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DEPARTMENT OF PUBLIC HEALTH AND FOOD SAFETY

SERO-PREVALENCE OF SMALL RUMINANT BRUCELLOSIS AND ASSOCIATED RISK FACTORS IN ENDERTA DISTRICT, SOUTHEASTERN ZONE OF TIGRAY

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DECLARATION

I, the undersigned, declare that this thesis is my work and all sources of material used for the thesis have been fully acknowledged.

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Signature **________________**Date **____________**

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By Sisay Fiseha

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LIST OF ABBREVIATIONS

CFT= Complement Fixation Test

RBPT= Rose Bengal Plate Test

SAT= Serum Agglutination Test

OIE= Office International des Epizootics

DNA= Deoxyribonucelicacid

ELISA= Enzyme Linked Immunosorbent Assay

WHO= World Health Organization

PA=Peasant Association

H_2S=Hydrogen sulfide

MRT=Milk Ring Test

OR=Odds Ratio

CI=Confidence Interval

CSA=Central Statistics Agency

PCR=Polymerase Chain Reaction

CO_2=Carbon dioxide

ABSTRACT

Small ruminant brucellosis is a significant zoonotic disease that poses a threat to both animal and human health. This study was aimed to determine the sero-prevalence of small ruminant brucellosis and identify associated risk factors in Enderta district of Tigray Region, Northern Ethiopia. A cross-sectional research design was employed from May, 2021 till October, 2023, and blood samples were collected from small ruminants managed under an extensive production system. Purposive sampling technique was followed to select district and peasant associations, and simple random sampling technique was also followed to select animals and attendants. The Rose Bengal Plate Test (RBPT) and the Complement Fixation Test (CFT) were used as screening and confirmatory tests, respectively. A questionnaire survey was also conducted on100 animal owners' to assess the knowledge, attitude, and practices regarding brucellosis. A total of 384 animals were sampled, and the overall sero-prevalence of brucellosis was determined. The RBPT and CFT results showed that 24 animals tested positive for brucellosis, resulting in an overall sero-prevalence rate of 6.3% (95% CI: 0.042-0.092). The sero-prevalence rates were further analyzed in relation to potential risk factors. The presence of abortion history and older age greater than 2years in the flock was significantly associated with sero-positivity ($p<0.05$). Logistic regression analysis revealed that the odds of sero-positivity were higher in flocks with a history of abortion (OR=36, 95% CI: 13.30-97.39, $p<0.001$) and the odds of sero-positivity were also higher in animals with older age greater than 2years (OR=20.13, 95% CI: 2.68-150.62, $P<0.003$). The questionnaire survey revealed that 83 % of flock owners associated abortion with disease conditions. However, some flock owners were observed practicing activities that expose to brucellosis. About 5 % consume raw milk, 95 % do not bury aborted materials and 96 % assist their animals at delivery with bare hands. The low level and inadequate preventive practices among flock owners highlights the need for targeted awareness campaigns and improved disease management strategies. These findings can inform the development of effective control and prevention measures to reduce the burden of small ruminant brucellosis in the study area.

Keywords: small ruminant brucellosis, sero-prevalence, risk factors, zoonotic disease, Enderta district, Tigray Region, Ethiopia.

CHAPTER I

1.1. Introduction

Ethiopia is one of the African countries with the largest small ruminant populations on the continent (Abebe, 2013). According to 2014 census, the country is home to approximately 27.35 million sheep and 28.16 million goats (CSA, 2014). The majority of these animals are concentrated in pastoralist areas of the country (CSA, 2012). Small-ruminant production plays a significant role in the agricultural activities of the livestock sub-sector. These animals are predominantly local breeds that are raised under an extensive production system, characterized by low productivity and limited commercial off-take (Seleem et al., 2009; Sonawane *et al*., 2011).

Small ruminants play a crucial role in livestock keeping, particularly in developing countries, especially in Sub-Saharan Africa. They are primarily kept for various purposes such as immediate cash sources, milk, meat, wool, manure, and as a means of saving or risk distribution (Gobena, 2016). Sheep and goats offer several advantages over large ruminants for smallholder farmers. These advantages include lower feed costs, faster turnover, easier management, and an appropriate size at slaughter. Additionally, small ruminants demonstrate greater tolerance to less favorable conditions, experiencing lower mortality rates during periods of drought compared to large ruminants. Breeders also prefer sheep and goats due to the high risk associated with losing large ruminants (Zahra et al., 2014).

In spite of the significance of small ruminants in the livelihoods of producers, their productivity in developing countries, including Ethiopia, remains low (Gizaw, 2010). Several factors contribute to this, including prevalent animal diseases, feed scarcity in terms of quality and quantity, limited genetic potential, and management challenges. Among these factors, infectious diseases are the primary obstacles to improving small ruminant production globally (Getahun, 2008; Gizaw, 2010; Kaur *et al*., 2013).

Brucellosis is widely recognized as one of the most significant zoonotic diseases worldwide, posing a major threat to both human and animal populations (Mensal *et al*., 2011; Kelkay *et al*., 2017). Despite its substantial impact on human and animal health, as well as the economy, the World Health Organization has classified it as a neglected zoonosis due to inadequate control programs in many countries (Holt *et al*., 2011). Brucellosis hinders the trade of animals and their products, and its associated reproductive wastage and infertility have enormous consequences (Godfroid *et al*., 2011).

Sheep and goat brucellosis, excluding Brucella ovis infection, which is not pathogenic to humans, is a zoonotic infection that significantly affects public health, animal health, and production, and is prevalent in many regions around the world (Alton, 1990). While the disease has been eradicated in most industrial countries, particularly in Europe, through intensive control and eradication schemes, its occurrence is increasing in developing countries, exacerbating the epidemiological situation. This is due, in part, to the practice of importing exotic high-production breeds without the necessary veterinary infrastructure and appropriate socioeconomic conditions for animal holders (Radostits *et al*., 2007).

In small ruminants, brucellosis causes significant economic losses due to reproductive impairments such as abortion, stillbirths, weak lambs and kids, neonatal mortality, and infertility. In humans, brucellosis is often misdiagnosed as other febrile syndromes like malaria and typhoid fever, leading to mistreatments and underreporting Transmission to humans occurs through breaks in the skin following direct contact with infected animals' tissues, blood, or secretions (Swai and Schoonman, 2009).

The traditional lifestyle, beliefs, and poor knowledge of the community about the disease create favorable conditions for the spread and transmission of brucellosis (Thrusfield, 2005). Therefore, having adequate knowledge of the epidemiology of brucellosis is of great public health importance, particularly among livestock workers and consumers of animal products. Such knowledge would greatly assist in developing strategies for its control. Despite the presence of a large population of small ruminants in different regions of Ethiopia, limited research has been conducted on small ruminant brucellosis, despite its endemic status in the country.

In Ethiopia, there have been few published studies on small ruminant brucellosis. Compared to brucellosis in cattle, small ruminant brucellosis is less commonly reported in sub-Saharan Africa. Similarly, there is limited published information on small ruminant brucellosis, which is mainly focused on a few districts in Ethiopia. The lack of scientific information on the prevalence of this significant disease, which poses a public health hazard and causes heavy economic losses, highlights the need for further research and future control measures in Ethiopia (Tekelye and Kasali, 2014).

The status of small ruminant brucellosis in Ethiopia is not well understood and there is limited information available. This may be attributed to the lack of attention given to the small ruminant production sector. The absence of research activities in animal diseases, poor veterinary development, and a lack of awareness about the economic and zoonotic impact of the disease have contributed to the insufficient amount of information. However, limited sero-surveillance studies conducted so far indicate that brucellosis may be a significant disease in communities that raise small ruminants. The disease causes significant reproductive losses in animals, including abortions, placentitis, stillbirths, and the birth of weak offspring in females, as well as epididymitis and orchitis in males (OIE, 2009). A sero-surveillance study conducted in different regions of Ethiopia clearly demonstrated the presence of the disease in small ruminants (Ferede *et al*., 2011).

Tigray Region is located in the Northern part of Ethiopia, has limited documented on sero-surveillance of small ruminant brucellosis. Specifically, in Enderta district, Southeastern Zone of Tigray, there are no reports on the disease, although farmers in the area have observed issues such as abortions, premature births, infertility, and retained placenta as priority problems. Therefore, the objectives of this study were as follows:

1. To determine the sero-prevalence of small ruminant brucellosis in the area.
2. To identify associated risk factors contributing to the occurrence of the disease in small ruminants.
3. To assess the knowledge, attitude, and practices of flock owners regarding the zoonotic importance of the disease in the study area.

1.2. Literature Review

1.2.1. History and Importance of Brucellosis

Brucellosis is known by various synonyms, including Melitococcosis, undulant fever, Malta fever, and Mediterranean fever in humans. In animals, it is referred to as contagious abortion, infectious abortion, and epizootic abortion, while in cattle it is sometimes called Bang's disease. The bacterium Brucella melitensis, which is responsible for brucellosis, was initially isolated by Sir David Bruce in 1887 from the spleen of a British soldier on the island of Malta. At that time, it was named Micrococcus melitensis. In 1920, Meyer and Shaw renamed the bacterium Brucella melitensis in honor of Dr. Bruce. This particular strain of Brucella causes significant problems in humans and in goats and sheep worldwide. Goats are highly susceptible to infection and serve as the primary hosts for Brucella melitensis. Infected goats are the main source of human brucellosis, which is a zoonotic disease with a global distribution (Khan and Zahoor, 2018).

1.2.2. Characteristic of the Causative Agent

The genus Brucella is taxonomically classified into ten species and further divided into biovars. The six well-known classical species include Brucella abortus (affecting cattle, with biovars 1-6 and 9), Brucella melitensis (affecting goats and sheep, with biovars 1-3), Brucella suis (affecting pigs, reindeer, and hares, with biovars 1-5), Brucella ovis (affecting sheep), Brucella canis (affecting dogs), and Brucella neotomae (affecting desert wood rats). More recently, new members of the genus have been identified, including Brucella ceti and Brucella pinnipedialis (affecting dolphins/porpoises and seals, respectively), Brucella microti (affecting voles), and Brucella inopinata (with an unknown reservoir). Among these species, Brucella melitensis poses the greatest risk for human infection, followed by Brucella suis, Brucella abortus, and Brucella canis. However, several other species within the genus have also been shown to be virulent for humans (Godfroid *et al.*, 2011).

Brucella organisms are Gram-negative coccobacilli and facultative intracellular pathogens. They are typically found in the reticulo-endothelial and reproductive systems. Brucella organism is strictly aerobic, non-encapsulated and catalase, and oxidase-positive. They do not ferment carbohydrates and may exhibit variable urease activity. These organisms have a lipopolysaccharide coat that is less pyrogenic compared to other Gram-negative organisms, which explains the relatively rare occurrence of high fever in brucellosis (Rust, 2012).

1.2.3. Brucellosis in small ruminants

Brucella melitensis primarily affects small ruminants such as sheep and goats. While the bacteria exhibit a strong host preference for these animals, they also have the ability to cross-infect other species. This means that Brucella melitensis can be found in other animal species as well. However, the bacteria's pathogenicity and infectivity are particularly high in sheep and goats, posing a significant risk to people who live in close proximity to these animals. In general, the disease caused by Brucella melitensis is more severe and prolonged in goats compared to sheep. This is because goats tend to have a higher susceptibility to Brucella melitensis infection compared to sheep (Quinn *et al*., 2002). Therefore, the presence of Brucella melitensis in goat populations increases the risk of transmission to humans and underscores the importance of effective control measures to prevent the spread of the disease (Corbel *et al*., 2006; OIE, 2009).

1.2.4. Epidemiology

1.2.4.1. Geographic distribution

Small-ruminant brucellosis has been documented worldwide and is primarily found in Mediterranean countries, the Middle East, Africa, India, China, Mexico, and parts of Latin America (Smith and Sherman, 1994). In sheep, the infection appears to be endemic in the Mediterranean region, particularly along its northern and eastern shores, extending through Central Asia to the Arabian Peninsula and as far east as Mongolia. Parts of Latin America, including Mexico, Peru, and northern Argentina, are also significantly affected. The disease is also prevalent in Africa and India. However, it's worth noting that North America (excluding Mexico) is considered free from small-ruminant brucellosis, as are Northern Europe (with sporadic incursions from the south), Southeast Asia, Australia, and New Zealand (FAO, 2010). Additionally, the disease does not occur in the United Kingdom or in countries where bovine brucellosis has been successfully eradicated (OIE, 2012).

1.2.5. Risk factors for transmission

1.2.5.1. Host factor

1.2.5.1.1. Age factor

The prevalence of small-ruminant brucellosis is higher in adult sheep and goats compared to younger animals (Walker, 1999). Sexually mature and pregnant animals are more susceptible to Brucella infection and brucellosis than sexually immature animals of either sex (Quinn *et al*., 1999).

The disease primarily affects sexually mature animals, with the reproductive tracts of both males and females being the preferred sites of infection. In particular, the pregnant uterus is highly susceptible to Brucella colonization. This susceptibility may be attributed to the fact that sex hormones and erythritol, which promote the growth and multiplication of Brucella organisms, tend to increase in concentration with age and sexual maturity (Radostits *et al*., 2000).

1.2.5.1.2. Sex factor

Male animals are generally less susceptible to Brucella infection compared to females, and this is attributed to the lower concentration of erythritol present in males relative to females (Hirsh and Zee, 1999 as cited in Tilahun *et al*., 2016). The susceptibility to Brucella infection is influenced by the sex and reproductive status of the individual animal, with sexually mature and pregnant animals being more vulnerable to the organism compared to sexually immature animals. During the later stages of pregnancy, placental trophoblasts produce increasing amounts of erythritol. This coincides with the period when pregnant sheep and goats are more susceptible to infection with Brucella melitensis (Sangari *et al*., 2000). The presence of erythritol provides a favorable environment for the growth and multiplication of Brucella organisms, thereby increasing the risk of infection during pregnancy.

1.2.5.1.3. Species and breed factor

Goats are at a higher risk of acquiring Brucella infection compared to sheep. This increased risk may be attributed to the greater susceptibility of goats to Brucella infection. Additionally, goats have been found to excrete the organism for a longer duration compared to sheep. This prolonged shedding of the bacteria by goats reduces the potential for disease spread among sheep flocks (Radostits *et al*., 2000).

The receptivity of ewes/female sheep to Brucella melitensis infection can vary depending on the breed. Milk-producing ewes are generally more receptive to infection compared to sheep raised for slaughter. Among goat breeds, most are fully susceptible to Brucella infection. In contrast, there is a significant variation in the susceptibility of different sheep breeds to the disease. Sheep breeds raised for milk production tend to be more susceptible compared to those raised for meat production (Corbel and Brinley-Morgan, 1984).

1.2.5.2. Reservoir factor

Carrier animals play a significant role in the transmission of brucellosis. They contribute to the spread of the disease by contaminating the environment and serving as a site for the multiplication of Brucella organisms within their bodies. These carrier animals excrete the bacteria, which can then infect other animals and humans, posing risks to both health and the economy of a country. Carrier animals include dogs, cats, and wild carnivores such as foxes and wolves. These animals can act as mechanical disseminators of infection by carrying away infected materials like died fetus or fetal membranes. This behavior enhances the viability of

Brucella organisms in the environment, thereby increasing the chances of infecting susceptible animals (Gall *et al*., 2001 as cited in Mustefa and Bedore, 2019). It highlights the importance of controlling brucellosis transmission not only within domestic livestock but also among carrier animals to mitigate the spread of the disease.

1.2.5.3. Environmental and husbandry factors

The systems of husbandry and environmental conditions have a significant impact on the spread of brucellosis infection. For example, lambing or kidding in dark and crowded enclosures provides a more favorable environment for the spread of the disease compared to lambing or kidding in open-air settings with dry conditions. The close proximity and limited ventilation in crowded enclosures contribute to the higher risk of transmission. The movement or gathering of infected animals is often associated with the spread of infection between flocks. When infected animals are introduced into a previously non-infected area, it poses a major risk for introducing the disease. The purchase of infected animals is identified as one of the main routes for the introduction of brucellosis into a previously disease-free area (EU commission, 2001).

1.2.6. Clinical Manifestation

Middle to late-term gestation abortion, stillbirths, and the delivery of weak offspring, often followed by the retention of fetal membranes, are characteristic clinical signs of brucellosis in small ruminants. These signs may be the only noticeable indicators of the disease. However, it's important to note that these clinical signs are not specific to brucellosis, and it is crucial to consider differential diagnoses with other potential causative agents. Achieving a definitive diagnosis can sometimes be challenging in the clinical field (Igwebuike, 2006 as cited in Rossetti *et al*., 2022).

In small ruminants, vertical transmission of brucellosis can occur through the ingestion of contaminated colostrum and milk, which may be a more common route compared to in utero infection. Lambs and kids born to infected dams may passively acquire neutralizing antibodies, providing some level of protection. However, these offspring can still become latent carriers of the disease until sexual maturity. Some of them may develop clinical signs of brucellosis, while others may remain asymptomatic carriers (Diaz, 2013).

In non-pregnant small ruminant females, natural *Brucella melitensis* infection typically does not show any symptoms (Mazlina et al., 2021 as cited in Rossetti *et al*., 2022). Similarly, adult ewes and rams infected with Brucella ovis rarely exhibit systemic signs of the disease. Rams with subclinical disease can become carriers or shedders of Brucella ovis and should be diagnosed through culture or routine serological tests and subsequently removed from the flock (Rajendhran, 2021).

In some cases, infected bucks and rams with Brucella melitensis may present unilateral or bilateral epididymo-orchitis and can shed the bacteria in their semen for up to a year (Ahmad and Niaz, 1998; Ali *et al*., 2019; Chand *et al*., 2002 as cited in Rossetti *et al*., 2022). Although the venereal route is usually considered of low epidemiological importance under natural conditions, the use of Brucella melitensis-contaminated semen in artificial insemination could potentially be a source of infection (Diaz, 2013). Both Brucella melitensis and Brucella ovis have a strong affinity for the lactating udder and supra mammary lymph nodes. However, clinical findings are usually limited to decreased milk yield and slight enlargement of the lymph nodes (Grilló *et al*., 1999; Meador *et al*., 1989 as cited in Rossetti *et al*., 2022).

Human brucellosis is characterized by a variable incubation period, ranging from several days up to several months. Common clinical signs include symptoms of continued, intermittent, or irregular fever of varying duration. Other associated symptoms may include headaches, weakness, profuse sweating, chills, depression, and weight loss. In pregnant individuals, brucellosis can lead to abortion during the early trimesters of pregnancy. In its chronic form, the disease can result in serious complications, affecting the musculoskeletal, cardiovascular, and central nervous systems (Mustefa M. and Bedore B., 2019).

Fortunately, the mortality rate associated with brucellosis is relatively low, particularly when patients receive appropriate antibiotic treatment. However, it's important to note that access to adequate healthcare and antibiotics may be limited, especially in low-income countries (Corbel, 2006). This can potentially impact the outcome for individuals affected by brucellosis in these regions.

1.2.7. Pathogenesis

Brucellae are facultative intracellular parasites that primarily infect the reticulo-endothelial system. The virulence of Brucella varies among different species and strains. Infection typically occurs through the mucous membranes of the oropharynx, upper respiratory tract, conjunctiva, and genital tract. In pregnant animals, the uterus is invaded by the bacteria, leading to abortions. The udder is also a significant site of infection for Brucella melitensis (EU commission, 2001).

Once Brucella organisms enter the bloodstream of a female animal, they can reach the placenta and subsequently infect the fetus. The preferential localization of Brucella within the reproductive tract of pregnant animals is thought to be influenced by factors present in the allantoic fluid. One such factor is erythritol, a four-carbon alcohol that becomes elevated in the placenta and fetal fluid around the fifth month of gestation. The initial localization of Brucella within erythrophagostic trophoblasts of the placentome, adjacent to the chorioallantoic membrane, leads to the rupture of these cells and ulceration of the membrane. This damage to the placental tissue, along with fetal infection and the resulting stress, can induce maternal hormonal changes and ultimately lead to abortion (Gul and Khan, 2007).

1.2.8. Laboratory Diagnosis

The diagnostic tests for brucellosis can be categorized into two main types: those that directly detect the presence of the Brucella organisms and those that indirectly detect the immune response to their antigens. It is important to perform laboratory diagnosis of brucellosis using direct and indirect methods whenever there are clinical signs or epidemiological evidence suggestive of the disease. Accurate and standardized diagnostic procedures are crucial for the successful control and eradication of brucellosis. Additionally, the identification of the specific Brucella species involved is of great epidemiological importance (Alton *et al*., 1975 as cited in Saavedra *et al*., 2019).

1.2.8.1. Direct Diagnosis

Direct diagnostic tests for brucellosis involve bacterial isolation and identification, as well as molecular methods. Cultural methods, which involve growing the bacteria in the laboratory, can be time-consuming and expensive. Molecular methods, such as Polymerase Chain Reaction (PCR), have become increasingly popular for diagnosing brucellosis in both human and veterinary medicine. PCR-based methods, in particular, have shown success in rapid diagnosis and differentiation of various bacterial species, including slow-growing ones. These molecular methods offer advantages in terms of speed, sensitivity, and safety compared to traditional bacterial isolation techniques. Isolation and identification of the Brucella bacteria is considered a definitive method for diagnosing brucellosis. It requires skilled and experienced laboratory personnel. The classical microbiological identification of Brucella strains involves examining their colonial morphology, microscopic appearance, and various biochemical properties, such as CO_2 requirement, H_2S production, urea hydrolysis, sensitivity to basic fuchsin and thionin, as well as agglutination with monospecific sera and phagetyping (Alton et al., 1988 as cited in Saavedra et al., 2019).

For bacterial culture, samples from uterine discharges, aborted fetuses, udder secretions, or specific tissues such as lymph nodes and male and female reproductive organs should be aseptically collected and promptly cooled or frozen if there will be a delay of more than 12 hours before culturing. Brucella isolation and culture are typically performed on solid media, using different basal media supplemented with 2-5% bovine or equine serum, with or without appropriate antibiotics to inhibit the growth of contaminant organisms (OIE, 2016).

1.2.8.2. Indirect Diagnosis

Indirect methods, also known as immunological methods, are used to detect the immune response to Brucella antigens. These methods are commonly employed due to their simplicity of execution and interpretation and are based on the detection of antibodies. Immunological diagnostic tests can be used to detect Brucella-specific antibodies in milk or serum samples (Garin-Bastuji *et al*., 2006 as cited in Saavedra *et al*., 2019).

Antibody detection tests are considered the most cost-effective approach for screening and detecting Brucella melitensis-infected herds, especially in resource-limited settings. Since no single serological test is sufficient for all epidemiological situations, it is strongly recommended to use at least two different serological techniques simultaneously, tailored to the specific epidemiological context, to assess the brucellosis status in small ruminant herds (OIE, 2018).

Common screening tests include macro-agglutination tests utilizing buffered smooth Brucella antigen, such as the Buffered Plate Agglutination Test (BPAT) or the Rose Bengal Test (RBT), as well as indirect Enzyme-Linked Immunosorbent Assays (iELISA). Complementary or confirmatory tests such as Fluorescence-Polarized Antigen (FPA) or the Complement Fixation Test (CFT) are often employed. In areas where *Brucella melitensis* is absent, the Brucellin Skin Test (BST) is the preferred diagnostic test to confirm false-positive serological results caused by infection with cross-reacting bacteria (like *Yersinia enterocolitica* O:9, Escherichia coli O157 or

Salmonella urbana). The Agar Gel Immunodiffusion (AGID) test is used in the field to serologically differentiate infected from vaccinated goats and sheep (Erdenebaatar, *et al*., 2002).

1.2.8.2.1. Rose Bengal Plate Test

The Rose Bengal Plate Test (RBPT) is a commonly used screening test for the detection of Brucella agglutinins. The principle of the test involves mixing sera collected from animals with an antigen and examining it for agglutination (Nielsen and Dunkan, 1990). The RBPT is considered a valuable screening test that is relatively easy to perform.

The RBPT is a rapid agglutination test that typically lasts for 4 minutes. It is carried out on a glass plate using an acidic-buffered antigen with a pH of 3.65 ± 0.05. This test has been implemented as the standard screening test in many countries due to its simplicity and perceived sensitivity (Greiner *et al*., 2009).

1.2.8.2.2. Complement Fixation Test

The Complement Fixation Test (CFT) is widely regarded as the most commonly used test for the serological confirmation of brucellosis in animals. It is a blood serum test that is considered both sensitive and specific, particularly when conducted by experienced users. The CFT is utilized as a definitive or confirmatory test for the detection of *Brucella abortus, Brucella melitensis,* and *Brucella ovis* infections. The CFT is known for its high accuracy and is recommended by the World Organization for Animal Health as the reference test for international animal transit. It is often employed when the initial screening test, such as Rose Bengal Plate Test (RBPT), produces positive results (OIE, 2009).

1.2.9. Prevention and Control Measures

After *Brucella melitensis* infection, three different options are recommended for control and eradication: test and slaughter of positive animals, massive vaccination, or pre-pubertal female vaccination with test and slaughter of infected animals (Alton, 1987).

Eradication of brucellosis by test-and-slaughter is unfeasible in developing countries because of limited resources to compensate farmers whose animals are slaughtered during such screening programs (Godfroid *et al*., 2011). It is usually accepted that a program of eliminating brucellosis by test and-slaughter policy is justified on economic grounds only when the prevalence of infected animals in an area is about 2% or less. For the implementation of such a program it is essential that the flocks are under strict surveillance and movement control. Animals must be individually identified and an efficient and well-organized veterinary service for surveillance and laboratory testing must be in place (Nicoletti, 1993).

However, a mass vaccination strategy (avoiding pregnant animals in mid-gestation) may be applied to control brucellosis in developing countries. The herd/flock level control of animal brucellosis may be achieved using some general principles: reducing the exposure to Brucella species and increasing the resistance to infection of animals in the populations (Corbel, 2006).

The Rev.1 live Brucella melitensis vaccine is the most widely used vaccine in control programs against brucellosis in small ruminants. When properly used the Rev.1 vaccine confers a long-lasting protection against field infections in a high proportion of animals. This vaccine however shows a considerable degree of virulence and induces abortions when the first vaccine dose is administered during pregnancy. The antibody response to vaccination cannot be differentiated from the one observed after field infection, and this therefore impedes control programs. Attempts have been made to develop new vaccines based on rough strains or genetically modified strains of the Brucella species. Those vaccines await further evaluation in field experiments (EU commission, 2001).

In an infected herd, the placenta, any abortion products and contaminated bedding should be removed promptly and destroyed, and contaminated fomites should be disinfected. Establishing a dedicated lambing or kidding area allows the site to be cleaned and disinfected more readily between births. The offspring of infected animals should not be used as herd replacements due to the risk that they may be latently infected (OIE, 2018).

In Ethiopia at regional levels, no strategy is in place to control brucellosis. But everybody has own responsibility to keep his environment, animals and own health care. To lower risk of getting brucellosis from a natural source it is important to avoid eating or drinking unpasteurized milk, cheese, or ice cream; check the label to make sure it says pasteurized and do not eat it if you aren’t sure, do not handle sick or dead animal bodies. But if you must, then use gloves plus face and eye protection, cook meat thoroughly. It is always a good idea to wash your hands regularly and avoid touching your eyes, nose, and mouth and disinfecting of the area where the animals are aborted (Haileleul, 2012 and Shimeles, 2008).

CHAPTER II: MATERIALS AND METHODS

2.1. Description of Study area

The study was conducted from May, 2021 till October, 2023 in Enderta district, Southeastern Zone of Tigray Regional State, Northern Ethiopia. As per the current administrative structure of districts in Tigray Region, Enderta is geographically bounded by Afar Regional State to the East, DeguaTemben district to the West, Hintalo district to the South, and Agulae district to the North. The district's absolute location is approximately 13° 29' 59.99" North Latitude and 39° 39' 59.99" East Longitude. In terms of livestock population, the district approximately consists of 125,933 cattle, 79,270 sheep, 135,350 goats, 636 horses, 48,639 donkeys, 2,008 mules, and 96,940 poultry. The total area coverage of the district is 141,051 hectares. The administrative center of the district is Kuiha, which is located 13 kilometers away from Mekelle city and 772 kilometers far from the capital city of Addis Ababa. Within the district, there are twenty four (24) Peasant Associations (PAs), and for the purpose of the study on brucellosis-like diseases, five PAs were selected. These selected PAs were Dergajen, Lemlem, Menbereqdusan, Meseret, and Addiazmera. These PAs collectively had a total of 22,603 small ruminants, including 4,182 sheep and 18,421 goats. The breakdown of small ruminant population in each selected PA was as follows: Dergajen had 1,184 sheep and 5,002 goats, Lemlem had 1,653 sheep and 3,027 goats, Menbereqdusan had 138 sheep and 2,693 goats, Meseret had 707 sheep and 6,399 goats, and Adiazmera had 500 sheep and 1,300 goats (Tigray Bureau of Agriculture and Rural Development and Personal communication with the district livestock experts).

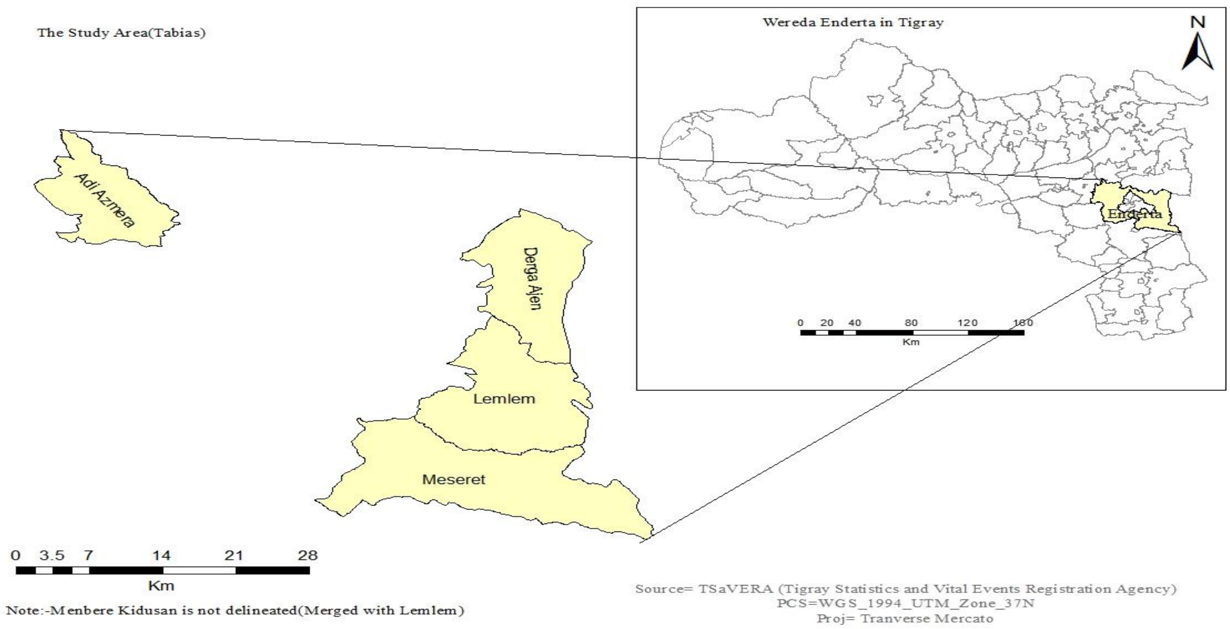

Figure 1: Map of peasant Associations of Enderta district, Southeastern cone, Tigray Regional; State, Northern Ethiopia.

2.2. Study population

The study focused on small ruminants that were managed under extensive production system, specifically the local breed of sheep and goats that had not been vaccinated against brucellosis. Animals of both sexes that were older than 6 months were included in the study, as the disease was not commonly observed in animals younger than 6 months due to the presence of maternal antibodies. During data collection, information on individual animal's age, species, breed, sex category, abortion history and flock size was recorded. The flock size per household was categorized as either ≤ 20 or > 20. Additionally, based on their sexual maturity, the study population was divided into two age groups: young animals, which were ≤ 2 years old, and adult animals, which were older than 2 years.

2.3. Study design

A cross-sectional research design was utilized to assess the sero-prevalence of brucellosis in small ruminants within Enderta district. This research design was chosen for its convenience in conducting a study at a single point in time, allowing for the collection of data from a representative sample of the population.

2.4. Sampling techniques

A combination of purposive and random sampling methods was employed to select the district, Peasant Associations (PAs), and individual animals for the study. The district and the five PAs (Dergajen, Lemlem, Menbereqdusan, Meseret, and Addiazmera) were purposively selected based on reports from animal owners regarding prevalent reproductive problems such as abortion, repeated breeding, birth of weak or dead offspring, retained fetal membranes, and infertility were prevalent in the study area..

Simple random sampling was used to select animals from each PA, taking into account the proportion of the animal population present in each PA. Additionally, a questionnaire survey was conducted to collect data from approximately 100 flock owners who owned either sheep or goats, or both species. The participants for the survey were selected using a simple random sampling technique, ensuring a representative sample to identify the disease and its associated risk factors.

2.5. Sample size determination

The sample size for the study was determined using the formula recommended by (Thrusfield, 2007) for simple random sampling, considering a 95% confidence interval and a desired absolute precision of 5%. Since there was no previous study conducted in the study area, an expected prevalence of 50% was used. The minimum sample size required, as per the aforementioned formula, was calculated to be 384 animals.

The formula used to calculate the sample size is as follows:

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

Where:

n = total sample size
d = absolute precision
Pexp = expected prevalence

2.6. Data collection

2.6.1. Blood sample collection

After properly restraining the animals, approximately 7-10 ml of blood samples were collected aseptically from the jugular vein of each animal using plain vacutainer tubes. All samples were carefully labeled and identified in a sequential manner. The tubes were then left tilted overnight at room temperature to facilitate clotting of the blood.

The following morning, the serum component was separated from the clotted blood by gently decanting and transferring it to sterile cryovials. The cryovials were appropriately labeled and the collected sera were stored at a temperature of -20°C in Mekelle Regional Veterinary Laboratory until they were needed for both the Rose Bengal Plate Test (RBPT) and the Complement Fixation Test (CFT) serological tests.

2.6.2. Questionnaire data collection

A questionnaire survey was conducted through face-to-face interviews with 100 flock owners in order to gather data. The objectives of the survey were twofold: first, to collect information pertaining to animal risk factors such as species, breed, sex, age, flock size, and history of abortion; second, to assess the flock owners' knowledge, attitudes, and practices regarding brucellosis infection.

The questionnaire was initially prepared in English and then translated into Tigrigna, the local language spoken by the research participants in the study area. Subsequently, it was translated back into English to ensure accuracy and consistency. The questionnaire consisted of a combination of closed-ended and open-ended questions, addressing various aspects related to brucellosis. The questionnaires were distributed to the flock owners, who also provided small ruminant blood samples for testing, as part of the study.

2.7. Serological tests

Two types of serological tests were utilized to detect Brucella antibodies: the Rose Bengal Plate Test (RBPT) as a screening test and the Complement Fixation Test (CFT) as a confirmatory test. The RBPT was conducted at Mekelle Regional Veterinary Laboratory, while the CFT was performed at the National Animal Health Diagnostic and Investigation Center (NAHDIC) in

Addis Ababa, Ethiopia. These two tests were chosen based on their established effectiveness in detecting Brucella antibodies and their widespread use in brucellosis diagnosis.

2.7.1. Rose Bengal Plate Test (RBPT)

The serum samples were subjected to screening using the Modified Rose Bengal Plate Test (MRBPT) to identify the presence or absence of agglutination, following the guidelines outlined by the OIE (2009). Prior to conducting the test, the required test sera and antigen for each day were removed from cold storage and allowed to equilibrate to room temperature for approximately 30 minutes.

For the MRBPT, 25µl of RBPT antigen and 75µl of test serum were carefully placed side by side on a plate. The mixture was thoroughly mixed using a plastic applicator, and then the plate was rotated for duration of 4 minutes. The resulting agglutination reactions were interpreted and classified into four categories: 0, +, ++, and +++, corresponding to the degree of agglutination observed. Specifically, 0 indicated the absence of agglutination, + indicated barely visible agglutination, ++ indicated fine agglutination, and +++ indicated coarse clumping.

Samples that exhibited no agglutination (0) were recorded as negative, while those showing +, ++, or +++ were considered positive and recorded accordingly. This test was conducted at Mekelle Regional Veterinary Laboratory.

2.7.2. Complement Fixation Test (CFT)

All serum samples that tested positive on the Rose Bengal Plate Test (RBPT) under went further confirmation using the Complement Fixation Test (CFT). A known antigen was incubated with a test and control sera to see formation of immune complexes. A defined amount of complement was added to the reaction mixture. In positive reactions of antigen and antibody create immune complexes, and the complement would be fixed or consumed. In negative sera, there was no immune complex formation and there was no consumption of the complement. In the second reaction step, red blood cells and their specific antibodies were added and form complexes. In the positive sera, no complement was left to hemolyze red blood cells. In negative sera, the complement will cause visible hemolysis of red blood cell. This test was conducted at National Animal Health Diagnostic and Investigation Center (NAHDIC), Addis Ababa, Ethiopia. A standard Brucella abortus antigen S99 and Brucella abortus positive control serum containing

5120 IU/ml, supplied by the Animal Health Veterinary Laboratories Agency (AHVLA) in New Haw, Addlestone, Surrey KT15 3NB, United Kingdom, were utilized. Prior to the test, 3% suspension of sheep red blood cells was prepared. The preparation of this reagent was evaluated through titration and performed following the protocols recommended by the World Organization for Animal Health (OIE, 2009).

During the CFT, sera exhibiting a strong reaction, with 100% complement fixation (4+) at a dilution of 1:2, with more than75% complement fixation (2+) at a dilution of 1:4 and above, were classified as positive. Conversely, the absence of complement fixation or complete hemolysis was considered as a negative result. An individual animal was considered positive if its serum sample tested positive on both the RBPT and CFT tests

2.8. Data analysis

The obtained data from the serological test results were entered into a Microsoft Excel spreadsheet program. The analysis of the data was conducted using the STATA version 17 statistical software program. To determine the presence of any association between seropositivity and potential risk factors, the Pearson's chi-square ($\chi2$) test was applied. Univariable logistic regression was employed to calculate the odds ratio (OR) and 95% confidence interval (CI) as measures of the strength of the associations. Species and sex were not included in the model as no brucellosis was reported in sheep and males. Significance was considered at a level of $P \leq 0.05$, and a confidence level (CL) of 95% was used for all analyses.

CHAPTER III: RESULTS

3.1. Sero-prevalence of small-ruminant brucellosis

A total of 384 serum samples (71 sheep and 313 goats) were examined to detect the presence of brucella antibodies. Out of these samples, 24 were found positive for brucella antibodies using RBPT (Rose Bengal Plate Test). Further testing was conducted using CFT (Complement Fixation Test), and all 24 samples tested were positive for brucella antibodies.

Based on these findings, the overall sero prevalence of brucellosis in the study area was determined to be 6.3% (24/384; 95% CI 0.042-0.092).

Table 1: Proportion of Brucella Sero-positive animals

Total samples	No of Positives	Prevalence (%)	CI (95%)
384	24	6.3	0.042 - 0.092

Table 2 presents the findings from a Chi-square ($\chi 2$) analysis investigating the relationship between selected risk factors and Brucellosis sero-positivity. The table provides a comprehensive breakdown of the variables and their respective categories, as well as the number of samples and the number of positive cases for each category. Regarding species, out of 71 samples of sheep, none tested positive, while out of 313 samples of goats, 24 tested positive. In terms of sex, out of 307 female samples, 24 tested positive, whereas none of the 77 male samples showed positive results. The analysis also considered age as a variable, revealing that out of 169 samples younger than 2 years, only 1 tested positive (0.59%), while out of 215 samples older than 2 years, 23 tested positive (10.7%). Furthermore, the presence of an abortion history showed a significant association with Brucellosis sero-positivity, as out of 276 samples with no abortion history, 8 tested positive (2.9%), whereas out of 31 samples with a history of abortion, 16 tested positive (51.6%). Finally, flock size did not seem to have a substantial impact, as both categories ($\leq$20 and >20) had similar proportions of positive cases (6.3% and 6.03%, respectively). The $\chi 2$ value and corresponding p-value were provided for each variable, indicating the strength and statistical significance of the associations found.

Table 2: Chi-square (χ^2) analysis of some selected risk factors with Brucellosis sero-positivity

Variables	Category	No. of samples	No. of Positives %	$\chi 2$	*P-value*
Peasant association	Dergajen	105	7(6.7)	3.2619	0.515
	Lemlem	80	5(6.3)		
	Menbereqdusan	48	3 (6.3)		
	Meseret	120	5(4.2)		
	Adi-Azmera	31	4(12.9)		
Species	Sheep	71	0	5.8070	0.016
	Goat	313	24		
Sex	Female	307	24	6.4208	0.011
	Male	77	0		
Age	≤2yrs	169	1 (0.59)	16.4929	0.000
	>2yrs	215	23 (10.7)		
Abortion history	No	276	8(2.9)	119.2667	0.000
	Yes	31	16 (51.6)		
Flock size	≤20	268	17(6.3)	0.0132	0.909
	>20	116	7(6.03)		

Table 3 presents the results of a univariate logistic regression analysis examining the association between selected risk factors and Brucellosis sero-positivity. The table provides information on the variables and their respective categories, the number of samples, the number of positive cases, the odds ratio (OR) with a 95% confidence interval (CI), and the p-value for each category. Dergajen is used as the reference category. Among the other peasant associations, Lemlem, Menbereqdusan, and Meseret did not show a statistically significant association with Brucellosis sero-positivity, as their odds ratios were close to 1 and their p-values were not significant. Adi-Azmera had a higher odds ratio of 2.01, but the p-value did not reach statistical significance. For the "Age" variable, samples younger than 2 years were used as the reference category. The odds ratio for samples older than 2 years was 20.13, indicating a significantly higher odd of Brucellosis sero-positivity in this age group. The p-value was significant at 0.003. The "Abortion history" variable showed a strong association with Brucellosis sero-positivity. The odds ratio for samples with a history of abortion was 36, indicating a significantly higher odd of sero-positivity compared to samples with no abortion history. The p-value was highly significant at <0.001. Lastly, the variable "Flock size" had no significant association with

Brucellosis sero-positivity, as both categories (≤20 and >20) had odds ratios close to 1 and non-significant p-values.

Table 3: Univariate logistic regression analysis of some selected risk factors with Brucellosis sero-positivity

Variables	Category	No. of samples	No. of Positives %	OR (95% CI)	*p-value*
Peasant associations	Dergajen	105	7(6.7)	Ref	
	Lemlem	80	5(6.3)	0.93(0.28 - 3.06)	0.909
	Menbereqdusan	48	3 (6.3)	0.93 (0.23 - 3.78)	0.923
	Meseret	120	5(4.2)	0.61(0.18 - 1.97)	0.409
	Adi-Azmera	31	4(12.9)	2.01(0.56 - 7.61)	0.271
Age	≤2yrs	169	1 (0.59)	Ref	
	>2yrs	215	23 (10.7)	20.13 (2.68 - 150.62)	0.003
Abortion history	No	276	8(2.9)	Ref	
	Yes	31	16 (51.6)	36 (13.30 - 97.39)	<0.001
Flock size	≤20	268	17(6.3)	Ref	
	>20	116	7(6.03)	0.95 (0.38 - 2.35)	0.909

3.2. Questionnaire survey result

The questionnaire survey was conducted to assess the knowledge, attitude, and practices of animal owners regarding brucellosis. A total of 100 respondents participated in the survey, and the results are summarized in Table 4.

In terms of milk consumption habits, 27% of the respondents reported not consuming milk at all. However, the majority, accounting for 66% of the respondents, consumed treated milk, indicating a positive practice. A small percentage, 7%, admitted to consuming raw milk, which can pose health risks.

When it came to the perception of brucellosis causing abortions, a significant proportion of respondents, 83%, related abortion with disease conditions. The remaining 17% attributed abortions to other factors, suggesting a lack of awareness or misinformation.

Regarding the occurrence stage of abortions, 37% of the respondents reported experiencing them at an early stage, while the majority, 63%, reported late-stage abortions. This information can be valuable for understanding the timeline of disease manifestation and implementing appropriate control measures.

The handling of aborted materials also varied among the respondents. Only 5% reported burying the materials, which is a recommended practice for preventing the spread of infection. Alarmingly, the majority, 95%, admitted to simply throwing the materials, which can contribute to the transmission of brucellosis to other animals or humans.

The mating system among animal owners showed a significant imbalance, with 85% practicing uncontrolled mating. Only 15% reported following a controlled mating system, which could be an area for improvement in disease prevention and breeding management.

In terms of assisting animals at delivery, a staggering 96% of respondents admitted to using bare hands, which increases the risk of transmitting infections. Only 4% reported using protective measures, highlighting the need for education and awareness regarding proper hygiene practices during delivery assistance.

The ownership of grazing and watering points was predominantly communal, with 97% of respondents indicating shared access. Only a small percentage, 3%, reported owning their own grazing and watering points, which could have implications for disease control and management strategies.

Table 4: Knowledge, Attitude and Practice of animal owners about brucellosis (n=100)

Parameter	Category	Number of respondents	Percentage (%)
Milk consumption habit	No consumption	27	27
	Yes treated	66	66
	raw	7	7
Perception on cause of abortion	Disease	83	83
	Other	17	17
Abortion occurrence stage	At Early	37	37
	Late	63	63
Handling of aborted materials	Burying	5	5
	Throw	95	95
Mating system	Controlled	15	15
	Uncontrolled	85	85
Assisting animals at delivery	Bare Hand	96	96
	With Protective	4	4
Grazing and watering points	Own	3	3
	Communal	97	97

CHAPTER IV: DISCUSSION

The overall sero-prevalence of small ruminant brucellosis recorded in this study area was 6.3% (24/384; CI: 0.042-0.092). This finding is moderately comparable to previous studies conducted in different regions of Ethiopia. For instance, Yohannes *et al*. (2013) reported a prevalence of 9.6% in Yabello pastoral area, and Negash *et al*. (2012) reported a prevalence of 9.11% in Dire Dawa. However, the current prevalence obtained in this study area is higher than the prevalence reported in and around Kombolcha 0.7% by Tewodros and Dawit, (2015), in Berbere district of Bale Zone 2.97% by Miftha *et al*., (2021), in selected export abattoirs of Addis Ababa 2.7% by Nigatu *et al*., (2014), in Werer Agricultural Research Center Afar 2.25% by Bezabih and Bulto, (2015), in Southern Zone of Tigray 3.5% by Teklue *et al*., (2013) and in Tselemti district of Tigray Region 1.79% by Kelkay et *al*., (2017). On the other hand, it is lower than the prevalence reported in Borana pastoral area 17.36% by Dereje *et al*., (2022), in Afar region 12.35% by Anteneh *et al*., (2014), in Afar Region 16% by Teshale *et al*., (2006), and also in Afar Region 13.6% by Adugna *et al*., (2013). The observed differences in the prevalence rates between the current study and previous studies may be attributed to various factors, including differences in management practices, sample size, animal species, awareness of the community, Agro-ecological differences and production systems.

In this study, brucellosis was found in goats with no report for sheep. This finding is consistent with the studies conducted by Mohammed et al. (2017), Wedajo *et al*. (2015), Aloto *et al*. (2022), and Negash *et al*. (2012), which also reported a higher prevalence in goats. It is also in agreement with Radostits *et al*. (2000), who stated that goats are at a higher risk of acquiring Brucella infection than sheep due to their greater susceptibility. Additionally, goats have been found to excrete the organism for a longer period, which reduces the potential for disease spread among sheep flocks. However, it is important to note that the inconsistency among various studies could be influenced by differences in the proportion of sheep and goats sampled, which could affect the reported prevalence of the disease.

Regarding the sex of animals, the disease was detected only in females. Similar trends have been reported by Ferede *et al*. (2011), Tsehay *et al*. (2014) and Kelkay *et al*. (2017). The absence of male sero reactor animals in this study could be attributed to the smaller number of males tested compared to females. Additionally, male animals are few in number and typically kept in the herd for a shorter period, which decreases their exposure to the disease. It is worth noting that male animals are generally less susceptible to Brucella infection than females, possibly due to the lower concentration of erythritol in males relative to females Hirsh and Zee, 1999 as cited in Tilahun *et al*., (2016). Moreover, it has been reported that the serological response of male animals to *Brucella* infection is limited and tests of infected male animals were usually observed to be non-reactors or showed low antibody titers Crawford *et al*., (1990).

Regarding the age of animals the odds ratio for samples older than 2 years was 20.13, indicating a significantly higher odd of Brucellosis sero-positivity in this age group. The p-value was significant at 0.003. This finding is consistent with the findings of Tsegaye *et al*. (2015) and Mohammed *et al*. (2017) reported higher prevalence in older age groups than in younger groups. This finding is also in agreement with previous studies by Quinn *et al*. (1999) and Radostits *et al.* (2000) reported sexually mature and pregnant animals are more prone to Brucella infection and brucellosis than sexually immature animals, the predilection sites being the reproductive tracts of the males and females, especially the pregnant uterus. This may result from the fact that sex hormones and erythritol, which stimulate the growth and multiplication of Brucella organisms, tend to increase in concentration with age and sexual maturity. Similarly Gul and Khan, (2007) expressed age is likely the most significant risk factor for brucellosis as it is closely correlated with the probability of infection due to sexual maturity, higher coital chances and increasing frequency of interaction with other animals with age. Therefore, it is important to consider the age of the animal when analyzing the factors influencing the occurrence of the disease.

Furthermore, animals with a previous history of abortion were found to be significantly more likely to be seropositive for brucellosis compared to animals without a history of abortion. This finding is in line with previous studies by Tekle *et al*. (2019) and Kelkay *et al*., (2017) which also reported a higher prevalence of brucellosis in animals with a history of abortion. Abortion is a common clinical manifestation of brucellosis in small ruminants, and infected animals that have experienced abortion can serve as a source of infection for other susceptible animals in the herd.

This is because abortions can release large numbers of infectious organisms into the environment, which can contaminate the area and infect other healthy animals in the flock. Animals infected with brucellosis often excrete Brucella species through vaginal discharges during lambing or kidding, which can contaminate the environment and could serve as an animal infection source. Insects such as flies may also serve as mechanical vectors for the spread of Brucella species throughout the farm atmosphere Coelho *et al*., (2019).

In this study, the prevalence of brucellosis was slightly higher in large flock sizes than small flock sizes, although the difference was not statistically significant. This finding is consistent with the study conducted by Wedajo *et al*. (2015) in the Afar region. Larger flock sizes and higher animal densities have been shown to be directly related to disease prevalence and can complicate infection control in a population, as reported by Walker (1999). Similarly, herds that included other livestock species alongside sheep and goats had higher brucellosis prevalence compared to herds that solely consisted of sheep and goats. This observation aligns with previous studies and suggests that the presence of other livestock species may contribute to increased disease transmission or exposure to Brucella organisms.

This study provides valuable insights into the sero-prevalence of small ruminant brucellosis in the study area. The overall prevalence of 6.3% indicates the presence of brucellosis among small ruminants in the region. Goats and older animals, as well as those with a history of abortion, were found to be at a higher risk of sero-positivity. These findings highlight the importance of implementing control and prevention measures targeting these high-risk groups to reduce the transmission and impact of brucellosis in small ruminant populations. Further studies are needed to explore the molecular epidemiology and risk factors associated with brucellosis in this region, which can guide the development of effective control strategies.

CHAPTER IV: CONCLUSION AND RECOMMENDATIONS

The study conducted on the sero-prevalence of small-ruminant brucellosis in the study area yielded important conclusions. The overall sero-prevalence of brucellosis was found to be 6.3% (24/384), with goats exhibiting a higher sero-prevalence 7.7% (24/313) compared to sheep (0%). This indicates that brucellosis is present in small-ruminant population in the study area, this may result in considerable economic loss in terms of reproductive wastages of animals and public health importance, thus requires attention for effective control and prevention measures. The analysis further revealed significant associations between brucellosis sero-positivity with age, abortion history, and species. Animals older than 2 years had a significantly higher risk of sero-positivity, emphasizing the importance of considering age as a risk factor. Additionally, individuals with a history of abortion were found to have a significantly higher risk, highlighting the need for targeted control measures focused on animals with abortion history.

The identification of these risk factors provides valuable insights for the prevention and control of brucellosis in the study area. By targeting older animals and those with a history of abortion, surveillance programs can be designed to detect and intervene in cases of brucellosis more effectively. Vaccination programs also become crucial, particularly for animals at higher risk, to reduce the prevalence of brucellosis. These findings underscore the significance of age and abortion history as key factors in disease transmission and emphasize the need for tailored measures to combat brucellosis effectively.

Furthermore, the questionnaire survey conducted to assess the knowledge, attitudes, and practices of animal owners’ revealed gaps in understanding and suboptimal practices related to brucellosis. Although a majority of respondents correctly identified diseases as causes of abortions, a small proportion attributed abortions to other factors, indicating a need for increased awareness and education. Additionally, improper handling of aborted materials, uncontrolled mating practices, and inadequate hygiene during delivery assistance were prevalent among respondents. These practices can contribute to the transmission and spread of brucellosis, necessitating educational programs to improve knowledge and promote good practices. In conclusion, the study provides valuable insights into the prevalence, risk factors, and knowledge, attitudes, and practices related to small-ruminant brucellosis in the study area.

The identification of risk factors such as age and abortion history enables targeted surveillance and control measures. Furthermore, addressing the gaps in knowledge and promoting good practices among animal owners through educational campaigns is crucial for effective prevention and control of brucellosis. By implementing the recommended measures, it is possible to reduce the prevalence and impact of brucellosis, improve animal health and welfare, and safeguard public health in the study area.

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ANNEXES

Annex 1.

The questionnaire format was prepared for the assessment of risk factors on the occurrence of small ruminant brucellosis on 100 flock owners in five selected peasant associations of Enderta district Southeastern Zone of Tigray region, Northern Ethiopia with the objectives of assessing knowledge, attitude and practice of attendants on brucellosis disease. This format was first developed by Tigray Bureau of Agriculture and Rural Development. For the purpose of this study the researcher adapted the questionnaire.

Part I

1. Zone: _____________

   District: _____________

   Peasant Association: _____________

2. Name of respondent___________________, Age___________, sex______________,

Educational level__________

Part II

3. Agro ecology

Highland__________

Midland__________

Lowland__________

4. Is livestock rearing the major activity on your farm?

A. Yes B. No

5. What type of breed of goat/sheep do you have?

A. Improved B. Local C. Both

6. What type of grazing do you use?

A. Own pastures B. Communal pasture C. Both

7. Do you keep separately small ruminants from other domestic animals?

A. Yes B. No

8. Do you keep separately sheep and goat?

A. Yes B. No

9. What type of watering do you use?

A. Public watering B. Own well

10. Do you sell breeding females/males of sheep/goats?

A. Yes B. No

If yes, for what reason?

A. Disease B. Infertility C. For cash income

11. Where do you get your replacement flock?

A. From your own B. From market C. From both

12. Did you introduce new animals to your flock in the past two years?

A. Yes B. No

13. Do you share rams/bucks with other flocks?

A. Yes B. No

14. Did you see any abortion in your flock of sheep/goats?

A. Yes B. No

15. Do you keep your animals separated from other flocks during grazing and watering?

A. Yes B. No

16. Do you use separate housing for small ruminants and other animals?

A. Yes B. No

17. Do you consume milk of sheep/goats?

A. Yes B. No

18. If yes, how do consume milk?

A. Raw B. Treated C. Other

19. Do you know any disease transmitted to human by drinking raw milk?

A. Yes B. No

20. In which species of animals did you see more abortions?

A. Sheep B. Goats

21. At what stage of pregnancy do the small ruminants face abortion?

A. Early B. Late

22. Have you seen any retention of placenta in your flock after birth?

A. Yes B. No

23. What do you think the cause of abortion in your flock of sheep/goats?

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24. What is the local name for the disease that causes abortion? If any

25. How do you manage aborted materials?

A. Throw on fields/bushes B. Burying

26. Do you keep aborted sheep or goat separately from the health once?

A. Yes B. No

27. Have you seen any birth of weak/dead fetus in your flock of sheep/goats?

A. Yes B. No

28. Have you seen repeat breeding in your flock? A. Yes B. No

Animal level

ID ______

1. District ______________

2. Peasant Association ______________

3. Species A. Goat B. Sheep

4. Sex A. Male B. Female

5. Age A. ≤ 2years B.>2 years

6. Flock size A. ≤ 20 B.>20

7. Abortion history A. No B. Yes

If yes early/late

8. Udder swelling A. No B. yes

9. Testicular swelling A. No B. Yes

10. Birth of weak/dead fetus A. No B. Yes

11. Retained placenta A. No B. Yes

12. Repeat breeding A. No B. Yes

Annex 2.

Principle, equipment, reagent and procedure of RBPT

The principle of the test:

The Rose Bengal Plate Test is one of the buffered *Brucella* antigen tests. It is a rapid agglutination test which is an effective screening test of brucellosis (OIE, 2012).

Equipment

- Applicator
- Micropipette
- Micropipette tips
- Plates

Reagent:

Rose Bengal-stained antigen

Positive and negative control sera

Test procedure

- ✓ Bring the sera samples and antigen to room temperature only sufficient for the day's tests should be removed from the refrigerator and placed at room temperature for at least 30 minutes before the test performed.
- ✓ Shake the antigen bottle well, but gently and Place 75µl of each serum sample and 25µl of antigen on a white enamel or plastic plate.
- ✓ Immediately mix the serum and antigen thoroughly (using a clean glass or plastic rod for each test) to produce a circular or oval zone approximately 2 cm in diameter.
- ✓ The mixture was agitated gently for 4 minutes at ambient temperature.
- ✓ Read for agglutination immediately after the 4-minute period was completed.
- ✓ Any visible reaction was considered to be positive.
- ✓ Finally the result of each test was read by looking the presence or absence of agglutination and the degree of agglutination was also appreciated in a very good light source and when necessary magnifying glass was used and any visible agglutination was considered positive.

Reaction was identified as 0, +, ++, +++ according OIE (2009)

+=barely visible agglutination (seen using magnifying glasses)

++=fine agglutination and

+++=coarse agglutination

Those samples with no agglutination (0) were recorded as negative while those with +, ++, and +++ were recorded as positive.

Annex 3.

Principle, material, and test proper of CFT

Principle of test:

The complement is fixed in the presence of antigen-antibody complex. This reaction is detected by the addition of sensitized SRBC (hemolysin and sheep red blood cells complex) used as an indicator system. The presence of immune complex is revealed by the absence of hemolysis (positive reaction). The absence of immune complex is revealed by hemolysis (negative reaction).

Materials

- 96 well U-bottomed micro plates
- Mono-channel, multi-channel micropipettes which able to dispense 25µl
- Water bath (NICKEL ELECTRO)
- Incubator-Shaker +37°C (INSL-England)
- Trough
- Refrigerator +4°C
- Bench top centrifuge (SIGMA 4-10)
- PH meter
- Sheet of plate lay out for records
- Shaker (R 100 ROTATEST SHAKER)

Reagents

Antigen, Complement (C), Sheep Red Blood Cells (SRBC), Haemolytic serum/Amoceptor, Reference serum/Haemolytic system, Veronal calcium magnesium (VCM), Positive & Negative control serum, Test serum.

Test procedure (proper)

De-complement test sera including positive and negative controls in hot water bath at 58°C ± 2°C for 30 minutes.

- Prepare a U-shaped 96- well microtiter plate.
- Dilute the test serum 1:2 (100µl test serum in 100µl VCM).
- Use column 1through 12 for test samples.
- Use each well of row A for anti-complementary control.

- Dispense25µl of VCM by using a hand-held 12 channel micropipette in to wells of rows A, C, D, E, F, G, and H.
- Dispense 25µl of diluted sera (1:2) in wells of rows A, B, C and homogenize wells of row C (column 1-12) of the test plate and pick up 25µl from row C of the test plate and deliver to the wells of row D (column 1-12) continue this serial dilution to row H (column 1-12) from which after homogenization 25µl is picked up and discarded.
- Add 25µl of diluted antigen in to all the wells of rows B, C, D, E, F, G, H and wells antigen and positive & negative control wells.
- Add 25µl diluted complement in the wells of rows A, B, C, D, E, F, G, H and all control wells except hemolytic system.
- The plate covered with sealer and incubates at +37°C under constant agitation on incubator shaker for 30 minutes.
- Add 25µl hemolytic system to all wells including control wells.
- The plate covered with sealer and incubated at +37°C under constant agitation on incubator shaker for 30 minutes.
- Reading: centrifuge at 2500 rpm for five minute or put in refrigeration at +4°C for 1 hour to let non-haemolysized SRBCs sediment. Then read the result over view box for degree of sedimentation or hemolysis.

  Interpretation of CFT result scores 1-4.

  0=100% hemolysis of SRBC no button/pellet of SRBC at bottom uniformly red supernatant.

  1=75% hemolysis of SRBC small button/pellet of SRBC at bottom of well, higher red supernatant.

  2=50% hemolysis of SRBC bigger button/pellet of SRBC at bottom of well medium red supernatant.

  3=25% hemolysis of SRBC still bigger button/pellet of SRBC at bottom of well higher supernatant, almost clear supernatant.

  4=0% hemolysis of SRBC larger button/pellet of SRBC at bottom of well supernatant clear like water.