SERO-EPIDEMIOLOGY OF *BRUCELLA* INFECTION AND ITS PUBLIC HEALTH SIGNIFICANCE IN FENTALE DISTRICT OROMIA REGION, ETHIOPIA

PhD Dissertation

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SERO-EPIDEMIOLOGY OF *BRUCELLA* INFECTION AND ITS PUBLIC HEALTH SIGNIFICANCE IN FENTALE DISTRICT OROMIA REGION, ETHIOPIA

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Sero-epidemiology of *Brucella* Infection and Its Public Health Significance in Fentale District Oromia Region, Ethiopia

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Dedication

This thesis/dissertation manuscript is dedicated to my mother Banche Gerbi, and my father Demissie Gizaw, for nursing me with affection and love and for their dedicated partnership in the success of my life.

Statement of author

First, I declare that this thesis/dissertation is my bonafide 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 requirements for a PhD degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under 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

AGID	Agar gel immunodifusion
AMOS	Acronym for *Brucella* ***a****bortus*, *B.* ***m****elitensis*, *B.* ***o****vis*, and *B.* ***s****uis*
BBAT	Buffered *Brucella* antigen test
bp	Base pair
Bv	Biovar
c-ELISA	Competitive enzyme-linked immunosorbent assay
CFSPH	Center for food security and public health
CFT	Complement fixation test
DNA	Deoxyribo nucleic acid
EU	European Union
FAO	Food and Agriculture Organization
FPA	Fluorescence polarization assay
GDP	Gross domestic product
i-ELISA	Indirect ELISA
Ig	Immunoglobulin
IS	Insertion sequence
LFA	Lateral flow assay
MLVA	Multiple-locus variable-number tandem-repeat analysis
OIE	International office for epizootics

PCR	Polymerase chain reaction
RBT	Rose Bengal plate test
rRNA	Ribosomal ribonucleic acid
SAT	Serum agglutination test
sLPS	Smooth lipopolysaccharide
SNP	Single nucleotide polymorphism
WHO	World health organization
WOAH	World organization for animal health

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List of Articles

Paper I (Published)

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Paper III (drafted)

Dagnachew Demissie, Tesfu Kassa, Gobena Ameni, Peter Baaro Gathura, Esayas Gelaye, and Gezahegne Mamo. Sero-prevalence of cattle brucellosis and its risk factors in Kereyu pastoral area of Oromia Region, Ethiopia

Paper IV (drafted)

Dagnachew Demissie, Tesfu Kassa, Gobena Ameni, Peter Baaro Gathura, Esayas Gelaye, and Gezahegne Mamo. Human brucellosis and associated risk factors in the Kereyu pastoral area of Oromia Region, Ethiopia

ABSTRACT

Brucellosis is a highly neglected, economically devastating zoonotic disease caused by *Brucella* species. In pastoral settings like Fentale, disease transmission is profoundly linked to close interaction between humans and livestock, the consumption of raw dairy products and unsafe handling of abortion cases. Despite its public health and economic importance, the specific sero-prevalence and associated risk factors of brucellosis in Fentale district remain under-documented. A cross-sectional study was conducted in Fentale district, east Shewa zone of Oromia Region, Ethiopia to investigate the sero-prevalence and associated risk factors of brucellosis in camels, small ruminants, cattle and humans from December, 2018 to October, 2022. A total of 324 camels, 384 sheep and goats, 384 cattle and 210 human serum samples were collected and tested for brucellosis antibodies using serological tests (Rose Bengal plate test, complement fixation test and competitive enzyme-linked immunosorbent assay). Questionnaires were also administered to livestock herders and human patients to assess their knowledge about brucellosis and other zoonotic diseases, the habits of animal product consumption and handling, and dead animal/aborted fetus disposal practices. The overall individual animal-level sero-prevalences were 9.6% (95% CI: 6.5 13.0), 2.6% (95% CI: 1.0-4.2) and 1.3% (95% CI: 0.3-2.6) in camels, small ruminants and cattle, respectively. The sero-prevalence recorded in febrile human patients visiting health centers was 2.4% (95% CI: 0.5-4.8). Herd size in camels, sex and flock size in small ruminants, and parity and history of abortion in cattle were found significantly associated risk factors with brucellosis sero-positivity *(p* <0.05). Accordingly, camels and small ruminants in the large herd/flock category were found at higher risk of *Brucella* infection than their respective categories. Similarly, cattle with multiple parity and history of abortion were more tested positive for brucellosis than their respective categories. In humans, higher education level was surprisingly found associated with higher sero-positivity (*p* <0.05). This suggests that even educated individuals, despite possessing some knowledge about the risks and transmission of the disease, are less likely to comply with practices that minimize *Brucella* infection. However, these results should be interpreted cautiously given the limited sample size. The questionnaires survey in camel owners indicated that

98.5% of them did not know about brucellosis, they usually consume whole milk without boiling and abortion materials are handled with bare hands, and they do not safely destroy these materials. Similarly, in cattle owners about 74.5% of the respondents did not know that they can get any disease from animals, 86.4% and 90% of them have no information on brucellosis as animal disease and human disease, respectively. Moreover, 96.4% of the cattle owners drink raw milk and almost all informed abortion materials are handled with bare hands, and they do not safely discard these materials. Abortion materials are usually left for dogs and wild carnivores. In the case of human patients sampled at health centers, 70.0% of them had no knowledge on brucellosis and its means of transmission, and 44.3% and 36.2% of them have the habit of consuming raw and raw/boiled milk, respectively. Similar to animal owners, human patients informed that abortion materials are handled with bare hands and not safely discarded. In conclusion, this study demonstrated that brucellosis is prevalent both in livestock and humans, and hence brucellosis should be considered in the differential diagnosis of febrile patients. Moreover, traditions such as unsafe handling and disposal of abortion materials and consumption of raw milk are likely to play roles in the transmission of brucellosis in the study area. Therefore, there is a need for implementation of better management practices, typing of infecting strains, culling of positive animals and increasing public awareness on brucellosis.

Keywords: Bovine brucellosis; camel brucellosis; c-ELISA; CFT; Fentale; human brucellosis; RBT; risk factor; sero-prevalence; small ruminant brucellosis.

1. CHAPTER ONE

1.1. General Introduction

Brucellosis is one of the most important neglected zoonotic diseases with significant effect on productive and reproductive performances, agricultural economy, international trade, and public health especially in low-income nations (Franc *et al*., 2018; Deka *et al*., 2024). It is an infectious disease caused by *Brucella* species (Khan and Zahoor, 2018). The disease primarly affects livestock (cattle, goats, sheep, and camels) but readily transmissible to humans (WHO, 2020).

In 1887, David Bruce isolated *Brucella melitensis* from the spleen of five British soldiers who died from Malta fever (Wyatt, 2013; Bakri *et al*., 2018), and in 1897, Bernard Bang discovered *B. abortus* to be the causative agent of Bang's disease (Colville, 2007). Later, the relationship between Malta fever and Bang's disease was established and the genus was named as *Brucella* in honor of David Bruce who first described the disease (Tan and Davis, 2011).

Infection with *Brucella* in cattle is primarly caused by *B. abortus* and occasionally by *B. melitensis* and *B. suis*. *Brucella melitensis* mainly infect sheep and goats, *B. suis* primarily infect pigs and *B. canis* infects dogs (WOAH, 2022). Camels are susceptible to *B. melitensis* and *B. abortus* (Gwida *et al*., 2012). *Brucella abortus, B. melitensis, B. suis, B. canis, B. pinnipedialis, B. ceti* and *B. inopinata* can be transmitted between humans and animals, *B. melitensis* being the most common zoonotic *Brucella* species, followed by *B. abortus, B. suis*, and *B. canis* (OIE, 2016; Deepak *et al*., 2019; Attia *et al*., 2024; Cloeckaert *et al*., 2024).

Brucellosis occurs worldwide, except in countries where bovine brucellosis (*B. abortus*) has been eradicated (Tabar and Jafari, 2014). Brucellosis has been largely eliminated in developed regions, including much of western and northern Europe, Canada, Japan, Australia, and New Zealand (WOAH, 2025). Even those countries that have already eradicated/controlled the disease are considering *Brucella* to be a potential bioterrorism threat leading to an increased interest in those countries (Cetaruk, 2024).

1.2. Literature Review

1.2.1. The Genus Brucella

Members of the genus *Brucella* are small, coccobacilli/rods, Gram-negative, nonmotile, facultative intracellular, aerobic but may need added CO_2 (5-10 percent) (Ferooz and Letesson, 2010; Percin, 2013; López-Goñi and O'Callaghan, 2012; Ghssein *et al*., 2025). They produce oxidase, catalase, nitrate reductase, and urease (except *Brucella ovis*); fail to produce indole; are nonhemolytic; do not liquefy gelatin; and have negative methyl red and Voges-Proskauer tests. (Alton and Forsyth, 1996; Khurana *et al*., 2021). They are taxonomically categorized in the phylum Proteobacteria, class Alphaproteobacteria, order Rhizobiales and family Brucellaceae (Araj, 2019; Jiao *et al*., 2021).

Genomic studies have shown high degree of genetic similarity among *Brucella* species (Paulsen *et al.,* 2002; Gee *et al.,* 2004; Halling *et al.,* 2005) that a monospecies designation for the genus has been proposed and the previously designated *Brucella* species were treated as biovars (Verger *et al.*, 1985). However, in 2005 the International Committee on Systematic Bacteriology, Subcommittee on the taxonomy of *Brucella* re-approved the classical system with recognized biovars (Osterman and Moriyón, 2006).

The classical taxonomic designations of *Brucella* are thus: genus, species, and biovar. Strains are further variants of biovars based on lesser mutations. Accordingly, the genus *Brucella* comprises 13 well-classified species that are recognized based on preferential host specificity (Rebollada-Merino *et al*., 2026). These include six classical *Brucella* species: *Brucella abortus, B. melitensis, B. suis, B. canis, B. ovis* and *B. neotomae* (Whatmore and Foster 2021), and seven new named species: *B. microti* (Scholz *et al*., 2008), *B. inopinata* (Scholz *et al*., 2010), *B. papionis* (Whatmore *et al*., 2014), *B. vulpis* (Scholz *et al*., 2016), *B. ceti, B. pinnipedialis* (Foster *et al*., 2007) and *B. pseudogrignonensis* (Li *et al*., 2021). In addition, there are four unnamed *Brucella* species (Hull and Schumaker, 2018). Of the 13 *Brucella* species, four cause almost the entire human infections: *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*, with *B. melitensis* being the most virulent (Whatmore *et al*., 2016). Some species contain biovars, for example: *B. suis*

have five (1–5), *B. melitensis* three (1–3) and *B. abortus* eight (1–7, 9) biovars (WOAH, 2022). Other *Brucella* species have not been differentiated into biovars (Matle *et al*., 2021).

While the "classic" species (*B. melitensis, B. suis*, *B. abortus* and *B. canis*) remain the most common causes of human brucellosis globally and economically important (Bukhari, 2018; Saddique *et al*., 2019), other species especially those from marine mammals and rodents can cross the species barrier and cause disease in humans. These *Brucella* species of zoonotic importance include, *B.ceti* (Attia *et al*., 2024), *B. pinnipedialis* (Deepak *et al*., 2019), *B. inopinata* (Cloeckaert *et al*., 2024) and *B. neotomae* (Vergnaud *et al*., 2024) as illustrated in below. Table 1.1 is partly adapted from El-Sayed and Awad (2018).

Table 1.1: List of *Brucella* species and their natural host.

***Brucella* species**	**Colony type**	**Natural host***	**Zoonoses**
B. melitensis (bv1-3)	Smooth	Goat and sheep	+++
B. abortus (bv 1-6, 7, 9)	Smooth	Cattle	++
B. suis biovar**			
1-3	Smooth	Pig	++
2	Smooth	Wild boar, Hare	+
4	Smooth	Reindeer, Caribou	++
5	Smooth	Rodent	–
B. ovis	Rough	Sheep	–
B. neotomae	Smooth	Desert rat	+
B. canis	Rough	Dog	+
B. ceti (B. delphini)	Smooth	Dolphins	+
B. pinnipedialis (B. phocae)	Smooth	Seals	+
B. microti	Smooth	Wild voles	(?)
B. inopinata	Smooth	Human	++
B. papionis	(?)	Baboons (*Papio* spp.)	(?)
B. vulpis	(?)	Red foxes (*Vulpes vulpes*)	(?)
*B. pseudogrignonensis****	Smooth	Environmental	+

*Nearly all *Brucella* species can infect other mammals beside their primary host with the exception of *B. ovis*. In such cases, the infection is mostly mild and even self-limiting.
**Different *B. suis* biovars vary in their zoonotic potential, while biovars 1, 3 and 4 are more pathogenic to human than *B. abortus* but less than *B. melitensis*, other *B. suis* biovars have obviously limited potential to infect humans.
***B. pseudogrignonensis* (formerly known as Ochrobactrum pseudogrignonense) is newly named *Brucella* species as an emerging pathogen in immunodeficient and immunocompetent patients (Li *et al*., 2021).

Brucella organisms can survive in the environment outside the animal for several months, particularly in cool moist conditions. They remain infectious to other animals which become infected by ingesting the bacteria. The bacteria also colonise the udder and contaminate the milk (WOAH, 2025).

1.2.2. The Disease Brucellosis

Brucellosis is a widespread and economically important zoonotic disease of animals and humans caused by a number of hosts adopted species of the genus *Brucella* (Franc *et al*., 2018; Lokamar *et al*., 2020; Lai *et al*., 2021; Qureshi *et al*., 2023). The disease causes tremendous economic losses through infertility, delayed heat, abortion, reduced meat and milk production (McDermott and Arimi 2002; Lokamar *et al*., 2020; El-Diasty *et al*., 2021), culling (Bardhan *et al*., 2020; Kidanu *et al*., 2026) and international trade bans (Franc *et al*., 2018) as well as a potentially debilitating infection in man (Dean *et al*., 2012).

Brucellosis remains heavily neglected in the developing world historically due to systemic underreporting, lack of public awareness, and the non-specific nature of its symptoms in humans (Franc *et al*., 2018). Small ruminants and camels are bred in resource poor areas of the world that lead to negligences compounded by poverty, geographical isolation, and a lack of access to veterinary infrastructure and health education (Qureshi *et al*., 2023).

In wildlife, brucellosis remains a critical scientific and epidemiological blind spot, largely because differentiating true reservoirs host from incidental spillover hosts remains difficult (Simpson *et al*., 2021). Besides, the lack of a standardized diagnostic test for brucellosis in wildlife is a major hurdle in epidemiology (Godfroid *et al*., 2010). Moreover, randomly selecting animals of all ages—including young animals not in the breeding age —for disease screening often leads to an underestimation of disease prevalence (Christensen and Gardner, 2000).

Transmission of brucellosis between animals usually takes place through direct or indirect interaction with diseased animals or their discharges most often through ingestion, respiratory exposure, conjunctival inoculation and infection through damaged skin or mucosal membranes (Güngür *et al*., 2002; WHO, 2020; WOAH, 2026). Brucellosis can also be transmitted during breeding and lactation (Radostits *et al*., 2007). In human, brucellosis is almost exclusively due to zoonosis, either through direct contact with infected animals or animal products, or through ingestion of infected meat and dairy products, or inhalation of aerosols (López-Santiago *et al*., 2019). Veterinarians, abattoir, farm and laboratory workers are known risk groups for *Brucella* infection (Pereira *et al*., 2020; Rossouw *et al*., 2025).

In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy, and epididymitis and orchitis in the male (Tassew, 2023). Brucellosis in humans may occur in acute, sub-acute or chronic forms (Roushan *et al*., 2016). However, these clinical signs are not pathognomonic and diagnosis is dependent upon demonstration of the presence of *Brucella* species, either by isolation of the bacteria or detection of their antigens or genetic material, or by demonstration of specific antibody or cell-mediated immune responses (Corbel, 2006; Shenoy *et al*., 2016; Yagupsky *et al*., 2019).

Brucellosis is common in rural areas because farmers live in close contact with their animals and often consume raw animal products. The retailing of dairy products also brings the disease to urban areas (Mantur and Amarnath, 2008). Under Ethiopian context, livestock of different species usually share pastures and dwellings. This could play a role in maintenance and transmission of endemic diseases such as brucellosis (Amenu *et al.,* 2010).

1.2.2.1. Camel brucellosis

The livelihoods of pastoral communities in the arid and semi-arid environmrnt of the world are dependent on camels which are well-adapted to harsh desert conditions (Adah *et al*., 2023). Camels contribute to the economy and food security of people (Suliman *et al*., 2019; Swelum *et al*., 2020) which include use of camel as a source of meat, milk, leather and wool, exportation of live camels, as well as use of camels for packing, transport and riding (Faye, 2016; Abu-Seida *et al*., 2024). However, camel production and productivity are constrained by infectious diseases, such as brucellosis, which is a highly infectious bacterial disease that affects camels and humans worldwide (Zhu *et al*., 2019; Waji and Neja, 2023). Moreover, camel milk is widely consumed raw (Shimol *et al*., 2012; Yaseen and Hanee, 2019) and hence poses a public health threat (Zhu *et al*., 2019).

Camel brucellosis is primarily caused by *Brucella abortus* and *B. melitensis* (Shabbir *et al*., 2020; Dadar *et al*., 2022), although *B. ovis* and *B. suis* have also been detected in camels (WOAH, 2022; Sahban *et al*., 2023). Because camels are not the primary hosts, they frequently contract the infection from cohabiting ruminants, making the disease rare in herds kept separate from infected sheep, goats, or cattle. Once exposed, the bacteria can enter the body through inhalation, ingestion, mucous membranes, broken skin, conjunctiva, or venereal contact (Waji and Neja, 2023)

The organism is transported to the thoracic duct via lymphatic channels and then disseminates to parenchymal and joint tissues through the bloodstream (Seleem *et al*., 2010). *Brucella* organisms cause various pathological changes in the pregnant uterus, udder, testicles, accessory male sex glands, lymph nodes, joint capsules and bursae (Wernery, 2014). Affected uterus shows reddening, edema and necrotic foci in the epithelium, fibrosis of the endometrium and atrophy of the uterine glands. Ovariobursal adhesions and hydrobursitis are also evident (Sprague *et al*., 2012).

The spread of brucellosis is increasingly concerning due to its asymptomatic nature in infected animals, and informal milk distribution chains in developing countries encourages the supply of

unprocessed, raw milk (Wareth *et al*., 2014). Clinical signs in breeding camelids are similar to those in bovines and small ruminants, but with fewer abortions reported (Wernery, 2014). Camel brucellosis causes prolonged calving, stillbirth, abortion, infertility, mastitis, and reduced milk and meat production (Abbas and Agab, 2002; Dadar *et al*., 2022). Orchitis and epididymitis in males and placentitis in females are also common (Wernery, 2014).

1.2.2.2. Small ruminant brucellosis

Sheep and goats are mojor domestic animals especially in pastoral areas (Degen, 2007; Adams *et al*., 2021). However, the losses associated with infectious diseases such as brucellosis in small ruminants is significant (Tewodros and Dawit, 2015; Geletu *et al*., 2021). Moreover, brucellosis associated with these animals is a significant concern for human health (Aloto *et al*., 2022).

Small ruminant brucellosis is primarlly caused by *Brucella melitensis* and *B. ovis* and occasionally by *B. abortus* (Rossetti *et al*., 2022). Sporadic cases caused by *B. abortus* and *B. suis* have also been observed in sheep and goats, but cases of natural infection are rare (WOAH, 2022). *Brucella melitensis* is also the most virulent *Brucella* species for humans, responsible for a severely debilitating and disabling illness (Rossetti *et al*., 2017).

Small ruminants often acquire *B. melitensis* by contact with organisms in vaginal discharges and birth products (e.g., placenta, fetus, fetal fluids). Disease transmission is primarly by direct contact with aborted fetuses, placental membranes, vaginal discharges, or ingestion of contaminated water, pastures or colostrums. *Brucella melitensis* infection under natural exposure is transmitted through ingestion or inhalation (Alemneh and Akeberegn, 2018; Erkyihun and Kassa, 2023; Desa, 2025). In addition, the organism exhibits potential for transmition through the conjunctival mucosa, broken skin and sexual contact (CFSPH, 2023).

Entry of *Brucella melitensis* by ingestion or inhalation enables the bacteria to colonize, proliferate and persist in the lymph nodes (LNs) of the head. Failure to eliminate *B. melitensis* on this primary line of defense results in escape of the bacteria through the efferent lymphatic vessels to the distal LN or via blood to the general circulation. In pregnant females, *B. melitensis*

colonizes placenta and induces abortion or stillbirth. *Brucella melitensis* also has affinity for lactating udder. Significant numbers of *B. melitensis* are excreted in vaginal discharges, aborted fetus, placenta and milk (Rossetti *et al*., 2022).

Small ruminant brucellosis mainly affects the reproductive tract of animals which is manifested by middle- to late-term abortion, retention of placenta, stillbirth and delivery of weak offspring in the case of female animals, and epididymitis and orchitis in males (Rossetti *et al*., 2017; Kebkiba *et al*., 2021), with excretion of the organisms in uterine discharges and in milk (OIE, 2009b).

1.2.2.3. Bovine brucellosis

Cattle are among the most important livestock species contributing significantly to agriculture, economy and culture. They provide food (meat, milk), raw materials (leather, wool, tallow), fertilizer (manure), labor (draught power), and even medicinal products (insulin) serving livelihoods of millions, especially in rural areas, and driving GDP through vast global markets for dairy and beef products (Mufareh, 2023; Huang, 2024; Getiso and Mijena, 2024). Their production, however, is affected by many diseases which are transmitted to humans. Among these, brucellosis is a highly infectious disease of zoonotic importance (Pal *et al*., 2017).

Bovine brucellsos is usually caused by biovars of *B. abortus* (WOAH, 2022). It can also be caused by *B. melitensis* and *B. suis* in areas where cattle are kept in close association with sheep/goats and pigs, respectively (CFSPH, 2018a, 2018b; OIE, 2018). *Brucella suis* causes a chronic infection in the mammary gland of cattle, but it has not been reported to cause abortion or spread to other animals (OIE, 2018).

Animals contract the infection by ingestion or inhalation of contaminated materials (Khurana *et al*., 2021) and organisms shed in the milk of infected animals may transmit the infection to the newborn (Tulu, 2022). Expossure to aborted fetuses, fetal membranes and discharges from uterus can also act as a means of transmission (Ferrero *et al*., 2014). Artificial insemination or natural service by infected bulls may also spread the infection (Li *et al*., 2020).

The pathogenesis of *Brucella* involves its ability to endure and mltiply inside cells (including phagocytes) thereby evading the immune system (Rehman *et al*., 2025). After multiplying in the host cells, the bacteria reach trophoblasts and the mammary gland through circulation, and very expansively multiply to induce abortion. While in non-pregnant animals, bacteria continue to multiply and shed in the environment through various body secretions and excretions (Khurana *et al*., 2021). The capacity of *Brucella* to endure various environmental conditions, including water, soil, dairy products, and meat, is an additional feature that enhances its ability to cause illness and spread (Rehman *et al*., 2025).

Bovine brucellosis is usually asymptomatic in young animals and non-pregnant cows. Following infection, pregnant adult females develop a placentitis usually resulting in abortion between the fifth and ninth month of pregnancy (WOAH, 2022). Infection may last long in cows during subsequent pregnancies; the recurrence of abortion is rare, but uterine and mammary infections commonly recur (Pappas *et al*., 2005). In infected animals, subsequent pregnancies are accompanied with reduced numbers of organisms in birth products and milk (Banai *et al*., 2021; Islam *et al*., 2023). Adult male cattle may develop orchitis and brucellosis may be a cause of infertility in both sexes. Hygromas, usually involving leg joints, are a common manifestation of brucellosis in some tropical countries and may be the only obvious indicator of infection; the hygroma fluid is often infected with *Brucella* (WOAH, 2022).

1.2.2.4. Human brucellosis

Brucellosis primarily affects livestock but it is readily transmissible to humans, causing acute febrile illness (undulant fever) with 1.6-2.1 million new human cases annually (Laine *et al*., 2023). With the renewed threat of biological warfare and agricultural terrorism, it is worth mentioning that brucellosis is considered a potential type B bioweapon (Shakir, 2021).

The disease in humans is caused mainly by *B. melitensis* as the most pathogenic species, followed by *B. suis*, *B. abortus* and *B. canis* (Galińska and Zagórski, 2013; Głowacka *et al*., 2018). Newly recognized species such as *B. pinnipedialis* (Deepak *et al*., 2019), *B. ceti* (Attia *et*

al., 2024) and *B. inopinata* (Cloeckaert *et al*., 2024) have also been reported to cause disease in humans.

The source of infection in humans are infected domestic and wild animals, and among humans, the highest exposure is observed in veterinarians, veterinary assistants, insemination service employees, zoo technicians, farmers, employees of dairy and meat processing enterprises, hunters, and personnel in microbiology laboratories (Galińska and Zagórski, 2013; Mirambo *et al*., 2018; Pereira *et al*., 2020). Infections in humans generally result from consumption of contaminated and/or unpasteurized animal products, direct contact with infected animal parts, and inhalation of infected aerosolized particles (Akhvlediani *et al.,* 2010). Human-to-human transmissions including vertical, sexual, blood transfusion, bone marrow transplantation and aerosol from an infected patient have also been reported (Tuon *et al*., 2017). Transmission from lactating mothers to their breastfed infants has also been reported (Al Dahouk *et al*., 2013).

Once *Brucella* organisms penetrate the host, they replicate in a wide variety of cells particularly within macrophages, dendritic cells, and placental trophoblasts (de Figueiredo *et al*., 2015). This intracellular replication helps the pathogen to evade host immune response (Martirosyan and Gorvel, 2013) and sequesters the organism from some antibiotics. The unique features of pathology in infected hosts typically include the incubation phase before clinical symptoms are evident, the acute phase during which time the pathogen invades and disseminates in host tissue, and the chronic phase that can eventually result in severe organ damage and death of the host organism (de Figueiredo *et al*., 2015).

Human brucellosis has various clinical manifestations including intermittent fever, sweating, headache, fatigue, myalgia, abortion, depression, anorexia, weight loss and general weakness (Baud and Greub, 2011; Dean *et al*., 2012; Madut *et al*., 2018b; Jiang *et al*., 2019). Brucellosis in human also produces serious complications affecting the musculoskeletal, cardiovascular, and central nervous systems (OIE, 2009a). As some of these signs also resemble other febrile illnesses such as malaria, typhoid, rheumatic fever and viral diseases, the disease is usually misdiagnosed and under-reported in most countries in sub-Saharan Africa (Bouley *et al*., 2012; Crump *et al*., 2013).

1.2.3. Epidemiology of Brucellosis

1.2.3.1. Geographical distribution

Brucellosis in animals occurs worldwide, except in those countries such as Australia, Canada, Cyprus, Denmark, Finland, The Netherlands, New Zealand, Norway, Sweden, and the United Kingdom where brucellosis has been eradicated (Khurana *et al*., 2021).

It remains endemic in the Mediterranean region (Abdeen *et al*., 2019; Rodríguez-Alonso *et al*., 2021; Mazzeo *et al*., 2025), the Middle East (Nejad *et al*., 2020; Al-Amr *et al*., 2022; Dadar *et al*., 2025), Central Asia (Charypkhan and Rüegg, 2022; Kydyshov *et al*., 2022; Qurbonov *et al*., 2024), parts of Latin America (Rossetti *et al*., 2017; Avila-Granados *et al*., 2019) and Africa (Ducrotoy *et al*., 2017; Simpson *et al*., 2021; Wagaba *et al*., 2025) particularly in east Africa including Ethiopia (Djangwani *et al*., 2021; Sibhat *et al*., 2022; Asrie *et al*., 2025) where the resources to establish control programs are lacking. In human it is often unrecognized and frequently goes unreported (Kalule *et al*., 2020).

Brucellosis is widespread across sub-Saharan Africa, with livestock animal-level prevalence ranging from 0.2% to 43.8% in cattle, 0.0% to 20.0% in goats, and 0.0% to 13.8% in sheep. Consequently, human prevalence rates in the region reach up to 55.8%, highlighting a major public health challenge (Djangwani *et al*., 2021; Qureshi *et al*., 2023). Recent studies reveal a higher global incidence than previously estimated, with 1.6–2.1 million new human cases annually (Laine *et al*., 2023).

1.2.3.2. Risk factors of brucellosis

The acquisition and occurrence of brucellosis in animals and humans are influenced by several factors that can either related to animals or humans.

Risk factors in animals

The acquisition and occurrence of brucellosis in animals are influenced by several factors. These factors have been associated with the biology of the agent, characteristics of host populations and management system (Coelho *et al.*, 2015).

Agent factors: *Brucella* organisms are intracellular pathogens that are able to survive and replicate within phagocytic cells (Jansen *et al*., 2020). They can persist on fetal tissues and soil or vegetation for 21-81 days depending on month, temperature, and exposure to sunlight (CFSPH, 2023). *Brucella* bacteria can also survive in cold areas and froozen meat for long periods of time (Pal *et al*., 2020).

Host factors: Susceptibility of livestock to *Brucella* infection is influenced by species (Getachew *et al*., 2025), breed of the animal (Coelho *et al*., 2015; Eticha *et al*., 2018; Sima *et al*., 2021), age (Ashagrie *et al.,* 2011; Megersa *et al.,* 2011b; Borba *et al.,* 2013) and sex (Bekele *et al*., 2011; Demissie *et al*., 2025b; Getachew *et al*., 2025). Health status of the animals, unvaccinated animals in infected herds and sexual maturity are all host factors associated with the spread of brucellosis (Gemechu, 2017).

In farm animals, the prevalence of brucellosis appears to be lower in small ruminants than large ruminants and in sheep than goats. In this last case, the vaginal excretion of *Brucella* spp. in goats is greater and more prolonged than sheep (Radostits *et al*., 2000). In small ruminant, Maltese and South American sheep breeds seem to present a greater resistance to brucellosis compared to the sheep breeds of southwest Asia and the Mediterranean, such as the Awassi breed (Coelho *et al.*, 2015).

The prevalence of brucellosis is higher in older animals than young animals that are in breeding age, and this might attributes to higher chance of acquiring the infection due to longer exposure time during the animals' life. The odds of infection by brucellosis in ruminants are higher in female than male animals and this is associated with presence of erythritol in female reproductive tract which stimulates the growth of the organism (Godfroid *et al*., 2010).

Management factors: The transmission of brucellosis between herds, and the maintenance and spread of infection within herds are influenced by herd size and composition, hygienic status of the farm, rate of contact between infected and susceptible animals and movement of infected animals from an infected herd into a non-infected susceptible herd (farm biosecurity) (Radostits *et al*., 2007; Luelseged *et al*., 2018). Other key derivers also include consumption of contaminated feed/water, poor herd management, reproductive failures (abortions, retained placenta), population density, health status of the animals, unvaccinated animals in infected herds, use of maternity pens, and close living with wild animals (Gemechu, 2017; Bedore and Mustefa, 2019). Some of these factors are attributed to increased contact between susceptible and infected animals. For example, crowding of a large number of mixed ruminants at watering points facilitates spread of the disease (Radostits *et al*., 2007).

Brucellosis sero-positivity is higher in large herds and/or flocks and this has been largely reported in animals (Ibrahim *et al*., 2010; Megersa *et al*., 2011b; Demissie *et al*., 2025a, 2025b). In large herds, there are higher odds of detecting at least one sero-positive animal, increase of transmission of the disease by contact among them, utilization of communal pasture areas or improper cleaning and disinfection procedures and increased cattle movement for replacement and trade (Coelho *et al*., 2015; Engdawork, 2025). On the other hand, small-sized flocks usually graze at pastures near or contiguous to the farm, avoiding contact with other flocks or utilization of common paths and/or roads. Moreover, cleaning, disinfection and manure removal procedures are easier and less time consuming to the farmer in small herds.

Farming several species in the same herd has been described as a risk of infection due to multiple sources of infection. For example, presence of dogs in large herds may spread *Brucella* species into the environment mechanically or through urine, vaginal secretions, aborted fetuses or feces (Coelho *et al*., 2015).

Environmental and climatic factors: The survival of the organism in the environment plays an important role in the epidemiology of the disease (Beruktayet and Mersha, 2016). *Brucella* may retain infectivity for several months in cool and wet conditions (Khurana *et al*., 2021).

Risk factors in humans

Occupational Exposure: Brucellosis is an occupational hazard. Humans living in close proximity with animals or handling them; laboratory workers, abattoir workers, farmers, and veterinarians are particularly at high risk of acquiring the infection (Addis, 2015; Pal, 2018; Bodenham *et al*., 2020; Mubanga *et al*., 2021). In general, handling aborted materials, attending retained placenta or dystocia without protective gear, and contact with infected animals and animal products and/or byproducts pose risk of brucellosis in humans (Musallam *et al*., 2016; Khalid, 2023; Narimisa *et al*., 2024).

Consumption of raw animal products: Raw milk and other dairy products are the main source of infection to humans (Onyango *et al*., 2021; Dadar *et al*., 2024; Dagnaw *et al*., 2024; Sharma *et al*., 2025). This is particularly important for people who do not have direct contact with animals (SCAHAW, 2001; FAO, 2006). Raw or undercooked meat and meat product consumption may also be a major source of infection for brucellosis caused by *Brucella abortus***,** *B. melitensis*, and *B. suis* (Ghugey *et al*., 2021; Faddane *et al*., 2022; Narimisa *et al*., 2024).

Age and sex: Human brucellosis most commonly affects adults and male individuals due to occupational exposure (Assafi *et al*., 2019; Alkahtani *et al*., 2020; Mubanga *et al*., 2021; Hassan *et al*., 2022). While males are often more affected (Tumwine *et al*., 2015; Alkahtani *et al*., 2020; Kahariri *et al*., 2021; Faddane *et al*., 2022), women in pastoral and rural community face higher risk due to household roles like processing unpasteurized dairy, handling raw meat and milking animals (Muturi *et al*., 2018; Assafi *et al*., 2019), highlighting that gendered roles drive transmission rather than biology alone.

Pregnancy and breastfeeding: Brucellosis is one of the diseases that poses health hazards during the course of pregnancy and carries the risk of adverse pregnancy outcomes in humans (Al Dahouk *et al*., 2013; Arenas-Gamboa *et al*., 2016; Alsaif *et al*., 2018). Human-to-human transmission from mothers to their infants through breastfeeding has also been reported (Palanduz *et al*., 2000; Apa *et al*., 2013). The most common unfavorable outcomes during brucellosis in pregnancy are abortion or intrauterine fetal death depending on the stage of

pregnancy. Other unfavorable outcomes due to brucellosis include congenital/neonatal brucellosis, low birth weight, development delay, or even death in infants, various clinical course of brucellosis in mother, and exposure of delivery team (Bosilkovski *et al*., 2020; Huy, 2024).

Seasons: In humans, the prevalence of the disease is high in the spring and summer season (Assafi *et al*., 2019; Faramarzi *et al*., 2019; Chen *et al*., 2023). Brucellosis transmission in human is significantly affected by temperature, humidity, rainfall, sunshine duration, and evaporation and higher values of these factors usually increase disease incidence (Faramarzi *et al*., 2019; Liu *et al*., 2020; Huang *et al*., 2024).

1.2.4. Brucellosis in Ethiopia

Despite the large number of livestock in Ethiopia, endemic diseases such as brucellosis threaten its productivity and trade (NAIP, 2022). Brucellosis is widely distributed and is among the top five (rabies, anthrax, brucellosis, Rift Valley fever and highly pathogenic avian influenza) neglected zoonotic diseases in Ethiopia (Erkyihun *et al*., 2022).

Ethiopia has diverse agro-ecological zones with different agricultural production systems and over 90% of agriculture is characterized by mixed crop–livestock farming systems (Duguma and Janssens, 2021). The distribution and prevalence of brucellosis in Ethiopia varies by location and time, owing to differences in animal production and management systems, multiple livestock species per holding, community living standards, awareness levels as well as agro-ecological conditions.

Camel brucellosis: Camels are the most important livestock species uniquely adapted to arid and semi-arid areas that are not hospitable to other domestic animals (Gebreyohanes and Assen, 2017). They are very important means of livelihood, food security, and transportation for pastoral community. However, camel production is hindered by many diseases, such as brucellosis. The first published data on camel brucellosis in Ethiopia were in 1977's (Domenech and Lefèvre, 1974; Domenech, 1977) and in general, it is less studied in Ethiopia. A systematic review and meta-analysis in Ethiopia estimated the pooled sero-prevalence of camel brucellosis

at 3.0% (Ambaw *et al*., 2026). Table 1.2 shows some reports of camel brucellosis in various areas of Ethiopia over the past fifteen years.

Small ruminant brucellosis: Most reports on small ruminant brucellosis in Ethiopia were from pastoral livestock production systems and the highest prevalence report (17.36%) was from Oromia pastoral areas (Teshome *et al*., 2022). Systematic review and meta-analysis of small ruminant brucellosis in Ethiopia estimated the pooled sero-prevalence at 3.0% (Zemene *et al*., 2024). Table 1.3 indicated small ruminant brucellosis sero-prevalence in Ethiopia over the past fifteen years from different geographical areas.

Bovine brucellosis: Multiple studies in the past ten years have reported the widespread distribution of bovine brucellosis (Table 1.4). Higher individual sero-prevalence of bovine brucellosis has been recorded in cattle managed under intensive management systems than extensive. Systematic reviews and meta-analyses of 20 years published data in Ethiopia estimate the pooled individual animal-level sero-prevalence at roughly 2.6% while herd-level prevalence is significantly higher (16.3%). The prevalence being higher in the pastoral/agro-pastoral system compared to mixed crop-livestock and intensive systems (Sibhat *et al*., 2022).

Human brucellosis: Data on human brucellosis in Ethiopia are sparse and are focusing on patients with febrile illnesses at health centers. The prevalence of human brucellosis varied significantly across regions, with the highest (48.3%) observed in Afar (Tschopp *et al*., 2021) (Table 1.5). The national sero-prevalence of human brucellosis in Ethiopia is estimated at an overall pooled prevalence of 14.0% (Asrie *et al*., 2025).

Table 1.2: Sero-prevalence of camel brucellosis reported from various regions in Ethiopia (2011-2026).

Study area		No. of samples	Type of test	Prevalence and 95% CI based on CFT or ELISA test		Reference
Region/City	Specific location			Prevalence (%)	95% CI	
Oromia	Borana	756	RBT, CFT	2.2	1.4-3.7	Megersa *et al*., 2011a
Oromia	Borana	1073	RBT, CFT	1.8	1.1-2.8	Megersa *et al*., 2012
Dire Dawa	In and around Dire Dawa	646	RBT, CFT	1.5	-	Warsame *et al*., 2012
Afar	Awash-Fentale and Amibara	768	RBT, CFT	7.6	-	Zeweld and Haileselassie, 2012
Afar	Afambo, Aysaita, Teru and Aura	461	RBT, CFT	5.4	-	Bekele *et al*., 2013
Somali and Oromia Regions	Pastoral areas	458	RBT, CFT	0.9	0.3-2.7	Gumi *et al*., 2013
Afar	Mille, Chifra and Dubti districts	1152	RBT, CFT	4.1	2.9-5.3	Hadush *et al*., 2013
Somali	Jijiga and Babile	822	RBT, CFT	2.43	1.3-3.8	Tilahun *et al*., 2013
Tigray	Mehoni district	300	RBT, CFT	5.78	-	Tassew and Kassahun, 2014
Tigray	Mehoni	415	RBT, CFT	3.37	-	Habtamu *et al*., 2015
Afar	Aysaita, Mille, Awash-Fentale and Amiber	813	RBT, CFT	2.09	-	Zeru *et al*., 2016
Addis Ababa	Akaki Abatoir	201	RBT, CFT	4.5	-	Abebe *et al*., 2017
Oromia	Yabello	384	RBT, CFT	3.1	1.3-4.9	Admasu and Kaynata, 2017
Afar	Dubti and Chifra	245	RBT, CFT	4.1	-	Gizaw *et al*., 2017
Oromia	Yabello and Gomole	368	RBT, i-ELISA	3.0	-	Jara *et al*., 2020
Somali	Adadle	171	RBT, i-ELISA	0.6	0.0-3.2	Ibrahim *et al*., 2021
Afar	7 districts	617	c-ELISA	9.0	6.6–12.2	Tschopp *et al*., 2021
Somali	6 districts	117	c-ELISA	1.8	0.6–6.9	Tschopp *et al*., 2021
Afar	Amibara	250	RBT, CFT	3.2	1.63-6.2	Wegi *et al*., 2021
Oromia	Borana	315	RBT, i-ELISA	2.86	13.15–53.54	Giro *et al*., 2022
Dire Dawa	In and around Dire Dawa	350	RBT, CFT	2.0	-	Waktole *et al*., 2022
Somali	Gurabakaksa, Guradhamole, and Dolo Ado	450	RBT, c-ELISA	2.9	1.5-4.9	Ahad *et al*., 2024

Somali	Fik, Hamaro and Laghida	450	RBT, CFT	4.8	2.8-6.8	Mohomed *et al*., 2024
Oromia	Elwayye	240	RBT, i-ELISA	3.7	1.94-7.05	Wario *et al*., 2024
Oromia	Dire	460	RBT, i-ELISA	4.78	3.17-7.17	Ambaw *et al*., 2025
Oromia	Gumbi Bordode	384	RBT, i-ELISA, CFT	1.30 (CFT)	-	Asmelash *et al*., 2025
Oromia	Fentale	324	RBT, c-ELISA	9.6	6.5-13.0	Demissie *et al*., 2025a
Oromia	Arero	313	RBT, i-ELISA	2.24	-	Edema *et al*., 2025
Oromia	Gomole and Dhas	160	RBT, i-ELISA	3.8	-	Getachew *et al*., 2025
Oromia	Dire	390	RBT, i-ELISA	4.36	2.72-6.91	Ambaw *et al*., 2026

Table 1.3: Sero-prevalence of small ruminant brucellosis reported from various regions in Ethiopia (2011-2025).

Study area		No. of samples	Type of test	Prevalence and 95% CI based on CFT or ELISA test		Reference
Region/City	**Specific location**			**Prevalence (%)**	**95% CI**	
South Ethiopia	Hammer and Dasenech districts	384	RBT, CFT	4.2	-	Ashagrie *et al*., 2011
Somali	Jijiga district	730	RBT, CFT	1.5	-	Bekele *et al.*, 2011
Amhara	In and around Bahr Dar	500	RBT, CFT	0.4	-	Ferede *et al*., 2011
Oromi	Borana	1248	RBT, CFT	1.6	1.0-2.5	Megersa *et al*., 2012
Dire Dawa	Dire Dawa	384	RBT, CFT	9.11	6.43-12.45	Negash *et al*., 2012
Afar	Afambo, Assaiyta, Teru and Awura	324 (sheep)	RBT, CFT	7.1	-	Adugna *et al*., 2013b
Afar	Afambo, Assaiyta, Teru and Awura	726 (goats)	RBT, CFT	13.64	-	Adugna *et al*., 2013b
South and Central Ethiopia	South and centeral Ethiopia	3,315	RBT, CFT	1.9	1.5-2.4	Asmare *et al*., 2013a
Oromia	Yabello district	384	RBT, CFT	1.56	-	Dabassa *et al*., 2013
Somali and Oromia	Pastoral areas	510	RBT, CFT	9.6	5.2-17.1	Gumi *et al*., 2013
Tigray	Spouthern zone	-	CFT	3.5	-	Teklue *et al*., 2013
Tigray	Mehoni district	150	RBT, CFT	3.56	-	Tassew and Kassahun, 2014
Oromia and Somali	Moyale Oromia. Moyale, Somali, Dillo and Dolo Ado districts	420	RBT, CFT	3.6	-	Tsehay *et al*., 2014
Oromia	Tiyo, Dodota and Adami Tulu-Jido Kombolcha	840	RBT, i-ELISA	4.6	-	Deddefo *et al*., 2015
Oromia	Ada'a-Liben, Ambo and Fentale	1119	RBT, CFT	3.57	2.49–4.66	Gebremedhin, 2015
Oromia	Debre-Zeit and Modjo town	450	RBT, CFT	1.11	-	Mohammed *et al*., 2015
4 regions	25 districts and eight pastoral zones	6,201	RBT, CFT	2.2	1.8–2.6	Sintayehu *et al*., 2015
Amhara	in and around Kombolcha town	714	RBT, CFT	0.7	-	Tewodros and Dawit, 2015
Afar and Oromia	Awash Fentale and Fentale	327 (cattle & goats)	ELISA	22.8	5.98-29.5	Tschopp *et al*., 2015

Oromia	Debrezeit and Modjo	853	RBT, CFT	1.76	-	Tsegay *et al*., 2015
Afar	Tellalak	414	RBT, CFT	13.7	-	Wedajo *et al*., 2015
Afar	Chifra and Ewa	1190	RBT, CFT	12.35	-	Tegegn *et al*., 2016
South Ethiopia	Mirab Abaya	389	RBT, CFT	5.1	-	Dulo, 2017
Tigray	Tselemti	558	RBT, CFT	1.79	-	Kelkay *et al*., 2017
Somali	Awbarre, Jijiga and Gursum.	291	RBT, CFT	1.37	0.02-2.72	Mohammed *et al*., 2017
Amhara	6 districts	2409	RBT, CFT	4.89	3.24-6.9	Addis and Desalegn, 2018
South Ethioia	Nechisar National Park	246	RBT, ELISA, CFT	4.5	2.25-7.86	Chaka *et al*., 2018
Dire Dawa	8 peasant associations	424	RBT, CFT	2.59	-	Teshome *et al*., 2018
Oromia	Babile	384	RBT, CFT	0.78	-	Atlaw and Girma, 2019
Oromia	Yabello	283	RBT, c-ELISA	8.1	5.2-11.9	Demena, 2019
Somali	Jigjiga and Gursum	172	RBT, CFT	1.7	-	Lakew *et al*., 2019
Oromia	Gomole, Elewoye, Dubuluk, and Miyo	882	RBT, c-ELISA	3.2	2.1-4.6	Edao *et al*., 2020
Oromia	Limu Seka and Chora Boter districts	804	RBT, CFT	4.7	-	Tulu *et al*., 2020a
Oromia	Yabello and Dire districts	228	RBT, c-ELISA	8.8	5.4-13.2	Zewdie, 2020
Oromia	Dallo-Manna and Haranna-Bulluk	384	RBT, CFT	2.9	-	Adem *et al*., 2021
Oromia	Chiro, Hirna, and Mieso	2070	RBT, CFT	0.24	-	Geletu *et al*., 2021
Oromia	Berebere district	470	RBT, CFT	2.97	-	Muhidin *et al*., 2021
Afar	7 districts	1486 (goats)	c-ELISA	9.7	8.3-11.7	Tschopp *et al*., 2021
Afar	7 districts	613 (sheep)	c-ELISA	8.9	6.7-11.9	Tschopp *et al*., 2021
Somali	6 districts	980 (goats))	c-ELISA	9.5	7.3-12.3	Tschopp *et al*., 2021
Somali	6 districts	243 (sheep)	c-ELISA	6.6	4.0-10.6	Tschopp *et al*., 2021
South Ethiopia	Konso and Gamo zones	788	RBT, CFT	4.1	3.7-4.4	Aloto *et al*., 2022
Oromia	Elwaya, Moyale and Yabello	789	c-ELISA	17.36	14.78-20.19	Teshome *et al*., 2022
South Ethiopia	Kolme and Abala Abaya	690	RBT, CFT	3.33		Dosa *et al*., 2023
Somali	Doboweyn, Kebridahar, and Sheygosh	324	RBT, CFT	1.23	0.0-2	Hussen *et al*., 2023
Oromia	Chiro and Burka Dhintu	444	RBT, i-ELISA	6.5	4.6-9.3	Wubaye *et al*., 2024
South Ethioia	Nyangatom	768	RBT, CFT	12.9	-	Asrat *et al*., 2025
Oromia	Fentale	384	RBT, c-ELISA	2.6	1.0-4.2	Demissie *et al*., 2025b

Oromia	Yabello and Elweye	541	RBT, CFT	2.4	1.41-4.07	Desa *et al*., 2025
Afar	Dubti	300	RBT, i-ELISA	6.7	4.15-7.95	Dubie *et al*., 2025
Oromia	Gomole and Dhas	330 (sheep)	RBT, i-ELISA	1.5	-	Getachew *et al*., 2025
Oromia	Gomole and Dhas	330 (goats)	RBT, i-ELISA	10.0	-	Getachew *et al*., 2025
Oromia	Wachile district	324	RBT, i-ELISA	1.23	-	Gumi and Wudneh, 2025

Table 1.4: Sero-prevalence of bovine brucellosis reported from various regions in Ethiopia (2010-2025).

Study area		Number of samples	Test used	Prevalence and 95% CI based on CFT or ELISA test		Reference
Region	**Specific location**			**Prevalence (%)**	**95% CI**	
Oromia	Jimma	1595	RBT, CFT	3.1	-	Ibrahim *et al*., 2010
Oromia	Jimma	950	RBT, CFT	1.1	-	Tolosa *et al*., 2010
Oromia	Merti district	370	RBT, CFT	0.05	-	Degefa *et al*., 2011
Somali	Kebribeyah, Awbare, Gursum and Harshin districts	435	RBT, CFT	1.38	-	Degefu *et al*., 2011
Tigray	Welkait, Tsegede, and Humera districts	1354	RBT, CFT	6.1	-	Haileselassie *et al*., 2011
South Ethioia	Six zones	1623	RBT, CFT	3.5	2.4-4.5	Megersa *et al*., 2011b
Addis Ababa	Addis Ababa	1202	RBT, CFT	1.5	-	Tesfaye *et al*., 2011
Oromia	Yabello district	290	RBT, CFT	5.9	3.2-8.6	Adane *et al*., 2012
Oromi	Borana	575	RBT, CFT	8.0	6.0-10.6	Megersa *et al*., 2012
Oromia	Guto-Gi district	406	RBT. CFT	1.97	-	Yohannes *et al*., 2012
Benishangul Gumuz	Dibate and Wembera districts	1152	RBT, CFT	1.0	0.5-1.7	Adugna *et al*., 2013a
Different regions	-	2334	RBT, CFT	1.9	1.2-2.6	Asmare *et al*., 2013b
Somali and Oromia	Pastoral areas	862	RBT, CFT	1.4	0.8-2.6	Gumi *et al*., 2013
Oromia	Tiyo, Digelu Tijo, Munessa, and Lemmu Bilbilo districts	584	RBT, i-ELISA	1.7	0.8-3.5	Tschopp *et al*., 2013
Oromia	Debre Zeit	300	RBT, CFT	2.0	-	Alemu *et al*., 2014
Oromia	Adami Tulu	690	RBT, CFT	4.3	-	Tibeso *et al*., 2014
Amhara	Debre-Birhan	415	RBT, CFT	0.7	-	Bashitu *et al*., 2015
Oromia	Jimma town and Chora Botor	348	RBT, CFT	0.3	-	Dirar *et al*., 2015
Afar and Oromia	Awash Fentale and Fentale	327 (cattle & goats)	ELISA	4.8	1.2-17.1	Tschopp *et al*., 2015
Amhara	In and around Gonder town	406	RBT, CFT	4.9	-	Alehegn *et al*., 2016
Oromia	in and around Alage	804	RBT, c-ELISA	2.4	-	Asgedom *et al*., 2016
Oromia	Asela and Bishoftu	570	Card test, RBT, i-ELISA, CFT	1.4	0.24-3.46	Geresu *et al*., 2016
Oromia	Six districts of north Shewa	384	RBT, CFT	0.0	-	Pal *et al*., 2016
Oromia	Asela town and Robe district	756	RBT, CFT	2.6	-	Tsegaye *et al*., 2016
Eastern Ethiopia	Eastern Ethiopia	967	RBT, CFT	1.3	0.72-2.2	Terefe *et al*., 2017

South Ethiopia	Nechisar National Park	268	RBT, CFT	9.7	6.44-13.8	Chaka *et al*., 2018
Addis Ababa	Addis Ababa	1550	CFT	0.06	-	Edao *et al*., 2018
Oromia	Agarfa and Berbere districts	768	RBT, CFT	4.95	-	Elemo and Geresu, 2018
Oromia	Asela	304	RBT, CFT	9.87	-	Eticha *et al*., 2018
South Ethiopia	Awassa	370	RBT, CFT	2.7	-	Abera *et al*., 2019
Oromia	Becho	384	RBT, ELISA	1.04	-	Teka *et al*., 2019
Oromia	Around Addis Ababa	503	RBT, CFT	0.4	-	Bifo *et al*., 2020
Oromia	In and around Adama town	384	RBT, CFT	1.04	0.02-2.62	Bulcha *et al*., 2020
Oromia	Borena	750	RBT, ELISA	2.4	1.4-3.7	Edao *et al*., 2020
Gambella	Jikow district	384	RBT, CFT	14.6	-	Negash *et al*., 2020
Oromia	Limu Seka	423	RBT, CFT	4.3	2.15-5.89	Robi and Gelalcha, 2020
Oromia	Jima zone	423	RBT, CFT	4.02	-	Tulu *et al*., 2020b
Oromia	Jimma	80	CFT	22.5	-	Ararsa *et al*., 2021
Somali	Adadle	135	RBT, i-ELISA	1.5	0.2-5.2	Ibrahim *et al*., 2021
Southern Ethiopia	Maji, Menitshasha and Menitgoldiya	772	RBT, CFT	1.94	0.97-2.92	Kenea and Megersa, 2021
Afar	Dubti, Asyta, Chifra	420	RBT, CFT	5.7	3.8-7.94	Negash and Dubie, 2021
Oromia	Diga, Guto, Gida, and Sibu Sire	1152	i-ELISA	1.82	-	Sima *et al*., 2021
Afar	7 districts	488	i-ELISA	7.2	5-10.2	Tschopp *et al*., 2021
Somali	6 districts	116	i-ELISA	6.9	3.5-13	Tschopp *et al*., 2021
Oromia	Horro Guduru Animal Production Centre	812	RBT, c-ELISA, CFT	0.73	0.241-3.461	Garoma, 2022
Southwest Ethiopia	Semen-Bench, Shei Bench, Sheko, Meinet Shasha and Goldiya	461	RBT, CFT	7.14	4.44-9.01	Robi *et al*., 2023
South Ethiopia	Chencha and Bonke districts	384	RBT, CFT	1.5	-	Shurbe *et al*., 2023
South Ethiopia	Malle, Bena Tsemay and south Ari	614	RBT, CFT	2.8	1.72-4.41	Demissie *et al*., 2024
Oromia	Chiro, Daro Labu, Mieso, and Burka Dhintu	384	RBT, i-ELISA	3.1	-	Aklilu and Weldemariam, 2025
Amahar and Oromia	Angolela, Tera and Kimbibit	511	RBT, CFT	0.98	0.41-2.34	Engdawork *et al*., 2025
Oromia	Gomole and Dhas	490	RBT, i-ELISA	1.4	0.6-2.9	Getachew *et al*., 2025

Table 1.5: Sero-prevalence of human brucellosis reported from various regions in Ethiopia (2011-2026).

Study area		No. of samples	Type of test	Prevalence and 95% CI based on CFT or ELISA test		Reference
Region/City	**Specific lacotion**			**Prevalence (%)**	**95% CI**	
Tigray	West Tigray	246	RBT, CFT	1.2	-	Haileselassie *et al*., 2011
Oromia	Adami Tulu	93	RBT, CFT	2.15	-	Tibeso *et al*., 2014
Oromia	Jimma town and Chora Botor	48	RBT, CFT	0.0	-	Michael *et al*., 2016
Oromia	Debre Zeit and Modjo	149	RBT, CFT	1.34	0.16-4.76	Tsegay *et al*., 2017
South Ethipia	Arba Minch town and around	254	RBT	10.6	7.0-14.0	Workalemahu *et al*., 2017
Afar	Amibara	650	RBT, CFT	4.4		Zerfu *et al*., 2018
Somali	Jigjiga and Gursum	211	RBT, CFT	0.4	-	Lakew *et al*., 2019
Oromia	Gomole, Elewoye, Dubuluk, and Miyo	341	RBT, c-ELISA	2.6	1.2-5	Edao *et al*., 2020
Oromia	Yabello and Dire	86	RBT, c-ELISA	25.6	16.8-36.1	Zewdie, 2020
Somali	Adadle	190	RBT, i-ELISA	2.8	0.9-6.4	Ibrahim *et al*., 2021
Afar	Awra and Gulina	444	RBT, ELISA	15.8	12.7-19.7	Mehari *et al*., 2021
Afar	7 districts	594	IgG ELISA	48.3	43.9-52.7	Tschopp *et al*., 2021
Somali	6 districts	215	IgG ELISA	34.9	28.1-42.4	Tschopp *et al*., 2021
Afar	Amibara	120	RBT, CFT	3.33	-	Wegi *et al*., 2021
Oromia	Holeta town, Wolmera district, and Adea Berga EIAR dairy farm	166	RBT, CFT	1.2	0.32-4.27	Getahun *et al*., 2022
Somali	Gurabakaksa, Guradhamole, and Dolo Ado	250	RBT, c-ELISA	2.0	0.2-3.7	Ahad *et al*., 2024
Southern Ethiopia	Myale Primary Hospital	293	RBT, ELISA	13.0	9.5-16.5	Sileshi *et al*., 2024
Oromia	Dire	390	RBT, i-ELISA	3.08	1.75-5.35	Ambaw *et al*., 2026

1.2.5. Diagnosis of Brucellosis

Generally, the common diagnostic tests used for laboratory diagnosis of brucellosis are of three types: indirect diagnosis through serological tests, direct diagnosis through culture (biotyping), and quick diagnosis utilizing molecular techniques (genotyping) (Yagupsky *et al*., 2019; Di Bonaventura *et al*., 2021; Elbehiry *et al*., 2023; Qureshi *et al*., 2023; Loubet *et al*., 2024).

Serological tests remain the primary diagnostic tools in endemic areas due to their simplicity and cost-effectiveness, especially under resource-limited settings (Rossetti *et al*., 2022). The main problem in the serological tests, however, is differentiating between the serological response to natural infection and that to vaccines (WOAH, 2022). Besides, other Gram negative bacteria such as *Yersinia enterocolitica* O:9 and *Escherichia coli* O157:H7 may cross-react with *Brucella* species (Bonfini *et al.*, 2018).

Cultural biotyping is the "gold standard" for the diagnosis of brucellosis, however, it requires high security laboratory facilities (biosafety level-3), highly skilled personnel, an extended time for results and it is considered a hazardous procedure (Nielsen and Yu, 2010; Ronai *et al*., 2015). Molecular genotyping using polymerase chain reaction (PCR) and its variants have been explored in order to overcome some of these difficulties and to collect relevant information for identification of species and biotypes of *Brucella* species, allowing differentiation between virulent and vaccine strains (Daugaliyeva *et al*., 2024).

1.2.5.1. Serological tests

In serological methods inactivated whole bacteria or purified fractions (i.e. lipopolysaccharides or membrane proteins) are used as antigens for detecting antibodies generated by the host during the infection. Serological tests can be classified as screening tests, monitoring or epidemiological surveillance tests, and complementary or confirmatory tests (Geresu and Kassa, 2016; Gürbilek *et al*., 2017; Loubet *et al*., 2024). No single serological test is appropriate in all epidemiological situations and all animal species. Moreover, combining screening and confirmatory or

complementary tests in diagnostic procedures improve diagnostic accuracy (WOAH, 2022; Andrade *et al*., 2024; Loubet *et al*., 2024).

In cattle usually a combination of a quick screening test and confirmatory test are commonly used (Gürbilek *et al*., 2017). The buffered *Brucella* antigen tests (BBAT), i.e. Rose Bengal test (RBT) and buffered plate agglutination test (BPAT) are suitable tests for screening herds and individual animals. However, the reactivity of samples that are positive in screening tests should be assessed using an established confirmatory and/or complementary strategy such as 2-mercaptoethanol, complement fixation test (CFT), ELISAs (e.g. c-ELISA), and fluorescence polarization assay (FPA) (Muktaderul *et al*., 2011; Kaltungo *et al*., 2013). The indirect ELISA (i-ELISA) or milk ring tests performed on bulk milk samples are effective for screening and monitoring dairy cattle for brucellosis (OIE, 2009a; Novoa *et al*., 2022).

RBT and CFT are usually used in combination in many countries for their higher sensitivity and higher specificity, respectively (OIE 2012). However, CFT is known for its prozone effect (Corbel, 2006) and it is associated with negative results in early stage of infection due to low IgG titers (Godfroid *et al*., 2010; Khan *et al*., 2017; Legesse *et al*., 2023). CFT is progressively being replaced by ELISAs as they are technically simpler to perform and more robust. For example, competitive ELISA differentiates between antibodies due to infection and vaccination, it is rapid (faster than CFT), it can be automated and it can be applied on samples that have deteriorated (Perrett *et al*., 2010).

The most widely used serum-testing procedures for the diagnosis of smooth *Brucella* infections in sheep and goats are RBT and CFT (De Massis *et al*., 2005; Setianingrum *et al*., 2025). Indirect ELISA and FPA have shown similar diagnostic performance (Nielsen *et al*., 2004). The bulk milk ring test, which has been very useful in cattle, is ineffective in small ruminants. The BBAT is not completely specific, but is adequate as a screening test for detecting infected flocks or for guaranteeing the absence of infection in brucellosis-free flocks. However, due to the relative lack of sensitivity of both tests, discrepancies between results obtained using the RBT and the CFT are not rare in infected sheep and goats. The results of the two tests should therefore be considered simultaneously to increase the likelihood of detecting infected individuals and to

improve control of the disease in areas where it has not been completely eradicated (Bosseray, 1992).

When, for practical or economic reasons, the CFT cannot be used simultaneously with the RBT in eradication programs, it is recommended to improve the sensitivity of the RBT by using three volumes of serum and one volume of antigen (e.g. 75 µl and 25 µl, respectively) instead of an equal volume of each. This simple modification increases RBT sensitivity and minimizes the discrepancies between RBT and CFT results (Blasco *et al*., 1994). Because antibodies induced after Rev.1 vaccination cannot be differentiated in both tests from those induced by *B. melitensis* infection, RBT and CFT results should be carefully interpreted according to the vaccination status in the flock. In addition, both tests are not specific enough to discriminate serological reactions due to *B. melitensis* from the false-positive reactions due to cross-reacting bacteria such as *Yersinia enterocolitica* O:9 (OIE, 2009b).

Good diagnostic results have also been obtained in sheep and goats with indirect (i) or competitive (c) enzyme-linked immunosorbent assays (ELISAs) using various antigens, but generally the ELISAs that use antigens with a high content of smooth lipopolysaccharide (sLPS) are the most useful (OIE, 2009b). Compititve ELISA provides similar sensitivity to the classical tests, RBT and CFT, and i-ELISA has greater sensitivity. Some ELISAs have potential advantages in sensitivity and/or specificity with respect to both BBAT and CFT (Garin-Bastuji *et al*., 1998). Preliminary c-ELISA studies with a periplasmic protein from *B. abortus* (Rossetti *et al*., 1996) or *B. melitensis* (Cloeckaert *et al*., 1996) as antigen have been applied in sheep and reported to be promising in differentiating Rev.1 vaccinated from *B. melitensis* infected animals (Debbarh *et al*., 1996; Cloeckaert *et al*., 2001).

Several countries have adopted various standard diagnostic techniques for *B. ovis*, but the only test prescribed by the OIE and the European Union for international trade is the CFT. The agar gel immunodifusion (AGID) test shows similar sensitivity to the CFT, and it is a simpler test to perform. Although standardization is lacking, i-ELISA is more sensitive and specific than either the CFT or AGID test, and with further validation and standardization studies, i-ELISA could become a suitable candidate for future designation as a prescribed test for *B. ovis* diagnosis.

Combinations of tests such as i-ELISA plus AGID usually provide more accurate results than single tests (OIE, 2009c).

Apart from the culture test, the most popular serologic tests in the diagnosis of human brucellosis are serum agglutination test (SAT), RBT, Coombs' test (CT) and ELISA (Al Dahouk and Nöckler, 2011). These tests are used to diagnose acute brucellosis and do not work in chronic brucellosis (http://en.wikipedia.org/wiki/User:Dgrtvwnp). The CFT and FPA are least commonly used methods in human brucellosis in clinical laboratories (Konstantinidis *et al*., 2007; Al Dahouk and Nöckler, 2011).

Additional diagnostic methods for brucellosis include Coombs' test (CT) (Haghdoost *et al*., 2021); the *Brucella* Coombs' gel test (BCGT) (Hanci *et al*., 2017); Dipstick assay (Smits *et al*., 1999); Brucellacapt (BCAP) (Yagupsky *et al*., 2019); Lateral flow assay (LFA) (Pfukenyi *et al*., 2020) and Brucellin allergic skin test (BST) (Krasteva *et al*., 2025).

1.2.5.2. Diagnosis through culture

Although serological tests and very sensitive nucleic acid amplification techniques have become increasingly common for the laboratory diagnosis of brucellosis, cultures remain the "gold standard" due to their clinical and epidemiological relevance (Di Bonaventura *et al*., 2021). Isolation of *Brucella* species is challenging and various techniques that directly detect the pathogen in tissues or blood have been attempted. However, none of these technologies has proven more reliable and effective than the isolation of bacteria itself (Moreno *et al*., 2022).

Brucella species are fastidious and require nutrient-rich media supplemented with antibiotics (Polymixin B 5,000 UI/L; bacitracin 25,000 UI/L; cyclohexamide 100 mg/L; nalidixic acid 5 mg/L; nystatin 100,000 UI/L and vancomycin 20 mg/L) to inhibit contaminants in primary cultures (De Miguel *et al*., 2011). Various factors also contribute to the rate of bacterial isolation, including the stage of the disease, the clinical specimen, previous antibiotic use, and culture technique (Al Dahouk *et al*., 2003; Christopher *et al*., 2010). Moreover, *Brucella* isolation

requires large number of viable bacteria in clinical samples, proper storage and quick delivery to the diagnostic laboratory (Seleem *et al*., 2010; Hadush and Pal, 2013).

Besides, *Brucella* isolation has risk of acquiring an infection from laboratory due to laboratory accidents and aerosolization of microorganisms during routine identification activities (Singh, 2009; Sam *et al*., 2012). Hence, a well-trained personnel and appropriate laboratory conditions (biosafety level-3) are very important.

For isolation of *Brucella* species, the choice of samples usually depends on the clinical signs observed (OIE, 2018). The samples of choice from animals include aborted fetus (stomach contents, spleen, lung and bronchial lymph nodes), fetal membranes (particularly the placental cotyledons), milk, vaginal secretions (swabs after abortion or parturition), semen and arthritis or hygroma fluids (Shrimali *et al*., 2017; Zainor *et al*., 2017; OIE, 2018; Islam *et al*., 2019a).

From animal carcasses, the preferred tissues for culture are those of the reticulo-endothelial system (i.e. head, mammary and genital lymph nodes and spleen), the pregnant or early post-parturient uterus, and the udder (OIE, 2018; Mazwi *et al*., 2024). In humans, blood is the most common sample, but bone marrow is considered the gold standard for high sensitivity, along with other tissues like bone tissue, urine, pleural and synovial fluid aspirates, liver biopsy specimens, cerebrospinal fluid and abscesses. It is possible to isolate *Brucella* species using blood clot cultures (blood clots in which leukocytes containing phagocytosed organisms are cultured) if serological tests are still negative or inconclusive (Tang *et al*., 2017; Di Bonaventura *et al*., 2021).

All samples should be cooled immediately and transported to the laboratory by the most rapid means. If they are to spend more than 12 hours in transit, all samples apart from vaginal swabs, should be frozen. Growth normally appears after 3-4 days, but the incubation needs to be prolonged up to 7 days in automatic blood culture systems (Raj *et al*., 2014) or even up to 4 weeks in blood culture bottles along with blinded subculture (Yagupsky *et al*., 2019). No specific transport medium has been demonstrated to improve *Brucella* survival in animal samples (OIE, 2018).

Standard culture practices need to be modified due to delayed growth, low blood concentration, and carbon dioxide requirement of *Brucella* species (Yagupsky *et al*., 1997) by incubating samples in 5% CO_2 at 35°C for up to two weeks or inoculating specimens into broth media (Wang *et al*., 2018). Enrichment media containing selected antibiotics can improve the sensitivity (Her *et al*., 2010; De Miguel *et al*., 2011).

Usually, solid media such as dextrose agar, tryptose agar, and trypticase soy agar, are recommended for primary isolation of *Brucella*, but *B. ovis* and *B. canis* require addition of 5-10% of sterile bovine or equine serum to the culture media. *Brucella* species can also be isolated mostly from blood agar and chocolate agar media after 24-72 hour of incubation (Di Bonaventura *et al*., 2021). In the case of blood or milk, biphasic media such as Castañeda's medium is recommended for improving sensitivity (Poester *et al*., 2010).

The presumptive identification of *Brucella* is based on colonial morphology (small, 1-2 mm, convex, nonpigmented, nonhemolytic with an entire edge, and frequently smooth/rough variants can occur with *B. canis*); Gram staining appearance (faintly stained Gram-negative very small coccobacilli resembling fine sand, often clustered in microcolonies); specific traditional phenotypic reactions (such as positive oxidase, catalase, and urease activities); no fermentation of sugars; hydrogen sulphide production (H_2S) production; growth in the presence of thionine and basic fuchsin; oxidative metabolic tests; and lack of motility (Adem and Duguma, 2020; Di Bonaventura *et al*., 2021).

Morphology and agglutination test help to differentiate *Brucella* from other bacteria. Oxidase test, urease test and phage typing help in the identification of *Brucella* species. Whereas, carbon dioxide requirement, hydrogen sulphide production, effect of dyes on *Brucella* growth, the agglutination test using antisera A, M and R, the oxidation of amino acids and carbohydrates, the analysis of the fatty acids of *Brucella* and the susceptibility of *Brucella* to antimicrobial testing are used in the identification of *Brucella* biovars (http://en.wikipedia.org/wiki/User:Dgrtvwnp).

Besides conventional phenotypic methods, the identification of *Brucella* to the species level can be also performed by Matrix-Assisted Laser Desorption Ionization-Time Of Flight mass

spectrometry (MALDI–TOF) technology, although its accuracy is sometimes contradictory (Mesureur *et al*., 2018; Poonawala *et al*., 2018). The presumptive identification of *Brucella* should be confirmed by a molecular method (Di Bonaventura *et al*., 2021).

1.2.5.3. Molecular genotyping

Genotyping of *Brucella* is a rapidly developing field. It can potentially allow a rapid evaluation and understanding of the molecular structure of *Brucella* and the relationships between species, biovars and strains not possible with traditional biotyping methods. It can also increase understanding of the relationship of *Brucella* to other bacteria and provide information on the phylogenetic family tree. Moreover, the risk of laboratory-acquired infections can be effectively eliminated in the process of genotyping due to the use of killed *Brucella* (Greenfield *et al*., 2002).

Molecular techniques can be utilized to detect brucellosis, both in animals and humans. These methods primarily relying on polymerase chain reaction (PCR) and its variants, and offer rapid, highly sensitive, and specific detection of *Brucella* DNA (Khaliq *et al*., 2025). In molecular approaches, the findings should be carefully interpreted, keeping in mind the relevant clinical and epidemiological contexts at all times. This is because, positive result does not always mean that an infection is active or ongoing. The detected DNA could be from a significantly reduced bacterial inoculum in animals which have had adequate treatment, DNA from non-viable organisms or phagocytosed bacteria (Elbehiry *et al*., 2023).

There are wide varieties of DNA amplification methodologies. Real-time polymerase chain reaction (PCR), quantitative reverse transcription polymerase chain reaction (RT-PCR), multiplex RT-PCR, nested PCR, in-house PCR, PCR-enzyme immunoassay in a microplate format, and traditional PCR procedures are some of the DNA amplification methodologies used (Navarro *et al*., 2015; Yagupsky *et al*., 2019).

Multiplex PCR assay has many advantages in comparison with previously developed PCRs, the main one is its ability to distinguish between *Brucella* species and vaccine strains (S19, RB51,

and Rev. 1) in a single step (OIE, 2009a). A multiplex PCR assay which is often termed the Bruce-ladder was also developed which can differentiate between all the six classical *Brucella* species, new marine mammal *Brucella* species, *B. abortus* vaccines S19 and RB51 and *B. melitensis* vaccine Rev 1 (García-Yoldi *et al*., 2006). The Bruce-ladder PCR worked equally well irrespective of the cultural conditions, DNA extraction methods, or thermocyclers used (Lo´pez-Gon˜i *et al*., 2008).

In addition to the Bruce-ladder, a number of different PCR techniques have been developed to identify *Brucella* species. The most widely accepted of these is AMOS-PCR which is based on IS711, an insertion sequence (IS) present in the *Brucella* genome which is specific to the genus. This method was developed by Bricker and Halling (1994). AMOS-PCR is an acronym related to the species of *Brucella* which it identifies (*B. abortus* bivars 1, 2 and 4, *B. melitensis*, *B. ovis* and *B. suis* biovar 1). The assay depends on the polymorphism arising from species-specific localization of the genetic element IS711 on the *Brucella* chromosome (Halling *et al*., 1993; Ouahrani *et al*., 1993) which is unique to *Brucella*.

Besides, loop-mediated isothermal amplification (LAMP) has been developed for the detection of *Brucella* species. Compared to PCR, the LAMP assay offers superior results due to its ease of use, ease of assembly, rapid response time, visual recognition, more cost-effective, and high sensitivity and specificity (Moeini-Zanjani *et al*., 2020; Soroka *et al*., 2021). It can be used in areas where resources are limited but the need for quick and accurate diagnosis is high. Moreover, a LAMP test can be performed without the need for electrophoretic analysis (Notomi *et al*., 2000).

The sequencing of the *Brucella* genome has allowed the identification of unique loci which exhibit hetrogenicity among the different *Brucella* genomes and this is the basis for the development of rapid tests such as DNA-DNA hybridization (Verger *et al*., 1985), pulsed-field gel electrophoresis (Allardet-Servent *et al*., 1988), 16S ribosomal RNA gene sequencing (Gee *et al*., 2004), multilocus sequencing analysis (Whatmore *et al*., 2007), single nucleotide polymorphism (SNP) typing (Foster *et al*., 2008), multilocus variable number of tandem repeat

analysis (MLVA) (Scholz and Vergnaud, 2013) and polymerase chain reaction (PCR) typing (García-Yoldi *et al*., 2006) to identify *Brucella* species, biovars, and strains.

Additionally, sequencing (metagenomic next-generation sequencing and whole-genome sequencing), makes possible the discovery of molecular mechanisms that explain variations of biotypes and evolutionary relationships for human brucellosis (Eko *et al*., 2022). Identifying these targets could be a significant step forward in the development of diagnoses and vaccinations aimed at combating and treating brucellosis (Islam *et al*., 2019b).

As there is no specific test for definitively identifying a bacterium, the integration of multiple techniques for the diagnosis of *Brucella* is necessary (Eko *et al*., 2022; Liu *et al*., 2022). Although there are valid and reliable molecular techniques that can ensure a high degree of accuracy and precision of results, indirect methods of serology and conventional methods of culture continue to be the primary methods of diagnosing brucellosis and following up on infections caused by *Brucella* species (Di Bonaventura *et al*., 2021).

1.2.6. Treatment of Brucellosis

Treatment of brucellosis in animals is not applicable as all positively tested animals should be culled. In humans, attempts have been made to control the illness and prevent complications, relapses and sequelae. Antibiotic treatment should be implemented as early as possible and the use of combination regimes and prolonged treatment duration are recommended (Pasquau-Liaño *et al*., 2022). However, there are reports of relapses of *Brucella* infections after well-timed treatment (Bahabri *et al*., 2025). Moreover, the emergence of antibiotic-resistant *Brucella* strains present significant challenges (Cassini *et al*., 2019; ARC, 2022; Wareth *et al*., 2022).

Antibiotics that possess activity in the intracellular acidic environment are essential for the treatment of brucellosis (Bosilkovski *et al*., 2021). Doxycycline and rifampin are commonly used antibiotics for the treatment of brucellosis (Patra *et al*., 2022). Doxycycline is the preferred antibiotic due to its dosing frequency and fewer gastrointestinal side effects. Rifampicin is another effective alternative. A six-week oral administration of both doxycycline and rifampicin

showed similar efficacy in treating uncomplicated brucellosis (Qureshi *et al*., 2023). To minimise the risk of relapse, aminoglycosides are often added during the initial 2-3 weeks of therapy (Głowacka *et al*., 2018; Bosilkovski *et al*., 2021). Fluoroquinolones are considered secondary alternatives because of their high efficacy and ability to act intracellularly under acidic conditions (Khanam, 2020; Qureshi *et al*., 2023).

1.2.7. Prevention and Control of Brucellosis

1.2.7.1. Prevention and control of brucellosis in animals

Control strategies of animal brucellosis in the developed and the developing worlds differ. In the developed world, most emphasis is put on eradication and on risk analysis to avoid the re-introduction of *Brucella*. After the incidence of brucellosis is sufficiently reduced by compulsory vaccination, vaccination is gradually restricted and eventually prohibited and a "test and slaughter" policy comes to effect in the developed world (Zhang *et al*., 2018).

Various vaccines have been developed for animals (Lalsiamthara and Lee, 2017; Qureshi *et al*., 2023). Two *Brucella abortus* vaccines, S19 and RB51 in cattle and one *Brucella melitensis* vaccine, Rev.1 in sheep and goats have been successfully used worldwide (Heidary *et al*., 2022). With the development of precise molecular techniques and accurate understanding of the mechanism of *Brucella* pathogenesis, new genetically-engineered vaccines have been developed and replaced traditional vaccines (Hou *et al*., 2019). There is no vaccine available for humans (de Figueiredo *et al*., 2015; Li *et al*., 2017).

Brucellosis control programs are rarely implemented in the developing world (Godfroid *et al*., 2011). With the exception of countries like Botswana, Namibia, Lesotho and South Africa (McDermott and Arimi, 2002), this is particularly true in parts of sub-Saharan African where vaccination is rarely done.

Vaccinations in animals, however, have drawbacks: they have limited efficacy (Yang *et al*., 2013), they can cause abortions in animals and infections in humans due to residual virulence (Heidary *et al*., 2022), they can produce false sero-positive results (Ibarra *et al*., 2023) and are often unsuitable for remote, pastoral areas due to technical and logistical constraints, and poor post-vaccination monitoring (Kidanu *et al*., 2026). Additionally, pastoralists sometimes lack trust in vaccines, which could hamper such control strategy (Tschopp and Kidanu, 2024).

Segregation and test-and-slaughter strategy, coupled with a multi-faceted strategy that includes training, education, disease awareness, improved veterinary services, and strong collaboration between health authorities and communities, is an alternative to vaccination and is likely more feasible and culturally accepted in the developing world (Kidanu *et al*., 2026).

Generally, prevention and control strategies should answer the question: what are the consequences of acting, or failing to act, on policy options of selected intervention scenarios (e.g., vaccination, test-and-slaughter, vaccination combined with test-and-slaughter, versus a status-quo scenario) (Kiiza *et al*., 2023).

1.2.7.2. Prevention and control of brucellosis in humans

Humans can contract brucellosis from infected animals or contaminated animal products. The most common ways of contracting brucellosis include consuming of *Brucella* contaminated raw dairy products, inhaling air contaminated by *Brucella*, and contact with infected animals (Libers, 2023). Therefore, general prevention of brucellosis in humans relies on controlling the disease in animal populations, avoiding contaminated food sources, practicing strict occupational hygiene, strengthening and enforcing inspection of meat and animal products, public awareness and education, use of personal protective equipment (PPE) and the One Health approach are essential (Kanu, 2024).

The One Health approach recognizes the interconnectedness of human, animal, and environmental health and promotes early detection and surveillance, integrated control measures,

research and innovation, and collaboration and information sharing between multiple sectors to address health issues comprehensively (Charypkhan and Rüegg, 2022).

1.2.8. Socio-economic and Public Health Impacts of Brucellosis

Endemic brucellosis in low-income countries of sub-Saharan Africa and south Asia has multiple soscio-economic implications across agriculture and public health (Pal *et al*., 2017). Brucellosis is an economically important disease of livestock causing reproductive wastage through infertility, delayed heat, late-gestation, abortion, fetal death and decreased productivity (reduced meat and milk production) (Poester *et al*., 2013; Lokamar *et al*., 2020; El-Diasty *et al*., 2021). Economic losses in animals are also related to loss of draught power and reduced weight gain from chronic infections and ill-thrift (Franc *et al*., 2018), culling of unproductive stock (Godfroid, 2017), veterinary costs associated with diagnostics and vaccination (Kiiza *et al*., 2023), and trade restrictions (Naseer *et al*., 2023).

It has been estimated that around 3.5 billion people have a permanent risk of acquiring brucellosis, resulting in significant public health impacts and economic losses due to treatment costs and reduced work efficiency (McDermott *et al*., 2013; Rossetti *et al*., 2017; Franc *et al*., 2018; Bosilkovski *et al*., 2021). Non-healthcare costs include expenses related to a patient's access to care and the prevention of disease, transport to and from medical facilities, housing accommodations while care is being received, and a loss of workdays or leisure time when seeking medical attention can amount to significant expenses. Reduced tax revenue as well as gender inequality through reduced production of livestock and livestock products are other forms of socio-economic impacts of brucellosis (McDermott *et al*., 2013; Franc *et al*., 2018).

A study that did not capture the system-wide impacts of brucellosis including on human health in Tanzania estimated an annual loss of approximately 19 million US Dollar due to livestock (cattle, sheep and goats) production losses attributable to brucellosis in a specific study area (Mendes *et al*., 2025).

Recent studies indicate indemicity of brucellosis in the Afar, Somali, and south Omo regions of Ethiopia, where intimate human-animal interaction and consumption raw milk and dairy products are common (Tschopp *et al*., 2021; Tschopp *et al*., 2022; Sileshi *et al*., 2024; Demissie *et al*., 2024; Sibhat *et al*., 2024; Tschopp and Kidanu, 2024). Human brucellosis is very difficult to diagnose based on clinical symptoms only, as it resembles many other febrile diseases that are prevalent in those areas (Tschopp *et al*., 2021).

1.3. Statement of the Problem

Brucellosis is a persistent neglected zoonosis that typically transmits from livestock to humans through consumption of unpasteurized milk and meat, or direct handling of infected animals and placental tissues (López-Santiago *et al*., 2019; Berhanu and Pal, 2020). The disease is endemic across sub-Saharan Africa. In Ethiopia, brucellosis continues to pose a severe veterinary health challenge due to high sero-prevalence rates in livestock. Furthermore, the infection is widespread among humans, with human prevalence reaching 48.3% and over half (56.3%) of households in heavily affected pastoralist communities having at least one infected individual (Tschopp *et al*., 2021). Economically, it causes massive reproductive wastage, including abortions, infertility, and lowered milk and meat yields (Lokamar *et al*., 2020; El-Diasty *et al*., 2021).

The public health burden of brucellosis is characterized by "undulant" fever, arthritis, chronic fatigue, and long-term disability, which frequently drain household economies. Brucellosis in humans is usually misdiagnosed as malaria or as fever of unknown origin, contributing to the hidden burden of disease (Zerfu *et al*., 2018; Mehari *et al*., 2021).

Understanding the local epidemiology of brucellosis in Ethiopia is significantly hindered by absence of routine national surveillance, lack of standardized diagnostic testing, underreporting in rural health centers, lack of molecular confirmation to track specific *Brucella* strains, the highly mobile nature of pastoralist communities and poor integration between veterinary and public health information systems. Thus, the true burden of the disease remains unknown.

Without intervention, uncontrolled transmissions of brucellosis persist and resulting in massive economic strain, food insecurity, and severe public health crises that would place vulnerable occupational groups (herders, veterinarians, abattoir workers) at perpetual risk (Pereira *et al*., 2020). Communities in the fentale district are vulnerable to brucellosis due to their heavy reliance on livestock farming and pastoralist economies. Cultural consumption of raw milk and unsafe handling of abortion cases compounded by lack of awareness on brucellosis increase daily exposure risks making the area a critical hotspot for zoonotic spillover. Therefore, this study will generate concrete location-specific evidence on brucellosis prevalence in livestock and humans. The findings will help to establish evidence-based policy, to improve integrated veterinary and medical control strategies, and to establish targeted awareness campaigns which ultimately, will safeguard animal health, optimize agricultural productivity, and protect human livelihoods in the region.

1.4. Research Questions

The study critically investigated research questions regarding,

1. the sero-epidemiology of brucellosis in camels which are incidentally infected, small ruminants which often carry *Brucella melitensis* and cattle which are the traditional hosts for *B. abortus*
2. the sero-epidemiology of brucellosis in humans who could get the infection primarily through the consumption of raw animal products, or direct contact with excretions, aborted fetuses, or placenta of infected animals
3. the risk factors associated with the oocurrence of brucellosis in livestock (camels, small ruminants, cattle) which enhance transmission
4. the risk factors associated with the oocurrence of brucellosis in humans

Hypothesis

1. Hypothesis: The prevalence of *Brucella* antibodies in camels in the study area is high ($\geq$3.0%) (Objective 1)

2. Hypothesis: The prevalence of *Brucella* antibodies in small ruminants in the study area is high (≥3.0%) (Objective 2)
3. Hypothesis: The prevalence of *Brucella* antibodies in cattle in the study area is high (≥2.6%) (Objective 3)
4. Hypothesis: The prevalence of *Brucella* antibodies in humans in the study area is high (≥14.0%) (Objective 4)

1.5. General Objectives

With the intention of filling the knowledge gaps mentioned in the statement of the problem, the general objectives of this PhD research project were:

1. Sero-epidemiological investigation of brucellosis in livestock (camels, small ruminants and cattle) in Fentale district of Oromia Region, Ethiopia.
2. Sero-epidemiological investigation of brucellosis in humans in Fentale district of Oromia Region, Ethiopia.

1.5.1. Specific Objectives

1. Investigation of the sero-prevalence and associated risk factors of brucellosis in camels
2. Investigation of the sero-prevalence and associated risk factors of brucellosis in small ruminants (sheep and goats)
3. Investigation of the sero-prevalence and associated risk factors of brucellosis in cattle
4. Investigation of the sero-prevalence and associated risk factors of brucellosis in humans

1.6. Significance of the Study

The studies conducted in this PhD research project are significant for identifying the prevalence, distribution, and transmission risk factors of brucellosis in livestock (camels, small ruminants and cattle) and humans. The studies determine the magnitude of *Brucella* infection at individual (animal/human) and herd/flock levels, confirming endemicity; identify risk factors contributing to transmission; highlight the risk of zoonotic transmission to humans, particularly in high-risk

occupations like pastoralists, aiding in the design of prevention strategies; enable monitoring of the disease in the study area to guide control strategies.

Moreover, these studies provide crucial data to inform public health policies, develop targeted control measures, and understand zoonotic risks. Understanding the sero-prevalence of human brucellosis and identifying associated risk factors are crucial steps in improving diagnostic strategies and guiding public health interventions.

1.7. Structure of the Dissertation

This dissertation has three major parts: The first is a sero-epidemiological study of brucellosis in livestock with parallel questionnaire surveys in livestock owners. This part is subsectioned into chapter 2, chapter 3 and chapter 4. Livestock included in the study were camels, sheep, goats and cattle. The second part of the dissertation is presented under chapter 5 and consisted of a sero-epidemiological study of brucellosis in humans and determining the risk factors associated with the acquisition and occurrence of the disease. The third part consists of chapter 6 under which the general discussion, conclusion and recommendation were included.

Because the attempts to isolate *Brucella* from human and livestock clinical samples (milk, swabs, and whole blood) were unsuccessful, this section was omitted.

2. CHAPTER TWO: SERO-PREVALENCE OF CAMEL BRUCELLOSIS AND THE ASSOCIATED PUBLIC HEALTH RISKS IN KEREYU PASTORAL AREA OF OROMIA REGION, ETHIOPIA

Abstract

Camel brucellosis has been reported in almost all camel-rearing countries in Africa and Asia. A cross-sectional study was carried out with the objective of estimating sero-prevalence of brucellosis among camels and identifying risk factors associated with sero-positivity to *Brucella* infection in Kereyu pastoral area of Oromia Region of Ethiopia. A total of 324 sera were collected randomly from apparently healthy camels in Fentale district, east Shewa zone of Oromia Region, Ethiopia. Data related to risk factors such as sex, age, herd size, and herd composition were collected and camel herders were interviewed to assess their awareness about brucellosis on the sampling day. The Rose Bengal plate test (RBT) was used as a screening test and those RBT-positive serum samples were further confirmed by complement fixation test (CFT) and competitive enzyme-linked immunosorbent assay (c-ELISA). Out of the collected sera, 21.0% (68/324, 95% CI: 16.4-26.2) were positive by the RBT and of the RBT-positive samples, 29 (9.0%, 95% CI: 5.9-12.3) and 31 (9.6%, 95% CI: 6.5-13.0) sera samples were positive by CFT and c-ELISA, respectively. Based on c-ELISA, the animal- and herd-level prevalence in the study area were 9.6% (95% CI: 6.5-13.0) and 35.4% (95% CI: 23.1-43.5), respectively. While sex, age and livestock composition had no significant effect on the prevalence of camel brucellosis ($p > 0.05$), herd size was the major risk factor for the presence of the disease. Accordingly, camels kept in large herds were 4 times higher at risk for *Brucella* infection than camels kept under small herd size (OR = 4.024; 95% CI: 1.667-9.716; $p < 0.005$). Out of 65 camel herders interviewed, 64 had no awareness about the zoonotic importance of brucellosis. This study demonstrated that brucellosis has a moderate occurrence in the study area and may pose health risks to the public. Therefore, there is a need for implementation of better management practices such as regular testing of all animals, typing of infecting strains, culling of positive animals and increasing public awareness on brucellosis.

Keywords: Camel brucellosis; c-ELISA; CFT; RBT; Risk factor; Sero-prevalence.

2.1. Introduction

Global camel population is estimated to be more than 40 million with countries of east Africa, such as Ethiopia, Sudan, Kenya and Somalia having more than 60% of the world dromedary camel population (Faye, 2020). In pastoral areas of Ethiopia, camels are vital livestock with a population of approximately 4.8 million (Kena, 2022). Camels are used for income, food security, and transportation. However, their production and productivity are affected by infectious diseases, such as brucellosis, which can cause loss of newborns due to abortion, birth of weak newborns that die soon after birth, impaired fertility, decrease milk yield and loss of man-hours in infected people (Matope *et al*., 2010; Mai *et al*., 2012; OIE, 2016). The spread of brucellosis is increasing as infected animals do not show pathognomonic symptoms and milk distributions chains in developing countries encourage supply of unprocessed milk (Wareth *et al*., 2014) and, hence spread of diseases (Racloz *et al*., 2013).

Recent data analyses suggest that the global burden of human brucellosis reaches at least 2.1 million cases per year (Laine *et al*., 2023). Infected camels and their products could be a source of human brucellosis, leading to severe arthritis, fever, infertility, and in some cases, chronic infections following misdiagnosis (Liu *et al*., 2021). Most of the time, human cases are linked to direct occupational exposure to livestock in rural areas and consumption of unpasteurized dairy products in urban environments (Obradovic´ and Velic´, 2010; Perrett *et al*., 2010).

Camels are not known to be primary hosts of *Brucella*. However, they are susceptible to *B. melitensis* and *B. abortus* (Gwida *et al*., 2012). Camels become infected with *Brucella* especially when they are in contact with infected large and small ruminants. Though brucellosis is rare in camels not in contact with ruminants, it remains a concern in pastoral areas due to lack of awareness and consumption of raw milk (Islam *et al*., 2023).

Brucellosis can be diagnosed by assessing specific cell-mediated or serological responses to *Brucella* antigens. A primary test, Rose Bengal plate test (RBT) and a confirmatory test, the complement fixation test (CFT) are widely used serum-testing procedures for the diagnosis of *Brucella* infections. Though no validation for camel sera (Gwida *et al*., 2011), serological tests

used for the diagnosis of *Brucella* infection in cattle may also be adequate for the diagnosis of brucellosis in camels (OIE, 2016). Competitive enzyme-linked immunosorbent assay (c-ELISA) is also one of the commonly used confirmatory tests for brucellosis in a variety of animal species (Abdel-Hamid *et al*., 2017).

Many countries, such as the United Kingdom, Australia, Japan, as well as some countries in north and west Europe have successfully eradicated brucellosis through intensive health control measures. However, camel brucellosis remains a widespread disease in camel producing countries such as Middle East (Franc *et al*., 2018; Alrawahi *et al*., 2019; Mohamed *et al*., 2019; Abedi *et al*., 2020; Al-Sherida *et al*., 2020; Manivannan *et al*., 2021; Alhussain *et al*., 2022), west Africa (Tanimoun *et al*., 2021; Akinyemi *et al*., 2022), north Africa (Ahmed *et al*., 2015; Benfodil *et al*., 2022; Selmi *et al*., 2024), and north-east Africa (Kadle *et al*., 2017; Lokamar *et al*., 2022; Hazem *et al*., 2023; Mohammed *et al*., 2023). In east Africa, the highest rate of camel brucellosis (40% herd level prevalence) was recorded (Omer *et al*., 2010).

Brucella infections in animals and humans have been reported in Ethiopia and the findings were quite varying. Brucellosis prevalence of 2.90% (Ahad *et al*., 2024), 2.86% (Giro *et al*., 2022) and 0.90% (Gumi *et al*., 2013) were recorded using c-ELISA, indirect ELISA and ELISA, respectively, in different regions. Other reports range from 2.00%-7.60% in different parts of Ethiopia using CFT (Megersa *et al*., 2011a, 2011b; Zeweld and Haileselassie, 2012; Tilahun *et al*., 2013; Tassew and Kassahun, 2014; Zeru *et al*., 2016; Wegi *et al*., 2021; Waktole *et al*., 2022). Similarly, in human 3.33% (Wegi *et al*., 2021), 2.0% (Ahad *et al*., 2024), 15% (Zeweld and Haileselassie, 2012), 35% (Zerfu *et al*., 2018) prevalences were reported in pastoral areas of Ethiopia by CFT. Moreover, Mehari *et al*. (2021) and Ibrahim *et al*. (2021) reported 15.8% and 2.8%, respectively, in different regions. Close physical contact with animals and the tradition of consumption of unpasteurized milk are risk factors for contracting brucellosis in pastoral communities (Abbas and Agab, 2002).

There is insufficient awareness and knowledge of brucellosis among livestock owners and herders in Africa and Asia (Zhang *et al*., 2019). In some pastoral areas of Ethiopia, livestock herders had no awareness on zoonotic importance of brucellosis, usually drink raw milk and

discard abortion materials in the open surroundings with minimal protection (Wegi *et al*., 2021; Tschopp *et al*., 2022). Moreover, they lack clear understanding about brucellosis as one of the diseases that cause abortion in their animals (Legesse *et al*., 2018). Generally, the tradition of mixing different livestock species (Wegi *et al*., 2021), occurrence of brucellosis in different animal species (Gumi *et al*., 2013; Ibrahim *et al*., 2021; Ahad *et al*., 2024) and habits of consuming raw milk (Wegi *et al*., 2021; Tschopp *et al*., 2022) in the pastoral areas urge the need for routine investigation of brucellosis. Hence the present study was designed to investigate sero-prevalence of camel brucellosis, identify potential risk factors for camel brucellosis and assess the awareness of Kereyu pastroalists on zoonotic brucellosis in Fentale district of Oromia Region, Ethiopia.

2.2. Materials and Methods

2.2.1. Study Area and Study Animals

The study was conducted in Fentale district (Figure 1). Fentale is located in the Awash lowland area of the east Shewa zone of Oromia Region, Ethiopia. The administrative center of Fentale district, Metahara town, is located 193 km east of Addis Ababa on the main highway joining Ethiopia with Djibouti. The area falls within an altitude range of 800-1100 meter above sea level (masl). The district takes its name from Mount Fentale, which has high peaks reaching elevations of up to 2,007 masl (Gedda, 2003). The Kereyu are Oromo speaking transhumant pastoralists and are the indigenous inhabitants of the Metehara Plain and Mount Fentale areas of this district. Fentale district is currently composed of 20 kebeles, 18 rural peasant associations and two urban kebeles. The livestock population of the district comprises about 53,682 cattle, 106,931 sheep, 129,424 goats, 20,298 camel, 13,005 equine and 6,446 poultry (Lemma and Fufa, 2023). The main watering point is the Awash River and the main common grazing areas of the district are also in the valleys of this river. The area is affected by recurrent droughts due to disrupted rainfall patterns. The annual rainfall ranges from 400 to 700 mm. Temperature ranges from 29 to 38°C.

Fentale district shares borders with the Afar Region and encompasses the Awash National Park. Livestock of the district are heavily intertwined with adjacent Afar pastoralist herds through

shared grazing lands (often near the Awash River) and cross-border trade at the Metehara town markets. This continuous contact creates critical transmission pathways for transboundary and zoonotic diseases.

The study animals were non-vaccinated camels (*Camelus dromedarius*) kept under extensive husbandry. Only camels older than 6 months of age were sampled.

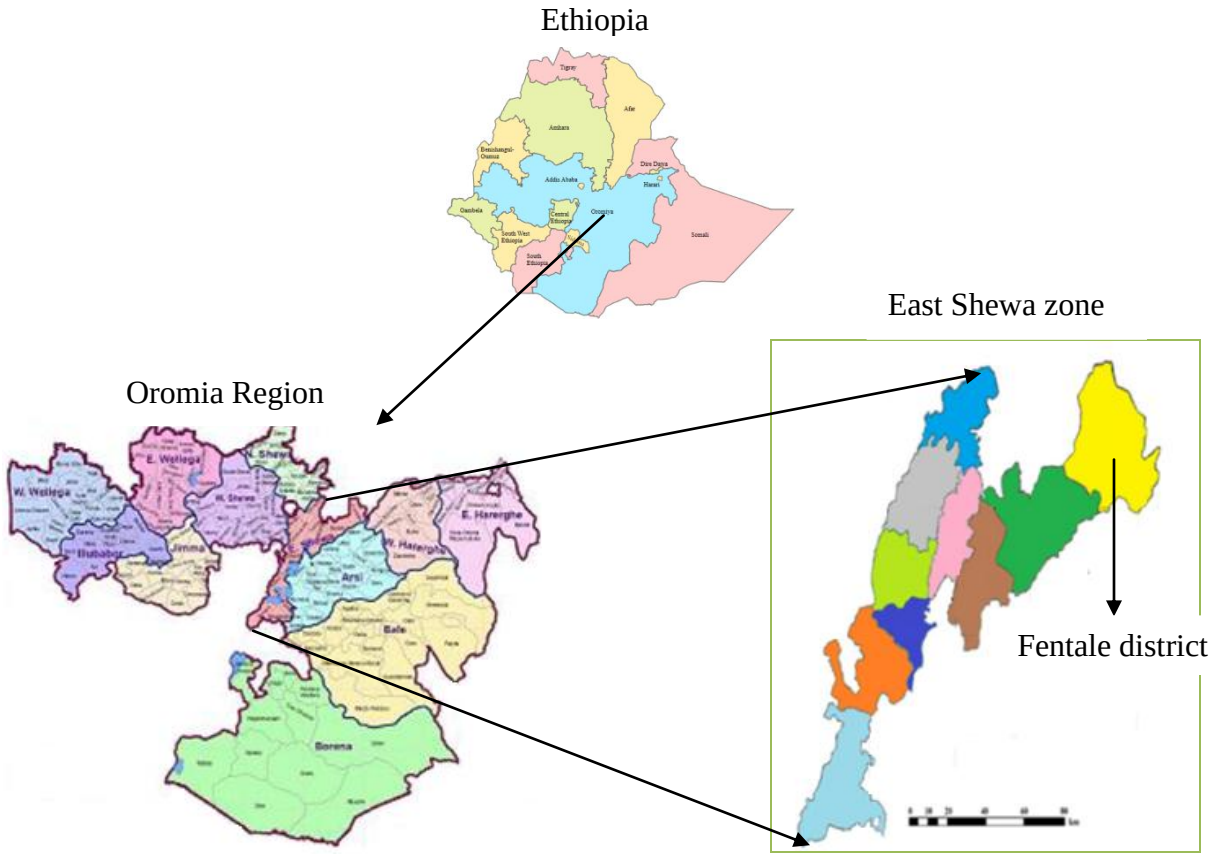


Figure 1: Map of the study area (modified and adopted from Bahru *et al*., 2021).

2.2.2. Study Design and Sample Size Determination

A cross-sectional study was conducted from December, 2018 to October, 2020 to estimate the sero-prevalence of *Brucella* infection in camels and to identify potential risk factors associated with sero-positivity in Fentale district. The sample size was determined by the formula $N = (1.96)^2 \times P \times Q/D^2$, where N = sample size, P = expected prevalence, Q = 1-P and D = level of precision (5%) (Thrusfield, 2005). Taking the previous report of 7.6% sero-prevalence of camel

brucellosis in Awash-Fentale district (Zeweld and Haileselassie, 2012), the calculated sample size was, $N = (1.96)^2 \times (0.076) \times (1 - 0.076)/(0.05)^2 = 108$. A total number of 324 camels were sampled to increase the samples size.

Random sampling technique was used in selecting the households. From the 18 peasant associations (PAs) found in Fentale district, 5 PAs were selected based on accesses to transportation and security reasons. Then, a total of 65 households were randomly selected from these 5 PAs (13 households from each PA). In randomly selected households, all camels were sampled from a herd with less or equal to five animals. In herds with more than five animals, only five animals were randomly selected using systematic random sampling. All camels of both sexes above the age of six months were sampled from the randomly selected herds. Based on their age, camels were grouped into young (camels below 4 years of age) and adult (camels at 4 years of age and older) according to Ahad *et al*. (2024). Moreover, camel herds were classified as small (<20 camels), medium (21-50 camels) and large (>50 camels) according to Gizaw *et al*. (2017) and Ahad *et al*. (2024) with some modifications.

2.2.3. Sample Collection

About 10ml blood samples were collected from the jugular vein of each animal using plain vacutainer tubes. The blood containing tubes were given identification numbers. The blood samples were allowed to clot overnight at room temperature. The serum was separated from the clotted blood by decanting to cryovial tubes. The separated sera were stored at -20°C until tested. Data including the owner's names, sex, age, herd size, livestock composition and other relevant information were recorded at the time of blood collection.

2.2.4. Serological Examination

The Rose Bengal plate test (RBT) and complement fixation test (CFT) were conducted at the National Veterinary Institute, Bishoftu, Ethiopia for the detection of brucellosis antibodies. All RBT-positive sera samples were also shipped to University of Nairobi, Nairobi, Kenya and further tested using competitive enzyme-linked immunosorbent assay (c-ELISA).

Rose Bengal plate test (RBT)

Following the procedure described by Staak *et al*. (2001), all serum samples were tested using RBT at the National Veterinary Institute, Bishoftu, Ethiopia. Briefly, 30µl of RBT antigen was mixed with equal amount of serum on a plate. The plate was rocked by hand for 4 minutes and the test was read by comparing with the positive and negative control sera by examining for agglutination in natural light. Magnifying glass was used to detect micro-agglutination. Results of RBT were interpreted as 0, +, ++ and +++ as described by Staak *et al*. (2001). 0 = no agglutination; + = barely visible agglutination (seen by using magnifying glass); ++ = fine agglutination and +++ = coarse agglutination. Samples with no agglutination (0) were recorded as negative while those with +, ++ and +++ were recorded as positive. The results were recorded and stored in Microsoft Excel.

Complement fixation test (CFT)

All RBT-positive samples were confirmed with a complement fixation test using the OIE protocol (OIE, 2016) at the National Veterinary Institute, Bishoftu, Ethiopia. Antigen standardization was done with dilution strength of 1:20. *Brucella* antigen, complement and 3% sensitized sheep red blood cells were added to serially diluted test sera on microtitre plates after serial dilution. The plates were incubated at 37°C for 30 minutes. At 1:10 dilution, positive results were considered for partial fixation (50% hemolysis) or complete fixation (no hemolysis). The negative control serum exhibited complete hemolysis, while the positive control serum exhibited inhibition of hemolysis.

Competitive enzyme-linked immunosorbent assay (c-ELISA)

All the RBT-positive sera samples were further tested using a commercial COMPELISA, a competitive ELISA kit for the detection of antibodies against *Brucella* in serum samples (APHA Scientific, Weybridge, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom) at Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Nairobi, Kenya. The test was conducted according to the manufacturer's protocol using 96-well polystyrene plates pre-coated with *Brucella* lipopolysaccharide antigen.

2.2.5. Case Definition

Animals were considered positive to brucellosis when they tested positive on either RBT/CFT or RBT/c-ELISA tests. Similarly, a herd was considered sero-positive when at least one animal in a herd tested positive. Since there is no history of vaccination against brucellosis in Ethiopia, sero-positivity observed in this study was considered to be due to natural infection.

2.2.6. Questionnaire Survey

During the interview, the objectives of the survey were explained to camel owners and verbal consent was obtained. A structured questionnaire format was developed and administered to camel herders in the local language (Afaan Oromo). The questionnaire focused on knowledge about brucellosis and other zoonotic diseases, the habits of animal product consumption and handling, and dead animal/aborted fetus disposal practices. All 65 camel herders whose animals were bled for serological examination were interviewed.

2.2.7. Data Management and Analyses

Data obtained during sample collection and serological tests were stored in a Microsoft Excel spreadsheet (Microsoft Corp.). Sero-prevalence was calculated as a percentage by dividing the number of positive samples for *Brucella* to the total number of samples. Animal- and herd-level sero-prevalence was calculated by dividing the number of positive test results by the total number of animals and herds sampled, respectively.

The data were analyzed using SPSS 20 statistical package. The presence of associations between explanatory variables (risk factors) such as sex, age, herd size, livestock composition and positive sero-prevalence were analyzed using logistic regression. Non-collinear variable that presented *p*-value of <0.25 in univariable logistic regression analysis was offered to the multivariable regression model. The degree of associations between the disease and risk factors was assessed using odds ratio (OR). A *p*-value of less than 0.05 was considered for statistically significant difference.

2.3. Results

Of the total 324 sera samples examined, using RBT screening test, 68 (21.0%, 95% CI: 16.4-26.2) camels were found positive for brucellosis. However, of the 68 sero-positive reactors further tested, using CFT and c-ELISA, 29 (9.0%, 95% CI: 5.9-12.3) and 31 (9.6%, 95% CI: 6.5-13.0) camels, respectively, were confirmed to be sero-positive for brucellosis, showing that the apparent prevalence of camel brucellosis in the study area was 9.6%. The herd level sero-prevalence of brucellosis based on c-ELISA was 35.4% (95% CI: 23.1-47.7). The herd-level prevalence of antibodies against *Brucella* spp. was 18.2% (95% CI: 4.5-36.4), 16.7% (95% CI: 0.0-33.3) and 54.8% (95% CI: 38.7-71.0) in small, medium and large camel herds, respectively, using c-ELISA.

Higher prevalence in females than males (9.1% vs. 5.9% by CFT and 9.8% vs. 5.9% by c-ELISA, respectively) were recorded in this study. However, sero-positivity among the sex groups was not statistically significant (p = 0.600). Both CFT and c-ELISA showed higher prevalence of brucellosis in adults than young camels (10.0% vs. 7.5% by CFT and 11.6% vs. 6.7% by c-ELISA, respec tively) with no statistical significance (p = 0.147) (Table 2.1).

Herd size was found statistically associated with camel brucellosis (p <0.05) with higher prevalence rate in large herds (22.9%, 95% CI: 13.0-31.4), followed by medium (7.6%, 95% CI: 3.5-11.8) and small (3.6%, 95% CI: 0.9-7.3) herds using c-ELISA. Despite the fact that the highest (20.0%, 95% CI: 0.0-50.0) brucellosis sero-prevalence was found in herds composed of camel only, followed by camel-cattle (14.0%, 95% CI: 4.3-24.0), camel-small ruminant (12.0%, 95% CI: 5.1-19.0) and camel-cattle-small ruminant (6.1%, 95% CI: 2.4-9.8), livestock composition was found not statistically significant in determining sero-positivity (p >0.05) (Table 2.1).

Table 2.1: Univariable logistic regression analysis of camel brucellosis by risk factors.

Risk factors	No. tested	c-ELISA positive (%, 95% CI)	Univariate	
			OR (95% CI)	*p*-value
Sex				
Male	17	1 (5.9, 0.0-17.6)		
Female	307	30 (9.8, 6.5-13.4)	1.733 (0.222-13.530)	0.600
Age				
Young (<4 year)	134	9 (6.7, 3.0-10.4)		
Adult (≥4 year)	190	22 (11.6, 7.4-17.3)	0.550 (0.245-1.235)	0.147
Herd size				
Small (<20 camels)	110	4 (3.6, 0.9-7.3)		
Medium (21-50 camels)	144	11 (7.6, 3.5-11.8)	7.852 (2.502-24.640)	0.000
Large (>50 camels)	70	16 (22.9, 13.0-31.4)	3.582 (1.562-8.218)	0.003
Livestock composition				
Camel-cattle-small rum	164	10 (6.1, 2.4-9.8)		
Camel-small rum	100	12 (12.0, 5.1-19.0)	0.476 (0.198-1.147)	0.098
Camel-cattle	50	7 (14.0, 4.3-24.0)	0.399 (0.143-1.110)	0.078
Camel only	10	2 (20.0, 0.0-50.0)	0.260 (0.049-1.389)	0.115
Total	**324**	**31 (9.6, 6.5-13.0)**		

No. = number, OR = odds ratio, CI = confidence interval

In general, sex, age, and livestock composition had no effect on sero-positivity to brucellosis (p >0.05). Herd size, however, was found to have a significant effect on the prevalence of brucellosis in individual animals. To measure the degree of association between sero-positivity and risk factors, multivariable logistic regression analysis was carried out (Table 2.2). The association between sero-positivity and herd size was found statistically significant (p <0.005). Higher sero-positivity to brucellosis was found in large herds (22.9%) than medium (7.6%) and small (3.6%) camel herds. Accordingly, camels kept in large herds were 4 times higher at risk for *Brucella* infection than camels kept under small herd size (OR = 4.024; 95% CI: 1.667-9.716) (Table 2.2).

Table 2.2: Multivariable logistic regression analysis for the association of camel brucellosis and risk factors.

Risk factors	**No. tested**	**c-ELISA positive (%)**	**OR (95% CI)**	***p*-value**
Age				
Young	134	9 (6.7)		
Adult	190	22 (11.6)	2.123 (0.906-4.978)	0.083
Herd size				
Small	110	4 (3.6)		
Medium	144	11 (7.6)	8.546 (2.609-27.997)	0.000
Large	70	16 (22.9)	4.024 (1.667-9.716)	0.002
Livestock composition				
Camel-cattle-small rum	164	10 (6.1)		
Camel-small rum	100	12 (12.0)	0.429 (0.171-1.077)	0.072
Camel-cattle	50	7 (14.0)	0.421 (0.145-1.226)	0.113
Camel only	10	2 (20.0)	0.741 (0.128-4.286)	0.738
Total	**324**	**31 (9.6)**		

The questionnaire survey revealed that 98.5% (64/65) did not know about brucellosis, and that their practices could potentially expose them to the disease. All the 65 camel herders interviewed said they usually consume whole milk without boiling. All of them informed abortion materials are handled with bare hands, and they do not safely destroy these materials. Abortion materials are usually left for dogs and wild carnivores. Milk collected from camels kept around nearby towns and cities (Olenchiti, Adama and Modjo) was being marketed at big cities such as Adama and Addis Ababa.

2.4. Discussion

The disparity between results of CFT and c-ELISA is due to the variation in sensitivity of the tests. For the diagnosis of brucellosis, the c-ELISA test is more sensitive, it can be used on a variety of animal species and it is suitable to use on poor-quality samples such as those affected by hemolysis as compared to CFT (Perrett *et al*., 2010). CFT, on the other hand, is prone to prozone effect which could lead to false negative results (Corbel, 2006). Moreover, CFT is

associated with negative results in early stage of infection due to low IgG titers (Khan *et al*., 2017; Legesse *et al*., 2023).

In Ethiopia, previous serological surveys showed that camel brucellosis is endemic and widespread (Zeweld and Haileselassie, 2012; Tilahun *et al*., 2013; Tassew and Kassahun, 2014; Zeru *et al*., 2016; Wegi *et al*., 2021; Waktole *et al*., 2022; Waji and Neja, 2023; Ahad *et al*., 2024). The 9.6% sero-prevalence recorded in current study is comparable with the 9.23% overall prevalence of camel brucellosis reported by Dadar *et al*. (2022). Similarly, Osoro *et al*. (2015) reported 11.1% sero-prevalence in camels in Marsabit County, Kenya. Zeweld and Haileselassie (2012) also recorded, comparably, moderate prevalence, 7.6% in Afar Region. The current finding is much lower than the 17% (Mohammed *et al*., 2023) and 23.8% (Musa *et al*., 2008) reported in Sudan, 20% (Lokamar *et al*., 2022) reported in Baringo County, Kenya and 19.4% (Dawood, 2008) reported in Jordan. However, it is higher than that of Hadush *et al*. (2013) who reported 4.1% in Afar Region. Kadle *et al*. (2017) also reported lower sero-prevalence (3.9%) in Somalia. The variation in the prevalence rates between the current finding and similar studies undertaken in Ethiopia could be due to differences in herd size, absence or presence of *Brucella*-infected herds, sample size, and sensitivity and specificity of tests used. Whereas, the observed differences in the current and related studies from other countries might be due to differences in management and husbandry practices (Al-Majali *et al*., 2008) and coverage and quality of veterinary services (Mohammed *et al*., 2023).

Brucellosis is considered as a disease of herd importance. The 35.4% (23/65) herd-level sero-prevalence of camel brucellosis recorded in this study is in agreement with the 35.1% (Al-Majali *et al*., 2008) and 31.3% (Kadle *et al*., 2017) herd-level sero-prevalence reported in Jordan and Somalia, respectively. The high herd-level sero-prevalence recorded in this study might be related to the frequent migration of camels that facilitates the sharing of grazing sites and watering points. Direct interactions between herds also increase their exposure to brucellosis.

Brucellosis prevalence of 9.8% in female and 5.9% in male camels was recorded in the present study with no statistical significance (p >0.05). This statistical insignificance between sexes regarding sero-positivity is against the established fact. The small number of male camels

randomly sampled, as the camel herders in the study area were keeping very small breeding males, might predictably bias the statistical analysis.

The current finding, however, was supported by similar trends observed by Ahad *et al*. (2024) who reported higher prevalence in females (4%) than males (0.7%) and Waktole *et al*. (2022) who reported higher prevalence rate in females (9.2%) than males (7%) with no statistical significances. Brucellosis infected male animals usually show clinical signs such as epididymitis and orchitis and could be culled more quickly leading to lower brucellosis prevalence in males. Moreover, male camels are kept for short period; they are usually fed and sold off, except for few individuals that are kept for breeding and transport purposes (Salisu *et al*., 2018). On the other hand, female animals are kept for longer period for breeding purposes and have more physiological stress than males which can be considered as risk factors for brucellosis. Conversely, Bekele *et al*. (2013) reported a higher prevalence in male camels than females and this could be attributed to the differences in the proportion of male and female animals included in the study.

In this study, adult camels had a higher prevalence (11.6%) of brucellosis compared with young camels (6.7%). Similar trends were observed by Hughes and Anderson (2020), Alhussain *et al*. (2022) and Dadar *et al*. (2022). This can be explained by the fact that brucellosis is a disease of adult animals since susceptibility increases after sexual maturity and pregnancy as erythritol sugar in these animals trigger expression of virulence traits in *Brucella* (Petersen *et al*., 2013). Young animals have short-time contact with infected animals or with the environment which also contributes to the lower prevalence of brucellosis in young (Megersa *et al*., 2011a).

Sero-positivity to brucellosis was higher in large herds (22.9%) than medium (7.6%) and small herds (3.6%). The difference in the sero-positivity in the three herd categories was found to be statistically significant (p <0.005). Accordingly, camels kept in large herds were 4 times higher at risk for *Brucella* infection than smaller herds (OR = 4.024). Zeweld and Haileselassie (2012) and Gizaw *et al*. (2017) have shown herd size to significantly affect prevalence of brucellosis. The current finding was also in agreement with that of Omer *et al*. (2010) who reported high prevalence in large herds than medium and small herds in Sudan. However, in a study conducted

by Wegi *et al*. (2021) no statistically significant difference in *Brucella* sero-positivity was observed among different herd groups of camels which disagrees with the current finding. The higher prevalence of brucellosis in large herds could be the result of easy contact among the animals in the herds favoring higher chances of brucellosis transmission (Yawoz *et al*., 2012).

Highest sero-prevalence was recorded in camels kept alone (20.0%), and no significant difference observed in the prevalence of brucellosis between camels kept alone and camels co-herded with cattle (14.0%), small ruminants (12.0%) and cattle and small ruminants (6.1%) in this study. This looks contrary to the established fact that camels co-herded with other livestock species is a contributing risk factor to brucellosis (Al-Majali *et al*., 2008). In the study area, although milk producing camels were kept separate around cities and towns for the whole lactation period, they could come in contact with ruminants from other households at communal pasture and watering points. Besides, other non-lactating camels which usually migrate alone very far in search of feed and water could also come in contact with ruminants from other households.

Milk producing camels in the study area are kept around nearby towns and cities for the whole lactation period. This is practiced to access milk markets as far as the capital, Addis Ababa. This tradition was observed during sample collection and could also be observed by anyone through the number of camels seen along the road.

Regarding questionnaire survey, almost all interviewed camel herders (98.5%) had no clear knowledge on zoonotic importance of brucellosis and this may predispose the community to the disease. Traditions such as bare-hand handling of aborted fetuses and fetal membranes, and the consumption of raw milk are risks for the transmission of the disease. Moreover, aborted materials are left for dogs and wild carnivores, and this is likely to play a role in the transmission of brucellosis in the study area. Waji and Neja (2023) reported that over 75% of animal owners are unaware of the risks of zoonotic camel brucellosis, and over three-quarters of pastoralists engage in at least one activity that increases the risk of transmission. In a study conducted by Hassan *et al*. (2022), 13 (39.4%) brucellosis diagnosed patients had history of contact with aborted materials and 28 (23.9%) consumed undercooked meat or unpasteurized milk. Similarly,

drinking raw milk from aborted animals, touching aborted materials or fetuses, and occupation were among the risk factors for human brucellosis (Dagnaw *et al*., 2024).

Limitations of this study were, some camel herders refused to allow blood sample collection from their lactating camels contending that this practice could reduce milk production. In those cases, other households from neighbors were replaced. Besides, the kebeles surveyed were limited to areas with less security concerns.

2.5. Conclusion and Recommendations

In conclusion, the findings of this study showed that brucellosis is prevalent in camels in the study area. Among the potential risk factors assessed, herd size was found significantly associated with *Brucella* sero-positivity. The presence of brucellosis in milk-producing camels poses a significant risk to human health as milk collected from such camels is consumed raw and even distributed to big cities like Adama and Addis Ababa. Moreover, unsafe handling and disposal of aborted materials is a common practice. Thus, further extensive epidemiological studies involving all domestic and wild animals and humans need to be undertaken in the study area. Moreover, awareness on public health importance of camel brucellosis and its prevention is quite necessary.

3. CHAPTER THREE: SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF SMALL RUMINANT BRUCELLOSIS IN KEREYU PASTORAL AREA OF OROMIA REGION, ETHIOPIA

Abstract

This study reports the prevalence and risk factors of brucellosis in small ruminants in the Fentale district of Ethiopia. For this purpose, a total of 384 serum samples—192 each from sheep and goats—were collected from 77 flocks using a random sampling method. All samples were initially screened for *Brucella* antibodies using the Rose Bengal plate test (RBT), and those tested positive were further confirmed using the complement fixation test (CFT) and competitive enzyme-linked immunosorbent assay (c-ELISA). Multivariable logistic regression analysis was employed to assess the association between potential risk factors and *Brucella* infection. Out of the 384 serum samples tested using RBT, 3.6% (95% CI: 2.1-5.8) were found positive. Further examination of the RBT-positive samples using CFT and c-ELISA demonstrated animal level sero-prevalence of 2.3% (95% CI: 0.9 4.2) and 2.6% (95% CI: 1.0-4.2), respectively. Based on c-ELISA results, the herd-level prevalence in the study area was 11.7% (95% CI: 6.4-22.1). Sex and flock size were found to be significantly associated with *Brucella* sero-positivity (p <0.05), while, species, age, and parity were not (p >0.05). Animals in the large herd category were found to be at significantly higher risk of *Brucella* infection compared to those in medium-sized herds. The current study found that brucellosis is prevalent in small ruminants in the study area. *Brucella* organisms circulating in the area need to be investigated. Moreover, pastoralists in the study area need to be aware on the importance of small ruminant brucellosis.

Keywords: c-ELISA; CFT; Fentale; RBT; Small ruminant brucellosis.

3.1. Introduction

Meat from sheep and goats makes up about 25% of the value of meat produced in Ethiopia (Shapiro *et al*., 2017). Small ruminants also provide significant contributions to the national economy through international trade. In 2018/19, Ethiopia earned 92.65 million USD from approximately 20,000 tons of meat exports. From this, about 80.35 million USD (86.7%) was gained from sheep and goat meat (Mamo, 2019). The sector, however, is primarily affected by diseases such as brucellosis.

Brucellosis is one of the most economically important zoonoses worldwide with multiple economic losses attributed to livestock health, production and public health (Dadar *et al*., 2021). Brucellosis remains endemic in low and middle-income nations such as in sub-Saharan Africa (Kimera *et al*., 2025), the Middle East (Musallam *et al*., 2016), India (Saxena *et al*., 2018; Shome *et al*., 2021), central Asia (Zhang *et al*., 2018; Qurbonov *et al*., 2024), Mexico (Marin *et al*., 2016) and central and South America (Hernández-Mora *et al*., 2017) where goats and sheep are the major livestock species. Although it is eradicated in northern European countries, brucellosis is also quite common in the Mediterranean basin (Ducrotoy *et al*., 2015; Wareth *et al*., 2020).

The disease is a major cause of production and reproductive losses in livestock, characterized by abortion, loss of offspring, decreased meat and milk production, weight loss, inflammation of the reproductive organs and fetal membranes as well as export restrictions imposed on animals and their products (McDermott *et al*., 2013; Noakes *et al*., 2019; Lokamar *et al*., 2020).

In domestic small ruminants, brucellosis is primarily caused by *Brucella melitensis*. Sporadic cases of brucellosis caused by *B. abortus* or *B. suis* have also been reported in sheep and goats. *Brucella ovis* is more commonly associated with epididymitis or orchitis in sheep (Rossetti *et al*., 2022; WOAH, 2022). However, there have been reports of *B. ovis*-specific antibodies in goats (Costa *et al*., 2016). *Brucella melitensis, B. abortus* and *B. suis* are readily transmissible to humans (WOAH, 2022).

Immunological methods remain the most cost-effective approach for the screening and detection of *Brucella* infection, especially under resource-limited settings (Rossetti *et al*., 2022). The Rose Bengal plate test (RBT) is a screening test and the complement fixation test (CFT) and competitive enzyme-linked immunosorbent assay (c-ELISA) are considered as suitable confirmatory tests (WOAH, 2022).

Sero-prevalence results have been documented in small ruminants across different production systems and regions of Ethiopia (Asmare *et al*., 2013a; Gebremedhin, 2015; Wedajo *et al*., 2015; Tewodros and Dawit, 2015; Tschopp *et al*., 2015; Tsegay *et al*., 2015; Wakene *et al*., 2017; Tulu *et al*., 2020a; Aloto *et al*., 2022; Teshome *et al*., 2022; Dosa *et al*., 2023; Hussen *et al*., 2023). However, the current status of small ruminant brucellosis in pastoralist communities of the Fentale district is missing. Therefore, the present study was carriedout with the objectives of determining the sero-prevalence of brucellosis in sheep and goats in the Kereyu pastoral area of Oromia Region and to identify potential risk factors associated with the disease.

3.2. Materials and Methods

3.2.1. Study Area

The study was conducted in Fentale district which is situated in the Awash lowland area of east Shewa zone of Oromia Region, Ethiopia (Figure 1). The district is located at 80°42ˈ to 90°00ˈ north latitudes and 30°03ˈ to 40°01ˈ east longitudes. The area falls within an altitude range of 800-1100 meters above sea level (masl). There are high peaks on Mount Fentale from which the district derives its name, reaching up to 2007 masl (Gedda, 2003). Fentale district is inhabited by the Kereyu people who are indigenous Oromo-speaking transhumant pastoralists.

The livestock populations of Fentale district are 53,682 cattle, 106,931 sheep, 129,424 goats, 20,298 camel, 13,005 equine and 6,446 poultry (Lemma and Fufa, 2023). Awash River and Lake Baseka are the main water bodies of this district. Common grazing areas of the district are located in the valleys of Awash River. Recurring droughts due to disrupted rainfall patterns affects the area and the district receives an annual rainfall ranging from 400 to 700mm.

Temperature ranges from 29 to 38°C. The Kereyu people are agro-pastoralists practicing both rain-fed and irrigated agriculture mainly as a response to the subsequent weakening of their pastoral means of livelihood in addition to livestock herding (Ayalew, 2001).

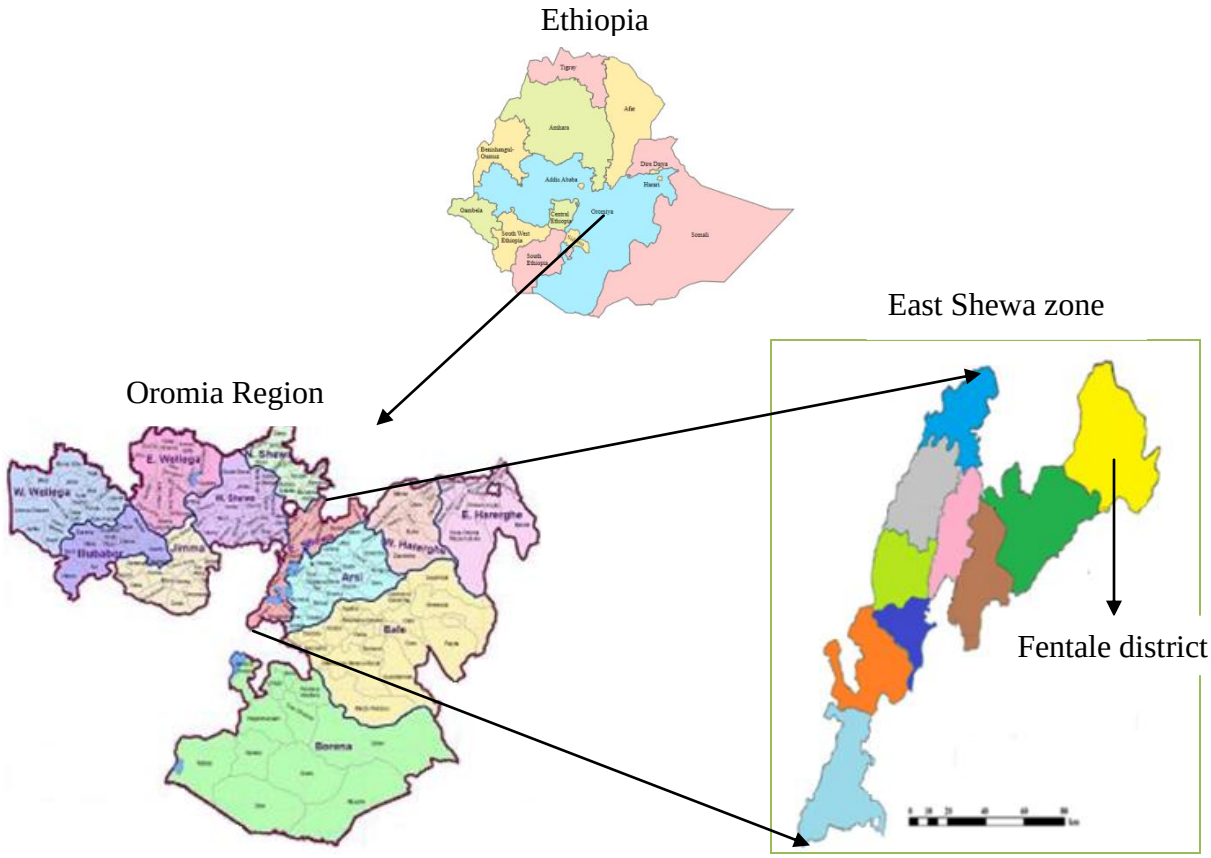


Figure 1: Map of the study area (Bahru *et al*., 2021).

3.2.2. Study Design and Sample Size Determination

A cross-sectional study was used to determine the sero-prevalence and associated risk factors of small ruminant brucellosis in the Fentale district from December, 2019 to October, 2021. Five kebeles, namely Benti, Ilala, Gelcha, Alge and Dega Adu were selected based on the willingness of the owners, security reasons, accessibility to transportation and density of small ruminant population, whereas animals were randomly selected following systematic random sampling. The sample size was calculated based on the formula $N = (1.96)^2 \times Pexp \times (1\text{-}Pexp)/D^2$, where N = sample size, Pexp = expected prevalence, and D = level of precision (5%) (Thrusfield, 2005).

On the basis of 50% expected prevalence, 5% level of precision and 95% confidence interval, the calculated sample size was 384. Hence, a total of 384 (192 sheep and 192 goats) serum samples from 77 flocks were collected. In 76 flocks, random samples of five animals per flock were sampled. Four additional animals were randomly sampled from the remaining one flock to obtain the total sample size. Sheep and goats above 6 months of age were included in the study. There was no history of vaccination against brucellosis in the study area.

3.2.3. Sample Collection

The objectives of the survey were explained to animal owners and verbal consent was obtained. From each animal, approximately 8ml of blood was aseptically collected from jugular vein using plain vacutainer tubes. The blood samples were left at room temperature overnight to allow clotting for serum separation. Sera were then collected in sterile cryogenic vials, and transported to the National Veterinary Institute (NVI), Bishoftu, Ethiopia using ice box containing ice blocks, and were stored in the laboratory at -20°C until tested.

Data on species, sex, age, parity, and flock size were also recorded. The age of animals was categorized as young (≤3 years) and as adult (>3 years) according to Dosa *et al*. (2023). Parity was categorized as zero parity, mono parity and multi-parity (Gumi and Wudneh, 2025). Similarly, flock size was categorized into small (<20 heads), medium (20-50 heads) and large (>50 heads).

3.2.4. Serological Examinations

The Rose Bengal plate test (RBT) and complement fixation test (CFT) were conducted at the National Veterinary Institute, Bishoftu, Ethiopia for the detection of small ruminant brucellosis antibodies. All RBT-positive serum samples were also shipped to University of Nairobi, Kenya for further testing using competitive enzyme-linked immunosorbent assay (c-ELISA).

The diagnostic performance of some enzyme-linked immunosorbent assays (ELISAs) is comparable with or better than that of the CFT, and because they are technically simpler and more robust, they may be used as complementary or confirmatory tests (WOAH, 2022). In a study comparing c-ELISA and CFT for detection of brucellosis in cattle, the sensitivity of c-ELISA ranged from 92.31% to 100%, in comparison with 77.14% to 100% for CFT (Perrett *et al*., 2010). Competitive-ELISA showed comparable (Perrett *et al*., 2010) or higher specificities (97.5%) than that of CFT in detecting cattle brucellosis (Samartino *et al*., 1999).

Rose Bengal plate test (RBT)

Following the procedure and interpretations described by Staak *et al*. (2001), all serum samples were first screened by RBT. The RBT antigen and serum samples were brought to room temperature before the test. About 75µl of each serum sample was placed on the agglutination plate. The antigen bottle was gently shaken and 25µl of RBT antigen was placed near each serum sample on the agglutination plate. The antigen and the serum were, then, mixed thoroughly using a plastic rod for each test to produce a circular zone of approximately 2cm in diameter. The mixture was agitated gently for 4 minutes at room temperature, and the result was read for the presence or absence of agglutination by comparing with the positive and negative control sera in natural light. Samples identified with no agglutination were regarded as negative and those with agglutination were regarded as positive.

Complement fixation test (CFT)

Sera positive for the RBT were further tested with CFT for confirmation using the WOAH (2022) protocol. A sheep red blood cell (SRBC) of 2% suspension was prepared. The complement, antigen and SRBC were mixed with the serum samples and positive reactions were indicated by sedimentation of SRBC and absence of hemolysis. Negative reactions were revealed by hemolysis of SRBC. Serum 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 regarded as positive.

Competitive enzyme-linked immunosorbent assay (c-ELISA)

Similarly, all the RBT-positive serum samples were also tested using a commercial COMPELISA 160 & 400, a competitive ELISA kit for the detection of *Brucella* antibodies in serum samples (APHA Scientific, Weybridge, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom). The test was conducted according to the manufacturer's protocol using 96-well polystyrene plates pre-coated with *Brucella* lipopolysaccharide antigen. The lack of color development indicated positive sample. A positive/negative cut-off was calculated as 60% of the mean of the Optical Density (OD) of the 4 conjugate control wells. Any test sample giving an OD equal to or below this value was regarded as positive.

3.2.5. Data Management and Analysis

The data were checked for completeness, clarity, consistency, and accuracy. The data were then cleaned, coded, summarized, and entered in a Microsoft Excel spreadsheet and analyzed using SPSS® Version 20. Sero-prevalence was calculated on the basis of RBT, CFT and c-ELISA positive results. C-ELISA was considered as the final confirmatory test and samples positive to both RBT and c-ELISA were taken for subsequent analysis. The presence of associations between risk factors and positive sero-prevalence was analyzed using logistic regression. In the analyses, a 95% confidence interval and *p*-value ($p < 0.05$) were set for significance of statistical associations between the dependent and independent variables.

3.3. Results

3.3.1. Sero-prevalence at Individual Animal and Flock Levels

The overall sero-prevalence of brucellosis in small ruminants was 3.6% (95% CI: 2.1-5.8), 2.3% (95% CI: 0.9-4.2) and 2.6% (95% CI: 1.0-4.2) using RBT, CFT and c-ELISA, respectively (Table 3.1).

Table 3.1: Sero-prevalence of *Brucella* antibodies by species and test type.

Category		Animal No. Tested	RBT result		CFT result		c-ELISA result	
			Animal No. Positive	Prevalence (95% CI)	Animal No. Positive	Prevalence [95% CI]	Animal No. Positive	Prevalence [95% CI]
Species	Sheep	192	6	3.1 (1.0-5.3)	2	1.0 [0.0-2.7]	3	1.6 [0.0-3.6]
	Goats	192	8	4.2 (1.6-7.8)	7	3.6 [1.0-6.3]	7	3.6 [1.6-6.3]
Total		**384**	**14**	**3.6 (2.1-5.8)**	**9**	**2.3 [0.9-4.2]**	**10**	**2.6 [1.0-4.2]**

No. = number

Of the 77 flocks sampled, the overall true herd sero-prevalence was 11.7% (9/77) (95% CI: 6.4-22.1) (Table 3.2). Only one flock had more than one animal testing positive for brucellosis. All the herds sampled had a mixture of sheep and goats; hence comparing sero-positivity at the herd level by species was not applicable.

Table 3.2: Individual and flock level sero-prevalence of small ruminant brucellosis based on c-ELISA.

Flock size	Individual-level prevalence			Flock-level prevalence		
	Animal No. Tested	Animal No. Positive	Prevalence (95% CI)	Animal No. Tested	Animal No. Positive	Prevalence (95% CI)
Small flock (<20 heads)	55	-	-	11	-	-
Medium flock (20-50 heads)	285	1	0.4 (0.0-1.1)	57	1	1.8 (0.0-6.1)
Large flock (>50 heads)	44	9	20.5 (6.8-38.3)	9	8	88.9
Total	**384**	**10**	**2.6 (1.0-4.4)**	**77**	**9**	**11.7 (6.4-22.1)**

No. = number; CI = confidence interval

3.3.2. Association of Brucellosis Positivity with Risk Factors

In this study, the overall sero-prevalence of brucellosis in small ruminants was 3.6% by RBT, 2.3% by combined RBT and CFT, and 2.6% by combined RBT and c-ELISA. For subsequent analysis, the individual-level sero-prevalence of 2.6%, based on c-ELISA, was used (Table 3.3).

Table 3.3: Logistic regression analysis and association of risk factors with *Brucella* sero-positivity based on c-ELISA test.

Variable	Category	Animal No. tested (sero-positive)	Prevalence (95% CI)	Univariable analysis	
				OR (95% CI)	*p*-value
Species	Sheep	192(3)	1.6(0.0-3.6)	Ref.	
	Goats	192(7)	3.6(1.5-6.3)	2.38(0.61-9.36)	0.213
Sex	Male	37(3)	8.1(0.0-18.9)	Ref.	
	Female	347(7)	2.0(0.6-3.7)	0.23(0.06-0.94)	0.041
Age	Young (≤3 years)	341(7)	2.1(0.6-3.8)	Ref.	
	Adult (>3 years)	43(3)	7.0(0.0-16.3)	3.58(0.89-14.39)	0.073
Parity	Zero parity	24(0)	0		
	Mono parity	63(2)	3.2(0.0-9.5)	Ref.	
	Multiple parity	260(5)	1.9(0.4-3.8)	1.11(0.30-4.15)	0.878
Flock size	Small (<20)	55(0)	0		
	Medium (20-50)	285(1)	0.4(0.0-1.1)	Ref.	
	Large (>50)	44(9)	20.5(11.3-31.9)	73.45(9.09-593.38)	0.000
Total		**384(10)**	**2.6(1.0-4.2)**		

No. = number; Ref. = reference category; OR = Odds Ratio; CI = Confidence Interval

The sero-prevalence of brucellosis was 1.6% in sheep and 3.6% in goats; however, this difference was not statistically significant (p >0.05).

In the logistic regression analysis, sex and flock size were found to be significantly associated with sero-positivity. Females and animals in the large herd category were found to be at significantly higher risk of *Brucella* infection compared to male animals and those in medium-sized herds, respectively (Table 3.3).

3.4. Discussion

As no single serological test is sufficient in all epidemiological contexts, the simultaneous use of at least two different serological methods is strongly recommended for assessing brucellosis status in small ruminant herds (WOAH, 2022). The RBT, CFT and c-ELISA were used for the diagnosis of brucellosis and samples positive to both RBT and c-ELISA were taken for subsequent analysis in this study.

The complement fixation test (CFT) could lead to false negative results and is associated with negative results in early stage of infection (Khan *et al*., 2017; Legesse *et al*., 2023). On the other hand, the c-ELISA test has more sensitivity and comparable specificity to the CFT, and can be used on poor-quality samples (Perrett *et al*., 2010).

The current study revealed a 2.6% and 11.7% overall and flock-level sero-prevalence of small ruminant brucellosis, respectively, in the study area. The study also disclosed higher sero-prevalence of brucellosis in males (8.1%) than females (2.0%) (p <0.05). None of the animals in zero parity and small flock category tested positive for *Brucella*.

The 2.6% overall sero-prevalence found in the present study is in line with the 2.6% and 2.97% reported by Teshome *et al*. (2018) and Muhidin *et al*. (2021), respectively, in different parts of Ethiopia. However, it is relatively higher than results of other previous studies conducted in Ethiopia (Kelkay *et al*., 2017; Mohammed *et al*., 2017; Hussen *et al*., 2023). On the other hand,

the present finding is lower than reports of Asmare *et al*. (2013a), Gebremedhin (2015) and Tschopp *et al*. (2015) from Ethiopia and Awais *et al*. (2024) from Pakistan. The 11.7% flock-level small ruminant sero-prevalence recorded in this study is comparable to that of Lone *et al* (2013). It is, however, higher than the 6.35% reported by Hussen *et al*. (2023), and lower than the 22% and 17.5% reported by Adem *et al*. (2021) and Muhidin *et al*. (2021), respectively, in Ethiopia. It is also lower than the 28.12% recorded by Awais *et al*. (2024) in Pakistan.

Contrary to the present finding, higher sero-prevalence was observed in goats than sheep in Ethiopia (Aloto *et al*., 2022; Dosa *et al*., 2023; Hussen *et al*., 2023) and Kenya (Muema *et al*., 2022), and in sheep than in goats in Ethiopia (Muhidin *et al*., 2021), Nepal (Gompo *et al*., 2021), Libya (Alshekh *et al*., 2024) and Pakistan (Awais *et al*., 2024). Similar to the current finding, Wedajo *et al*. (2015) reported higher sero-prevalence in males than female animals. However, it is difficult to draw a strong conclusion as number of male animals sampled in the present study (37 males versus 347 females) and the other study (35 males versus 379 females) mentioned are much smaller. In previous studies, Muhidin *et al*. (2021) and Teshome *et al*. (2022) reported lower sero-prevalence in males than females with statistically significant difference. Other studies, contrary to our finding, reported no statistically significant association between sex of the animal and *Brucella* sero-positivity (Asmare *et al*., 2013a; Gebremedhin, 2015; Dosa *et al*., 2023; Hussen *et al*., 2023).

Although statistically non-significant, adult animals were found higher at risk for *Brucella* infection than young animals. Dosa *et al*. (2023) and Hussen *et al*. (2023) recorded higher sero-prevalence in old than young animals with significant difference among age groups. Moreover, Tulu *et al*. (2020a) and Muhidin *et al*. (2021) reported the odds of brucellosis sero-positivity to be higher among old than young small ruminants. On the other hand, Wedajo *et al*. (2015) reported higher sero-prevalence of brucellosis in young than old animals. In line with the current findings, higher sero-prevalences of small ruminant brucellosis were reported in animals within large flock than medium and small flocks (Geletu *et al*., 2021; Dosa *et al*., 2023).

The differences seen in the overall sero-prevalence in the present study and other studies mentioned might be caused by variations in sample size, serological tests used, agro-ecological

location and animal management practices. The relatively high flock-level sero-prevalence recorded in the present study might be correlated with the free animal movement and aggregation of animals at common pastures and watering points. This aggregation might increase the transmission of brucellosis from animal to animal or from contaminated environment.

A possible reason for the finding of higher *Brucella* sero-prevalence in male than female animals in the present study could be due to the sharing of male animals for breeding purposes between villages which could have led to increased likelihood of infection. It could also be due to the small number of male animals included in the study as compared to the female animals. In addition, inclusion of young animals that didn't reach breeding age could also explain the difference. Although a lot of literature state that most breeds of goats are fully susceptible to brucellosis (Radostits *et al*., 2010) and goats excrete *Brucella* organisms for longer periods (Quintas *et al*., 2019) increasing the potential for disease spread among goats, the difference observed among species in the present study was not statistically significant. The existence of higher brucellosis sero-prevalence in large flocks as compared to small flocks could be due to close contact between animals in large flocks which facilitates easy transmission of the disease within the flock.

The limitations of the study include that the kebeles surveyed were limited to areas with less security concerns, and some were conveniently selected based on the density of small ruminant population, willingness of the owners and accessibility to transportation.

3.5. Conclusion and Recommendations

In conclusion, the observed sero-prevalence of 2.6% at the animal level and 11.7% at the flock level indicates that small ruminant brucellosis is present and of epidemiological significance in the Fentale district of Oromia Region, Ethiopia. Sex and flock size are significantly associated with brucellosis sero-positivity, and the sero-prevalence of brucellosis is higher in males and in animals within large flocks. Further epidemiological studies involving humans and other animal species, along with the isolation and molecular characterization of the *Brucella* species

circulating in the area are essential for effective control of brucellosis. Additionally, raising awareness among pastoralists about small ruminant brucellosis is crucial for the success of prevention and control efforts.

4. CHAPTER FOUR: SERO-PREVALENCE OF CATTLE BRUCELLOSIS AND ITS RISK FACTORS IN KEREYU PASTORAL AREA OF OROMIA REGION, ETHIOPIA

Abstract

Brucellosis constitutes a serious health threat for livestock and the public in the Kereyu pastoral communities of Ethiopia. Public health risk is high as pastoralists in the study area are accustomed to risky practices. Public health risk is particularly high in these communities, as their way of life is highly linked with their herds. This study was conducted to estimate the sero-prevalence and identify potential risk factors of cattle brucellosis in Kereyu pastoral areas of Oromia Region, Ethiopia. A total of 384 serum samples from cattle above six months old were collected. Data on hypothesized risk factors were also collected using a semi-structured questionnaire. The screening of the sera for *Brucella* antibodies was done using Rose Bengal plate test (RBT) and positive sera were further subjected to complement fixation test (CFT) and competitive enzyme-linked immunosorbent assay (c-ELISA). The overall animal level sero-prevalence of brucellosis was 1.3% (95% CI: 0.3-2.6) while herd level prevalence was 6.3% (95% CI: 1.3-12.7). Among the risk factors considered, sero-prevalence was higher in cattle of older age groups (3.7%), in cattle from larger herds (3.8%), in herd composed of cattle-small ruminant-camel (2.6%) and in cattle from agro-pastoral production systems (1.5%) than cattle in the respective categories with statistically non-significant difference. However, higher sero-prevalence of brucellosis was observed in cows with multiple parity (3.4%) and in cows with history of abortion (28.6%) than their respective categories (p <0.05).

Keywords: cattle brucellosis; c-ELISA; CFT; Fentale; RBT.

4.1. Introduction

Brucellosis is an infectious disease of domestic and wild mammals and humans. While some countries have succeeded in preventing or eradication of this zoonotic disease (WOAH, 2022), it remains endemic in the low- and middle-income countries across the Mediterranean region (Abdeen *et al*., 2019), Africa and Asia (McDermott *et al*., 2013; Djangwani *et al.*, 2021), central and South America (Hernández-Mora *et al*., 2017) and the Middle East (Musallam *et al*., 2016; Nejad *et al*., 2020).

Brucellosis is caused by several species of the genus *Brucella* which localize in the reproductive organs of host animals, causing abortions, death of young animals, stillbirth, retained placenta or birth of weak calves, delayed calving, reduction in milk yield and infertility (Garofolo *et al*., 2016; Arif *et al*., 2017; Franc *et al*., 2018). The disease is known to cause abortion in the last stage of pregnancy and infertility in succeeding pregnancies in cattle (Robi, 2020).

Transmission of brucellosis between animals is commonly mediated by direct or indirect contact with aborted materials, contaminated materials, contaminated products or byproducts (e.g., milk, meat, and cheese) (Godfroid *et al*., 2005; Ferrero *et al*., 2014; Ibrahim *et al*., 2021) through ingestion, respiratory exposure, conjunctival inoculation, damaged skin or mucosal membranes (Poester *et al*., 2013; Tadesse, 2016). In humans, brucellosis is mainly transmitted through direct contact with infected animals, consumption of infected raw milk and dairy products (Pappas *et al*., 2005). Airborne transmission through inhalation of infected aerosolized particles can also occur in humans (Zhang *et al*., 2020).

So far, 13 *Brucella* species have been identified (Rebollada-Merino *et al*., 2026) and cattle brucellosis is usually caused by biovars of *Brucella abortus* (WOAH, 2022). Cattle are also infected with *B. melitensis* and *B. suis* when they graze together with infected small ruminants and pigs, respectively (Godfroid *et al*., 2011; Constable *et al*., 2017; Robi and Gelalcha, 2020).

Although Ethiopia possesses the largest livestock population in Africa, this resource remains underutilized due to various constraints. Brucellosis is among the foremost impediments to cattle

production and causes extensive economic losses in the country (Asgedom *et al*., 2016; Abera, 2024).

A number of studies (Asfaw *et al*., 2016; Asgedom *et al*., 2016; Tulu *et al*., 2020b; Robi and Gelalcha, 2020; Kenea and Megersa, 2021) in Ethiopia have indicated the endemicity of bovine brucellosis, leading to high economic losses in cattle production. Most of these studies, however, were carried out in cattle under crop-livestock mixed farming (Asfaw *et al*., 2016; Pal *et al*., 2016; Tsegaye *et al*., 2016; Robi and Gelalcha, 2020; Tulu *et al*., 2020b). Brucellosis constitutes a serious public health threat in pastoral settings as pastoralists' way of life is closely interlinked with their large livestock herds (Tschopp *et al*., 2022). In particular, there is scarcity of information on cattle brucellosis in pastoral areas of east Shewa zone, Ethiopia which derived the initiation of this study. Therefore, the objective of this study was to determine the prevalence of cattle brucellosis and associated risk factors in Fentale district of Oromia Region, Ethiopia.

4.2. Materials and Methods

4.2.1. Study Design and Sample Size Determination

A cross-sectional study design was conducted from December, 2020 to October, 2022 to determine the sero-prevalence of *Brucella* infection in cattle and to identify potential risk factors associated with sero-positivity in Fentale district. The sample size was calculated based on expected prevalence of 50% bovine sero-prevalence according to Thrusfield (2005) at 95% confidence level and 5% required precision using the formula, $N = (1.96)^2 \times P \times Q/D^2$, where N = sample size, P = expected prevalence, Q = 1-P and D = level of precision (5%). Accordingly, $N = (1.96)^2 \times 0.5 \times (1 - 0.5)/(0.05)^2 = 384$. Hence, a total of 384 cattle were sampled for the sero-prevalence study of bovine brucellosis in the district.

For this study, five kebeles (Ilala-karari, Kobo, Kanifa, Turo-badanota and Golala) were purposively selected based on security, access to road and their representativeness of the pastoralist and agro-pastoralist communities. Ilala-karari and Kobo from kebeles inhabited by pure pastoralists, Kanifa, Turo-badanota and Golala from kebeles inhabited by agro-pastoralists were included in the study. A total of 79 cattle herds (41 from agro-pastoralist kebeles and 38

from pastoralist kebeles) were randomly selected. In a herd with less or equal to five animals, all were sampled. In herds with more than five animals, random samples of five animals were taken. Cattle older than six months of age were sampled from the randomly selected herds. Age of animals were categorized into < 3, 3–6 and > 6 years (Ibrahim *et al*., 2010); herd size was categorized into < 15, 15–29 and > 29 heads of cattle (Megersa *et al*., 2011a) and parity number was categorized as nullparous, monoparous and pluriparous (Ibrahim *et al*., 2010).

4.2.2. Serum Sample Collection

About 10ml blood samples were collected from the jugular vein of each animal using plain vacutainer tubes. The blood containing tubes were given identification numbers. The blood samples were allowed to clot overnight at room temperature. The serum was separated from the clotted blood by decanting to cryovial tubes. The separated sera were stored at -20°C until tested. Data including the owner's names, sex, age, herd size, livestock composition and other relevant information were recorded at the time of blood collection.

4.2.3. Serological Examination

The Rose Bengal plate test (RBT) and complement fixation test (CFT) were conducted at the National Veterinary Institute, Bishoftu, Ethiopia for the detection of brucellosis antibodies. All RBT-positive sera samples and 94 (25%) randomly selected RBT-negative bovine sera were also shipped to University of Nairobi, Nairobi, Kenya and further tested using competitive enzyme-linked immunosorbent assay (c-ELISA).

The Rose Bengal plate test (RBT) was conducted following the procedure described by Staak *et al*. (2001). All RBT-positive samples were confirmed with a complement fixation test CFT) using the OIE protocol (OIE, 2016). Again, all the RBT-positive sera samples and 94 (25% of 377) randomly selected RBT-negative bovine sera were further tested using a commercial COMPELISA, a competitive ELISA kit for the detection of antibodies against *Brucella* in serum samples (APHA Scientific, Weybridge, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom) at Department of Public Health, Pharmacology and Toxicology, University of

Nairobi, Nairobi, Kenya according to the manufacturer's protocol using 96-well polystyrene plates pre-coated with *Brucella* lipopolysaccharide antigen.

4.2.4. Case Definition

Animals were considered positive to brucellosis when they tested positive on either RBT/CFT or RBT/c-ELISA tests. Similarly, a herd was considered sero-positive when at least one animal in a herd tested positive. Since there is no history of vaccination against brucellosis in Ethiopia, sero-positivity observed in this study was considered to be due to natural infection.

4.2.5. Questionnaire Survey

Verbal consent was obtained from the respondents, and the objectives of the survey, including the potential benefits, were explained to them before the start of the interview. Structured questionnaire format were developed and used in this study. The interviews were conducted in the local language (Afaan Oromo). The questionnaires administered were focusing on knowledge about zoonotic diseases, the habits of animal product consumption and handling, and dead animal/aborted fetus disposal practices. In total, 110 pastoralists (79 pastoralists whose animals were sampled and 31 cattle owners visiting veterinary clinic at Metahara town) were interviewed.

4.2.6. Data Management and Analysis

The data were analyzed using SPSS 20 statistical package. Data obtained during sample collection and serological tests were stored in a Microsoft Excel spreadsheet (Microsoft Corp.). Sero-prevalence was calculated as a percentage by dividing the number of RBT and c-ELISA positive animals by the total number of animals tested. Herd-level sero-prevalence was calculated by dividing the number of herds with at least one reactor in RBT and c-ELISA by the total number of herds tested.

The presence of associations between risk factors such as sex, age, herd size, livestock composition and positive sero-prevalence were analyzed using Chi-suare (χ^2) statistics, with Fisher's exact test. A *p*-value of less than 0.05 was considered for statistically significant difference.

4.3. Results

4.3.1. Sero-prevalence of Bovine Brucellosis and Associated Risk Factors

A total of 384 sera samples were examined using RBT screening test and 7 (1.8%, 95% CI: 0.5-3.4) were found positive to this test. The RBT-positive sera samples were further tested using CFT and c-ELISA. No sero-reactor animal was found by CFT whereas 5 out of the 7 RBT-positive sera were positive by c-ELISA (1.3%, 95% CI: 0.3-2.6). All the 94 RBT-negative sera samples subjected to c-ELISA were also found negative. Sera that were positive to both RBT and c-ELISA were considered true sero-positive and used for subsequent data analysis. The sero-prevalence of bovine brucellosis in the study area was thus 1.3% (95% CI: 0.3-2.6) based on c-ELISA test. The overall sero-prevalence of *Brucella* antibodies recorded in pastoral and agro-pastoral production systems were 1.1% (95% CI: 0.0-2.7) and 1.5% (95% CI: 0.0-3.1), respectively. The herd level sero-prevalence of brucellosis based on c-ELISA was 6.3% (5/79). Sero-prevalence in the agro-pastoral system was 7.3% at the herd level, while in the pastoral system, these figure was 5.3% (herd level) (Table 4.1).

Table 4.1: Animal level and herd level prevalence of bovine brucellosis in the study area.

Production system	**Animal level**				**Herd level**			
	No. tested	**RBT positive (%, 95% CI)**	**CFT positive (%)**	**c-ELISA positive (%, 95% CI)**	**No. tested**	**RBT positive (%, 95% CI)**	**CFT positive (%)**	**c-ELISA positive (%,95% CI)**
Pastoral	190	4(2.1, 0.4-4.2)	0(0.0)	2(1.1, 0.0-2.7)	38	4(10.5, 2.6-23.7)	0(0.0)	2(5.3, 0.0-13.2)
Agro-pastoral	194	3(1.5, 0.0-3.6)	0(0.0)	3(1.5, 0.0-3.1)	41	3(7.3, 0.0-15.5)	0(0.0)	3(7.3, 0.0-17.1)
Total	**384**	**7 (1.8, 0.5-3.4)**	**0(0.0)**	**5 (1.3, 0.3-2.6)**	**79**	**7(8.9, 3.8-15.2)**	**0(0.0)**	**5(6.3, 1.3-12.7)**

Table 4.2 present prevalence of *Brucella* antibodies in different categories and results of Fisher's exact test for the association of the exposure variables and *Brucella* sero-positivity. According to the present study, 1.5% *Brucella* prevalence was detected in female animals and none of the male animals was found sero-positive. Higher sero-prevalence of *Brucella* antibodies were found in cattle of older age groups (3.7%), in cattle from larger herds (3.8%), in herd composed of cattle-small ruminant-camel (2.6%) and in cattle from agro-pastoral production systems (1.5%) than cattle in the respective categories, however the differences were statistically non-significant. A significantly higher sero-prevalence of *Brucella* antibodies was observed in cattle with multiple parity (3.4%) and a history of abortion (28.6%) (p <0.05).

Table 4.2: Prevalence of bovine brucellosis on the basis of the potential risk factors

Variables	**Category**	**No. tested (No. positive)**	**Prevalence (95% CI)**	**Fisher exact test**	
				χ^2	***p*-value**
Sex	Male	41(0)	0		
	Female	343(5)	1.5 (0.3-2.9)		
Age	<3 years	89(0)	0		
	3-6 years	188(1)	0.5 (0.0-2.1)	6.714	0.059
	>6 years	107(4)	3.7 (0.9-7.5)		
Herd size	<15 cattle	170(1)	0.6 (0.0-1.8)		
	15-29 cattle	134(1)	0.7 (0.0-2.2)	4.550	0.123
	>29 cattle	80(3)	3.8 (0.0-8.8)		
Livestock composition	Cattle-small rum	267(2)	0.7 (0.0-1.9)	2.012	0.174
	Cattle-small rum-camel	117(3)	2.6 (0.0-6.0)		
Parity (only female)	No parity	86(0)	0		
	Single parity	108(0)	0	6.606	0.045
	Multiple parity	149(5)	3.4 (0.7-6.7)		
Abortion (only female)	Absent	329(1)	0.3 (0.0-0.9)	74.697	0.000
	Present	14(4)	28.6 (7.1-57.1)		
Production system	Pastoral	190(2)	1.1 (0.0-2.6)	0.164	1.000
	Agro-pastoral	194(3)	1.5 (0.0-3.6)		
Total		**384(5)**	**1.3 (0.3-2.6)**		

4.3.2. Results of Questionnaire Survey

Questionnaires were administered to 110 cattle owners to know their knowledge about zoonotic diseases, the habits of animal product consumption and handling, and dead-animal/aborted fetus

disposal practices (Table 4.3). About 74.5% (82/110) of the respondents did not know that livestock owners can get any disease from animals. Moreover, 86.4% (95/110) and 90% (99/110) of the respondents have no information on brucellosis as animal disease and human disease, respectively. In this survey, 96.4% of the respondents drink raw milk and 19.1% consume raw meat. Almost all of the respondents informed abortion materials are handled with bare hands, and they do not safely discard these materials. Abortion materials are usually left for dogs and wild carnivores.

Table 4.3: Response of pastoralists on the awareness and occupational risks of brucellosis in the study area.

Variable	**Response category**	**Frequency**	**%**
Know that pastoralists can get any disease from animals?	Yes	28	25.5
	No	82	74.5
Know brucellosis as animal diseases?	Yes	15	13.6
	No	95	86.4
Know brucellosis as human disease?	Yes	11	10
	No	99	90
Know about transmission of brucellosis from animal to human via raw milk and contact with aborted materials?	Yes	3	2.7
	No	107	97.3
Drink raw milk?	Yes	106	96.4
	No	4	3.6
Consume raw meat?	Yes	21	19.1
	No	89	80.9
Disposal of placental membrane and aborted fetus	Properly dispose	2	1.8
	Left on open field	108	98.2
Use protective glove during handling abortion cases?	Yes	0	0
	No	110	100

4.4. Discussion

For brucellosis, a combination of RBT and c-ELISA or CFT tests is the most widely used serial testing scheme (Asgedom *et al*., 2016). Though false positivity can occur due to cross-reactivity with non-target pathogens, RBT is a highly sensitive test (Díaz *et al*., 2011) and can be used in field conditions. The use of c-ELISA in addition to, or as a replacement for, conventional tests for brucellosis offers many benefits. It is highly specific and usually used as a confirmatory test (Muma *et al*., 2007). Competitive ELISA differentiates between antibodies due to infection and vaccination, it is rapid (faster than CFT), it can be automated, and it can be applied on samples that have deteriorated (Perrett *et al*., 2010). On the other hand, CFT is known for its prozone effect (Corbel, 2006) and it is associated with negative results in early stage of infection due to low IgG titers (Khan *et al*., 2017; Legesse *et al*., 2023). Therefore, the use of a combination of RBT, CFT and c-ELISA tests in this study could maximize the accuracy of the findings.

In the present study, the overall animal level sero-prevalence (1.3%) of bovine brucellosis was low but the 6.3% herd level sero-prevalence was moderately high. This pattern of low individual but high herd sero-prevalence is consistent with what has been described for endemic areas (Ducrotoy *et al*., 2017; Ntivuguruzwa *et al*., 2020). Other studies in Ethiopia also documented similar observations (Abera *et al*., 2019; Bulcha *et al*., 2020; Robi and Gelalcha, 2020; Robi *et al*., 2023; Demissie *et al*., 2024). The current 1.3% animal level prevalence is consistent with previous reports in different parts of Ethiopia. Gumi *et al*. (2013), Geresu *et al*. (2016), Terefe *et al*. (2017), Teka *et al*. (2019), Bulcha *et al*., (2020) and Sima *et al*. (2021), reported 1.4% in south eastern Ethiopia (using RBT and ELISA), 1.4 in Bushoftu and Asella (using card test, RBT, i-ELISA and CFT), 1.3% in eastern Ethiopia (using RBT and CFT), 1.04% in south west Shewa (using RBT and i-ELISA), 1.04% in and around Adama town (using RBT and CFT), and 1.82% in western Ethiopia (using i-ELISA), respectively, which agree with the current finding. The relatively low prevalence found in the current study area might be attributable to the extensive grazing conditions which could reduce contact between animals and contamination of pastures.

In contrast, there are reports of a higher sero-prevalence of bovine brucellosis, 2.2% in dairy and breeding ranches (Asmare *et al*., 2013b), 2.0% in eastern Shewa (Alemu *et al*., 2014), 4.3% in Adami Tulu (Tibesso *et al*., 2014), 2.6% in Arsi zone (Tsegaye *et al*., 2016), 9.87% in Asella organized dairy farm (Eticha *et al*., 2018), 2.7% in Hawassa town (Abera *et al*., 2019), 14.6% in Jikow districts (Negash *et al*., 2020), 4.3% in Jimma zone (Robi and Gelalcha, 2020), 7.14% in southwest Ethiopia (Robi *et al*., 2023) and 2.8% in south Omo (Demissie *et al*., 2024). The current finding was also lower than the 2.6% pooled sero-prevalence estimate reported by a comprehensive review of 20 years published data in Ethiopia (Sibhat *et al*., 2022).

Similarly, higher sero-prevalence rates were reported from Uganda (Makita *et al*., 2011), Nigeria (Mai *et al*., 2012), Sudan (Senein and Abdelgadir, 2012), Ghana (Folitse *et al*., 2014), Angola (Mufinda *et al*., 2015), South Sudan (Madut *et al*., 2018a) and Rwanda (Ntivuguruzwa *et al*., 2020). On the other hand, lower sero-prevalence rates such as 0.05% in Arsi Zone (Degefa *et al*., 2011), 0.2% in Debrebirhan and Ambo towns (Bashitu *et al*., 2015), 0.06% in Addis Ababa (Edao *et al*., 2018) and 0.73% in westren Ethiopia (Garoma, 2022) were reported. A decline in cattle brucellosis prevalence in the absence of control programs might be driven by spontaneous herd immunity and accidental culling.

The present 6.3% herd level sero-prevalence is consistent with the finding of Terefe *et al*. (2017) (6.8%). In other previous studies, herd level sero-prevalences as high as 45.9% (Asgedom *et al*., 2016) and as low as 0.8% (Edao *et al*., 2018) were recorded in Ethiopia. As compared to the 34% (Alehegn *et al*., 2016), 45.9% (Asgedom *et al*., 2016), 25.8% (Abera *et al*., 2019), 9.5% (Bulcha *et al*., 2020), 12.23% (Robi *et al*., 2023) and 11.3% (Demissie *et al*., 2024) reported in Ethiopia, and the 36% (Omer *et al*., 2000), 77.5% (Mai *et al*. (2012) and 40.1% (Mufinda *et al*. (2015) recorded elsewhere in Africa, the 6.3% herd level sero-prevalence observed in the present study could be regarded as low. However, it can be seen as evidence to the potential of the disease to flare up when conditions promoting transmission occur (Ducrotoy *et al*., 2017).

The differences in prevalence among studies may be explained by differences in geographic locations, diagnostic tests and production systems (Sibhat *et al*., 2022). In the absence of any control measures and awareness, the low herd level sero-prevalence reported in this study

demands further study on isolation and characterization of *Brucella* species circulating in the study area.

In the present study, all sero-positive animals were female. This result was in agreement with that of Berhe *et al*. (2007) who recorded no male sero-reactor. Similarly, Asgedom *et al*. (2016) reported that almost all (94.7%) of the sero-positive animals were female. Moreover, many previous studies in Ethiopia (Kebede *et al*., 2008; Moti *et al.*, 2012; Tibesso *et al*., 2014; Elemo and Geresu, 2018; Eticha *et al*., 2018; Sima *et al*., 2021) and elsewhere (Junaidu *et al*., 2011) documented higher sero-prevalence among female than male animals. The lower prevalence (0%) of male reactors in the present report could be as a result of smaller number of male animals tested as compared to females. It was also reported that the serological response of male animal to *Brucella* infection is limited due to low level of erythritol (Kebede *et al*., 2008). Female animals are more susceptible to *Brucella* organism due to the presence of erythritol in female reproductive tract which stimulates the growth of the organism (Godfroid *et al*., 2010).

This study revealed that animals over six years old had high sero-prevalence compared to their younger counterparts (3.7% vs. 0.5% and 0%). Though age was non-significantly associated with sero-positivity for *Brucella* infection in the current study, similar findings in Ethiopia (Tolosa *et al*., 2010; Megersa *et al*., 2011a; Asgedom *et al*., 2016; Robi and Gelalcha, 2020; Robi *et al*., 2023) and elsewhere (Kushwaha *et al*., 2016; Ali *et al*., 2017) identified age as a major risk factor for *Brucella* infection in cattle.

Higher prevalence of brucellosis in older cattle may be attributed to higher chance of acquiring the infection due to frequent exposure over time to the pathogen. In addition, age-related increases in the concentration of sex hormones and erythritol make older animals more susceptible to brucellosis. Moreover, younger animals tend to be more resistant to infection and recover from it more frequently (Radostits *et al*., 2007).

Larger herd sizes showed higher infection rates (3.8%) than medium (0.7%) and small (0.6%) herd sizes with statistically insignificant association. In previous studies, herd size was also reported as an important factor for brucellosis transmission (Ibrahim *et al*., 2010; Megersa *et al*.,

2011b; Teklue *et al*., 2013; Lindahl *et al*., 2014; Terefe *et al*., 2017; Abera *et al*., 2019; Kenea and Megersa, 2021; Robi *et al*., 2023). This is mainly due to higher density of animals in large herds that facilitate close contact and environmental contaminations leading to higher chances of transmission, especially, during cases of abortion and parturition (Constable *et al*., 2017). Moreover, large herd size enhances the exposure to *Brucella* infection through increased contact at common feeding and watering points. The likelihood of exposure and maintenance of *Brucella* after an abortion increases with larger herd sizes due to increased contact in shared feeding and watering areas, facilitating the spread of *Brucella* organisms (Terefe *et al*., 2017; Robi and Gelalcha, 2020).

Sero-positivity to brucellosis is higher (2.6%) in cattle from households with a mix of small ruminants and camel compared to those kept with small ruminants only (0.7%) but with statistically insignificant association. Though the herd composition and cattle sero-positivity showed no significant association, there is increased chance of *Brucella* transmission between cattle and camel when they are herded together. In support of this, Demissie *et al*. (2025a) reported a sero-prevalence of 9.6% brucellosis in apparently health camels in the current study area. Moreover, Legesse *et al*. (2024) detected a prevalence of 5% *Brucella abortus* from apparently healthy camels originating from the current study area and other places and slaughtered at Akaki slaughter house.

Parity was significantly associated with *Brucella* sero-positivity. A sero-prevalence of 3.4% was found among cattle with multiple parities whereas, no *Brucella* sero-positivity was observed in cattle with single parity and null parity. Hence, parity can be considered as one of the potential risk factors in the study area as there is frequent exposure to fetal materials and vaginal discharge from infected cows there by increasing the likelihood of *Brucella* infections. In agreement with this, different authors (Elemo and Geresu, 2018; Ndazigaruye *et al*., 2018; Abera *et al*., 2019; Sima *et al*., 2021) from Ethiopia and elsewhere reported parity as one of the potential risk factor for bovine brucellosis.

In this study, *Brucella* sero-prevalence was significantly associated with the occurrence of abortion. This finding is in line with those of Ibrahim *et al*. (2010), Haileselassie *et al*. (2011),

Tesfaye *et al*. (2011), Lindahl *et al*. (2014) and Kenea and Megersa (2021) who reported significant association between abortion and sero-positivity to brucellosis. This could be explained probably by the fact that abortion is the typical outcome of brucellosis infections (Constable *et al*., 2017; Ntirandekura *et al*., 2020).

The present study revealed comparable sero-prevalences of bovine brucellosis in agro-pastoral (1.5%) and pastoral (1.1%) systems with statistically non-significant difference. Previous studies by Dinka and Chala (2009) and Adane *et al*. (2012) noted higher prevalence of cattle brucellosis in pastoral than agro-pastoral production systems; however they lacked statistical significance. The current somewhat higher prevalence in the agro-pastoral than purely pastoral might be associated with the commingling and aggregation of cattle herds in the agro-pastoral areas as they search for feed from agricultural residues. This was also evident during data collection. Agro-pastoral systems often involve cattle grazing on agricultural residues, leading to greater aggregation of herds and potentially increased transmission of the disease.

Regarding results of the questionnaire survey, most of the respondents were not aware of zoonotic diseases transmitted from animals to humans. More than 86% of the respondents had no information on brucellosis as animal disease and human disease. Almost all of the respondents had the habit of drinking raw milk, handling abortion cases with bare hands and dumping aborted materials on open field. Similarly, a study conducted in Ethiopia indicated about 80% of farmers did not know the disease and most of them were handling placental membranes and aborted fetuses with bare hands (Bulcha and Etefa, 2023). With all these practices and lack of awareness, it is obvious that pastoralists are putting their health at risk (Radostits *et al*., 2007) and they do not take precautions to prevent spread of the disease. A study from Tanzania indicated that family members in sero-positive livestock household were 3.3 times more likely to be sero-positive than those with sero-negative (Shirima *et al*., 2010).

4.5. Conclusion and Recommendations

In conclusion, this study revealed low individual level and moderately high herd level sero-prevalence of bovine brucellosis in the study area. In view of the infectious and contagious

nature of brucellosis, moderately high herd level prevalence, lack of awareness, consumption of raw milk and risky handling of abortion cases by pastoralists can result in further spread of the disease in the herd and to pastoral community. Hence, there is a need to investigate the status of brucellosis in humans and wildlife in the study area. Moreover, isolation and identification of the circulating *Brucella* biotype in the study area is crucial.

5. CHAPTER FIVE: HUMAN BRUCELLOSIS AND ASSOCIATED RISK FACTORS IN THE KEREYU PASTORAL AREA OF OROMIA REGION, ETHIOPIA

Abstract

This study was conducted to estimate health centers-based sero-prevalence and identify potential risk factors of human brucellosis in Kereyu pastoral areas of Oromia Region, Ethiopia. A total of 210 serum samples from human out patients visiting Alge and Ilala health centers were collected. Data on hypothesized risk factors were also collected using a semi-structured questionnaire. The preliminary screening of the sera for *Brucella* antibodies was done using Rose Bengal plate test (RBT) and positive sera were further subjected to complement fixation test (CFT) and competitive enzyme-linked immunosorbent assay (c-ELISA). The overall sero-prevalence of human brucellosis was 2.4% (95% CI: 1.0-4.8) by c-ELISA. All the sero-positive samples were participants in the age group 20–35 years and whose income was related to livestock production. Among the risk factors considered, sero-prevalence was higher in individuals with some level of education than illiterate ones. More than 70.0% of participants had no knowledge on brucellosis and its means of transmission. In conclusion, the 2.4% sero-prevalence of human brucellosis among patients visiting the two health centers and the high sero-positivity found in participants despite having knowledge of brucellosis highlight the need for improving community awareness and strengthening public health education on safe animal handling and safe animal product consumption. Healthcare providers must include brucellosis in the differential diagnosis of acute febrile illnesses to ensure timely treatment.

Keywords: c-ELISA; CFT; Fentale; human brucellosis; RBT.

5.1. Introduction

Brucellosis is an important human disease in many parts of the world. It is common in Europe (Qureshi *et al*., 2023; ECDC, 2024), north and east Africa (Ahmed *et al*., 2015; Djangwani *et al*., 2021; Munyua *et al*., 2021; Faddane *et al*., 2022; Dagnaw *et al*., 2024; Asrie *et al*., 2025), the Middle East (Musallam, *et al*., 2016; Al-Amr *et al*., 2022; Dadar *et al*., 2024), Asia (Ali *et al*., 2018; Holt *et al*., 2021; Nawaz *et al*., 2021; Tao *et al*., 2021), North America (Fritz *et al*., 2021) and central and South America (Yasmin and Lone, 2015; Lemos *et al*., 2018) and often unrecognized and frequently goes unreported (Kalule *et al*., 2020).

The disease has major economic impact related to debilitating effects in humans and reduced production in animals (Franc *et al*., 2018; Kiiza *et al*., 2023). In human, the disease is characterized by nonspecific symptoms such as fever, sweating, arthralgia, headache, vomiting, diarrhea, fatigue, anorexia, myalgia, weight loss and miscarriage (Głowacka *et al*., 2018; Adetunji *et al*., 2019; Govindasamy, 2020; Zhang *et al*., 2022). Chronicity and recurring febrile conditions, as well as devastating complications in pregnant women are common consequence of brucellosis (Franc *et al*., 2018).

There are 13 named *Brucella* species (*B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis*, *B. neotomae*, *B. ceti*, *B. pinnipedialis*, *B. microti*, *B. inopinata*, *B. papionis* and *B. vulpis B. pseudogrignonensis*) (Li *et al*., 2021; Rebollada-Merino *et al*., 2026) and four unnamed isolates (Hull and Schumaker, 2018). *Brucella abortus*, *B. melitensis*, *B. suis* and *B. canis* which preferentially infect cattle, small ruminants, swine and dogs, respectively, are of great zoonotic and economic importance (Mathew *et al*., 2015; Tadesse, 2016; Saddique *et al*., 2019). *Brucella melitensis* has the greatest risk for human infection followed by *B. suis* and *B. abortus* (Laine *et al*., 2023). *Brucella canis* has the lowest zoonotic potential among the classic *Brucella* species (Santos *et al*., 2021). Other *Brucella* species of zoonotic importance include, *B.ceti* (Attia *et al*., 2024), *B. pinnipedialis* (Deepak *et al*., 2019), *B. inopinata* (Cloeckaert *et al*., 2024) and *B. neotomae* (Vergnaud *et al*., 2024).

Infection in humans is almost exclusively due to zoonosis, either through direct contact with infected animals or ingestion of contaminated dairy products (Kose *et al*., 2014; Dadar *et al*., 2019, Qureshi *et al*., 2023) and inhalation (Ferrero *et al*., 2020). Human-to-human transmission can occur through placenta, breastfeeding, and rarely sexual intercourse, organ transplantation and blood transfusions (Tuon *et al*., 2017; Laine *et al*., 2023).

In areas where the disease is endemic, consuming raw milk and dairy products made of unpasteurized milk poses the greatest risk to the general public. Individuals who handle infected animals/carcasses, and aborted fetuses or placentas have an occupational risk (Kutlu *et al*., 2014; Igawe *et al*., 2020; Holt *et al*., 2021). Brucellosis is also one of the most easily acquired laboratory infections (Traxler *et al*., 2013).

Diagnostic tools for brucellosis include serological assays, culture and molecular methods (Yagupsky *et al*., 2019; Jiang *et al.,* 2020). Serological tests are cost-effective and their lower risks of biological hazards make them preferable in *Brucella* endemic countries, particularly in resource-limited settings (Elrashedy *et al*., 2022; Qureshi *et al*., 2023). Moreover, the widespread availability and feasibility of serological tests encourage screening and surveillance efforts to ensure early detection and appropriate intervention (Yagupsky *et al*., 2019). The most commonly used serological tests include slide agglutination test, buffered acidified plate antigen test (BAPA), Rose Bengal plate test (RBT) and the enzyme-linked immunosorbent assay (ELISA) (Xu *et al*., 2020).

In Ethiopia, epidemiological data on human brucellosis from individuals visiting health centers remain scarce. It is usually left undetected and untreated. Studies conducted in human risk groups in different parts of Ethiopia revealed sero-prevalence ranging from 0.4% to 48.3% (Zeweld and Haileselassie, 2012; Zerfu *et al*., 2018; Lakew *et al*., 2019; Edao *et al*., 2020; Ibrahim *et al*., 2021; Mehari *et al*., 2021; Tschopp *et al*., 2021; Wegi *et al*., 2021; Getahun *et al*., 2022; Ahad *et al*., 2024; Sileshi *et al*., 2024). There are also reports of human brucellosis from febrile individuals visiting health centers (Tibesso *et al*., 2014; Feleke *et al*., 2015; Tadesse, 2016; Zerfu *et al*., 2018; Mehari *et al*., 2021).

Brucellosis poses more risk to farmers, animal handlers, abattoir workers and veterinarians (Godfroid *et al*., 2013; Laine *et al*., 2023) especially in areas with limited access to healthcare facilities (Wegi *et al*., 2021). The public health implication of brucellosis in the Kereyu pastoral area of Oromia Region, Ethiopia has not been studied and evaluated by diagnostic techniques. Therefore, this study was conducted to estimate the sero-prevalence of brucellosis in humans visiting health centers and to investigate potential risk factors associated with human brucellosis.

5.2. Material and Methods

5.2.1. Study Design and Method

A cross-sectional study was conducted in Fentalle district to determine *Brucella* sero-prevlence in humans. The sample size for human brucellosis was calculated based on the previously reported 4.8% sero-prevalence rate in Addis Ababa by Kassahun *et al*. (2006) using the formula: $N = (1.96)^2 \times P \times Q/D^2$, where N = sample size, P = expected prevalence, Q = 1-P and D = level of precision (5%) (Thrusfield, 2005). Accordingly, the calculated sample size was, $N = (1.96)^2 \times 0.048 \times (1-0.048)/(0.05)^2 = 70$. This sample size was inflated three times to have more precise estimates. Hence, a total number of 210 humans were sampled. Age of the patients was classified into <20 years, 20-35 years and >35 years.

5.2.2. Sample Collection

All patients visiting Ilala and Alge health centers with febrile diseases were considered. Patients who refuse to provide consent at the time of data collection were excluded from the study. Individuals from various socio-demographic backgrounds with extended sample collection period were included to ensure the inclusion of participants who might otherwise have been missed. Blood samples were collected from febrile volunteers in collaboration with health professionals working in the respective health centers.

About 5 ml of whole blood samples were collected from each human patient using plain tubes. The tubes were then tilted on a table overnight at room temperature to allow clotting of the

blood. Serum was collected passively by decanting and stored at -20°C until tested serologically by RBT, CFT and c-ELISA.

5.2.3. Serological Examination

The Rose Bengal plate test (RBT) and complement fixation test (CFT) were conducted at the National Veterinary Institute, Bishoftu, Ethiopia for the detection of brucellosis antibodies. All RBT-positive sera samples were also shipped to University of Nairobi, Nairobi, Kenya and further tested using competitive enzyme-linked immunosorbent assay (c-ELISA).

The Rose Bengal plate test (RBT) was conducted following the procedure described by Staak *et al*. (2001). All RBT-positive samples were confirmed with a complement fixation test CFT) using the OIE protocol (OIE, 2016). Again, all the RBT-positive sera samples were further tested using a commercial COMPELISA, a competitive ELISA kit for the detection of antibodies against *Brucella* in serum samples (APHA Scientific, Weybridge, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom) at Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Nairobi, Kenya according to the manufacturer's protocol using 96-well polystyrene plates pre-coated with *Brucella* lipopolysaccharide antigen.

5.2.4. Questionnaire Survey

For the determination of risk factors associated with brucellosis transmission, a structured and pre-tested questionnaire was used to collect information on: demographic characteristics, awareness on zoonosis, consumption of milk, source of income, etc (Annex 2).

5.2.5. Data Management and Analyses

Data obtained from serological tests and questionnaire surveys were stored in Microsoft Excel spreadsheet (Microsoft Corp.). Sero-prevalence was calculated as percentage by dividing the

number of positive samples for *Brucella* to the total number of samples. Human sera tested positive to c-ELISA were defined as sero-positive.

The data were analyzed by descriptive statistics, univariate and multivariate regression using the SPSS 20 statistical package. The presence of association between explanatory variables (risk factors) such as sex, age, marital status, level of education, knowledge of zoonosis, etc and positive sero-prevalence was also analyzed.

5.2.6. Ethical Consideration

The procedures for the human study were approved by Institutional Ethical Review Board of Aklilu Lemma Institute of Pathobiology, Addis Ababa University. A support letter was also obtained from Oromia Health Bureau.

Written consent form was prepared to make all participants fully informed regarding the study and what was being asked of them, including the potential benefits, to help them make a fully informed decision on whether or not to participate in the study. The written consent form and questionnaires were also translated to a local language, Afaan Oromo, in order to make participants more informed.

5.3. Results

5.3.1. Demographic Characteristics and Sero-prevalence

Among 210 study participants, 186 (88.6%) were rural residents, 159 (75.7%) were male and 118 (56.2%) married. The age range of the participants was from 5 to 60 years with a mean age of 27.5 (SD = ± 10.4) years. About four-fifth of the participants had no formal education (81.0%) and their dominant livelihood was related to livestock rearing (78.1%). Majority of participants (80.5%) consumed raw and/or boiled milk (Table 5.1).

Out of 210 human sera tested 5.2% (95% CI: 2.4-9.0) and 4.3% (95% CI: 1.4-8.1) were found positive by RBT and CFT, respectively. The overall sero-prevalence of human brucellosis was 2.4% (95% CI: 0.5-4.8) by c-ELISA (Table 5.1).

Table 5.1: Demographic characteristics and sero-prevalence of human brucellosis among participants.

Factors		**No. of samples (%)**	**Sero-prevalence**		
			RBT(%)	**CFT(%)**	**c-ELISA(%)**
Residence	Urban	24(11.4)	1(4.2)	1(4.2)	1(4.2)
	Rural	186(88.6)	10(5.4)	8(4.3)	4(2.2)
Sex	Male	159(75.7)	6(3.8)	4(2.5)	2(1.3)
	Female	51(24.3)	5(9.8)	5(9.8)	3(5.9)
Age	<20	41(19.5)	2(4.9)	1(2.4)	0(0.0)
	20–35	133(63.3)	8(6.0)	7(5.3)	5(3.8)
	>35	36(17.1)	1(2.8)	1(2.8)	0(0.0)
Marital status	Single	83(39.5)	3(3.6)	2(2.4)	1(1.2)
	Married	118(56.2)	7(5.9)	6(5.1)	4(3.4)
	Divorced	4(1.9)	0(0.0)	0(0.0)	0(0.0)
	Widow	5(2.4)	1(20.0)	1(20.0)	0(0.0)
Educational status	Illiterate	157(74.8)	8(5.1)	6(3.8)	2(1.3)
	Read and write	13(6.2)	1(7.7)	1(7.7)	1(7.7)
	Primary school	21(10.0)	2(9.5)	2(9.5)	2(9.5)
	Elementary school	13(6.2)	0(0.0)	0(0.0)	0(0.0)
	Secondary school	6(2.9)	0(0.0)	0(0.0)	0(0.0)
Income	Livestock rearing	117(61.3)	6(5.1)	6(5.1)	4(3.4)
	Crop farming	24(12.6)	2(8.3)	2(8.3)	0(0.0)
	Livestock and	47(24.6)	1(2.1)	1(2.1)	1(2.1)

	crop farming				
	Trade	3(1.6)	0(0.0)	0(0.0)	0(0.0)
Knowledge on zoonosis	No	149(71.0)	7(4.7)	5(3.4)	3(2.0)
	Yes	61(29.0)	4(6.6)	4(6.6)	2(3.3)
Knowledge on causes of zoonosis/retained placenta	No	187(89.0)	9(4.8)	7(3.7)	3(1.6)
	Yes	23(11.0)	2(8.7)	2(8.7)	2(8.7)
Knowledge on milk borne diseases	No	73(70.2)	4(5.5)	3(4.1)	2(2.7)
	Yes	31(29.8)	2(6.5)	2(6.5)	1(3.2)
Knowledge on meat borne diseases	No	154(73.3)	9(5.8)	7(4.5)	3(1.9)
	Yes	56(26.7)	2(3.6)	2(3.6)	2(3.6)
Milk consumption	Raw	93(44.3)	10(10.8)	8(8.6)	4(4.3)
	Raw/boiled	76(36.2)	0(0.0)	0(0.0)	0(0.0)
	Boiled	41(19.5)	1(2.4)	1(2.4)	1(2.4)
Health center	Alge	149(71.0)	4(2.7)	2(1.3)	1(0.7)
	Ilala	61(29.0)	7(11.5)	7(11.5)	4(6.6)
Total		**210**	**11(5.2; 2.5-8.1)**	**9(4.3; 1.9-7.1)**	**5(2.4; 1.0-4.8)**

5.3.2. Risk Factors Associated with Sero-positivity

Brucella sero-prevalence among the study participants was 2.4% (95% CI: 1.0-4.8). The sero-prevalence of human brucellosis was relatively high among female participants (3/51, 5.9%) and married ones (4/118, 3.4%) with statistically non significant differences. All the sero-positive samples were participants in the age group 20–35 years and whose income was related to livestock production. The sero-prevalence was also high in participants who usually drink raw milk (4/93, 4.3%). For variables with *p*-values less than 0.25 multivariable logistic regression model was fitted to find independent predictors of human brucellosis. Accordingly, educational status was found significantly associated with human brucellosis (Table 5.2).

Table 5.2: Logistic regression analysis for *Brucella* sero-positivity using c-ELISA test results.

Factors		No. of samples	Sero-prevalence (%)	Univariable analysis		Multivariable analysis	
				OR (95% CI)	p-value	OR (95% CI)	p-value
Residence	Urban	24	1 (4.2)	1			
	Rural	186	4 (2.2)	0.51(0.06-4.72)	0.549		
Sex	Male	159	2 (1.3)	1		1	
	Female	51	3 (5.9)	4.91(0.80-30.23)	0.086	3.48(0.24-51.45)	0.365
Age	<20	41	0 (0.0)				
	20–35	133	5 (3.8)				
	>35	36	0 (0.0)				
Marital status	Single	83	1 (1.2)	1			
	Married	118	4 (3.4)	2.88(0.32-26.22)	0.349		
	Divorced	4	0 (0.0)				
	Widow	5	0 (0.0)				
Educational level	Illiterate	157	2 (1.3)	1		1	
	Read and write	13	1 (7.7)	6.46(0.55-76.45)	0.139	5.05(1.39-1833.89)	0.032
	Primary school	21	2 (9.5)	8.16(1.09-61.32)	0.041	19.09(1.14-318.58)	0.040
	Elementary school	13	0 (0.0)				
	Secondary school	6	0 (0.0)				
Income	Livestock rearing	117	4 (3.4)	1			
	Livestock and crop farming	47	1 (2.1)	1.63(0.18-14.97)	0.667		
	Crop farming	24	0 (0.0)				

	Trade	3	0 (0.0)				
Knowledge on zoonosis	No	149	3 (2.0)	1			
	Yes	61	2 (3.3)	0.61(0.10-3.70)	0.589		
Knowledge on causes of zoonosis/retained placenta	No	187	3 (1.6)	1		1	
	Yes	23	2 (8.7)	0.17(0.03-1.09)	0.061	6.75(0.58-79.20)	0.128
Knowledge on milk borne diseases	No	73	2 (2.7)	1			
	Yes	31	1 (3.2)	0.85(0.07-9.68)	0.892		
Knowledge on meat borne diseases	No	154	3 (1.9)	1			
	Yes	56	2 (3.6)	0.54(0.09-3.30)	0.501		
Milk consumption	Boiled	41	1 (2.4)	1			
	Raw	93	4 (4.3)	0.56(0.06-5.12)	0.605		
	Raw/boiled	76	0 (0.0)				
Health center	Alge	149	1 (0.7)	1		1	
	Ilala	61	4 (6.6)	0.10(0.11-0.88)	0.038	13.99(0.77-253.22)	0.074

5.4. Discussion

Diagnosis of brucellosis based on culture presents significant biohazard risks to laboratory personnel as the bacteria can be easily transmitted through aerosols (Etemadi *et al*., 2019). Consequently, serological tests serve as a reliable and safer alternative for routine diagnosis. The Rose Bengal plate agglutination test (RBT) is a rapid screening test which is shown to be effective in the diagnosis of human brucellosis (Waringa *et al*., 2023). The complement fixation test (CFT) and competitive enzyme-linked immunosorbent assay (c-ELISA) are widely used confirmatory tests for brucellosis (WOAH, 2022). The c-ELISA is more sensitive and a rapid method for diagnosis of brucellosis with some advantages: it is capable of identifying all antibody isotopes (Nielsen *et al*., 2006), there is less sample purification needed and it has low variability (Kohl and Ascoli, 2017).

This health facility-based cross-sectional study identified an overall sero-prevalence of 2.4% by c-ELISA, indicating the public health importance of brucellosis in the study area. This is in agreement with the findings of Tibesso *et al*. (2014), Feleke *et al*. (2015) and Tadesse (2016). However, the current finding is lower than health facility-based sero-prevalences reported from febrile individuals in Ethiopia (Zerfu *et al*., 2018; Mehari *et al*., 2021) and other sub-Saharan African countries like Tanzania (Chipwaza *et al*., 2015), Uganda (Muloki *et al*., 2018), and Kenya (Kahariri *et al*., 2021). The possible explanation for the difference in the sero-prevalences could be due to the difference in the sampling design, the number of samples, exposure to *Brucella* species, and the type of diagnostic tests used.

Almost all socio-demographic risk factors were insignificantly associated with *Brucella* sero-positivity in humans. Studies in Ethiopia (Sileshi *et al*., 2024) and elsewhere (Mangtani *et al*., 2020; Almashhadany *et al*., 2022) have shown residence as a significant factor in human brucellosis, with rural populations facing a much higher risk than urban dwellers. The present study, however, indicated higher sero-positivity in urban (4.2%) than rural (2.2%) residents with statistically non-significant difference. This could be explained by the small number of urban participants included in the study (24 vs. 186). It might also show the actual burden of the disease in the urban area.

Though statistically not significant, the sero-prevalence of human brucellosis was higher in female (5.9%) than male (1.3%) participants. This was consistent with the finding of Tibesso *et al*. (2014) who reported higher sero-prevalence in females than males with statistically insignificant difference. Zerfu *et al*. (2018) also reported a statistically non-significant difference in the prevalence of human brucellosis among sexes. Women in pastoral and rural community face higher risk due to household roles like processing unpasteurized dairy products, handling raw meat, and milking animals (Muturi *et al*., 2018).

All the sero-positive individuals in the present study were participants in the age group 20-35 years. Age is a significant factor in human brucellosis, with the highest rates typically seen in middle-aged adults due to occupational exposure (Yuan *et al*., 2020). In the present study, married participants had higher prevalence (3.4%) than participants who are single (1.2%) with

non-significant difference. The relationship between marital status and human brucellosis was also reported as statistically insignificant across various studies (Tsegay *et al*., 2017; Majalija *et al*., 2018; Wegi *et al*., 2021; Shayan *et al*., 2025). However, we could not find literature or scientific fact that associates marital status and human brucellosis.

This study revealed that more than 70.0% of participants had no knowledge on brucellosis and its means of transmission. Therefore, there is a need for awareness creation on transmission, prevention and risk factors of brucellosis to occupational risk groups. This is very important to reduce the risk of acquiring the disease. Higher sero-prevalence of human brucellosis was also recorded in participants whose dominant livelihood was related to livestock rearing (p >0.05). Studies in Zambia (Mubanga *et al*., 2021), Tanzania (John *et al*., 2010) and elsewhere (Pereira *et al*., 2020) have also showed occupation as a risk factor for acquiring brucellosis.

Consumption of unpasteurized milk and dairy products were reported to be risk factors for acquiring brucellosis in human (Ali *et al*., 2013; Islam *et al*., 2023). The present study indicated that 44.3% and 36.2% of participants had consumed raw and raw/boiled milk, respectively, although none of these practices were significantly associated with sero-positivity. Nonetheless, this habit of consuming raw milk suggests a higher risk of brucellosis transmission if the disease is present in the herd.

Regarding health centers, a higher sero-prevalence was recorded in Ilala than Alge with statistically significant difference (p <0.05). This observation might reflect a higher disease burden within the urban setting, as the convenient location of Ilala health center near Metahara town attracts a large number of urban residents.

The prevalence of brucellosis in the current study was found higher in participants with some level of education than illiterate ones (p <0.05). While educated people know the risks, occupational and economic demands often prevent them from implementing proper protective measures. In support of this, a study in Kenya indicated that nomadic pastoralists are less likely to comply with practices that would minimize *Brucella* infection despite having significant knowledge on risk and transmission of the disease, occupational hazards and cultural practices of

consumption of animal products (Njenga *et al*., 2020). Nevertheless, a lower level of education is generally associated with increasing risk of contracting brucellosis, as it often correlates with lack of awareness about disease transmission and prevention leading to a higher likelihood of engaging in risky practices (Mburu *et al*., 2021).

5.5. Conclusion and Recommendations

In conclusion, the 2.4% sero-prevalence of human brucellosis among patients visiting the two health centers cannot be ignored and brucellosis should be considered in the differential diagnosis of febrile patients. Moreover, the high sero-positivity found in participants despite having some knowledge of brucellosis indicates that even educated ones may still lack detailed knowledge of specific transmission modes and prevention methods. These findings highlight the need for improving community awareness and enhancing healthcare support for patients with brucellosis.

6. CHAPTER SIX

6.1. General Discussion, Conclusion and Recommedndations

The current sero-prevalence data, which is 9.6% in camels, 2.6% in small ruminants, 1.3% in cattle, and 2.4% in humans, highlights a high-risk zoonotic environment. In pastoral regions, this dynamic demonstrates how easily the bacteria jump across species, directly threatening both livestock economies and human health without a coordinated management strategy.

Ethiopia ranks first in Africa for livestock biomass and second for human population size. While pastoral communities in the arid and semi-arid drylands are critical drivers of the export economy (Duale, 2024), they endure significant socioeconomic hurdles. Chief among these is the high endemicity of zoonotic diseases, particularly brucellosis.

In Ethiopian epidemiological studies, most brucellosis prevalence is typically estimated using the Rose Bengal plate test (RBT) as an initial screen, followed by the Complement Fixation Test (CFT) for confirmation. Some studies have utilized individual assays (RBT or indirect ELISA), but using a single test in isolation often compromises specificity and generates false positives. This inaccuracy stems from cross-reactivity with non-target organisms like *Yersinia enterocolitica* O:9 and *Escherichia coli* O157:H7 (Bonfini *et al*., 2018).

In the present studies, combinations of RBT, CFT and c-ELISA were used. The use of c-ELISA in addition to conventional tests for brucellosis offers many benefits. Competitive ELISA is highly specific and usually used as a confirmatory test (Muma *et al*., 2007). It differentiates between antibodies due to infection and vaccination, it is rapid (faster than CFT), and it can be applied on samples that have deteriorated (Perrett *et al*., 2010). On the other hand, CFT is known for its prozone effect (Corbel, 2006) and it is associated with negative results in early stage of infection due to low IgG titers (Khan *et al*., 2017; Legesse *et al*., 2023). Therefore, the use of a combination of RBT, CFT and c-ELISA tests in these studies could maximize the accuracy of the findings.

This thesis examines the prevalence and risk factors of brucellosis in livestock and humans. The studies consist of the investigation of brucellosis sero-prevalence and associated risk factors in livestock (camels, small ruminants, cattle), followed by investigation of human brucellosis and associated risk factors in the Kereyu pastoral area of Oromia Region, Ethiopia.

Most brucellosis studies in Ethiopia usually targeted cattle, occasionally sheep and goats, and rarely camels and humans. In pastoral settings, pastoralsts are at higher risk of acquiring the infection as they live very close to their livestock. Moreover, brucellosis has not been considered as a differential diagnosis in human patients in areas where other infections are endemic. Sero-prevalence reports of brucellosis in cattle, small ruminants, camels and humans over the past ten years were reviewed (Chapter 1). The sero-prevalences in camels and humans varied from 0.6% (Ibrahim *et al*., 2021) to 9.6% (Demissie *et al*., 2025a) (Chapter 2) and 0.0 (Michael *et al*., 2016) to 48.3% (Tschopp *et al*., 2021), respectively. In cattle and small ruminants, sero-prevalences varied from 0.06% (Edao *et al*., 2018) to 22.5% (Ararsa *et al*., 2021) and 0.24% (Geletu *et al*., 2021) to 22.8% (Tschopp *et al*., 2015), respectively with higher reports from extensive and pastoral production systems.

The 9.6% camel brucellosis sero-prevalence recorded in the present study (Chapter 2) was higher than sero-prevalences so far reported in Ethiopia. Similarily, higher brucellosis sero-prevalences in dromedary camels were reported from neighboring countries, Sudan (Bashir *et al*., 2022) and Kenya (Muturi *et al*., 2021). This could be the result of a combination of endemic, environmental, and management-related factors. In the present study, several camel herders usually keep lactating camels together around nearby towns and cities (e.g. Adama, Modjo) to access milk markets as far as Addis Ababa, thereby facilitating disease transmission. This tradition could be observed by anyone through the number of camels seen along the road.

The 2.6% animal level and 11.7% flock level sero-prevalences recorded in small ruminants (Chapter 3; Table 3.2) and the 1.3% animal level and 6.3% herd level sero-prevalences recorded in cattle (Chapter 4; Table 4.1) are of epidemiological significance. The aggregation of animals around Awash River for watering and in the agro-pastoral areas for feed of agricultural residues could further spread the disease in the flock/herd and to pastoral community.

For efficient prevention and control methods, awareness of local community regarding brucellosis is very crucial (Buttigieg *et al*., 2018; Ntirandekura *et al*., 2018). This study indicated that 98.5% (Chapter 2); 86% (Chapter 4) and 71% (Chapter 5) of participants had no information on brucellosis as animal disease and human disease. Majority of the respondents had the habit of drinking raw milk (>80%) and all were handling abortion cases with bare hands and dumping aborted materials on open field. These findings are consistent with other studies in Ethiopia and elsewhere (Bosilkovski *et al*., 2020; Onyango *et al*., 2021; Hassan *et al*., 2022; Avalos *et al*., 2023; Waji and Neja, 2023; Dagnaw *et al*., 2024). In view of the infectious and contagious nature of brucellosis, lack of awareness, consumption of raw milk and risky handling of abortion cases by pastoralists can result in further spread of the disease.

Pastoralists in the Fentale district generally practice a mixed-species livestock production system, rearing cattle, sheep, goats and camels together as a strategy to maximize the use of available resources and mitigate drought risks. In the present studies, herd/flock size was found to be a significant risk factor for brucellosis in camels and small ruminants (Chapter 2; Table 2.2 and Chapter 3; Table 3.3). Cattle with multiple parity and cattle with history of abortion also showed higher sero-prevalence, with statistically significant association (Chapter 4; Table 4.2). In multiparous animals, the periparturient period constitutes a high-risk window that significantly elevates disease vulnerability and transmission, a risk compounded by inadequate biosecurity. The likelihood of exposure and maintenance of *Brucella* after an abortion increases with larger herd sizes due to increased contact in shared feeding and watering areas, facilitating the spread of the disease (Terefe *et al*., 2017; Robi and Gelalcha, 2020). In some households where multiple species sampled, there were more than one sero-positive animal species (camels and cattle) indicating a high probability of *Brucella* transmission across species.

Brucellosis in humans is almost exclusively due to zoonosis, either through direct contact with infected animals or from contaminated animal products (Kose *et al*., 2014; Qureshi *et al*., 2023). Hence, the disease poses more risk to farmers/pastoralists, animal handlers, abattoir workers and veterinarians (Godfroid *et al*., 2013; Laine *et al*., 2023). The present health facility-based cross-sectional study revealed an overall sero-prevalence of 2.4% human brucellosis (Chapter 5; Table 5.1), signifying its public health importance. There are reports in Ethiopia (Zerfu *et al*., 2018;

Mehari *et al*., 2021) and other sub-Saharan Africa (Chipwaza *et al*., 2015; Muloki *et al*., 2018; Kahariri *et al*., 2021) with even higher sero-prevalences in febrile individuals. Thus, brucellosis is a critical differential diagnosis for fever of unknown origin in humans, especially those in contact with animals or those consuming unprocessed animal products (Pathak *et al*., 2014; Saddique *et al*., 2019; Deniz *et al*., 2024).

Lower level of education in general is significantly associated with an increased risk of contracting brucellosis. This correlation is primarily driven by a lack of awareness regarding disease transmission, which leads to a higher likelihood of engaging in risky practices (Mburu *et al*., 2021). However, the higher brucellosis prevalence observed in individuals with some formal education in the present study (Chapter 5; Table 5.2) should be generalized with caution, given the small sample size. Conversely, a higher prevalence among educated individuals may stem from two key factors. First, they might assume that milk from their own herd is inherently safe, leading them to bypass vital safety precautions like boiling. Second, educated individuals tend to seek medical care more promptly when experiencing fevers, which results in higher recorded diagnosis rates compared to less educated populations, who may have lower trust in or financial access to formal healthcare. In support of this, a study in Kenya indicated that nomadic pastoralists are less likely to comply with practices that would minimize *Brucella* infection despite having significant knowledge on risk and transmission of the disease (Njenga *et al*., 2020).

Although Ethiopia has prioritized brucellosis as among the top-five animal diseases of economic importance, a definitive national control policy and strategy remain absent. Control of diseases in Ethiopia, particularly in pastoral community, relays on reactive (emergency-based measures), rather than proactive (preventative), measures. This is partly due to lack of better understanding of the burden of the disease in livestock and public health sectors. The present study revealed that brucellosis is endemic in livestock and human alongside associated risk factors in Kereyu pastoral area. Livestock herders exhibited a critical knowledge deficit regarding brucellosis and other zoonotic diseases which was compounded by unsafe practices such as raw animal product consumption, unsafe handling and disposal of abortion materials. Hence public awareness and education and the One Health approach that recognize the interconnectedness of human, animal,

and environmental health are essential in the control and prevention of brucellosis in particular and other zoonoses in general.

Based on the major findings of these studies, the following recommendations are forwarded:

- ✓ Combined passive monitoring of clinical signs (abortion) with active serology-based screening is indispensable
- ✓ Controlling animal movement in pastoral and communal farming settings is vital to prevent the spread of brucellosis between herds and across regions
- ✓ Proper handling and prompt disposal of abortion materials is critical for brucellosis control
- ✓ Adoption of One Health approach that foster collaboration between veterinary and public health sectors to manage zoonotic transmission is very essential
- ✓ Integrating mandatory reporting, robust diagnostics, and intersectoral collaboration (public health and veterinary) is paramount for effective brucellosis control
- ✓ Brucellosis screening should be integrated into the standard diagnostic workup for febrile illnesses at healthcare facilities and staff should be trained to detect and address cases
- ✓ Targeted education and training on identifying signs of brucellosis in animals, with strong emphasis on its zoonotic risk to humans, is very important
- ✓ The use of personal protective equipment (gloves, masks) during animal births, abortions and handling placentas is crucial
- ✓ It is vital to consume only pasteurized dairy and thoroughly cooked meat to avoid infection

6.2. Future Research Directions and Interventions

These multi-species findings—with human sero-prevalence at 2.4% and livestock herd sero-prevalence reaching 35.4% in camels, 11.7% in small ruminants, and 6.3% in cattle—indicate that brucellosis is broadly endemic rather than a localized issue. To effectively mitigate this threat, we must isolate circulating *Brucella* strains, pinpoint primary transmission hotspots, and clarify the ecological role of wildlife in disease dynamics using a One Health framework.

6.3. Limitations of the Study

Key limitations of this study include restricted site selection due to security and logistical constraints, and potential bias from convenience sampling based on accessibility. Furthermore, the cross-sectional design may be confounded by seasonal camel migration. Lastly, the failure to isolate and characterize specific *Brucella* strains in both livestock and humans prevents a deeper epidemiological analysis.

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8. APPENDICES

Annex 1. Questionnaires to livestock owners

I. General information

1. Owner's name:_____
2. Address

   Kebele: _____, Village name/House No.:_____, Telephone: _____
3. Sex (male/female): _____
4. Age: _____
5. Educational status of the owner/manager: 5.1. Illiterate 5.2. Read and write

   5.3. Elementary school 5.4. High school complete 5.5. Professional

   If professional: 5.5.1. Related to animal production 5.5.2. Not related to animal production
6. Source of income (in the order of importance) 6.1. Livestock rearing 6.2. Crop farming 6.3. Craft 6.4. Trade 6.5. Other (specify): _____
7. What types and number of livestock do you keep?

   7.1. Cattle _____7.2. Sheep _____ 7.3. Goats _____ 7.4 Camel. _____.

   7.5. Other _____
8. Livestock breeds according to local classification

   8.1. Cattle _____ 8.2. Sheep _____ 8.3. Goats _____
9. Grazing habit of the livestock

   9.1. Grazing all neighbors livestock together as one herd Yes ☐ No ☐

   9.2. Grazing house hold herd separately Yes ☐ No ☐

   9.3. Mixing at watering points Yes ☐ No ☐

   9.4. Herds not mixed at watering/watered at different site Yes ☐ No ☐
10. Source of water for animals

    10.1. Communal watering points (pond) Yes ☐ No ☐

    10.2. Individual sources Yes ☐ No ☐

10.3. Rivers and springs Yes ☐ No ☐

11. Types of livestock management system 11.1 Mixed farming ☐

11.2. Agro pastoral ☐ 11.3. Transhumant ☐

11.4. Pastoral ☐

12. Which kind of animal health services is available in the area?

12.1. How does the service response to your requests (timely, delayed no response)?

II. Clinical signs/diseases

13. What kind of clinical signs do you observe in your herd/flock?

Briefly list and describe them

14. What kind of livestock diseases do you know?

Briefly list and describe them

15. Which disease did you assume or describe symptoms if you do not know the disease?

15.1. At what time of the year was it?

16. Have there been any abortion cases in your herd/flock? Yes ☐ No ☐

16.1. Camels/cattle/small ruminants and number affected?

16.2. Origin of aborted animals (born in the herd/introduced from other areas)

16.3. What do you do with the aborted fetus?

17. What do you do with animals with retained fetal membrane?

18. Do you had/have any livestock in your herd, which suffered for a long time (more than one month) of any disease? Yes ☐ No ☐

18.1. Camels/cattle/small ruminants and number affected?

18.2. Which disease did you assume or describe symptoms?

18.3. What do you do with the milk of such an animal?

18.4. What do you do if such animal dies?

19. Have there been any animal with swollen joints in your herd/flock? Yes ☐ No ☐

19.1. Camels/cattle/small ruminants and number affected?

19.2. Origin of animals with swollen joints (born in the herd/introduced from other areas)

19.3. What do you do with the swollen joints?

III. Zoonotic aspect

20. Do you know any of diseases, which could be transmitted to human beings?

    Yes ☐ No ☐

    20.1. If yes, how is it transmitted and do you know how to avoid transmission?

21. Do you know in your family or in your herd/flock any case of disease, which could be originated in animals of your herd/flock? Yes ☐ No ☐

22. How do you consume the milk produced at your farm?

    23.1. How do you sell the milk?

    23.2. Do you know that some infections are transmitted by milk?

    Yes ☐ No ☐

23. Do you know that some animal diseases can affect the quality of meat?

    Yes ☐ No ☐

    24.1. If yes which and what did you observe?

24. What do you think is it possible, that animal diseases can be transmitted via the meat through man?

    25.1. If yes, do you know any disease or symptom of such disease?

25. Do you visit a doctor when you feel sick or do you use your own treatment?

Annex 2. Questionnaires to human patients sampled at the health centers

1. Sex (male/female): _____
2. Age: _____
3. Educational status of the owner: 3.1. Illiterate 3.2. Read and write 3.3. Elementary school 3.4. High school complete 3.5. Professional

   If professional: 3.5.1. Related to animal production 3.5.2. Not related to animal production
4. Source of income (in the order of importance) 4.1. Livestock rearing 4.2. Crop farming 4.3. Craft 4.4. Trade 4.5. Other (specify): _____
5. What kind of livestock diseases do you know?

Briefly list and describe them

6. Do you know any of animal diseases, which could be transmitted to human beings?

   Yes ☐ No ☐

   6.1. If yes, how is it transmitted and do you know how to avoid transmission?

7. Do you know any disease which can cause abortion and/or retained placenta in animal?

   7.1. What do you do with the aborted fetus?

   7.2. What do you do with animals with retained fetal membrane?

8. How do you consume the milk produced at your farm or purchased from the market?

9. Do you know that some infections are transmitted by milk? Yes ☐ No ☐

10. Do you know that some animal diseases can affect the quality of meat?

    Yes ☐ No ☐

    11.1. If yes which and what did you observe?

11. What do you think is it possible, that animal diseases can be transmitted via the meat through man? Yes ☐ No ☐

    11.1. If yes, do you know any disease or symptom of such disease?

Annex 3: Procedures of Rose Bengal plate test (RBT)

1. Bring the RBT antigen and samples (sera) to room temperature before beginning the test.
2. Place 30µl of each serum sample on the agglutination plate.
3. Shake antigen bottle gently before use and place 30µl of RBT antigen next to the serum sample on agglutination plate.
4. Mix the antigen and the serum.
5. Shake the plate for 4 minutes and read.

   Magnifying glass are used to detect micro-agglutination

Result: No agglutination indicates negative sample. Agglutination indicates positive sample.

NB Slight agglutination is also considered as a positive result.

Annex 4: Procedures of complement fixation test (CFT)

Using standard 96-well microtitre plates with round (U) bottom, the technique is usually performed as follows:

1. Volumes of 25 µl of diluted inactivated test serum are placed in the well of the first and second rows. The first row is an anti–complementary control for each serum. Volumes of 25 µl of VBS are added to the wells of the first row (anti–complementary controls) to compensate for lack of antigen. Volumes of 25 µl of VBS are added to all other wells except those of the second row. Serial doubling dilutions are then made by transferring 25 µl volumes of serum from the third row onwards; 25 µl of the resulting mixture in the last row are discarded.

2. Volumes of 25 µl of antigen, diluted to working strength, are added to each well except wells in the first row.

3. Volumes of 25 µl of complement, diluted to the number of units required, are added to each well.

4. Control wells are set up to contain 75 µl total volume in each case; the wells contain

   a) diluent only,

   b) complement + diluent,

   c) antigen + complement + diluent.

   A control serum that gives a minimum positive reaction should be tested in each set of tests to verify the sensitivity of test conditions.

5. The plates are incubated at 37°C ± 2°C for 30 minutes or at 5°C ± 3°C overnight, and a volume (25 or 50 µl according to the techniques) of sensitised SRBCs is added to each well. The plates are reincubated at 37°C ± 2°C for 30 minutes.

6. The results are read after the plates have been centrifuged at 1000g for 10 minutes at 5°C ± 3°C or left to stand at 5°C ± 3°C for 2–3 hours at least to allow unlysed cells to settle. The degree of haemolysis is compared with standards corresponding to 0, 25, 50, 75 and 100%

lysis. The titre of the serum under test is the highest dilution in which there is 50% or less haemolysis.

Interpretation of the results: Sera giving a titre equivalent to 50 ICFTU/ml or more are considered to be positive.

Annex 5: Procedures of COMPELISA (c-ELISA)

1. Warm the diluting buffer to room temperature. It is recommended that the diluting buffer is warmed in a water bath at 23°C (± 3°C). Mix the conjugate concentrate (BM40) thoroughly and dilute to working strength in the warmed diluting buffer according to the instructions on the conjugate ampoule. This solution cannot be strored.

   NB When removing the conjugate from -20°C it is advisable to hand warm the ampoules before adding to the warmed diluting buffer. When emptying the conjugate vial, a small volume of conjugate can remain in the vial. Use a fine tip pipette to remove the remainder out; failure to do this may affect the outcome of the test.

2. Add 20µl of each test serum per well. Leave columns 11 and 12 for controls (see microtitre plate layout page 7).

3. Add 20µl of the positive control to wells F11, F12, G11, G12, H11 and H12.

4. Add 20µl of the negative control to wells A11, A12, B11, B12, C11 and C12.

5. The remaining wells in columns 11 and 12 have no serum added and act as the conjugate control. D11, D12, E11, E12.

6. Immediately dispense into all wells 100µl of the prepared conjugate solution. This gives a final serum dilution of 1/6.

7. The plate is then vigotously shaken (on the microtitre) for two minutes in order to mix the serum and conjugate solution. Cover the plate with a lid and incubate at room temperature (21°C ± 6°C) for 30 minutes on a rotary shaker at 160 revs/min.

If neither shaker is available the plate should be initially shaken for 30 seconds followed by 10 second hand-shakings every 10 minutes for a total of 1 hour.

Caution – care should be taken when hand shaking to keep liuid in their respective wells.

8. Shake out the contents of the plate before washing each plate 5 times with either washing solution or drinking water from a tap under low pressure (keep tap water at a steady, soft flow). Dry plate by tapping firmly onto a few layers of absorbent towel until no more liquid is removed.

   NB Hand washing is the recommended method although a plate washer may be used if proven to be fit for purpose; the number of washes may be adjusted accordingly. Validation of the plate may be required to ensure the use of this equipment is suitable for the test.

9. Immediately before use, prepare the substrate and chromogen solution by dissolving one tablet of urea H_2O_2 in 12ml of distilled water. When dissolved, add the OPD tablet and mix thoroughly.

   This can take a few minutes; the use of magnetic stirrer will greatly increase the speed with which it dissolves. This solution cannot be stored.

   NB OPD solution is sensitive to light and should be made up immediately before use and kept in an opaue container.

10. Add 100µl of OPD solution to all wells. Incubate the plate at room temperature (21°C $\pm$ 6°C) for a minimum of 10 minutes and a maximum of 20 minutes.

    NB Gently tap the plate approximately at five minutes intervals during the substarte incubation. Caution – care should be taken when tapping to keep the liquid in their respective wells.

11. Switch on microplate reader and allow the unit to stabilize for 10 minutes.

12. Slow the reaction by adding 100µl of stopping solution to all wells.

13. Remove condensation from the bottom of the plate with absorbent paper towel. Read plate at 450nm. NB Read plate within 10 minutes.

    If a microtitre plate reader is not available, a visual inspection of the plate may be used to determine whether a sample is positive or negative.

    Analysis of results: The lack of color development indicates that the sample tested was positive. A positive/negative cut-off can be calculated as 60% of the mean of the optic density (OD) of the 4 conjugate control wells. Any test sample giving an OD equal to or below this value should be regarded as being positive.

Annex 6: Puplished papers

1. Dagnachew Demissie, Tesfu Kassa, Gobena Ameni, Peter Baaro Gathura, and Gezahegne Mamo. 2025. Sero-prevalence of camel brucellosis and the associated public health risks in Kereyu pastoral area of Oromia Region, Ethiopia. *Ethiop. Vet. J.*, 29(1), 69-90. https://doi.org/10.4314/evj.v29i1.5.
2. Dagnachew Demissie, Tesfu Kassa, Gobena Ameni, Peter Baaro Gathura, Esayas Gelaye, and Gezahegne Mamo. 2025. Sero-prevalence and associated risk factors of small ruminant brucellosis in Kereyu pastoral area of Oromia Region, Ethiopia. *Ethiop. Vet. J.*, 29(2), 31-46. https://doi.org/10.4314/evj.v29i2.3.

Annex 7. Individual animal data recording sheet

No.	Animal ID	Sex	Age	Species	Parity	Herd/flock size	Livestock composition	History of abortion	RBT	CFT	c-ELISA	Remark
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												

Annex 8. Individual human data collection sheet

No.	Sample ID	Residence	Sex	Age	Marital status	Educational level	Source of income	Knowledge on zoonosis	Knowledge on causes of zoonosis/retained placenta	Knowledge on milk borne diseases	Knowledge on meat borne diseases	Milk consumption	RBT	CFT	c-ELISA	Remark
1																
2																
3																
4																
5																
6																
7																
8																
9																
10																
11																