JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

SEROPREVALENCE; RISK FACTORS AND ASSESSMENT OF KNOWLEDGE, ATTITUDE, AND PRACTICE OF OWNERS TOWARDS SMALL RUMINANT BRUCELLOSIS IN SELECTED DISTRICTS OF ILUBABOR ZONE, ETHIOPIA

MSc THESIS

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JUNE, 2024

JIMMA, ETHIOPIA

Jimma University
College of Agriculture and Veterinary Medicine
School of Veterinary Medicine

Seroprevalence; Risk Factors, and Assessment of Knowledge, Attitude, and Practice of Owners towards Small Ruminant Brucellosis in Selected Districts of Ilubabor Zone, Ethiopia

MSc Thesis Paper

Submitted to Postgraduate Studies, College of Agriculture and Veterinary Medicine, in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Veterinary Public Health

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June, 2024
Jimma, Ethiopia

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Thesis Submission Request Form (F-07)

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DEDICATION

I dedicate this thesis manuscript to my lovely parents, my beloved wife Mrs. Damme Getachew, and my children Moa Debela and Milki Debela, for their patience, encouragement, and support throughout the course of this work.

STATEMENT OF THE AUTHOR

I have acknowledged and proclaimed that this thesis is my original work by signing below. I prepared, gathered, analyzed, and finished this thesis in accordance with all scholarly ethical guidelines. Every academic reference in the thesis has been acknowledged with a citation. I certify that every source I utilized to create this paper has been cited and referenced. The production of this thesis has been done with the utmost care to prevent plagiarism.

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BIOGRAPHICAL SKETCH

The author was born to his father, Terefa Jambare, and his mother, Gadise Nurfata, on January 10, 1992. G.C. in Nunu Kumba district of East Wollega zone, Oromia Regional State, Ethiopia. He completed his primary schooling at Adare Primary School from 1999–2006 G.C. and attended secondary school from 2007–2008 G.C. at Nunu Gaba Robi Secondary School. He joined Alage TVTE College in 2009, graduated from Animal Health in 2011, and joined Nunu Kumba Animal and Fishery Production Office as an animal health worker for two years. He joined Jimma University in 2016 and graduated with a BVSc degree in veterinary science in June 2020 G.C. Soon after his graduation, he joined Jimma University in September 2021 to pursue his graduate studies leading to a Master of Science Degree in Veterinary Public Health. He has been conducting his MSc thesis on seroprevalence, risk factors, and assessment of knowledge, attitude, and practice of owners towards small ruminant brucellosis in selected districts of Ilubabor Zone, Southwestern Ethiopia, and now he is ready to present his research work.

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ACKNOWLEDGEMENTS

First and foremost, I extend my deepest gratitude to the Almighty God for the guidance, strength, and assistance provided throughout the numerous challenges encountered, which enabled me to accomplish the completion of my thesis successfully.

I would like to express my sincere appreciation to my advisors, Dr. Mukarim Abdurahaman and Dr. Mekonnen Addis, for their invaluable intellectual guidance, astute commentary, and the sharing of their experiences. Their unwavering commitment and the time they dedicated to meticulously reviewing my research paper were instrumental. The objective perspectives, assistance, and astute suggestions they provided were pivotal in the successful completion of this research.

My gratitude extends to the technical and administrative staff at Bedele Regional Veterinary Laboratory for their cooperation and support, which were crucial in providing the necessary kits and processing my samples.

I extend my heartfelt thanks to Jimma University College of Agriculture and Veterinary Medicine for the time and resources provided to me during the preparation of this research paper. My profound gratitude goes to my family, whose unwavering support, love, and care have been my cornerstone. Additionally, I am grateful to my friends for their invaluable assistance and encouragement throughout the writing process.

LIST OF ABBREVIATIONS

CFSPH	Center for food security and public health
CFT	Complement fixation test
CSA	Central Statistical Agency
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
HRP	Horseradish Peroxidase
IBM	Interim Brucellosis Manual
I-ELISA	Indirect Enzyme Linked Immune Sorbent Assay
ILRI	International Livestock Research Institute
KAP	Knowledge, attitude and practice
MoARD	Ministry of Agriculture and Rural Development
PCR	Polymerase Chain Reaction
RBPT	Rose-Bengal-Plate Test
SAT	Serum agglutination test
SPSS	Statistical Product for Service Solutions
TMB	Tetra methyl Benzidine
WHO	World Health Organization
WOAH	World Organization for Animal Health

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ABSTRACT

Brucellosis is a zoonotic disease that affects both animals and humans, leading to reproductive issues and public health concerns. It is endemic among livestock in Ethiopia. In selected districts of the study area, there was a lack of published data on the prevalence and awareness of small ruminant brucellosis among small ruminant owners. A cross-sectional study was conducted in selected districts within the Ilubabor zone of Ethiopia, from April 2023 to January 2024. The objectives of the study were to estimate seroprevalence; and assessment of knowledge, attitude, and practice of participants towards small ruminant brucellosis. A total of 422 sera from 298 sheep and 124 goats were randomly selected using simple random sampling techniques. The research assessed the knowledge, attitudes, and practices of farmers regarding brucellosis through the use of a structured questionnaire. Antibodies against brucellosis within serum samples were tested using the I-ELISA method. SPSS version 23 and binary logistic regression were used to evaluate the correlation between seropositivity and risk factors. The study revealed an overall seroprevalence of brucellosis in small ruminants of 2.4% at the individual level and 9.1% at the flock level. The seroprevalence was significantly associated with species (OR = 5.5, 95% CI: 1.013–29.753), pregnancy status (OR = 6.4, 95% CI: 1.071–38.337), history of abortion (OR = 9.1, 95% CI: 1.523–54.24), and retained fetal membranes (OR = 9.02, 95% CI: 1.496–54.452) at the individual animal level. At the flock level, it was associated with flock size prevalence (OR = 14.5, 95% CI: 1.589–132.150) and history of abortion (OR = 5.5, 95% CI = 1.02–29.080). A survey revealed that 74.2% of small ruminant owners lack knowledge, 70.8% have negative views, and 80.5% engage in risky practices. The study suggests a moderate prevalence of small-ruminant brucellosis in the area. The community should be educated about brucellosis transmission, symptoms, and preventive measures, promote safe handling practices, implement biosecurity, and use personal protective equipment.

Keywords: *I-ELISA, Ilubabor zone, Seroprevalence, Small ruminant brucellosis*

1. INTRODUCTION

1.1. Background

In developing countries, small ruminants serve a variety of social and cultural purposes that vary depending on the culture, socioeconomic system, agro-ecology, and geographic region in tropical and subtropical Africa (Gobena, 2016). In Africa, Ethiopia is home to the greatest number of livestock: 70.3 million cattle, 42.9 million sheep, 52.5 million goats, and 8.1 million camels, according to a CSA (2021) livestock survey study.

Infectious and non-infectious diseases are the main causes of these animals' reduced production outputs. According to Melese *et al*. (2016), brucellosis is endemic in Ethiopia and affects livestock, with prevalence varying from 3% to nearly 50% depending on the species and geographical location. Brucellosis is one of the most common zoonotic diseases, affecting hundreds of thousands of people and animals worldwide. In animals, *Brucella* infection has a drastic impact on health and productivity, especially in farm animals, which constitute the source of staple food for many local communities and parts of the global trade (Franc *et al*., 2018). Brucellosis is caused by bacterial species of the genus Brucella (Alemu *et al*., 2014). Today, this genus is considered to contain different species, including *Brucella abortus, B. melitensis, B. suis, B. ovis, B. canis, B. ceti, B. pinnipedialis, B. neotomae, B. microti, and B. inopinata* (CFSPH, 2018).

Brucella organisms are gram-negative, small, non-motile, non-spore-forming, and facultative intracellular coccobacilli that occur in a wide variety of animals, including domesticated cattle, sheep, goats, camels, pigs, and other livestock, as well as humans (Haggag, 2016; Tekle *et al*., 2019). Sheep and goats are the primary hosts for *Brucella melitensis* (*B. melitensis),* which is a common Brucella species in humans. *B. ovis* attacks only rams and occasionally infects ewes. In addition to these agents, *B. abortus* and *B. suis* have been reported sporadically in small ruminants (Kelkay *et al*., 2017). In Brucella-infected animals, the levels of immunoglobulin isotopes (IgM, IgG, and IgA) significantly increased in the serum. Chronic granulomatous lesions develop in infected tissue, whereas macrophages, neutrophils, and lymphocytes respond to Brucella antigens (Radostits *et al*., 2007).

Brucella melitensis is the causal organism for Undulant, or “Malta fever," which has the greatest risk for human infection, followed by *B. suis* and *B. abortus; however*, several other species have been shown to be virulent for humans. It causes a systemic infection with clinical manifestations such as undulant fever, headache, sweating, fatigue, and joint pain in humans (Godfroid *et al*., 2013).

When a female animal has brucellosis, abortion, or gives birth to a weak one, she may also have fluids coming out of her body that have bacteria in them. If other animals touch or eat these fluids, they can get brucellosis too. This can happen easily if there are many animals together. Brucella is a bacteria that can cause a disease called brucellosis in animals and people. Sometimes, these bacteria can live outside of the body of an animal or a person for a long time. They can stay alive in different places, such as dead animals, soil, paper, or blood. This means that they can infect new animals or people who come in contact with them (Franc *et al*., 2018). Pastoralists and farmers keep mixing different species of animals, including sheep, goats, cattle, camels, and equine species. In view of the widespread risk factors for the establishment and transmission of pathogen infection due to high stock density and multi-species composition, a favorable climate, and a lack of controlling measures (Megersa *et al*., 2013), In both pastoral and agro-pastoral livestock production systems, people live closely with livestock, making contact with small ruminants vaginal discharges and aborted fetuses. Consumption of unpasteurized contaminated milk led to a high incidence of acquiring Brucella infection (Habtamu *et al*., 2015).

The knowledge, attitude and practices of communities on small ruminant brucellosis are low in pastoralist and agro-pastoral areas of low-income countries with conducting risks practices. These practices include consuming raw animal products and not employing protective measures when handling parturient animals or disposing of placenta and other aborted materials (Zinsstag *et al*., 2006). This lack of precaution is often due to their limited knowledge about brucellosis which consequently facilitates potential transmission of zoonotic Brucella pathogens from animals to humans (Addis, 2013). In Ethiopia, researches conducted in different parts of the country indicated that communities in various districts display poor awareness of small ruminant brucellosis. For instance, knowledge rates were found to be 11%

in Yabello district (Wubishet *et al*., 2018), 30% in Dallo-Manna and Haranna-Bulluk (Adem *et al*., 2021) and 15% in the South Eastern Somali Region (Ahad, 2021).

A very important approach to the control of brucellosis that is gaining more and more recognition around the world in recent years is the One Health approach to control and prevent human and animal brucellosis requires multidiscipline efforts (Gemachu, 2017). Brucellosis is controlled and eradicated in developed countries, but it endemically distributed in developing countries (McDermott *et al*., 2013).

Even though brucellosis is the endemic nature of disease in Ethiopia, there is no plan to prevent and control strategy at national level (Bruktayet and Marsha, 2016). As different seroprevalence studies carried out small ruminant brucellosis endemically exists in various parts of Ethiopia (Tadeg *et al.,* 2015). The disease is highly prevalent in pastoral and agro-pastoral husbandry practices where aggregation of animals favors the agent (Tadeg *et al.,* 2015). Furthermore, the detailed status of brucellosis and its associated risk factors were not studied in small ruminants, particularly in the Ilubabor zone. Therefore, the recent finding could help to introduce the existence of brucellosis and associated risk factors and to assess the knowledge, attitude, and practices of farmers regarding small ruminant brucellosis in the study area.

1.2.Statement of the Problem

In several parts of Ethiopia, small-ruminant brucellosis is still endemic. However, some researchers have serologically demonstrated evidence of brucellosis in different parts of the country (Melese, 2016; Tulu *et al*., 2020; Tegegn *et al.,* 2023; Tasew *et al.,* 2023; Negash *et al*., 2012; Tadeg *et al*., 2015; Wubishet *et al*., 2018; Adem *et al*., 2021; Ahad, 2021). Researchers from different backgrounds have reported flock-level seroprevalence in Ethiopia's small ruminant population. Higher estimate of flock level seropositivity of (57.7%) in the sheep and goat flocks from the Afar region, (28%) from southern Tigray (Teklue *et al.,* 2013), (26%) in the sheep and goat flocks in Arsi and East Shewa area, and (22.3%) in the shoat flocks in the Amhara region. According to Asmare *et al.* (2013), goats from sedentary and agro-pastoral production systems in the southern and central regions of Ethiopia had flock-level seroprevalence of 3.6% and 13.2%, respectively.

Among several zoonotic diseases affecting humans and animals, brucellosis is known to be an important zoonosis and economically important disease, posing a considerable cause of reproductive losses in animals (Terefe *et al*., 2017). It is also defined as a contagious systemic bacterial disease primarily of ruminants, characterized by inflammation of the genital organs and fetal membranes, abortion, sterility, and the formation of localized lesions in the lymphatic system and joints (Awah-Ndukum *et al*., 2018).

The primary causes of brucellosis could include low awareness, a lack of knowledge about the disease, a lack of control policies, and a lack of resources (Ismail *et al*., 2019). In Ethiopia, there was limited information on the prevalence of small ruminant brucellosis and the KAP of the communities about brucellosis (Legesse *et al*., 2018). The serostatus, risk factors, and stakeholders' perceptions about brucellosis in small ruminants are not well documented in the southwestern parts of the country (especially the Ilubabor zone).

In the Ilubabor Zone of Ethiopia, there was a lack of published data regarding the prevalence of small ruminant brucellosis, as well as the level of awareness among small ruminant owners about this disease and its associated risk factors. The study estimates the prevalence of small-ruminant brucellosis, identifies risk factors, and evaluates the knowledge, attitudes, and practices of small ruminant owners in selected districts of Ilubabor Zone. There was a risk of Brucella infection in areas where abortion and retained fetal membranes were common in sheep and goats. To address this gap, this study was undertaken with the following objectives:

1.3. Objectives of the Study

- To estimate the seroprevalence of small ruminant brucellosis in selected districts of the Ilubabor zone.
- To identify the major risk factors associated with the occurrence of brucellosis in the study area at the individual animal level and flock level.
- The study aims to assess the knowledge, attitudes, and practices of participants regarding small ruminant brucellosis.

2. LITERATURE REVIEW

2.1. History of Brucellosis

Brucellosis has a long history dating back to when humans first came into touch with animals. Research has indicated that the disease has been present in humans as well as animals for a very long time (Akpınar, 2016). The isolation and identification of *Brucella melitensis* (*Micrococcus melitensis*) in the 1880s does not, therefore, mark the beginning of the history of brucellosis. Prior to this, brucellosis may have been the cause of many historical reports of illnesses, such as fever in humans and animal abortion epidemics. The paleo-pathological evidence from the partial skeleton of the late Pliocene *Australopithecus africanus* suggests that brucellosis occasionally affected our direct ancestors 2.3-2.5 million years ago. The pathological, molecular (DNA analysis) and electron microscopy findings from the human skeletal buried cheese remains also suggested the presence of brucellosis *a* long time ago (3000–1200 B.C.) in Bahrain, the Persian Gulf, 2100–1550 B.C. in Palestine and Jordan, and 79 A.D. in the Roman towns of Pompeii and Herculaneum. 1260–1020 A.D. in Butrint, Albania (Gebretsadik *et al*., 2016).

The causative agent of brucellosis, *"Micrococcus melitensis*" (also known as *Brucella melitensis*), was identified in the livers of sick soldiers on the Mediterranean island of Malta in 1887 by Maltese microbiologist doctor Giuseppe Caruana-Scicluna, his wife Mary Elizabeth Steele, and British surgeon captain David Bruce. Following this finding, Maltese physician Temi Zammit disclosed that contaminated milk was the means by which people contracted Malta fever from infected goats. The Danish scientist Bernhard Bang discovered "*Bacillus abortus*" (also known as *Brucella abortus*) in 1897 from aborted bovine fetuses, 10 years after "*Micrococcus melitensis*" was discovered (Rahman, 2015). The condition was then given the extra moniker "Bang's disease" (Gebretsadik *et al*., 2016).

In 1968, Carmichael and Bruner isolated Brucella canis from beagle dogs. In 1994, Ewalt, Ross, and associates separated *Brucella pinnipediae* and *Brucella cetacea* from seals, whales, and dolphins. Last but not least, Scholz *et al.* (2008) recovered *Brucella microti* from field mice in Central Europe and the mandibular lymph nodes of a wild red fox (Rahman, 2015).

In 2008, researchers isolated a new species, *Brucella microti*, from common voles and red foxes. Two more strains were later found in humans in 2010. The first was extracted from an infected human breast implant and named *Brucella inopinata*. The second strain, which showed similarities to *Brucella inopinata*, was isolated from a patient with chronic lung disease. Two recently discovered species are *Brucella papionis*, taken from two baboons with retained placenta by Whatmore *et al* in 2014, and *Brucella vulpis*, isolated in Austria from the mandibular lymph nodes of two red foxes by Scholz *et al* in 2016 (Rajala, 2016).

2.2. Etiology, Taxonomy, and Characteristics of Brucellosis.

Brucellosis is a bacterial disease affecting both animals and humans, and is attributed to the Genus Brucella within the family Brucellae. This genus belongs to the order Rhizobiales, class Alphaproteobacteria, and phylum Proteobacteria. Brucella species are facultative intracellular, slow-growing, gram-negative, aerobic, and non-spore-forming. These bacteria are small cocco-bacilli or short rods that are partially acid-fast and typically found as individual cells or in small groups, lacking capsules, endospores, or native plasmids (Wernery, 2014; Mustefa and Bedore, 2019).

Brucella can survive and reproduce within epithelial cells, placental trophoblasts, dendritic cells, and macrophages in both livestock and humans. In livestock, brucellosis primarily impacts the reproductive organs, leading to abortion, reduced fertility, and decreased milk production (De Figueiredo *et al*., 2015).

Carbon dioxide is an essential element for the growth of Brucella organisms, especially *B. abortus*. These organisms, which require carbon dioxide for their growth, are called capnophilic organisms. Brucellae are quickly and effectively inactivated by an acidic pH of less than 3.5. They can also be destroyed by moist heat at 121°C (250°F) for at least 15 minutes, dry heat at 320-338°F (160-170°C) for at least 1 hour, gamma irradiation, and pasteurization. Boiling for 10 minutes is generally effective for liquids (CFSPH, 2018).

The twelve closely related species of the genus *Brucella* are as follows: *B. melitensis* (sheep and goats), *B. abortus* (cattle), *B. suis* (swine), *B. canis* (dogs), *B. ovis* (sheep), *B. neotomae* (desert wood rats), *B. cetacea* (cetacean), *B. pinnipedia* (seal), *B. microti* (voles), and *B.*

inopinata (unknown) (CFSPH, 2018). This genus is formed by preferential host specificity. Spillover transmission, however, is conceivable (Jemal *et al*., 2019 and Saleem *et al*., 2019). While goat breeds are more susceptible than sheep, *B. ovis* is a major cause of orchitis and epididymitis in rams and can also infect ewes on occasion. The most prevalent strains were *Brucella abortus* biovar1, next to *B. melitensis* biovar 3. All animal species are susceptible to infection (Hashem *et al*., 2020; Rabah *et al*., 2022).

B. melitensis, B. abortus, B. suis, B. ovis, B. neotomae, and *B. canis* are the six classical species. According to Rajala (2016), *Brucella suis, B. abortus*, and *B. melitensis* are further divided into biovars. The primary cause of caprine and ovine brucellosis is *Brucella melitensis*, which is extremely pathogenic to humans and one of the most dangerous zoonoses in the world (Ferede *et al*., 2011).

From a socioeconomic perspective, the most significant members of the genus Brucella are *B. melitensis, B. abortus, B. suis*, and *B. ovis*, which predominantly infect sheep and goats, cattle, pigs, and sheep, respectively. According to Asnake *et al*. (2017), the first three species are primarily to blame for brucellosis in humans in addition to lowering animal productivity. Except for *B. ovis*, Brucellae produce urease, oxidase, catalase, nitrate reductase, and hemolytic activity. They do not create indole, liquefy gelatin, and have negative results on the methyl red and Voges-Proskauer tests. With the exception of *B. ovis*, the majority use glucose as a fuel source. Due to their aerosol-spreading ability and lack of human immunizations, Brucella species have been categorized as possible bioterrorism agents (Gemechu, 2017).

The world's most dangerous zoonoses, *Brucella melitensis* biovars (1-3), *B. suis* biovars (1-5) and *B. abortus* biovars (1-7, 9), are said to be highly pathogenic for humans, according to Hadush and Pal (2013). Although its preferred host is still unknown, *B. inopinata* was initially isolated from a human patient. It is not harmful to humans for *B. ovis, B. neotomae*, or *B. microti.*

Table 1. The species, biotypes, host preferences and zoonotic potentials of Brucella (Godfroid *et al*., 2013)

Species	Biovars	Colony type	Host tropism	First reported country	First Described	Zoonotic potential
B. melitensis	1-3	Smooth	Goat, sheep, camels, cows	Malta	Bruce, 1887	High
B. abortus	1-6, 9	Smooth	Cattle, buffalo, camels, bison, elk, yaks	Denmark	Bang, 1897	High
B. suis	1-3		Pigs			High
	2	Smooth	Wild boar, hare			No
	4		Reindeer, Caribous	USA	Traum, 1914	High
	5		Rodents			No
B. neotomae	-	Smooth	Desert wood rat	USA	Carmichel and Bruner, 1968	No
B. pinnipedia	-	Smooth	Seal	Scotland	Ewalt *et al.*, 1994	Mild
B. ceti	-	Smooth	Dolphin, porpoise, whale	Scotland		Mild
B. microti	-	Smooth	Vole, fox, (soil)	Czech Republic		Unknown
B. inopinata	-	Smooth	Unknown	Australia		Mild
B. ovis	-	Rough	Sheep	New Zealand	Van Drimmelen, 1953	No
B. canis	-	Rough	Dog	USA		Mild

2.3. Epidemiology of Small Ruminant Brucellosis

2.3.1. Geographical distribution

The geographic range of brucellosis is intricate and subject to fluctuations. Microorganisms can survive in populations due to their broad host range, environmental resistance, and host immune system. It remains a major source of disease in domestic animals and humans (Yasmin and Lone, 2015).

World Organization for Animal Health (WOAH, 2018) estimates that the illness continues to spread and impacts about 50% of the world's livestock population. The most virulent species in the Brucella genus, *B.melitensis*, is also the one that is isolated in small ruminants most commonly throughout the Mediterranean, the Middle East, and Latin America. In addition to being a dangerous zoonosis, brucellosis hinders the trade in animals and animal products and results in large abortion losses. For the broad and intense animal production systems found in the tropics, *B. melitensis* and *B. ovis* pose an economic challenge. There is no trustworthy record indicating that the organism has ever been eliminated from small ruminants in any industrialized country, despite the fact that the disease has been eliminated in the majority of them (Addis, 2015).

2.3.2. The source of the infection and how it spreads.

Large livestock populations, readily available resources, and the mixing of different livestock species can make it easy for uninfected animals to come into contact with diseased animals, both directly and indirectly. Numerous researches have also shown that mixed farming systems, particularly those that include small ruminants in addition to cattle, increase the danger of Brucella transmission between other animal species. Purchasing animals from unscreened sources and merging flocks owned by different people can both facilitate the spread of disease (Godfroid *et al*., 2013).

There are both vertical and horizontal routes for brucellosis transmission in animals. Ingestion of contaminated feed and water, contact through intact skin and conjunctiva, fetal fluids, and vaginal discharges generated from infected ewes and does after miscarriage or full-term parturition are the routes of horizontal transmission. According to Godfroid *et al*. (2013), this

is the main way that small ruminant brucellosis spreads naturally. In a vertical manner, newborns and lambs may contract an infection from their mothers' infected milk or by breastfeeding, and during the nursing stage; they may excrete *B. melitensis* in their feces. Young animals that contract the bacteria may develop into chronic carriers. Sheep and goats that are chronically infected either become latent carriers or self-limiting (Selim *et al*., 2015).

Small ruminants are susceptible to contracting *B. melitensis* when they come into contact with flocks and herds owned by various people or when they buy animals from unreliable sources. The exchange of male breeding stock throughout farms facilitates the spread of illness. In certain places, transhumance summer grazing and animal interactions at markets are important factors that contribute to development.

It may be common practice to keep animals close together in cold climates, which promotes the spread of infection (Habtamu *et al*., 2015).

During infection, the infected animal spreads these bacteria through milk, feces, urine, and vaginal discharge. Infertility may develop in the affected mothers in later life. Goats' vaginal secretions can shed microorganisms, which could take two to three months or longer. Compared to goats, sheep have far less infection in their milk and uterine exudates. According to studies, direct skin and mucous membrane contact accounts for 70–90% of *Brucella* infections (Franc *et al*., 2018).

Infected small ruminants remain unknown, serve as a source of infection for humans, and continue to spread disease, report Terefe *et al*. (2017). Among the several zoonotic illnesses that can affect both humans and animals, brucellosis is recognized as a significant zoonosis and a disease with significant economic implications that can lead to significant reproductive losses in animals (figure 1).

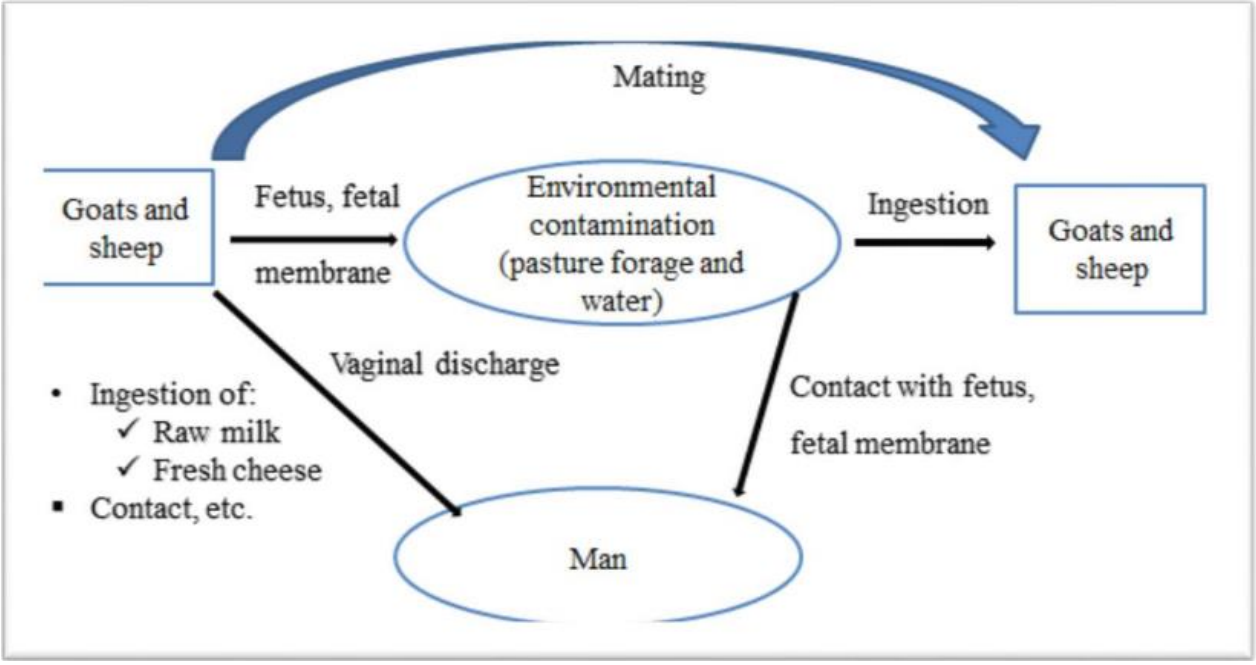

Figure 1: Transmission mode of brucellosis in small ruminants (Franc *et al*., 2018).

2.3.3. Related risk Factors

Numerous risk factors for brucellosis are associated with production methods, the biology of the individual host, and environmental factors. A number of factors related to the disease's transmission between flocks can be categorized as affecting the epidemiology of brucellosis in any given geographic area. A number of related variables, such as agent factors, host factors (age, sex, and breed), and extrinsic (environmental) factors (management and ecology), affect the maintenance and spread of Brucella infection within the herd (Guven *et al*., 2013).

Risk factors related to agents: Brucella is an intracellular pathogen that can survive and replicate within phagocytic cells. It can persist on fetal tissues and soil or vegetation for 21-81 days depending on month, temperature, and exposure to sunlight. B. abortus field strain persisted for up to 43 days in oil and vegetation at naturally contaminated bison birth or abortion sites (Gemechu, 2017). Brucella bacteria can also survive in cold areas and freeze meat for long periods (Addis, 2015). It has been recovered from the fetuses and from manure that has remained in a cool environment for > 2 months. Brucella abortus field strain persisted for up to 43 days in soil and vegetation at naturally birth-contaminated birth or abortion sites.

The organisms can survive within host leukocytes and may utilize both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanisms during the period of hematogenous spread (Mustefa and Bedore, 2019).

Risk factors related to the host: The biology of the individual host and production systems are among the risk variables that affect the prevalence of brucellosis. Exotic breed animals as well as hybrids are shown to be more susceptible. This could be linked to more skilled farmers and closely monitored (Rossetti *et al*., 2017). It is also widely accepted that susceptibility the disease is most frequently occurring in adult sheep and goats than younger ones. Male and female small ruminants' reproductive tract is the predilection site for replication, especially increases in pregnant uterus (Guven *et al*., 2013).

Risk factors related to the environment: The environment and husbandry practices have a big impact on how brucellosis spreads. It is easier for diseases to spread when lambs are raised in crowded, dark cages as opposed to outside in a dry climate. Farmers may unwittingly buy fresh animals from contaminated farms during restocking, which might spread infection throughout their farms. This is particularly common on farms, where biosecurity protocols and procedures are often lacking (Khan *et al*., 2018).

Large livestock populations and the mixing of different livestock species may make it easier for uninfected animals to contract the disease from a variety of sources, including direct contact with sick animals and abortion discharges. Numerous studies have found that raising goats and sheep alongside cattle, in particular, and combining flocks at pasture and sheltering the animals at night increase the risk of Brucella transmission between various animal species (Godfroid *et al*., 2013).

The primary method by which infection spreads among flocks is through movement or gathering of diseased animals. The biggest risk factor for spreading the disease to previously uninfected areas is the purchase of sick animals. The frequency of brucellosis in small ruminants and the livestock trade practice are strongly correlated in many countries (Khan *et al.*, 2018).

Management: The disease is transferred from one flock to another and from one location to another by means of diseased animals moving from an infected flock into a susceptible non-

infected flock. Therefore, the control of animal movement from one area to another, inadequate hygienic procedures, and effective husbandry management all contribute significantly to the rise in brucellosis prevalence (Ashagrie *et al*., 2011). The low transmission rates are often indicative of brucellosis in large systems, and intensification raises the risk of transmission due to higher birth index, higher stocking density, and more animal contact (Mc Dermott *et al*., 2013).

Work occupations at increased risk: Individuals who handle animals or come into touch with blood that has been contaminated are more susceptible to brucellosis. Individuals who work in slaughterhouses, dairies, ranches, microbiologists, veterinarians, and hunting are at risk (Gemechu, 2017). Additionally, workers in endemic areas and those conducting artificial insemination run the risk of contracting an illness. Brucella is currently thought to be one of the world's possible bioweapons (Bauerfeind *et al.*, 2020).

2.4. Clinical Findings

The reproductive tract in any flock is the primary site of clinical manifestation for brucellosis in naturally infected sheep and goats. The most obvious signs or symptoms of a *Brucella* infection are abortion, which typically happens during the third and fourth month of pregnancy; other symptoms include stillbirths, the birth of weak offspring, lameness, endometritis, and infrequently mastitis (Rerkyusuke *et al*., 2022). Most infected animals only fail to deliver once, but with subsequent pregnancies, the uterus may reopen and organisms may shed. Orchitis and epididymitis in male can lead to infertility later on (Bauerfeind *et al.*, 2020).

Many non-pregnant sheep and goats are asymptomatic from the disease, and following the initial episode of abortion caused by Brucella, the ewes frequently give birth to healthy offspring. Though there are fewer organisms in milk and uterine secretions, uterine and mammary infections still reoccur (WOAH, 2018). When an animal is aborted or has an infection in its udder after giving birth normally, the amount of milk produced is sharply decreased. However, mastitis seldom manifests clinically. Male can have acute orchitis and epididymitis, and both sexes can suffer from arthritis on rare occasions Poester *et al*. (2013).

Small ruminants are often the home of *Brucella melitensis*, a highly pathogenic organism for humans that causes one of the worst zoonoses in the world. Acute brucellosis brought on by *B. melitensis* presents with flu-like and largely vague symptoms. Fever with waves is the most common symptom seen in humans. One of the most prevalent symptoms in patients is fever; it occurs sporadically in 60% of cases of acute brucellosis and continuously in 40% of cases of sub-acute brucellosis. The nonspecific and imprecise symptoms of chronic brucellosis might be mistaken for other illnesses impacting different organ systems (Kelkay *et al*., 2017). Brucellosis increases the risk of spontaneous abortion early trimesters of pregnancy, premature delivery, miscarriage and intrauterine infection with fetal death in humans as well, which is accompanied with malaise, fatigue and arthritis (Kose *et al*., 2014).

2.5. Public Health and Economic Importance

Brucella melitensis infection has major veterinary and human importance in areas where it occurs and causes considerable economic losses associated with abortion, and neonatal death (Rajala *et al*., 2016). Public health importance: Brucellosis has been reported in 100 different countries worldwide and is a serious threat not only to livestock but also to human health globally. Currently, half a million cases of brucellosis occur in humans around the world annually (Pal, 2018). It is a significant health risk for the entire community since there is close contact between humans and their livestock through sharing the same housing enclosures. The expansion of animal industries, the lack of hygienic measures in animal husbandry, and poor food handling partly account for brucellosis as a significant public health hazard (Addis, 2015). Economic importance of brucellosis: Brucellae cause both direct and indirect economic losses in the livestock sector. The direct effects of brucellosis include bovine abortion, reduced milk quantity and quality, decreased weight gain due to chronic infection, slower growth rate, premature death, culling of non-productive stock, and poor animal welfare (Franc *et al*., 2018). The economic losses of brucellosis include losses due to abortion in the affected animal population, diminished milk production, Brucella mastitis and contamination of milk, culling and condemnation of infected animals due to breeding failure, endangering animal export, human brucellosis causing reduced work capacity through sickness, government costs on research and eradication schemes, and losses of financial investments (FAO, 2003).

2.6. Diagnostic Methods of Brucellosis

Although the flock history may be useful, the disease's clinical indications are not pathognomonic in small ruminants. As a result, laboratory diagnosis is necessary to identify and eradicate affected animals. Isolating Brucella organisms is the most dependable and exclusive method for diagnosing animal brucellosis. Accurate identification of Brucella species infection is critical to managing disease in animals and, by extension, humans. (Poester *et al*., 2013)

In order to prevent the reintroduction of brucellosis through the importation of infected animals or animal products, diagnostic tests are used for certification, screening or prevalence

research, surveillance, and confirmatory diagnosis (Godfroid *et al*., 2013). Most control and eradication programs rely on these techniques, making them essential for laboratory diagnosis of brucellosis. For the purpose of making an assumption about brucellosis diagnosis, the most favored way is to isolate and identify the causal agent from abortion material, udder secretions, or postmortem tissues. For large-scale programs, however, bacteriological diagnosis is not a viable or reliable method of diagnosis because it is dangerous and time-consuming (Ali *et al*., 2014). The direct bacteriological testing culture of Brucella, isolated from body fluids (blood, cerebrospinal fluid, urine, and others) or tissues, is the "gold standard" for diagnosing brucellosis (Smirnova *et al*., 2013).

2.6.1. Direct detection of the causative agents

Smears are useful for making the first, conclusive bacteriological diagnosis. Pre-scapular lymph nodes, supra mammary, Parotid, medial and internal iliac, retropharyngeal, and aborted fetuses are among the tissues from animal carcasses that are desired for culture. A modified Ziehl-Neelsen (Stamp) stain procedure was used to stain an optimum sample from vaginal swabs, milk, placentas, or vaginal discharge in a live infected host. It takes from days to weeks for Brucella culture to develop (Rahman, 2015). Hanci *et al*. (2017) state that the only "gold standard" technique for diagnosing brucellosis is the identification of Brucella organisms through cultural isolation or detection from the diseased person. In order to get around limitations and challenges with bacterial culture and serological assays, molecular technologies such as Polymerase Chain Reaction (PCR) are a novel technique and are used in many diagnostic works (Plumb *et al*., 2013).

The most effective way to isolate and culture Brucella is to use solid media, which makes it possible to clearly identify and isolate the growing colonies. There are many commercially available dehydrated basal media that can be employed, including blood agar base (Oxoid), serum dextrose agar (SDA), glycerol dextrose agar, and tryptose soy agar (TSA). But for other species, such *B. ovis* and *B. canis*, the culture media must contain 5–10% sterile bovine or equine serum (Poester *et al*., 2013).

2.6.2. Detection of host body response to brucellosis

Certain species of Brucella cause the host to develop antibodies. Antibodies targeting smooth Brucella species, such as *B. abortus, B. melitensis*, and *B. suis*, exhibit cross-reactivity with antigen preparations derived from *B. abortus*, while those targeting rough Brucella species, such as *B. ovis* and *B. canis*, exhibit cross-reactivity with antigen preparations from *B. ovis*. Though they weren't officially approved for use in sheep and goats, the current serological assays utilized for the diagnosis of *B. melitensis* and *B.ovis* in small ruminants were first created for the diagnosis of *B. abortus* in cattle. For the serological diagnosis of brucellosis in small ruminants, particularly RBPT, CFT, and more recently ELISA, have been frequently utilized (WOAH, 2016).

Rose Bengal Plate Test (RBPT): In field screening, RBPT is typically used for brucellosis, and it may also be used in conjunction with CFT (Abdalla *et al*., 2019). In endemic locations, the use of RBPT should be carefully examined due to its overall sensitivity of 92.9%. Mostly in those who have had brucellosis exposure and in people who have already contracted Brucella. It is based on the detection of specific antibodies of the IgM and IgG types but more effective in detecting antibodies of the IgG1 type than the IgG2 and IgM types. Due to its simplicity and low cost, it is the most common test used for brucellosis screening purposes, especially in laboratories with limited resources (WOAH, 2018; Legesse *et al*., 2023). The drawbacks of RBPT include low sensitivity particularly in chronic cases, relatively low specificity in endemic areas and prozones make strongly positive sera appear negative in RBPT (Díaz, 2013).

Complement Fixation Test (CFT): It is the most popular confirmatory test and WOAH recommends using it. The foundation of CFT is the identification of particular IgM and IgG1 antibodies that fix complement. It's a lengthy process that calls for specialized laboratory facilities and highly skilled workers. Although its specificity is crucial for the management and eradication of brucellosis, it may not work when IgG2 antibodies prevent complement fixation (Gebretsadik *et al*., 2016; Legesse *et al.*, 2023).

2.6.3. Enzyme-Linked Immune Sorbent Assay

The enzyme-linked immunosorbent assay test (ELISA) is a highly effective technique for determining the acute vs chronic stages of the disease and screening large populations for Brucella antibodies. For complex, local, or chronic cases especially when other tests come back negative and the condition is highly suspicious it is the test of choice. Within 4–6 hours, ELISA has a high sensitivity and specificity for detecting both total and individual-specific immunoglobulins (IgG, IgA, and IgM). ELISA can identify Brucella-specific IgG subclasses and additional Brucella immunoglobulins, such as IgE, in addition to immunoglobulin classes (Hailu, 2017).

2.6.4. Molecular Diagnosis

Polymerase chain reaction (PCR)-based techniques have been developed in recent years and are in use as alternative diagnostic tests for brucellosis. They are based on the detection of specific sequences of Brucella spp. DNA in clinical samples. PCR techniques have lower diagnostic sensitivity and higher specificity than culture methods; hence, the best results are obtained when the two are combined. Molecular techniques are important tools for diagnosis and epidemiologic studies, providing relevant information for the identification of species and biotypes of Brucella spp. and allowing differentiation between virulent and vaccine strains. Molecular detection of Brucella spp. can be done directly on clinical samples without previous isolation of the organism. In addition, these techniques can be used to complement the results obtained from phenotypic tests. Despite the high degree of DNA homology within the genus Brucella, several molecular methods, including PCR, PCR restriction fragment length polymorphism (RFLP), and Southern blot, have been developed that allow, to a certain extent, differentiation between Brucella species and some of their biovars (Mfune, 2015).

2.7. Treatment

Due to the organisms' intracellular sequestration in the mammary gland, reproductive organs, and lymph nodes, brucellosis treatment is ineffective. The facultative intracellular bacteria known as Brucella species are able to proliferate and live inside the macrophage system's cells. Although brucellosis is a challenging disease to treat because to its complexity, long-

term antibiotic treatment is believed of is helpful. Combining antibiotics has been shown to be more effective against infections in the majority of cases (Moon, 2014).

Meng *et al*. (2018) state that rifampicin with doxycycline administered in combination for six weeks is sufficient to eradicate Brucella infection. Experimental evidence supports this medication combination (Yang *et al*., 2018). The scientific community is still working to find an effective treatment; hence, Lamiaceae has been studied in conjunction with doxycycline (Saxena *et al*., 2018). The full treatment of brucellosis needs a successful therapeutic approach, despite the fact that various therapies are now in use and help to manage the condition (Khan *et al*., 2018).

Treatment of brucellosis in humans often faces challenges, with a low success rate and frequent instances of infection relapse. Despite these difficulties, certain conventional antibiotics have been widely employed in clinics to combat the disease. These antibiotics include tetracycline, trimethoprim-sulfamethoxazole, aminoglycosides, rifampicin, quinolones, chloramphenicol, doxycycline, and streptomycin (Mehmet *et al*., 2002). Following the recommendations issued by the World Health Organization (WHO) for human brucellosis treatment, a common approach involves the use of doxycycline at a dosage of 100 mg twice daily for duration of six weeks. This is combined with either rifampicin, administered at a dosage of 600-900 mg daily for six weeks, or streptomycin, given at a dosage of 1 g daily for two to three weeks (Ariza *et al*., 2007).

2.8. Strategies for Preventing and Manage Brucellosis

Understanding regional and local differences in social standards, infrastructure, animal husbandry techniques, and epidemiological patterns of the disease might help develop practical approaches to brucellosis management and prevention (Dorneles *et al.,* 2015). Common strategies for brucellosis control include immunizing animals that test negative for the disease and quarantining imported stokes (Radostits *et al*., 2007).

In a flock, *Brucella melitensis* can be eliminated via depopulation based on the severity of the disease's prevalence and the nation's economic standing, or by properly disposing of aborted fetuses, fetal membranes, and discharges and disinfecting the contaminated area afterward.

Programs to remove this organism from a nation also involve tracking and monitoring infected animals, as well as mobility restrictions on infected herds. Any flock that contracts *B. melitensis* in a region where it is not endemic is typically depopulated (Musallam *et al*., 2016).

Vaccination is considered a strategy for lowering the prevalence of brucellosis in areas where eradication by test and slaughter is feasible (Hasannia *et al*., 2015). According to global experience, immunization is typically the only workable way to prevent brucellosis in sheep and goats (Musallam *et al.,* 2016).

2.8.1. Immunization, testing, and killing

Strong immunization would be the most effective way to prevent and manage the common intra- and interspecies brucellosis infection in animals (Aznar *et al*., 2017). Immunizations can aid in the management of clinical symptoms in infected flocks. In an effort to lower the prevalence of *B. melitensis*, they have also been used in control efforts. Small ruminants are typically immunized with the Rev-1 vaccine, while some nations have also used the *B. suis* S2 and *B. melitensis* M5 vaccinations. The Rev-1 vaccine interferes with serological tests, particularly when it is injected subcutaneously, but Conjuctival administration to lambs and kids between the ages of three and five months minimizes (WOAH, 2018). Immunization with effective vaccines helps to get the infection under control, limit its spread, prevent human infections and reduce economic losses (Musallam *et al*., 2016).

The primary issue with using the Rev. 1 vaccine is that most of the animals that were vaccinated had abortions after receiving the full standard dose of the vaccine subcutaneously. The use of lower vaccine dose may resolve this safety problem in goats, cost of decreasing the level of immunity conferred by the standard doses (Smits, 2012). Small ruminants are the main reservoirs of human brucellosis because absence of pragmatic sign to protect humans from such infected animals. Therefore, the goal must be to control the disease in the small ruminant population (Grahn, 2013). There is no human vaccine available to protect workers directly, but there are effective *B. melitensis* vaccines for small ruminants (Guven *et al*., 2013).

Eliminating *B. melitensis* from a flock needs the cooperation of animal owners and involves the rapid death of animals that test positive. Depopulation is a costly method as well. Movement restrictions on infected herds, surveillance, and animal tracing are further measures used in programs to eradicate this bacterium from a country. Every herd that contracts *B. melitensis* in an area where it is not endemic is frequently depopulated (WOAH, 2018). Regulation, economics, and prevalence are taken into account before deciding whether to slaughter test-positive animals. It takes several herd or flock tests to verify eradication and lower the incidence of brucellosis (Hasannia *et al*., 2015).

2.9. Status of Small Ruminant Brucellosis in Ethiopia

There have been reports of brucellosis in humans and animals in Ethiopia from a variety of locations. Since brucellosis was first identified in Ethiopia in the 1970s, it has been recognized as a significant livestock disease in the country (Geresu *et al*., 2016). Small ruminant brucellosis is endemic in Ethiopia, according to stated prevalence rates. There is not enough progress on this in Ethiopia. This can be the result of the sector producing small ruminants receiving insufficient attention. Afar and Somali regional states were found to have the majority of cases of small ruminant brucellosis (90/204 = 44.11%). However, a few studies have been carried out on the irregular distribution of isolated *B. ovis* and *B. melitensis* throughout the nation (Ferede *et al.*, 2011).

Among recently growing seroprevalence of small ruminant brucellosis in Ethiopian Dedeffo *et al*. (2015) tested sera from 840 sheep and goats in pastoral regions of Ethiopia and documented 4.6 % positive using mRBPT and upon further testing, all were found to be positive by I-ELISA. In Jijiga Mohammed *et al*. (2017) screened 291 serum samples (100 from sheep and 191 from goats) and the result revealed 1.72% and 1.37% positivity using RBPT and CFT, respectively. In addition, Tadeg *et al*., 2015 indicated that 414 serum samples (272 from sheep and 142 from goats) collected from Tellalak district, Afar Region showed 17.7% tested positive for brucellosis using RBPT and 13.7 % counter positive were found using the confirmatory test CFT. In central and northeast Ethiopia by Mustefa and Bedore (2019) revealed that from 2409 blood samples sheep collected all sera samples screened by modified Rose Bengal Test (mRBT) positive reactors 4.9% tested positive 4.89% in CFT.

According to the current Sero surveillance findings of the disease in the country, low infection rate was recorded at Bahir Dar Town of Amhara Regional State by Ferede *et al.* (2011) and the highest was reported at Tellalak District of Afar Regional State by Tadeg *et al*. (2015) (Table 2). According to Asmare *et al.* (2013) finding revealed that 1.9% overall seroprevalence in different production systems. In sedentary production system, the observed seroprevalence was 0.6% while 1.9% and 7.6% were the proportion of seroreactors for agro pastoral and pastoral production systems, respectively. At the flock level analysis, 11.2% of the flocks sampled had at least one seropositive goat among themselves. Like individual animal level analysis, the highest prevalence of 32.5% was recorded for pastoral production system, followed by agro-pastoral, 13.0 % and sedentary production system, 3.6%. This may be due to the presence of large number of small ruminant population in pastoral areas than agro pastoral areas.

A cross-sectional study was conducted in pastoral areas by Sintayehu *et al.* (2015) from a total of 6,201 sampled animals (3,694 sheep and 2,507 goats) were tested 320 (5.2%) and 134 (2.2%) were positive for brucellosis by using RBPT and CFT, respectively. All Afar zones Borena, Jigjig and S. Omo administrative zones, 84% of the districts and 58% of the kebele surveyed were found to be affected with brucellosis, but the distribution was not uniform. Standardized seroprevalence values 3.3% and 2.8% had recorded in Borena and Afar zones, respectively than Jijiga 0.07% and 0.26% South Omo prevalence values (Sintayehu *et al*., 2015).

According to Melese (2016) based on serological tests, the individual animal level seroprevalence of brucellosis was 4.3%, 3.8% and 3.7% using mRBPT, ELISA and CFT reagents, respectively. Flock level seroprevalence across the two districts was 10.81% for small, 37.84% for medium and 51.4% for large flock sizes. Using direct detection of the causative agents Gebretsadik *et al*. (2016) isolated 13.8% positive out of the 65 bacteriological samples cultured on Brucella selective agar medium. Among the overall isolates, 10.7% and 21.4% were from milk and vaginal swabs, respectively.

Table 2: Seroprevalence of ovine and caprine species brucellosis in Ethiopia.

Region	Specific district	species	No tested	RBPT	CFT/ ELISA	Authors
SNNP	Arba Minch and Mirab Abaya	Both	1000	4.3	3.8	Melese (2016)
Afar	Chifra and Ewa	Both	1190	13	12.35	Tegegn *et al*.(2016)
Tigray	Tselemti district	Ovine	145	1.38	0.69	Kelkay *et al*.(2017)
		caprine	413	2.66	2.18	
Oromia	Yabello	Ovine	99	6.1	6.1	Wubishet *et al*.(2017)
		caprine	184	9.8	9.2	
Somali	Three selected districts of Jijiga zone	Ovine	100	1	1	Mohammed *et al*. (2017)
		Caprine	191	2.09	1.57	
Oromia	Limu Seka and Chora Boter districts	both	804	6.3	4.2	Tulu *et al*.(2020)
Oromia	Dallo Manna& Haranan Bulluk districts	both	384	6.5	2.9	Adem *et al*.(2021)
SNNP	Kolme &Abala Abaya districts	both	690	4.1	3.33	Dosa *et al*.(2023)
Oromia	Berbere district	both	468	3.61	2.97	Muhidin *et al*.(2021)
Afar	Amibara & Dubti districts	both	638	11.751	10.03	Tegegne *et al*.(2023)

2.10. Knowledge, Attitude, and Practice Concerning Brucellosis

A knowledge, attitude, and practice (KAP) study on brucellosis conducted in Egypt and conducted in African countries revealed a relatively high general knowledge of the disease, but there was still a high risk of vulnerability among livestock owners. The authors concluded that this could be a factor in the high Sero-prevalence of brucellosis among livestock in the region (Holt *et al*., 2011). An additional study conducted in Uganda revealed that agro

pastoralists knew more about human and animal brucellosis than pure pastoralists did, with pastoral responses knowing only little about the disease (Kansiime *et al*., 2014). On the other hand, a study conducted in Kenya found that pastoralists knew only a reasonable amount about the primary symptoms of brucellosis in humans and animals, but not much about the agents that cause the disease. The study also revealed an average level of general understanding, a negative attitude, and inadequate brucellosis procedures (Obonyo, 2018).

3. MATERIAL AND METHODS

3.1. The Study Area

The Ilubabor Zone is located in Ethiopia's Oromia Region, in the western part of the country. It is bordered by the South West Region of Ethiopia to the south, the Gambella Region to the southwest, the Kelem Wollega Zone to the west, the West Wollega Zone and Benishangul-Gumuz Region to the north, the East Wollega Zone to the northwest, and Jimma to the east. Ilubabor Zone is located in the south-western part of Ethiopia, 600 kilometers from Addis Ababa, the country's capital city. The zone is situated between 7^{o} 7'40" and 9^{o} 2'10" North and 34^{o} 52'12" and 41^{o} 34'55" East. This zone covers a total area of 163315656 hectares of land, 10% of which is highland, 67% of which is medium land, and 23% of which is low land, with elevation varying from 500 to 2575 meters above sea level. The yearly rain fall is between 1500 and 2200mm, and the temperature ranges from 18°C to 24°C. Rainfall is unimodal, falling primarily between May and September. The zone still has a lot of its original vegetation, with woods and woodlands covering 65% of the area. There are 14 administrative districts in the zone. The zone is home to 1.6 million people, 88% of whom live in rural areas (Ilubabor Zone Agricultural and Natural Resource Management Office, 2022/2023). This study was conducted in three districts (Yayo, Bilo Nopa, and Didu) of the Ilubabor Zone.

Yayo District is one of the 14 districts in the Ilubabor Zone of the Oromia National Regional States. Yayo is located in the southwest part of Ethiopia. It is located 564 kilometers from Addis Ababa. Astronomically, the district is located between 7^{0}52'34"-7^{0}58'21"N and 38^{0}41'01"-38^{0}44'31"E. Yayo shares boundaries with Chora district in the western part, Hurumu district in the eastern part, Dorani district in the southern part, and Sigmo/Jimma district in the northern part. The farming system of the area was mixed farming, with 87% of the total population engaged in agriculture. Crop and livestock sales are important sources of income for all wealth groups. The human population of the district is 57,938; the number of households is 29311. The livestock populations of the district were estimated to be 69412 cattle, 30164 sheep, 12551 goats, 83200 poultry, and 7572 equines (Yayo District Agricultural and Natural Resource Management Office, 2022–2023).

Bilo-Nopa district is found in the Ilubabor zone of Oromia Regional State, about 615 km from Finfinne (Addis Ababa) and 18 km from Mettu, which is the administrative seat of the Ilubabor zone. The total land area of the district is 37,009 hectares, and the district is composed of 16 kebeles. The agro-ecological zone of the district consists of highland and lowland. The total population of the district is 39,848; from this, the number of households is 22,269. Bilo Nopa district is located at the latitude of the area, ranging from 07°05.33' to 08°45.33' to the north, while the longitude of the area ranges from 033°47.57' to 036°52.33' east. This district comprises a livestock population of 45160 cattle, 29575 sheep, 12307 goats, 67900 poultry, and 5320 equines (Bilo Nopa District Agricultural and Natural Resource Management Office, 2022–2023).

Didu District, which is located in southwest Ethiopia, is in the Oromia regional state Ilubabor zone. The Didu district is located along the region’s longitude, which extends from the latitude of 35° 35' 48.270' to a longitude of 7° 5' 02.662'. The district is one of the 14 districts in the zone and is located 59 km from Mettu, the zone capital city. The total population of the district was 43,289; from this, the number of households is 28740, of which the highest percentages are farmers. The district has 16 rural kebeles and one urban kebele. The agro-ecological zone of the district is 62.5% of Woreda's agro-ecological zone, and the remaining 37.5% is temperate. The total livestock populations of the Didu district were estimated to be 1033510 cattle, 22916 sheep, 9536 goats, 81700 poultry, and 4242 equines (Didu District Agricultural and Natural Resource Management Office, 2022–2023).

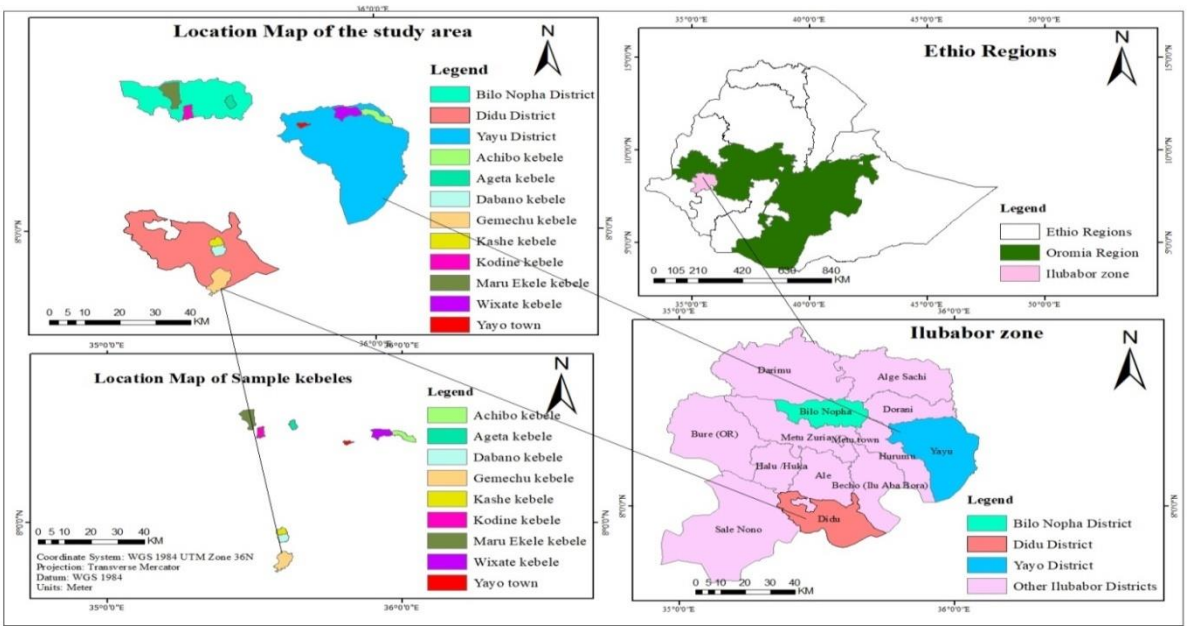

Figure 2: Map of the study Area (taken by GIS)

3.2. Study population

The study animals were indigenous breeds of sheep and goats managed under an extensive system. Small ruminants over six months of age were included in the study to avoid the potential presence of maternal-derived antibodies, which could be acquired through colostrum from naturally infected ewes or does (Quinn *et al*., 2004). The individual animal-level risk factors considered were species, sex, age, flock size, pregnancy status, parity number, history of abortion, and history of retained fetal membranes. At the flock level, risk factors included the origin of animals, flock size, history of abortion, and history of retained fetal membranes. Flock sizes per household were classified as small (1–10), medium (11–20), and large (>20) (Deddefo *et al*., 2015). In this context, 'flocks' refers to a group of domestic animals, specifically sheep and goats, kept together. Age was determined using dentition methods and categorized as less than 2 years (young), 2–5 years (adult), and over 5 years (old) (Tegegn *et al*., 2016). Parity numbers were categorized as nulliparous (zero parity), 1-2 parities, and 3-4 parities (Tegegn *et al*., 2016). Abortion was defined as the loss of a fetus or fetuses before 140 days of gestation (Margatho *et al*., 2019).

3.3. Study Design and study period

A cross-sectional study was conducted from April 2023 to January 2024 in selected districts of the Ilubabor Zone to investigate the seroprevalence, risk factors, and the knowledge, attitudes, and practices of owners regarding small ruminant brucellosis. The densities of animal populations, herd sizes, management practices, and environmental factors are believed to be significant determinants of the infectious dynamics among herds (Omer *et al*., 2000). Additionally, a questionnaire survey was carried out to assess farmers' perceptions of brucellosis and its economic and public health implications.

3.4. The Inclusion and Exclusion Criteria for the Study

3.4.1. Inclusion Criteria

Indigenous breeds of sheep and goats, from various age groups but all older than six months and appearing healthy, were included in the study. Small ruminant owners aged at least 18 years, residents in selected kebeles, and those who were able to communicate verbally in the local language, Afan Oromo, were interviewed face-to-face, and farmers who owned at least one goat or sheep were eligible to participate.

3.4.2. Exclusion Criteria

Goats and sheep that had recently been treated with antibiotics, those with specific health conditions, and participants under the age of 18 were excluded from the study.

3.5. Sampling Method and Sample Size Determination

Districts were selected purposively based on their sheep and goat population density, transport facilities, and relative security during sample collection. The study kebeles (the smallest administrative unit), which were the primary sampling units, were selected using simple random sampling methods. The total number of kebeles in the districts was 16 for Yayo, 16 for Bilo Nopa, and 17 for Didu; these were used as the sampling frame. Nine kebeles (3 from each district) were taken, and the list of kebeles was obtained from the Agricultural and Natural Resource Management Office of the district. For every kebeles, the total number of samples needed was allocated based on the proportionality of the animal population.

A total of 110 households, which constituted the secondary sampling unit, were chosen using the simple random sampling method. These households were selected based on their ownership of goats and sheep and their extensive experience in rearing small ruminant animals. Subsequently, individual animals, representing the 3 tertiary sampling units, were also selected through simple random sampling.

The sample size for small ruminants was determined using Thrufield's formula (2005) with an acceptable error of 5% and a 95% confidence interval. Since there was no reported seroprevalence of brucellosis in the study areas, an estimated prevalence of 50% and a 95% confidence level were assumed. Consequently, the calculated sample size was 384.

$$n = \frac{z^2 * P\,exp\,(1-P\,exp)}{d^2}$$

Where n = sample size required; z = is the selected critical value of desired confidence level; P exp= expected prevalence; d = desired absolute precision/ desired margin of error

However, to increase precision, the sample size was increased by 10% of the calculated sample size, determined as 422. Thus, a total of 422 small ruminants (298 sheep and 124 goats, according to their relative population) were sampled and examined.

The sample size for the questionnaire survey was determined using the method provided by Arsham (2002), which is given by the formula $N = 0.25/SE^2$, where N is the sample size and SE (standard error) is 5%. Therefore, the initially calculated sample size was 100. However, an additional 10% was added to account for potential non-response rates, resulting in a total sample size of 110 flocks. Households owning more than one sheep or goat were selected. Within each Kebele, the number of households chosen corresponded to the predetermined sample size for each household or flock. 20% of the flock was selected for individual animals using the random sampling procedure.

3.6. Questionnaire survey

To gather relevant information about the knowledge, attitudes, and practices (KAP) related to small ruminant brucellosis, a structured questionnaire was utilized. Before administering the questionnaire, it was translated into the local language, the Afan Oromo. The questionnaire was administered through face-to-face interviews with the animal owners. Separate sets of questions were prepared to assess the socio-demographic characteristics of respondents, knowledge of brucellosis, attitudes toward brucellosis, and practices relating to small ruminant husbandry, disposal of aborted material, and contact with animals. The questionnaire survey was closed-ended and contained binary and multiple choices. If the respondents' answers were above the mean or average, their KAP was considered good; otherwise, it was considered poor.

3.7. Sample Collection and Laboratory Tests

3.7.1. Blood sample collection

After carefully handling the animal and disinfecting the site with 70% alcohol, approximately 5-7 ml of blood was aseptically drawn from the jugular vein of each animal into Vacutainer tubes and needles, which were free of anti-coagulants or preservatives. All samples were serially identified, correctly labeled using a permanent marker, and transported to the district's laboratory. The blood samples were positioned at a slant and allowed to clot overnight at room temperature to separate the serum. Then, 2 ml of the serum samples were decanted, and the purified serum was transferred into sterile screw-capped cryovials, in accordance with WOAH (2009) guidelines, and transported to the Bedele regional veterinary investigations laboratory in an ice box. They were stored at −20°C until serologically tested for the presence of Brucella antibodies.

3.7.2. Indirect enzyme-linked immunosorbent assay (I-ELISA)

Indirect enzyme-linked immunosorbent assay (I-ELISA) is an immunological assay used to detect Brucella antibodies directed against *B. abortus, B. melitensis,* and *B. suis* LPS antigens (WOAH, 2018). Positive and negative controls provided along with the kit were used for the

validation of the assay. The testing procedure allows all reagents to be kept at room temperature. Then, by adding 190µl of Dilution Buffer-2 to all wells, add 10µl of negative control to wells A1 and B1, add 10µl of positive control to wells C1 and D1, and add 10µl of specific antibody (serum) to the remaining wells. Then incubate for 45 minutes. Wash three times and add 100µl of enzyme-conjugated secondary antibody, then incubate for 30 min. wash the solution three times and add 100 µl of substrate solution to each well. Incubate for 15 minutes in a dark place, and add 100 µl of stop solution to each well to stop the reaction. The resulting color depends on the amount of specific antibody present in the sample to be tested. Finally, plates were read using an ELISA micro plate reader with a 450 nm wavelength (Lab System, USA). The percent value (S/P%) was calculated using the formula recommended by the kit manufacturer (ID Vet, France) for lot BRUS-MS ver 1014GB kit protocol.

For each Sample positive percentage (S/P %) was calculated according to below.

$$\text{S/P \%} = \frac{\text{ODsample} - \text{ODNC}}{\text{ODPC} - \text{ODNc}} \text{X100}$$

The results were interpreted as follows: If the percentage of inhibition was 110 or lower, the outcome was categorized as negative. In cases where the inhibition percentage was greater than 110 and less than 120, it was regarded as uncertain or doubtful. If the inhibition percentage was 120 or greater, the result was recorded as positive, in accordance with the manufacturer's guidelines.

3.8. Data Management and Statistical Analysis

All gathered data were entered into a Microsoft Excel spreadsheet, version 2010. The data were then reviewed for errors, which were corrected as needed. Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 23 (SPSS Inc., IBM, Chicago, Illinois, USA). Descriptive statistics, such as frequency and proportion, were used to analyze the demographic characteristics of the participants and to describe the prevalence of the disease. Seroprevalence at both the flock level and the individual animal level was calculated by dividing the number of positive test results by the total number of

flocks and animals sampled, respectively. Logistic regression was employed to identify potential risk factors for seropositivity in sheep and goats, with the odds ratio (OR) and 95% confidence intervals indicating the strength of association. Univariable logistic regression was performed to assess the relationship between each independent variable and the dependent variable.

All collinear variables from the univariable logistic regression with a *P*-value less than 0.25 were subsequently analyzed using multivariate logistic regression. For all analyses, a p-value of less than 0.05 ($P < 0.05$) was considered statistically significant. The seroprevalence of brucellosis was assessed through descriptive statistics, while logistic regression was employed to evaluate the association between risk factors and the occurrence of brucellosis.

3.9. Ethical Clearance Certificate

Ethics approval and consent to participate in this research were approved by Jimma University and the JUCAVM institutional ethical review board animal research ethical committee with reference number A13/APR/2023. This protocol was presented to the initial and continuing review boards. Before any attempt was made to gather data, the small ruminant owners who were taking part in the study were made aware of its goal. Following a brief discussion with the owners regarding the study's goals and the necessity of the research, the study was conducted in accordance with their oral consent. Blood samples were collected under aseptic conditions and with the necessary safety measures. Since the study involves domestic animals, ethical approval for the study was obtained from the Ethical Research Board (ERB) of the JUCAVM for domestic animals.

4. RESULTS

4.1. Overall Seroprevalence of Brucellosis in Small Ruminants

The total number of small ruminants sampled was 422, which included 298 sheep (n=298) and 124 goats (n=124), from 110 randomly selected flocks. At the animal level, the test results showed that 10 (2.4%) were seropositive for brucellosis, with a (95%CI; 1.22-4.16) (Table 3).

Table 3: Overall animal and flock-level brucellosis seroprevalence across study districts

Study districts	Animal level		Flock level	
	Total animal examined	Seroprevalence(%) (95% CI)	Total animal examined	seroprevalence(%)(95% CI)
Didu	151	2(1.3)(2.8-4.18)	41	2(4.9)(1.03-14.74)
Yayo	154	3(1.9)(0.5-5.11)	37	3(8.1)(2.34-20.08)
B/nopa	117	5(4.3)(1.65-9.11)	32	5(15.6)(6.22-30.9)
Overall	422	10(2.4)(1.22-4.16)	110	10(9.1)(4.77-15.53)

CI: confidence interval

4.2. Risk Factors Analysis

4.2.1. Animal level risk factors analysis

The results of the univariable logistic regression analysis are presented in Table 4. The analysis revealed that the prevalence of Brucella antibodies was 4.8% in goats (n=124) and 1.3% in sheep (n=298). Females had a higher infection rate of 3.2% (n=248) compared to males at 1.15% (n=174). Seroprevalence rates of 0.99% (n=101), 1.03% (n=194), and 5.5% (n=127) were observed for small, medium, and large flock sizes, respectively. The infection rate was higher in the elderly at 3.9%, compared to 1.25% in adults and 1.2% in the young age group. Pregnant shoats had a seroprevalence of 8.1%, which was higher than the 1.1% in non-pregnant shoats. Among the 248 females, the prevalence of brucellosis was 11.8% in those with a previous history of abortion and 1.02% in those without, 10.9% in those with a

previous history of retained fetal membranes and 1.04% in those without. The disease prevalence was 1.2% for zero parity, 1.9% for 1-2 parity, and 8.2% for 3-4 parity.

After performing univariable logistic regression analysis, all variables who were *P*-values were ≤0.25 were considered potential risk factors and thus subjected to multivariable logistic regression analysis.

Table 4: Seroprevalence of small ruminant brucellosis in relation to different risk factors using univariable logistic regression analysis

Risk factors	Category	Tested animal	Number positive	Seropos i-vity (%)	COR	95% CI	*P*-value
Species	Caprine	124	6	4.8	3.7	1.036-13.483	0.044
	Ovine(ref)	298	4	1.3			
Sex	Female	248	8	3.2	2.9	0.601-13.667	0.186
	Male(ref)	174	2	1.1			
Age	Young(ref)	83	1	1.2			
	Adult	160	2	1.25	1.04	0.093-11.617	0.976
	Old	179	7	3.9	3.34	0.404-27.574	0.263
Flock size	Small(ref)	101	1	0.99			
	Medium	194	2	1.03	1.04	0.093-11.628	0.974
	Large	127	7	5.5	5.8	0.706-48.212	0.102
Pregnancy	Pregnant	74	6	8.1	7	1.495-38.526	0.014
Status	Not pregnant(ref)	174	2	1.1			
Parity number	0(ref)	81	1	1.2			
	1-2	106	2	1.9	1.5	0.137-17.268	0.727
	3-4	61	5	8.2	7.1	0.812-62.811	0.07
HA	Yes	51	6	11.8	13	2.540-66.536	0.002
	No(ref)	197	2	1.02			
HRFM	Yes	55	6	10.9	11.7	2.289-59.731	0.003
	No(ref)	193	2	1.04			

COR=crude odd ratio; **CI**=confidence interval; **HA**=history of abortion, **HRFM**=history of retained fetal membrane, **Ref**=reference

After performing univariable logistic regression analysis, it was observed that factors such as species, pregnancy status, history of abortion, and history of retained fetal membrane exhibited significant associations with small ruminant brucellosis (P<0.05) (Table 5). Consequently, these significant factors were chosen for subsequent analysis using multivariable logistic regression to evaluate their independent impact on the occurrence of small ruminant brucellosis.

Table 5: Multivariable logistic regression analysis of potential risk factors associated with Brucella seropositivity using I-ELISA

Risk factors	Category	Tested animal	Number positive	Seroposi-vity (%)	AOR	95% CI	*P*-value
Species	caprine	124	6	4.8	5.5	1.013-29.753	0.048
	ovine	298	4	1.3			
Status of pregnancy	Pregnant	74	6	8.1	6.4	1.071-38.337	0.042
	No pregnant	174	2	1.15			
HA	Yes	51	6	11.8	9.1	1.538-54.236	0.015
	No	197	2	1.02			
HRFM	Yes	55	6	10.9	9.02	1.496-54.452	0.016
	No	193	2	1.04			

AOR= adjusted odd ratio; **CI**=confidence interval, **HA**=history of abortion, **HRFM**= history of retained fetal membrane.

4.3.2. Flock Level Risk Factors Analysis

Out of 110 flocks studied, (9.1%) were positive for brucellosis using I-ELISA, with a (95%CI; 4.77–15.53). The flock-level risk factors considered in the study included flock size, origin of animals within flocks, history of abortion, and history of retained fetal membranes, summarized in Table 6. A higher seroprevalence was observed in larger flocks (30.4%) and medium-sized flocks (4.8%) compared to small flocks (2.2%). Flocks with a history of

abortion exhibited a higher seroprevalence (19.5%) than those without such a history (2.9%). Similarly, flocks with a history of retained fetal membranes had a higher seroprevalence (18.4%) compared to those without (4.2%). Additionally, flocks with purchased animals had a higher seroprevalence (16.2%) compared to those with only born-in animals (5.5%). All variables in univariable logistic regression analysis at the flock level were subjected to multivariable logistic regression analysis (P≤0.25) (Table 6).

Table 6: Analysis of potential risk factors for small ruminant brucellosis at flock level by univariable logistic regression

Risk factors	Category	Number of flocks	Infected flocks	(95% CI)	COR	*P*-value
Flock size	Small	45	1(2.2%)			
	Medium	42	2(4.8%)	0.192-25.199	2.2	
	Large	23	7(30.4%)	2.194-168.936	19.5	0.004
Origin of new animal	purchased	37	6(16.2%)	0.879-12.677	3.39	0.077
	Born	73	4(5.5%)			
HA	Yes	41	8(19.5)	1.632-40.40	8.12	0.011
	No	69	2(2.9)			
HRFM	Yes	38	7(18.42%)	1.259-21.429	5.19	0.023
	No	72	3(4.2%)			

COR: crude odds ratio; **CI**: confidence interval, **HA**= history of abortion, **HRFM**: history of retained fetal membrane

In Table 7, the results of the multivariable logistic regression analysis identify significant risk factors for Brucella seropositivity in flocks. Accordingly, flock size and history of abortion were the only factors significantly associated with flock-level Brucella seropositivity ($P < 0.05$).

Table 7: Analysis of potential risk factors for small ruminant brucellosis at flock level by multivariable logistic regression

Risk factors	Category	Number of flocks	Infected flocks	(95% CI)	AOR	*P*-value
Flock size	Small	45	1(1.49%)			
	Medium	42	2(3.85%)	0.208-29.070	2.5	
	Large	23	7(23.08%)	1.589-132.150	14.5	0.020
HA	Yes	41	8(19.5%)	1.02-29.080	5.5	0.047
	No	69	2(2.9%)			

AOR: adjusted odds ratio; **CI**: confidence interval, **HA**= history of abortion

4.3. Questionnaire Survey

4.3.1. Socio-demographic profile of the respondents

The sociodemographic profile of the respondents presented in Table 8 indicated that 82 (74.5%) were males and 28 (25.5%) were females, of whom 42 (38.2%) were between the ages of 31 and 40, while 50 (40.9%) had not attended any education (illiterate).

Table 8: Demographic Profiles of the Respondents who participated in the study (n = 110).

Study Parameters	**Category**	**Number of respondents**	**Percentage**
Sex	Male	82	74.5
	Female	28	25.5
Age	18-30 Years	17	15.5
	31-40 Years	42	38.2
	41-50 Years	37	33.6
	>50 Years	14	12.7
Educational level	Illiterate	45	40.9
	Basic education	30	27.3
	Primary	18	16.4
	Secondary	11	10.0

	College and above	6	5.5

4.3.2. Knowledge, Attitudes, and Practices of the Owners about Brucellosis

A total of 110 respondents were interviewed to assess their knowledge, attitudes, and practices regarding brucellosis. The livestock reared in the area comprised cattle, goats, sheep, donkeys, horses, mules, and poultry. All the households studied were involved in rearing small ruminants.

The study found that 83.6% of communities were unaware of brucellosis, with 66.7%) not knowing about its impact on sheep and goats, and 72.2% lacked knowledge about its control and prevention.

Table 9: Knowledge Related to Brucellosis in the Study Area.

Study Parameters	Category	NR (%)	NFS (%)
Do you know about brucellosis?	Yes	18(16.4)	3(16.7)
	No	92(83.6)	7(7.6)
Do you know brucellosis affects sheep and goats?	Yes	6(33.3)	1(16.7)
	No	12(66.7)	2(16.6)
Do you know about the prevention and control of brucellosis?	Yes	5(27.8)	1(20)
	No	13(72.2)	2(13.4)

NR=Number of respondents, **NFS**=Number of flocks seropositivity, **%**=Percentage

Eighty-three percent (83.3%) of respondents did not believe personnel with exposed does or ewes are at high risk of Brucella infection, but. 61.1% don't recognize isolating delivered does or ewes to prevent brucellosis, and 61.1% prefer selling aborted sheep and goats. Most of the owners (77.8%) have a habit of assisting sheep and goats during parturition without using protective gloves, and they shared three positive flocks (Table 10).

Table 10: Attitudes Related to Brucellosis in the Study Area.

Study Parameters	Category	NR (%)	NFS (%)
Is there a high risk of infection for you or your family	Yes	3(16.7)	0()
members who work with sheep and goats? (18)	No	15(83.3)	3(20)
Do you think that segregating rooms for deliveries	Yes	7(38.9)	0()
does and ewes are crucial for preventing disease? (18)	No	11(61.1)	3(27.3)
Do you believe it is appropriate to sell often-aborted	Yes	11(61.1)	2(18.2)
does or ewes? (18)	No	7(38.9)	1(14.3)
Do you think it's essential to use protective clothing	Use	4(22.2)	0()
when working with animals? (18)	protective		
	Bare hand	14(77.8)	3(21.4)

NR=Number of respondents, **NFS**=Number of flocks seropositivity, **%**=Percentage

Seventy-six percent (76%) of respondents disposed of aborted material after birth, resulting in five seropositive flocks. 77.3% of flocks had potential contact with other flocks, and all had contact with animals and products. A significant majority did not wash hands after contact, and 84.5% came into contact with fetal membranes and fluids (Table 11).

Table 11: Practices Related to Brucellosis in the Study Area.

Study Parameters	Category	NR (%)	NFS (%)
Do flocks come into contact with other flocks at grazing or watering points?	Yes	85(77.3)	8(9.41)
	No	25(22.7)	2(8)
Have you had contact with animals or animal products? (birth product, blood, blood or meat)	Yes	110(100)	10(100
	No	0	0
Do you wash your hands after having contact with anima1product?	Yes	17(15.5)	2(11.8)
	No	93(84.5)	8(8.6)
Do you know RFM?	Yes	38(34.5)	3(7.9)
	No	72(65.5)	7(9.7)
Have you had contact with RFM or fetal fluids? (38)	Yes	25(65.8)	5(20)
	No	13(34.2)	2(15.4)
How should aborted material be removed? (25)	Burial/burning	6(24)	0()
	Throwing into field	19(76)	5(15.6)
Do you know about abortion?	Yes	41(37.3)	4(9.7)
	No	69(62.7)	6(8.7)
Assisting during an abortion? (41)	Yes	25(60.9)	3(12)
	No	16(39.1)	1(6.3)
How is the handling of aborted material done? (25)	Bare hand	16(64)	3(18.7)
	Protected hand	9(36)	0()
Removal or disposal of aborted	Burial/burning	2(8)	0()

material? (25)	Throwing into field	23(92)	3(13.1)

NR=Number of respondents, **NFS**=Number of flocks seropositivity, **%**=Percentage

5. DISCUSSION

In the current study, out of a total of 422 serum samples collected from three districts, ten samples were positive for brucellosis, resulting in an individual animal seroprevalence of 2.4%. The study found that independent variables such as age, sex, flock size, and parity number had no significant impact on an animal's risk of testing positive for Brucella at the individual level ($P > 0.05$). However, factors including species, pregnancy status, history of abortions, and retained fetal membranes were statistically associated with brucellosis in small ruminants ($P < 0.05$).

The results of this study closely align with those of previous serological studies: 2.3% at the Werer Agricultural Research Center in Afar (Bezabih and Bulto, 2015), 2.6% in selected settlements of the Dire Dawa Administrative Council Area in Eastern Ethiopia (Haile *et al*., 2018), and 2.7% in specific export abattoirs in Addis Ababa (Nigatu *et al*., 2014). These similarities may be attributed to the areas' comparable agro-ecological conditions, diagnostic methods, and animal management practices.

However, higher prevalence rates have been reported than our results in other regions of Ethiopia;13.7% in the Tallalak district of the Afar region (Tadeg *et al*., 2015), 12.4% in the Chifra and Ewa districts of the Afar region (Tegegn *et al*., 2016), 8.1% in the Yabello districts of the Borena Zone, Oromia Regional State, Southern Ethiopia (Wubishet *et al*., 2018), and 7.5% in selected Kebeles of the Amibara district of the Afar region (Gebretsadik *et al*., 2016). The possible reasons for these variations might include differences in agro-ecology, animal management systems, diagnostic tests used, production systems, and husbandry practices. In pastoral regions, large numbers of different species of animals move unrestrictedly in search of pasture and are raised on communal grazing lands with limited watering areas, especially

during the dry season. In contrast, the livestock management and production system in the current study area was characterized by mixed farming, where fewer animals are raised and kept separately.

Our study's prevalence was higher than the seroprevalence rates of 0.7% in and around Kombolcha, Amhara Regional State, North-Eastern Ethiopia (Tewodros and Dawit, 2015), 0.8% in Babile Woreda, Eastern Hararghe, Ethiopia (Yeshibelay and Teferi, 2019), and 0.71% in and around Bule Hora District, Western Guji Zone, Oromia Regional State, Southern Ethiopia (Debano, 2023). The variations between the current and previous studies could be attributed to various factors, such as differences in diagnostic assays, sampling techniques, study areas, and sample sizes.

Goats were five times more likely to be seropositive than sheep in the research area (AOR = 5.5, 95%CI: 1.013–29.735), P = 0.048), indicating a statistically significant difference in seropositivity between the two species ($P < 0.05$). The findings of Teshome *et al*. (2018), Tegegn *et al*. (2016), and Tegegne *et al*. (2023) demonstrate a significantly higher prevalence in goats compared to sheep, which aligns with this observation. This disparity was attributed to goats being more susceptible to Brucella infection than sheep and excreting the organisms for a longer duration (ILRI, 2013). However, these results contrast with those of Wubishet *et al*. (2018), Adem *et al*. (2021), Dosa *et al*. (2023), and Seid *et al*. (2021). The variations between the current and previous studies could be due to factors such as differences in diagnostic assays, sampling techniques, study areas, and sample sizes.

Pregnant sheep and goats were six times more likely (AOR = 6.4, 95%CI: 1.071–38.33, P = 0.042) to exhibit Brucella seropositivity compared to their non-pregnant counterparts. The increased risk of contracting Brucella infection during pregnancy was attributed to the Brucella organisms' preference for the uterus. Components of allantoic fluid, such as erythritol, may enhance the growth of Brucella, leading to an increase in placental and fetal fluids starting from approximately the second trimester of pregnancy (Coetzer and Tustin, 2004; Radostits *et al*., 2007). This finding was consistent with reports by Tulu *et al*. (2020), Deddefo *et al*. (2015), Yohanis *et al*. (2013), and Ashagrie *et al*. (2011), which link

brucellosis to the reproductive status of sheep and goats. However, these results contrast with those reported by Kelkay *et al*. (2017) in the northern Ethiopian district of Tselemti.

A statistically significant difference in the prevalence of small ruminant brucellosis was observed between animals with a history of abortion (11.8%) and those without (1.02%) in the study area. Animals with a previous history of abortion had nine times higher odds of being seropositive for Brucella (AOR = 9.1; 95%CI: 1.538–54.236, P = 0.015) compared to those without such a history. This difference was statistically significant (P < 0.05).

The statistically significant difference observed in the prevalence of small ruminant brucellosis between having a history of abortion and not having a history of abortion was suggested to be attributed to the production of sugar erythritol, which stimulates the growth and multiplication of Brucella organisms during consequent pregnancies (Coetzer and Tustin, 2004; Radostits *et al*., 2007). These results align with the findings of Wakene *et al*. (2017), Tegegne *et al*. (2023), Dosa *et al*. (2023), and Adem *et al*. (2021), which highlight the importance of these risk variables. However, they contradict the findings of Deddefo *et al*. (2015), Wubishet *et al*. (2017), Muhidin *et al*. (2021), and Tulu *et al*. (2020), who reported no association between the risk of Brucella seropositivity and abortion. The variation could be attributed to the impact of sexual and reproductive hormones on the development of brucellosis in small ruminants.

The odds of being seropositive to Brucella were 9 times higher in sheep and goats with a previous history of retained fetal membrane (AOR = 9.02; 95% CI: 1.496–54.452, P = 0.016) compared to those without such a history. This difference was statistically significant (P < 0.05). These findings align with the research by Kelkay *et al*. (2017), Adem *et al*. (2021), Tadeg *et al*. (2015), and Muhidin *et al*. (2021). However, they contrast with Tulu *et al*. (2020). The difference may be due to the influence of sexual and reproductive hormones on the pathogenesis of brucellosis in small ruminants.

The higher seroprevalence of brucellosis in females, 8 (3.2%), compared to males, 2 (1.1%), might be attributed to the high concentration of erythritol, which was scarcely produced in male reproductive organs (Adugna *et al*., 2013). According to the present study, the

seropositivity of Brucella infection between sexes was not statistically significant (P > 0.05). Supporting these findings, Dosa *et al*. (2023) and Tegegne *et al*. (2023) also reported no observable difference in the prevalence of brucellosis between male and female sheep and goats. The variation may arise from the impact of sexual and reproductive hormones on the pathogenesis of brucellosis in small ruminants, due to the smaller sample size of males (n = 174) and the practice of keeping males in the flock for a shorter period, which may decrease their exposure to the disease. In addition, male animals are less susceptible to Brucella infection due to the absence of erythritol (Dima and Dadar, 2020). In contrast to our findings, Sintayehu *et al*. (2015) and Mohammed *et al*. (2017) reported that male small ruminants were more susceptible than females.

The analysis indicated no statistically significant difference in Brucella seropositivity among different age groups of sheep and goats (P > 0.05). This aligns with the findings of Kelkay *et al*. (2017), Yohanis *et al*. (2013), Gebretsadik *et al*. (2016), and Abebe *et al*. (2023), who also reported no significant correlation with age. “Nonetheless, the study observed that seroprevalence was higher in older age groups (3.9%) compared to adult (1.25%) and young (1.2%) groups. Sexually mature animals of both sexes were more likely to contract Brucella than sexually immature ones. Younger animals, however, often exhibit a higher resistance to infection and can overcome established infections more readily (Gyles and Prescott, 2004). Contrary to these observations, Dosa *et al*. (2023), Tulu *et al*. (2020), Mudihin *et al*. (2021), and Adem *et al*. (2021) reported a significant association between age and seropositivity. The prolonged presence of adult sheep and goats in the flock increases their chances of contact with Brucella organisms, while young animals are often sold quickly for family expenses, potentially reducing their exposure.

In the study area, there was no statistically significant difference (P > 0.05) in seropositivity among different parities. The seropositivity of female sheep and goats with a history of no parity, 1-2 parity, and 3-4 parity was (1.2%), (1.9%), and (8.2%), respectively. This contradicts the findings of Yohannes *et al*. (2013), Negash *et al*. (2012), Melese (2016), and Tegegne *et al*. (2023). It might be due to the fact that animals’ frequent exposure to parturition and other physiological stressors increases the risk of contracting a Brucella infection.

Since brucellosis was regarded as a disease that affects flocks, large flock sizes had significantly higher levels of Brucella seropositivity (5.5%), compared to 1.03% and 0.99% for medium and small flock sizes, respectively. This was attributed to the fact that a larger flock size allows for increased animal contact and a higher animal density per flock, which in turn increases the number of bacteria in the environment, raising the likelihood of brucellosis spreading. However, the results of the current investigation did not show a statistically significant correlation ($P > 0.05$) between flock size and the disease's seroprevalence. Tadeg's (2015) earlier investigation also indicated no statistically significant differences in flock sizes. In contrast, Tegegne *et al*. (2023), Tulu *et al*. (2020), and Muhidin *et al*. (2021) reported a statistically significant difference ($P< 0.05$) among flock sizes.

The overall flock-level seroprevalence of small ruminant brucellosis was 9.1% (95% CI: 4.77–15.53) were positive using I-ELISA, which aligns with the seroprevalence rates of 5.8% reported by Yohannes *et al*. (2013), 6.35% by Hussen *et al.* (2023), and 7.3% by Tsegaye *et al*. (2016) in extensive management systems. However, higher seroprevalence rates have been documented in other regions of Ethiopia within similar management systems, as indicated by Teklue *et al*. (2008), Jergefa *et al*. (2009), Asmare *et al*. (2013), and Kiros *et al*. (2016).

Small ruminants categorized in larger flock sizes have a higher prevalence (OR = 14.5, 95% CI: 1.589–132.150, $P = 0.20$) than those in medium and small flock sizes. This result was consistent with the reports of Asmare *et al*. (2013), Adugna *et al*. (2013), Wakene *et al*. (2017), Melese (2016), and Muhidin *et al*. (2021), who recorded a high seroprevalence of brucellosis at the flock level. The reason was that an increase in flock size was usually accompanied by an increase in stocking density, which was one of the determinants of exposure to Brucella infection, especially following an abortion. This was due to the close proximity contributing to the contagious nature of the infectious agent, allowing it to affect a large number of animals according to Radostits *et al*. 2007.

In this study, higher seroprevalence was recorded in flocks of animals with a history of abortion than in those without a history of abortion. The prevalence among flocks with a

history of abortion was five times higher (OR = 5.5, 95% CI = 1.02–29.080, P = 0.047) than among those without a history of abortion. The presence of abortion in flocks was one of the risk factors for small-ruminant seropositivity. Brucellosis causes late-term abortions, which increases the risk of the disease spreading to other animals in the flocks while they graze on the contaminated pasture lands because aborting animals typically shed the bacteria into the environment. Abortion represents the major complaint attributed to Brucella infections in livestock (Perez and Berhe, 2021; Kabagambe *et al.*, 2001; Muma *et al*., 2007; Musa *et al*., 2008; and Osoro *et al*., 2015). It is known that females infected with brucellosis shed considerable amounts of the pathogen in milk, placental membranes, and aborted fetuses. Such females have been reported to shed organisms for several months (Perez and Berhe, 2021; Radostits *et al*., 2007; Osoro *et al*., 2015). This finding was consistent with reports by Muhidin *et al*. (2021) and Megersa *et al*. (2011) in Ethiopia, who reported more positive reactions in animals with a history of abortion than in animals with no history of abortion. This causes environmental contamination, which increases the risk of pathogen transmission between animals in the same herds as well as other herds during free mixing in grazing and watering areas.

A survey was conducted to assess the sociodemographic profiles of small ruminant owners and their knowledge, attitudes, and practices regarding brucellosis in selected districts of the Ilubabor zone. The educational background of most participants was low, with many having no formal schooling or only basic to secondary education. This low level of educational attainment was often linked to a reduced adoption of technological advancements such as improved forage cultivation, advanced breeding methods, animal vaccination, and artificial insemination all crucial for enhancing animal productivity (Getahun *et al*., 2022; Abraham and Pal, 2014). Additionally, standard safety measures, such as wearing protective gloves during animal birth, isolating animals in pens, properly disposing of materials from abortions, and utilizing dedicated birthing pens, were largely overlooked. These findings were consistent with previous research indicating similar trends in extensive livestock production systems (Adugna *et al*., 2013; Getahun *et al*., 2022).

The majority of the community, 83.6%, participating in this study were not aware of a disease known as brucellosis, and seven seropositive flocks were identified. The respondents were

also asked to describe whether brucellosis affects sheep and goats. Most of the respondents (66.7%) had no knowledge of how brucellosis affects sheep and goats, and two seropositive flocks were identified. Most of the participants, 72.2%, who were aware of brucellosis lacked knowledge about its control and prevention, with two seropositive flocks found among them. These findings align with studies conducted in Ethiopia by Muhidin *et al*. (2021), Kenya, and Tajikistan (Kangethe *et al*., 2008; Lindahl *et al*., 2018). However, they contrast with research from Egypt and Jordan, which indicated higher disease awareness (Holt *et al*., 2011; Musallam *et al*., 2015). Among those familiar with brucellosis, understanding of its causes, transmission routes, and prevention methods was limited. Half of these respondents did not know which animals were susceptible to the disease.

Many of the respondents, 83.3%, did not believe that personnel working primarily with does or ewes exposed to Brucella infection are at high risk of infection, and three of their flocks were found to be seropositive. The majority of respondents, 61.1%, did not believe that isolating delivered does or ewes in a separate room was important for disease prevention, and three flocks were found to be seropositive. Approximately 61.1% of the participants in the study area responded that most small ruminant owners would sell aborted sheep and goats to neighbors or in the market rather than keep them in their flocks. Among these owners, two seropositive flocks were identified. This finding aligns with studies conducted in Ethiopia (Adem *et al*., 2021), Egypt (Holt *et al*., 2011), and Jordan (Musallam *et al*., 2015), which indicated that most farmers chose to sell diseased animals at the market rather than keep them in their flocks. These differences in behavior may be attributed to variations in disease knowledge (Holt *et al*., 2011). Conversely, a survey by Hegaz *et al*. (2016) found that the majority of shepherds retained animals that had aborted in their flocks, with only a small percentage considering selling them at the market. Most of the owners (77.8%) have a habit of assisting sheep and goats during parturition without using protective gloves, and they shared three positive flocks.

The common practice of disposing of aborted material after birth by throwing it into the field was reported by 76%, from which five seropositive flocks were identified. 77.3% of the flocks were recognized as having the potential for contact with other flocks at grazing and watering points, sharing eight seropositive flocks. All respondents (100%) had contact with animals

and animal products, which led to the identification of ten seropositive flocks. A significant majority (84.5%) did not wash their hands after contact with animal products, correlating with eight positive flocks. 65.8% of the study population came into contact with fetal membranes and fluids, resulting in five seropositive flocks. 60.9% assisted during abortion procedures, identifying three seropositive flocks. Most respondents (64%) who handled aborted materials barehanded without using protective equipment during lambing or kidding were associated with three seropositive flocks. This may also be partly due to a lack of access to protective gloves, which would have to be purchased at the farmer's expense. Our findings are consistent with results reported from Tajikistan, Egypt, Jordan, and Ethiopia, suggesting that glove use is not a common practice in many lower-income countries (Holt *et al*., 2011; Lindahl *et al*., 2015; Musallam *et al*., 2015; Muhidin *et al*., 2021).

The study yielded important insights into the seroprevalence of small ruminant brucellosis, as well as the associated risk factors, and the knowledge, attitudes, and practices of farmers within the Ilubabor zone's selected districts. Despite these significant findings, the study faced several constraints. The limited budget restricted the coverage of all kebeles in the chosen districts. Moreover, logistical challenges such as transportation issues, along with a shortage of reagents and laboratory kits, posed difficulties in data collection. These limitations may have impacted the study's comprehensiveness and the generalizability of the results.

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6. CONCLUSION AND RECOMMENDATIONS

The study found that small ruminant brucellosis was moderately prevalent in the area, with a seroprevalence of 2.4% at the animal level and 9.1% at the flock level. Seropositivity in individual animals was significantly influenced by species, pregnancy status, abortion history, and retained fetal membranes, while flock size and abortion history were also significant at flock level. The survey revealed that 74.2% of small ruminant owners lack knowledge about brucellosis, 70.8% was at a higher risk due to their negative attitudes, and 80.5% of individuals engage in risky practices, such as handling aborted materials with bare hands, discarding on environment, and not taking adequate protective measures. These practices contribute to the maintenance and spread of pathogens, as well as environmental contamination. The recommendation is formulated based on the research findings.

- In order to enhance community awareness about brucellosis, it is essential to educate individuals about its transmission, symptoms, and preventive measures. Additionally, promoting safe handling practices for aborted materials is crucial to minimize risks.
- Implementing biosecurity measures involves isolating and quarantining new animals, maintaining regular disinfection of equipment and facilities, restricting farm access to prevent contamination, and ensuring proper management of aborted materials.
- Raise awareness among farmers and workers regarding the significance of using personal protective equipment (PPE), including gloves for handling animals or aborted materials, as well as appropriate clothing to avoid direct contact with bodily fluids.
- Effective control and prevention of brucellosis rely on close collaboration among veterinarians, public health authorities, and livestock owners.
- Further research is needed to isolate and characterize the Brucella species found in livestock in the study districts.

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8. ANNEXES

Annex 1. Blood sample collection format for individual sheep and goat

Region_____Zone_____District_____Kebele_____Date_____

GPs: Longitudinal_____, Latitude_____Alt_____,

Purpose of collection_____

Number	Owner Name	Flock Size	Origin of animal	Blood Sample	Code	Age	Sex	PARITY	Pregnant status	Hist. of abortion	Freq of abortion	Gestation stage of abortion	HRFM	Lab.result (+/-)	
														RPT	iELISA
1															
2															
3															

Annex 2. Age and Dentition

Number of permanent incisors	Estimated age range	
	Sheep	Goat
0 pair	Less than 1 year	Under 1 year
1 pair	1-1½ years	1-2years
2 pair	1½-2years	2-3 years
3 pair	2½-3years	3-4 years

4 pair	More than three years	More than four years
Broken mouth (teeth missing or worn down)	Aged	Aged

Source: ESGPIP (2009) and Payne, W.J.A (1990).

Annex 3. Questionnaires survey use for assessment of risk factors of brucellosis (Structured questionnaire)

Questionnaire number_____Date of interview_____District:_____

__kebele: _____ Number of owned Sheep_____ Goats_____

1. Name of Interviewer: _____
2. Socio-demographic profile of respondent

Study Parameters	Category	Remark
Sex	Male	
	Female	
Age	18-30 Years	
	31-40 Years	
	41-50 Years	
	>50 Years	
Educational level	Illiterate	
	Basic education	
	Primary	
	Secondary	
	College and above	

Knowledge, Attitude and Practices of Community about Brucellosis

Knowledge Questions	Categories	Remark
Flock size	Small	
	medium	
	Large	
Have you heard of brucellosis?	Yes	
	No	
Which animals are affected by brucellosis?	Cattle	
	Wild animals	
	Sheep and goat	
	Don't know	
Does sheep and goats can affect by brucellosis?	contaminated feed	
	Contacts with wild animal	
	Coitus	
	Don't know	
Are you aware of the history of abortion?	Yes	
	No	
Have you heard of the history of RFM?	Yes	
	No	
Do you know the prevention and control of brucellosis?	Yes	
	No	

Attitude questions	Categories	Remark
Do you believe there is a significant risk of infection from	Yes	

Brucella for individuals or their family members who work with sheep and goats, given that these animals are highly susceptible to the disease?	No	
	Don't know	
Do you believe that the segregation of rooms designated for delivered does and ewes is essential in preventing disease transmission?	Yes	
	No	
Do you think that it is ethically acceptable to sell does or ewes that have a history of frequent abortions?	Yes	
	No	
Do you consider the use of protective clothing to be essential when working with animals?	Yes	
	No	
	Don't know	
What is your decision on aborting a shoat?	Sell	
	Keep	

Practices Related to Brucellosis in the Study Area	**Category**	**Remark**
Do your flocks come into contact with other flocks while grazing or at the watering point?	Yes	
	No	
Have there been any contacts with animals or animal products such as birthing products, blood, or meat?	Yes	
	No	
Do you wash your hands after having contact with animal products?	Yes	
	No	
Have you had contact with retained fetal membranes (RFM) or fetal fluids?	Yes	
	No	
Who manages RFM?	Veterinarian	
	Traditional healers	
	Owners itself	

How should aborted material be removed or disposed of?	Burying	
	Feed to the dogs	
	Throwing into the field	
Are you assisting during the abortion?	Yes	
	No	
How is aborted material handled?	Bare hand	
	Protected hand	
How is the removal or disposal of aborted material carried out?	Burying	
	Feed to the dogs	
	Throwing into the field	

Annex 4. Testing procedure of indirect (I-ELISA)

Indirect ELISA for the detection of the antibodies directed against *B.abortus, B.melitensis* and *B.suis* in serum and plasma for individual samples or pools up to 10 bovines serum and plasma. This diagnostic kit is designed to detect antibodies directed against *B.abortus* (bovine), *B.melitensis (*ovine and caprine) and *B.suis* (pigs).It can be used in bovine, ovine, caprine and pigs individual serum and plasma or in bovine pools up to 10.

Wells are coated with purified *Brucella abortus* LPS. Specimens to be tested and the controls are added to the micro wells diluted at1/20. Anti-Brucella anti-bodies, if present, form an anti-body-antigens complex. A multi-species horseradish peroxidase (HRP) conjugate is added to the micro wells. It fixes to the anti-Brucella anti-bodies, forming an antigens-antibody

conjugate-HRP complex. After washing in order to eliminate the excess conjugate, the Substrate solutions (TMB) are added. The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested. In the presence of the anti-bodies, a blue solution appears which becomes yellow after additions of the stop solution. In the absence of the anti-bodies no colorations appears (IDVet, Louis Pasteur).

Allow all reagents to come to room temperature ($21^{O}C\pm5$) before uses homogenize all reagents by inversion or vortex.

1. Add

- 190 µl of dilution buffer 2 to all wells
- 10 µl of the **negative control** to wells A1 and B1
- 10 µl of the **positive control** to wells C1 and D1
- 10 µl of each sera sample or pools of 10 sera to be tested to the remaining wells

2. Incubate 45 min (±4min) at $21^{o}{}_{C}(\pm5^{O}{}_{c})$

For individual serum samples only, it's also possible to incubate overnight, between 16 and 20 hours, at $21^{0}C$ ($\pm5^{O}C$).

3. Empty the wells. Wash each well 3 times with approximately 300 µl of the wash solution. Avoiding drying of the wells between washings.

4. Prepare the **conjugate** by diluting the **concentrated conjugate** 10X to 1/10(short incubation) or 1/20(overnight incubation) in **dilution buffer** 3.

5. Add 100 µl of the **conjugate** 1X to each well.

6. Incubate 30 min±3min at 21 ^{OC} ($\pm5^{OC}$) in the dark

7 Empty the wells. Wash each well 3 times with approximately 300 µl of **wash solution**

8. Add 100 µl of the **substrate solution** to each well.

9. Incubate 15 min ±2 at 21^{OC} (±5oc)

10. Add 100 µl of the **stop solution** to each well in in order to stop the reaction.

11. Read and record the O.D. at 4500 nm.

Interpretation

For each sample, calculate the S/p percentage(S/P %) as follows using the sample and control well values

Sample positive percentage(S/P %) $=\frac{\text{ODsample}-\text{ODNC}}{\text{ODPC}-\text{ODNc}}$X100

S/P% = $\frac{\text{OD of sample - OD of negative control}}{\text{OD of positive control - OD of negative control}}$ X 100

Sample with S/P%:

- ✓ Less than or equal to 110% are considered negative
- ✓ Greater than 110% and less than120% are considered doubtful
- ✓ Greater than or equal to 120% are considered positive

Result	Status
$S/P\% \leq 110$	Negative
$110\% > S/P\% < 120\%$	Doubtful
$S/P\% \geq 120$	Positive

Annex 5. Photos showing the whole from blood collection up to Confirmatory test

During interview the respondent

During sample taking

Labeling the sample

During harvest the serum.

During ELISA procedure in the laboratory.

During ELISA reading the sample