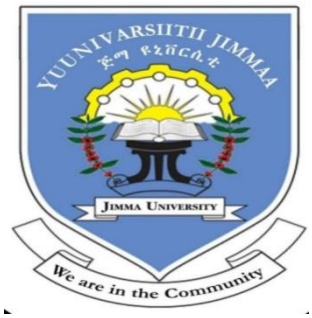

JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

SEROPREVALENCE OFSMALL RUMINANTBRUCELLOSIS, AND KNOWLEDGE, ATTITUDE AND PRACTICE OF HOUSEHOLDS IN ELWOYA AND GOMOLE DISTRICTS OF BORENA ZONE, ETHIOPIA

BY

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JUNE, 2024

JIMMA, ETHIOPIA

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Seroprevalence of small Ruminants Brucellosis, and knowledge, Attitude and practice (KAP) of Households in Elwoya and Gomole Districts of Borena zone, Oromia Region, Ethiopia

By
Yohannis Teshome

Thesis Submitted to School of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University in Partial Fulfilment of Requirements for the Degree of Master Of Science in Veterinary Public health

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June, 2024

Jimma, Ethiopia

SCHOOL OF GRADUATE STUDIES

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We, the undersigned, member of the Board of Examiners of the final open defense by **Yohannis Teshome** have read and evaluated his/her thesis entitled **"Seroprevalence of small Ruminant brucellosis, and Knowledge, Attitude and Practice of Households In Elwoya and Gomole Districts of Borena Zone, Ethiopia"** and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree Master of Science in Veterinary Public Health

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DEDICATION

This manuscript is dedicated to my beloved father Teshome Gebre Mariam and my beloved mother Ayelech Urgessa He'ii & my brother Solomon Teshome.

ACKNOWLEDGMENTS

Firstly, I glorify the almighty God, the super master of the world, the most gracious and merciful. Secondly, I would like to express my sincere gratitude to my advisor, **Dr**. **Mukarim Abdurahaman** and Co-Advisor Dr. **Tadele Kabeta for** his intellectual guidance, constructive suggestion, endurance and patience for correcting this Thesis paper.

We are very thankful to Yabello Regional Veterinary Laboratory for supporting and equipping us with neces-sary diagnostic equipment in this study. And also, many appreciations go to Elowaya and Gomole district animal health workers specially **Dr. Kula Jilo**, for their unforgettable effort with us during the Data analyasing period. We also acknowledge all Yabello Regional Veterinary Laboratory (serology) professionals who participated in this research activity**.**

I cannot get a word to express my feeling and gratitude to my brothers **Sintayew Getacho/Boss/** for his generous support and provision of all the necessary materials and his incalculable and unreserved technical support. I greatly acknowledge to Jimma University College of Agriculture and Veterinary Medicine internet service that help me to get different data sources.

STATEMENT OF THE AUTHOR

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

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BIOGRAPHICAL SKETCH

The Author was born in Oromia Region Borena Zone yabelo from his mother Ayalech Uregessa and his Father Teshome G/Mariam On January 10, 1996. He attended his primary school at Yabelo junior Primary school, Secondary School at Yabelo high school in 2004 he joined Oromia pastoralist OPTVET College graduated with diploma in Animal health. Soon after graduation he was hired by the Yabelo woreda Livestock development Office as an Animal health assistant. In May 2021 he joined in jimma University College of Agricultural and veterinary Medicine graduated with a Bachelor degree in veterinary science (BVSc) And also 2014 He again joined post graduate program at Jimma University in 2022 to peruse his career in Veterinary Public Health (MVPH).

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LIST OF ABBRIVATIONS

µl	Micro Liter
ASS	Agricultural sample survey
C-ELISA	Competitive Enzyme-Linked Immuno-Sorbent Assay
CFT	Complement fixation test
CSA	Central Statistical Agency
DNA	Deoxyribose Nucleic Acid
FAO	Food and Agricultural Organization
IBM	Interim Brucellosis Manual
I-ELISA	Indirect Enzyme-Linked Immuno-Sorbent Assay
IgGs	Immunoglobulins G
KAP	Knowledge, Attitude, Practices
MRBPT	Modified Rose Bengal Plate Test
PA	Peasant Associations
PH	Power of Hydrogen
RBPT	Rose Bengal Plate Test
SPSS	Statistical Package for Social Sciences
SRBC	Sheep Red Blood Cell
USA	United State of America
WHO	World health organization

ABSTRACT

Brucellosis is an infectious bacterial disease of severe public healath threat and economic lossess. Data on seroprevalence and Kwoldege Attitude Practice of the disease in pastoral areas Thus, a cross-sectional study was conducted between March to July, 2023 with the aim to determine prevalence and risk factors of brucellosis in small ruminant and to asses Kwoldege Attitude Practice of pastoralists in Gomole and Elwoya districts of Borana zone. A total of 240 serum samples of randomly selected small ruminants were screened by RBPT and confirmed with I-ELISA test. Semi-structured questionnaire survey was used to conduct face to face interview with pastoralist (N=200) in study districts. Logistic regression analysis was used to determine the association of hypothesized risk factors. Final I-ELISA analysis revealed the overall seroprevalence of 3.33% (95% CI: 1.44- 6.46) of which 3.47 and 3.12% were goats and sheep respectively. District wise insignificantly varied seroprevalence of 4.17 and 2.50 % were recorded in Gomole and Elwoyya district respectively (COR=1.20; CI: 0.29- 4.95; P=0.79). By Multivariate logistic regression analysis likelihood of seropositivity for brucellosis was significantly higher in female [(AOR=1.48; CI=1.01-3.51; P=0.021], animal with previous history of abortion [AOR=4.01; CI=3.33-6.51; P=0.031] and retained fetal membrane [AOR=5.31; CI=1.34-7.13; P=0.003]. Regarding questionnaire survey, about three fourth (75%) of the herd had no awareness about public health and economic effects of the brucellosis. Additionally, the highest proportion of respondents are extremely exposed to hazardous risk factors of brucella infections such as: unhygenic water (78%), consumption of raw animal products (77.5%) and unprotected fetal handlings (66%).Therefore, given the preveailing of the disease, hazardious consumption habits, unprotected animal handlings and inaccessibility of hygienic water in these districts; health education about brucellosis and exposing risk factors, provision of hygienic water for human and animals and sanitary measures of water sources are also of paramount importance to tackle the economic and public health consequences of *Brucella spp*. Furthermore, further studies that encompass both animals and humans should be conducted to obtain a real figure of brucellosis distribution in pastoral areas.

Keywords: *Brucellosis; Small ruminants; Pastoral area; Borana*

1. INTRODUCTION

1.1 Backgrounds

Sheep and Goats are important domestic animals capable of surviving in harsh environments such as common in arid and semi-arid areas (Tharwat and Al-sobayil, 2017). In Borana pastoral areas, they are the second most important livestock species after cattle and serve the society mostly as source of cash and collaterals for the family to cover school fees for children and other family expenses (Teshome *et al*., 2019). The optimal utilization of sheep and goats, however, is hampered by infectious diseases. One possible disease is brucellosis, which has been recognized as one of the neglected tropical zoonotic diseases of worldwide public health importance (Olufemi *et al*., 2018). It is a sub-acute or chronic disease caused by *Brucella* species (Gondal *et al*., 2017). In livestock it is mainly caused by *Brucella abortus* (*B. abortus*), *Brucella melitensis* (*B. melitensis*), *Brucella suis* (*B. suis*), *Brucella canis* (*B. canis*) and *Brucella ovis* (*B. ovis*). Among these species, *B. melitensis* and *B. ovis* are the common cause of brucellosis in sheep and goats (Tekle *et al*., 2019). Although several species of *Brucella* can infect sheep and goats, *B. melitensis* is the primary cause of sheep and goat brucellosis (Constable *et al*., 2017). There are three biovars of *B. meltensis* that have differing geographic distribution, but no difference in pathogenicity or host preference. *Brucella melitensis* infection has major veterinary and human importance in areas where it occurrs and causes considerable economic losses associated with abortion, neonatal death (Rajala *et al*., 2016), reduced fertility and decreased milk production (Ran *et al*., 2018).

The considerable costs of preventive programs and restrictions on trade of animals and their products constitute an important loss to infected countries. In addition, there is a huge loss associated with human illness (Constable *et al*., 2017). In Ethiopia, the occurrence of brucellosis in goats has been known for long time. Conservatively the overall pooled prevalence was estimated to be about 5.3 % (95 % CI = 3.5, 7.5) as reported by Tadesse (2016). The presence of *Brucella* species among sheep and goat herds has also been confirmed using bacteriological methods (Tekle *et al*., 2019). The nationwide average prevalence, however, does not reflect the situation in pastoral areas since pastoralism is a distinct production system from mixed crop-livestock farming. Within the pastoral areas, depending on the local husbandry system, the prevalence can vary. For example, a prevalence of 1.7 % was

observed in Somali pastoral region of Ethiopia (Lakew *et al*., 2019) whereas a prevalence of 4.8 % was reported in sheep and goats in Afar pastoral region (Ashenafi *et al*., 2007). In one study comparing seroprevalence of brucellosis in sheep and gaots in Somali and Afar regions, a prevalence of 13.2 % was recorded in Afar where commingling of animals due to communal grazing is the common practice. From Borana pastoral area, only few serological studies have been reported. In addition information on factors affecting its occurrence as well as knowledge, attitude and practices of the pastoralists is scarce (Edao *et al*., 2020). The previous studies employed mostly complement fixation test (CFT) and other screening tets such as rose bengal plate test (RBPT) and indirect ELISA (Curro *et al*., 2012; Mohseni *et al*., 2017). An alternative diagnostic approach is the coompetetive ELISA (Yahaya *et al*., 2019). The sensitivity of the test ranged from 92.31 %–100 %, in comparison with 77.14 %–100 % for the complement fixation test (Perrett *et al*., 2010). There is empirical evidence of the frequent occurrence of unconfirmed cases of abortion and stillbirth in goats in pastoralist areas of Southern Ethiopia. Most of those cases are being handled by farmers and community animal health workers. Hence, reliable information is needed on the disease prevalence and the risk factors to reduce the veterinary and public health impacts of brucellosis in sheep and goats. Therefore, this study was conducted to estimate the prevalence of brucellosis using I- ELISA in sheep and goats and identify associated risk factors as well as KAP about the disease in Borana pastoral area. pastoralism (nomadic pastoralism) is the main means of livelihood for the Borana people (Gelagay *et al*., 2007). Cattle, goats, sheep and camels are important livestock species raised in the area .

1.2 The Statement of Problem

The economic and public health impact of *Brucella* infection remains the problem in developing countries (Roth*et al*.,2003).Brucellosis results in an obstacle to trade of animals, animal products and animal movement. It causes economic losses because of abortion or breeding failure in the affected animal population, diminished milk production and inhuman brucellosis result in reduced work capacity through the sickness of the affected people (Robinson ,2005). *B. melitensis* is considered to have the highest zoonotic potential followed by *B. abortus* and *B. suis.* The disease is also categorized as one of the neglected zoonoses with serious veterinary and public health important throughout the world (WHO,2006;OIE,2009). *B. melitensis* and *B. abortus* are zoonotic pathogens which cause brucellosis in human (Pappas*et al*.,2006 ;Radostits *et al*.,2007).

The states of brucellosis of the small ruminant in Ethiopia are well researched but no more. This may be due to the lack of attention given to small ruminant production sector. The less of research activity in animal diseases, poor veterinary development, lack of awareness of the economic and zoonotic impact of the disease have contributed to the less amount of information observed. Though limited, zero-surveillances carried out so far indicate that brucellosis may be one of the important diseases in goat rising communities. A sero-surveillance study carried out in small ruminants in different regions clearly demonstrated that the disease exists in Ethiopia (Teshale *et al.,* 2006; Teklue *et al*.,2013; Tadeg*et al.,* 2015; Kelkay *et al.,* 2017; Wubishet *et al.,* 2018; Adem *et al*., 2021).

According to the current sero-surveillance findings of the disease in the country, lowest 0.4% infection rate was recorded at Bahir Dar Town of Amhara Regional State (Ferede *et al.,* 2011) and the highest was reported at Tellalak District of Afar Regional State was 16% (Tadeg *et al.,* 2015). In Ethiopia, there is paucity of published data on the status of small ruminant brucellosis. Among few reports, from Tigray and tested sera from 2000 sheep and goats in pastoral regions of Ethiopia and documented 1.9% (n = 38) positive using RBPT and 9.7% (n = 193) positive by I-ELISA (Teshale *et al.*,2006 and Teklue *et al*.,2013),4.6% by Deddefo *(-et al*., (2015) from East Arsi and Showa zone,1.79% (Kelkay *et al*., 2017) from Tigray in

Tselemeti disricts,6.2% by(Wubishet *et al.,* 2018) inYabello districts of Borena Zone and 2.9%(Adem *et al*., 2021) in Dallo Manna and Harrana Bulluk districts of Bale zone.

Study on risk factors about brucellosis is lacking in much of the previous studies. However, understanding the risk factors, community perception of the disease is crucial, thus consideration of the baseline survey level of infection is therefore essential for the formulation of appropriate control strategies (Hegazy *et al*.,-2009). Moreover, theidentification of risk factors for infection and spatial heterogeneities in the disease distribution could allow control efforts. However, no research has been done to quantify and document the actual prevalence of brucellosis in study areas.

Therefore, there is aserious need to know the status of the disease both in human and small ruminant for better response to the impact of the disease. Furthermore, livestock keepers in the study area are likely to be prone to that disease due to close cohabitation, handling animal case and their eating habit. The knowledge of the community regarding of these diseases, there attitude and practice predispose them to zoonosis has not been studied previously in the study area, but it is important for future public health education and training. Particularly, there is no data on small ruminant brucellosis in the study area. On the other hand, there is high population of sheep and goat in the study area.Brucellosis is a common problem of sheep and goats in pastoral areas of the Ethiopia such as Afar, Borana, Omo Valley and in the lowlands of Tigray where there is large population of goats (Teshale *et al*., 2006). In spite of the aforementioned prevailing situation and the presence of a number of problems due to brucellosis in small ruminants there is dearth of well-documented information on the occurrence of the disease among small ruminants in Gomole and Elwoyya districts of Borana zone.

Therefore, the objective of this study was designed;

1.3 Objectives of the study

- ✓ To determine seroprevalence of small ruminant brucellosis inElwoya and Gomole Districts.
- ✓ To evaluate the risk factors associated withthe occurrence of brucellosis in sheep and goat.
- ✓ To Asses Knowledge, attitude and practice of households about Brucellosis in Elwoya and GomoleDistricts.

2. LITERATURE REVIEW

2.1 Etiology

Brucella species are facultative intracellular gram-negative cocco-bacilli, non-spore-forming and non-capsulated. Although *Brucella* species are described as non-motile, they carry all the genes except the chemotactic system, necessary to assemble a functional flagellum. Nine *Brucella* species are currently recognized, (Table1) seven of them that affect terrestrial animals are: *B. abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae, and B. microti* (Sriranganathan *et al*., 2010) and two that affect marine mammals are: *B. ceti* and *B. pinnipedialis* (Foster *et al.,* 2007).The first three species are called classical *Brucella* and within these species, seven biovars are recognized for *B. abortus*, three for *B. melitensis* and five for *B. suis*.(Seleem *et al*., 2008; Sriranganathan *et al., 2010).*

Table 1*:* Brucella species and their hosts

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

Source: Radostits *et al*., 2006).

2.2 Epidemiology

2.2.1 Geographical distributions

The geographical distributionof brucellosis is constantly changing, with new foci emerging or reemerging. The epidemiology of human brucellosis has drasticallychanged over the past few years because of various sanitary, socioeconomic, and political reasons, together with increasedinternational travel. New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries ofthe Middle East is rapidly worsening (Pappas *et al* 2006). Brucellosis is a disease ofworldwide distribution occurring in domestic as well as wild animals.It has been reported wherever animals are raised all over the world (Seifert, 1996). Although some of the industrialized countries in Europe andAmerica have achieved the eradication of brucellosis in domesticanimals through intensive control and eradication schemes, thedisease is still a serious problem in developing countries (Warner,2001 and Ragan,2002). *B. melitensis* is the most virulent species of the *Brucella* genusand the ones isolated most frequently in small ruminants inthe Mediterranean, the Middle East and Latin America Lucero *et al*,.20008 ; Blansco and Molina,2011).Brucellosis is a barrier to trade in animals and animal products andcauses significant losses from abortion, as well as being a seriouszoonosis (Benkirane ,-2006; Banai ,-2007 and Seleem *et al*.,2010).

2.2.2 Modes of transmission

The primary route of infection is through ingestion of contaminated feed and water, inhalationduring overcrowding, contact through intact skin andconjunctiva, lambs may be infected while in the uterus or by suckling infected milk of their mother. Venereal transmissions by the infected ram tosusceptible ewes appear to be rare. Transmission may occur by artificial insemination (Radostits *et al*., 2007). Sources of infection include aborted fetuses, fetal membranes, vaginal discharges and milk from infected sheep and goat. Studies indicate that 70-90% cause of Brucella infection occurs via the skin and mucus membrane by direct contact (Franc *et al.,* 2018).

2.3 Risk Factors

2.3.1 Host factors

It is widely accepted that susceptibility increases with sexual development and pregnancy (Guven *et al*., 2013). Kids and lambs may become infected before or soon after birth, and tend to become free from infection before reaching breeding age, occasionally infection persist much longer (WHO, 2006). *Brucella melitensis* infection causes disease only in adult (sexually mature) females and males. Young animals may be infected but do not show any clinical sign and generally show only a weak and transient serological response (Radostits *et al*., 2007). In *B. melitensis* infection males of sheep and goat are less susceptible than females. *Brucella ovis* has a great affinity for the reproductive tract of the male than the female. Breeding ewes with infected rams seldom cause the disease in ewe and incidence of abortion is low. Most breeds of goats are fully susceptible to B. melitensis. There is great variation in the susceptibility of different breeds of sheep, where Malta sheep are very resistant whereas fat-tailed sheep are very susceptible (WHO, 2006)

2.3.2 Pathogen risk factor

Brucella is intracellular bacteria, hence has protection from the innate host defense and from therapeutics, moreover in quiescent state does not cause formation of humeral antibodies (Guven *et al*., 2013). *Brucella*survives for up to 4 months in milk, urine, water and damp soil under proper environmental condition (WHO, 2006). Disinfectants like caustic soda, formalin 2%, and Lysol 1% destroy *Brucella* (Radostits *et al*., 2007).

2.3.3 Environmental and climatic factors

The survival of the organism in the environment plays a great role in the epidemiology of the disease (Beruktayet and Marsha C,2016). *Brucella*may retain infectivity for several months in water, aborted fetuses and fetal membranes, feces and liquid manure, wool, hay, on buildings, equipment and clothes. *Brucella* is also able to withstand drying particularly in the presence of extraneous organic material and will remain viable in dust and soil (CSA,2013). Humidity and PH of the environment influence the survival of *B. melitensis.* The organism is sensitive to direct sunlight, disinfectant, and pasteurization *Brucella* survives for up to 4 months in milk, urine, water and damp soil under proper environmental condition (WHO, 2006).

2.3.4 Pathogenesis

B. melitensis can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, the gastrointestinal tract, and reproductive tracts. In the alimentary tract, the epithelium covering the ileal Peyer's patches is the preferred site for entry (Tabak *et al*., 2008). Organisms are rapidly ingested by polymorphonuclear leukocytes, which generally fail to kill them and are also phagocytosed by macrophages. In macrophages, *B. melitensis* inhibits the fusionof phagosome and lysosome and replicates within compartments that contain components of the endoplasmic reticulum (Pizarro *et al*., 1998). In ruminants, *Brucella* organisms bypass the most effective host defenses by targeting embryonic and trophoblastic tissue. In cells of these tissues, the bacteria grow not only in the phagosome but also in the cytoplasm and the rough endoplasmic reticulum. In the absence of effective intracellular microbicidal mechanisms, these tissues permit exuberant bacterial growth, which leads to fetal death and abortion Seifert.,1996).

2.4 Clinical Signs

The clinical features of *B. melitensis* infection in sheep and goats vary according to the biovar involved (Xavier *et al*.,2009 and Kose *et al*.,2014). Brucellosis suspected in any flock with a history of abortion during the last stage of pregnancy, infertility, orchitis, epididymitis, stillbirths and neonatal mortality (Poester *et al*.,2010). The major clinical sign in the first stage of the disease is abortion, but other signs due to localization of the organism may be observed. These signs include orchitis, epididymitis, hygroma, arthritis, metritis, and subclinical mastitis among others However, numerous animals develop a self-limiting infection or they may become asymptomatic latent carriers and potential excretors (Radostits *et al*., 2007).

Brucellosis is not established if the female is exposed to the organism at the end of the pregnancy. The second stage is characterized by either elimination of *Brucella* or more frequently, by persistent inflammation of mammary gland and supramammaryand genital lymph nodes, with constant or intermittent shedding of the organisms in milk and genital secretions. Animals generally abort once during the mid-third of gestation but re-invasion of the uterus occurs in subsequent pregnancies with shedding in fluids and fetal membranes. The pregnancy can also get to full- term (Poester *et al*., 2010). Females that are born into an

infected area and get infected generally abort less than others. This explains the high level of abortions in newly infected herds and their relatively low frequency in herds where the infection is enzootic. The udder is a very important predilection site for *Brucella*organisms. Infection in lactating, none pregnant goats is likely to lead to colonization of the udder with excretion of *Brucella* organisms in the milk (Radostits *et al*.,2007).Retention of placenta and metritis are common sequels to abortion. Females usually abort only once, presumably due to acquired immunity. In general, abortion with retention of the placenta and the resultant metritis may cause prolonged calving interval and permanent infertility (walker, 1999).

2.5 Diagnosis

2.5.1 Bacteriological methods

Isolation of the organism is considered the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, which is relevant under an epidemiological point of view (Bricker,2002; Al Dahouk *et al*.,-2003). However, in spite of its high specificity, culture of Brucella spp. is challenging. Brucella spp. is a fastidious bacterium and requires rich media for primary cultures. Furthermore, its isolation requires a large of viable bacteria in clinical samples, proper storage and quick delivery to the diagnostic laboratory (Seleem *et al*., -2013);Hadush and Pal,-2013).

Specimen of fetal stomach, lung, liver, placenta, cotyledon and vaginal discharges are stained with Gram stain and modified ZiehlNeelsen stains. Brucella appears as small red-colored, coccobacili in clumps. Blood or bone marrow samples can be taken culturedin 5-10% blood agar is used. To check up bacterial and fungal contamination; *Brucella selective media* are often used. The selective media are nutritive media, blood agar based with 5% seronegative equine or bovine serum. On primary isolation it usually requires the addition of 5 -10% carbon dioxide and takes 3-5 days' incubation at 37^{O}C-for visible colonies to appear(Maguire *et al*.,-2002). There are a range of commercially available culture media for growing *Brucella;* the most common basal media in use are triptcase soy, bacto tryptose, triptic soy and tryptone soya. Frequently, field samples are contaminated with other bacteria, thus, selective media should be used to avoid overgrowth by fast growing agents. The use of selective culture media is needed to increase the probability of success of bacterial culture, and it is compulsory for the adequate

bacteriological diagnosis of brucellosis. Any basal media mentioned above with agar may be used to prepare selective media. The most widely selective media used are the kuzdas, Morse and farrell´s mediums. (Fernando *et al*.,2010& Miguel *et al*., 2011).

2.5.2 Serological methods

Serological tests are crucial for laboratory diagnosis of brucellosis since most of control and eradication programs rely on these methods. Inactivated whole bacteria or purified fractions (i.e., lipopolysaccharide or membrane proteins) are used as antigens for detecting antibodies generated by the host during the infection. Antibodies against smooth Brucella species (e.g., *B. abortus* , *B. melitensis* , and *B. suis*) cross react with antigenpreparations from *B. abortus* , whereas antibodies against rough Brucella species (e.g., *B. ovis*and *B. canis*) cross react with antigen preparations from *B. ovis*(Poester *et al.*,-2010).

Although several serological methods are currently available, these tests can be classified as screening tests (e.g., buffered antigen plate agglutination-BPAT), monitoring or epidemiological surveillancetests (e.g., milk ring test), and complementary or confirmatory tests (e.g., 2-mercaptoethanol, complement fixation, ELISAs, and fluorescence polarization assay). Selection of a given test should take into account the species affected as well as local regulations (Nielsen K 2002; Poester*et al*., 2010).

2.5.3 Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assay (ELISA) has become popular as a standard assay for the diagnosis of brucellosis, serologically. It measures IgG, IgA and IgM antibodies and this allows a better interpretation of the clinical situation. The diagnosis of brucellosis is based on the detection of antibodies against the smooth LPS.Detection of IgG antibodies is more sensitive than detection of IgM antibodies for diagnosing cases of brucellosis but specificity iscomparable (Agasthya *et al*.,*-2012*).

Compared to the conventional agglutination methods, ELISA is more sensitive in acute and chronic cases of brucellosis and it offers a significant diagnostic advantage in the diagnosis of brucellosis in endemic areas. For case detection and an accurate diagnosis of suspected cases, the combination of ELISA IgM and IgG tests should be used as this combination of laboratory tests bhas been shown to be the most efficient technique in the detection and diagnosis of

brucellosis. For follow-up and monitoring of prognosis, ELISA Ig M and 2- mercapto ethanol (2-MET) are more promising (Mantur *et al*.,2010; Asaad AM and Alqahtani JM ,2012).

Enzyme linked immunosorbent assay (ELISA) is an excellent method for screening large populations for *Brucella* antibodies and for differentiation between acute and chronic phases of the disease(Gall,-2003) It is the test of choice for complicated, local or chronic cases particularly when other tests are negative while the case is under high clinical suspicion. It can reveal total and individual specific immunoglobulins (IgG, IgA and IgM) within 4-6 hours with high sensitivity and specificity. In addition to the detection of immunoglobulin classes, ELISA can also detect *Brucella*-specific IgG subclasses and other *Brucella* immunoglobulins such as IgE (Christopher *et al*., 2010).

The indirect ELISA (I-ELISA) has been used for serologic diagnosis of brucellosis in sheep, goats and pigs. It has also been used for diagnosis using serum or milk from cattle (Gall, 2003; Di Febo *et al*., 2012). ELISA-i has been usually used for smooth LPS *Brucella*spp., and it is sensitive and specific for *B. abortus* or *B. melitensis*, but it is not capable of differentiating antibodies induced by the vaccine strains S19 or Rev1 (KO *et al*.,2012). Sensitivity of I-ELISA varies from 96 to 100% and its specificity from 93.8% and 100% (Gall D, 2001 and Gall, 2004).

The competitive ELISA (c-ELISA) with smooth *Brucella* LPS as antigen is used for detection of anti-Brucella in serum samples from cattle, sheep, goats, and pigs. This test is capable of differentiating vaccine antibody response from actual infections, and its sensitivity varies from 92 to 100%, whereas the specificity ranges from 90 and 99% (Godfroid *et al*., 2010; Perrett *et al*., 2010).

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2.5.4 Rose Bengal plate test

The Rose Bengal Test (RBT) is a rapid, slide-type agglutination assay performed with a stained B. abortus suspension at pH of 3.6-3.7 and plain serum. Its simplicity made it an ideal screening test for small laboratories with limited resources. The drawbacks of RBT include: low sensitivity particularly in chronic cases, relatively low specificity in endemic areas and prozones make strongly positive sera appear negative in RBT (Díaz *et al*., 2011).

The overall sensitivity is 92.9%, so the use of RBT should be considered carefully in endemic areas, particularly in individuals exposed to brucellosis and those having history of Brucella infection (. Ruiz-Mesa *et al.,* 2005).. Rose Bengal plate test [RBT] is an agglutination test that is based on reactivity of antibodies against smooth lipopolysaccharide (LPS). As sensitivity is high, false negative results are rarely encountered. To increase specificity, the test may be applied to a serial dilution (1:2 through 1:64) of the serum samples (Christopher *et al*., 2010).

The present World Health Organization (WHO) guidelines recommend the confirmation of the RBT by other assays such as serum agglutination tests (Araj GF, 2010; Christopher*et al*.,2010).

2.5.5 Indirect Elisa test

Enzyme linked immunosorbent assay (I-ELISA) is an excellent method for screening large populations for *brucella* antibodies and for differentiation between acute and chronic phases of the disease(gall,2003) it is the test of choice for complicated, local or chronic cases particularly when other tests are negative while the case is under high clinical suspicion. it can reveal total and individual specific immunoglobulins (igg, iga and igm) within 4-6 hours with high sensitivity and specificity. in addition to the detection of immunoglobulin classes, Elisa can also detect *brucella*-specific igg subclasses and other *brucella* immunoglobulins such as ige (christopher *et al*., 2010).

2.6 Treatments

Due to intracellular localization of *Brucella* and its ability to adapt to the environmental conditions encountered in its replicative niche e.g., macrophage (Seleem *et al.,* 2008).treatment failure and relapse rates are high and depend on the drug combination and patient compliance. The optimal treatment for brucellosis is a combination regimen using two antibiotics since monotherapies with single antibiotics have been associated with high relapse rates (Pappas*et al*.,2006).The combination of Doxycycline with Streptomycin (DS) is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis (Alp *et al*.,2006).

Neither streptomycin nor doxycycline alone can prevent multiplication of intracellular *Brucella* Although the DS regimen is considered as the gold standard treatment, it is less practical

because the streptomycin must be administered parenterally for 3 weeks. A combination of doxycycline treatment (6 weeks duration) with parenterally administered gentamicin (5 mg/kg) for 7 days is considered an acceptable alternate regimen. Although DS combinations had been considered by the WHO to be the standard therapy against brucellosis for years, in 1986 the Joint FAO/WHO Expert Committee on Brucellosis changed their recommendations for treatment of adult acute brucellosis to rifampicin (600–900 mg/day orally) plus doxycycline (200 mg/day orally) DR for 6 weeks as the regimen of choice. However, the studies that compared the effectiveness of DR regimen with the traditional DS combination concluded that DR regimen is less effective than the DS regimen especially in patients with acute brucellosis (Glynn *et al*.,-2008).

2.7 Public Health and Economic Impact of Small Ruminant Brucellosis

2.7.1 Public health impact

Human brucellosis is widely distributed all over the world. It is considered by theFAO, the WHO and the OIE as one of the widest spread zonooses in the world(Acha and Szyfers ,2001).Almost all human cases of brucellosis are acquired from animals, in particular goats and sheep. In humans, ovine/caprine brucellosis caused by *B. melitensis* is the most important clinically apparent disease and remains one of the most common zoonotic diseases worldwide, with more than 500,000 human cases reported annually (Pappas *et al*.,2006;Garcell *et al*., 2016).

The disease is primarily an occupational risk in exposed professions, i.e. veterinarians, farmers, laboratory technicians, abattoir workers, and others who work with animals and their products. The primary source is the animal and infection is transmitted either by direct or indirect contact through the skin or mucous membranes, inhalation of infectious aerosols with invasion occurring through the mucosa of the upper respiratory tract and ingestion of contaminated products, especially consumption of fresh unpasteurized milk from sheep and goats. *Brucella spp*. persists for several days in milk (even when it turns sour). It may also persist for weeks in ice cream and months in butter. Meat of animals with brucellosis may also be a source of infection if eaten when insufficiently cooked. The maximum danger is during the lambing or kidding period when infected animals, whether they have aborted or not, may discharge billions

of bacteria from the uterus in birth products and the discharges that follow. Abattoir workers handling infected sheep or goats are also at risk, especially from the contents of the uteri and udders (WHO,2006).Inhalation is often responsible for a significant percentage of cases in abattoir employees. Contamination of skin wounds may be a problem for persons working in slaughterhouses or meat packing plants or for veterinarians(Godfrod *et al*.,2005; . Johansen *et al*.,-2017;Li *et al.*,2017).

2.7.2 Economic significance impact

Brucellosis results in an obstacle to trade of animals, animal products and animal movement. It causes economic losses because of abortion or breeding failure in the affected animal population, diminished milk production and in human brucellosis result in reduced work capacity through the sickness of the affected people (Robinson ,2005).Brucellosis alsopresents a significant impediment to the economic potential of the large population of small ruminants. Since small ruminants and their products are an important export commodity, detaining seropositive animals in quarantine has a negative economicimpact (Ifa *et al.*,1996 and IBM,2013). Thecommon sequel of infertility increases the period between lactations in infected herds, and the average inter-calving period may be prolonged by several months. Costs include production loss associated with infection in animals, preventive program, and in the human disease cost of treatment and absenteeism from work brings many economicimpacts (Gul and Khan,2007).

2.8 Control and Prevention

As the ultimate source of human brucellosis is direct or indirect exposure to infected animals or their products, prevention must be based on elimination of such contact. The obvious way to do this-elimination of the disease from animals is often beyond the financial and human resources of many developing countries. The technical and social difficulties involved in eradicating B. melitensis from small ruminants have even taxed the resources of some developed countries.In many situations, there is little alternative but to attempt to minimize the impact of the disease and to reduce the risk of infection by personal hygiene, adoption of safe working practices, protection of the environment and food hygiene. The lack of safe, effective, widely available vaccines approved for human use means that prophylaxis currently plays little part in the prevention of human disease (WHO, 2006).

Prevention and control of brucellosis can be adopted realistically through an understanding of local and regional variations in animal husbandry practices, social customs, infrastructures and epidemiological patterns of the disease (Dorneles *et al*., 2015). The common approaches used to control brucellosis includes quarantine of imported stoke and decide for or against immunization of the negative animals (Radostits*et al.,* 2007).Eradication by test and slaughter principles are based on the magnitude of disease prevalence and economic status at countries and handling hygienic disposal of aborted fetuses, fetal membrane and discharges with subsequent disinfection of contaminated area (WHO, 2006).Control measures must include hygiene at lambing and the disposal of infected or reactor animals. Separate pens for lambing ewes, which can be cleaned and disinfected, early weaning of lambs from their dams, and their environment and vaccination, are recommended.

In endemic areas, all placentas and dead fetuses should be buried as a routine practice. The need to test and cull, introduced and resident animalslikely to be carriers is recommended, but difficult to be effective because of the inaccuracy of the tests. Because of the possibility that lambs may be infected at birth and carry the disease for life, it may be more economical to dispose off the entire flock (Radostits *et al*., 2007). The experience from all over the world, that vaccination is in most situations the only practical method of control of brucellosis in sheep and goats.

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). Most workers agree that the smooth live organisms of *B. abortus* strain 19 and *B.melitensis* Rev 1have many advantages over inactivated vaccines. Their limitations, including interference with diagnostic tests, are well known. However, they provide good protection on a herd per flock basis by reducing clinical symptoms (exposure potential) and elevating to induce sexually mature animals. The reduced doses also reduce the physiologic and serologic effects. It is illogical to restrict the use of vaccines among mature animals where there are no controls on infected populations (Donev, 2010).

2.9 Current Status of Small Ruminant Brucellosis in Ethiopia

The pastoral and agro-pastoral production system represent approximately 45-55% of the cattle, 75% of the small ruminants, 20% of the equines and 100% of the camels of the total national livestock population. The main mobile pastoralists in Ethiopia are the Somalis (Somali region) in the east, the Afars (Afar region) in the northeast, the Borena Oromos (Oromiya region) in the south and south-east and the Southern Omo people (SNNPS region) in the south and partly in the Gambela and Benishangul regions and around the Dire Dawa Administration. Despite the large size of the regional livestock population, its economic contribution to the regional and national economy is not significant, mostly due to natural and human limitations (Amaha, 2006).

Most of the studies which conducted on brucellosis were entirely based on estimation of seroprevalence of the disease. There are very few reports on the Seroprevalence of brucellosis, which are conducted on different livestock species and human in pastoral and agro-pastoral areas of the Ethiopia. Additionally, pastoral households often keep a diverse composite of livestock species as part of a coping mechanism for uncertainties and risks. Such conditions certainly increase aggregation and interaction of different animals at villages, grazing fields and water points, thus, facilitate transmission of the disease. The dynamics and frequent migration of pastoral flocks might increase the chance of coming into contact with other potentially infected flocks and exposure to geographically limited or seasonally abundant diseases. Mobility also increases the opportunity of interactions with wild animals. This has already been confirmed by (Samui *et al*., 2007; Megerssa *et al*., 2011) in that flocks coming into contact with wildlife had higher likelihood of acquiring infection than those without contact.

A sero-surveillance study carried out in small ruminants in different regions clearly demonstrated that the disease exists in Ethiopia.According to the currentsero-surveillance findings of the disease in the country, low infection rate was recorded at Bahir Dar Town of Amhara Regional State (Ferede *et al.,* 2011) and the highest was reported at Tellalak District of Afar Regional State (Tadeg *et al*., 2015).(Table 2).

Table 2. Prevalence of small ruminant brucellosis in different pastoral and agro pastoral area of Ethiopia

Study Region	**Prevalence (%)**	**Authors and years**
Amhara	0.4 %	Ferede *et al*., 2011
SNNP and Oromia	1.9 %	Asmare *et al*., 2013
Oromia	4.6%	Deddefo *et al*.,2015
Afar	13.7%	Tadeg *et al.,* 2015
Tigray	1.79%	Kelkay *et al*., 2017
Somali	1.37%	Mohammed *et al*., 2017
Oromia	6.2%	Wubishet *et al*., 2018
Oromia	2.9%	Adem *et al*.,2021

3. MATERIAL AND METHODS

3.1 Description of the Study Area

The study was carried out in Elwoya and Gomole districts of the Borena zone. Elwaye district is five newly established districts of Borena zone of Oromia regional state by the year 2009 E.C. The district is located geographically east to the Taltelle and Konso, west of Yabello, south of Burji and north of Dillo district. The district has arid climate with almost dual annual rainfall: Ganna season running from March to May and Hagayya season running from September to November. The district has an area of 334210 hek and 598 km far from capital Finfinne and and 29 km from Yabello town, the capital city of Borena zone. Located at altitude of 971-1875m Latitude $3^{0}18^{1}64^{,-}$ $10^{0}09^{1}04^{,,}$North, Longitude $3^{0}18^{1}03^{,,}$ - $43^{0}04^{1}24^{,,}$ East.

Predominant species of livestock in this district include cattle, goat, sheep and camel with few practices of poultry and equine production (cattle = 65,314 goat = 152,237 sheep = 84,521 donkey = 13,122 mule = 123 camel = 10,151 and poultry=37,416). Elwoya and Gomole District pastoralism livelihood is the way of life where migration is the main mode of escaping drought and shortage of water and pasture.

The agro-ecology of the district is characterized by highland (13%), mid-highland (55%) and lowland (32%). The altitude of the district is between 1,400-2,200 meters above sea level.
Gomole district is located 533 km away from Addis Ababa in south direction and 40 km from Borena zone capital city. The zone has latitude ranging between 943 and 2,400 meters above sea level with average annual rain fall of 400 to 1100 mm exhibiting a bimodal rainfall (long and short rainy seasons). The Long rainy season (Ganna) extends from March–May while short rainy season (Hagayya) extends from September–November. The annual temperature varies from 19–42^{0}C.

Gomole is among 13 administrative districts Borana zone, which has highest livestock population specially shoat and camel population and 533 km far from capital city Addis Ababa to the south and 40 km far from capital city Yabello to the north. The district is located at altitude 500-1420 meters above sea level, 400-600 mm annual rain fall and 17-32 °C daily annual temperature and about 47,829 camels. The district was found 533 kilometers from

capital Addis Ababa at latitude 5.143813 and longitude 38.294105. The district was established in 2009 according to Ethiopian calendar.

The livelihood of district population was purely pastoral and small mixed farming activities So the district constitutes largest livestock especially shoat and camel population such that about 233,249 head of goats and 178,281 head of sheep, 243,123 cattle, 68,926 camel, 78,896 poultry, 1377 mule, 477 horse, 23,456 donkey and totally about 836,785 livestock populations was estimated according to updated data in 2013 Ethiopian calender. But livestock population in the Area can be larger than estimated because the introduction of new herds from different direction of the district.

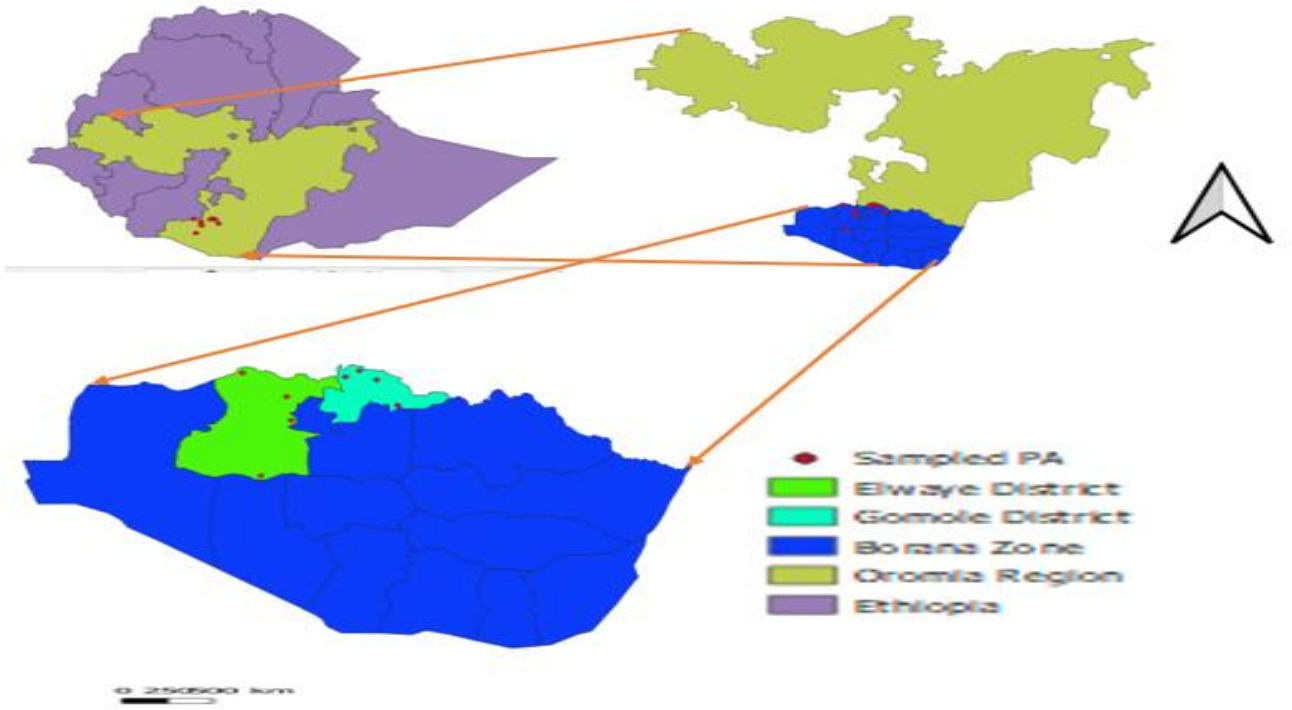

Figure 1 Map of study Area

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3.2 Study Populations

Population of interest was sheep and goats in the area managed under refering to intensive and semi intensive pastoral production system for dual purpose (milk and meat) featured by

communal grazing land and flock owners/herders in Gomole and Elwoyya district of Borana zone.

3.3 Study Design and Sample Size Determination

3.3.1 Study design

A cross-sectional study was carried out from March to July, 2023 in purposively selected (accessibility) Elwoya and Gomole districts, to determine seroprevalenceand potential risk factors associated with the seropositivity of brucellosis in small ruminats and to assess knowledge, attitudes and perceptions of pastoralists and their exposure to the risk factors in study areas. Four PAs (E/magala, Sabba, Cari rufay and A/galichat from Elwoya district) and (S/Magala, Haro Bake, Bildim and Harboro from Gomole district) were randomly selected from each district where three households were purposely selected from each PAs depending on possessing a minimum sampling animal (more than six goats and four sheep in flock).

3.3.2 Sampling method

The multistage sampling method was used to select sampling units in purposively (accessibility) selected two districts (Elwoya and Gomole) of Borana zone. From each district four PAs were randomly selected, where three households having a minimum of six heads of goats and four sheep were purposevely selected for sampling. The proportion of species was set by proportional sampling based on cumulative small ruminants' proportion in the two districts using update small ruminat data from the two districts. Thus as 60% of cumulative small ruminants of the two districts comprise of goats 60% of total sample (144 heads of goats) was covered by goats and the remaining 40% (72 heads of sheep) of sample was taken from sheep. Therefore, proportionally 6 goats and 4 sheep where randomly sampled from 3 households in each PAs (eight PAs).

Regarding questionnaire survey, a total of 200(100 from each district) pastoralists were interviewed about knowledge, attitude and perceptions about brucellosis.

3.3.3 Sample size determination

The sample size was calculated using the formula recommended by (Thrusfield, 2007) for simple random sampling considering 1.56 % expected prevalence (Dabasa *et al.* 2013) with 95% confidence interval level and 5% desired absolute precision using the following formula:

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

Where:

n-the required sample size

P_{exp}-expected prevalence rate

CI-confidence interval and

d-desired absolute precision

Sampling Method and Sample Collection

3.3.4 Sample Collection

3.3.4.1 Blood Sample collection

About 8 ml of whole blood was aseptically collected from the jugular vein of the randomly selected sheep and goat using disposable plain vacutainer tubes and needles. Then each sample tube was properly labeled and dispatched to Yabello regional veterinary laboratory stored at at -20 ^{O}C.

3.3.4.2 Questionersurvey

After verbal consentof the respondents were agreed the structured questionnaire survey was conducted via face-to-face interview with flock owners and other pastoralists involved small ruminnat rearing to assess socio-demographic, herd characterstics, knowledge, attitude and practice about brucellosis, the habit of consuming raw milk and meat, handling of aborted fetuses and contaminated materials.

3.3.5 Rose Bengal plate test

The collected blood the samples were allowed to stay for 24 hours at room temperature to for clotting and serum separation. Then serum was decanted in to cryovials tubes. Then The RBPT test carried out according to the method recommended by OIE, (2004).

The antigen used for RBPT, was RBPT antigen (Institut Pourquier 325, rue de la galèra 34097 Montpellier cedex 5, France).Antigen and sera required for each day for serological testing was taken out from the cold storage (in refrigerator at -4^{0}C) and brought to room temperaturfor 30 minutes before testing takes place. Briefly 30μl of stained rose Bengal antigen wasdispensed on to card plate and then 30μl of sera samples dropped alongside the stained Rose Bengal brucella antigen. By using the tip of the automatic micropipette tips, the sera and the stained rose Bengal brucella antigen were mixed and examined for agglutination. Positive and negative controls wasemployed for interpretation of the results. Agglutinations was recorded as 0, +, ++ and +++ according to the degree of agglutination (Nielson, 2002). A score of 0, +, ++ and +++ indicates the absence, barely visible, fine and coarse of agglutination, respectively. Then those samples with no agglutination (0) and with agglutination either +, ++ or +++ were recorded as negative and positive for brucella infection, respectively (appendix 1).

3.3.6 Indircet Elisa test

The Indirect elisa (I-ELISA) has been used for serologic diagnosis of brucellosis in sheep, goats and pigs. it has also been used for diagnosis using serum or milk from cattle (gall, 2003; di febo *et al*., 2012]. o elisa-i has been usually used for smooth lps *brucella* spp., and it is sensitive and specific for *b. abortus* or *b. melitensis* , but it is not capable of differentiating antibodies induced by the vaccine strains s19 or rev1(ko *et al*.,2012). sensitivity of I-ELISA varies from 96 to 100% and its specificity from 93.8% and 100% (Ggall d, 2001 and Ggall, 2004).

An animal considered positive if the serum specimen tested positive on both RBPT and I-ELISA, whereas a herd considered positive if at least a single serum specimen from an animal within the herd tested positive on both RBPT and I-ELISA.

3.4 Data Analysis

Data generated from laboratory investigation and questionnaire survey was recorded in a Microsoft Excel spreadsheet (Microsoft Corporation), coded and analyzed using STATA version 13. The seroprevalence was calculated as the number of serologically positive samples divided by the total number of samples tested. Descriptive analysis was applied to describe study population in relation to risk factors and variables. The association of the assumed risk factors and for *Brucella spp.* seropositivity was analyzed by univariate logistic regression

analysis. Variables with P-value < 0.25 in univariable analysis were offered to the final multivariable model. By final multivariate logististic regression analysis risk factors or variables having p<0.05 were considered significant.

3.5 Ethical Consideration

The study was approved by the research ethics committee and the letter of clearance was obtained from Jimma University College of Agriculture and Veterinary Medicine Ref No- A14/MAY/2024 and Yabelo Regional Veterinary labartory Ref No- LFBY/302/2016 The data was collected after verbally informed consent is made with all study participants.

4. RESULTS

4.1 Overall Seroprevalence

In the present study out of 240 small ruminants examined 8 were found positive for brucellosis by I-ELISA test with overall seroprevalence of 3.33% (95%CI: 1.44- 6.46) where prevalence of 4.17 and 2.50% were recorded in Elwoyya and Gomole district respectively (COR=1.20; CI:0.29- 4.95; P=0.79) (Table 3). At PA level the highest seroprevalence was detected in Sabba (Elwoyya district) and Haro Bakke (Gomole district) with equal prevalence of 6.67% whereas 0% prevalences were observed from Surupha magala and Bildim PAs of Gomole district (Table 3 and 4).

Table 3: Overall seroprevalence of brucella infection in small ruminants in study areas

		RBPT Result		I-ELISA Result	
District	PA	NE	NP(Pr%)	NP(Pr%)	X^2(P-value)
Elwoyya	E/magala	30	3(10)	1(3.33)	0.32
	A/galichat	30	4(13.33)	1(3.33)	0.09
	Sabba	30	4(13.33)	2(6.67)	0.06
	Carii rufay	30	3(10)	1(3.33)	
Gomole	H/Bakke	30	7(23.33)	2(6.67)	0.079
	S/magala	30	2(6.67)	0(0.00)	1.06 (0.30)
	Bildim	30	0(0.00)	0(0.00)	0.067
	Harboro	30	2(6.67)	1(3.33)	
	Total	**240**	**25(10.41)**	**8(3.33)**	

PA=Pastoral association; NE=Number Examined; NP=Number positive; Pr=Prevalence; X^2=Chi square

Table 4: Seroprevalence of Brucellosis in sheep and goats of study at PAs

	Goat			Sheep			X2(P-value)
	RBPT Result		I-ELISA Result	RBPT Result		I-ELISA Result	
PA	NE	NP (%)	NP(Pr)	NE	NP (Pr%)	NP(Pr%)	0.32
E/magala	18	2(11.11)	1(5.56)	12	1(8.33)	0(0.00)	
A/galichat	18	2(11.11)	0(0.00)	12	2(16.67)	1(8.33)	0.07 (0.79)
Sabba	18	2(11.11)	2(11.11)	12	2(16.67)	0(0.00)	
Carii rufay	18	2(11.11)	0(0.00)	12	1(8.33)	1(8.33)	
H/Bakke	18	3(16.67)	1(5.56)	12	4(33.33)	1(8.33)	0.79
S/magala	18	1(5.56)	0(0.00)	12	1(8.33)	0(0.00)	
Bildim	18	0(0.00)	0(0.00)	12	0(0.00)	0(0.00)	
Harboro	18	2(11.11)	1(5.56)	12	0(0.00)	0(0.00)	
Overall	144	14(9.72)	5(3.47)	96	11(11.45)	3(3.12)	

PA=Pastoral association; NE=Number Examined; NP=Number positive; Pr=Prevalence; X^2=Chi square

PA=Pastoral association

4.2 Risk Factors

By primary univariate logistic regression analysis five variables such as species of animal (p=0.79), age (p=0.029), stillbirth (p=0.95), sterility (p=0.59) and the source of drinking water (p=0.71) were droped (p >0.25) (Table). However, sex (p=0.016), flock size (p=0.021), history of abortion(p=0.011) and Retain placenta(p=0.002) were considerd by the final multivariable model of logistic regression analysis(p<0.25) (Table 5). Finally, sex, history of abortion and retained fetal membrane were found potetial risk factors significaltly associated with seropositivity of brucellosis in study animals.Accordingly, the odds of acquiring Brucellosis infection were 1.48 times higher in female animals than male (AOR=1.48; CI=1.01-3.51; P=0.021) (Table 6). Regarding the history of abortion, the animal with the previous history abortion were found 4 times more seroreactor than those animals with out abortion record (AOR=4.01; CI=3.33-6.51; P=0.031) (Table 6). Additionally, retained fetal membrane was also found significant predictor of brucellosis seropositivity; as animals with history of retained fetal membrane were 5.31 time more seropositive to brucellosis than animals without sign of retained fetal membrane (AOR=5.31; CI=1.34-7.13; P=0.003) (Table 6).

Table 5: Seroprevalence of Brucellosis and univariate logistic regression analysis output of risk factors and variables

Risk factors		NE (RBPT +ve)	I-Elisa +ve (Prevalence %)	COR (95% CI)	P-Value
District	Elwoya	120(14)	5(4.17)	0.48(0.11- 1.99)	0.32
	Gomole	120(11)	3(2.50)	Ref*	
Species	Goat	144(14)	5(3.47)	1.20 (0.29- 4.95)	0.79
	Sheep	96(11)	3(3.12)	Ref*	
Age	Adult	136(17)	6(4.41)	2.02(1.45- 8.10)	0.029
	young	104(8)	2(1.92)		
Sex	female	182(19)	7(3.84)	1.75(1.03-4.27)	0.016
	male	58(6)	1(1.78)		
Flock	small	16(6)	3(18.75)		
size	large	8(5)	3(37.50)	2.41(1.97-5.73)	0.021
Abortion	Present	157(22)	7(44.58)	4.2(3.47-7.61)	0.011
	Absent	83(3)	1(12.05)		
Stillbirth	Present	92(13)	6(6.52)	0.94(0.11- 7.84)	0.95
	Absent	148(12)	2(1.35)		
Retain	Present	68(15)	4(5.88)	7.49(1.52-9.73)	0.002
placenta	Absent	172(10)	4(2.32)		
sterility	Present	73(11)	3(4.10)	1.8(0.210-15.35)	0.59
	Absent	167(14)	5(2.99)		

Water	Well	70(8)	3(4.28)	1.47(0.175-2.49)	0.71
	Lake	120(12)	3(2.50)	1.20(0.26-1.95)	
	pipe	50(5)	2(4.0)	Ref*	

NE=number examined, COR =crude odd ratio, CI =Confidence Interval

Table 6: Seroprevalence of Brucellosis and multivariate logistic regression analysis output of risk factors and variables

Risk factor/ variable		NE (RBPT +ve)	I-ELISA +ve (Prevalence)	AOR (95% CI)	P-Value
Age	Adult	136(17)	6(4.41)	1.03(0.78- 4.92)	0.06
	young	104(8)	2(1.92)		
sex	female	182(19)	7(3.84)	1.48(1.01-3.51)	0.021*
	male	58(6)	1(1.78)		
Flock	small	16(6)	3(18.75)		
size	large	8(5)	3(37.50)	1.8(0.37-3.81)	0.086
Abortion	Present	157(22)	7(44.58)	4.01(3.33-6.51)	0.031*
	Absent	83(3)	1(12.05)		
Retain	Present	68(15)	4(5.88)	5.31(1.34-7.13)	0.003*
placenta	Absent	172(10)	4(2.32)		

NE=number examined, AOR= adjusted odd ratio, CI =Confidence Interval

4.3 Questionnaire Survey

The questionnaires were administered to a total of 200 householdsin two districts to capture awareness level about potential exposure risk factors of the disease. The result revealed that pastoral community in the current study area is extremely at risk of brucellosis and other zoonotic diseases due to combined effects of minimal awareness level, high illiteracy rate, insufficient improved water sources, overrated human-animal intimacy, and raw consumption habit of animal products.

showed that 90% of the respondents were rural residents and 86% were uneducated. About 77% of the respondents were male where 43% fall in 25-25-36 age range. Of the repsondents, 78% were use surface water for drinking purpose. Additionally, the majority of respondents 80 and 75% consumed raw milk and meat respectively. Regarding appropriate fetal handling, about 66% of the interviewees did not apply protective handling. Moreover, more than three fourth of the respondents were not aware of the zoonotic nature of the disease (Table 7).

Table 7:-Summary of the findings of the questionnaire survey regarding possible risk factors and awareness level of community about brucellosis in study area

	District			
	Elwoyaa		**Gomolee**	
Variable	**Frequency**	**Percentage**	**Frequency**	**Percentage**
Knowledge about brucellosis (transmission prevention and control)	67	75.6	6	7.5
Consumption of raw milk and meat	83	52.3	86	86.3
No	75	71.4	9	9.5
Yes	30	28.6	86	90.5
Attitude				
Awareness about zoonotic nature of brucellosis	7	4.7	65	79.5
fetal membrane and during assisting delivery	99	94.3	10	10.5
No	6	5.7	85	89.5
Yes	99	94.3	10	10.5
Practice				
Disposing of aborted fetus/fetal membrane	57	56.2	5	95.8
Experience of using protective while handling aborted fetus	43	43.8	91	95.8
No	59	56.2	4	4.2
Yes	96	98.1	91	89.8

5. DISCUSSIONS

Sheep and Goats remain valuable resources for the pastoral community inhabiting fragile and marginal lands. Optimum utilization of these resources requires control of infectious diseases such as brucellosis, which is highly communicable, resulting in considerable economic loss due to reproductive wastages (Rashid *et al*., 2017) and is a risk for public health. In pastoral areas of Ethiopia, different seroprevalence of brucellosis in people have been reported and seem occupationally linked. A prevalence of 2.6 % was reported in Borana by Edao *et al*. (2020), however higher prevalences of 48.3 %, and 34.9 % were found in Afar and Somali regional state respectively by Tschopp *et al*. (2021), 21.1 % pooled prevalence by (Tadesse, 2016) and as well as 34.9 % and 29.4 % in Borana and South Omo communities respectively (Megersa *et al*., 2011). The overall sheep and goat seroprevalence of 3.33% was in agreement with the 3.2% reported by Edao *et al*. (2020) and the 3.3% reported by Sintayehu *et al*. (2015) in the Borena zone. Additionally, this study's prevalence was greater than that of Tesfaye *et al*. (2020) in small ruminant brucellosis and in the Borena zone of the Oromia area, both of which reported a prevalence of 1.56% Dabasa *et al*. (2013).

The prevalence was also lower than that which was reported in the Yabelo district of the Borena zone by Zewdie *et al*. (2018), and in the Yabelo and Dire district of the Borana zone by Zewdie (2020), both of which reported 8.8%. the variation in the prevalence could be due to differences in flock structure, the laboratory tests used and the sample size used for the investigation. The present study documented serological evidence of brucellosis in sheep and goats and low level of awareness of brucellosis in occupationally exposed household members in two selected districts Elwoya and Gomole of Borena zone in Southern Ethiopia. Since we employed I-ELISA and RBPT Accordingly, among many; sex, history of abortion and retained fetal membrane were found significant predictors of brucella spp. infections in small ruminants. The prevalence difference between districts is considerable, ranging from 2.50 % in Gomole to 4.17 % in Elwaya districts. The high seroprevalence observed shows the widespread occurrence of brucellosis in sheep and goat population in an area where there is no control program in place. It is comparable to the results of authors who reported brucellosis in sheep and goats from different parts of Ethiopia such as Teshale *et al*. (2006); Ashenafi *et al*. (2007) and Tsehay *et al*. (2014) The pastoralits often mix animals of different ages and species in communal

grazing areas and during night enclosures. Such activities favor transmission and spread of Brucella among animals. The difference in seroprevalence could be attributed to difference in knowledge, attitude and practice of the households in the study area and the types of laboratory tests used for the detection of evidence of infection. The statistically significant difference observed in the prevalence of brucellosis between female and male sheep and goats was suggested to be attributed to the production of a sugar erythritol, which stimulates the growth and multiplication of *Brucella* organisms during consequent pregnancies (Radostits *et al*., 2010). A statistically significant difference in the prevalence of small ruminant brucellosis was observed. Current study significant Accordingly among ; sex, history of abortion and Retained fetal membrane were found significant predictors of brucella spp. brucellosis in small ruminants For instance, sex wise, the odds of acquiring Brucella spp infection were 1.48 times higher in female animals than male (AOR=1.48; CI=1.01-3.51; P=0.021) This disparity in sero reaction might be due to physiological difference between the two sexes, as pregnant and lactating animals might suscebtible for the infection.

That abortion and retained fetal membrane are prominent clinical manifestation of brucellosis in small ruminants. In the same manner, in this study, animals with the previous history of abortion were found 4 times more sero reactors than those without previous history of abortion (AOR=4.01; CI=3.33-6.51; P=0.031) . Additionally, animals with the history of retained fetal membrane were also found 5.31 time more seropositive to Brucella spp. infections than animals without sign of retained fetal membrane (AOR=5.31; CI=1.34-7.13; P=0.003). These variations in sero status would be due to difference in exposure to the brucella spp infections as naïve aniamls may reveal negative sero reactions. This observation is in agreement with the reports of Rahman *et al*. (2011). In female sheep and goats the occurrence of reproductive problems is associated with infection with *Brucella*. Reproductive loss due to abortion, birth of weak offspring, and infertility are recorded as the common clinical signs of brucellosis in infected hosts (Radostits *et al*., 2010). The high prevalence of brucellosis observed in adult goats in this study is due to the biology of the *Brucella*, which is associated with the sexual maturity of the hosts. There is also a commulative effects of age in which older animals are more likely to be exposed over lifetime. That is, adult animals have greater chance of coming into contact with other animals and become infected due to continued exposure to *Brucella* as they remain in the

herds over a long period of time serving as breeding stock. In the pastoral production system young animals are sold for immediate family expenses while adult animals, especially females, remain to produce offsprings. This observation has been reported in Ethiopia and elsewhere in the world (Bertu *et al*., 2010; Radostits *et al*., 2010; Zubairu *et al*., 2014; Asmare *et al*., 2013; Olufemi *et al*., 2018; Edao *et al*., 2020). In addition, adult animals move frequently from place to place during the dry season in serach of pasture and water. This increases the chance of contact with other animals and the chance of infection. Larger flock size was found to be significantly associated with seropositivity to *Brucella* in sheep and goats. This can be described to the fact that an increase in flock size is usually accompanied by an increase in stocking density, one of the determinants for exposure to *Brucella* infection especially following abortion or parturition. The association of flock size with the prevalence of anti-*Brucella* antibody has also been previously reported (Teklue *et al*., 2013; Asmare *et al*., 2013).

In this study, seropositivity to *Brucella* infection in sheep and goats was significantly associated with history of reproductive problems. Although the proportion of *Brucella* infected sheep and goats developing reproductive problems remains to be explored in the future, the observed association reveals the possible effects of brucellosis on the optimal utilization of the sheep and goats by pastoralists and the nation. In general, the distribution of anti-*Brucella* antibodies among different districts and herds was found to be variable. This could be associated with variability of the herd sizes and the geographical locations of the districts. Borana pastoralists trek their livestock, with the exception of lactating and few pregnant animals, to different districts, or even crossing national borders by traveling several kilometers in search of pasture, water and sometimes market. This results in massive concentration of animals in areas with relatively better pasture and watering points. This in turn, may contribute to increased transmission of *Brucella* organisms among different herds and districts. This situation also contributes to wider spread of the infection since there is no official brucellosis control program in Ethiopia.

Questionnaire survey result The questionnaires were administered to a total 200 pastoralist households in two districts to capture awareness about the disease. 83.1 %, 67.3 % and 64.2 % of the respondents in Elwaya and Gomole respectively did not have knowledge on transmission, prevention and control of sheep and goat brucellosis. Also in Elwoya (81.6 %),

Gomole (84.9%) repsondents were not aware of the zoonotic nature of the disease. Of the interviewed respondents 75.6 % in Elwaya, 80.3 % in Gomole consumed raw milk regularly. In addition to raw milk; 10.2 %, 3.8 % and 6.1 % of the respondants in Elwaya and Gomole respectively consume raw meat. None of the respondents indicated to either bury or burn aborted fetuses and fetal membrane; rather 76.3 %, 94.3 % and 40.8 % of them were throw them on the field, while 23.7 %, 5.7 % and 59.2 % in Elwaya and Gomole respectively were feeding them to dogs. A large proportion of participants, 100 % in Elwaya, 98.1 % in Gomole and 89.8 % normally assist delivery of kids. However, the majority of the respondents, 94.5 % in Elwaya, 96.8 % in Gomole do not use protection while assisting delivery or disposing of aborted fetus as well as fetal membrane.

6. CONCLUSION AND RECOMMENDATIONS

The study revealed that brucellosis is prevalent and well-established infection among goats and sheep in the study area. Sero-prevalence of brucellosis was higher in sheep than goats and animals within dense and large herd size species of animals. Collectively, sex, the history of abortion and reatained fetal membrane are underpinned as significant predictors of *Brucella spp,* seropositivity in small ruminants. It could be concluded that the positive animal can be a potential risk for both animals and humans in the area. In addition, communities in study area had no awareness about zoonotic importance of the disease. This could increase risk of human brucellosis additionally; the highest proportions of respondents are extremely exposed to hazardous risk factors of brucella infections such as: unhygenic water, consumption of raw animal products and unprotected fetal handlings. Despite, high risk of human brucellosis exists; physicians are not considering brucellosis while diagnosing patients with suggestive clinical sign of brucellosis. Therefore, we recommend that

- National brucellosis control and prevention strategy should be developed and applied
- Community should be educated about zoonotic importance of the disease and Human and animal health professionals; have to work together on zoonotic disease; like
- Brucellosis for successful prevention and control of the disease both in human and animals.
- The Ethiopian government should in farmer training center /FTC/ brucellosis control measures based on vaccination, as well as awareness creation for the community on the economic and public health implication of brucellosis for the contribution to successful prevention and control of the disease

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8. APPENDIXES

Appendixes 1 Rose Bengal Blate Test Procedure (RBPT) reagent material and equipment and procedure

Rose Bengal Blate Test Procedure

Sera (control and test sera) and antigen for the use will be left at room temperature for half an hour before testing since active materials straight from the refrigerator react poorly.

1. 30µl serum will be mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameters.

2.The antigen and serum will be mixed thoroughly using an applicator stick (a stick being used only once).

3.Rock plate by hand for four about 4 minutes.

4.Examine for agglutination in a good light.

5.Use magnifying glass when micro agglutination suspected.

Interpretation

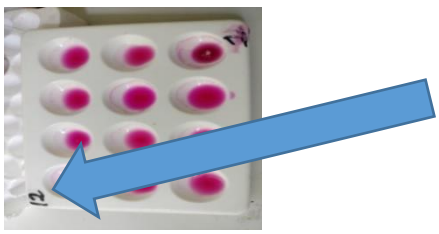
0=no agglutination

+=barely perceptible

++= fine agglutination, some clearing

+++=coarse clumping, definite clearing

Those samples identified with no agglutination will be recorded as negative those with +, ++, +++, ++++ **will be as recorded as positive**



Appendixe Figure 1Arrows indicates RBPT- Postive reaction

Appendixes 2 Indirect Elisa Test

ELISA TEST PROCEDURE
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} ($\pm 5^{OC}$) 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.

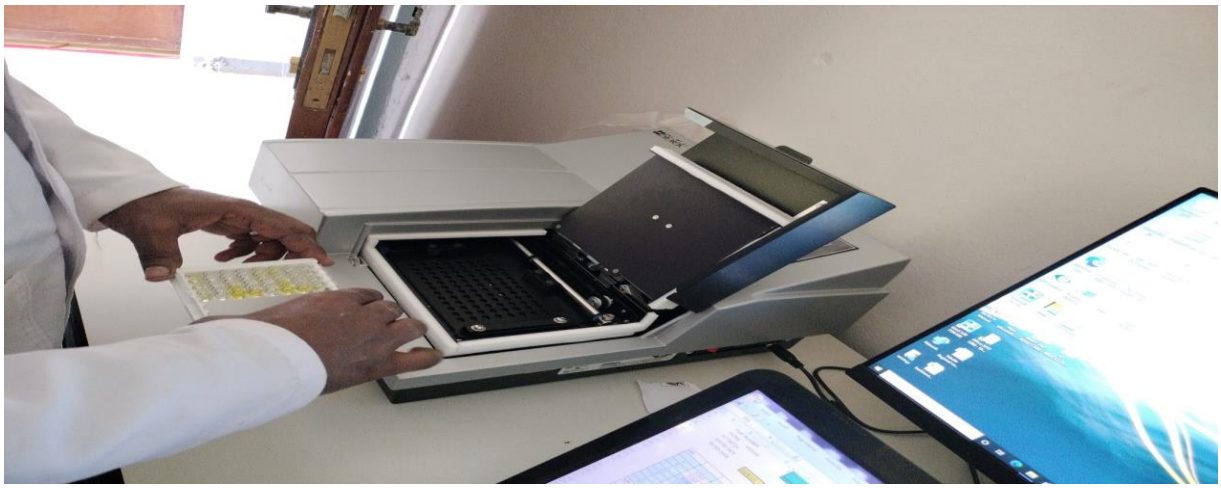
11. Read and record the O.D. at 4500 nm.

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 %) $= \frac{\text{ODsample} - \text{ODNC}}{\text{ODPC} - \text{ODNc}} \text{X}100$

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



Appendixe Figure 2 Indirect ELISA technic

Appendixes 3 Questioner of data collection

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Annexes 1; Questionnaires format for the survey of sheep and goat brucellosis

Date of interview_____

1. Demographic characteristics of respondents

1.1 Name of owner_____

District _____ PA_____ (code)_____

Sex_____ Age_____

1.2 Occupation

Owner _____ shepherd _____

2. Disease condition

2.1. Do you know Brucellosis?

Yes_____ No_____

2.2. If yes from where do you get information of brucellosis?

From health care workers_____

From neighbors, friends or relatives_____

From Traditional Healer _____

2.3. Which species of livestock it affects? (make circle)

Goat _____Sheep_____ Camel _____ Cattle _____ Wild Animals_____

2.4. Which of the following symptoms infected animals with brucellosis show?

Abortion_____ Swollen leg joints_____

Stillbirth_____ Retain placenta_____ Infertility _____ other _____

2.5. How can animal become infected with brucellosis?

Direct contact with aborted material/animal _____

Sharing the same pasture/water point with brucellosis infected herd_____

Introducing brucellosis infected animal into a herd_____

Mixing with brucellosis infected large/small ruminants _____

Mixing with wild animals _____

I have no idea _____

2.6. At what age the disease is more common?

<3 years (kids) _____Between 3 to 4 years (Young)_____

>4 years (Adult)_____

2.7. In which season disease is more prevalent

Major Rainy Season (“Ganna”) _____

Cool Dry Season (“Adololessa”) _____

Short Rainy Season (“Haggaya”) _____

Major Dry Season (“Bona”) _____

2.8. Why do you think the disease occur in that specific season/s?

_____

_____

2.9. Do you Know brucellosis as human disease (zoonosis)?

Yes_____ No_____

2.10. Which of the following symptoms infected human with brucellosis show?

Headache _____

Joint pain _____

Fever _____

2.11. If yes how can people become infected with brucellosis?

Consuming raw milk and its products_____

Consuming raw meat/ blood _____

Contact with aborted fetus or placental membrane_____

Assisting animals during parturition_____

Slaughtering animals_____

I have no idea _____

2.12. Do you use personal protective clothes during assisting delivery animals or contact with aborted material and fetal membrane?

Yes _____No_____

2.13. Do you destroy/buries aborted material and fetal membrane?

Yes _____No_____

2.14. What intervention you take if animal is affected by brucella ?

Separate from other _____ cull_____ give treatment_____

Take to clinic_____ Do nothing_____

2.15. Do you consume raw milk?

Yes _____No_____

2.17. Do you consume raw meat/organ?

Yes _____No_____

2.18. Purpose of milk production?

For sell_____

For family consumption_____

3. Control and prevention Measures

3.1. What are the possible sources of brucellosis?

A) Water point B) Grazing point C) New introduction from marketing

3.2 Risk factors

A) Flock size B) Farming practices C) Source of breeding stock

3.3. What measures do you take to combat the disease?

A) Treatment B) Destocking C) Vaccination D) Isolation E) Slaughtering

Appendixe Figure 3 Face to face in questioner at Pa level

Appendix table 4**.** Serum Sample Collection Format

Region _____Zone _____District _____Date _____

S. No.	Owner's name (Household)	PA	Sex of the animal	Age (Months)	Health status	Any clinical sign	Source	
							Purchased	Born

Appendixe Figure 4 Blood sample decant proceses in lab

Appendixe Figure 5 Decant blood sample