellosis that utilize the selectivity and sensitivity of polymerase chain reaction (PCR) which could be easily used for routine diagnosis. Recently Faham *et al*., (2015), reported that detecting Brucella in blood and lymph node specimens from camels by the use of real-time PCR

is to be effective in detecting *B. abortus* and *B. melitensis* in blood and lymph samples, respectively.

2.1.6. Treatment

Treatment of brucellosis in animals is rarely recommended or effective when it is undertaken. Among domestic food animals including camel, treatment is not an option given disease eradication goals; thus, infected animals are slaughtered. Exceptions for wildlife would be rare and only potentially feasible for protected species in captive zoo settings. Disadvantages of treatment include the expense of the antimicrobials, the lengthy treatment period with potential for multiple required courses, declining owner compliance, uncertain results, and ongoing public health risks (Hollett, 2006).

The essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics (Tetracyclines, doxycycline in combination with rifampin and streptomycin; and Aminoglycosides) for an adequate length of time. Antibiotic treatment should be implemented at as early a stage as possible, even in patients who appear to be showing a spontaneous improvement. In those patients with complications, additional treatment, including in some cases surgical intervention, will be necessary (Corbel, 2006).

2.1.7. Prevention and Control

Persistence risk factors of camel brucellosis are various and complex. The most exposed populations to contract camel brucellosis are those linked to livestock breeding. The illiteracy, the lack of sanitary consciousness, the humans' wrong habitudes and the search to satisfy sensorial desires, are the main causes of brucellosis persistence in third world countries (Mammeri, 2015).

Since camels suffer from lack of attention and negligence in numerous countries, the control of brucellosis in camels is severely hampered. Several approaches could be applied to improve the situation and significantly reduce the occurrence of brucellosis in camels, among them are: encouragement of closer interaction between animal keepers and veterinary personnel, as well as

the establishment of a common vocabulary for camel diseases and conditions; introduction of hygiene procedures and correct disposal of aborted materials and the use of disinfectants (Megersa, 2010).

Successful eradication programmes of brucellosis have always been costly, long, and hard to carry through. The difficulty in controlling and eradicating camel brucellosis stems from a variety of issues. The most important of which is the animal management conditions (extensive breeding, transhumance, co-existence of several livestock species, etc.). In almost all countries, effective prevention of brucellosis among humans and other animals including camels is based on disease control programs in domestic animals involving vaccination and slaughter of infected animals.

Several vaccines for use in nonhuman animals have been developed over the years, the most effective of which are live attenuated Brucella vaccines like live *B abortus* strain 19; live *B abortus* strain RB51 and live B.melitensis strain Rev.1. Generally, each has efficacy against a specific Brucella spp and only in certain animal species. Since there is no vaccine available for humans, prevention of human brucellosis relies on its control in the animal reservoir (Blasco, 2006).

A sensible intersectional collaboration between public health and veterinary sectors based on the concept of ‘one medicine’ which would greatly improve the health status both in animals and humans; intergovernmental cooperation between trading countries to prevent cross border transmission; mass testing with appropriate techniques and culling accompanied by adequate compensation (livestock), introduction of rational vaccination schemes and application of antibiotics. Although these suggestions have been made by numerous scientists for many years, hardly any have been implemented outside from study set ups and non-governmental organization (NGO) projects and the general situation have not greatly improved (Ahmed *et al.,* 2010).

2.2.Public health importance

In times of increasing human population, more resources are needed and camels are an ideal asset. Not only do they function as power suppliers (drawing water, grinding wheat, ploughing, etc.) and act as transport medium, they are vital sources of milk and inexpensive meat. Despite its capabilities to withstand against environmental harsh conditions, camels like other domestic animals are susceptible to different diseases including brucellosis. In a recent publication, the International Livestock Research Institute identified the 13 zoonoses that are most important to poor livestock keepers including Ethiopia, because of their impacts on human health, animal health and livelihoods. Brucellosis is one of the top zoonotic diseases in this list (ILRI, 2012).

Occupational acquired brucellosis is of special concern for public health because of the high risk of direct transmission from infected animals to persons being employed in animal husbandry. This exposed group includes slaughter men, dairymen, herdsmen and veterinary clinicians. In humans, the disease, which is often referred to as 'undulant fever' or 'Malta fever' is a serious public health problem. It is a debilitating disease with acute and chronic illness, resulting in physical incapacity and loss of manpower and death in some cases in the endemic regions Human brucellosis remains one of the most common zoonotic diseases worldwide, with more than 500,000 new cases annually. Infection prevalence in the animal reservoirs determines the incidence of human cases (Faham *et al.*, 2015).

Brucella melitensis and *B. abortus* are the two species most commonly found in human cases, and *B. melitensis* is responsible for the most serious infections. The main modes of transmission are contact through skin with infected animal tissues, blood, urine, vaginal discharge, aborted fetuses and, especially, placentas, and by consuming raw milk and other unheated dairy products. Airborne infections occur in animal pens, stables, laboratories and abattoirs .Some cases have also occurred from accidental self-inoculation with live vaccines (Saleem *et al.*, 2010).

Humans are at risk through consumption of unheated milk or through handling Brucella-positive animals. Shimol *et al.*, (2012), described a brucellosis outbreak that affected 15 people who consumed unpasteurized camel milk. Affected people suffered mainly from arthralgia and fever and 50% had positive blood culture for *B. melitensis*, whereas 60% had serum agglutination titres of 1:60 or higher. Extreme care must be exercised when working with Brucella organisms in

laboratories. It is estimated that up to 2% of all diagnosed brucellosis cases are laboratory acquired infections, mainly through inhalation when handling diagnostic specimens (Desta *et al.*, 2015).

Moreover, it was also shown by Bradenstein *et al* (Bradenstein *et al.*, 2002), that Rev 1 vaccine strain can cause human infections. In their study humans became infected after consuming milk from vaccinated adult pregnant animals which excreted the vaccine strain in milk for a long period of time. The high and increasing herd and animal prevalences of camel brucellosis in many countries is of grave concern; therefore, veterinary authorities, consumers, camel owners and camel keepers, as well as responsible persons in the Ministry of Health and Agriculture of each country, should make every effort to address this issue.

In humans, the incubation period lasts from five to 60 days, but can also be longer. The disease occurs as an acute and chronic illness, leading high rates of fetal loss (up to 40%) in pregnant women at early stage and orchitis and epididymitis in men. It also results a physical incapacity and loss of manpower and death in some cases in the endemic regions. Brucella melitensis DNA persists in human blood for many years after infection despite appropriate treatment and apparent recovery (Vrioni *et al.*, 2008).

In order to estimate human morbidity and mortality losses, disability-adjusted life-years are applied, which are based on data obtained from human brucellosis control programs. Despite the advances made in surveillance and control, the prevalence of brucellosis is increasing in many developing countries, due to various sanitary, socioeconomic, and political factors (Pappas *et al.*, 2006).

Brucellosis is not only a major zoonotic problem but is also linked with bioterrorism. *Brucella species* are listed by the Centers for Disease Control and Prevention (CDC) as Category B (second-highest priority agent) bioterrorist agents. The severity of this disease, their relative stability in aerosol form, lack of vaccines suitable for use in man and frequent failure of clinical laboratories to correctly identify isolates led to the investigation of Brucella as an agent for bioterrorism. The impact is likely to be greatest in those areas in which the disease is not

endemic. *B. melitensis* and *B.suis* have been developed experimentally as biological weapons by state sponsored programmes (Yagupsky and Baron, 2005).

Before 1954, when Britain was focusing on anthrax, brucellosis was the first microorganism chosen by the United States to develop as a weapon. This microorganism could be effectively disseminated in four pound bombs. Indeed, the American military weaponized *B. suis* in 1954; however, changing global politics resulted in abandonment of these efforts following the biological and toxic weapons convention in 1972. Thus Health and veterinary authorities should be aware of this potential source of infection (Corbel, 2006).

In Ethiopia, Based on the findings of Habtamu *et al.,* (2015), an estimated economic loss due to camel brucellosis was found to be 429,351.48 ETB (21,467.56 US $) for each individual infected camel with age above 4 years. The disease can generally cause significant loss of productivity through late first calving age, long calving interval time, low herd fertility and comparatively low milk production in camels. The disease poses a barrier to export and import of animals constraining livestock trade and is an impediment to free animal movement (Zewolda and Hailleselassie, 2012).

Above all, camel brucellosis is prevalent in Ethiopia and there is a need for planning and implementation of joint programs by stakeholders in prevention and control of the disease as well as raising public awareness, particularly to animal keepers and consumers of camel products, in decreasing the distribution of the disease and its public health implications (Catley *et al.*, 2005).

2.3. Status of Camel Brucellosis in Ethiopian Pastoral and Agro-pastoral Systems

There are about 50 to 100 million pastoralists globally and the majorities are confined to Africa. Ethiopia has the largest pastoral population of 7 to 8 million where the majorities of these people are living in the Ethiopian Somali and Afar administrative Region. Ethiopian Somali pastoralists who suffered from political and geographical marginalization in the past, live in an arid and semi-arid parts of the country (Bekele *et al.*, 2013).

In times of increasing human population, more resources are needed and camels are an ideal asset. Not only do they function as power suppliers (drawing water, grinding wheat, ploughing, etc.) and act as transport medium, they are vital sources of milk and meat. Despite its capabilities to withstand against environmental harsh conditions, camels like other domestic animals are susceptible to different diseases including brucellosis. In a recent publication, the International Livestock Research Institute identified the 13 zoonoses that are most important to poor livestock keepers including Ethiopia, because of their impacts on human health, animal health and livelihoods. Brucellosis is one of the top zoonotic diseases in this list (ILRI, 2012).

In Ethiopia, brucellosis has been reported in camels from pastoral areas, where the prevalence was quite vary (ranging between 2 and 5% were reported from most pastoral areas in Ethiopia) from area to area and from herd to herd, due to the variation in animal husbandry and management systems by which the people in the area were practicing. Camels are not known to be primary hosts of Brucella, but they are susceptible to *B. abortus*, *B. melitensis* and *Brucella*

ovis. Consequently, the prevalence depends upon the infection rate in primary hosts being in contact with them (Gwida *et al.,* 2011).

Brucellosis has great losses in livestock of developing countries by causing tremendous economic losses due to abortion, premature birth, decreased milk production, reduced fertility, mortality and cost of treatments and cross transmission to other animal species. On the other hand it has an obvious impact on human health by causing a debilitating disease with acute and chronic illness, resulting in physical incapacity and loss of manpower and death in some cases in the endemic regions.

Persistence risk factors of camel brucellosis are various and complex. The most exposed populations to contract camel brucellosis are those linked to livestock breeding. The illiteracy, the lack of sanitary consciousness, the humans' wrong habitudes and the search to satisfy sensorial desires, are the main causes of brucellosis persistence in third world countries (Mammeri, 2015).

Ethiopian pastoralists' people keep different species of animals together at several conditions where they keep camels either with both sheep and goats or either of the two or with cattle. They consume raw milk, which contributes to disease transmission. Above three-quarters of the pastoralists are practicing at least one activity considered to be at risk for the transmission of zoonotic diseases and more than 75% of the animal owners do not know about zoonotic camel brucellosis (Gwida *et al.,* 2011).

Moreover, the mixing of the different species during migration, at watering or in night enclosures (resting), between camels and other ruminants is visible. Mobility also increases the opportunity of interactions with wild animals. 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).

Ethiopian Somali pastoralist, consume raw milk and they did not use any protective materials during handling parturient camels, removing placenta and/or other aborted materials since most of the people had poor knowledge about brucellosis . Most of the camel owners believed that

camel milk to possess superior storage life, medicinal properties (against dropsy, jaundice, diabetes, glycaemia) and has an aphrodisiac effect. However, most of them didn't have any knowledge about the transmission of brucellosis from consumption of raw milk. Furthermore, they also use a common breeding camel bull for different herd groups (Bekele *et al.*, 2013).

Bekele *et al.*, 2013; who observed at herd level, a seroprevalence of 24% in the camels from Afar, reported that most Afar people did not know about the transmission of zoonotic diseases, and that their practices could potentially facilitate the transmission of zoonotic pathogens including Brucella bacteria and they recommended that implementing control measures and increasing public awareness in the prevention methods of camel brucellosis in the area is urgently needed.

Above all, despite the advances made in surveillance and control, the prevalence of brucellosis is increasing in many developing countries including Ethiopia due to various sanitary, socioeconomic, and political factors (Pappas *et al.*, 2006). Thus there is a need for planning and implementation of joint programs by stakeholders in prevention and control of the disease as well as raising public awareness, particularly to animal keepers and consumers of camel products, in decreasing the distribution of the disease and its public health implications (Catley *et al.*, 2005).

3. MATERIALS AND METHODS

3.1.Description of the study area

The study was conducted in three purposively selected districts namely JigJiga, Babile and Gursum of Fafan zone, Ethiopian Somali regional state. Fafan administrative zone, previously known as JigJiga zone is located in the northern part of the Ethiopian Somali Region, at 9°20' North latitude; 45°56'° East longitude, about 630 km East of Addis Ababa. Currently Fafan zone consists of nine districts namely, Jigjiga, Babile, Gursum, Awbare, Kebribayah, Harshim, Tuli-Guled, Haroorays and Der-wanaje (Sherif *et al.*, 2012). It shares border with Shinile zone to the North, the Hararghe highlands of Oromia Region to the West, Degahbur to the South, and Somalia to the East. It covers a total land area of 40.86 km^2 with altitude ranging from 500 to 1,650 meter above sea level (masl) (Degefu *et al.*, 2011). It is characterized by a semi-arid climate with unreliable and erratic rainfall with a precipitation ranging from 300 to 600 mm per annum and an average daily temperature of 16°C to 20°C (Keskes *et al.*, 2013).

The zone is divided into three separate livelihood production system and life style as sedentary agriculturalists, agro pastoralists and pastoralists. Agro-pastoralist is a dominant production system in Fafan Zone. Farming system of the area includes mixed crop livestock production. The vegetation of the area includes different plants where sorghum, maize, barely, wheat and bean are the most important agricultural crops (SCUK, 2004). The estimated livestock population of the zone includes 248,435 cattle, 666,130 sheep, 503,881 goats 72,390 camels and 10,548 poultry (CSA, 2011/2012). According to the report of Birhan (2013), cattle, sheep, goats and

camels are the main productive livestock reared in the area, and camel population was estimated to be 85,000.

Jigjiga district is located 9° 35’ N latitude and 42° 8’ E longitude and has an elevation of 1,609 mass above sea level (masl) (htpp//populationmongabay.com/). Babile district is located 8°40’ 0” N latitude and 42° 25’ 0” E longitude and has an altitude ranging from 950 to 2000 masl (htpp// population mongabay.com/). Gursum district is located 9°29’ 0” N latitude and 42° 60’ 0” E longitude and has an altitude ranging from 976 to 1369 masl (htpp// population mongabay.com/).The districts are inhabited by different tribes of Somali communities of which the “*Yebere*”, “*Abskul*”, “*Gedebursi*”, “*Malingur*”, “*Bartire*”, “*Geri*”, “*Hawiye*” and “*Jaarso*” are known camel rearing tribes.

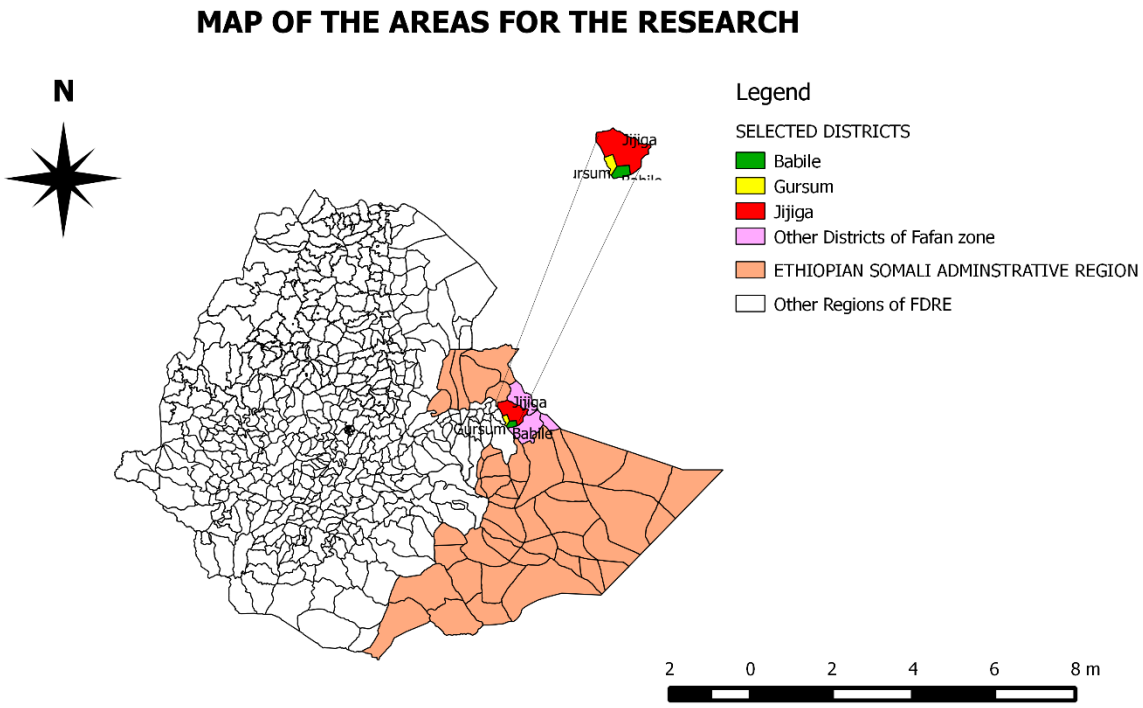

Figure.1. Map of the study areas.

3.2. Study animal population

The target study population was local Fafan zone camels managed under extensive pastoral production system by the pastoralist. The three districts Jigjiga, Gursum and Babile had an estimated camel population of 28,134 (CSA, 2011/2012). Based on camel population of the districts proportionally sample size was distributed. Camels aged two and above 2years old were included in this study. Those camels above four years of age were considered matured (at age of puberty), while camels that were four and less than 4 years old were considered sexually immature. Herd consisting 3-15, 16-25 and >25 camels were also considered as small, medium and large herds, respectively.

3.3. Study design

A cross-sectional study was conducted on 450 one humped camels, in selected pastoral and agro-pastoral residences of the Jigjiga, Babile and Gursum districts of Fafan zone, Ethiopian Somali region, from October, 2016, until April, 2017, 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 (Kebeles) (5 Kebeles from Babile, 3 Kebeles from Gursum and 2 Kebeles from Jigjiga 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, 45 willingly selected camel owners (20 from Babile, 15 from Gursum and 10 from Jigjiga, districts), living in the selected peasant associations, whose animals were tested for brucellosis have been included in the questionnaire survey.

3.4. Sample size determination

Sample size was determined according to Thrusfield (2005) for random sampling and calculated using the expected prevalence of 2.43% (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 P_{exp} = expected prevalence (2.43%). The minimum sample size calculated was 36 however; it was inflated to 450 for better precision. Proportional distribution of the sample was carried out depending on the camel population in the study areas.

3.5.Questionnaire survey

Two questionnaire formats (Annex 8.5 and 8.6), prepared in Somali language, one for the serum sampled individual animal history and the other with a structured questionnaire format for the herders on brucellosis perception and its zoonotic risk factors, was developed and used in this study. In the questionnaire survey the animal feeding and housing practices, knowledge about zoonotic diseases particularly brucellosis and its zoonotic risk factors, the habits of camel product consumption and the ways of handling and disposal practices for dead animal or aborted fetus, that have possible associations with the occurrence of camel brucellosis was investigated and used to support the serological results.

3.6. Blood sample collection

Approximately 10ml of blood sample was collected from the jugular vein of each animal using plain vacutainer tubes, needles and needle holders. Each sample was labeled by using codes describing the specific animal. The blood samples were left at room temperature overnight, to allow clotting, for sera separation. Then, the sera was separated from the clotted blood by decanting to other tubes and were stored at −20°C until serologically tested. Modified Rose Bengal plate test (mRBPT) and complement fixation test (CFT) were used for screening and confirmatory test of sera respectively.

3.7. Serological 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 RBPT antigen (Institut Pourquier 325, rue de la galèra 34097 Montpellier cedex 5, France). This test was carried out at Jigjiga regional diagnostics and research laboratory and Addis Ababa University, College of Veterinary Medicine and Agriculture, Immunology laboratory, Bishoftu campus. Antigen and sera required for each day for serological testing was taken out from the cold storage (in refrigerator at -4^0C) and brought to room temperature for 30 minutes before testing takes place. Briefly $30\mu l$ of stained rose Bengal antigen was dispensed on to card plate and then $30\mu l$ of sera samples were dropped alongside the stained rose Bengal brucella antigen. By using the tip of the automatic micropipette tips, the sera and the stained rose Bengal brucella antigen were mixed and examined for agglutination. Positive and negative controls were employed for interpretation of the results. Agglutinations were recorded as 0, +, ++ and +++ according to the degree of agglutination (Nielson, 2002). A score of 0, +, ++ and +++ indicates the absence, barely visible, fine and coarse of agglutination, respectively. Then those samples with no agglutination (0) and with agglutination either +, ++ or +++ were recorded as negative and positive for brucella infection, respectively.



Figure.2: 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).

Complement fixation test (CFT):

All sera which were tested positive by the RBPT were further retested, using the CFT, for confirmation and the CFT test was done at National Veterinary Institute (NVI). Standard *B. abortus* antigen for CFT (from the Veterinary Laboratories Agency, Addle stone, United Kingdom), Amboceptor and sheep red blood cells (SRBCs), National Veterinary Institute (NVI), Debre Zeit, Ethiopia were used to detect the presence of brucella antibodies against brucella antigen in the sera. Similarly, the control sera and complement used in this test were also obtained from NVI, Debre Zeit, Ethiopia. As an interpretation the test serum having SRBCs sedimentation at a dilution of ≥1:5 were considered to be positive for the disease; camel Brucellosis.

3.8. Data analysis

The generated data on serum sampled individual animals and questionnaire were carefully stored and entered on to Microsoft Excel spreadsheet (Microsoft Corporation) as database. Data on serum sampled individual animals that were 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 seroprevalence 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. Univariate logistic regression analysis was employed to determine the associations of risk factors with occurrence of camel brucellosis in the study areas. Odd ratio (OR) was used to indicate the degree of risk factor association with the disease occurrence signified by 95% confidence intervals.

All risk factors having $p < 0.2$ on univariate analysis were subjected to multivariable analysis using logistic regression to determine the major risk factors. A stepwise approach (forward selection and backward elimination) was constructed to analyze those factors having putative effects on disease occurrence, based on a p-value < 0.20 as the significance threshold for entry or removal. For statistical inference a confidence level of 95% and a *P*-value less than 5% was considered significant. Questionnaire data were analyzed by descriptive statistics using *Microsoft Excel*

4. RESULTS

4.1. Seroprevalence of camel Brucellosis

Accordingly, in the current study, the overall sero-prevalence of camel brucellosis was 4.8% (95% CI: 0.02−0.068) based on RBPT confirmed by CFT and Rose Bengal plate test alone detected 56 (12.4 %, 95% CI: 0.093−0.155) of the samples as seropositive. Upon further testing by CFT only 22 (4.8 %, 95% CI: 0.02−0.068) sera were left positive.

Table. 1: Camel brucellosis by RBPT and CFT

	RBPT	**CFT**

Location	N	No. positive	%	No. positive	%	95%, CI
Babile	210	21	10	4	1.9	0.007-.052
Gursum	140	18	12.8	8	5.7	0.92- 10.57
Jigjiga	100	17	17	10	10	1.7-18.7
Total	450	56	12.4	22	4.8	0.02−0.068

N = number of camels examined; No. = number

Table. 2: Seroprevalence of Brucellosis in relation to different risk factors by univariate logistic regression analysis

Variables	Categories	No. sera Tested	No. sera Positive	Prevalence with 95%, CI	OR	P-value
Sex	Female	321	20	6.2 (4.2-10.4)	**Ref**	**Ref**
	Male	129	2	1.6 (0.05-1.02)	0.23	0.05*
Age	≤4 years	154	2	1.3 (0.003-0.053)	**Ref**	**Ref**
	5-7years	187	12	6.4 (1.14- 23.65)	5.2	0.03*
	>7 years	109	8	7.3 (1.25- 28.92)	6.0	0.02*
Districts	Babile	210	4	1.9 (0.007-0.052)	**Ref**	**Ref**
	Gursum	140	8	5.7 (0.9-10.5)	3.1	0.06

	Jigjiga	100	10	10 (1.7-18.7)	5.7	0.000**
Parity	No parturition	62	2	3.2 (0.008-0.134)	**Ref**	**Ref**
	Single parity	77	12	15.5 (1.2-26.1)	5.6	0.02*
	Mult parity	182	6	3.3 (0.2-5.2)	1	0.96
Herd size	Small herd	355	6	1.7 (0.007-0.039)	**Ref**	**Ref**
	Medium herd	38	6	15.7 (3.3-35.7)	10.9	0.000**
	Large herd	57	10	17.5 (4.3-35.6)	12.3	0.000**
ICWR	Yes	304	21	6.9 (1.43- 80.8)	10.7	0.02*
	No	146	1	0.68(0.001-4.9)	**Ref**	**Ref**
AFAC	Yes	66	12	18.2 (2.67- 17.5)	6.86	0.000**
	No	255	8	3.23(0.008-0.134)	**Ref**	**Ref**

*= Significance, **= Strongly significance

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

AFAC= Abortion in Female Adult Camels; OR= Odds Ratio; CI= Confidence Interval

Results of univariate logistic regression analysis of potential risk factors at animal level in relation to Brucellosis revealed that all the variables investigated (except between Gursum and Babile district (p = 0.06) and also multiparous adult female camels comparing to females with no history of parturition (p=0.96)) had significant association with Brucella seropositivity (P< 0.05) (Table.2).

In this study, in a univariate logistic regression, the highest seroprevalence was found in females 6.2%, (CI: 4.2-10.4) (20 of 321) than males 1.6%, (CI: 0.054-1.029) (2 of 129) with statistically marginal significance (p= 0.05). Similarly, higher reactor rate was recorded in female animals with a single parity (15.58%, CI: 1.2-26.2) and there was statistically significance difference in univariate logistic regression. On the other hand lower seroprevalence of (3.3%, CI: 2.03- 5.25) and (3.2%, CI: 0.008- 0.134) was observed in adult multi-parous female camels and adult female

camels with no history of parturition, respectively (Table.2). But, multivariable logistic regression has showed that sex and parity had no effect for the occurrence of the disease in the area (Table.3).

Likewise, camel brucellosis was statistically detected in all of the three districts and the highest sero-prevalence was recorded in Jigjiga (10%, CI: 1.7-18.7). A sero-prevalence of (5.7%, CI: 0.92- 10.57) was observed in Gursum, while Babile has got the lowest sero-prevalence of (1.90%, CI: 0.007-.052) in relation to the other two districts. Also, the observation shows that seroprevalence of brucella in female camels with history of abortion was 18.2%, (CI: 2.7- 17.6) whereas those without history of abortion was 3.1%, (CI: 1.6 - 6.5). In univariate logistic regression analysis the seroprevalence of brucella in relation to these two risk factors was statistically significance ($p<0.05$) difference (Table.2). Nonetheless, similarly to sex and parity multivariable logistic regression has showed that location and abortion had no effect for the occurrence of camel Brucellosis in the study area (Table.3).

Table. 3: Multivariable, stepwise approach logistic regression analysis of risk factors (sex, age, districts, herd size and ICOR) for seroprevalence of camel Brucellosis

Variables		**No. sera tested**	**No. sera positive**	**Crude OR**	**Adjusted OR**	**95%, CI**	**P-value**
Sex	Female	321	20				
	Male	129	2	0.23	0.30	0.06-1.45	0.138
Age	≤4 years	154	2	-	-	-	-
	5-7years	187	12	5.2	3.14	1.5-6.6	0.003*
	>7 years	109	8	6.0	-	-	-
Districts	Babile	210	4	-	-	-	-
	Gursum	140	8	3.1	1.02	0.4-2.1	0.952

	Jigjiga	100	10	5.7	-	-	-
Herd size	Small herd	355	6	-	-	-	-
	Medium	38	6	10.9	4.37	2.1-8.9	0.000**
	Large herd	57	10	12.3	-	-	-
ICOR	Yes	304	21	10.7	11.8	1.4-93.7	0.020*
	No	146	1	-			

*= Significance, **= strongly significance ***= very strongly significance

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

OR= Odds Ratio; CI= Confidence Interval

Table. 4: Multivariable, stepwise approach logistic regression analysis of risk factors (sex, age, herd size and ICWR) on seroprevalence of camel Brucellosis

Variables	**Categories**	**No. sera tested**	**No. sera positive**	**Crude OR**	**Adjusted OR**	**95%, CI**	**P-value**
Sex	Female	321	20	-	-		
	Male	129	2	0.23	0.30	0.06-1.45	0.138
Age	≤4 years	154	2	-	-	-	-
	5-7years	187	12	5.2	3.14	1.49- 6.61	0.003*
	>7 years	109	8	6.0	-	-	-
Herd size	Small herd	355	6	-	-	-	-

	Medium	38	6	10.9	4.44	2.54-7.74	0.000**
	Large herd	57	10	12.3	-	-	-
ICWR	Yes	304	21	10.7	11.74	1.48-92.61	0.019*
	No	146	1	-	-	-	-

Table.5: Putative effects of advance in age, herd size and ICOR on seroprevalence of camel Brucellosis.

Variables	**Categories**	**No. sera tested**	**No. sera positive**	**Crude OR**	**Adjusted OR**	**95%, CI**	**P-value**
Age	≤4 years	154	2	-	-	-	-
	5-7years	187	12	5.2	3.27	1.58 - 6.74	0.001**
	>7 years	109	8	6.0	-	-	-
Herd size	Small herd	355	6	-	-	-	-
	Medium	38	6	10.9	4.64	2.66 - 8.10	0.000***
	Large herd	57	10	12.3	-	-	-
ICOR	Yes	304	21	10.7	11.46	1.39 - 85.46	0.023*
	No	146	1	-	-	-	-

In this study multivariable logistic regression analysis of risk factors showed that the age, herd size and keeping camels closely together with other ruminants as the major risk factors for the occurrence of seropositivity to *Brucella* infection in camels ($p < 0.05$) (Table.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, shows that increasing age and herd size together with keeping camels closely with other ruminants had significantly joint effect on seropositivity 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).

The questionnaire analysis showed that an extensive management system was practiced in the current study area; where most of the camels were kept together with other species of animals

and mainly reared for milk production, transport, cultural and social value. The highest proportion (82%) of the camel herds kept together with other species of animals while 8% of camel herds were kept alone. The camel rearing experience of pastoralists ranged from 7 to 45 years. According to camel herd composition pregnant and lactating female camels were quietly proportionate as 23.7% and 22% respectively.

On the other hand non-lactating female adult camels accounted for 17.5%. Camel bulls in the herds of the study area were relatively quite low as 8.4% of the herd. Females were constituted about 63.2% of the entire herd while young immature camels of both sexes accounted for 28.3% of the herd. Similarly, pastoralists people in the study area were mainly keep camels for milk production (80%) where the rest particularly camel bulls were kept for draft power (10%) and other purposes like social value 4% and herd accumulation (6%).

In the study area, 75% of the total milk production from Babile and Gursum, was sold to the private milk collecting centers, which were established in Fafan (*Dhegahle*) Centre, 28kms to the west of jigjiga city, where later taken to Jigjiga town , capital city of Ethiopian Somali region, to generate income. The remaining 25% milk was used for home consumption. According to the respondents, all most all of the herders (100%) consumed fresh raw milk without any heat treatment. Also people in the study area consume milk after mixing with boiled tea so called "*Caddeys*" in Somali language. Camel meat was consumed as cooked. However, some of the respondents said that they consume hump of camel as raw.

In relation to the respondents, herding and watering were the activities done by young and adult males while adult and young males were belong to the milking of camels in a proportion of 43% and 34% respectively. Likewise, females in the study area share same activity, with the males of both age groups, i.e. milking of camels as 23%. As per the respondents, during dry season, Camel owners in the study area were used traditional wells (71%) and ponds (29%) made by the Ethiopian, Somali Regional Government, as the main water sources for their camels. Where as in rainy season, Jerer and Fafan rivers were the main source of water for camels. According to the respondents, the prevalent camel diseases in the study area were included pasteurellosis (85%) trypanosomosis (91%), anthrax (92%), pneumonia (64%), camel pox (63%) and abortion (45%).

People in the study area give local names for the above mentioned diseases as "*Cuna barar*", "*Dhukaan*", "*Kud*", "*Oof*", "*Furuqa geela*" and "*Dhicis*" respectively. Besides these localized abscess (48%), GIT parasites (51%), camel mange (61%), wound and tiger bite (19%) were declared by the respondents as commonly occurred diseases and events in the area. Additionally, trypanosomosis (48%), pasteurellosis (12%), camel pox (10%), Anthrax (21%), sunstroke (4%) and pneumonia (5%) were the diseases that respondent individuals in the questionnaire replied as the causes of abortion in camels.

Activities like delivery and mating assistance to camels were the main responsibilities of adult males accounting about (95%) while young males did only (5%) regarding to this family activity. In this study, most of the respondents told that aborted camels were removed from the herd mainly by means of selling, where the Aborted fetus, placenta and discharges were either left on the ground or threw to the dogs of the herders. Similarly, using communal village bull among different herds was not uncommon and about 35% of the herders were practicing this. The other 65% of camel herders were used breeding bull from their own herd. In the study area 45% and 55% of camel owners were grazing their camels separately and together with other ruminants, respectively. On the other hand, in this study, 78% and 22% of camel herds had separate night resting area and camel herds shared night enclosures with other ruminants, respectively.

5. DISCUSSION

The overall seroprevalence of camel brucellosis was 4.8% by RBPT and CFT combined test while it was 12.4% by RBPT alone. This result is compatible to the previous reports of 4.1%, (Hadush 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. Also the current result was in covenant with higher than the observation of Habtamu and fisseha (2014), who reported a seroprevalence of 3.67% in camel brucellosis from south Eastern of Tigray region.

Likewise the present result was lower than the observation of Mohamed *et al*., (2015) and Bekele *et al.,* (2013) who reported a seroprevalence of 5.5% and 5.4% of Brucella seropositivity in

camels from Khartoum state, Sudan and Afar national region, respectively. Similarly it is in promise with Mohamed *et al.,* (2014), who reported 4.1% in Libya. Moreover our result was in agreement with the findings of Abbas and Agab (2002) who reported low seroprevalence (< 5%) in nomadic or extensively kept camels.

However, the observed seroprevalence of Brucella in this study was higher than the results, recorded by Omer *et al.,* (2000), Robayo and Esubalew, (2017), Gumi *et al*., (2013), Tilahun *et al.,* (2013) and Megersa *et al.,* (2011) who reported 3.1%, 1.5%, 0.9%, 2.4% and 1.8% in Eritrea, Ethiopian Somali region, in and around Dire Dawa, southeast Ethiopia, Ethiopian Somali region and Borana respectively. Correspondingly, the result of the recent study was higher than that of Mohamed *et al.*, (2011), who reported a lower seroprevalence of 1.6% in camels from in and around Dire Dawa town. Furthermore the observed seroprevalence of this study was higher than that of Teshome *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.

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, 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).

This is true for Ethiopian Somali 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 applied test 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).

On the other hand, the current study indicated that it is relatively lower than the seroprevalence of, 5.7%, 7.6%, and 5.4%, recorded by Teshome *et al.,* (2003) Zewold and Hailleselassie (2012),

and Bekele *et al.,* (2013) in Afar region. This difference might be due to the variation in herding practices. In afar region mixing of the animals from various areas is common at communal grazing and watering areas (Teshale *et al*., 2006) while in Somali region 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.

Moreover sadiq *et al.,* (2011), Gameel *et al.,* (1993) and Mohamed *et al.,* (2014) reported a higher sero prevalence of camel brucellosis in 9.4%, 5.8% and 5.8% from Nigeria, Kenya and Sudan, respectively than the present study. Also relatively, higher seroprevalence of 30.5%, 23.8% and 7.3% in camel brucellosis has been recorded by Omer *et al.,* (2007), Musa *et al.,* (2008) and El-Boshy *et al.,* (2009) in Sudan, Darfur (Western Sudan) and in Egypt, respectively. The differences in the prevalence of camel brucellosis from different countries may be attributed to varying husbandry and management practices. Sample selection bias also might affect serological findings.

The variation of brucellosis in relation to sex is an established fact that male animals are less susceptible to Brucella infection, due to the absence of erythritol (Hirsh and Zee, 1999). Nonetheless, multivariable logistic regression showed statistically a non-significance difference for the occurrence of brucella in camels regarding to sex. The lack of the statistical significance difference between male and female camels may be due to the presence of effects for confounding factors. Furthermore, this should not be overemphasized since very few numbers of male positive cases was observed which makes it difficult to make comparisons.

In this study, even though statistically significant difference in seroprevalence ($P<0.05$), of brucellosis was observed, between Jigjiga and Babile district where the highest seroprevalence was found in sera of camels from Jigjiga (10%), with a likelihood disease occurrence of 5.7 times higher in jigjiga than sera of camels from Babile district there was no statistical significance difference for brucella seropositivity between Gursum (5.7%) and Babile (1.90%) district. Moreover location of camels did not show statistical significance difference after doing multivariable logistic regression analysis. This could be attributed to the similarities in agro-

ecological location, animal husbandry and herd management and production systems in the areas.

Similar to sex and location, parity and brucella related reproductive problems (Abortion) had an effect on seropositivity of brucellosis in adult female breeding camels under univariate logistic regression analysis but, Multivariable logistic regression analysis determined statistically non-significance difference for seroprevalence ($P<0.05$) of Brucella..

Infection may occur in animals of all age groups, but persists commonly in sexually mature animals of both sexes. The present study revealed a higher seroprevalence of camel brucellosis in the adult age groups (6.4%), than in the young age groups (1.3%). This higher seropositivity was statistically significance between the two age groups in both univariate and multivariate logistic regression analysis where animals with 5-7 years old had 5.2 times higher risk of likelihood disease occurrence than animals with an age of $\leq$ 4 years old. This is in agreement with the observation of Habtamu and Fisseha (2014), who reported a significantly higher occurrence of brucellosis in adult camels (>4 years) (6.5%) than young camels (6 month to 4 years) (0%) with a likelihood odds ratio (OR) of 9.6, from Mehoni district, south eastern of Tigray region, Ethiopia. Also the current study was 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.

Similarly, according to the present study animals above 7 years of age had higher seroprevalence of 7.3% compared to young camels and this was statistically significant, where the odds ratio (OR) was indicating 6 times higher risk for the probability of disease occurrence in old camels than young individuals. This higher seroprevalence of brucellosis in older camels (7.3%) 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.

Tefera and Gebreab (2001) recorded age at puberty and first calving to be 4 and 5 years, respectively for females whereas males had age of 5 years at puberty in eastern Ethiopia. Wossene (1991) also reported the same age for puberty and first calving in Ogaden female

dromedaries. It has been reported that sexually mature and pregnant animals are more prone to Brucella infection than sexually immature animals of either sex (Radostits *et al*., 2007).

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).

Since, brucellosis is considered as a disease of herd importance, considerably high level of 1.7%, 15.7%, and 17.5%, Brucella seropositivity was observed in small, medium and large herd sizes, respectively. The likelihood risk of Brucella positivity occurrence was 10.9 times higher in medium and 12.4 times higher in large herds compared to small herd sizes. Thus, herd size was statistically identified to be one of the major risk factors for Brucella occurrence in the study areas ($p = 0.001$) for both univariate and multivariable logistic regression analysis. 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. Also Mohamed *et al.*, (2013), who reported 4.4% ($p = 0.000$) seroprevalence of camel Brucellosis in Abu Dhabi was documented, as herd size increases, the chance of contact between animal increases too, which leads to more chances of infection. Similarly Zewold and Hailleselassie, (2012) shown that herd size was highly related to the brucella occurrence among camels in the area. These 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) was observed the same effect of herd size for seroprevalence of camel brucellosis in Khartoum state Sudan and stated that multivariable analysis was 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 *et al.,* (2013) OR= 3.05 with p = 0.01, in Afar region, North Eastern of Ethiopia and Adamu *et al.,* (2014) OR= 7.8 with P=0.0003 in North-Eastern Nigeria.

Regarding, to the interaction of camel herds with other ruminants the seroprevalence was 6.9% and 0.68% in animals kept close contact with other ruminants and those without contact with other ruminants, respectively. 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.

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, keeps 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. Mohamed *et al.,* (2011), reported that the seroprevalence of brucellosis in camels in eastern Ethiopia kept without ruminants, with small ruminants and with large ruminants was 1%, 4.3% and 5.3% respectively. Also Chauhan, *et al.,* (2017) observed that rearing of multispecies in same herd may leads 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. 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.

Correspondingly, the mixing of the different species during migration, at watering or in night enclosures (resting) among camels and other ruminants was recorded. Contributing factors to the spread of the camel by brucellosis may be movement of animals for grazing and watering, as aggregating the animals around watering points will increase the contact between infected and healthy animals and there by facilitate the spread of the disease.

Furthermore there is public health hazards and high risk human 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 that had observed in this study 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. Besides these the knowledge about Brucellosis is nil among herdsmen. Similar result was observed by Tilahun *et al*., (2013) who reported that all the herders (100%) from Jigjiga and Babile districts consumed fresh raw milk without any heat treatment. 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. These can put the public health of the area at risk. Furthermore, Zewold and Hailleselassie (2012), reported that camel herders of afar Region were keeping livestock in close contact and consume raw camel milk and milk products and also assist animals during parturition and grooming livestock as well. These were contributed for potential risk factors associated with human brucellosis. Consequently, Zewold and Haileselassie (2012), reported 15% (30 out of 200) Brucella seropositivity in clinical patients from two health centers of Awash-Fentale and Amibara districts in Afar region, north east Ethiopia.

6. CONCLUSION AND RECOMMENDATIONS

The result of the present study shown a seroprevalence of 4.8% and is moderately higher than the other previous studies of camel brucellosis in the region. Multivariable logistic regression analysis of presumed risk factors indicated that age, herd size and camels closely kept with other ruminants were the major associated risk factors for the likelihood 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 will intensify the condition and increase the prevalence of brucellosis. On the other hand there is a possible risk of brucella spread to people in the area, since the livelihood of pastoral community is mainly depends on camel species, 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 of Fafan zone, eastern Ethiopia and the risk factors that had potentially contribute to the occurrence of the disease in camels and also the possible zoonotic significances in human beings.

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

- In order to design and implement control measures through vaccination programmes aiming at preventing further spread of camel brucellosis in the region, advanced research work with more emphasis on the isolation and identification of the Brucella biotypes circulating in camels in the study area is crucial.
- Further epidemiological studies with more accent on role of other ruminants for the occurrence of camel brucellosis infection 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.

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8. ANNEXES

8.1.Rose Bengal Plate Test (RPBT)

All the sera from the studied animals were exposed to RBPT for screening of the presence of brucella antibodies within the sera. This test was done in National Veterinary Institute (NVI), DebreZeit, Ethiopia. For doing the test, the procedure described by Nielson (2002) was followed.

2.1. Materials and Reagents Required

- Applicator stick
- Plate of wet enamel
- Ag dispenser(30µl)
- Serum dispenser(70µl)
- Rose Bengal stained brucella antigen
- Positive and negative control sera

2.2. Procedure

The antigen, control and sera were removed from the refrigerator and left at room temperature for about 30minutes before testing. Briefly 30µl of the stained brucella antigen was placed on the test plate along the side of 70µl of the test serum and it was thoroughly mixed using the tip of the micropipette pits. After all the serum and antigen have been mixed the plate was shaken gently for 4minutes and watched under a good light for the presence of agglutination.

2.3. Interpretation

- Reactions were identified as 0, +, ++ and +++ according to Nielson (2002).That is:
  - ✓ 0= No agglutination
  - ✓ += Barely precipitate agglutination
  - ✓ ++= Fine agglutination
  - ✓ +++= Coarse clumping
- Those samples with no agglutination (0) were recorded as negative (-Ve) while those with +, ++ and +++ were recorded as positive (+Ve) for the presence of brucella antibodies.

8.2.COMPLEMENT FIXATION TEST (CFT)

All sera that were positive, by RBPT were subjected to CFT for confirmation. This serological test was done at National Veterinary Institute, DebreZeit, Ethiopia. Preparation of the reagent and the CFT proper were done according to the test protocol recommended by OIE (2004).

8.2.1. Materials and Reagents Required:-

- Water bath, incubator and agitator.
- U-shaped micro-plates
- Multi-channel and Single-channel micropipettes,
- Micropipette tips
- Complement
- Sheep Red Blood Cells (SRBC)
- Trough
- Syringe
- Veneral buffer with calcium and magnesium (VCM buffer)
- Alsever's solution
- Amboceptor (Anti SRBC antibody)
- Brucella antigen
- Positive and Negative control sera
- Distilled water
- Arranged test sera and sheets of plate lay out for record.

8.2.2. Procedure

8.2.2.1. Preparation of sheep and red blood cells (SRBCs)

- Firstly a blood was drawn from the jugular vein of male sheep freely in to a syringe containing Alsever's solution in a ratio of 7.5ml of sheep blood to 12.5ml Alsever's solution
- Next small amount of crystalline penicillin was added in to the content, to avoid bacterial contaminants.
- Then the blood was stored at $+4^0$c overnight, where in which it can be used for about 2 weeks.

N. B. Sheep blood for CFT should be at least one day old.

8.2.2.2. Preparation of Hemolytic System (HS) (indicator)

- In this technique the Alsever's solution was discarded very gently or sipped out by using micropipette.
- Secondly the sheep blood was washed in three times at a dilution of 1:10 by adding VCM at P^H 7.2 and centrifuged at 2,500 rpm for 5 minutes.
- Then discarding of supernatant followed by resuspension of the SRBCs in VCM where it was mixed gently and centrifuged as above was carried. This step was repeated three times, that is three total wash.
- Next to this another tube of identical size was taken, holding it next to the centrifuged tube and then packed cell volume of the SRBCs was measured through addition water, until the meniscus of SRBCs (volume of SRBCs in ml) was reached.
- Then SRBCs was diluted in VCM to 1 % (eg.1ml PCV of SRBCs).
- Finally reconstitution of the freeze dried Amboceptor with 1ml distilled water, followed by keeping at $+4^oc$ was performed. In this procedure working dilution of the Amboceptor was 1:1000 (Always drawing of the Amboceptor sterile from the bottle was adapted. Along with this step, the reconstituted Amboceptor in a 1:1000 dilution was added in to the 1% SRBCs there by mixing with constant gentle agitation during incubation for 30 minutes (sensitization) at room temperature.

8.2.2.3. Evaluation of Complement

- For accomplishment of this evaluation firstly 25µl VCM was dispensed in to all well of rows A, B, C and D of U-shaped micro-plates.
- Secondly 25µl of complement at a starting dilution of 1:2 was added in to the first wells of row A, B and C (A1, B1 and C1) but row D was left as hemolytic system (HS) control where we expect 100% participation of SRBCs
- Next to this two fold dilution of the complement was made by transferring 25µl of the mixture after through homogenization to the next wells until A12, B12 and

C12 respectively. In this step up on doing so, the last 25µl was discarded after mixing.

- Then 25µl of the hemolytic system, indicator (Amboceptor + SRBCs) was dispensed in all wells of rows A, B, C and D and then it was incubated in moist chamber at 37^0c with constant agitation for 30minutes.
- Finally the last dilution's column showing complete hemolytic and 50% haemolysis of SRBCs was read and recorded by comparing with the HS control. Then the average titres was taken and multiplied by the international unit (2.5-5) to get the working dilution of complement.

8.2. 2.4. The Test Proper

First of all before testing 200µl of sera was transferred in to the U-shaped micro-plate, then it was sealed by a plate sealer where it was de complemented in a water bath at 58^0c for 30minutes.

For CBPP and CCPP the sera are tested at 1:10 dilution but for Brucellosis the sera is tested at 1:5 dilution. The column A, B, C, D, E, F, G and H is made four rows 4×12=48 specimens can be tested in plate (see Annex 3.1).

The one-fifth dilution of serum for Brucellosis was prepared as follows: - Firstly 40µl of VCM was added in to row A1-A2, C1-C2, E1-E2, and G1-G2 and then 10µl of serum was transferred from the U-shaper micro plate, making the content a total of 50µl. Secondly it was homogenized and by 12 multichannel micropipettes 25µl of the solution was transferred in to B1-B2, D1-D2, F1-F2 and H1-H2 respectively. Based Anti-complementary controls (Ac), the test samples were always checked for anti-complementary reactions (No antigen was added in these wells as above).

After preparing one-fifth dilution of the sera the following steps were carried for accomplishment of the test as: - Firstly 25µl of diluted antigen at working dilution was added to the wells of rows B, D, F and H while 25µl of VCM was added to the wells of rows A, C, E and G; then the plates were covered by micro-plate sealers, to prevent evaporation and incubated at 37^0C in moist chamber for 30 minutes with constant agitation. Secondly a 25µl of complement at working diluted was added in to all wells and incubated for 30 minutes as above. Next to this a

25µl of HS (indicator) was added to all wells of the plate and again incubated at 37^0C for 30 minutes at constant agitation. Then the micro-plates were put in a refrigerator overnight and the result was read.

8.2.2.5. Interpretation

As an interpretation the test serum having SRBCs sedimentation at a dilution of ≥1:5 were considered to be positive for the disease; camel Brucellosis.

8.2.2.6. CFT plate layout on a U bottomed Micro Plate

	1	2	3	4	5	6	7	8	9	10	11	12		
A	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	No Ag	Row 1
B	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	Has Ag	
C	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	No Ag	Row 2
D	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	Has Ag	
E	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	No Ag	Row 3
F	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	Has Ag	
G	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	No Ag	Row 4
H	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	Has Ag	

8.3.Questionnaire formats for serum sampled individual camels

Formats 1: Serum sample collection format for individual camels

Region …… Zone…… District ……PA……Date……..

Code No.	Sex	Age	Herd size	Owner experience (year)	Breeding female history in the herd			Remarks
					calving	abortion	stillbirth	

8.4. Structured questionnaire format for camel owners

Formats 2.Questionnaires for individual camel owners Date…….. ……

I. General information

Region ……Zone ……District …… PA ……. Village……..

Name of respondent…….Age……Sex……Herd size: ……Owner experience (years) ……Family size……

II. Comparative importance of camels and its products

1. Types of livestock kept and purpose

Species	Purpose	More liked	Relative importance	Count
Cattle				
Camels				
Sheep				
Goats				
Equines				

2. What is the purpose of camel production?

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

3. Rank the use of camels:

a) Milk production …….
b) Transportations ……
c) Draught power ……
d) Cash income by sale……
e) e. Meat consumption……..

4. Herd composition or Herd inventory

a) Breeding females dry (non-pregnant)……pregnant…… Lactating……
b) Breeding bulls…… Castrated male……
c) Non-breeding males (below 5 years) ……non-breeding females……
d) UN weaned males……UN weaned females……

5. What is amount of camel milk used for?

5.1.Home consumption? (percent from total)

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

5.2 cash income by selling?

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

6. Milk consumption and preservation means

Descriptions	Fresh	Boil	Sour
Usually consumed			
Rarely consumed			
Delicacy / more liked			

6.2. What is the shelf life of camel milk (days)? ……

7. Do you slaughter camel at home? (Yes / No)……..if yes for what reason?

    a) for home consumption
    b) group share
    c) ceremony
    d) emergency slaughtering

8. How do you consume camel meat
    a) Cooked
    b) Raw
    c) other treatment

III. Herd dynamism

9. Animal entered the herd (born, purchased, gift in) or left the herd (Sold, dead, gift out,

Slaughtered, predator)

Animal Types	Animals entered the herd				Animals left the herd				Remarks
	This year		Last year		This year		Last year		
	No.	Reason	No.	Reason	No.	Reason	No.	Reason	
Breeding Female									
Young (F)									
Young (M)									
Adult (M)									
Calf (M)									
Calf (F)									

10. Do you do delivery assistance? **Y☐ ☐o**

If yes how do you do?

a) Hand pulling
b) Other means……

11. How do you take care for new born?

a) Cleaning newborn
b) Hand feeding of weak calves
c) Carrying newborn to home

IV. Herd management and health care

12. . Activities and labor divisions

Youngsters(Male, Female)	Adult(Male, Female)	Activities
		Herding
		Watering
		Delivery assistance
		Milking
		mating assistance

13. Water points in different seasons

Water Sources	Seasons (Dry, Wet)	Frequencies (days)
River Ponds		
Traditional wells		
others		

14. What is the main means of health care for your camels?

a. Traditional healer
b. self-administered vet drugs
c. Vet clinic

14.1 List and prioritize ten top camel diseases ……

……..

15.

Reproductive disease events in the herd (indicators of Brucellosis)	Events in the camel herd(Since one years since three years Yes or no)	Number	Remarks
Abortion			
Birth to weak calf			

Still birth			
Cycling female			
Bull with swollen tests and joints			
Retained placenta			

15.1 What do you think that cause abortion in camels?……

15.2 What do you do with camels that frequently abort?

a. Sell
b. Slaughter
c. Keeping
d. Others ……

15.3 How do you manage aborted fetus/ fetal membrane?

a. Leave in the field
b. Disposing
c. Give to dog
d. Others……

15.4 What do you do with female that doesn't conceive?

a. Sell
b. Slaughter
c. Keeping
d. Other ……

16. What is the source of bull?

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

17. How do you herd Camels?

a. Separately
b. with village herd

c. with cattle
d. with small ruminants

17.1 How is night resting?

a. Separate
b. share with cattle
c. Share with small ruminants

17.2. Have you ever sold breeding females? (Yes/No)…….. If yes what was the reason of selling

a. Disease
b. Infertility
c. Short age of money
d. Others……..

V. Breeding aspects

18. Comparative age of male and female (years)

Parameters	Weaning age	Age at first mating	Reproductive life	Life span
Male				
Female				

1.8.1 Age at first calving (Years)…… Gestation length (Month)……

Calving interval (Month)…… Lactation length (Month)…….

19. Seasonal variations of reproductive traits

Parameters	Calving (yes/no)	Mating (yes/no)	Milking frequencies	Milk off take per day (liters)	Fixing breeding time (yes/no)
Dry season					
Wet season					
both seasons					

8.5. Translated, Questionnaire formats for serum sampled individual camels, in to Somali language

9. Qaab dhismeedka suaalaha ee geela looxushay in laga qaado sambalada serumta ` ah

10. Shaxda 1: Qaab dhismeedka ururinta xogaha, geela looxushay in laga qaado sambalada serumta ah

11. Deegaan……Gobol……Degmo……Tuullo……
Taariikh……..

Sumad	Jinsiyadda	Da'da	Tirada xoolaha	Khibradda xoolo dhaqdaha (Sannad)	Hasha dhaloodiga ah xogteeda laxidhiidha dhalmada			Faallo
					Dhalmo	Dhicis	stillbirth	

8.6. Translated Structured questionnaire format for camel owners, in to Somali language

QAAB DHISMEEDKA SUAAL WEYDIINTA EE SHAKHSIYAADKA ISKA LEH GEELA LOOXUSHAY IN LAGA QAADO SAMBALADA SERUMTA AH

Shaxda 2: Qaab Dhismeedka Ururinta Xogaha, Ee Shakhsiyaadka Iska Leh Geela Looxushay In Laga Qaado Sambalada Serumta Ah

Date……

I. Warbixinta Guud

Deegaan……..Gobol……Degmo……PA……. Village……

Magacaxogbixiyaha……...Da'da…….Jinsiga……..Tiradaxoolaha……Khibrad da xoola dhaqdaha (Sanado) ……Baaxadda qoyska……

II. Isbarbardhigga faaiidooyinka geela iyo waxsoosaarkooda

1. Noocyada xoolaha iyo ulajeedooyinka loodhaqdo

Nooca faraca ee xoolaha ladhaqdo	Ujeedada loo dhaqdo	Syaabaha ugu badan ee looga faaiideysto	Siyaaba laxidhiidha siyaaba ugu badan ee looga faaiideysto	Tirada
Lo'da				
Geela				
Idaha				
Riyaha				
Fardaha iyo dameeraha				

2. Darajee ama ukala hormari sidey ukala muhimsanyihiin, siyaabaha looga faaiidaysto geela:

f) Waxsoosaarka caanaha …….
g) Muur ahaan…….
h) Iibin ayadoo lacag caddaan ah lagu bedelanayo……
i) Waxsoosaarka hilibka…….

3. Qaybaha ay ka kooban yihiin amma ay isugu jiraan xooluhu

e) Dhedigga aan rimaneyn……dhedigga riman…… dhedigga irmaan…….
f) Labka rimin kara dhedigga……labka la dhufaanka ah……
g) Labka aan wax rimin kara (eek a sareeya sadd)……..dhedigga madhalayska ah……
h) Labka aan laga gudhin naasaka……dhedigga aan laga gudhin naasaka

4. Waa intee qiyaas ahaan boqolkiiba caanaha aad ka heshaan geela, islamarkaana u isticmaashaan?

4.1.Qoyska dhexdiisa (boqolkiiba xaddiga guud ee caanaha)

e) 100%
f) 75%
g) 50%
h) 25%

4.2. Suuq geyn si looga helo lacag cadaan ah?

e) 100%
f) 75%
g) 50%
h) 25%

14. Iticmaalka caanaha iyo siyaabaha loo keydin karo

Noocyada isticmaalka	Lis ahaan	La karkariyey	La dhanaaniyey ama la gadhoodhiyey
markasta			
Mar mar			

14.1. Muddo intee le'eg ayaa la kaydinkaraa caanaha geela (maalmo)? …….

15. Geela ma ku gowracdaa gurigaaga? (Haa/ Maya)…….. haddii jawaabtaadu ay tahay haa sabab?

e) qoyska laf ahaantiisa oo quud ahaan uisticmaala
f) In loo wadaago koox ahaan
g) Marka ay jirto xaflad ama casuumad

h) xilliyada gurmadka degdegga ah

16. Sideed u isticmaashaa hilibka geela

Heerka da'da ee neefka	Animals entered the herd				Animals left the herd			
	This year		Last year		This year		Last year	
	No.	Reason	No.	Reason	No.	Reason	No.	Reason
Dhedigga loo xushay taranka(Dh)								
Da'yarta dhedig (Dh)								

d) Isagoo la kariyey
e) Caydhiin ahaan
f) Qaabab kale

III. isbedbedelka tirada xoolaha muddo laba sano gudahood ah

17. Neefafka lagusoo biiray xoolaha (dhashay, gatay,lasiiyey) ama laga saaray (iibiyey, dhintey, bixiyey, la gawracey, la ugaadhsadey)

Da'yarta lab (L)								
Qaangaadhka lab(L)								
(L)								
weysha (Dh)								

III. Maareynta iyo xannaaneynta xoolaha

18. Ma siisaa caawimo xilliga neefku dhalayo? ☐ **Ha** ☐ **Maya** haddii jawaabtaadu tahay haa sabab?
    c) Gacmaha kaga soo jiidid
    d) Siyaabo kale……

19. Sideed u xannaaneysaa neefafka cusub ee kuu dhasha?
    d) nadiifin
    e) gacmo ku quudin
    f) in looqaado neefka dhashay xagga guriga

20. qaybsiga hawlaha la xidhiidha dhaqashada xoolaha

Da'yarta qoyska (lab, dhedig)	Qaangaadhka qoyska (L, Dh)	hawlaha
		Xannaaneyn
		Waraab geyn
		ukaalmeyn xilliga dhalmada
		Ka lisid ama maalid
		Ukaalmeyn xilliga is abaahinta

21. Meelaha biyaha laga helo xilliyada kala duwan ee sannadka

Ilaha biyaha	Xilliyada (jiilaalka, Guga)	Inta jeer (maalmo)
barkadaha		
Ceel biyoodka		
kuwakale		

22. Waamaxay adeega caafimaad ee ugu muhiimsan ee ay geelaagu helaan?

d. Daawo dhaqameed
e. Isticmaalka daawooyinka xoolaha si iskaa ah
f. xarumaha daaweynta xoolaha

13.1. Tax islamarkaana kala hormari 10 xanuun ee ugu sareeya xanuunada geela ku dhaca.

14.

Dhacdooyinka laxidhiidha xanuunada ku dhaca habdhiska taranka ee xoolaha (indicators of Brucellosis)	Jiritaanka dhacdooyinkan ee geela (hal illaa saddex sano) (Haa ama Maya)	Tirada dhacdooyinka	Faallo
Dhicis			
Ilmo tabcaan ah			
Still birth			
Cycling female			
Dhikilo iyo kalagoys barar ku dhaca dibiga			
Madheer ku hadh			

14.1 Maxaad u malaynaysaa waxa sababa dhiciska geela?……

14.2 Maxaad ku samaysaan geela dhiciya markasta?

e. iibin
f. Gawrac
g. Siihayn
h. Kuwakale ……

14.3 sideed u maaraysaa neefka yar ee dhiciska ah ama xuubabka neefka dhiciska ah?

e. Waxaan uga tagnaa qaaliga
f. tuuris
g. Eydaa lasiiyaa
h. Kuwakale……

14.4. Maxaad kusmaysaan neefka dhedig e rimi waaya kaddib marka uu labku abaahiyo?

e. iibin
f. Gawrac
g. Siihayn
h. Kuwakale

15.xaggeed ka keentaan dibiga?

d. Isla xoolaha dhexdooda
e. tuullada
f. kuwa kale……..

16. Sideed u daajisaa geela?

e. Gaar
f. Wadajir (Iyaga iyo geela kale ee tuulada)
g. Wadajir (Iyaga iyo lo'aha kale)
h. Wadajir (Iyaga iyo adhyaha kale)

17.1 Waa sidee nasinta xooluhu xilliga habeenkii?

II. Gaar
III. Wadajir (Iyaga iyo geela kale ee tuulada)
IV. Wadajir (Iyaga iyo lo'aha kale)
V. Wadajir (Iyaga iyo adhyaha kale)

17.2. weligaa ma iibisay neef dhedig oo dhaloodi ah? (Haa/maya)……..haddii jawaabtaadu tahay haa maxay ahayd sababta aad u iibisay.

e. Cudur
f. Madhaleys
g. Lacag yaraan
h. Kuwa kale……..

V. Qaybaha tarminta xoolaha

18. Isbarbardhigga da'da labka iyo dhedigga (sanado)

halbeegyada	Da'da ka gudhinta	Abaahinta u horreysa	Nolosha habdhiska taranka	Life spanheerka nolosha amma cimriga
Lab				
Dhedig				

1.8.1 Da'da neefka dhedig xilliga uu dhalo neefka u horreeya (Sanado))…….

Mudada uurka (bilo)……..……

Mudada udhaxaysa labada dhalmo ee isxiga (bilo)……..

Mudada irmaanaanshaha (bilo)…….…….

19. kala duwanaanshaha xilliyada ee hidaha taranka neefka dheddig

Halbeegyo	Dhalmo (haa/maya)	abaahin (haa/maya)	Intajeer ee la liso	Qiyaasta Caanaha laga liso maalintiiba (Litirro)	Xilliga la cayimay ee tarminta xoolaha (haa/maya)
Jiilaal					
G'u					
labadaba					