# SEROPREVALENCE OF BRUCELLOSIS IN SMALL RUMINANTS AND PUBLIC AWARENESS TOWARDS BRUCELLOSIS IN TWO DISTRICTS OF WEST GUJI ZONE, SOUTHERN OROMIA, ETHIOPIA

**BY**

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**DECLARATION**

I declare that this thesis is my original work and that all sources of materials used for this thesis have been accordingly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc. degree at Hawassa University. I sincerely declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Name: Miessa Banata Deresso
Signature: _____
Place: Hawassa, Ethiopia
Date of submission: _____

# TABLE OF CONTENT

**CONTENTS** **PAGES**

## ACKNOWLEGDGMENTS

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# LIST OF TABLES

# LISTS OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| CFT | Complement Fixation Test |
| FAO | Food and Agricultural Organization |
| IFNs | Interferons |
| I-ELISA | Indirect Enzyme Linked Immuno-Sorbent Assay |
| LPS | Lipopolysaccharides |
| MZN | Modified Ziehl-Neelsen's |
| NK | Natural Killer |
| OIE | Office International des Epizooties |
| PAs | Peasant Associations |
| PCR | Polymerase chain reaction |
| RBPT | Rose Bengal Plate Test |
| TNF- | Tumor Necrosis Factor |
| TLR | Toll-like receptors |
| WAHIS | World Animal Health Information System |
| WHO | World Health Organization |
| TB | Tuberculosis |

# LIST OF ANNEXES

# ABSTRACT

Brucellosis is one of the most widespread zoonotic illnesses in the world. A close human-animal contact and tradition of raw animal product consumption make zoonosis among the major public health hazards, with particular implication to pastoral area such as Wet Guji zone. Lack of sufficient awareness regarding the disease in the area is another considerable issue. A cross sectional study was conducted with the objectives of estimating seroprevalence of small ruminants' brucellosis, assessing associated risk factors and understanding the community awareness towards the disease. Systematic sampling methods were used to select the study animals. Accordingly, 324 small ruminants (132 sheep and 192 goats) and 52 human sera samples were collected. These samples were first screened by Rose Bengal plate taste (RBPT) and then positive ones were finally confirmed by I-ELISA. Out of which 34 small ruminants and 13 human samples were positive using RBPT of these 23 ruminants and 10 human samples were confirmed using I-ELISA. An overall seroprevalence of brucellosis was 7.1% and 19.2% in small ruminants and humans respectively. Univariable logistic regression showed that risk factors such as the sex, abortion history, age group, flock size, management, BCS, and parity were significantly associated ($p<0.05$) with increased seropositivity in small ruminants. Whereas in multi variables logistic regression age group, abortion history, flock size, BCS and parity showed significant difference. Adult age, animal with abortion history, large flock size, poor body condition and multiparous animals were more likely infected than their respective counterparts. Seroprevalence in humans was high in adult, females and those with problem of sanitation. So, these results provided evidence of the importance of brucellosis in humans and small ruminants in the study area. Overall, the existence of brucellosis, the community's daily practice of uncontrolled movement of animals, and the livelihood nature of pastoralists suggest the need for public health education on the zoonotic importance of brucellosis continuously in the study area.



***Key words:*** *Brucellosis, Dugda Dawa, Seroprevalence, Small Ruminant and Suro Berguda*

# 1.NTRODUCTION

## 1.1. Background of the Study

One of the most widespread zoonotic illnesses in the world, brucellosis, is diagnosed in over 500,000 people annually. The worldwide illness load on cattle is likewise significant. The Near East, the peninsula of the Balkans, Central Asia, and portions of Africa and South America are all plagued by the disease. The most current reviews of the brucellosis prevalence in humans worldwide were conducted by Khan and Zahoor, (2018).

Brucella species are the primary source of the zoonotic bacterial illness known as brucellosis, which mostly affects animals, while humans serving as accidental hosts. Despite being a significant public health issue, the illness is often ignored across the world. It is the second-most significant zoonotic disease next to salmonellosis in the world, according to OIE, (2018). The illness is more significant in developing nations and has significant negative effects on the economy and public health (Carbel, 2006). It is an industrial disease that mostly affects butchers, farmers, veterinarians, stock inspectors, employees of abattoirs, and laboratory workers. The World Health Organization (WHO), has designated the illness as one of the world's top "neglected zoonotic diseases" because of the impact it exerts particularly on low-income nations (Franc *et al.*, 2018).

The existence of small ruminant brucellosis in sub-Saharan Africa has been confirmed, but the full economic and zoonotic effects of the illness have only been partially studied, if at all (Ducrotoy *et al*., 2014). Additionally, almost all of reports have relied on serological data. Due to its vast distribution and effects on several species of animals, especially cattle, sheep, goats, pigs, and people, the disease is one of the highest priority diseases in sub-Saharan Africa and other developing nations (McDermott and Arimi, 2002).

According to the available serological data, the prevalence has been discovered to vary from location to location based on agro ecology, management, flock size, as well as host-related variables (Megersa *et al*., 2011; Teklue; *et al.,* 2013) discovered a link between reproductive inefficiencies and Brucella exposure, whereas Asmare *et al.,* (2013) revealed that prevalence tends to increase in adults kept in larger flocks. On the other hand, because the etiological agents have not been discovered in Ethiopia (Edao *et al*., 2023; Tekele *et al.,* 2019 and Asmare *et al*.,

2014), exposure research involving zoonosis (Kassahun, 2006) could not be linked to any of the known *Brucella* species.

Bovine, ovine, caprine, swine, and several other domestic animals, including camels, are often affected animals and have an increased risk of abortions in the third trimester of pregnancy due to infection (Melaku and Tessema, 2013). Animals' chief symptoms of infection include abortions in females and epididymitis and orchitis in males. Only laboratory testing, which may even detect latent infections, may definitively diagnose an infection (Carbel, 2006). The illness has been compared to TB as a result of this as well as the granulomatous form of the lesions (Sanjuan-Jimenez *et al*., 2013).

It is a direct contributor to economic losses due to clinical illness, abortion, neonatal deaths, decreased fertility and reduced milk production. According to Renukaradhya *et al*. (2002), breeding inefficiency, lamb and young loss, and decreased wool, meat, and milk production are the main causes of financial losses in small ruminants. It also plays a significant role as a barrier for international trade of live animals by being used as an impediment to free animal movement and export (Coelho *et al*., 2007; Yuguda *et al.*, 2019).

The predilection locations for brucellosis are the male and female reproductive systems, particularly the uterus during pregnancy. It is mainly a disease of sexually active animals. Most *Brucella* is stimulated to proliferate by allantoic stimuli. These factors include erythritol, possibly steroid hormones and other substances (Meles and Kibeb, 2017).

The close contact between humans and animals as well as the custom of eating raw animal products make zoonosis one of the biggest risks to public health, with specific implications for pastoral communities. This necessitates a comprehensive epidemiological analysis that takes into account identifying the key risk variables that predominately impact the development of the illness and helps to build effective and workable national control plans.

Depending on the animal species involved, *Brucella* Spp. management techniques, and the accessibility and effectiveness of vaccinations, brucellosis in farm animals can be controlled and prevented. Immunization, testing, removal, and better management methods and movement control are all approaches for controlling the illness. The one health approach to manage and prevent human and animal brucellosis, however, is a crucial strategy for brucellosis control that has gained increasing global attention in recent years (Bedore and Mustefa, 2019).

## 1.2. Statement of the Problem

According to the OIE, (2018), *Brucella ovis* and *Brucella melitensis* are the main causes of the deadly illness brucellosis in small ruminants, which mostly affects goats. According to Radostits *et al. (*2007), it is a condition that affects sexually mature animals and has a preference for placentas, fetal fluids, and testicles. The disease is one of the most pressing health issues in sub-Saharan Africa and other developing countries because of its wide distribution and impact on many species of animals, including cattle, sheep, goats, pigs, and people (McDermott and Armi, 2002).

In Ethiopia different Studies showed 3.6–16% seroprevalence of brucellosis in small ruminants in pastoral areas of Afar, Oromia and Somalia regional states (Yibeltal, 2005; Ashenafi *et al*., 2007; Tsehay *et al*., 2014). In addition, a study in Ethiopia by Tschopp *et al*., (2015) showed poor community's knowledge about brucellosis and high risk for *Brucella* infection among pastoralist communities adjacent to Awash National Park.

Thus, in Ethiopia a number of studies showed individual seroprevalence ranging from 0.1–15.2% in different parts of the country (Berehe *et al*., 2007; Regassa, 2009; Asmare *et al*., 2010; Hailesillasie *et al*., 2010; Megersa *et al.*, 2011). More importantly, a close human-animal contact and tradition of raw animal product consumption make zoonosis among the major public health hazards, with particular implication to pastoral area. The livelihood of pastoral communities is mostly dependent on small ruminant production. This requires a thorough investigations including due consideration to identifying the major risk factors that predominantly influence the disease occurrence. Moreover, there is lack of sufficient information at the west Guji zone regarding the disease. Therefore, the current study was under taken to asses sero-prevalence of Brucellosis in small ruminants and public awareness towards brucellosis in two districts of West Guji Zone.

## 1.3. Significance of the Study

Animals can contract the highly infectious, economically significant bacterial illness known as brucellosis from members of the genus *Brucella*. Animals most frequently have reproductive losses, but humans might experience a crippling non-specific sickness or localized organ involvement. Each *Brucella* species typically has a particular animal host, but other species can get the infection, particularly if they are kept in close proximity to one another (Teklue *et al.*,

2013). The typical hosts for *Brucella melitensis* include sheep and goats. According to some estimation, *B. melitensis* is the most prevalent species of *Brucella* in human diseases and is to blame for 70% of all infections. According to Megersa *et al.,* (2011), the majority of individuals get this bacterium through direct contact with sick animals or their tissues or by consuming contaminated dairy products.

The expense of surveillance to stop the reintroduction of *B. melitensis* is high in countries where it is not present. There are also worries that a bioterrorist strike would employ these bacteria. In many under developed nations throughout the world, brucellosis not only poses a serious threat to livestock's health but also results in significant economic losses (Renukaradhya *et al.,* 2002).

It is anticipated that this study will provide information for veterinarians in and around Dugda Dawa and Suro Berguda district of West Guji zone that will help in the planning and implementation of effective awareness programs and strategies to prevent or minimize small ruminant brucellosis. Additionally, it could close any gaps in the literature and serve as a foundation for future research.

## 1.4. Limitation of this Study

Absence of well-equipped laboratory nearer to study area for zoonotic disease diagnosis and investigation limited my study. Others issues like financial problem to collect sufficient samples or samples with the best representation of brucellosis situation in community also limited my scope

## 1.5. Research Questions

In this study, the following research questions were tried to be answered. These are:

- ✓ What is the sero-prevalence of small ruminant brucellosis in the West Guji zone?
- ✓ What is the associated risk factor for transmission of brucellosis in small ruminants in West Guji zone?
- ✓ What is the public awareness importance of the small ruminant brucellosis in the study area?

## 1.6. Objective of the Study

### 1.6.1. General objective

The general objective of this study was: -

- ❖ To assess the seroepidemiology of small ruminants' brucellosis in West Guji zone, southern Oromia, Ethiopia.

### 1.6.2. Specific objectives

The Specific objectives of this study were: -

- ✓ To estimate the seroprevalence of brucellosis in the study area.
- ✓ To assess risk factors associated with seropositivity of brucellosis in small ruminants in the study area.
- ✓ To assess awareness of small ruminant owners about brucellosis and its public health risk.

# LITERATURE REVIEW

## 1.2. Definition

Brucellosis is a contagious bacterial zoonotic disease of veterinary and public health importance in developing countries. *Brucella* species are non-spore-forming, non-capsulated facultative intracellular gram-negative coco-bacilli. Despite being classified as non-motile, *Brucella* species possess all the genes needed to put together a functioning flagellum, with the exception of the chemotactic system (Sanjuan-Jimenez e*t al.*, 2013). According to Debassa, (2013) it is a zoonotic bacterial illness that affects animals and causes large reproductive losses. A gram-negative, facultative intracellular bacterium of the genus *Brucella* causes these zoonotic illnesses, which have an impact on both human health and animal productivity (Ariza *et al.*, 2007).

Usually organized individually, but occasionally in pairs or small groups, *Brucella* is intracellular coccobacilli or short rods. *Brucella* organisms, particularly *B. abortus*, depend on carbon dioxide for their development, which is why these organisms are referred to as capnophilic organisms. Agents of *Brucella* are incapable of surviving at pH< 4 (Poester *et al.*, 2010).

## 2.2. Brief History of Brucellosis

The first-time humans came into touch with animals is when brucellosis initially emerged. Studies have shown that the sickness has been present in people and animals for a very long time (Akpınar, 2016). Thus, the isolation and identification of *Brucella melitensis* (*Micrococcus melitensis*) in the 1880s did not mark the beginning of brucellosis. Numerous historical descriptions of illnesses from before this period, such as animal abortion out breaks and fever in humans, might really be describing brucellosis (D'anastasio *et al.*, 2011). The fragmentary skeleton of the late Pliocene Australopithecus Africanus provides paleo-pathological evidence that brucellosis occasionally afflicted our ancestors 2.3–2.5 million years ago (Gebretsadik, 2016). Jeffery Allen Marston published the first account of brucellosis in 1860. Marston was the first to identify brucellosis; he described the illness as "gastric remittent fever" in his thorough writings (Akipinar, 2016)

However, the brucellosis-causing organism, "*Micrococcus melitensis*" (i.e., *Brucella melitensis*), was identified in 1887 from the liver of sick soldiers on the Mediterranean island of Malta by the Maltese microbiologist's doctor Giuseppe Caruana-Scicluna and the British surgeon captain (Gebretsadik, 2016). After making this finding, Maltese physician Temi Zammit demonstrated that the microorganisms that causes Malta fever spreads from sick goats to people through tainted milk. The illness was given the extra name "Bang's disease" because the Danish scientist Bernhard Bang discovered "*Bacillus abortus*" (i.e. *Brucella abortus*) in 1897 from bovine aborted fetuses, 10 years after "*Micrococcus melitensis*" was discovered (Rahman, 2015; Gebretsadik , 2016).

After finding particular antibodies to *Brucella melitensis* in both humans and animals, Wright and Smith argued in 1897 that brucellosis was a zoonotic disease (Scholz *et al*,.2008). After that, in 1905 Themistocles Zammit (1864-1935) found *Brucella melitensis* from goat's milk and urine, and in Indiana, the United States and Jacob Traum discovered Brucella suis in the livers, stomachs, and kidneys of prematurely born piglets. Then, in 1957, Stoenn and Blackman in Utah, in the United States, isolated Brucella neotoma from a wood tick. Carmichael and Bruner isolated *Brucella canis* in beagle dogs in 1968. In 1994, finally, (Pappas, 2006) identified *Brucella microti* from mandibular lymph nodes of a wild red fox and field mice in Central Europe.

## 2.3. Etiology

*Brucella* infection is caused by species of the bacterial genus *Brucella* (Morgan and MacKinnon,1979). Biovars are an additional classification for *Brucella melitensis, B. abortus*, and *B. suis* (Rajala, 2016). According to Ferede *et al*., (2011), *Brucella melitensis* is the primary cause of caprine and ovine brucellosis and is one of the most dangerous zoonosis in the world. *B. ceti*, *B. pinnipedialis*, and a *Brucella* species isolated from the common vole (*B. microti*) are among the other species that infect marine animals that have been discovered and categorized (Foster *et al*., 2007).

The most significant strains of *Brucella* are *B. melitensis, B. abortus, B. suis*, and *B. ovis*, which predominantly infect sheep and goats, cattle, pigs, and sheep, respectively. The first three species are mostly to blame for brucellosis in humans in addition to lowering animal production (Asnake *et al.,* 2017). Comparatively little is known about the elements influencing the Brucella's persistence in the host and proliferation inside phagocytic cells since they lack the traditional

virulence genes that encode capsules, plasmids, pili, or exotoxins. According to Seleem *et al*. (2010) many facets of the relationship between *Brucella* and its host are yet unknown.

## 2.4. Taxonomic Classification

The family Brucellaceae, order Rhizobiales, class Alphaproteobacteria, and phylum Proteobacteria are all home to the genus *Brucella*. Proteobacteria are a phylum of Gram-negative, lipopolysaccharide-primarily, Gram-negative bacteria (Garrity *et al.*, 2005). The genus Brucella was once thought to include six "classical" species, which were classified based on antigenic variation and the principal host species from which they were obtained. According to Adams *et al*. (2010), these traditional species include *B. abortus, B. melitensis, B. suis, B. ovis, B. canis, and B. neotomae.*

In addition to these classical *Brucella* species, other species infecting marine mammals have been identified and classified including *B. ceti and B. pinnipedialis*, and a Brucella species isolated from the common vole (*B. microti*) (Foster *et al*., 2007). They are not truly acid-fast, but are resistant to decolorisation by weak acids and thus stain red by the Stamp's modification of the Ziehl–Neelsen's method and false positive results in the Stamps's method because other organisms that cause abortions *Chlamydophila abortus* (formerly Chlamydia psittaci) and *Coxiella burnetii* are difficult to differentiate from *Brucella* organisms hence, positive or negative result should be confirmed by culture (OIE 2012). In a woman who had clinical indications of brucellosis, a breast implant infection was recently shown to be caused by Brucella inopinata (Scholz *et al*, 2010).

## 2.5. Morphology

According to Morgan and MacKinnon, (1979), Brucella are Gram-negative coccobacilli or short rods that are non-motile, non-spore-forming, non-capsulated, non-flagellated, aerobic, facultative intracellular bacteria that can invade, survive, and multiply inside epithelial cells, placental trophoblasts, dendritic cells, and macrophages. It is mostly found in the lungs, liver, spleen, bone marrow, and synovium, all of which have a large number of macrophages (Akhvlediani *et al*., 2010).

The bacteria are often organized individually; pairs or small groups of bacteria are less common. With the exception of ancient cultures, where pleomorphic forms could be visible, Brucella's

morphology is largely stable. Although they are not really acid-fast, they may still be stained red using the Stamp's adaptation of the Ziehl-Neelsen's procedure since they are resistant to decolorisation by mild acids (Gorvel, 2008). When plates are viewed in the daylight via a transparent medium, they appear translucent and a light honey hue. Colonies seem convex and pearly white when viewed from above. Colonies get bigger and a little bit darker later (OIE, 2012).

With the exception of ancient civilizations, when pleomorphic variants may be visible, Brucella is generally consistent (European Commission, 2001). In particular, with subcultures, smooth Brucella cultures, notably those of *B. melitensis*, show a propensity to change during development and dissociate to rough (R) forms and occasionally mucoid (M) forms. Colonies then exhibit a significant reduction in transparency, a more granular, dull surface (R) or a sticky, gelatinous feel (M), and a color spectrum ranging from matt white to brown in reflected or transmitted light (OIE, 2004).

## 2.6. Epidemiology

### *2.6.1. Occurrence of the disease*

The prevalence and incidence of brucellosis in sub-Saharan African nations are poorly documented, and the majority of reports submitted to the Office International des Epizooties (OIE) currently named the World Organization for Animal Health are limited to serological surveys that are primarily conducted on cattle and less frequently on sheep and goats. (McDermott and Arimi 2002) referred to a great variation in prevalence in sub-Saharan Africa (ranging from 4.8 to 41%) in pastoral systems. It is commonly transmitted to other animals by indirect or direct contact with infected animals or their discharges (OIE, 2009). The geographical distribution of brucellosis is constantly changing, with new foci emerging or re-emerging (Hailu, 2017). New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening (Pappas *et al.*, 2006).

According to Godfroid *et al.*, (2005); the illness is more prevalent in small ruminants in the Mediterranean region, which includes southern Europe, West and Central Asia, South America, and Africa, with significant regional and geographic diversity. According to Belasco and Molina Flores., (2011), *B. melitensis*, the most dangerous species in the Brucella genus, has

three biovars, with biovars 1 and 3 being most commonly isolated in small ruminants in the Mediterranean, the Middle East, and Latin America.

It is a major zoonosis, a barrier to commerce in animals and animal products, and it causes large abortion-related losses (Benkirance, 2006; Banai, 2007). The main clinical symptoms of brucellosis in ruminants are abortion and stillbirths, which typically happen in the final third of pregnancy after infection and typically only occur once throughout the animal's lifetime (Elzer *et al*. 2002; and Belasco and Molina, 2011). In terms of pathology and epidemiology, *B. melitensis* infection in small ruminants is similar to *B. abortus* infection in cattle.

*2.6.2. Source of infection.*

Aborted fetuses, fetal membranes, vaginal secretions, and milk from sick animals are all potential sources of infection (Adugna *et al*., 2013). Environment borne contamination, occupational exposure often brought on by close contact with sick animals, and food borne transmission are all possible sources of infection (WHO, 2006). The placenta, fetal fluids, and vaginal discharges released by infected ewes following abortion or full-term parturition are the main sources of Brucella transmission. At the moment of parturition or abortion, extremely huge numbers of organisms are shed (Glenn *et al*., 2005). It can pass through mucous membranes and the skin that are healthy or damaged (El-Sayed and Awad, 2018).

According to OIE, (2009), materials ejected from the female vaginal tract serve as the primary source of organisms for transmission to other animals and humans. Transmission generally happens in the same manner in sheep, goats, and cattle. Inhalation, udder contamination during milking, skin penetration through the conjunctiva, licking the discharge from an animal, a newborn calf, or a retained fetal membrane are all examples of ways that horizontal transmission can happen (Gul *et al.*, 2007). Additionally, venereal infections are possible and are typically caused by *B. suis* infections. The significance of venereal transmission varies according on the species; for *B. ovis*, *B. suis*, and *B. canis*, it is typically the main method of transmission. Despite the fact that *B. abortus* and *B. melitensis* may be identified in semen, these organisms seldom spread during sex (Ashenafi *et al*., 2007).

As one of the world's most dangerous zoonosis, *B. melitensis* is regularly seen in sheep and goats and is extremely pathogenic for humans (Gall *et al*., 2001). According to Nielsen and Duncan, (2017), the expulsion of organisms from the vagina is lengthy and abundant in goats

(usually lasting 2 to 3 months) and occurs 3 weeks after abortion or full-term parturition in sheep. According to Beruktayet and Mersha, (2016), the illness has caused the small ruminant sector to suffer significant financial losses.

By coming into touch with the blood, body tissues, or bodily fluids of infected animals, humans can get brucellosis. The most popular technique is to consume dairy products and unpasteurized milk. When handling contaminated animal tissues, fractures in the skin may result in infections. Brucella can be spread by aerosols, contact with lab cultures and tissue samples, inadvertent administration of live Brucella vaccines, and presumably also in slaughterhouses (Hegazy *et al*., 2011). Although transmission from person to person is uncommon, it can occur through close physical or sexual contact, blood donation, tissue transplantation, and bone marrow donation (Wakene *et al*., 2017).

*2.6.3. Risk factors*

Numerous production systems related risk factors, host biology-related variables, and environmental risk factors all have an impact on brucellosis. Age, sex, species, reproductive state, herd size and composition, farm hygiene, the frequency with which infected and vulnerable animals come into contact, farm biosecurity, geographical location, and climatic conditions are a few of these factors (Hailu, 2017). Animals reared in complex systems have been observed to have a greater sero-prevalence. The danger of virus exposure was further heightened by large herd numbers and inadequate housing (Mfune, 2015). Insufficient manure removal and cleaning, poor management of aborted materials, the, introduction of new animals from herds that were not free of brucellosis or of unknown status, herds kept in close confinement, and mixed herds are additional risk factors that have been mentioned (Deka *et al*.. 2019).

***A. Agent risk factors****: B. Melitensis* is a facultative intracellular bacterium that may grow and survive inside the phagosome of the host cell. Polymorph nuclear leucocytes phagocytoze the organisms, some of which survive and proliferate. Because it can live in phagolysosome, the organism can survive within macrophages. A novel non-endotoxin lipopolysaccharide found in the bacteria confers resistance to antimicrobial agents and regulates the host immune response. For Brucella to survive and replicate in the host, these characteristics make lipopolysaccharide an essential virulence factor (Radostits, 2006). The timing of the early IgA and IgM types of antibody responses to Brucella relies on the method of exposure,

the dosage of bacteria, and the animal's state of health. IgG1 and IgG2 antibodies are produced quickly after the IgM response (Nielsen, 2002).

*B. Host factors*

**Age factor:** Small ruminants are susceptible to brucellosis, which mostly affects sexually mature animals. Predilection sites include both male and female reproductive systems, particularly the uterus during pregnancy. This may be due to the fact that erythritol and sex hormones, which promote *Brucella* organism development and multiplication, tend to concentrate more with advancing age and sexual maturity (Radostits *et al*., 2000). Although latent infections might happen, younger animals often have higher infection resistance and commonly recover from an existing illness (Mekonnen, 2016). Kids and lambs may contract the virus before or soon after birth, and they often recover before they reach reproductive age, however occasionally the infection can last much longer (WHO, 2006).

**Species and Breed Factor**: Breed and species are two additional risk factors for brucellosis seropositivity, according to several experts, such as (Muma *et al*., 2007). Compared to sheep, goats are more likely to get Brucella infection. This could be as a result of goats being more susceptible to *Brucella* infection. As a result there is a lower chance of disease transmission within sheep herds (Radostits *et al*., 2000). Sheep reared for meat production are less responsive than milk-producing ewes (Corbel, 2006). Due to their increased output and intense management, exotic breeds and their hybrids are proven to be more vulnerable (Garrido *et al*., 2001).

**Sex factor**: Compared to male ruminants, female ruminants had increased probabilities of contracting brucellosis (Coelho *et al*., 2019). Male goats are less vulnerable to *B. melitensis* infection than females are. Male goats are less sensitive to *B. melitensis* infection than females are (FAO, 2003).

**Health status:** Animals who have received vaccinations and are free of disease are less vulnerable to infection than those who have not had vaccinations, impaired immune systems, or diseases. The health state of the animals may also play a significant influence in acquiring the infection. Compared to unvaccinated and immune-compromised ill animals, vaccinated and disease-free animals are less vulnerable (Radostits *et al*., 2007).

**Reservoirs:** By contaminating the environment, harboring *Brucella* organisms in their bodies, and excreting these agents, carrier animals greatly aid in the spread of the disease. The excreted

organisms then infect humans and other animals, endangering public health and the nation's economy. The ability of animals to mechanically spread infection by transporting infected material, such as fetuses or fetal membranes, increases the viability of the organisms in the environment, increasing the likelihood that susceptible animals will contract the disease (FAO, 2006). Dogs, cats, and wild carnivores like foxes and wolves are the carriers. These animals may be crucial as mechanical dispersers of infection by carrying away infected material like fetuses or fetal membranes, which increases the viability of the organisms in the environment and increases the likelihood of infecting susceptible animals (Gall *et al*., 2001).

**Flock size:** Herd size increases the likelihood of animal interaction, which increases the risk of infection, especially after calving or abortion (Zeru and Hadush, 2016). According to (Radostits and Gay, 2006), herd size and animal density are closely correlated with illness prevalence and the difficulties of controlling infection in the population.

**Stage of Pregnancy:** Animals that are pregnant are more likely to contract the organism than animals that are still developing sexually. As the gestational stage advances, susceptibility also rises (Fulasa, 2004). During the later stages of pregnancy, placental trophoblasts manufacture erythritol in increasing levels, which is also the time when pregnant sheep and goats are increasingly vulnerable to *B. melitensis* infection. Erythritol enhances the development of several *Brucella* strains. (Sangari *et al*., 2000).

**Management risk factors:** Lack of clean water, insufficient manure removal and cleaning, poor management of aborted materials, introduction of new animals from herds that were not free from brucellosis of unknown status, herds kept in close confinement, and mixed herds were other factors that increased the risk of exposure to infection (Deka *et al*., 2018).The primary reason brucellosis eradication operations fail is the uncontrolled movement of cattle from infected herds or regions to herds or areas that are brucellosis-free. Once the herds are infected, big herd size, active abortion, and loose housing lengthen the time needed to recover from brucellosis. The passage of an infected animal from an infected herd into a non-infected susceptible herd is usually invariably what causes the illness to spread from one herd to the next and from one location to another. Animals housed in complex systems have been observed to have a greater sero prevalence. The danger of virus exposure was further heightened by large herd numbers and inadequate housing (Mfune, 2015).

**Occupational risk factors**: The danger of contracting brucellosis is increased for those who work with animals or who come into contact with contaminated blood. A few examples are:

veterinarians, dairy farmers, ranchers, slaughterhouse employees, hunters, microbiologists, and farmers (Gemechu, 2017) Also at danger are people conducting artificial insemination and those working in endemic regions. As of late, Brucella was thought to be a possible bioweapon (Bauerfeind *et al*., 2016). Direct contact with infected animals or exposure to a highly contaminated environment puts farmers, farm workers, animal attendants, stockmen, shepherds, sheep shearers, goatherds, pig keepers, veterinarians, and inseminators at risk (WHO, 2006), If the illness is present in domestic animals, this may imply that rural populations and animal health professionals are also quite likely to catch it (Minja, 2002).

**Environmental Risk Factor:** Bacterial survival is prolonged at low temperatures and organisms will remain viable for many years in frozen carcass. The organisms in aqueous suspensions are readily killed by most disinfectants (Walker ,1999)

A 10g/l solution of phenol will kill *Brucella* in water after less than 15 min exposure at 37°C. Formaldehyde solution is the most effective of the commonly available disinfectants, provided that the ambient temperature is above 15°C. W.H.O, (1986).

The aggregation of numerous mixed ruminants near water sources promotes the transmission of illness (Radostits *et al*., 2007).The capacity of the organisms to survive in the environment, which raises the risk of infection in animals that are prone to it (Islam *et al*., 2013).

*2.6.4. Transmission*

Ingestion of contaminated pasture, feed, fodder, water, and contact with aborted fetuses, uterine discharges, and newly born calves are the most prevalent routes of transmission. These sources of infection are crucial because they contain high dosages of infectious organisms. However, infections typically spread through damaged or healthy skin, respiratory system mucosa, and conjunctiva (Teka *et al*., 2019). Additionally encouraging the spread of infections between farms is the exchange of male breeding stock. In some regions, transhumance of summer grazing and animal mixing at markets or fairs are important promotional factors. In frigid areas, it may be customary to keep animals near to one another, which makes it easier for diseases to spread (Habtamu *et al*., 2015).

**B.** *abortus, B. melitensis, and B. suis* are the major agents that cause brucellosis in humans and are also the main agents that cause brucellosis in cattle, goats/sheep, and pigs. Human brucellosis sometimes referred to as "undulant fever," "Mediterranean fever," or "Malta fever," is a zoonosis that is nearly always spread by contact with sick animals or their waste

products. All age groups are affected, albeit both sexes and those under the age of 14 are less vulnerable (Carbel, 2006). The risk of contracting brucellosis rises when people are exposed to contaminated animals and animal products (Makita *et al*., 2011).

As indicated in Figure 1: *B. abortus, B. melitensis, and B. suis* are the major agents that cause brucellosis in humans and are also the main agents that cause brucellosis in cattle, goats/sheep, and pigs. As a result of their frequent interaction with animals and their products, residents of pastoral communities are at a significant risk of contracting an illness. For urban populations, food borne transmission is typically the predominant cause of brucellosis. For the majority of populations, consuming fresh milk or dairy products made from warmed milk is the main cause of illness. *B.* Six Brucella species, including *B. melitensis, B. suis, B. abortus, B. canis, B. ceti*, and *B. pinnipedialis,* can infect people.

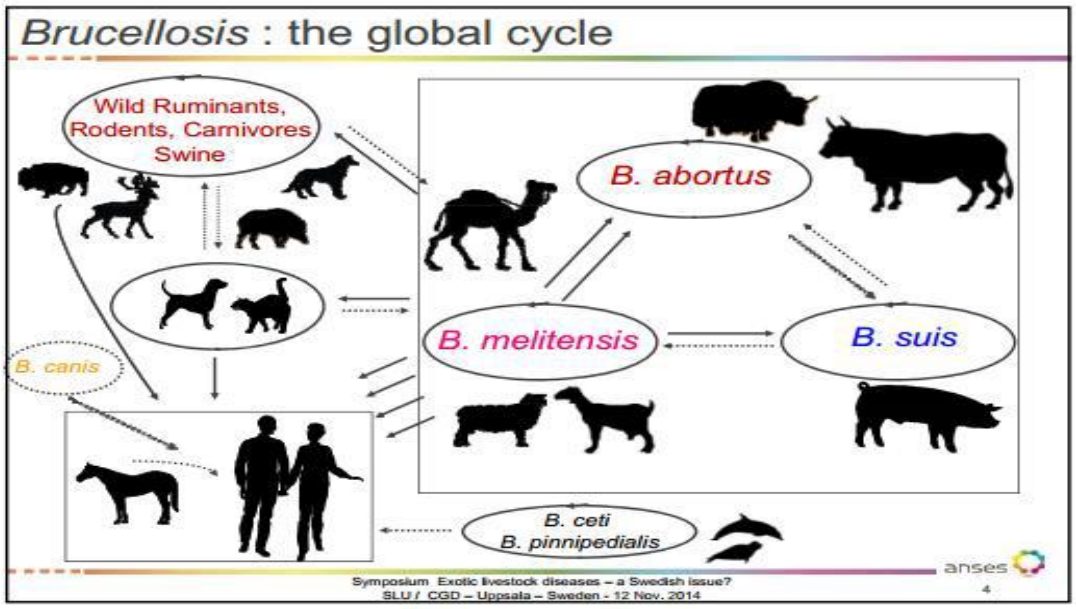


Figure 1: Transmission of brucellosis within the host range, (Garin *et al*., 2014)

## 2.7. Pathogenesis

According to Mustafa and Bedore, (2019), the severity of a *Brucella* infection relies on the animal's inherent resistance to the pathogens, the virulence of the *Brucella* species, and the exposure dose. In contrast to many other infections, *Brucella* spp. enter host cells without invoking the innate immune system's defense mechanisms, and they subsequently manage to fend of intracellular death in order to survive in the host (Gorvel and Moren, 2002).

The primary entrance point for *B. melitensis* is the mucosal surface of the digestive system (Carlos *et al*., 2011). Brucella enters the mucosal epithelium and is either taken up by phagocytic cells or transmitted as free bacteria. It proliferates, disseminates homogenously, and localizes in the reticulo endothelium after penetration and localization to local lymph nodes (Adem and Duguma, 2020). The placenta and ultimately the fetus are reached by organisms that go through the hematogenous pathway. The presence of the allantoic fluid components that would promote Brucella development accounts for the preferred localization to the reproductive tract of the pregnant animal. One of the factors that increased in the placenta and fetal fluid at the end of the second trimester of pregnancy caused damage to the placental tissue, along with fetal infection and fetal stress, which led to changes in the mother's hormones that resulted in an abortion (Mustefa and Bedore, 2019).

The chorionic trophoblasts of the placenta in pregnant cattle get colonized when *Brucella* spp. is found there (Corbel, 2006). The chorioallantoic membrane becomes ulcerated as a result of the placentitis brought on by reproducing bacteria, while the uterine endometrium is unaffected (Radostits *et al.* 2010).

By focusing on embryonic and trophoblastic tissue in ruminants, *Brucella* organisms get past the most potent host defenses. The bacteria develop not only in the phagosome but also in the cytoplasm and rough endoplasmic reticulum of the cells that make up these tissues. These tissues allow for abundant bacterial growth in the absence of functional intracellular microbicidal systems, which results in embryonic mortality and abortion (Ferreira *et al*., 2003). The host and Brucella species will determine how the infection turns out. According to Xavier *et al*., (2009), the Brucella species that infect animals are host-specific.

### *2.7.1 Entry into the host and evading immune system.*

The most effective host defensive mechanisms are overcome by *Brucella* organisms by concentrating on embryonic and trophoblastic tissue in ruminants. The cells that make up these tissues' cytoplasm and rough endoplasmic reticulum are also where the bacteria grow in addition to the phagosome. Due to the abundance of bacteria that can proliferate in these tissues in the absence of working intracellular microbicidal mechanisms, abortion and embryonic death occur (Ferreira *et al*., 2003). The outcome of the infection depends on the species of *Brucella* and the host. The Brucella species that infect animals are host-specific, according to (Xavier *et al*., 2009).

The most crucial component of Brucella ecology is their capacity to create an intracellular replicative niche and avoid being attacked by the host immune system since they are unable to replicate outside of their mammalian hosts (Bargen *et al*., 2012). Due to the absence of traditional virulence factors like toxins, fimbriae, and capsules, it is possible that Brucella use more subtle and specialized methods to enter host cells, overcome host defenses, change intracellular trafficking to avoid degradation and killing in lysosomes, and modify the intracellular environment to promote long-term intracellular survival and replication (Delrue *et al*., 2004)

In comparison to other Gram-negative bacteria, Brucella exhibits a high degree of hydrophobicity and LPS with a non-canonical structure that has a delayed inflammatory response and less stimulatory action on TLR4 receptors. The major histocompatibility complex class II molecules and the LPS's O side chain can combine to produce complexes that prevent macrophages from presenting foreign proteins. Due to their inability to circumvent the host defensive mechanism, the rough (vaccine) strains (i.e., strains with lipopolysaccharide lacking the O-side chain) are less virulent (Rittig *et al*., 2003).

### *2.7.2. Survival inside host cell*

As one of the world's most dangerous zoonosis, *B. melitensis* is regularly seen in sheep and goats and is extremely pathogenic for humans (Gall *et al*., 2001). According to Nielsen K and Duncan JR (2017), the expulsion of organisms from the vagina is lengthy and abundant in goats (usually lasting 2 to 3 months) and occurs 3 weeks after abortion or full-term parturition in sheep. According to Bauerfeind *et al*., (2016), the illness has caused the small ruminant sector to suffer significant financial losses.

The propagation of Brucella in cells includes two stages: The stable stage and the exponential stage are the two phases of Brucella infection in cells. While the exponential stage is employed to reproduce under the proper environmental circumstances, this adaptive regulation is finished by molecular mechanism. The physiological state of the stable stage is beneficial for Brucella to adapt to the tough living conditions in the phagocytosis body. According to research, the VirB operon expresses strongly throughout the exponential growth stage but becomes repressed once the cell reaches the stable stage (Roop, 2003).

Brucella no longer needs to collect energy storage molecules since they have adjusted to their intracellular lifestyle (Chai *et al*., 2005). Opsonin-mediated phagocytosis cannot stop Brucella

from replicating in cells. Brucella's ability to adapt to intracellular survival depends in large part on the presence of cytochromes with high oxygen affinity, such as the cytochrome bc1 complex or hydroquinone( Bellaire *et al*., 2003).Acute, chronic, and incubation stages can be used to categorize Brucella infections (Figueiredo *et al.*, 2015).

The aforementioned procedures make up the incubation phase, during which no clinical symptoms are yet visible. In this stage, Brucella enters the host, moves past the mucosal barrier, and infiltrates dendritic cells and macro phages that are connected to the mucosa. Brucella must pass through the mucosal barriers of the digestive, genitourinary, and respiratory tracts in order to reach its target cells. This causes the spread of the organism to lymphoid and reproductive organs. Brucella that is still alive creates a place to reproduce within the phagocytes of the host. The infection has now been restricted to the area where the mucosal barrier was penetrated. The acute phase of the illness, during which Brucella disseminates in host tissues, starts if these phases of the infection process are effective (Rossetti *et al.*, 2013). Understanding the relationship between *Brucella* and various macrophage subsets is essential to comprehending Brucella cell viability and chronic infection (Barbier *et al*., 2005).

According to Mohammed and Sanousi, (2013), Brucella organisms use a number of strategies to prevent phagolysosome fusion, thwart phagocytes' ability to kill bacteria, and decrease the myeloperoxidase $H_2O_2$ halide system. They enter the phagosome, block the fusion of the phagosome and lysosome, and prevent the death of the host cell through apoptosis. They spread to various organs, particularly into the cells of the reticulo endothelial system, liver, urogenital tract, spleen, and skeletal muscle where they cause granulocytic inflammation with or without necrosis or caseation (Mustafa and Bedore, 2019).

### *2.7.3. Survival outside host cell*

For some time, *Brucella* could survive in the environment. According to Saegerman *et al*., (2010), cold temperatures and moisture outside the mammalian host are beneficial, while high temperatures, dryness, and direct solar exposure are detrimental. The capacity of the organism to survive in its environment is influenced by temperature, humidity, and pH (Radostits *et al*., 2007). The capacity of the organisms to survive in the environment, which raises the risk of infection in animals that are prone to it (Islam *et al*., 2013).

## 2.8. Host Immune Response against Brucellosis

However, investigations on ruminants and humans are required to describe the abnormalities in host immunological response brought on by Brucella (Baldwin and Parent, 2002).

### *2.8.1. Innate immune response*

The innate immune system will serve as the first line of defense for the host, stopping the microbe from reproducing, lowering its initial population, and eliminating it in addition to setting the stage for an efficient adaptive immune response (Higgins, 2015). It is essential for both impeding bacterial initial replication and affecting the emergence of a defense enhancing adaptive immunological response Atluri *et al*., (2011). The phagocytosis of pathogens by cells like neutrophils, macrophages, and DC, death by natural killer (NK) cells, secretion of cytokines and chemokines, recognition of molecules typical of a microbe PAMPs by PRRs, and activation of the complement system are all parts of this first line of defense (Diacovich and Gorvel, 2010).

**Neutrophils:** One of the first cells to react to the invasion of gram-negative bacteria is the neutrophils. Brucella is readily internalized by marine, human, and bovine neutrophils, who internalize far more bacteria than macrophages (Barquero *et al*., 2007). Normally, neutrophils are crucial to the innate immune response to bacterial infections; however, Brucella only slightly activates neutrophils and has the capacity to evade their killing processes. Neutrophils suppression by monoclonal antibody therapy did not affect the bacterial burden in the spleen, demonstrating in murine experiments that neutrophils play a little role in Brucella infection (Barquero *et al*., 2009).

Since hepatic cell apoptosis was significantly increased by stimulation with supernatants from Brucella infected neutrophils, human neutrophils have also been implicated in potential mechanisms of tissue damage during liver brucellosis. In humans, the persistence of Brucella in the neutrophils during early infection has been observed, suggesting that the transportation of Brucella to lymphoid tissues can be mediated by neutrophils (Delpino *et al*., 2010).

**Macrophages:** According to (Figueiredo *et al*., 2015), the host species, the Brucella species, and a variety of characteristics unique to the sick person all affect how effective these direct and indirect mechanisms are against Brucella. In order to kill pathogens directly, macrophages use a variety of techniques, such as phagocytosis and autophagy, which are then degraded by

hydrolytic lysosomal enzymes, an oxidative burst, which is then killed by reactive oxygen and nitrogen species, and antimicrobial peptides with a variety of mechanisms of action (Levine *et al*., 2011) LPS, TNF-, and IFNs all work to activate macrophages (Barquero-Calvo *et al*., 2009), which increases the macrophages' capacity to kill bacteria.

***Dendritic cells (DCs*)**: The majority of cells that deliver antigens to immature T lymphocytes are DCs. As a result, DCs serve as a link between innate and adaptive immunity and play a crucial role in determining the nature of a protective Th1 response (Billard *et al*., 2005). TLR signaling works to fuel the adaptive immune system by triggering phagocytes' bactericidal capabilities, inducing cytokine release, and improving DCs' antigen-presenting abilities (Atluri *et al*., 2011).

*2.8.2. Adaptive Immunity*

According to Ko and Splitter, (2003), the antigen-specific identification of pathogens by T- and B-lymphocytes and antibody secretion is the definition of adaptive immunity. Three main pathways are used by the adaptive immune response to try to control Brucella infection. IFN-, which is generated by CD4+ and CD8+ T cells, first activates macrophages and increases their potential to kill bacteria. Second, infected macrophages are directly killed by cytotoxic CD8+ T cells. Third, according to Skendros and Boura, (2013), B cells release an antibody that has little impact on how an infection turns out but is helpful for diagnosing sickness. As shown in Figure2: immune defense line, plays a very important role in the process of protecting the body from pathogens.

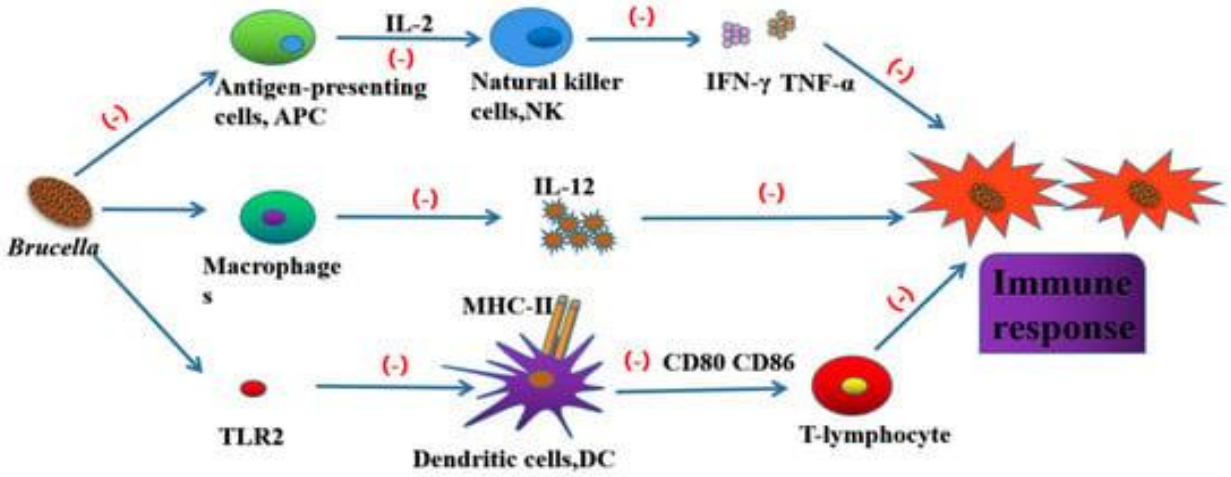


Figure 2: *Brucella* interferes with immune recognition and response of host, (Oliveira *et al., 2008)*

## 2.9. Brucellosis in Small Ruminants

Animals that generate food, such as cattle, sheep, goats, camels, and other animals, are more susceptible to brucellosis than other species, including humans (Ghanem and El-khodery, 2009) The infection normally causes placentitis in sexually developed animals' reproductive systems, followed by abortion in the female during the latter third of pregnancy and epididymitis and orchitis in the male. Because of the disease's high morbidity, sexually mature animals suffer large losses in productivity and reproduction (Pappas *et al*., 2006).Pastoral flocks may be more likely to encounter other possibly infected flocks and become exposed to regionally or seasonally numerous illnesses due to their dynamics and frequent movement (Megersa *et al*., 2011).

One of the three biovars of *Brucella melitensis* is the main cause of brucellosis in sheep and goats, with the exception of *Brucella ovis* infection. Zoonotic pathogens that may infect people include *B. melitensis, B. abortus*, and *B. suis*. (uterus, epididymides and testes), as well as from arthritic lesions (OIE, 2016). (Sheep and goats are *B. melitensis* preferred hosts, while goats are its traditional and natural host. Abortion and stillbirths, which often occur in the latter third of pregnancy after infection and typically only occur once in an animal's lifetime, are the predominant clinical sign of brucellosis in ruminants (Elzer *et al.*, 2002).

### *2.9.1. Status of small ruminant brucellosis in Ethiopia*

The US Navy Medical Research Unit’s Veterinary department first documented brucellosis in Ethiopia in 1970 (Meles and Kibeb, 2017). In Ethiopia, where it is endemic, it is quite common in small ruminants, cattle, camels, and pastoral and agro-pastoral regions (Gemechu, 2017). It affects a variety of animal species, including humans, but is particularly common in cattle, sheep, goats, and other food-producing animals (Ashagirie *et al.*, 2011). According to reports, communal usage of grazing pasture by farmers results in a substantially greater frequency of small ruminant brucellosis than flock/herd based on clan. This might be as a result of the communal grazing and irrigation systems mixing animals from different habitats (Yibeltal, 2005).

The infection frequently causes placentitis followed by abortion in pregnant females, commonly during the latter third of pregnancy, and epididymitis and orchitis in males, localizing in the reproductive system in sexually mature animals (Yohannes *et al*., 2013). Because of the disease's high morbidity, sexually mature animals suffer large losses in productivity and reproduction (Tsegaye *et al*., 2015).

The zoonotic pathogens *B. melitensis, B. abortus, and B. suis* may infect humans. Immuno-suppressed people may get infections from *B. canis* (Lemu D *et al*., 2014). As indicated table1: studies on small ruminant brucellosis in Ethiopia have shown that seroprevalence of the disease varies from location-to-location (Bekele *et al*., 2011). This variation in sero-prevalence may be caused by variations in animal production and management systems as well as a reasonable variation in the agro-ecological conditions of the study locations. According to reports, community grazing pasture use by farmers' results in considerably higher rates of small ruminant brucellosis than clan-based flock/herd segregation regions (Yibeltal, 2005).

Table 1: Sero-prevalence of small ruminant brucellosis in different regions of Ethiopia.

| Location | Test used | Sample size | Over all prevalence (%) | Reference |
|---|---|---|---|---|
| Afar and Somali pastoral of East | RBPT, I ELISA | 2000 | 1.9,9.9 | Teshale *et al*. (2006) |
| South East Somali and Oromia | RBPT | 510 | 9.6 | Gumi *et al*. (2013) |
| Boku export farm | RBPT CFT | 2030 | 0.8,0.6 | Girmay*et al*. (2013) |
| Yabellodistrict Borena zone | | | 8.1 | Wubishet *et al (*2018) |
| Amibara District, Afar, Ethiopia | RBPT, CFT | 226 | 12.4, 7.52 | Muluken (2016) |
| East Showa, Oromia region | RBPT | 384 | 1.56 | Lemu *at el*. (2014) |
| Gamo Gofa southern Ethiopia | RBPT, CFT | 1000 | 4.3, 3.7 | Melese (2016) |
| pastoral and agro pastoral low lands of Ethiopia | RBPT, CFT | | 5.2, 2.2 | Sintayehu *et al*. (2015) |
| Yabello district of Borena Zone | RBPT, CFT | | 8.5, 3.6 | Wakene *et al*. (2017) |
| Yabello district of Borena Zone | RBPT, CFT | 384 | 2.34,1.56 | Debassa *et al*. (2013) |
| Dire Dawa | RBPT, CFT | 384 | 9.38,9.11 | Negash *et al*. (2012) |
| Arsi and East Shewa | RBPT, CFT | 384 | 4.6 | Abiot *et al*. (2015) |
| Jijiga | RBPT, CFT | 730 | 1.51 | Bekele (2011) |
| Borena | RBPT, CFT | 882 | 2.6 | Edao *et al*. (2020) |

## 2.10. Status of Human Brucellosis in Ethiopia

As shown in Table 2; comparatively little is known about human brucellosis in Ethiopia, and much less is known about the risk factors for human infection. Studies were carried out in high-risk groups in the Amhara Regional State, including farmers, veterinarians, meat inspectors, and technicians who perform artificial insemination (Mussie *et al*., 2007). By screening sera from 238 and 336 people, respectively, (Kassahun *et al*., 2006; and Kassahun *et al*., 2007), reported a seroprevalence of 5.30%, and 4.8%. According to (Ragassa, 2009), the difference in prevalence between studies and may be the result of different milk consumption patterns among study population and test technique sensitivity.

Table 2: Sero-prevalence of human brucellosis in different regions of Ethiopia

| Location | Prevalence | Source |
|---|---|---|
| Jimma University hospital | 3.6 | Tolosa *et al*. (2007) |
| Borana | 34.9% | Genene *et al*. (2009) |
| North Western Ethiopia | 2.6% | Animut *et al*. (2009) |
| Metema | 3.0% | Genene *et al*. (2009) |
| Hamer | 29.4% | Genene *et al*. (2009) |
| East Shoa | 6.0 | Mekonen (2016) |
| Abattoir workers at Debrezeit and Modjo export abattoir | 1.34 | Tsegay *et al*. (2017) |
| Hamer | 16.5 | Hailu (2017) |
| Yabello and Dire | 25.6 | Wubishet *et al*. (2018) |
| Borena | 2.6 | Edao *et al*. (2020) |

### *2.10.1. Economic impact of brucellosis*

According to Perry and Grace, (2009), brucellosis is frequently regarded as one of the most economically significant zoonosis in the world. The analysis of the economic effects of brucellosis is divided into three main sections, with a focus on the low-income nations of Asia and Africa. The first outlines a general framework for evaluating the economic costs and benefits of various disease prevention initiatives. The second section thoroughly examines any available

animal, human, and combined burden estimates from research done in these areas. When available, estimates of various expenditures related to brucellosis sickness and its control are given in the third part (Zamri Saad and Kamarudin, 2016).

Premature miscarriages, decreased fertility, and decreased milk production in animals are caused by Brucellosis, causing significant economic losses (WHO, 2006). Visible losses include animal miscarriage, decreased milk production, lost draught power, decreased weight increase due to chronic illnesses and malnutrition, early mortality or culling of unproductive stock, veterinary expenditures for diagnostics and vaccinations, and lowered animal welfare. According to (Franc *et al*., 2018). Brucella spp. can significantly lower herd production in endemic locations, putting farmers' livelihoods and access to extra meat, dairy, and progeny from their animals at risk.

*2.10.2. Public health importance of the diseases*

According to (Makita *et al*., 2011), human brucellosis is a multisystem illness that can be fatal and has a wide range of vague symptoms. Due to acute and chronic sickness, physical disability, and manpower loss, brucellosis in humans is a serious public health risk that has an impact on social and economic development in many nations (Corbel, 2006). Animals, particularly goats and sheep, are the primary source of nearly all human cases of brucellosis (Bayu, 2018).

Six Brucella species, including *B. melitensis, B. suis, B. abortus, B. canis, B. ceti*, and *B. pinnipedialis,* can infect people. *B. melitensis* is the most invasive and pathogenic species for humans, and *B. suis, B. abortus, and B. canis* are the next most dangerous species. The Centers for Disease Control and Prevention of the United States have also identified *B. melitensis, B. suis, and B. abortus* as possible bioweapon. This is because all three species are extremely contagious and easily aerosolize (Asnake *et al*., 2017).

Usually, it has a work-related or food-borne etiology. The three types of unpasteurized milk or milk products most frequently linked to food-borne brucellosis are camel, small ruminant, and cow milk. Due to the low pH of the latter items, Brucella persists more in soft cheeses, butter, and ice cream than in hard cheeses and yogurt (Godfroid *et al.*, 2005). By direct contact with the skin or mucosa during parturition or abortion, by direct contact with specimens containing Brucella spp. handled in a lab, or by direct contact with infected animals (FAO, 2006).

As shown in Figure 3: Six Brucella species, including *B. melitensis, B. suis, B. abortus, B. canis, B. ceti*, and *B. pinnipedialis,* can infect people. Fever, asthenia, myalgia, arthralgia, sweats, lymphadenopathy, hepatomegaly, and splenomegaly are the most typical signs and symptoms of human brucellosis (Pal, 2007). The most prevalent types of localized illness are *Oste-oarticular* symptoms, such as peripheral arthritis, sacroiliitis, and spondylitis (Madkour, 2001).

## 2.11. Diagnostic Techniques of Brucellosis

It is very difficult to make a diagnosis based on clinical signs despite abortions in the third trimester being indicative of brucellosis; this is because other infectious diseases such as leptospirosis, rift valley fever and listeriosis can also cause abortion storms (Mfune *et al*., 2021). Diagnostic tests are applied for the following purposes: confirmatory diagnosis, screening or prevalence studies, certification, and, surveillance in order to avoid the reintroduction of brucellosis (in countries where brucellosis is eradicated) through importation of infected animals or animal products (Godfroid *et al*., 2011).

### *2.11.1. Direct diagnosis*

**Microscopic staining:** Bacteria in a smear demonstration served as confirmation. Smear created with the Modified Ziehl-Neelsen's, (MZN) stain from fetal abdominal, placenta, fetal stomach fluid, and vaginal discharge. You can obtain an impression smear from recently cut and blotted tissue. The bacteria show as red intracellular coco bacilli when allowed to air dry and heat fix smears, whereas the majority of other bacteria stain blue (Nielsen *et al*., 2004). They stain red on a blue backdrop, but are resistant to decolorisation by mild acids. Due to the fact that Brucella organisms stain red on a blue background when observed under a light microscope, the Stamp's modified Ziehl Nelsen staining is used to detect them (Mfune, 2015).

**Culture:** The isolation or identification of Brucella organisms in culture from the diseased patient is the sole "gold standard" approach for determining the presence of brucellosis (Gebretsadik, 2016). Human blood, bone marrow, joint fluid, semen, and cerebral spinal fluid are acceptable clinical samples for culture, as are aborted fetuses, fetal membranes, vaginal secretions, sperm, milk, and hygroma fluid in animals. The most typical cultivation method for possible species of Brucella from tissue samples is Farrell's medium. These media include a number of antibiotics that can stop other bacteria detected in clinical samples from growing (Higgins, 2015). Growth appears after 1-2 days, and after 2-3 weeks of incubation, it is only

regarded as negative. Brucella are finicky slow growers; therefore, the culture method is time consuming and has limitations since they may easily be overtaken by other bacteria, which frequently results in misdiagnosis (Mfune, 2015).

**Polymerase chain reaction:** The only procedure that provides a diagnosis with confidence is Brucella species isolation or PCR detection of Brucella species DNA. Due to the low bacterial density in clinical samples and the inhibitory effects of the matrix, direct detection of Brucella DNA in brucellosis patients is difficult. Basic sample preparation techniques should concentrate the bacterial DNA template and reduce inhibitory effects (Colmenero, *et al*., 2010).

*2.11.2. Indirect Diagnosis*

**Rose Bengal Plate test (RBPT):** Rose and Roekpe (1957) created the Rose Bengal test (RBPT) to distinguish specific Brucella agglutinins from non-specific components for diagnosing bovine brucellosis. According to (Gebretsadik, 2016), this test is widely recognized as the screening method of choice for brucellosis in small ruminants and cattle. The buffered Brucella antigen tests, of which the RBPT is one, are based on the idea that IgM antibodies' capacity to bind to antigen is significantly diminished at low pH levels. The test involves mixing droplets of dyed antigen and serum on a plate, and any agglutination that occurs denotes a positive response. Although the test is a great screening tool, it may be oversensitive for diagnosis in some animals, especially those who have had vaccinations (Asnake *et al*., 2017).

**Complement fixation test (CFT):** The most popular confirmatory test is the complement fixation test (CFT), which is also advised by OIE. According to (Gebretsadik, 2016), the CFT is based on the identification of certain IgM and IgG1 antibodies that fix complement. The CFT has high sensitivity and specificity, but it is a difficult procedure to carry out and necessitates top notch lab facilities and skilled personnel. It can be highly pleasant if these are accessible and the test is conducted periodically with careful attention to quality assurance (Corbel, 2006).

**Enzyme linked immuno-Sorbent assay:** For usage in smaller facilities, the Enzyme linked ImmunoSorbent assay (ELISA) is more suited than the CFT, and ELISA technology is being employed for the diagnosis of a variety of illnesses in both animals and people. Although ELISAs may be used to analyze blood from all animal and human species in theory, findings may differ between labs depending on the precise approach employed (Corbel, 2006). It is a great way to identify acute and chronic illness stages and screen huge populations for antibodies to Brucella (Gall e*t al*., 2003).

When other tests are negative and the case is surrounded by strong clinical suspicion, it is the test of choice for complex, local, or chronic situations. It has a high sensitivity and specificity and can detect total and individual IgG, IgA, and IgM immunoglobulins within 4-6 hours. The Enzyme Linked Immuno-sorbent technique (ELISA) has gained popularity as a recognized technique for the serological diagnosis of brucellosis. This provides for a better evaluation of the clinical condition because it analyzes IgG, IgA, and IgM antibodies (Araj, 2010).

The serologic diagnosis of brucellosis in sheep, goats, and pigs has been performed using the indirect ELISA (i-ELISA). Additionally, it has been applied to diagnose conditions using cow's milk or serum. According to (Gall and Nielsen, 2004), i-ELISA has been used most frequently for smooth LPS Brucella spp. and is sensitive and specific for *B. abortus or B. melitensis.* The i-ELISA's specificity ranges between 93.8% and 100% and its sensitivity ranges from 96 to 100% (Godfroid, 2010).

### *2.11.3. Clinical manifestation of brucellosis*

As a huge number of germs are shed in the birth fluids or fetus, placenta, and abortion secretions of infected females, healthy animals can be exposed to *Brucella* infection in a variety of ways (Banai, 2007). Any herd with a history of late-term abortions, infertility, orchitis, epididymitis, stillbirths, neonatal mortality, and hygroma might be questionable (Poester *et al*., 2010).

Infected females who are born in a region with an outbreak tend to have fewer abortions than other women. This explains why abortion rates are high in herds that have recently contracted the disease and comparatively low in herds where the illness is enzootic. A crucial preference location for Brucella organisms is the udder. According to (Radostits, infection in lactating, non-pregnant goats is likely to result in colonization of the udder and excretion of *Brucella* organisms in the milk. In several tropical nations, hygromas, which often involve the leg joints, are a common symptom of brucellosis; the fluid around the hygroma is frequently contaminated with Brucella (OIE, 2004).

Depending on the severity of the lesions, infection in men might cause either temporary or permanent infertility. When orchitis does emerge, it generally affects just one testis, though both might be affected. It is occasionally accompanied by vesiculitis and epididymitis. Total testicular necrosis is the result of many foci of necrosis coming together (Foster *et al*., 2007).

Since human brucellosis mimics many other illnesses, such as malaria, typhoid, rheumatic fever, joint ailments, and other conditions producing pyrexia, it presents a wide clinical spectrum and a variety of diagnostic challenges (Andriopoulos *et al*.,2007) Brucellosis commonly presents in people as a variety of non-specific clinical symptoms. The disease's acute and chronic symptoms can cause large workday losses, which can have a negative impact on the socioeconomic standing of the afflicted and their families (Franc *et al*. ,2018). Pregnant *Brucella* infected women frequently experience spontaneous abortion throughout the first and second trimesters of their pregnancies (Dubie *et al*., 2014).

*2.11.4. Treatment*

Treatment regimes for human brucellosis require combination of antibiotics like rifampicin or gentamicin and doxycycline twice daily is the combination most often used, and appears to be efficacious (Yohannes *et al*., 2013). The combination of doxycycline with streptomycin is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis (Seleem *et al*., 2008). Pregnant women can safely be treated for brucellosis with rifampicin with or without Trimethoprim-sulfamethoxazole (960 mg twice daily for 6 weeks) (Ozbay and Inanmis, 2005: Baldwin and Parent, 2002).

*2.11.5. Control and Prevention*

A disease's influence on human health and its financial costs are minimized by an animal management program (Godfroid *et al*., 2005). Animal items (including bone marrow) that are uncooked or undercooked and dairy products that have not been pasteurized should not be ingested. When helping with a birth, conducting a necropsy, or slaughtering an animal for sustenance, precautions should be taken to prevent skin contamination, inhalation of contaminants, and unintentional ingesting of contaminants. Risky agricultural practices including skinning aborted fetuses or breaking the umbilical cord of newborn cattle with the teeth should be avoided (WHO, 2009). The establishment of the various epidemiological settings within a nation, or even a region or district, should be the first step in any plan for the control or eradication of brucellosis, (Minas 200; and Blasco and Molina, 2011).

In small animals, control measures must include hygiene at lambing and the disposal of infected or reactor animals. Separate pens for lambing ewes, which can be cleaned and disinfected, early weaning of lambs from their dams, and their environment and vaccination, are recommended. In endemic areas, all placentas and dead fetuses should be buried as a routine practice. The need to

test and cull, introduced and resident animals likely to be carriers is recommended, but difficult to be effective because of the inaccuracy of the tests. Because of the possibility that lambs may be infected at birth and carry the disease for life, it may be more economical to dispose of the entire flock (Radostits *et al*., 2007). Animal health services provided by the public sector in sub-Saharan Africa have significantly reduced over the past 20 years as a result of a number of causes, including shrinking government funding, especially for operational expenses of disease control. As a result, many sub-Saharan nations do not execute programs that call for coordinated monitoring, information sharing, and the use of control measures (McDermott and Arimi, 2002).

**Vaccination** Comparatively to most other categories of non-sporing pathogenic bacteria, they have a very high ability to tolerate inactivation under natural settings. No vaccine has yet been authorized for the prevention of human *brucellosis*, despite significant research efforts Skalsky K, *et al*. (2008). An efficient way to lower the frequency of *brucellosis* among entire flocks or flocks in low-income countries and/or endemic nations is to provide the *B. melitensis* REV 1 vaccine, which is an attenuated strain of *B. melitensis* OIE (2009). Compared to animals who have received vaccinations, unvaccinated animals have a higher rate of abortions and shed significantly more germs from the tissues and birth fluids. The relevance of immunizing *small ruminants*, particularly in low-income countries, is increased by the aforementioned Corbel *et al*. (2006).

# 2. MATERIALS AND METHODS

## 3.1. Description of the Study Area

This study was conducted in West Guji Zone, which is located in the Southern part of Ethiopia. The capital town of the zone is Bule Hora, which is 467 km away from both the regional and national capital city of Ethiopia (Addis Ababa). The West Guji zone is bounded by SNNP and Sidama Regional State in the north, the Southern Ethiopia state in the west, the Guji zone in the East, and the Borena zone in the south. Geographically, the zone is located between 37° 56' and 38° 31' East longitude and 5° 26' and 5° 52' North latitude, at altitude ranging from 1500 and 2400 m above sea level.

Out of the nine districts that make up West Guji Zone, this research focused on Dugda Dawa and Suro Berguda. According to the Zone Livestock Resource and Development Office (2019), the West Guji zone 2,432,796 cattle, 1,230,518 sheep, 1,735,586 goats, 696,230 equines (horse 347,101, donkey 290,422, mule 58,707), and 2,212,928 poultry.

**Dugda Dawa**: The district is one of the pastoralist areas of the zone located 498 kilometers from Addis Ababa, and 30 kilometers from the zonal town of Bule Hora. The climate consists of 30% mid-altitude and 70% lowland, with an average annual temperature of 25.7–33°C and annual rainfall between 430–500mm. According to the District Livestock Resource and Development Office (2019), there are 118,272 chickens, 195,998 cattle, 6,348 sheep, 133,008 goats, 42,076 equines (38,694 donkeys, 1796 mules, and 1,568 horses), in the livestock population.

**Suro Berguda:** The District lies 495 km south of Addis Ababa. The average yearly temperature ranges from 19.8 °C to 28.7 °C. The average annual rainfall is between 450 and 500 millimeters, with lengthy and brief rainy seasons. The short rainy season lasts from mid-September to mid-November, whereas the long rainy season lasts from March to May. The district is located between 1500 and 2000 meters above sea level. The population of animals consists of 100,214 chickens, 27,694 donkeys, 1696 mules, 3,348 sheep, 112,005 goats, 30,076 horses, and 155,998 cattle

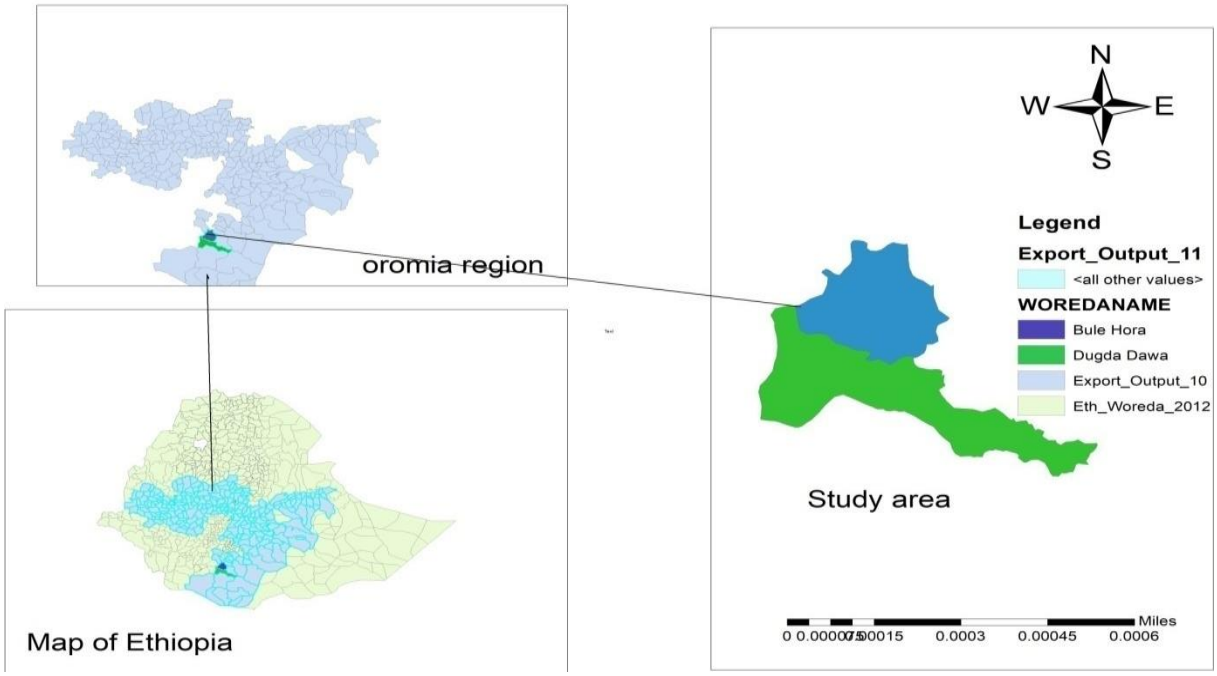


Figure 3: Map of Study Area

## 3.2. Study Populations

Sheep and goats that are 6 months of age and older are managed under extensive and semi-extensive pastoral production systems in the Dugda Dawa and Suro Berguda districts of West Guji and were designated as the study population. Samples were taken from human patients who had visited Bule Hora University Teaching Hospital and had frequent interaction with small ruminants.

## 3.3. Study Design

The study used a cross-sectional design in order to estimate the prevalence of brucellosis in small ruminants and humans in these two districts of West Guji zone, to identify potential risk factors linked to seropositivity, and gauge small ruminant owners' awareness of the disease's significance for public health, the study was carried out in Dugda Dawa and Suro Berguda. Following the collection of a list of the districts in the West Guji zone, two districts (Dugda Dawa and Suro Berguda) were chosen from a total of nine districts based on potential of small ruminant population and their proximity to a local veterinary laboratory. Target Kebeles (Jigesa, Arbicho, and Mokonisa from Dugda Dawa and Danbala Hara and Sororo Malka Jawe

from Suro Berguda) in the two districts were chosen based on their small ruminant populations, while communities were chosen more conveniently based on their ease of access by car.

## 3.4. Sampling Method and Sample size Determination

### *3.4.1. Sampling Method*

A combination of random, purposive, and convenient sampling techniques were applied for the selection of study animals (sheep and goats), study areas (district and kebele), and study villages respectively. Thus, the study districts and Kebeles were chosen purposively based on the geographic proximate of the location to the regional Veterinary laboratory and small ruminant population; Individual sheep and goats were selected using a systematic random sampling technique, and 52 human were chosen at random from the Bule Hora University Teaching Hospital to assess the public health importance of brucellosis in humans, according to Wubishet *et al*., (2018) the patient based on the presence of symptoms (fever, sweat, anorexia, malaise, weight loss, depression, headache, and joint pains) resembling that of brucellosis, on the history of consumption of unpasteurized milk, raw meat, contact with aborted animals or aborted materials, and handling of parturient animals (those who came from the Dugda Dawa and Suro Berguda districts).

Relevant individual's general information and flock level information such as sex, age flock size presence or absence of reproductive problems such as abortion history were also recorded. Based on literature of Solomon *et al*. (2007), flock size was grouped into three; large, medium, and small based on the number of animals in the flock. So that number of shoats greater than 30 (>30), number of shoats greater than or equal to 10 (≥10) but less than thirty (< 30), and number of shoats less than or equal to ten (≤ 10); were classified as large, medium and small flock size respectively.

### *3.4.2. Sample size determination*

**Small Ruminants:** Sample size was determined using the formula presented by Thrusfield (2018). Previous report prevalence of 6.1 and 9.2 % brucellosis in sheep and goats, respectively reported by Wubishet *et al*. (2018) were used to calculate sample size. Given a target absolute accuracy of 5% and a confidence interval of 95%, the required sample size was determined as:

$$N = \frac{1.96^2 \times Pexp(1-Pexp)}{d^2} = 216$$

Where, N is the sample size, P is the prevalence, and D is the required degree of accuracy (5%).

Based on the calculation above, the estimated sample sizes for sheep and goats were 88 and 128 respectively. To improve the accuracy of the result, the calculated sample size was increased to 192 goats and 132 sheep. So, 324 shoat in total were chosen for this investigation. Age, sex, parity, and a history of reproductive issues (abortion and retained fetal membrane) were documented for each animal.

**Humans:** fifty-two human samples were purposively collected from those patients who had visited Bule Hora University Teaching Hospital.

## 3.5. Blood Sample Collection

### *3.5.1. Humans*

Prior to collecting samples from humas, participants or their parents or legal guardians were informed of the study's objectives and given the opportunity to agree. Blood sample collection and humans' samples were both recorded. In humans, verbal consent was made before a sample of 5-7 ml of peripheral blood was taken from each respondent. Blood samples from the human subjects were taken by nurses working at the Teaching Hospital of Bule Hora University. To facilitate blood clotting, the sample was left to stand on the rack overnight. After that, the sera were poured into sterile cryovials and kept at -20°C until a lab test was done

### *3.5.2. Small ruminants*

After the animals were appropriately secured, an 8ml blood sample was aseptically taken using sterile plain vacutainer tubes from the jugular vein. To facilitate blood clotting, the sample was left to stand on the rack for the duration of overnight. After that, the sera were poured into sterile cryovials and kept at -20°C until a lab test was done. During blood collection, owners provided information regarding the size of the flock, the type of the animals, their ages and sexes, the

occurrence of abortions, and retain of fetal membranes, which was noted in the sample collection format were registered Annex (I).

## 3.6. Questionnaire Survey

The owners of small ruminants were interviewed using a semi-structured questionnaire about potential risk factors such as management practices, breeding practices, watering practices, age of the animals, sex and educational levels of respondents, awareness of the causes of reproductive disorders, keeping of animals in the dwelling house, and limitations on livestock production. The format, created for this purpose is used to record any detected reproductive abnormalities. The presence of symptoms suggestive of brucellosis in humans (fever, sweat, anorexia, malaise, weight loss, depression, headache, and joint pains), the practice of consuming unpasteurized milk, contact with aborted animals or aborted materials, and handling of parturient animals were all taken into consideration when determining the disease's significance for public health recorded at format forwarded.

## 3.7. Serological Diagnostic Tests

### *3.7.1. Rose Bengal Plate test (RBPT)*

All sheep and goats' serum samples were initially screened using a Rose Bengal Plate test using 75:25 µL, sera: antigen ratio, whereas, a 30:30 µL ratio (antigen: serum ratio) was used for humans. The sera and antigen kept in the refrigerator were taken out from the refrigerator and left at room temperature. Briefly, RBPT antigen of 25 µL was added onto a glass slide next to 75 µL of sheep or goats' serum. The antigen and the test serum were mixed thoroughly in a plastic applicator, shaken for 4 minutes and then the result was read immediately as described in (Annex VI). Any observed agglutination by the naked eyes was considered to be a positive reaction. Agglutination was recorded as 0, +, ++ and +++. A score of 0 indicates the absence of agglutination; + indicates barely visible agglutination; ++ indicates fine agglutination, and +++ indicates coarse clumping. Those samples with no agglutination (0) were recorded as negative while others were recorded as positive.

*3.7.2. Indirect enzyme-linked Immunosorbent assay (I-ELISA)*

Subsequently, the positive reactors to RBPT were re-investigated using indirect enzyme-linked immunosorbent assay (I-ELISA) for further confirmation and used to detect antibodies against Brucella at Yabello Regional Veterinary Laboratory for small ruminants and simultaneously human sera process in Bule Hora University Teaching Hospital. The taste was conducted in a microplate coated with activated antigens following manufacturer's instructions. One hundred micro litters of pre-diluted sera and controls (1:400) were added in to the microtiter plate and incubated for 45 min at 21°C. The plate was then washed 3 times. Then 100 µL of conjugate was added to each well followed by covering of the plates before incubating it for 30 min at 21 °C. The plates were washed 3 times. Finally, 100 µL of substrate was added to each and incubated at 21°C for 15 min. Then 100 µL of stop solution was added to each well and the result was read at a wavelength of 450 nm. Results were expressed as the percentage of the ratio between the corrected sample OD and positive control OD. The taste conducted according to the manufacturer guide lines (Annex VII).

## 3.8. Data Management and Analysis

The collected data were entered into Microsoft Excel® 2010 Spread Sheet; variables that are hypothesized to be connected to the epidemiology of brucellosis in humans and small ruminants were noted. Animal owners and attendants provided information on issues with reproduction, animal age, and parity numbers. Data on a person's age, gender, how they consume animal products, how they handle aborted fetuses, and how they help animals during parturition were also recorded. Microsoft Excel was used to capture and code the data.

**Ethical Clearance:** Ethical Clearance for animal sampling was obtained from the Hawassa University Faculty of Veterinary Medicine and Ethical Clearance for humans was approved by the Research Ethics Committee and the letter of clearance was obtained from the Hawassa University Ethical Committee. The sample was taken after written informed consent was made with all study participants. All the rights of privacy and confidentiality of participants are protected.

# 4. RESULTS

## 4.1. Seroprevalence of Brucellosis in Small Ruminants

Out of 324 blood samples collected from sheep and goat populations in Dugda Dawa and Suro Berguda districts (192 caprine and 132 ovine); 34 (10.49%) tested positive by RBPT. Further confirmatory test conducted for positive reactors using I-ELISA, of these 23 samples (animals) were positive for brucella infection (7.1%). Out of 147 tested sera in Dugda Dawa district, 15 was positive by I-ELISA (10.2%) and out of 177 tested in Suro Berguda district, 8(4.5%) were positive by I-ELISA, as shown in (Figure3).

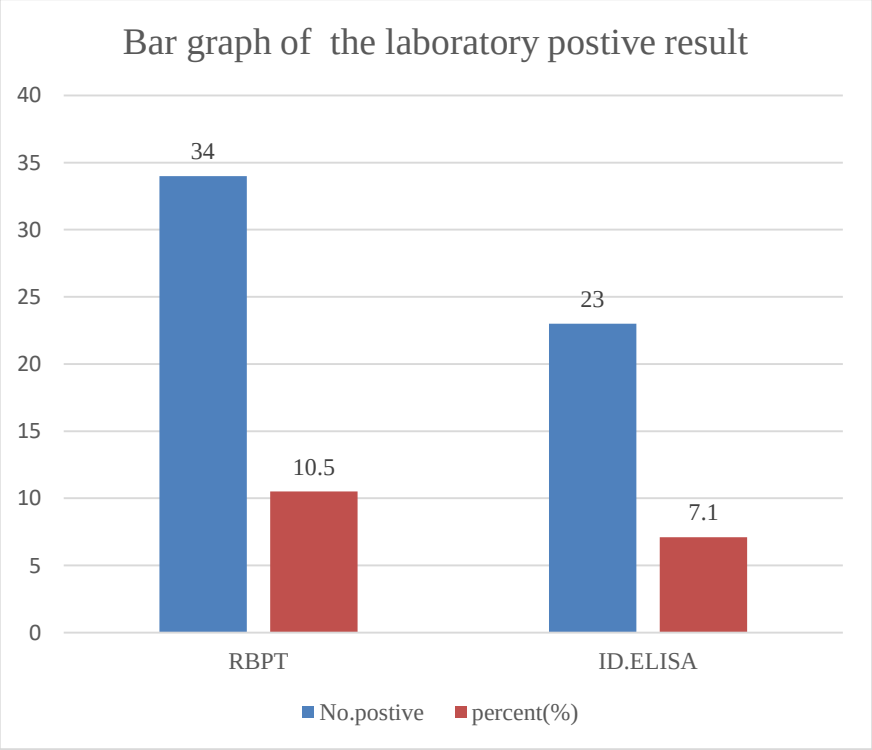


Figure 4: The overall sero-prevalence of small ruminants' brucellosis

## 4.2. Risk Factors Affecting Individual Animal Level Seroprevalence of Brucellosis

Descriptive analysis showing seroprevalence of small ruminants' brucellosis with associated risk factors including; district, Peasant Association, species, age, flock size, management, history of abortion, Body condition score, and parity numbers shown in Table 4. The individual animal level seroprevalence was higher in Dugda Dawa 15(10.2%) than in Suro Berguda 8(4.5%) and goats (8.85) than in sheep (4.54%) populations. The seroprevalence in the present study, in Table:4 shown that, the prevalence higher in females' animals (9.3%) than in males (2.75%), elders' animals (17.6%) than youngers (2.25%), larger flock size (26.6%) than smaller (1.37%), extensive management system (11.3%) than semi-extensive (3.4%), animals aborted (37.7%) greater than not aborted (2.2%), poor BCS (18.9%) greater than good BCS (2.4%) and animal with multiple parturition (25.5%) greater than non-parous (1.7%).

Table 3: Descriptive analysis of small ruminant brucellosis prevalence relative to different factors by I-ELISA

| Variables | Categories | No. of sampled | No of positive | Prevalence (%) |
|---|---|---|---|---|
| Districts | D. Dawa | 177 | 15 | 8.5 |
| | S. Barguda | 147 | 8 | 5.4 |
| Pa | Jigesa | 79 | 7 | 8.8 |
| | Arbicho | 67 | 7 | 10.4 |
| | Mokonisa | 31 | 1 | 3.2 |
| | D.Hara | 69 | 4 | 5.7 |
| | S/M/Jawe | 78 | 4 | 5.1 |
| Spp | Ovine | 132 | 6 | 4.54 |
| | Caprine | 192 | 17 | 8.85 |
| Sex | Male | 109 | 3 | 2.75 |
| | Female | 215 | 20 | 9.3 |
| Age | Young | 222 | 5 | 2.25 |
| | Adult | 102 | 18 | 17.6 |
| Flock size | Small | 145 | 2 | 1.37 |
| | Medium | 119 | 5 | 4.2 |
| | Large | 60 | 16 | 26.6 |
| Management | Se_extensive | 174 | 6 | 3.44 |
| | Extensive | 150 | 17 | 11.33 |
| Abortion | No | 279 | 6 | 2.2 |
| history | Yes | 45 | 17 | 37.7 |
| | Poor | 95 | 18 | 18.94 |
| BCS | Medium | 146 | 3 | 2.05 |
| | Good | 83 | 2 | 2.40 |
| | None | 229 | 4 | 1.74 |
| Parity | 1-3 | 48 | 7 | 14.58 |
| | Above 3 | 47 | 12 | 25.53 |

*4.2.3. Serological results of human brucellosis*

As shown below in Table 6, out of 52 serum samples of humans tested for RBPT only 13 were positives for agglutination test. 4 (16.6) males and 9 (27.2) females were, Analysis of laboratory results in Human sera indicates that human sera tested for I-ELISA 3(12.5 males and 7(21.2) females positive for this serological taste. The overall seroprevalence of human brucellosis among the blood samples tested was 19.2% in this study.

Table 3: Association of risk factors with brucellosis reactivity in humans

| Variables | Categories | No. of sampled | No.of positive | prevalence % |
|---|---|---|---|---|
| Districts | Dugda Dawa | 28 | 5 | 17.85 |
| | Suro Berguda | 24 | 5 | 20.83 |
| | Jigesa | 11 | 1 | 9.0 |
| Pa | Arbicho | 8 | 2 | 25 |
| | Mokonisa | 9 | 2 | 22 |
| | Danbala Hara | 11 | 2 | 18.2 |
| | Soror M/Jawe | 13 | 3 | 23 |
| Sex | Male | 24 | 3 | 12.5 |
| | female | 33 | 7 | 21.2 |
| Age | Adult | 33 | 9 | 27.9 |
| | Young | 19 | 1 | 5.26 |
| Help delivery | Yes | 37 | 8 | 21.6 |
| | No | 15 | 2 | 13.3 |
| Consume raw meat | Yes | 40 | 8 | 20 |
| consume raw milk | No | 12 | 2 | 18 |
| | Yes | 39 | 8 | 20.5 |
| Contact manure | No | 13 | 2 | 15.3 |
| | Yes | 37 | 7 | 18.9 |
| Wash hands with soap | No | 15 | 3 | 20 |
| | Yes | 25 | 2 | 8 |
| | No | 27 | 8 | 29 |

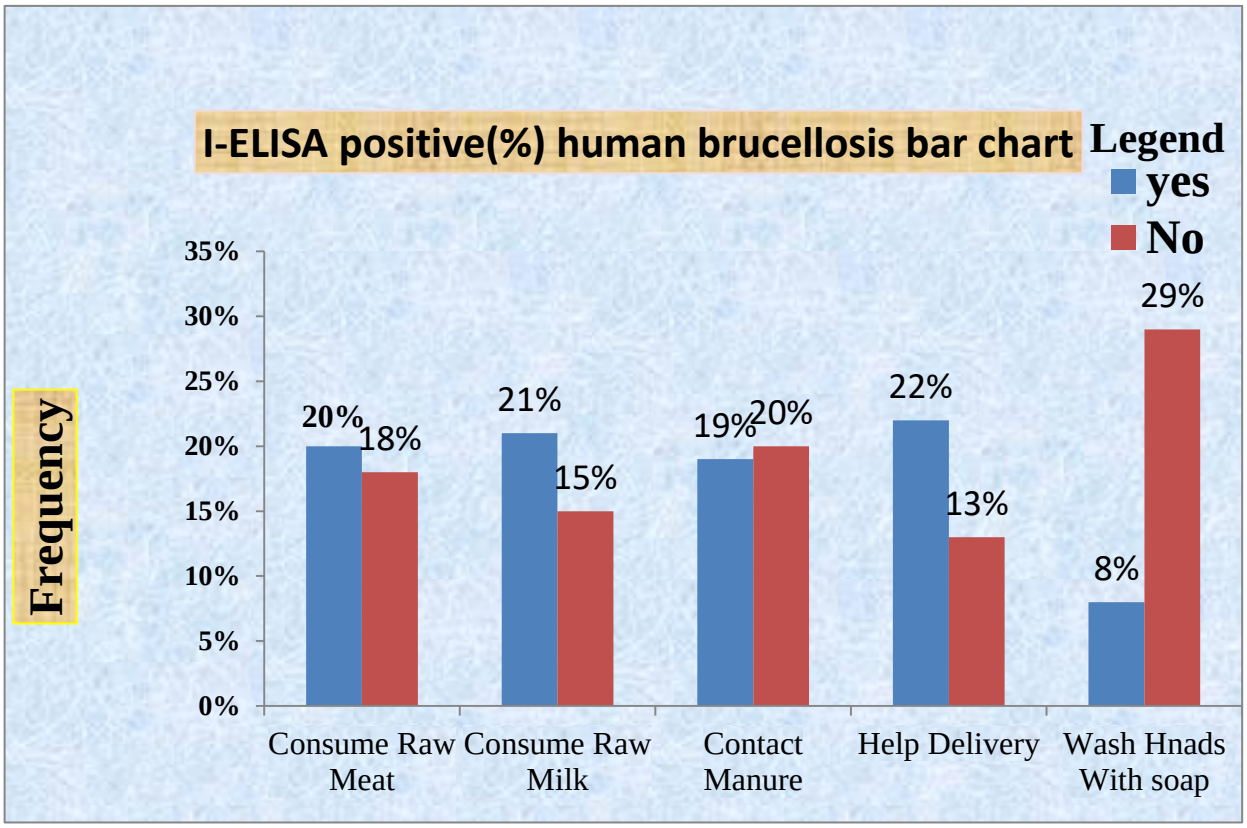


Figure 5: Laboratory result of human brucellosis using I-ELISA

### 4.3. Results of Questionnaire Survey

Interviewer administered face to face questionnaire was used to evaluate the knowledge and practice of the community on brucellosis. The questionnaire was pretested in the field. Finally, it was translated to local language Afan Oromo. As indicated in Table 7, a total of 50 proprietors of small ruminants from two districts and five Kebeles received the questionnaire. The findings showed that just 11 (22%) of these individuals had separate house for your sheep and goats**,** while the remaining 39 (78%) had no separate house for your sheep and goats. 46 (92%) of the pastoralists surveyed consume raw milk. Similarly, 37 (74%) of respondents were unaware that consuming raw milk may transmit brucellosis. The majority of 41 volunteers (82%) aided parturition without safety precautions. Regarding methods of handling and treating aborted fetuses and placentas, 29 (58%) left the placenta on the ground, while 21(42%) fed it to dogs. In response to the question of what they did with their animals when a they abort, 14 (28%) said they sold them, 20 (40%) said they treated animals themselves, and only 16 (32%) said that they brought the afflicted animal to a local veterinary clinic.

Table 4: Awareness of small ruminant owners about brucellosis

| Variables | Categories | Numbers | Percentage |
|---|---|---|---|
| Do you use separate house for your sheep and goats? | Yes | 11 | 22 |
| | No | 39 | 78 |
| Do you know disease which cause abortion? | Yes | 18 | 36 |
| | No | 32 | 64 |
| Do you separate aborted animal from others? | Yes | 0 | 0 |
| | No | 50 | 100 |
| Do you assist lambing or kidding? | Yes | 50 | 100 |
| | No | 0 | 0 |
| Do you assist by your bare hand? | Yes | 41 | 82 |
| | No | 9 | 18 |
| Do you wash your hands with soap and water after kidding or lambing? | Yes | 23 | 46 |
| | No | 27 | 54 |
| Means of meat consumption | Raw | 46 | 92 |
| | Cooked | 4 | 8 |
| Means of milk consumption | Raw | 37 | 74 |
| | Fermented | 13 | 26 |
| | Boiled | 0 | 0 |
| | Feed to dog | 21 | 42 |
| Means of managing aborted foetus/FM | Bury/Burn | 0 | 0 |
| | Leave on ground | 29 | 58 |
| Purpose of animal when aborted | Treated by their own | 20 | 40 |
| | Take to vet. clinic | 16 | 32 |
| | Sell | 14 | 28 |
| Existence of abortion and retained foetal membrane in your flock | Yes | 50 | 100 |
| | No | 0 | 0 |

# 3. DISCUSSION

In the study districts, the overall prevalence of small ruminants was 7.1%, while the prevalence at the species level remained at 4.54% for sheep and 8.85% for goats. This result is generally in line with the findings of Wubishet *et al*. (2018) and Ashenafi *et al*. (2007) from the Borena zone in the Oromia region and Afar region, respectively; And they reported an overall prevalence of 8.1%, with species level prevalence of 9.2% and 6.1% in goat and sheep in Borena zone and overall prevalence of 4.8%, species level prevalence of 5.8% in goats and 3.2% in sheep from Afar region, respectively.

The 7.1% overall seroprevalence found in the current study was significantly higher than the overall seroprevalence previously reported, which was 1.6% by Debassa *et al*., (2013), 3.3% by Sintayehu *et al.,* (2015) from the same agro ecology of Borena pastoralist area, 1.6% by Mengistu (2007) from Konso, Southern Ethiopia, and 3.5 by Teklue *et al*. (2013) from Southern Tigray. Furthermore, Teshale *et al*. (2006) from the Somali pastoral region reported 1.7% in goats and 1.6% in sheep, while Bekele *et al*. (2011) from the same region reported 1.9% in goats and 1.2% in sheep, both of which showed far lower species level seroprevalence than the current study. Additionally, the present finding was higher than the results from certain African nations. Benkirane (2006) from Eritrea measured 4.1% in goats and 1.6% in sheep. Animal level prevalence was also found to be 15% in sheep and 16.5% in goats from the Afar area by Yibeltal (2005). The observed difference in prevalence could be due to the variation in sensitivity and specificity of the various tests, sample size of the study (Omer *et al*., 2000).

Difference in seropositivity among sheep and goats was found in the current investigation, with goats, recording a higher seropositivity of 8.85% than sheep 4.54%. This situation matched the findings of earlier research by Ashenafi *et al*. (2007), which found 3.2% and 5.8% in sheep and goats from the Afar area of northern Ethiopia, respectively. These findings are also consistent with that of Aworh *et al*. (2017), who reported prevalence of 19.6% and 9.4% in caprine and ovine, respectively, from animals butchered at abattoirs in Abuja, Nigeria. They found that caprine had a higher seroprevalence of brucellosis than ovine. Compared to sheep, goats are more likely to get Brucella infection. This could be a result of goats being more susceptible to Brucella infection. It could possibly be partially because goats, as opposed to sheep, expel the organism over a longer period of time. As a result, there is a lower chance of disease transmission within sheep herds (Radostits *et al*., 2000).

In the current study, flock level seroprevalence was recorded at 26.6%, which was lower than the results of Wesinew *et al*. (2014) in Afar (50.51%), higher than the results of Edao *et al*. (2020), who recorded flock level prevalence at 22.7% from a Borena pastoralist in Southern Ethiopia, and comparable to Teklu *et al*., (2013) who reported 28.3% from the Southern zone of Tigray region in Northern Ethiopia. In the current investigation, an increase in flock size was directly correlated with an increase in animal seropositivity, and this relationship was statistically significant ($p<0.05$) when compared to a decrease in flock size. Teklu *et al.* (2013) further validated the phenomena of larger flock size being more susceptible to Brucella infection, finding a significant ($p<0.05$) association between higher flock size and Brucella seropositivity in small ruminants. According to Radostits *et al.* (2006), herd size and animal density are closely correlated with illness prevalence and the difficulties of controlling infection in the population.

Brucellosis seroprevalence varied depending on the age group of the investigated animals. According to the study, adult brucellosis rates in these settings were higher than those for young (2.25%), at 17.6%. The reports of Megersa *et al*., (2011) from the Borena pastoral region of Southern Ethiopia were generally consistent with the findings of this study. In 2007, Ashenafi *et al*. from the pastoral Afar area and Ashagirie *et al*. (2011) from the South Omo zone in Southern Ethiopia. Adugna *et al.* (2013) from Afar region, North East Ethiopia. Muma *et al*. (2007) found that being older increases the likelihood of contracting the Brucella bacteria. This may be explained by the fact that only adult, sexually mature males, and females contract the disease brought on by infection. Due to the influence of sex hormones and placental erythritol on the pathogenesis of brucellosis, which stimulates the growth and multiplication of Brucella, susceptibility does, however, increase with sexual maturity and pregnancy (Radostits *et al.*, 2007). However, it is also true that young animals have a higher level of infection resistance and typically recover from an existing illness, even though latent infections can happen (Quinn *et al*., 2013).

Male and female small ruminants varied statistically significantly regarding their seropositivity to Brucella. In the current study, female ovines or caprines had a greater seroprevalence of brucellosis (9.3%) than male ovines or caprines (2.75%). This result is consistent with those reported. Moti *et al*., (2013) found that brucellosis affected 3.2 and 1.2% of females and males, respectively, in Southern Ethiopia. A high quantity of erythritol, which is seldom formed in male reproductive organs, may be the cause of the high frequency of brucellosis in females (European Commission, 2001). Due to the formation of erythritol, a 4-carbon sugar in fetal and female reproductive tissues that promotes the growth of Brucella organisms, these tissues have a special

affinity for Brucella species, which is why female animals have different levels of Brucella antibodies. Additionally, female animals are often kept in the flock for a longer amount of time than males. Female farm animals live longer time than males do which increases their exposure to Brucella germs and increases their risk of infection (Radostits *et al*., 2007).

It was discovered that the prevalence of brucellosis was also higher in animals with a history of abortions and a previous history of retained fetal membrane (37.7%) and lower than the report by Wubishet (2018) who reported 50.0% in Yabello district of Borena Zone. Animals that are pregnant were more likely to contract the organism than animals that are still developing sexually. As the gestational stage advances, susceptibility also rises. This suggests that retained placenta and abortions or stillbirths are typical clinical sign of brucellosis (Radostits *et al*., 2007). It is a result of Brucella species' affinity for certain important target cells known as trophoblast. During the last stages of ruminant pregnancy, high concentrations of steroid hormones and erythritol appear to work synergistically to promote Brucella growth inside the trophoblast. The ability of trophoblasts to multiply quickly and widely can undermine the placenta's integrity and infect the fetus, leading to abortion or the birth of undeveloped children (Xavier *et al.*, 2009; OIE, 2012).

The correlation between animal body condition ratings and the prevalence of brucellosis was once again statistically significant ($p<0.05$). According to the study, animals with poor body condition were more likely (18.94%) to contract brucellosis under these conditions than those with good body condition (2.40%). Tsegay *et al*. (2015) from Ethiopia reported that there was a correlation association between Brucella seropositivity and body condition scores in the study conducted on small ruminants slaughtered at Debrezeit and Modjo export abattoirs that thin animals were more susceptible to Brucella infection than animals of medium and good body conditions. The reason for higher seropositivity observed in sheep and goats with good body condition scores in the current study could be that animals that were walked in search of water and pasture were more likely to come in contact with other infected flocks and therefore remain exposed. Animals that were not going to search for food or those grazed poor pastures around the homesteads came with a penalty in the form of malnutrition which may result in loss of body condition and this then reduced the risk of exposure likely to result from comingling with other infected animals. Nutrition plays a great role in immunity against various infectious diseases. Underfed animals are expected to have a decreased immunity that is manifested by poor body condition (Radostits *et al*., 2007).

Human brucellosis seroprevalence in the current investigation was 19.2% by I-ELISA. The study's recorded result is less than the result of 25.6% that was reported in the Borena Zone's Yabello and Dire Districts (Wubishet *et al.,* 2018), 34.1% and 29.4% in Borena and Hamer pastoral area of southern Ethiopia respectively (Genene *et al*., 2009) and 16.5% (Eshetu *et al*., 2008) in Chifra District, of Afar region, Ethiopia.

Because the prior study was done on pastoralists with febrile illnesses, it is possible that the current study's lower prevalence compared to the previous studies is due to changes in the study population. The current study's findings are higher than those of the majority of highland investigations. Higher incidence can be attributed to differences in the agroecology and local customs (handling aborted fetuses and fetal membranes, consuming raw milk and blood). These practices are thought to be the primary means of disease transfer from animals to humans. To, prevent infection, one should avoid consuming raw milk and blood, handling aborted fetuses or retained fetal membranes with one's bare hands, leaving or putting aborted materials in the environment, and touching vaginal secretions. This survey's findings are consistent with the research that has been published by Kassahun *et al*. (2006) Mussie *et al*. (2007) and Asmare *et al*. (2007).

According to studies by Ragassa *et al*. (2009), Tolosa *et al* (2007), and Asmare *et al*. (2007), there are likely many unrecognized cases of febrile diseases, Osteoarticular complications (joint problems), and other generalized complications in pastoral communities. Characterizing the bacterial species present in humans and small ruminants is beneficial, but I was unable to do this test owing to budgetary constraints and a lack of a nearby, well-equipped laboratory for the diagnosis and investigation of zoonotic diseases.

The current study's findings regarding the practice of handling aborted fetuses and aborted materials (placenta) with bare hands and drinking raw milk were consistent with findings from a related study area reported by Wubishet *et al.* (2018), who found that 120 (95.3%) pastoralists drank raw milk and handled aborted fetuses with bare hands. Similarly, Tegegn *et al*. (2016) stated a similar scenario with the study conducted on sheep and goats raised in urban, peri-urban, and rural regions of Niger overall, as stated by Boukary *et al.* (2013), revealed a similar scenario. The current findings showed that livestock owners in the studied areas were at significant risk of catching brucellosis from diseased animals. According to several experts, the consumption of raw livestock products and the fact that people become sick due to a lack of community knowledge about brucellosis might both contribute to the disease's continued spread (Muluken *et al*., 2017).

# 6. CONCLUSION AND RECOMMENDATIONS

The seroprevalence study conducted in this study in the West Guji zone, southern Oromia, Ethiopia's Dugda Dawa, and Suro Berguda Woredas, suggested that brucellosis could be one of the major diseases in areas where people and animals have close contact. In small ruminants, large flock size, poor body condition, female animals with a history of the retained fetal membrane and history of abortion are prone to be seropositive relative to other groups of animals. In this study, most individuals had close contact with small ruminants and known practices that put an individual at risk of brucellosis were common. This is in conformation with the general living standard and cultural conditions in Ethiopia that inherently predispose individuals to zoonotic diseases. In addition, animal husbandry is very traditional in Ethiopia and people most often live with their animals under the same roof, and health-care support for their animals is minimal. These factors were believed to support the spread of the disease between animals as well as from animal to humans in the study area. Increasing age, increasing flock level, poor management practices, and letting aborted material in the environment were also associated with higher prevalence. fit to support this. The prevalence of brucellosis in humans is greater than that in animals. Age and history of assisting animals' parturition were factors in the probability of brucellosis seropositivity in people.

Considering the above conclusion into account the following recommendations were forwarded:

- Health education about the mode of transmission of brucellosis and about the disease should be provided to the community regularly.
- Integration of different sectors should be focused on to take action against the community's exposure to brucellosis.
- Further research on the isolation and characterization of circulating *Brucella* species in small ruminants and humans should be conducted in the study area to propose effective control measures.

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## 8. ANNEXES

Annex 1: Individual animal data

Date: _____

1.Location: Region: _____ Zone: _____ District: _____

Keeble: _____Village: _____

2. Owner's name: _____

3. Animal I.D.: _____ Species: _____ Sex: _____

Age: _____ Parity: _____ Pregnancy Status: _____

4. History of the disease

4.1 History of abortion: Yes/ No;

4.2 History of stillbirth: Yes/ No;

4.3 History of retained placenta: Yes/ No;

4.4 History of testicular swelling: Yes/ No;

4.5 Body condition score: _____

Annex 2: Flock Level Data

Date _____

1. Location: Region _____ Zone _____ District _____Kebele _____Village _____

2. Respondent's detail: Name _____; Sex _____ Age _____

Responsibility: Owner/ Attendant; Occupation_____

Level of Education:

1. Tertiary /University; 2. Secondary School; 3. Primary school; 4. Illiterate

3. Species of animals on the farm?

A. Cattle B. Sheep C. Goats D. Equines E. Camels

No. of animal by species _____

5. Management/husbandry type _____; Watering source _____Grazing: Private/ Communal; Contact with other flock: Yes/ No; Contact with other species of animals: Yes/ No; Housing type _____; Breeding: Own sire/Neighbor's sire/Both

6. History of abortion: Yes/ No; History of stillbirth: Yes/ No; History of placental retention: Yes/ No

7. How do you dispose aborted fetus and placenta? A. Burry B. Burn C. Give to dogs D. Dispose in the immediate environment

8. Do you use separate housing for your sheep and goats? (from other animal species) Yes/ No

9. Do you separate aborted animal from others? Yes/ No

10. Do you use mixed grazing and watering with other animal species? Yes/ No

11. Do you migrate your animals to other area? Yes/ No

If yes: a. To where _____ b. In which season _____

Annex 3 Iyyaafannoo Haala Dhukubaa (Awareness, Attitude and Practice data)

Guyyaa (Date): _____

1.Bakka (Location): Naannoo (Region) _____Godina (Zone) _____ Aanaa (District) _____Ganda (Kebele) _____ Mandara (Village) _____

2. Haala walii Gala Gaafatamaa (Respondent's detail): Maqaa (Name) _____; Koorniyaa (Sex) _____; Umrii (Age)_____; Ogummaa (Occupation)_____

Sadarkaa Barnootaa (Educational background): A. Barreessuu fi Dubbisuu (Illiterate) B. 1-8(Elementary) C.9-12(Secondary) D. Dippiloomaa (Diploma) or Degree(degree)

Baay'ina maatii (Family size): _____

3.Beeyladoota ni tiksituu (Do you keep domestic livestock)? Eeeyyee (Yes / Lakkii/No?

Deebiin keessan eeyyee yoo ta'e beeylada gosa kam? If yes, which species?

A. Loon (Cattle) B. Hoolaa (Sheep) C. Re'ee (Goats) D. Kotte-Duudaa (Equines) E. Gaala (Camels)

4. Ni dhalchiistuu? Do you assist lambing/kidding? Yes/ No

A. Deebiin keessan eeyyee yoo ta'e harka qullaanii? If yes, do you manipulate with your naked hand? Eeyyee (Yes)/lakk (No)

B. Deebiin keessan lakkii yoo ta'e maal fayyadamtu? Maaliif? If not, what do you use for protection and why? _____

C. Yeroo dhalchiiftanu bishaanii fi saamunaa ni fayyadamtuu? Do you wash your hands with soap and water after assisting lambing or kidding? Eeyyee (Yes)lakk (No)

Maaliif? Why? _____

5. Beeylada kee keessa horiin garaa gatee beekaa? Have you encountered abortion in your flock? _____

6. Dhukkuba Hoolaa fi Re'ee gatachiisu ni beektuu? Do you know a disease, which could cause abortion in animals including sheep and goats? _____

A. Maal jedhama? What is the name of this disease? _____

7. Gatachisa ni beektuu? Do you know a disease by the name brucellosis? _____

8. Namootni akkamitti dhibee kanaaf saaxilamu? How do people become infected with brucellosis? _____

9.Beeyladoota irraa gara namaa ni dabraa? Can humans contract diseases from animals? Eeyyee (Yes)Lakkii (No)

Eeyyee yoo ta'e akkamitti? If yes, how? _____

10.Akkamiin dil'uu fi Sallessa gatuu dandeessu? How do you dispose aborted fetus and placenta?

A. Awwaaluu (Burry) B. Gubuu (burn) C. Sarootaaf kennuu (Give to dogs) (D. bakkuma argametti gatuu Dispose in the immediate environment)

11. Aannan mi'ii ni dhugduu/Do you consume raw milk? Eeyyee/Yes/Lakkii/ No

12. Foon dheedhii ni nyaattuu? Do you consume raw meat? Eeyyee/Yes/ Lakkii? No

Gatachiisaan akka nama hubu ni beektuu? Do you know that brucellosis can affect/infect humans? Yes/ No

13. Soorata akkasii kana soorachuudhaan miidhaan dhufu ni jiraa? Is there any risk associated with consumption of these products raw? Eeyyee/Yes/ Lakkii/No

14.Eeyyee yoo ta'e maal fa'a? If yes, what are these risks? _____

19. Beeyladoonni akkamitti Gatachiisaaf saaxilamu? How can animal become infected with brucellosis? _____

15. Mana keessanitti Aannan akkamitti Duguma? What is the main way milk is typically consumed in your household?

A. Mi'ii/ Raw milk B. Danfisanii/Boiled milk C. Itichanii/ Fermented

Annex 4: Questionnaire for Data Collection on Brucellosis in Humans.

A. Odeeffannoo walii galaa/General Information

Guyyaa/Date: _____

1.Bakka/ Location: Naannoo/Region _____ Godina/Zone _____ Aanaa/District _____ Ganda/Kebele _____ Mandara/Village _____

2.Haala odeeffatamaa/ Respondent's detail: Maqaa/Name _____; Koorniyaa/ Sex _____;

Umrii/Age _____; Ogummaa/ Occupation_____

Sadarkaa Barnntaa/Educational background: A. Barreessuu fi Dubbisuu/ Illiterate B.1-8/Elementary C.9-12/ Secondary D. Dippiloomaa/ Diploma or Degree/ degree

3. Oomisha dheedhii beeylada irraa ni soorattuu? Do you Consume Raw/ Unprocessed Shoat Products?

1. Eeyyee/ Yes 2Lakkii/ No

A. Maal jedhama? Name the shoat products consumed

1. Aannan/Milk. 2. Foon/ Meat. 3. Dhiiga/ Blood. 4. Kan biro (Others)

B. Soorachuun dura ni bilcheessituu? Do you process them before consumption?

1. Lakkii/No. 2. Eeyyee/Yes. 3. Yeroo tokko tokko/ Sometimes.

C. Hoolaa fi Re'ee Wajjiin Wal tuxxuqtuu? Do you have close contact with shoat?

1. Eeyyee/ Yes. 2. Lakkii/ No.

D. Eeyyee yoo ta'e akkamitti? If yes, how?

1, Naannoo tokko keessa jiraachuu/ Sharing compound 2, Mana tokko keessa jiraachuu/ Share house 3, bishaan Wajjiin dhuguu/ Sharing watering points.

4.Dhibee gatachiisuu fi maseensu argitanii beektuu? Have you encountered cases of infertility and abortions

1.Eeyyee/ Yes. 2. Lakkii/ No.

5. Sallessa akkam gootu? How do you handle aborted fetuses?

1, Ni nyaanna/Eat. 2, bakkuma argametti gatna/ throw away in bush. 3, Ni gubna/ Bury. 4sarootaaf laanna/ give dogs.

6.Gatachiisaan dhukkuba namaa ta'uusaa ni beektuu? / Do you Know brucellosis as human disease (zoonosis)?

Eeeyyee/Yes_____B. Lakkii/No_____

7. Namarratti mallattoo akkamii agarsiisa? / Which of the following symptoms infected human with brucellosis show?

A. Mataa bowwoo/ Headache _____

B. Dhukkubbii mitikaa/Joint pain _____

C, ho'ina/. Fever _____

8. Namootni akkamitti Gatachiisaaf saaxilamu? /How, can people become infected with brucellosis?

Aannan mi'ii dhuguun/Consuming raw milk and its products_____

B. Foon dheedhii nyaachuun/Consuming raw meat/ blood _____

C.Dil'uu ykn Sallessa tuttuquun/Contact with aborted fetus or placental membrane_____

D. Beeyladoota dhalchiisuun /Assisting animals during parturition_____

E. Beeyladoota qaluun/Slaughtering animals_____

F. Hin beeku/ I have no idea _____

9. Yeroo beeylada dhalchiiftanu uffata of eeggannoo ni uffattuu/ Do you use personal protective clothes during assisting delivery animals or contact with aborted material and fetal membrane?

A. Eeyyee/Yes B. Lakkii/No

10. Beeylada ni Gaansiftuu/ Do you assist mating?

Eeeyyee/Yes B. Lakkii/No

11. Beeylada dhibee Gatachiisaan qabanii fi deeggarsa akkamii gootu/What intervention you take if animal is affected by Brucella?

A. Adda baasuu/Separate from other B. Qaluu/ cull C. Wal'aansa kennuuf/give treatment_____

D. Kiliinika Geessu/ Take to clinic E. Mala aadaatiin wal'aanu/Treated by their own

Annex 5 Human blood sample collection format

Date____/_____/_____

Name of Study Area_____ Name of Health Institution_____

| Data code | Sample code | Age | Sex | Contact with animal | Contact with manure | Shoat blood consumption | Shoat raw meat consumption | Shoat raw milk consumption | Status of blood | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Positive | Negative |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |

Annex 6: Rose Bengal Plate Test procedure

**Intended use**

Brucella antigens are bacterial suspensions for use in slide agglutination tests to detect the presence of bacterial agglutinins associated with bacterial infection or previous exposure to a related organism. This slide test is recommended as a screening procedure only to establish the presence or absence of homologous antibody.

**Principle of the test**

In the course of animal infection with any pathogenic microbiological agent, a variety of antibodies are formed. Among these antibodies are the agglutinins. An agglutinin when combined with homologous antigen (agglutinogen) under the properly controlled conditions is capable of causing agglutination. A suspension of Brucella possessing active antigen will

agglutinate when exposed to homologous Brucella antibody. This agglutination forms clumps which become macroscopically visible.

**Reagents**

1- Rose Bengal Brucella Antigen (0.5% phenol)

2- Positive Control (0.01% sodium azide)

3- Negative Control (0.01% sodium azide)

**Materials required**

1. Brucella Antigen, Rose Bengal Stained.

2. Positive Control.

3. Negative Control.

4. Microtiter plate.

5. Plastic applicator stick

**Procedure**

1- Allow reagents and serum samples to reach room temperature for testing.

2- Shake the antigen bottle gently to insure a uniform suspension.

3- Place 30ul sample serum onto the selected ring of the slide.

4- Place one drop of the Rose Bengal antigen onto serum sample.

5- Mix serum sample with Rose Bengal antigen using Stirring stick.

6- Repeat these steps using the positive and negative controls instead of serum sample.

7- Gently rock the slide for 4 minutes

8- Observe for agglutination after 4 minutes from beginning of shaking (this is the optimum time limit).

**Results and interpretation**

The result is interoperated immediately after four minutes as follow:

O= no agglutination

+= Barely perceptible agglutination (seen using magnifying glass)

++= fine agglutination- some clearing

+++= course clumping- definite clearing

- ✓ Negative: No agglutination (0)
- ✓ Positive: (Presence of Specific Antibodies):
  Agglutination (+, ++, +++)

Annex 7: Indirect enzyme-linked Immuno sorbent assay(I-ELISA*)*

1. All reagents should equilibrate to room temperature 21°C (± 5°C) before use.
2. Add:

A. 190 µl of Dilution Buffer 2 to all wells.

B. 10 µl of negative control to wells A1 and B1.

C. 10 µl of positive control to wells C1 and D1.

D. 10 µl of each sample to the remaining well and Seal the plate.

3. Incubate for 45 minutes (± 4 minutes) at 21°C (± 5°C).
4. Prepare the wash solution (1X) by diluting the wash concentrate (20X) in distilled water.
5. Empty the wells. Wash each well 3 times with approximately 300 µl of the wash solution.
6. Prepare the conjugate by diluting the concentrated conjugate (10X) to 1/10 in dilution buffer3.
7. Add 100 µl of the conjugate 1X to each well and Seal the plate.
8. Incubate for 30 minutes ± 3 minutes at 21°C (± 5°C).
9. Repeat step #5.
10. Add 100 µl of the substrate solution to each well.
11. Incubate for 15 minutes ± 2 minutes at 21°C ( ± 5°C) in the dark.
12. Add 100 µl of Stop solution to each well in order to stop the reaction.
13. Measure the Optical density (OD) of the controls and samples at 450 nm in micro-plate photometer. Measure the OD within 15 minutes after the addition of Stop solution to prevent fluctuation in OD values.

<u>Validation</u>

The test is valid if:

- ❖ The mean value of the positive control OD ($OD_{PC}$) is greater than 0.350 and

- ❖ The ratio of the mean values of the positive and negative ($OD_{PC}/OD_{NC}$) controls is greater than 3.

Interpretation

For each sample calculate the Seropositivity percentage (S/P %) as follows using the sample and control values:

$$S/P\% = \frac{OD(sample) - OD(NC)}{OD(PC) - OD(NC)} * 100$$

- ✓ Less than or equal to 110% are considered negative.
- ✓ Greater than 110% and less than 120% are considered doubtful.

Greater than or equal to 120% are considered positive

Annex 8: Different figures taken during sample collection and laboratory examination