JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

SEROPREVALENCE OF CAMEL BRUCELLOSIS AND ITS ASSOCIATED RISK FACTORS IN GUMBI BORDODE DISTRICT OF WEST HARARGHE ZONE, ETHIOPIA

BY

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Seroprevalence of Camel Brucellosis and its Associated Risk Factors in Gumbi-Bordode District of West Hararghe Zone, Oromia Region, Ethiopia

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BIOGRAPHICAL SKETCH

The author, Zanaba Jambare was born on November 25, 1991, G.C. from his father, Jambare Ijige, and his mother, Wudinesh Kirub, in Hareto 01 Kebele, Jimma geneti District of Horo Guduru Wellega, and Oromia Regional State, Ethiopia.

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STATEMENT OF THE AUTHOR

First, I declare that this thesis is my *bona fide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirement for an advanced (MVSc) degree at Jimma University, College of Agriculture and Veterinary Medicine, and is deposited at the University/College library to be made available to borrowers under the rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS AND ACRONYMS

AOR	Adjusted odd ratio
cELISA	Competitive Enzyme-Linked Immunosorbent Assay
CFT	Complement fixation test
COR	Crude odd ratio
CSA	Central Statistical agency
FAO	Food and Agricultural Organization
GBDLRDO	Gumbi-Bordode district livestock Resources development office
iELISA	Indirect Enzyme-Linked Immunosorbent Assay
LPS	lipopolysaccharides
Mbp	Mega base pair
MOA	Ministry of agriculture
OIE	World Organization for Animal Health
PCR	Polymerase Chain reaction
RBPT	Rose Bengal Plate Test
RER	Rough endoplasmic reticulum
R-LPS	Rough lipopolysaccharide
SAT	Serum agglutination test
S-LPS	Smooth lipopolysaccharide
SRBCs	Sheep red blood cells
STAT	Standard tube agglutination test
WHO	World health organization

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ABSTRACT

Camel brucellosis is an infectious bacterial disease of global public health and economic significance. However, there is limited information regarding the status of this disease in camels in the Gumi Bordode district. Hence, this study aimed to determine the seroprevalence and associated risk factors of camel brucellosis in the study area. To realize this, a cross-sectional study design with a simple random sampling method was employed from June 2023 to January 2024 in the Gumbi Bordode district of West Hararghe Zone, Oromia region, Ethiopia. A total of 384 serum samples were collected from five randomly selected kebeles along a questionnaire survey was administered to the herdsmen to obtain the required information. The registered data were encoded and entered into Statistical Product and Service Solutions software version 24 for statistical analysis. The overall seroprevalence of camel brucellosis confirmed by Complement fixation test at the animal level and herd level was 4.95%, (95% CI: 3.19-7.6%), and 23.75 % (95% CI: 15.76-34.14) respectively. Multivariable logistic regression analysis of potential risk factors at individual animal level showed that the age OR= 3.2, CI: 1.05-9.75, P-value=0.04), sex OR=3.89, (CI: 1.29-11.68, P-value=0.015), herd size OR=13.56, CI: 2.62-70.1), P-value=0.002) and keeping camels closely together with other ruminants (P-value=0.004, OR= 4.741, CI:.65-13.63) were significant. At herd level only herd size OR=3.16(1.53-8.99), P-value=0.013) was found to be the potential risk factors that significantly affect the seropositivity to Brucella infection in camels. Generally, camel Brucellosis was identified as an important disease in the study area. animal and management factors (sex, age, herd size, and keeping camels with other ruminants) were the potential risk factors that affected camel brucellosis. Thus, further investigation on the isolation and identification of camel brucellosis should be implemented to design effective prevention and control strategies in the study area.

Keywords: *Brucellosis, Camel, Ethiopia, Gumbi-Bordode, Risk Factors*.

1. INTRODUCTION

1.1. Background

Camels (*Camelus dromedarius*) belong to the Camelidae family and play an effective socioeconomic role in different parts of the world with arid and semi-arid climatic conditions (Alamian and Dadar, 2019). Eastern Africa is known to be the heartland for camel production as 80% and 63% of the African and world populations, respectively produced in the areas. Camels are a subset of huge livestock resources in Ethiopia with the population estimated to be 8.1 million camels (CSA, 2021a). The productivity of traditional camel production is constrained by several factors such as; diseases, husbandry practices, nutritional deficiency, and livestock mortality rates (CSA, 2021).

Camels are infected with different diseases including brucellosis, particularly when they are in contact with other infected ruminants.. In camel, brucellosis is mainly caused by *B. abortus,* and *B. melitensis* (Dehkordi *et al.,* 2012). Based on the reports of different scholars, *B. abortus* and *B. melitensis* are the most frequently isolated *Brucella* spp. from milk, aborted fetus, and vaginal swabs of diseased camels. Even though camels are not known to be the primary hosts of *Brucella,* they are susceptible to both *B. abortus* and *B. melitensis,* and consequently, the prevalence depends upon the infection rate in primary hosts being in contact with them (Robayo and Esubalew, 2017). *Brucella* bacteria can enter the body of animals through inhalation, ingestion, and through mucous membranes or broken skin (Fehlhaber, 2014).

In camels, brucellosis is characterized by abortion, infertility, placentitis in females, and orchitis and epididymitis in males (Jafer, 2018). Brucellosis is found to be one of the diseases associated with reproductive wastage in camel-producing pastoral areas. Significant loss of productivity through delayed first calving age, prolonged calving interval, low herd fertility, and comparatively low milk production in camels was reported, 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. Additionally, this disease has restricted livestock trade and free movement of animals (Abebe *et al*., 2017). For an accurate diagnosis of camel brucellosis, serological tests like the rose Bengal plate test (RBP) are cheap and easy for herd-based screening of animals with high sensitivity and low specificity, whereas tests like Ezyme linked immunosorbent assay (ELISA) and Complement fixation test (CFT) are used for the confirmatory test (Ullah, 2015). The disease is one of the oldest recognized diseases of mankind and is controlled in most developed countries (Sprague *et al*., 2012). However, controlling brucellosis disease in developing countries like Ethiopia is difficult due to the nature of the disease and economic resources, this is largely a result of a lack of facilities and budget to run such a program. Moreover, many responsible bodies may not recognize the significance of brucellosis given the contradictory and sometimes low prevalence data (Pal *et al*., 2017).

1.2. Statement of Problem

The economic and public health impact of brucellosis remains of particular concern in developing countries. The disease poses a barrier to trade of animals and animal products, represents a public health hazard, and impedes free animal movement (Khurana *et al* ., 2021). The role of specific etiologic agents such as *Brucella* species in causing abortion and reproductive loss in camels has been well established, as documented by Radostits *et al*. (2000), but known causes of abortion and female infertility involve a wide range of etiologic agents.

Ethiopia is one of several resource-constrained developing nations that are now dealing with other urgent illnesses that are more dramatic and have not yet completely implemented programs with any kind of intervention for brucellosis. Hence, brucellosis remains endemic and continues to be a major public and animal health problem in developing regions of the world (Godfroid *et al*., 2005). The disease can generally cause significant loss of productivity through abortion, prolonged calving, kidding, or lambing interval, low herd fertility, and comparatively low milk production in farm animals (Edao 2021). The disease could seriously impair socioeconomic development for livestock owners, which represent a vulnerable sector in rural populations in general and pastoral communities in particular. It has a significant public health implication for a pastoral community as a consequence of lifestyles, feeding habits, close contact with animals, low awareness, and poor hygienic conditions that favor infections (Obonyo 2018).

Zoonotic diseases, especially brucellosis remain a serious obstacle to public health. Brucellosis is even more ignored in humans and most cases go undiagnosed and untreated, leading to considerable suffering for those affected (McDermott *et al*., 2012). Previously the evidence of Brucella infections has been serologically demonstrated by different authors in sera of different domestic animals (cattle, camel, sheep, and goats) indicating that brucellosis is a major threat to livestock production and public health concern in Ethiopia (Berhe *et al*., 2007; Kebede *et al*., 2008; Mekonnen *et al*., 2010, Teshome *et al*. 2003; Megersa *et al*., 2005, Zewold and Mekonnen, 2012).

The highest prevalence was recorded in the Afar region by Hadush *et al*., 2013, 5.0% (RBPT) and 4.7 % (CFT). Seroprevalence in selected Regions of Somali, Afar, and Oromia; Teshome *et al*., 2003 reported 8.2% (RBPT) and 4.2% (CFT). Seroprevalence in Southern Ethiopia region; reported by Gumi, 2013, 0.9%. Seroprevalence in Borena pastoral; Megersa *et al*., 2011 reported 2.2%. Seroprevalence in Jigjiga and Babile; Tilahun *et al*., 2013 reported 2.43%, and War same *et al*., 2012 reported 2% (RBPT) and 1.5% (CFT) in Dire Dawa. A study conducted by Getahun and Belay,(2002) on camel husbandry practice in the eastern part of the country indicated abortion rates and stillbirths of 9% and 4.3%, respectively, for which brucellosis is more likely to be incriminated. This is due to a large number of different species of animals raised together on communal pastures and watering points which allows close contact of infected and healthy animals of different species (Tilahun *et al*., 2013). So far, there have been no previous studies on seroprevalence and associated risk factors of camel brucellosis in the current study area. Hence, the availability of this study was designed to address this limited information in the study area and introduce proper control and prevention measures against camel brucellosis for animal owners and communities of the areas, with the following objectives:

1.1.1. General objective

- ❖ To assess the presence of camel Brucellosis in the Gumbi-Bordode districts of the west hararghe zone, Oromia regional State, Ethiopia

1.1.2. Specific objectives

- To estimate the seroprevalence of camel brucellosis
- To assess potential risk factors that are associated with the occurrence of camel Brucellosis in the study areas.

2. LITERATURE REVIEW

2.1. Etiology

Brucellosis is caused by species of the bacterial genus Brucella, which are Gram-negative coccobacilli or short rods measuring from 0.6 to 1.5 µm long and from 0.5 to 0.7 µm wide, non-motile, non-spore-forming, non-capsulated, non-flagellated, aerobic, facultative intracellular bacteria capable of invading, survive and multiply within epithelial cells, placental trophoblasts, dendritic cells and macrophages(Gebretsadik, 2016). To date, twelve different Brucella (B.) species have been described. The six classical species are *B. melitensis, B. abortus, B. suis, B. ovis, B. neotomae,* and *B. canis. Brucella melitensis, B. abortus, and B. suis* are further classified into biovars (Rajala, 2016). Brucella melitensis is the main causative agent of caprine and ovine brucellosis and it is highly pathogenic for humans causing one of the most serious zoonoses in the world (Ferede *et al*., 2011). The more recently, identified new members of *brucella* species include; *B. ceti* and *B. pinnipedialis, B. microti* (voles), and *B. inopinata* (Godfroid *et al.,* 2011).

Brucella species are distinguished based on host preference and phenotypic characteristics (Seleem *et al.*, 2010). However, host preference is not absolute and most of the species of *Brucella* bacteria have been isolated in multiple different hosts (Potter, 2013). For instance, some *Brucella* species like *B. abortus, B. melitensis*, *B. Suis,* and *B. canis* can affect a range of hosts in addition to their natural hosts resulting in hazards to the health of animals including humans. Due to this, infected countries are challenged and have been under difficulties to overcome or control brucellosis effectively (Gutema, and Tesfaye 2019). In camels *B. abortus* and *B.melitensis* are the major causative agents of brucellosis even though camels are not found to be their primary host. Complete genome sequences of *B. abortus*, *B.melitensis, B. Suis, B. canis,* and *B. ovis* are available showing, their similarity in size and genetic make-up (Meng *et al*., 2009).

Brucella are generally susceptible to heat, direct sunlight, acidic conditions and common disinfectants. 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 an 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).

2.2. Host range

Brucella can affect domestic animals such as cattle, sheep, goats, camels, and pigs, and creates a serious economic problem for both the intensive and extensive livestock production systems in the tropics, and also affects wild animals, rodents, sea mammals, and even freshwater fish (Degefu *et al*., 2011). The severity of the disease depends upon many factors such as previous vaccination, age, sex, and management such as herd or flock size and density. Abortions are more prevalent in unvaccinated animals and the numbers of organisms shed are much greater. The bacteria are found in tissues and fluids associated with pregnancy, the udder, and the lymph nodes which drain the relevant areas (WHO, 2006).

Brucellosis has been reported in the one-humped camel (*Camelus dromedarius*) and the two-humped camel (*Camelus bactrianus*), and in the South American *camelids*, *llama* (*Lama glama*), alpaca (*Lama pacos*), guanaco (*Lamaguinicoe*), and vicuna (*Vicugnevicugne*) related to contact with large and small ruminants infected with *Brucella abortus* or *Brucella melitensis (*OIE,2013). Brucellosis in cattle is usually caused by biovars of *Brucella abortus*. In some countries, particularly in southern Europe and western Asia, where cattle are kept in close association with sheep or goats, infection can also be caused by *Brucella melitensis,* and occasionally, *Brucella suis* may cause a chronic infection in the mammary gland of cattle, but it has not been reported to cause abortion or spread to other animals. The disease is usually asymptomatic in non-pregnant animals (WOAH, 2013).

The susceptibility of cattle to *Brucella abortus* infection is influenced by the age, sex, and reproductive status of the individual animal. Sexually mature, pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex. Abortions occur most commonly in outbreaks in unvaccinated heifers after the fifth month of pregnancy, and affected bulls show orchitis, epididymitis, and seminal vesiculitis (Radostitis *et al*., 2007). Small ruminant brucellosis is most commonly caused by *Brucella meltensi*, and also *Brucella ovis* is an important cause of orchitis and epididymitis in sheep but it is not recognized as a cause of natural infection in goats (Kahn, 2005).

Porcine brucellosis is an infection caused by biovar 1, 2 or 3 of *Brucella Suis*. It occurs in many countries where pigs are raised. Generally, the prevalence is low, but in some areas, such as South America and South-East Asia, the prevalence is much higher. Porcine brucellosis may be a serious, but presently unrecognized, problem in some countries (Pérez Sancho *et al*., 2015). The most common manifestation of brucellosis in female pigs is abortion, occurring very early or at any time during gestation (OIE, 2013). Brucellosis in horses is caused by *Brucella abortus* or *Brucella Suis*. Supportive bursitis, most commonly recognized as fistulous withers or poll evil, is the most common condition associated with brucellosis in horses (Kahn, 2005)

Table 1: *Brucella* species and their hosts.

Organism	**Host**
B. melitensis	Sheep, Goat and Camel
B. abortus	Buffalo, Cows and Camels
B. canis	Dog
B. suis	Pig
B. neotomaei	Rodent
B. ovis	Sheep
B. pinnipediae	Marine animals
B. cetaceae	Marine animals

Source: (Radostits, 2006).

2.3. Epidemiology of Camel Brucellosis

2.3.1. Global distribution of camel brucellosis

Brucellosis is reported from all the countries that rear camels for their milk and meat (Gwida *et al*., 2012). The occurrence of this disease in camels is closely related to husbandry and breeding practices (Omer *et al*., 2010). Brucellosis was reported in camels as early as in 1931 by Solonitsiun in Russia. Since then, serological evidence of brucellosis has been reported from the most important camel keeping countries (Bayasgalan *et al*., 2018). According to Gizaw *et al.*, (2017), a seroprevalence of camel brucellosis ranging between 2% to 5% was reported in most countries where camels are still kept by nomadic or transhumant pastoralists and extensive form of husbandry is practiced. Additionally, Gul and Khan, 2007 reported seroprevalence of camel brucellosis ranging from 0.0- 17.20% in Arabian and African countries where the disease also occurs in buffalo and other domestic animals. The differences in the prevalence of camel brucellosis husbandry and management practices, the number of susceptible camels, the virulence of the organisms, presence of reactor animals in the region, absence of veterinary service, lack of awareness about the disease in camels and continuous movement of infected camels into a susceptible herd (Gwida *et al.*, 2012).

2.3.2. Distribution of camel brucellosis in Africa

Many countries of Africa have also reported evidence for Brucellosis in camel populations. In the arid region of Nigeria, the camel population infected by Brucellosis was found to have overall prevalence of 7.6% and 11.9% by Complement Fixation Test and RBPT out of 768 camels screened by CFT and RBPT, respectively (Zewolda and Wereta, 2012). Brucellosis is prevalent in camels of East Africa at a rate of 1.9 - 40.5% (Wakene and Mamo, 2017). High prevalence among animals and in herds has been reported in different countries, which poses a severe risk to humans and other livestock. In countries such as Chad and Ethiopia having more extensive forms of camel husbandry, the seroprevalence of Brucellosis is 3.8% respectively (Wernery, 2014).

2.3.3. Distribution of camel brucellosis in Ethiopia.

Ethiopia has the largest pastoral population of 7 to 8 million and the majority of these people are living in the Ethiopian Somali and Afar administrative regions (Bekele *et al.*, 2013). In Ethiopia, brucellosis has been reported in camels from pastoral areas, where the prevalence was quite varied ranging between 0.73- 11.9% for RBPT and 0.53-9.6% for CFT (Yilma *et al.*, 2016). This variation in seroprevalence of camel brucellosis is attributed to the difference in animal husbandry and management systems practiced by pastoral societies (Awole *et al.*, 2002). In Ethiopia, pastoralists used to consume raw dairy products through infected raw or undercooked meat, unpasteurized milk, butter, and cheeses which contributed to the transmission of this disease among humans and animals. 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).

Table 2: Seroprevalence of Camel Brucellosis in Ethiopia

Study Area	Diagnostic test	Prevalence	References
Afar	RBPT	11.9%	(Zewold and Mekonnen, 2012)
	CFT	7.6%	
	mRBPT	5.4%	(Bekele *et al*., 2013)
	CFT		
	RBPT	5.8%	(Hadush *et al*., 2013)
	CFT	47%	
	RBPT	2.09%	(Gebrezgabher and Mohammed, 2016)
	CFT		
Somali, Afar and Oromia	RBPT	8.2%	(Teshome *et al*., 2003)
	CFT	4.2%	
Southern Ethiopia	RBPT	0.9%	(Gumi, 2013)
	ELISA		
Akaki	RBPT	6.5%	
	CFT	4.5%	(Abebe *et al*., 2017)
Jigjiga and Babile	RBPT	2.43%	(Tilahun *et al*., 2013)
	CFT		
Dire dawa	RBPT	2%	(Warsame *et al*., 2012).
	CFT	1.5%	
Borana	RBPT	2.2%	(Megersa *et al*., 2011)
	CFT		

2.4. Risk Factors

2.4.1 Host and pathogen risk factors

Brucella infection is influenced by the Age, Sex and reproductive status of individual animals. Sexually mature pregnant 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 a 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. The presence of organic matter and low temperature decrease the efficacy of disinfectants (Sprague *et al*., 2012).

2.4.2. Environmental and Management risk factors

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. Camel brucellosis caused by *B. aborts* or *B. melitensis* biovars can be encountered in all camel rearing countries. High individual animal and herd prevalence’s 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 the 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 the 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 herd with cattle and camels the prevalence rates of the disease were higher than those herded separately (Isam, 2016).

2.5. Transmission of Camel Brucellosis

The primary shedding routes of Brucella organisms remain uterine fluids and placenta expelled from infected animals. Due to this, both domestic and wild animals can contract brucellosis through direct contact with infected animals and their excreta. Many placental mammals, including herbivores, participate in placentophagy, with camels as a noted exception, which may contribute to the spread of Brucella bacteria through wind. Although parturition in camels generally occurred in a laying or standing position without extra help, they may deliver or abort on the pasture and the aborted material may spread over a wide area of the pasture by stray dogs and foxes. These play an important role for the transmission of the disease to other health animals (Gwida *et al*., 2012).

On the other hand, a close contact between infected and susceptible camels in a herd promotes the spread of diseases. The camels share the same watering points and pastures with other livestock and so it is not surprising to find a higher incidence of the disease among camels (Teshome *et al*., 2003). Generally, Animals become infected through feed, water, colostrum, contaminated milk and, especially, by licking or sniffing at placentas and aborted fetuses. In humans, brucellosis is transmitted by contact with infected animals, consumption of unpasteurized dairy products and undercooked meat, drinking camel urine (Salisu *et al*.,2018) including aerosol transmission (Minogue *et al*., 2014). For instance, consumption of traditional delicacies such as raw liver can cause human infection (Gwida *et al*., 2010).

2.6. 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 lymphadenitis which may persist for months. Once in the bloodstream, the organism disseminates to multiple organs, thereby displaying an affinity for reticuloendothelial tissues, such as the liver, spleen, the skeletal, and 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 by 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).

2.7. Clinical Sign

The clinical picture of brucellosis in camels can vary from asymptomatic to abortion (Musa *et al.*, 2008). According to various researchers, the clinical signs of brucellosis in breeding camels are the same as those in bovines and small ruminants, although infection in breeding camel causes fewer abortions than it does in bovines and small ruminants. Abortion in camel due to brucellosis usually occurs only once. Dams can develop ovario-bursal adhesions, hydrobursitis, and granulomatous endometritis. Placental retention, infertility, and delayed sexual maturity have also been reported (Rafieipour and Ziaei, 2011). Males may suffer from orchitis, infection of the accessory sex glands, arthritis accompanied by acute lameness (Sprague *et al*., 2012).

Some authorities feel that the most significant result of infection may be premature birth. Brucellosis also causes fetal death and mummification and reduced milk yield. It was reported that delayed service age and fertility are also another complication associated with brucellosis. However, placental retention is rare in camels due to the difference in the placental attachment as they possess a diffuse like placenta (Fowler *et al*., 2010).

2.8. Diagnosis

Establishment of adequate control programs against brucellosis in a population depends on the presumptive diagnosis of the infection. Brucellosis may be suspected based on clinical signs such as abortion, but confirmation can be made through serological tests. Since 1897, a considerable number of serological tests have been developed. A number of these tests were modified in various ways to increase performance (Nielsen, 2011). Serological tests offer best alternatives to culture and isolation methods of diagnosis since the tests are easy to perform, less risky and provide results within a short period. On the other hand, brucellosis can be diagnosed definitively by isolation and identification of the causative organism. This was first reported by Bruce and coworkers in 1887 when they isolated B. melitensis from military personnel in Malta (Nielsen, 2011).

The diagnosis of brucellosis by culture and isolation of organisms from clinical samples is the gold standard method. But this method is laborious, time consuming, and risky, whereas the outcome of the test depends on the competence of the laboratory personnel. In clinical brucellosis, valid samples to diagnosis the disease include aborted fetuses (stomach, spleen, and lung), fetal membranes, vaginal secretions, colostrum, milk, sperm, and fluid collected from arthritis or hygroma (Godfroid *et al*., 2010). At slaughter, in order to confirm suspected cases of acute or chronic brucellosis, the preferred tissues are the genital and oropharyngeal lymph nodes, the spleen, and the mammary gland and associated lymph nodes (Godfroid *et al*., 2010). The presence of anti Brucella antibodies suggests exposure to Brucella spp. But it does not indicate which Brucella spp induced production of those antibodies (Godfroid *et al*., 2010).

2.8.1. Bacteriological diagnosis

This refers to isolation and identification of Brucella from clinical samples. The morphology of the Brucella bacterial colonies is associated with the presence of lipopolysaccharides (LPS) in the external membrane of the bacterium. Smooth (S-LPS) and rough (R-LPS) phenotypes are differentiated. The S-LPS phenotype is found in most Brucella species, and only B. canis and B.ovis possess the R-LPS (Wernery, 2014).

Brucellosis is usually diagnosed in the laboratory by the culture of blood, milk or tissue or the detection of antibodies in sera. Brucella organisms can be recovered from the placenta, but, more conveniently, in pure culture from the stomach and lungs of aborted fetuses. For isolation of brucella, the recommended medium is Farrell's medium, which contains six antibiotics. But other selective Brucella media are also in use for the growth of this pathogen from fresh Camel milk and other tissue samples (Radwan *et al*., 1995).

2.8.2. Serological diagnosis

The majority of studies on camel brucellosis use serological methods for diagnosis. But none of the serological tests are validated for use in camels yet, as acknowledged by OIE. Similarly, none of the tests have been validated for the diagnosis of human brucellosis, according to (Yohannes *et al*., 2012). However, it was found that a combination of different serological tests can increase diagnostic efficacy in camels, although none of the serological tests can differentiate between a B. abortus or B. melitensis or B. suis infection. On the other hand, false-positive or unspecific reactions with various other bacterial species such as Yersinia enterocolitica serotype O: 9 can occur (Wernery, 2014).

Rose Bengal Plate Test (RBPT)

Among many types of serological test employed for diagnosis of brucellosis in camels and other domestic animals, RBPT is a widely used screening test for regulatory control and export requirements. Rose Bengal Plate Test (RBPT) is one of a group of tests known as the buffered Brucella antigen tests which rely on the principle that the ability of IgG antibodies to bind to antigens is markedly reduced at a low pH (Hotam Singh Chaudhary, 2011). RBPT is a very sensitive test and is suitable for screening herds for brucellosis, but it can give false positive results due to vaccination with B. abortus strain 19 vaccine or cross-reactions with other bacteria (Omer *et al.,* 2010). The RBPT has been reported to have high sensitivity; therefore false negative responses are reported to occur less frequently than false positive responses (Omer *et al.*, 2010).

It was reported by (Chachra *et al*., 2009) that, among the commonly used conventional serodiagnostic tests for brucellosis, RBPT and Standard tube agglutination test (STAT) may not be reliable. RBPT detected antibodies in the sera of 50% of the animals suspected of brucellosis whereas the standard tube agglutination test (STAT) could detect only 5.55% of cases according to (Chachra *et al*., 2009) report.

Complement fixation test (CFT)

The Complement Fixation Test (CFT) allows the detection of anti-Brucella antibodies that can activate complement. Many authors regarded the CFT as being the most sensitive and specific test for brucellosis diagnosis. Because CFT antibodies remain in the serum for a longer time than Serum agglutination test (SAT) antibodies (Njeru *et al.,* 2016). On the contrary, some authors disclosed that this test is not highly sensitive but shows an excellent specificity. In recent CFT is progressively being replaced by ELISAs since it is difficult to be standardized. Nevertheless, CFT is a “prescribed test for trade” by the WOAH (*Godfroid et al.*, 2010).

Enzyme Linked Immunosorbent Assay (ELISA)

ELISAs are divided into two categories, the indirect ELISA (iELISAs) and the competitive ELISA (cELISAs). Most iELISAs use purified smooth LPS as antigen and detect mainly IgGs or IgG sub-classes. Their main quality is their high sensitivity but they are also more vulnerable to non-specific reactions, notably those due to Yersinia enterocolitica serotype (YO9) infection (Godfroid *et al*., 2010). ELISA was first developed for the diagnosis of human brucellosis. The ELISA tests offer an excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form. A comparison with the SAT, ELISA yields higher sensitivity and specificity. ELISA is also reported to be the most sensitive test for the diagnosis of neuro-brucellosis (Miguel *et al*., 2006) .The omp28 protein is now being used in an indirect plate ELISA system and has been evaluated with good sensitivity and specificity on large number of clinical samples (Hotam Singh Chaudhary, 2011).

2.8.3. Molecular Methods

Polymerase Chain reaction (PCR)

The isolation of Brucella organisms is still the preferred method of diagnosis. But, the PCR method allows typing of the isolated strains. PCR based assays have been developed for brucellosis diagnosis and are based on the detection of specific gene sequences of the pathogens. One of the first PCR assays to differentiate among Brucella spp was called AMOS- PCR, developed by Bricker and Halling in 1994. This PCR uses a single reverse primer, targeting the Brucella specific insertion element IS711 (Ewalt and Bricker, 2000). Even though PCRs can distinguish between Brucella species and between wild and vaccine strains, it does not distinguish between Brucella biovars. In recent time new PCR techniques are being implemented for both identification and phenotypic biotyping (Ron-Román *et al*., 2019).

2.9. Treatment

As a general rule, treatment of infected livestock is not attempted because of the high treatment failure rate, cost, and potential problems related to maintaining infected animals in the face of ongoing eradication programs (Yousefi-Nooraie *et al*., 2012). In developed countries, treatment of infected animals is not a common practice. However, the infected animals are isolated, culled or slaughtered to prevent the spreading of infection to another herd. Even though the complex nature of brucellosis makes it difficult to treat, long term treatment with an antibiotic is thought to be beneficial to care for economically valuable breeding male animal and must be instituted before irreparable damage to the epididymis has occurred (Alemneh and Akeberegn, 2018). Humans are treated with antibiotics (doxicycline with rifamipicine) even though relapses are possible (Solis and Solera, 2012). Several conventional antibiotics including tetracyclines, trimethoprim, sulfamethoxazole, amino-glycosides, rifampicin, quinolones, chloramphenicol, doxycycline, and streptomycin are commonly used in clinics (Saltoglu *et al*., 2002). The World Health Organization recommends that acute brucellosis cases should be treated with oral doxycycline and rifampin (600 mg for six weeks) (Ersoy *et al*., 2005).

However, rifampin monotherapy is in common practice for treating brucellosis in pregnant women, and combined therapy of sulfamethoxazole and trimethoprim is recommended for children (Karabay *et al.*, 2004).

2.10. Public Health Importance of Camel Brucellosis

Brucellosis is a systemic infection that can involve any organ or organ system of the body. Since many cases of brucellosis go unrecognized, the true incidence of the disease is unknown. In human, the disease is common in rural and pastoral areas, because farmers or pastoralists live in close contact with their animals and often consume fresh unpasteurized dairy products. In addition, pastoralists handle aborted cases with bare hands which is the main predisposing factor of the disease in the area (Zewdie and Mamo, 2018). Food producing animals such as cattle, sheep, goats, pigs and camels are also the main sources of brucellosis to human beings (Potter, 2013). The type of Brucella to which an individual is exposed is a significant determinant factor of the risk of disease and its severity in humans. This will be influenced by the species of host animal acting as source of infection (Corbel, 2006).

Person to person transmission of brucellosis can rarely occur among innocent camel herders through close personal or sexual contact while occupational exposure usually is resulting from direct contact with infected animals, and food borne transmission (Zewdie and Mamo, 2018). Blood donation/tissue transplantation and bone marrow transfer are the prominent interpersonal transmission ways of brucellosis. Even though, *B.abortus, B. suis* and *B. canis* are considered as potential causative agents of brucellosis in human, *B. melitensis* is the most virulent brucella with a few organisms (10 to 100) being sufficient to cause a debilitating chronic infection(Xavier *et al*., 2014).

Brucellosis may present with acute or insidious onset, with continued, intermittent or irregular fever of variable duration, profuse sweating, fatigue, anorexia, weight loss, headache, arthralgia and generalized aching. Abscess formation is a rare complication (Seleem *et al*., 2010). Brucella endocarditis and neuro-brucellosis cause most deaths (Pendela *et al*., 2017).

Sometimes, the manifestations of brucellosis are more pronounced in a specific organ system. The most common local manifestations are: spondylitis, peripheral arthritis (especially of the hip, knee and shoulder) and epididymo-orchitis (Colmenero *et al*., 1996). Arthritis and joint pain are common and usually migratory in character, affecting mostly the large joints, with unilateral joint involvement being more common among the younger age group (Memish and Balkhy, 2004).

In human's brucellosis is essentially acquired by the oral, respiratory, or conjunctival routes, but ingestion of raw contaminated milk constitutes the main risk to the general public where the disease is endemic. Though camel milk ingestion is a known mechanism for brucellosis acquisition, only a few reports of sporadic cases have been published in the medical literature (Shimol *et al*., 2012). On the other hand, traditional types of food animal slaughtering in non-hygienic methods are common practices which definitely downgrade the hygiene, safeness and Wholesomeness of food of animal origin. Consumption of such contaminated food which may contain Brucella bacteria has the potential to cause an adverse health effect (Desta, 2016).

Somali regional state and Afar pastoralists do 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. So, these practices could potentially facilitate the transmission of zoonotic Brucella pathogens from camel to human. They also believed that camel milk to possess superior shelf life, medicinal properties (against dropsy, jaundice, diabetes and glycaemia) (Bekele *et al*., 2013). Generally, human brucellosis is increasing in Ethiopia like many other developing countries due to various sanitary, socio-economic, and political factors (Pappas *et al*., 2006). Thus, collaborative work of different stakeholders to prevent and control the disease as well as to enhance public awareness level of camel keepers is required (Catley *et al*., 2005).

2.11. Prevention and Control of Camel Brucellosis

According to Zhang *et al.,* 2018 report, brucellosis control or eradication programs, the implementation of the programs, and the control measures in different countries vary greatly depending on their national conditions. Even with the high economic burden of the disease in many low-income countries, the disease does not attract the appropriate attention of national health systems (Godfroid *et al*., 2013). Although brucellosis has been controlled in most industrialized nations, the disease has become a neglected zoonosis in some tropical or developing countries due to a lack of sustainability in the disease prevention and control programs (Ekere *et al*., 2018).

In the developed world, the control of animal brucellosis has been approached with a combination of procedures such as: vaccination, test, and slaughter programs whereas human brucellosis through milk pasteurization and hygienic measures coupled with effective disease surveillance and animal movement control (Godfroid *et al*., 2011). Control of camel brucellosis should be tailored to suit conditions in the particular countries where camels are raised. Most of these countries are poor and camels are raised by nomadic tribes. So control of camel brucellosis can be achieved through extending veterinary services to pastoral areas (Abbas and Agab, 2002).

2.11.1. Vaccination

Animal brucellosis control strategies differ in the developed and developing world. In the developed world, most emphasis is given to eradication and risk analysis to avoid the re-introduction of Brucella while information related to the prevalence of brucellosis is still scarce and control programs are rarely implemented in the developing world (Franc *et al.,* 2018). However, vaccination is the cornerstone of control programs to prevent brucellosis in livestock in both developed and developing world. So, serious efforts of vaccination have been made to prevent the infection through the use of vaccines (Wernery, 2014). Before vaccination is started in camels, thorough investigations are paramount important, in order to find out whether the animals are naturally infected by B. abortus or B. melitensis and this can only be determined by culture or PCR. An eradication campaign in camels may also be based on vaccination (Wernery, 2014).

Generally, the main approach in a long term control strategy of brucellosis is to vaccinate only female replacement camels less than 1-year-old. After several years, this strategy will establish an immunized herd and will not induce abortions. It will also protect these herds from brucellosis threat by surrounding positive livestock (Castillo *et al*., 2016).

2.11.2 Test and slaughter

Test and slaughtering of positive animals is only successful in reducing the incidence if the herd or flock prevalence is very low (Luelseged, 2019), which is feasible only in the developed world. The decision about slaughtering of test positive animals is made after regulatory, economic and prevalence factors are considered. In developing countries, the isolation of test positive animals is essential, especially during and after parturition since immediate slaughtering of test-positive animals is expensive and requires animal owner cooperation (Luelseged, 2019).This indicates that, camel producers in developing countries cannot afford the traditional test and slaughter approach especially when expensive animals with high genetic potential are involved (Radwan et al., 1995). Furthermore, the application of test and slaughter policies works well only under reliable diagnostic tests to avoid unnecessary decisions due to false positivity (Alp *et al*., 2006).

2.11.3. Hygienic prophylaxis

Application of hygiene measures to the control of brucellosis become successful through the reduction of exposure of susceptible animals to those that are infected, or to their discharges and tissues. This is a classical procedure in disease control. Factors such as the methods of animal husbandry (e.g., commingling of herds or flocks), patterns of commerce, type of facilities, and degree of dedication of the owners of animals, will also determine success. However, owners have poor understanding about the transmission route of brucellosis in camels so that separation of parturient animals can be difficult or even impossible to implement (Glynn and Lynn, 2008) which is a conspicuous existing gap.

2.12. Economic Impact

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 which are not only consumed locally, but also exported to numerous countries (Ahmed *et al*.,2010). Consequently, a disease like brucellosis, besides having a major impact on a country's economy, can have devastating effects on human health (McDermott and Arimi, 2002).The economic impact of brucellosis on camels can be estimated on the basis of losses due to morbidity and mortality and by estimating treatment costs (McDermott and Arimi,2002).

Brucellosis causes heavy economic losses in camel production as a result of abortions, sterility, mastitis, decreased milk production, veterinary attendance and more importantly due to interference of free animal and animal product trade ((Kulplulu and Sarimehtoglu, 2004; Al-Majali, 2005). The common sequel of infertility increases the period between lactations in infected herds, and the average inter-calving period may be prolonged by several months (Radostits *et al*., 2000). Added to the above losses is the economic impact of human brucellosis. The costs associated with medical care of Brucella infected humans and the duration of time the infected people are out of work account for financial losses (Refai, 2002).

Brucellosis seriously impairs public health and socio-economic development. This holds true especially for livestock owners, who represent a vulnerable segment of the community in many rural populations (Bochiroli *et al.*, 2003). Generally, heavy economic losses associated with brucellosis emanate from: Losses of calves due to abortion, reduced milk yield Culling of valuable animals because of reproductive problems, Endangering animal export / trade of animal products, Loss of man-hours and medical costs, Government cost incurred for research and eradication programs.

3. MATERIAL AND METHODS

3.1. Study Area

The study was conducted in the Gumbi-Bordode districts of west hararghe Zone, Oromia Regional State, Ethiopia. The study districts were selected purposely based on the camel population and accessibility to vehicles. Bordode town is located at a distance of 73 km from west Hararghe capital Chiro town, and 275 km from country centers, Addis Ababa. The district comprises about 29 Kebeles of which 11 (38%) kebeles and 18 (62%) kebeles of the people dwelling in and around the district practicing pastoral and agro-pastoral activities, respectively. Camels were sampled from five Kebeles namely Sama, Gumbi, B/anani, Gololcha, and Obensa. Pastoral communities mainly rear and derive most of their income from livestock; whereas, agro-pastoralists are segments of pastoral communities who promote opportunistic crop farming integrated into their livestock husbandry practices. The area was selected based on a gap of no previous published literature on the prevalence of camel brucellosis.

Geographically the district is located at a latitude of about 90°09’49″ N and longitude of about 39400°38’01″ E and an elevation ranging 800-2580 meters above sea level. The mean annual maximum and minimum temperatures are 40 and 15°C, respectively. The climate is generally semi-arid with annual average rainfall ranging from 400 mm in the south to >700 mm in the north. According to the West Hararghe Zone Department of Agriculture Bureau (unpublished), the total camel population of West Hararghe Zone and Gumbi-Bordode district were estimated to be about 232,589 and 58,896, respectively.

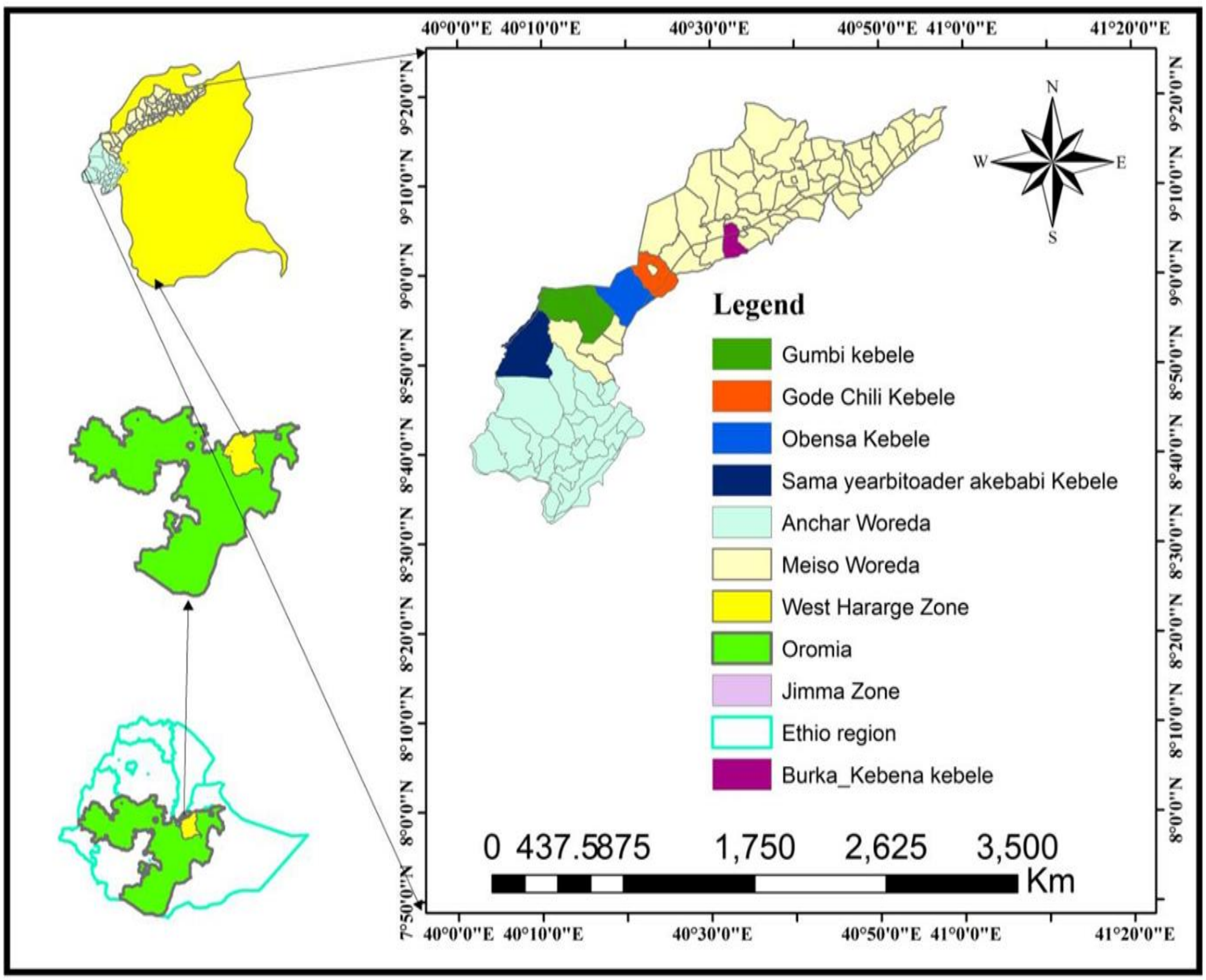


Fig 1. Map of study area

3.2. Study Design and Period

A cross-sectional study was carried out from June 2023 to January 2024 in Gumbi-Bordode districts, to estimate the seroprevalence of Brucella infection in camels in the selected districts and to identify the potential risk factors associated with the seropositivity. Sex, age, herd size, and contact with other ruminants' kebeles, body condition score at the individual level and herd size, the introduction of new animals, and history of abortion at the herd level were considered potential cause risk factors for brucellosis.

3.3. Study population

The sources of the study population were camels in the Gumbi-Bordode district. The total camel population of the district is estimated to be about 58, 8960. The camels under study were the local breeds with no history of vaccination against brucellosis. Both sexes and different age groups greater than six months were included in the study as the disease was not common in camels less than 6 months of age due to maternal antibodies. The camels were classified into two age groups; the young age group (less than 5 years old), and the adult age group greater than 5 years old (Kadim 1998). Herds consisting of 3-15, 16-25, and >25 camels were considered as small, medium, and large herds, respectively (Ghanem *et al*., 2009).

3.4. Sampling Method and Sample Size

3.4.1. Sampling method

The study districts were selected purposely based on the camels' population and accessibility to vehicles. A simple random sampling technique was used to select kebeles. However, a sample size of kebeles was calculated based on the number of the size of their camels' population proportionally and known camel-rearing pastoralist communities in the west hararghe zone. Thus the total population of camels was 10187, 8810,9908,2977,2918 in Sama, Gumbi, B/anani, Gololcha, and Obensa respectively. In this study 112, 98, 109, 33, 32 samples were taken from Sama, Gumbi, B/anani, Gololcha, and Obensa respectively. The households having greater than three camels from kebeles, and the number of household selected was dependent on the amount of sample determined for each kebeles, from each households/herd, One-fourth of camels was selected using the simple random procedure.

Therefore, 384 camels were sampled. Sampling was proportionally distributed based on the total camel population in the study kebeles. Moreover, a total of 80 willingly selected pastoralists were included in the study for the purpose of a questionnaire survey. Sera were collected from the camels, and then, questionnaires were administered to each randomly selected livestock owner

3.4.2. Sample Size Determination

The average expected prevalence rate was assumed to be 50% for the area within 95% Confidence Intervals (CI) at 5% desired absolute precision. Hence, sample size calculation was performed based on Thrusfield (2005), using the formula.

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

Where n = sample size, d = (0.05),

Pex = expected prevalence (50%), thus the

The desired sample size for Pex = 0.5 is n = 384.

3.5. Blood Sample collection

About 7 ml to 10 ml of whole blood was collected from the jugular vein of the randomly selected camels using disposable plain vacationer tubes and needles. Before commencing the draining of the blood sample, each animal was isolated and restrained properly to avoid unexpected personal injury and to minimize unnecessary stress that might happen to the animals. After shaving and disinfecting the site of the jugular vein, 10 ml of blood sample was collected into a sterile plain vacationer tube from each study animal. The sample was labeled by using code describing herd number, and kebeles were placed in the rack by maintaining protection from exposure to direct sunlight and extreme temperature and left for 24 hours at room temperature to allow clotting serum samples were separated after 24 hours gently transferring to cryovials and stored at -20 ^{O}C at Gumbi-Bordode district Livestock resource and development office veterinary clinic laboratory until submitted to Sebeta Animal Health Institute. Finally, the serum samples was transported in to sebeta animal health institute with an ice box and submitted for laboratory examination and serum samples was analyzed using

Rose Bengal plate test (RBPT) and complement fixation test (CFT) was be used for screening and confirmatory test of sera respectively.

3.6. Serological test

3.6.1. Rose Bengal Plate Test

The RBPT test was carried out according to the method recommended by OIE, (2004). The antigen used for RBPT, was RBPT antigen. Antigen and sera required for each day for serological testing were taken out from the cold storage (in a refrigerator at -40C) and brought to room temperature for 30 minutes before testing takes place. Briefly, 30µl of stained rose Bengal antigen was dispensed onto the card plate, and then 30µl 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 was 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.

3.6.2. Complement Fixation Test

All sera which was tested positive by the RBPT were further retested, using the CFT, for confirmation, and the CFT test was done once at Sebeta Animal Health Institute. Standard *B.abortus* antigen for CFT (from the Veterinary Laboratories Agency, Addle Stone, United Kingdom), Amboceptor and sheep red blood cells (SRBCs). The detailed procedure of CFT and RBPT was indicated in (Annex 3), one at Sebeta animal health Institute was used to detect the presence of brucella antibodies against brucella antigen in the sera. A titration of hemolysin and antigen was performed according to protocols recommended by World Organization for Animal Health (OIE, 2008). The minimum hemolytic dose was also estimated for each run. As for the interpretation of test results, positive reactions were indicated by sedimentation of Sheep Red Blood Cells (SRBC) and absence of hemolysis. Negative reactions were revealed by hemolysis of SRBC. According to OIE (2009) sera with strong reaction, more than 75% fixation of complement at a dilution of 1:10 and at least with 50% fixation of complement at a working dilution (1:5) was classified as positive.

3.7. Questionnaire survey

A questionnaire survey was administered to respondents and the objectives of the survey was explained to them before the start of the interview. The interviews were conducted in the local language (Afaan Oromo). Semi Structured questionnaire format for the herders was developed and used in this study. The questionnaire focused on camel's history such as herd size, and contact with other ruminants, composition of camel herds, camel rearing experience, camel management, milk consumption habits and purpose of camel rearing and knowledge about brucellosis. Pastoralists from Gumbi-Bordode whose animals tested for brucellosis was interviewed. In doing so, the risk factors that have possible associations with the occurrence of brucellosis was investigated and used to support the serological result.

3.8. Data Management and Analysis

The data were entered into Microsoft Excel and coded data were stored and finally the data collected through questionnaires and laboratory results transferred to SPSS® version 24 for statistical analysis. Descriptive statistics were used to analyze the sero-positivity of individual animals. The seroprevalence was calculated as the number of serologically positive samples divided by the total number of samples tested. Univariate analysis was to select factors with p-value less than 0.25 for multivariate analysis. Multivariable logistic regression was used to determine the association between explanatory variable (risk factor) and the dependent variable (serological status of the camels). Finally the degree of association was computed using odds ratio (OR), signified by 95% confidence intervals and 5% absolute precision by taking a p-value less than 0.05 as significant.

3.9. Ethical Consideration

The protocol for field studies and collection of blood sample from camel and materials was approved by the animal research ethical review committee of the Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) with certificate reference number A15/may/2023. For the questionnaire survey, pastoralists were informed, the aim of the study, and their verbal consent was sought before the commencement of data collection

4. RESULTS

4.1. Seroprevalence of Camel Brucellosis at Individual Animal Level

In the current study, the overall seroprevalence of camel brucellosis at individual animal level was 4.95% (95% CI=3.19-7.6) based on RBPT confirmed by CFT and Rose Bengal plate test alone detected 36 (9.37 %(95% CI=6.85-12.7) of the samples as seropositive. Upon further testing by CFT, only 19(4.95% (95% CI=3.19-7.6)) sera were left positive. (Table 3).

Table 3: Prevalence of camel brucellosis by RBPT and CFT concerning Kebeles

Kebeles	Animals	Positive		Prevalence with 95% CI	
	Tested	RBPT	CFT	RBPT	CFT
Gumbi	89	11	7	12.36(7.4-20.27)	7.87(3.86-15.36)
Obsinet	86	8	3	9.3(4.79-17.3)	3.45(1.19-9.76)
Gololcha	68	5	3	7.3(3.1-16)	4.41(1.51-12.19)
B/anani	60	6	3	10(4.66-20.15)	5(1.7-13.7)
Sama	81	6	3	7.4(3.44-15.23)	3.7(1.27-1.033)
Total	**384**	**36**	**19**	**9.37(6.85-12.7)**	**4.95(3.19-7.6)**

CI = confidence interval, B/anani=Burka anani.

Results of Univariate logistic regression analysis in kebeles (p=0.260) and Body condition score (p=0.497) had no significant association with Brucella seropositivity. (Table 4). This could be attributed to the similarity in agroecological conditions and livestock management system in the district. On the other hand, in this study body condition score and kebeles was statistically not significant (P>0.25). The rest of risk factors; age, p=0.014 sex, p=0.0031 herd size, p=0.006, and contact of camels with other ruminants p=0.003 in the current study have a significant association to brucella infection p< 0.25 in Univariate logistic regression analysis at individual animal level. (Table.4).

In the present study, multiple logistic regression analysis of risk factors showed that out of the 384 camels examined, only 6(2.59%) young were found infected as compared to adult camels 13 (8.49%). The difference was statistically significant (p < 0.05). The odds of seroprevalence of camel brucellosis were 3.2(1.05-9.75, P= 0.04) higher in adults than in young's (Table 4). Comparing the seroprevalence of brucellosis in the sex of camels in this study, a statistically significant association ($p < 0.05$) higher prevalence was detected in females (7.78%) than of males (2.76%). The odds of seroprevalence of camel brucellosis were 3.89(1.29-11.68, P= 0.015) higher in females than males. On the other hand, there were statistically significant differences in herd size ($P<0.05$) in seroprevalence of brucellosis from camels in the current study areas, higher prevalence was detected in large (8%) than that of medium (2.76%) and small (2%). The odds of seroprevalence of camel brucellosis were 8.72(1.84-41.32, P= 0.002) higher in large than small and in medium. Camels together keeping closely with other ruminants had a significant joint effect on seroprevalence of brucellosis ($p < 0.05$) higher prevalence was detected in Camel together keeping closely with other ruminants 4.24 (1.62-11.1,p=0.004) higher than that of separate from other ruminants at individual level. Thus, they were found to be the risk factors for the occurrence of camel brucellosis in the study area (Table 4).

Table 4: The seroprevalence of camel brucellosis in relation with different potential risk factors

Risk factor	**Categories**	**Animal tested**	**Positives**	**Proportion (%)**	**Logistic regression 95%CI**			
					Univariate		**Multivariate**	
					COR	**P-value**	**AOR**	**P-value**
Age	Young (R)	231	6	2.59	R		R	
	Adult	153	13	8.49	3.48	0.014	3.2(1.05-9.75)	0.04
Sex	Male (R)	217	6	2.76	R		R	
	Female	167	13	7.78	2.9	0.031	3.89(1.29-11.68)	0.015
PA	Gumbi (R)	89	7	7.8	R	R		
	Obensa	86	3	3.49	0.423	0.224		
	Gololcha	68	3	4.41	0.541	0. 386		
	B/anani	60	3	5	0.617	0.497		
	Sama	81	3	3.7	0.451	0.260		
BCS	Poor	77	4	5.2				
	Medium	169	4	2.37	0.633	0.48		
	Good(R)	138	11	7.97	0.280	0.33		
Herd size	Small (R)	159	2	1.26	R		R	
	Medium	135	8	5.93	4. 95	0.046	4.16(0.78-22.09)	0.094
	Large	90	9	10	8.72	0.006	13.56(2.62-70.1)	0.002
Contact with other ruminant	No (R)	267	7	1.23	R		R	
	Yes	117	12	10.26	4.24	0.003	4.74(1.65-13.63)	0.004

r= reference's, COR= crude odd ratio, AOR= adjusted odd ratio, CI= confidence intervals, BSC=body condition score, B/Anani= burka anani

4.2. Herd Level Seroprevalance of Camel Brucellosis

Overall seroprevalence of camel brucellosis at the herd level was 23.75 % (CI: 15.76-34.14). In the herd-level final logistic regression model, herd size was found to have a significant effect (p =0.032) on the seroprevalence of camel brucellosis in Univariate and (p=0.013) multivariate analyses. However, the study showed that there was no significant association (P > 0.25) between the herd-level seroprevalence of camel brucellosis and factors such as the introduction of a new animal, and history of abortion in Univariate logistic regression analysis. (Table 5). In the current study of 384 examined camels of five kebeles, 80 herds were examined. One-fourth of the total camels examined in the study were selected as herds.

Table 5: Seroprevalence of camel brucellosis concerning different potential risk factors at herd level.

Risk factors	Categories	Herd tested	Positive	Logistic regression			
				Univariate		Multivariate	
				COR	P-value	AOR	P-value
Herd size	Small	40	6	R		R	
	Medium	27	3				
	Large	13	10	1.91(1.05-3.42)	0.032	3.16(1.53-8.99)	0.013
Introduction of new animal	Yes	60	6				
	No	20	13	13.17(4.89-3546)	0.56		
Abortion history	Yes	60	7				
	No	20	12	17.22(6.07-48.8)	0.37		
Total		**80**	**19**				

4.3. Results of Questionnaire Survey

In this study, 80 individuals whose animals were sampled for blood collection were asked about awareness of brucellosis, the presence of regular veterinary service, grazing system and disposal of fetal membrane, handling aborted fetuses, the source of bull and night resting to find out the presence of associated risk factors with the occurrence of brucellosis. The majority of households used the same communal grazing area and do migrate seasonally in search of feed and water for their animals. Most of the respondents (50.9%) commonly leave the afterbirth on the ground while the remaining 35.1% just throw for dog and 14% disposing.

Almost all households do not properly take care of aborted fetus and fetal membranes. The purpose of camel rearing in the study area is mainly for milk (57.1%) and to some extent (33.8%) draught mitigation, herd accumulation (1.9%), and (7.8%)other social value. In all the sampled households, activities like herding and watering were done by young and adult males. Additionally, respondents identified various causes of abortion in camels, including trypanosomiasis (35.1%), brucella itself (24.7%), anthrax (18.2%), and pasteurellosis (22.1%). In this study, most of the respondents said that aborted camels were removed from the herd mainly through selling, where the aborted fetus, placenta, and discharges were either left on the ground or thrown to the dogs of the herders. Similarly, using communal village bulls among different herds was common and about 61.0% of the herders were practicing this. The other 39.0% of camel herders used breeding bulls from their herd. In the study area, 44% and 56% of camel owners were grazing their camels separately and together with other ruminants, respectively. Night resting practices for camels varied, with nearly half of the respondents (46.3%) indicating that camels rested with cattle, while others reported resting separately (13.0%) and with small ruminants (40.2%). Awareness of small ruminant brucellosis was very low among owners and further, a good proportion of owners also did not have a regular veterinary service.

Table 6: Questioner survey

Variable	Categories	Frequency	Percent
Age of respondent	<20	18	23.4
	20-40	25	32.5
	>40	34	44.2
Sex of respondent	Male	70	90.9
	Female	7	9.1
What is the purpose of camel production	Milk production	44	57.1
	Drought mitigation	26	33.8
	Herd accumulation	1	1.3
	Social value	6	7.8
How do you manage aborts fetus and the fetal membrane	Disposing	10	14
	Give to dog	27	35.1
	Leave in the field	40	50.9
What is the source of bull	From village	47	61.0
	From own herd	30	39.0
How is night resting	Separately	10	13.0
	With cattle	36	46.3
	With small ruminant	31	40.2
How do you herd camels	With cattle	27	35.1
	With cattle and small ruminant	14	18.2
	With small ruminant	17	22.1
	Separately	5	6.5
	With village herd	6	7.8
What was the reason for selling	Disease	25	32.5
	Infertility	43	55.8
	Shortage of Money	9	11.7
What is the means of health care for your camels	Self administrated vet. Drug	34	44.2
	Traditional healer	22	28.6
	Veterinary clinic	21	27.2
What do you think the cause of abortion in camels	Trypanosomosis	27	35.1
	Brucellosis	19	24.7
	Anthrax	14	18.2
	Pasturellosis	17	22.1

5. DISCUSSIONS

In the current study, the overall seroprevalences of Brucella antibodies determined with CFT and RBPT in Gumbi-bordode District were 4.95 % (95% CI=3.19-7.6) and 9.37(6.85-12.7) respectively at animal leve. This finding agrees with Fikru *et al*. (2017), from camels in Afar, Ethiopia, Hadush and Pal (2013) from camels in the Afar region, Ethiopia, Habtamu and Fisseha (2014), from camels in south Eastern Tigray, Ethiopia, Bekele *et al*. (2013) from a camel in Afar national region, Ethiopia, Chauhan *et al*. (2017) from camels in Gujarat, India, and Mohamed *et al*. (2013) from camels of Abu Dhabi Emirate who reported 4.1%, 4.1%, 3.67%, 5.4%, 4.5%, and 4.4% respectively.

Conversely, the current finding was lower than that of Mekonnen (2018) from camels in Fantale, Ethiopia, and Azmi, (2008) from camels in the south province of Jordan; who reported 9.1 and 12.3 %, On the other hand, the result of the current finding was to some extent higher than the reports of (Aden *et al.* 2022) in Selected Districts of Borana Zone, Southern Oromia, Ethiopia, Robayo and Esubalew (2017) in Somali, Ethiopian, (Gumi *et al*. 2013) in and around Dire Dawa, Southeast Ethiopia, Tilahun *et al*. (2013) in Somali, Ethiopian and Megersa *et al*. (2011) in Borana, Ethiopia, who reported 2.86%, 1.5%, 0.9%, 2.4% and 1.8% respectively. This difference might be due to geographical variation, sample size, and sensitivity of diagnostic methods (Radostits *et al*., 2007). The prevalence of brucellosis can vary according to climatic conditions, geography, sex, age, and diagnostic tests applied (Gul and Khan, 2007). The movement of animals may worsen the epizootic situation of brucellosis in the study area as the disease spreads from one herd to another due to the movement of an infected camel into a susceptible camel herd (Radostitis *et al*., 2007). This is true for the West Hararghe pastoral community in case of long dry seasons because camel herders move from place to place searching for feed and water for their animals. The difference in specificity and sensitivity of the applied test may also affect 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).

In the current study, a statistically significant difference ($P<0.05$) was observed between the two age groups, to some extent higher seroprevalence was found in those groups with adult age (3.2%), than those groups with young age. This result is in line with the findings of (Weldegebriel and Haileselassie 2012), in camels from selected districts of the Afar region, Ethiopia. Similarly, a higher seroprevalence (13.8%) in adults than (0%) in young camels in selected districts of the Afar region in Ethiopia (Sisay and Mekonnen, 2012). Age has been referred to as one of the factors associated with brucellosis in animals. Brucellosis is known as a disease of adult animals since susceptibility increases after sexual maturity and pregnancy (Bekele *et al*., 2011). This is since, *Brucella spp*. presents tropism to the reproductive tract due to the production of erythritol sugar in the fetal tissues (Paridah *et al.,* 2016). Long-time contact with infected animals or with the environment also contributes to the higher prevalence of brucellosis in adult animals, which is significantly seen in those herds without culling of positive animals (Megersa *et al*., 2011).

Sexually matured animals are more prone to Brucella infection than sexually immature animals of either sex (Radostits *et al*., 2007). On the other hand, it is also true that younger animals tend to be more resistant to infection and frequently clear an established infection, although latent infections can occur (Quinn *et al*., 2004). This may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of Brucella organisms, tend to increase in concentration with age and sexual maturity (Radostits *et al*., 2007). This study showed a higher seropositivity in females (3.89%), than in the males group. This finding shows a statistically significant difference($p<0.05$) in seroprevalence of camel brucellosis among sex groups and it is in agreement with the report by Bekele (2004) from Ethiopia, Agab *et al.* (1994) from Sudan, and Ajogi and Adamu (1998) from Nigeria who reported a significantly higher occurrence of Brucella species in female camels than males.

In camels, females are more susceptible to brucellosis than males. This relatively higher susceptibility of female camels could be due to the fact that they have more physiological stress than males (Bale et al., 2018). Male animals are less susceptible to brucella infection due to the absence of erythritol sugar, which is found in the uterus (Hirsh and Zee, 199). Also, female camels are kept longer in herds for breeding purposes than male camels, which are fattened and sold off, except for a few that are kept to service the females, for haulage, transport, and other such purposes

This study revealed that herd size was significantly associated with brucellosis in camels ($P<0.05$). The likelihood risk of Brucella positivity occurrence was 13.56% times higher in large herds compared to small herd sizes. This is following the findings of Bekele (2004) and Zewolda and Wereta (2012) in Borena and Afar, respectively. Herd size was statistically identified to be one of the major risk factors for Brucella occurrence in the study areas ($p = 0.002$) for multivariable logistic regression analysis. As herd size increases, the chance of contact between animals increases leading to more chances of infection which is particularly more important during calving or abortion when most brucellosis contamination occurs (Mohamed et al., 2013). Thus, herd size and density of animal population together with poor management are directly related to infection rate (Abou-Eisha, 2000; Wernery and Kaaden, 2002) In the area, camels are herding mainly in extended common grazing land separated from other species, but accumulation of camels are observed at water points like traditional wells and ponds during dry seasons. This might facilitate the spread of diseases including brucellosis, as the animals have contact with each other and direct access to water points which they contaminated by their discharge and increases the infection rates as has also been reported previously (Abbas and Agab, 2002).

Contact of camel herds with ruminants was 4.74% in animals that kept close contact with ruminants than those without contact with ruminants. This reflects the real situation of brucellosis among the two groups that pay attention to studying the role of ruminants (sheep, goats, and cattle) in transmitting brucellosis to camels and vice versa. The results of our study go parallel with the findings of (Adugna *et al*,.2013) and (Zeru *et al.*, 2016) in Afar and Tassew and Kassahun, 2014 in Mehoni District, South Eastern Tigray, Ethiopia (Zeru *et al*., 2016). 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. 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. Also (Chauhan, *et al*., 2017) observed that rearing of multispecies in the same herd may lead to close contact of animals, which may facilitate the exchange of various pathogenic microorganisms. In the present study area, higher seroprevalence was observed in camels reared with ruminants which might be the possible source of infection for camel.

In the current study, the overall seroprevalences of Brucella antibodies determined in the Gumbi-bordode District were 23.75 % (CI: 15.76-34.14)at the herd level, this finding is in agreement with (Adugna *et al*. 2013) who reported (24%) of camels at herd level in Afar National Regional State in northeastern Ethiopia. This finding was higher than that of Mohamed (2011) from camels in and around Dire Dawa who reported (10.23%) and (Alrawahi *et al*., 2019) who reported (1.44%). On the other hand, the result of the current finding was to some extent lower than the reports of (Abou *et al*. 2019) who reported (28%) of camels in Egypt. This variation in the prevalence could be attributed to an increase in stock density in large herd sizes, which facilitates the transmission of *Brucella spp*. infection during calving or abortion. Furthermore, this variation could be influenced by fluctuations in disease prevalence at the overall animal level and the herd size during the study period.

Herd size risk factors for *Brucella* seropositivity at the herd level were high with larger herds having 3.16% times of seropositivity than smaller herds in the current study. This finding is in agreement with (Ghanem 2009), who reported from Somaliland. This may be due to, the close contact of animals increasing the likelihood of transmission through direct contact, contaminated feed or water sources, or aerosol transmission. This can lead to a higher rate of infection within the herd. Detecting brucellosis in large herds can be more challenging due to the sheer number of animals involved. In small herds, it may be easier to notice symptoms or conduct regular testing, whereas in large herds, the disease might go unnoticed or undiagnosed for longer periods, allowing it to spread more extensively

6. LIMITATION OF THE STUDY

The study may have focused solely on camel-level factors and may not have considered broader contextual factors such as socio-economic status, cultural practices, or veterinary infrastructure, which could also influence the prevalence and transmission of camel brucellosis.

7. CONCLUSION AND RECOMMENDATIONS

The present study provides the baseline status of camel brucellosis in West hararghe zone Gumbi-Bordode district. Multivariable logistic regression analysis of presumed risk factors indicated that age, sex, herd size and camels closely kept with other ruminants at individual camels level as determinants of seropositivity. Herd size at the herd level was the major associated risk factor for the likelihood of occurrence and transmission of camel brucellosis in the study areas. Higher seropositivity was recorded in adult or matured; females, large herd size, and mixing with other animals. Therefore, the disease likely spreads to the unaffected animals and herds, given the extensive production system prevailing in the area, which may allow contact of animals during grazing and at watering points. Moreover, pastoralists are in close contact with their animals, and handling of aborted materials is common. As a result, the study's findings highlight the significance of camel brucellosis in the study area as well as a potential zoonotic implication of the disease.

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

- Encourage further research to better understand the epidemiology of camel brucellosis in the study area and collaborate with local and regional authorities, as well as international organizations, to develop comprehensive control and prevention strategies.
- Increase awareness about the zoonotic nature of camel brucellosis and educate the community about the importance of seeking medical attention in case of suspected exposure to the disease.
- Encourage improved herd management practices, such as separating camels from other ruminants to reduce the risk of disease transmission.
- Implement strategies to control the movement of camels and minimize mixing with other herds.

8. REFERENCES

Abbas, B., Agab, H., 2002: A review of camel brucellosis. *Prev. Vet. Med*., **55**(1):57-56

Abebe, G., Worku, Y., Mamo, G. and Nazir, S., 2017. Sero-prevalence and associated risk factors of brucellosis in camel at Akaki Abattoir, Central Ethiopia. *Journal of Animal Research*, **7**(4): 617-622.

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

Aden Giro, Teshale Sori and Dereje Tesfaye. (2022) "Sero-epidemiological Investigations of Camel Brucellosis and Community Perception in Selected Districts of Borana Zone, Southern Oromia, Ethiopia." *Journal of Agricultural Research Pesticides and Biofertilizers*, **3**(1): DOI:http;//doi.org/01.2022/1.1044.

Adugna, W., Sisay, T., and Keskes, S., 2013. Camelus dromedarius brucellosis and its public health associated risks in the Afar National Regional State in northeastern Ethiopia. *Acad. Vet*. Sci., 55:89

Ahmed, M.O., Elmeshri, S.E., Abuzweda, A.R., Blauo, M., Abouzeed, Y.M., Ibrahim, A., Salem, H., Alzwam, F., Abid, S., Elfahem, A. and Elrais, A., 2010. Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006–January 2008. *Eurosurveillance*, **15**(30):19625.

Alamian, S. and Dadar, M., 2019. Brucella abortus contamination of camel milk in two Iranian regions. *Preventive veterinary medicine*, **169:**104708.

Alemneh, T. and Akeberegn, D., 2018. A review on small ruminants brucellosis. *J. Med. Res*, **18**(2): 2249-4618

Alp, E., Koc, R., Durak, C., Yildiz, O., Aygen, B., Sumerkan, B. and Doganay, M., 2006. Doxycycline plus streptomycin versus ciprofloxacin plus rifampicin in spinal brucellosis [ISRCTN31053647]. *BMC Infectious diseases*, **6**(1):1-10.

Alrawahi, A., Robertson, I., Hussain, H., and Saqib, M., 2019. A cross-sectional seroepidemiological study of camel (Camelus dromedarius) brucellosis and associated risk factors in the Sultanate of Oman. *Open veterinary journal*, **9**(2): 133-139.

Al-Shamahy, A., Whitty M., and Wright G., 2000. Risk factors for human brucellosis in Yemen: a case control study. *Epidemiology & Infection*, **125(**2):309-313.

Awole, M., Gebre-Selassie, S., Kassa, T. and Kibru, G., 2002. Isolation of potential bacterial pathogens from the stool of HIV-infected and HIV-non-infected patients and their antmicrobial susceptibility patterns in Jimma Hospital, south west Ethiopia. *Ethiopian Medical Journal*, **40**(4):353-364.

Azmi, H., 2008. Brucellosis in Camels (Camelus dromedorius) in the south province of Jordan. *American Journal of Agricultural and Biological Sciences* **3** (3): 623-626.

Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J. and Skjerve, E., 2011. Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. *Tropical animal health and production*, **43**:651-656.

Bayasgalan, C., Chultemdorj, T., Roth, F., Zinsstag, J., Hattendorf, J., Badmaa, B., Argamjav, B. and Schelling, E., 2018. Risk factors of brucellosis seropositivity in Bactrian camels of Mongolia. *BMC veterinary research*, **14**:1-11.

Megersa, B., 2004. Sero-epidemiological study of brucellosis in camels (Camelus dromedarius) in Borena lowland pastoral areas, Southern Ethiopia. *Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia. Partial fulfillment of Degree of Master of Science in Tropical Veterinary Epidemiology*.

Bekele, A., Tessema, S., and Melaku, S, 2013. Camelus dromedarius brucellosis and its public health associated risks in the Afar National Regional State in northeastern Ethiopia. *Acta veterinaria scandinavica*, **55**(1):1-8.

Castillo, V., Pessina, P., Hall, P., Blatter, M.C., Miceli, D., Arias, E.S. and Vidal, P., 2016. Post-surgical treatment of thyroid carcinoma in dogs with retinoic acid 9 cis improves patient outcome. *Open Veterinary Journal*, **6**(1):6-14.

Catley, A., Leyland, T., Admassu, B., Thomson, G., Otieno, M. and Aklilu, Y., 2005. Communities, commodities and crazy ideas: changing livestock policies in Africa. *IDS Bulletin*, **36(**2):96-102.

Chachra, D., Saxena, M., Kaur, G. and Chandra, M., 2009. Comparative efficacy of Rose Bengal plate test, standard tube agglutination test and Dot ELISA in immunological detection of antibodies to Brucella abortus in sera. *J. Bacteriol. Res*, **1**(3):30-33.

Chauhan, H. C., Patel1, K. B., Patel1, S. I., Patel1, B. K., Chandel, B.S., . Bhagat, A. G., et al., (2017): Serological Survey of Brucellosis in Camel of Gujarat. *International Journal of Current Microbiology and Applied Sciences.* **6 (**4):1815-1821

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

CSA, J., 2021. Agricultural Sample Survey 2006-07. *Volume I, Addis Ababa*.

Degefu, H., Mohamud, M.,Hailemelekot, M. and Yohannes, M.J.E.V.J., 2011.Seroprevalence of bovine brucellosis in agro pastoral areas of Jijjiga zone of Somali National Regional State, Eastern Ethiopia.*Ethiopian Veterinary Journal*, **15**(1).

Dehkordi, F., Saberian, S. and Momtaz, H., 2012. Detection and segregation of Brucella abortus and Brucella melitensis in Aborted Bovine, Ovine, Caprine, Buffaloes and Camelid Fetuses by application of conventional and real-time polymerase chain reaction. *The Thai Journal of Veterinary Medicine*, **42**(1):13-20

Desta, A.H., 2016. Pastoralism and the issue of zoonoses in Ethiopia. *Journal of Biology, Agriculture and Healthcare*, **6**(7):21-27.

Ducrotoy, M., Bertu, J., Matope, G., Cadmus, S., Conde-Álvarez, R., Gusi, A.M., Welburn, S., Ocholi, R., Blasco, J.M. and Moriyón, I., 2017. Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. *Acta tropica*, **165:**179-193.

Edao, B.M., 2021. *Brucellosis in Ethiopia: epidemiology and public health significance* (Doctoral dissertation).

Ekere, S.O., Njoga, E.O., Onunkwo, J.I. and Njoga, U.J., 2018. Serosurveillance of Brucella antibody in food animals and role of slaughterhouse workers in spread of Brucella infection in Southeast Nigeria. *Veterinary World*, **11(**8):1171.

Ersoy, Y., Sonmez, E., Tevfik, M.R. and But, A.D., 2005. Comparison of three different combination therapies in the treatment of human brucellosis. *Tropical doctor*, **35**(4):210-212.

Faye, B., Bengoumi, M., Viateau, E., Tourret, M. and Chilliard, Y., 2001. *Adipocyte patterns of adipose tissue in camel hump and kidney*.

Ferede, Y., Mengesha, D. and Mekonen, G., 2011. Study on the seroprevalence of small ruminant brucellosis in and around Bahir Dar, North West Ethiopia. *Ethiopian Veterinary Journal*, **15**(2).

Fikru, G., Gizachew, F., Semu, M., Hailegebriel, B., Mahendra, Pal. and Venkataramana, K., 2017. Seroprevalence and Risk Factors of Brucellosis among Camels Belonging to Selected Districts of Afar, Ethiopia: Need for Public Awareness. *American Journal of Microbiological Research*, **5**(5): 94-100. doi: 10.12691/ajmr-5-5-1.

Fossi, M., 2014. Meat inspection and control in the slaughterhouses. *Suomen eläinlääkärilehti* **120** *(2014): 10*.

Fowler, M.E., Bravo, P.W., Fowler, M.E. 2010.Medicine and surgery of camelids. *Wiley Blackwell.Inc.*, Publication, 3rd Edition.

Franc, K., Krecek, R., Häsler, B., and Arenas-Gamboa, A., 2018. Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC public health*, **18**(1):1-9.

Gebretsadik, M.T. and BISHOFTU, E., 2016. Seroprevalence of Brucellosis and isolation of brucella from small ruminants that had history of recent abortion in selected kebeles of Amibara District. *Afar region, Ethiopia*.

Ghanem, Y., El-Khodery, S., Saad, A., Abdelkader, A., Heybe, A., Musse, A., 2009. Seroprevalence of camel brucellosis (Camelus dromedarius) in Somaliland. *Trop. Anim. Health Prod.*, **41**(8): 1779-86.

Glynn, M., Lynn, and T., 2008: Brucellosis. J. Am. *Vet. Med. Assoc*., 233: 900-8.

Godfroid, J., Al Dahouk, S., Pappas, G., Roth, F.,Matope, G., Muma, J., Marcotty, T., Pfeiffer, D.,Skjerve, E., 2013. A “One Health” surveillance and control of brucellosis in developing countries: Moving away from improvisation. *Comp. Immunol. Microbiol. Infect.Is*, **36:**241-248.

Godfroid.,J.,Scholz, H.,Barbier, T.,Nicolas, C.,Wattiau, P.,Fretin,D.,Whatmore, A., Cloeckaert, A., Blasco, J., Moriyon, I., Saegerman, C., Muma, J., Al Dahouk, S., Neubauer, H., Letesson, J.,2011. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Prev. Vet. Med.*, **102**:118-131.

Gul, S. T. and Khan, A. 2007: Epidemiology and Epizootology of Brucellosis: *A Review. Pakistan Veterinary Journal*. **27**:145-151.

Gutema, F. and Tesfaye, J., 2019. Review on camel brucellosis: public health importance and status in Ethiopia. *Academic Research Journal of Agricultural Science and Research*, **7**:513-529.

Gwida, M., Dahouk, A., Melzer, F., Rösler, U., 2010: Brucellosis - Regionally Emerging Zoonotic Disease. *Croat Med J.*, **51**(4):289-295

Gwida, M., El-Gohary, A., Melzer, F., Khan, I., Rösler, U. and Neubauer, H. 2011: Brucellosis in camels. *Research Veterinary Science.*, (in press).

Gwida, M., El-Gohary, A., Melzer, F., Khan, I., Rösler, U., Neubauer, H., 2012: Brucellosis in camels. *Res. Vet. Sci.vol.*, **92**:351-355.

Hotam Singh Chaudhary, A.S., 2011. *Journal of Chemical and Pharmaceutical Research preparations* .**3**: 773-776.

Isam, M. (2016): “Prevention of Camel Brucellosis Spreading to Humans: *A Real Challenge”. EC Bacteriology and Virology Research* **2**:1- 36

Jafer, M., Mengistu, S., Eshetu, A. and Belina, D., 2018. *Sero-prevalence of brucellosis in camels and febrile human patients attending health facilities in selected districts of eastern ethiopia* (Doctoral dissertation).

Kadim, I., Mahgoub, O. and Purchas, R., 2008. A review of the growth, and of the carcass and meat quality characteristics of the one-humped camel (Camelus dromedaries). *Meat science*, **80(**3):555-569.

Kahn, C., Line, S. and Aiello, S., 2005. The merck veterinary manual. Merck & Co. *Inc., Whitehouse Station, NJ*.

Karabay, O.,Sencan, I., Kayas, D., Şahin, I.,2004. Ofloxacin plus rifampicin versus doxycycline plus rifampicin in the treatment of brucellosis:A randomized clinical trial.*BMC Infect.Dis*.

Khamesipour, F., Doosti, A., Rahimi, E., 2015: Molecular study of Brucellosis in camels by the use of Taq Man® real-time PCR. Acta Microbiol. *Immunol. Hung*., **62** (4): 409-421

Khurana, S., Sehrawat, A., Tiwari, R., Prasad, M., Gulati, B., Shabbir, M., Chhabra, R., Karthik, K., Patel, S.K., Pathak, M. and Iqbal Yatoo, M., 2021. Bovine brucellosis–a comprehensive review. *Veterinary Quarterly*, **41**(1):61-88.

Kulplulu, O. and Sarimehmetoglu, B. 2004. Isolation and identification of Brucella from ice cream. *Food Control*, **15**:511-514

Gutema, F. and Tesfaye, J., 2019. Review on camel brucellosis: public health importance and status in Ethiopia. *Academic Research Journal of Agricultural Science and Research*, ***7***(7):513-529.

Luelseged, A., 2019. Review on Molecular Epidemiology and Public Health Significance of Brucellosis. *Anim.Res.Vet.Sci***., 2:**1-10.

McDermott, J., Arimi, S., (2012). Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *International Livestock Research Institute*

Mekonnen K. (2018). Prevalence study for camel and human Brucellosis in Mid Rift Valley. Glob. *J. Anim. Sci. Res*. **6**(1), 9-20. 2, 120–126

Mekonnen, H. and Sisay, W.Z., 2012. Seroprevalence of brucella infection in camel and its public health significance in selected districts of afar region, Ethiopia.

Memish, Z., Balkhy, H.H., 2004: Brucellosis and International Travel. *J. Travel Med.*, **11**:49-55

Meng, X., Lindsay, D., Sriranganathan, N., 2009. Wild boars as sources for infectious diseases in livestock and humans. *Philos Trans R Soc Lond B Biol Sci,* **364** (1530): 2697-2707

Miguel, P., Fernández, G., Vasallo, F., Hortas, M., Lorenzo, J., Rodríguez, I., Ortiz-Rey, J., Antón, I., 2006. Neurobrucellosis mimicking cerebral tumor: case report and literature review. *Clin. Neurol.Neurosurg.* **108** (4): 404-6

Musa, M. , Eisa, M., El Sanousi, E., Abdel Wahab, M.B., Perrett, L., 2008: Brucellosis in Camels (C.dromedarius) in Darfur, Western Sudan. *J. Comp. Pathol.*, **138 (**2-3**):**151-5.

Nielsen, K., 2011. Diagnosis of Brucellosis by serology.Vet. Microbiol., 90:1-13in certain nomadic localities in Sudan, Rev. *sci*. *tech. Off. int. Epiz*.**29 (3):** 663-9.

Njeru, J., Wareth, G., Melzer, F., Henning, K., Pletz, M.W., Heller, R. and Neubauer, H., 2016. Systematic review of brucellosis in Kenya: disease frequency in humans and animals and risk factors for human infection. *BMC public health*, **16(**1):1-15.

Nowak, R. and E. Walker, 1999. Walker's Mammals of the World, JHU Press.

O.I.E., 2008. Manual of diagnostic tests and vaccines for terrestrial animals. *Chapter*, **2**(13):304-323.

Obonyo, M., 2018. *Sero-Prevalence and Factors Associated with Brucellosis in Goats and Sheep and Assessment of Pastoralists, Knowledge Attitude and Practices towards Brucellosis in Garissa County* (Doctoral dissertation).

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L. and Tsianos, E.V., 2006. The new global map of human brucellosis. *The Lancet Infectious Diseases*, **6**(2):91-99.

Pal, M., Gizaw, F., Fekadu, G., Alemayehu, G. and Kandi, V., 2017. Public health and economic importance of bovine Brucellosis: an overview. *Am J Epidemiol*, **5**(2):27-34.

Pendela, S.V., Agrawal, N., Mathew, T., Vidyasagar, S. and Kudaravalli, P., 2017. An Uncommon presentation of Brucella endocarditis masquerading as neurobrucellosis. *Journal of Clinical and Diagnostic Research: JCDR*, **11(**2).

Pérez-Sancho, M., García-Seco, T., Domínguez, L. and Álvarez, J., 2015. Control of animal brucellosis is the most effective tool to prevent human brucellosis. *Updates on brucellosis*, **10**(61): 222.

Quinn, P., B. Markey, M. Carter, W. Donnelly and F. Leonard, 2004. *Veterinary Microbiology and Microbial Disease*, Blackwell Science.

Radostits, O., Gay, C., Hinchcliff, K. and Constable, P. eds., 2006. *Veterinary Medicine E-Book: A textbook of the diseases of cattle, horses, sheep, pigs, and goats*. Elsevier Health Sciences.

Radostits, O., Gay, C.C., Blood, D. and Hinchcliff, K., 2000. A textbook of the diseases of cattle, sheep, pigs, goats and horses. *Veterinary medicine*, **9**:603-700.

Radostits, O., Gay, C., Hinchcliff, K. and Constable, P., 2007. A textbook of the diseases of cattle, horses, sheep, pigs and goats. *Veterinary medicine*, **10**:2045-2050.

Rafieipour, A. and Ziaei, N., 2011. Study of brucellosis in serum of camels in southeast of Iran. *Veterinary Science Development*, **1**(1):14-e14.

Rajala, E., 2016. Brucella in Tajikistan-Zoonotic risks of urbanized livestock in a low-income country. *Swedish University of Agricultural Sciences*, .39-40.

Refai, M., 2002. Incidence and control of brucellosis in the Near East region. *Veterinary microbiology*, **90**(1-4):81-110.

Robayo, Y. and Esubalew, S., 2017. Seroprevalence and Associated Risk Factors of Brucellosis in Camels Kept Under Pastoral Management in Fafen Zone, Somali Regional State, Ethiopia. *Int. J. Livest. Res*, **7**(1).

Ron-Román, J., Ron-Garrido, L., Abatih, E., Celi-Erazo, M., Vizcaíno-Ordóñez, L., Calva-Pacheco, J., González-Andrade, P., Berkvens, D., Benítez-Ortíz, W., Brandt, J. and Fretin, D., 2019. Bayesian evaluation of three serological tests for detecting antibodies against Brucella spp. Among humans in the Northwestern Part of Ecuador. *The American Journal of Tropical Medicine and Hygiene*, **100**(6):1312.

Salisu, U.S., Kudi, C., Bale, J., Babashani, M., Kaltungo, B., Baba, A., Yusuf, M.S. and Jamilu, Y.R., 2018. Risk factors and knowledge of Brucella infection in camels, attitudes and practices of camel handlers in Katsina State, Nigeria. *Nigerian Veterinary Journal*, **39(**3):227-239.

Saltoglu, N., Tasova, Y., Inal, A.S., Seki, T. and Aksu, H.S., 2002. Efficacy of rifampicin plus doxycycline versus rifampicin plus quinolone in the treatment of brucellosis. *Saudi medical journal*, **23**(8):921-924.

Scholar, E. and H. Tarekegn, 2014. Sero - epidemiological Study of Camel Brucellosis in Mehoni District, South Eastern Tigray, Ethiopia. East Africa TIRI Research, **01**(3): 1-4.

Seleem, M.N., Boyle, S.M. and Sriranganathan, N., 2010. Brucellosis: a re-emerging zoonosis. *Veterinary microbiology*, ***140***(3-4):392-398.

Shimol, S., Dukhan, L., Belmaker, I., Bardenstein, S., Sibirsky, D., Barrett, C. and Greenberg, D., 2012. Human brucellosis outbreak acquired through camel milk ingestion in southern Israel. *Isr Med Assoc J*, **14**(8):475-8.

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

Tanko, P, Emikpe, B. O. and Sabri, M. 2013. Evaluation of the shedding routes and serological patterns in experimentally induced Brucella melitensis infection in Dexamethasone-treated and transport-stressed goats, *Veterinary World.* **6:**686-692

Tassew, H. and Kassahun, F. 2014. Sero-Epidemiological Study of Camel Brucellosis in Mehoni District, South Eastern Tigray, and Ethiopia. *J. Microbiol*. Res., **4**: 18-23

Teshome, H., Molla, B. and Tibbo, M., 2003. A seroprevalence study of camel brucellosis in three camel-rearing regions of Ethiopia. *Tropical Animal Health and Production*, ***35*,**.381-390.

Tilahun, B., Bekana, M., Belihu, K., Zewdu, E., 2013. Camel brucellosis and management practices in Jijiga and Babile districts, Eastern Ethiopia. *J. Vet.*, **5**:81-86

Wakene, W.Z. and Mamo, G., 2017. Review on Epidemiology of Camel and Human Brucellosis in East Africa, Igad Member Countries. *Science*, **6**(6):109-15.

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

Wernery, U., 2014. Camelid brucellosis: a review. *Rev sci tech*, **33**(3):839-57..

World Health Organization (WHO), 2006.Brucellosis in humans and animals. World Health Organization 20 Avenue Appia, 1211 Geneva 27, Switzerland. *WHO Press, WC310*.

Xavier, M., A Paixao, T., B den Hartigh, A., M Tsolis, R. and L Santos, R., 2014. Pathogenesis of Brucella spp. *The open veterinary science journal*, **4**(1).

Yilma, M., Mamo, G. and Mammo, B., 2016. Review on brucellosis seroprevalence and ecology in livestock and a human population of Ethiopia. *Achievements in the Life Sciences*, **10**(1):80-86.

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

Yousefi-Nooraie, R., Mortaz-Hejri, S., Mehrani, M., Sadeghipour, P., 2012: Antibiotics for treating human brucellosis. *Cochrane Database Syst. Rev.,* **10**:CD007179.

Zeru, F., Gebrezgabher, W., Dessalegn, K., Tilahun, S., Guben, Y., Mohammed, H. and Hadush A. (2016). Prevalence and Risk Factor of Brucellosis in Dromedaries in Selected Pastoral Districts of Afar, Northeastern Ethiopia. *J Natu Sci Res*.**6**:2224-3186.

Zewdie, W. and Mamo.G., 2018: Review on Epidemiology of Camel and Human Brucellosis in East Africa, Igad Member Countries. Sci. *J. Clin*. Med. **6**:109.

Zewolda, S.andWereta, M. 2012. Seroprevalence of Brucella infection in camel and its public health significance in selected districts of Afar region, Ethiopia. *J. Environ. Occupat. Sci.,* **1**: 91- 98.

9. ANNEX

ANNEX 1: Pictures during blood sample collection of camels.

ANNEX 2: Pictures during serum separation from the whole blood sample of camels and RBPT preparation.

ANNEX 3: laboratory procedure

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 Sebeta animal health Ethiopia. For doing the test, the procedure described by OIE (2005) was followed.

8.1.1 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

8.1.2. 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.

8.1.3. Interpretation

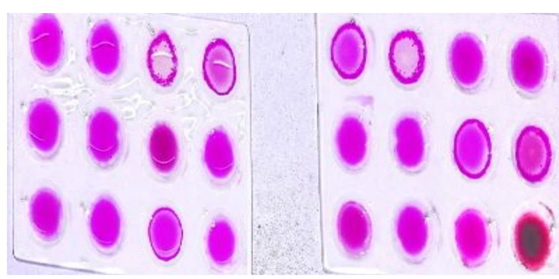
 Reactions were identified as 0, +, ++ and +++ according to Nielson (2002).That is:

 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 Sebeta animal health inistitute, 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 asyringe 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 +40c 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 1:10by adding VCM at PH 7.2 and centrifuged at 2,500 rpm for 5 minutes.
- Then discarding of supernatant followed by re suspension 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 +4oc 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 370c 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 580c for 30minutes.

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 into B1-B2, D1-D2, F1-F2, and H1-H2 respectively. Based on 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: - 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 370C in moist chamber for 30 minutes with constant agitation. Secondly, a 25µl of complement at working diluted was added into 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 370C 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.

Annex 4: Proportional sample size determination

Zone	District	Total kebeles	Selected kebeles	Total camel of the district	Name of the selected kebeles	Total camels of the selected kebeles	Sampled camels
West hararghe	Gumi-Bordode	29	5	58869	Sama	10187	112
					Gumbi	8810	98
					B/anani	9908	109
					Gololcha	2977	33
					Obensa	2918	32

Annex 5: Body condition scoring

	Back						Flank		
	Ischialt uberosit y	Scrotuber al ligament	Ano-genital region	Spinous apophys es	Coxal tuberos ity	Transverse apophyses	Hollow of the flank	Rib	Hump
0	Poor	Very concave	Very deep at the base of the tail	All visible	Very promin ent	Highly apparent	All prominent	All visible («skin on bones »)	No hump
1	Medium	Concave	Deep, the base of the tail is still proeminent	Proemin ent on the back	Proemi nent	Apparent	Proeminent	Visible	Very small hump
2	Good	Flat	Visible hollow	Visible on the back	Visible	visible	Visible all along the back	Visible in front	Small
3	Visible, low quantity of fat	Flat to convex	Slight hollow	Slightly apparen t	Slightl y visible	Very slight	Slightly visible	Invisible or slightly visible in front of the thorax	Mediu m size hump
4	Hardly visible and covered with fat	Convex	Filled	Well covered by fat	Almost invisib le	Almost invisible	invisible	invisible	Big hump
5	Disappe ared in fat	Convex	The base of the tail is covered by fat	Invisibl e	Invisib le	Invisible	Invisible and rounded back	Visible Fat cover	Very big hump coveri ng the back

Source: (Faye *et al.*, 2001)

ANNEX 6: Questionnaire format.

8.4 Structured questionnaire format for camel owners

Formats .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........................

1. What is the purpose of camel production?

a) High milk production

b) Drought mitigation

c) Bush encroachment control

d) Herd accumulation

2. Rank the use of camels:

a) Milk production..........

b) Transportations......

c) Draught power............

d) Cash income by sale......

e) e. Meat consumption........

3. Milk consumption and preservation means

a. Fresh

b. Boil

c. Sour

4. How do you consume camel meat?

a) Cooked

b) Raw

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

a. Traditional healer

b. self-administered vet drugs

c. Vet clinic

6. What do you do with camels that frequently abort?

a. Sell

b. Slaughter

c. Keeping

d. Others....................

7. How do you manage aborted fetus/ fetal membrane?

a. Leave in the field

b. Disposing

c. Give to dog

d. Others....................

8. What do you do with female that doesn‟t conceive?

a. Sell

b. Slaughter

c. Keeping

d. Other....................

9. What is the source of bull?

a. From own herd

b. village bull

c. Others.............

10. How do you herd Camels?

a. Separately

b. with village herd

c. with cattle

d. with small ruminants

11. How is night resting?

a. Separate

b. share with cattle

c. Share with small ruminants

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

a. Disease

b. Infertility

c. Short age of money

d. Others.............

: Sample collection format

Annex7: sample collection format.

Region___Zone___________District________kebeles__________Date__________Herd size_______________

SN	Code	Spp	Sex	Age	Owner Name	Type of sample taken
						Blood
1						
2						
3						
4						
5						
.						
.						