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SEROPREVALENCE OFBRUCELLA IN SMALL RUMINANTS ITS RISK FACTORS AND KNOWLEDGE, ATTITUDE AND PRACTICE OF COMMUNITY IN BERBERE DISTRICT OF BALE ZONE SOUTH EAST ETHIOPIA

MSc THESIS

BY

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Seroprevalence of Brucella in Small Ruminants, Its Risk Factors, Knowledge, Attitude and Practice of Community in Berbere District of Bale Zone South East Ethiopia

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DEDICATION

This thesis is dedicated to my Father Muhidin Mohammed and my Mother Muntaha Kamal who brought me up and taught me the value of education, an opportunity they themselves were unable to have.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my own work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree in Veterinary Public Health at Jimma University, College of Agriculture and Veterinary Medicine. I declare that I and other scholars or institution anywhere for the award of any academic degree does not submit this thesis. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the School of Graduate Studies when in his or her judgment the proposed use of the material is for scholarly interest. In all other instances, however, permission must be obtained from the author.

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BIOGRAPHICAL SKETCH

The author was born from his father Muhidin Mohammed and his mother Muntaha Kamal on November 11, 1993 G.C. in Berbere district of Bale zone, Oromia Regional State, Ethiopia. He completed his primary School at Haro Dumal Primary School from 2000-2008 G.C. and attended Secondary School from 2009-20010 G.C. at Haro Dumal Secondary School. He joined Alage TVTE Collage from 2011 graduated by Animal health in 2013 and joined Berbere district animal and fisher production office as animal health worker for two years, He joined Jimma University in 2016, and graduated with BVSc. degree in veterinary science in 2017 G.C. he joined Jimma University in September 2018 to pursue his graduate study leading to Master of Science Degree in Veterinary Public Health.

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LIST OF ABBREVIATIONS AND ACRONYMS

µl	Micro liter
CFT	Complement Fixation Test
CI	Confidence Interval
CSA	Central statistics Authority
ELISA	Enzyme Linked Immune Sorbent Assay
MOA	Ministry of Agriculture
NVI	National Veterinary Institute
OIE	Office International des Epizooties**,**
OR	Odd Ratio
PA	Peasant Association
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
RLPS	Rough Lipopolysaccharide
SLPS	Smooth Lipopolysaccharide
SOP	Standard Operational procedures

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ABSTRACT

Brucellosis is an important infectious disease responsible for reproductive losses in sexually mature animals and zoonotic importance. A cross sectional study was conducted from November 2018 to November 2019 in Berbere districts with the Objective of Assessing the burden of brucellosis in small ruminants, risk factors knowledge, attitude and practice of communityin study area. A total of 470 sera from 80 flocks were collected (Goat, n=306 and sheep, n=164) by Simple random methods. The sera were tested by using Rose Bengal Plate Test (RBPT) and sero-positive reactors confirmed by Complement Fixation Test (CFT) using serial interpretation. A sample was considered to be positive when both tests results were positive and a herd was considered positive when a single animal within the herd tested positive. Over all prevalence in both species by CFT at individual animal level was 2.97% (2.43% and 3.26%) in goat and sheep respectively. Herd-level prevalence was 17.5%. Individual animal level multivariable logistic regression analysis revealed that herd size (OR=3.83, 95% CI: 1.287 - 11.40, P=0.016), age (OR=8.374, 95% CI: 2.786 - 25.17, P=0.000), parity status (OR=8.499, 95% CI: 1.187 - 60.88, P=0.033) and history of retained fetal membranes (OR=12.896, 95% CI: 2.575-64.585, P=0.002) was significantly associated with Brucella infection in small ruminants. In herd level multivariable logistic regression analysis herd size (OR: 11.018, 95%CI: 2.582 -47.023, P=0.001), abortion (OR: 0.102 95%CI: .017 - 0.627, P=0. 014), and retention placenta (OR: 0. 127 95%CI: 0.021 - 0.759, P=0. 024) was also significantly associated Brucella seropositivity (P<0.05). The results of questionnaire survey revealed that the majority of the community do not have sufficient knowledge about brucellosis and they are in risk of acquiring the infection. Most of respondent was consuming row milk, milk by products, handling of aborted fetus and other aborted materials without protective clothes. In conclusion, the present serological test revealed that brucellosis is prevalent among small ruminants in the study area. Therefore, further extensive molecular studies of the isolates and appropriate controlling strategies are required to reduce zoonosis and its economic impact in study area. Awareness creation for animal owner, animal attendant and other stockholder about the disease through extension service on risk of consuming of raw milk / milk by product, handling of aborted fetuses, placenta and also the impact of improper disposing of those material.

Keywords: *Berbere, Brucellosis, CFT, Goat, Seroprevalence, Sheep,RBPT.*

1. INTRODUCTION

1.1. Background

Small ruminants, which account for more than half of the domesticated ruminants in the world, are important components of the farming systems in most developing countries (Gebremedhin *et al.,* 2015). Recent studies in different regions of the world indicate that the global population of small ruminants increased from 1.35 billion to 1.94 billion (Tedeschi *et al.,* 2011). Small ruminants are an integral part of livestock keeping in developing countries, especially in Sub-Saharan Africa that are mainly kept for immediate cash sources, milk, meat, wool, manure, and saving or risk distribution. Small ruminants also have various social and cultural functions that vary among different cultures, socio-economies, agro-ecologies, and locations in tropical and subtropical Africa (Gobena, 2016).

Brucellosis is a contagious bacterial zoonotic disease ofveterinary and public health importance. The disease affects domestic animals (cattle, sheep, goat, camels and pigs), humans and wildlife. It is caused by various*Brucella*species such as *B. melitensis* in small ruminants, B. *abortus* in cattle, B. *suis* in swine and *B.canis*dogs, while all the species are known to be of zoonotic importance.*Brucella*species are slow-growing, Gram negative, small cocobacilli and facultative intracellular bacteria that is capable to survive and multiply within epithelial cells, placental trophoblasts, dendritic cells and macrophages (Gorvel, 2008).*Brucella melitensis* is considered to have the highest zoonotic potential followed byB. *suis* and B. *abortus.* According to the Office for International des Épizooties (OIE), the disease is also classified as one of the neglected zoonosis with a serious veterinary and public health importance throughout the world (WHO, 2006; OIE, 2009).

Globally, it is estimated that nearly 500,000 cases of brucellosis occur in humans every year (Pappas *et al.,* 2006), and often persists in the poorest and most vulnerable populations (FAO, 2003). The economic and public health impact of brucellosis remains of concern in developing countries (Roth *et al.,* 2003). The disease poses a barrier to trade of animals and animal products, an impediment to free animal movement (Zinsstag *et al.,* 2011).

It also causes losses due to abortion or breeding failure in the affected animal population, diminished milk production and in human brucellosis causing reduced work capacity through sickness of the affected people (FAO, 2003). In Africa and central Asia, the incidence of brucellosis is generally considered higher in pastoral settings. However, because of the difficulty to access pastoral communities the occurrence and the control of brucellosis are poorly understood both in humans and their animals in the pastoral settings of the sub-Saharan Africa where the burden of the disease could be high (Mcdermott and Arimi, 2002). According to the Central Statistics Agency (CSA), Ethiopia is one of the developing countries with domestic small ruminant population estimated to be 27.35 million sheep and 28.16 million goats (CSA, 2014).

Small ruminants are the chief source of cash income to small holders (EPAIAT, 2003; Akabarmehr and Ghiyamirad, 2011). This is because sheep and goat provide rapid cash turnover (OIE, 2009; Godfroid *et al*., 2011). Most of the sheep and goat populations in Ethiopia are raised under pastoral conditions. These small ruminants and their milk/meat products represent an important export commodity, which significantly contributes to the national economy. At optimum off take rates, Ethiopia can export 700,000 sheep and 2 million goats per year and at the same time supply 1,078,000 sheep and 1,128,000 goats for the domestic market (Alemu and Markel, 2008). Even though these animals contribute much to the national economy, however, there production is hampered by different constraints in Ethiopian pastoral areas. Among many factors that limit economic return from small ruminants, reproductive diseases including brucellosis are the major disease affects pastoral areas (ILRI, 2006).

1.2. The Statement of Problem

Brucellosis in sheep and goats due to*Brucella melitensis* is the most important zoonosis in terms of presenting a serious hazard to public health. The reports from different parts of Ethiopia are indicating that the occurrence of livestock and human brucellosis is increasing. Studies on the prevalence of brucellosis have been carried out in many parts of Ethiopia by different researchers.Previous reports on the overall prevalence of small ruminant beucelosis is variable about 0.4% prevalence was report in study conducted in and around Bahir Dar (Ferede *et al.*, 2011), 13.7% as a pooled prevalence for a study conducted in the district of Tellalake in Afar Region (Tadeg, *et al.,* 2015) and overall prevalence of small ruminant 16% according to report of

(Yibelta, 2005) in selected sites of Afar and Somali Regions. The Flock-level seroprevalence among the small ruminant population of Ethiopia has been reported by six researchers. Higher estimate of flock level seropositivity of (57.7%) (26/45) in the flocks of sheep and goat (Tegene *et al.*, 2016) from Afar region, (32 %) for pastoral production system in southern Ethiopia, (Asmare *et al.*, 2013), (28%) by (Teklue *et al.*, 2013) from southern Tigray, (26%) (34/121) in flocks of sheep and goat in Arsi and East Shewa area, (22.3%) (61/274) flocks of Shoat in Amhara region specifically from South Wollo, North wollo, North Shewa. Relatively low flock level prevalence of (12.8%) flocks from Nechisar area (Chaka *et al.*, 2018). Similarly, (13.2%) and (3.6%) flock level seroprevalence were reported in goats from agro-pastoral and sedentary production systems from southern and central part of Ethiopia (Asmare *et al.*, 2013).

Study on risk factor about brucellosis is lacking in much of the previous studies. However, understanding the risk factor, community perception of the disease is critical, thus consideration of the baseline survey level of infection is therefore essential for the formulation of appropriate control strategies (Hegazy *et al.*, 2009). Moreover, the identification 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 small ruminant brucellosis in the present study areas. Thus there is an urgent need to know the status of the disease both in humans and animals (small ruminants) for better response to the impact of the disease. Furthermore, livestock keepers in the study area might be more prone to the disease due to close cohabitation, handling animal cases and their eating habit. The knowledge of the community regardingthe disease, their attitude and practice predispose them to zoonosis has not been studded previously in the study area, but it is important for future public health education and training.

1.3. Objective

1.3.1. General Objective

- To estimate seroprevalence of small ruminant brucellosis and risk factor in Barber district.

1.3.1. Specific Objectives

- ✓ To estimate seroprevalence of small ruminant brucellosis
- ✓ To identify risk factors associated with*Brucella* seropositivity at individual animal and flock level
- ✓ To assess community knowledge, attitude and practice of community to ward brucellosis in study area

2. LITERATURE REVIEW

2.1. Taxonomy of the causative agent

The etiological agent of brucellosis is a bacterium of the genus Brucella . Currently ten species are recognized including the better known six classical species comprised of *B.abortus, B. melitensis, B. suis, B. ovis, B. canis* and *B. neotomae*. More recently, new members to the genus include *B. ceti* and *B. pinnipedialis, B. microti* and *B.inopinata* (Godfroid *et al.,* 2011). Brucella are facultative intracellular coccobacilli belonging to the order Rhizobiales of the α-2 subgroup of Proteobacteria. The class alpha-proteobacteria includes organisms that are either mammalian or plant pathogens or symbionts (Garrity, 2001; Ficht, 2010). The Proteobacteria are a major phylum of bacteria, which include a wide variety of pathogens, such as *Escherichia, Salmonella, Vibrio, and Helicobacter*. All proteobacteria are Gram negative, with an outer membrane mainly composed of lipopolysaccharides (Bergey *et al*., 1994).

Within the family Brucellaceae, Ochrobactrum is the closest phylogenetic neighbour of*Brucella.* Historically, Brucella e are differentiated by host tropism, pathogenicity and phenotypic traits (Al Dahouk*et al.,* 2013).*Brucella*is taxonomically placed in the alpha-2 subdivision of the class Proteobacteria. The species of*Brucella*based on preferential host specificity: *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) (O 'Callaghan 2011). *B. melitensis* (small ruminants), *B. abortus* (cattle), *B. suis* (swine), and *B. canis* (dogs) are known to cause human disease. *B. neotomae* (desert wood rats) and B. *ovis* (sheep) are not pathogenic to humans. The majority of human cases worldwide are attributed to *B.melitensis*(Pappas *et al.,*2006).

Some Brucella specie like *B. abortus, B. melitensis, B. suis* and *B. canis* can affect a ranges of hosts in addition to their natural hosts resulting hazards on the health of animals including humans; due to this, infected countries are challenged and have been under difficulties to overcome or control brucellosis effectively. In addition to cattle, *B. abortus* can affect other animals like sheep, goats, horses, camels, swine, dogs and humans. Brucella *melitensis*also affects other animals like sheep, horses, swine, camels, dogs and humans. *B. suis* also affects different animal species suchas cattle, sheep, goats, dogs, camels, horses and humans. *B. ovis*

affects only ovine while *B.canis*affects dogs and humans (FAO *et al*., 2006). In general, *B. melitensis* and *B. suis* are more virulent for humans than *B. abortus* or *B.canis*(WHO, 2006). *B. melitensis*, *B. abortus*, and *B. suis* have 3, 8, and 5 biotypes, respectively (Whatmore, 2009). Sequencing and annotation of the genomes of *B. suis*, *B.melitensis*, and *B. abortus* has been completed; the majority of the open reading frames share greater than 99 percent sequence similarity between species (Paulsen *et al*., 2002; Halling *et al.*, 2005). The different synonyms of Brucellosis include: undulant fever, Malta fever, Mediterranean fever, enzootic abortion, epizootic abortion, contagious abortion, Bang‘s disease, Gibraltar fever, Cyprus fever, Rock fever and typhomalarial fever in animal and human. It was an important zoonotic disease and causes significant reproductive losses in sexually mature animals (Forbes and Tessaro, 1996; Mantur *et al*., 2007; Wadood *et al*., 2009).

2.2. Morphology of *Brucella*

Briucella species are slow-growing, Gram negative coccobacilli or short rods measuring from 0.6 to 1.5μm long and from 0.5 to 0.7μm wide, non-motile, non-spore forming, non-capsulated, non-flagellated, aerobic, facultative intracellular bacteria capable of invading, surviving and multiplying within epithelial cells, placental trophoblasts, dendritic cells and macrophages (Gorvel, 2008). The bacteria are usually arranged singly, and less frequently in pairs or small groups. The morphology of Brucella is fairly constant, except in old cultures where pleomorphic forms may be evident. They are not truly acid-fast, but are resistant to decolourisation by weak acids and thus stain red by the Stamp ‘s modification of the Ziehl-Neelsen‘s method. On suitable solid media, Brucella colonies can be visible after 2–3 days’ incubation at 37^{o}C. After 4 days ‘incubation, Brucella colonies are round, 1–2 mm in diameter, with smooth margins. They are translucent and a pale honey color when plates are viewed in the daylight through a transparent medium. When viewed from above, colonies appear convex and pearly white. Later, colonies become larger and slightly darker (OIE, 2009). The cellular and colonial morphology of the Brucella species are similar in most respect. All Brucella species possess smooth lipopolysaccharide (SLPS) in their outer cell wall except *B. ovis* and *B. canis,* which have rough lipopolysaccharide (RLPS) and protein antigens (Blasco *et al*., 1990). Smooth lipopolysaccharide contains an immune dominant O-polysaccharide which has been chemically defined as a homopolymer of 4, 6- dideoxy-4-formamide-Alpha-D mannose linked through

glycosidic linkages. Smooth Brucella cultures, especially *B. melitensis* cultures, have a tendency to undergo variation during growth, especially with subcultures, and dissociate to rough (R) forms, and sometimes mucoid (M) forms. Colonies are then much less transparent with more granular, dull surface (R) or a sticky gelatinous texture (M), and range in color from matt white to brown in reflected or transmitted light. Intermediate (I) forms between S, R and M forms may occur in cultures undergoing dissociation to the non-smooth state. Changes in the colonial morphology are generally associated with changes in virulence, serological properties and phage sensitivity (OIE, 2009).

2.3. Epidemiology of Brucellosis

2.3.1. Distribution

Brucellosis is a widespread disease and of major economic importance in most of the countries in the world, particularly among cattle. In small ruminants the disease is more restricted to the Mediterranean region including southern Europe, West and Central Asia, South America and Africa (Corbel, 1997; Godfroid *et al.,* 2005). With considerable variation between flocks and between areas and countries, *B. melitensis* is the most virulent species of the Brucella genus and has three biovars (biovars 1, 2 and 3) being the ones isolated most frequently in small ruminants in the Mediterranean, the Middle East and Latin America (Lucero *et al.,* 2008, Blasco and Molina-Flores, 2011).Goats are the classic and natural host of *B. melitensis* and together with sheep are its preferred hosts. In pathological and epidemiological terms, *B. melitensis* infection in small ruminants is similar to *B. abortus* infection in cattle. The geographical distribution of brucellosis is constantly changing, with new foci emerging or re-emerging.

The epidemiology of human brucellosis has significantly changed over the past few years because of various sanitary, socioeconomic, and political reasons, together with increased international travel. New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening(Pappas *et al.,* 2006). In Africa, the occurrence of brucellosis in sub-Saharan countries (either prevalence or incidence) is not well documented and reports submitted to the World Organization for Animal Health (Office International des Epizooties) are largely confined to serological surveys mainly conducted for cattle and less for sheep and goats (McDermott and Arimi, 2002). referred to a

great variation in prevalence in sub-Saharan Africa (ranging from 4.8 to 41%) in pastoral systems. In comparison with bovine brucellosis, brucellosis in sheep and goats caused mainly by *B. melitensis* has with only a few exceptions a low or sporadic degree of incidence throughout the African continent (McDermott and Arimi, 2002). Brucellosis is endemic in Ethiopia since 1970 (Tadele, 2004).There are many risk factors for occurrence of brucellosis in human beings and from these factors some of them are food consumption behavior, hygienic practices, occupational exposure, seasons, and health status of the veterinary professionals and lack of practicing bio security. Feeding behavior such as Consumption of unpasteurized milk and milk products from cows, small ruminants or camels is considered to be the risk factor of infection in human brucellosis. Occupational exposure is one of the risk factors that affect risk groups like veterinarians, laboratory workers, food processors and farmers who handle infected animals and aborted fetuses or placenta (OIE, 2009).

2.3.2. Risk Factors for Transmission

The epidemiological variables which are considered to affect the initiation, spread, maintenance and/or control of brucellosis can be categorized into those related to the animal population, to management, or to biology of disease (Nicoletti, 1980, Radostitis *et al.*,1994).The factors influencing the transmission of Brucella species in a geographical region can be classified into two categories: those associated with transmission of disease between flocks (purchase of infected animals, proximity of infected flocks to clean flocks, sharing pastures, dip tanks, watering points, and strays of infected animals into clean flocks), and those influencing the maintenance and spread of infection within flocks (unvaccinated animals in infected flocks, flock size, population density, method of housing and use of maternity pens)(Nicoletti, 1980, Radostitis *et al.*,1994) .

Host factors

The host factors, which are associated with spread of the brucellosis within a herd, include unvaccinated animals in infected herds, herd size, population density, age, sexual maturity and use of maternity pens. Large herd sizes are often maintained by the purchase of replacement small ruminant which may be infected. Population density (number of animal to land area) is attributed to increased contact between susceptible and infected animals. Health status of the

animals may also play a great role in acquiring the infection, hence vaccinated and disease free animals are less susceptible than unvaccinated and immune compromised or diseased animals (Radostits *et al.,* 2007).

The antibody against*Brucella*appears to be associated with age, as low prevalence in young stock has been reported than the adults. This low prevalence in young animals may be explained on the basis that the animal may harbor the organism without expressing any detectable antibodies until their first parturition or abortion (Jergefa *et al.,* 2009).It may be possible that after entry the organism localizes itself in the regional lymph nodes and enjoy there without provoking antibody production until the animal is conceived and start secreting erythritol, which stimulates and supports the growth of*Brucella* organisms. This is related to the fact that sex hormones and meso-erythritol (in male testicles and seminal vesicles) and erythritol in female, allantoic fluid stimulate the growth and multiplication of*Brucella*organisms and tend to increase in concentration with age and sexual maturity (Radostits *et al.,* 2007; Wadood *et al.,* 2009). A higher seroprevalence of small ruminant brucellosis in female than male was reported as the result of that male are kept for relatively shorter duration in breeding herd than female and thus the chance of exposure is lower for male and the spread of disease under natural condition is also not important. Moreover, female experience comparatively greater physiological stress during pregnancy and lactation due to which they are more susceptible to infection (Wadood *et al.,* 2009).

2.4.2. Reservoirs

Carrier animals facilitate transmission of brucellosis highly by contaminating the environment and also being site of multiplication for the*Brucella*organisms in their body and execrating such agents and again the execrated organisms infect animals and humans then bring hazards on health and economy of the country (Radostits, 2006). The carriers are dogs, cats and wild carnivores, such as foxes and wolves, which may be important as mechanical disseminators of infection by carrying away infected material such as fetuses or fetal membranes enhances the viability of the organisms in the environment, thus increasing the chances of infecting susceptible animals (FAO, 2006). It should be remembered that wild carnivorous like foxes and wolves, can acquire infection with *B.abortus, B. melitensis or B.suis* from aborted ruminants or

swine, usually by ingesting fetal or fetal membrane that left freely in the environment. These animals excrete these agents and contaminate the environment where other animals and human live and this may present a serious hazard to humans and domestic livestock; hence poor management of wastes disposal and lack of controlling pet animals plays a great role in the spread of brucellosis in animals and humans (Megerssa, 2004).

2.4.3. Environmental

The survival of the organism in the environment may play a role in the epidemiology of the disease under unsanitary condition where aborted fetuses are simply left everywhere where livestock, carnivorous animals and humans reach. Bovine infection presents a particularly serious problem due to excretion of large volume of infected material which contamination the environment. Even in single abortions or infected births and can produce large volume of infected milk by individual animal. Temperature, humidity and PH influence the organism's ability to survive in the environment.*Brucella*is sensitive to direct sun light, disinfectant and pasteurization. The congregation of a large number of mixed ruminants at water points facilitates disease spread (Radostits *et al.*,2007). The viability of the organisms in the environment, thus increasing the chance of infecting susceptible animals (Islam *et al.*, 2013; Baumann and Zessin, 1992).

2.4.4. Management

The spread of the disease from one herd to another and from one area to another is almost always due to the movement of infected animals from an infected herd into a non-infected susceptible herd. Hence, lack of strict movement control of animal from one area to another, lack of proper hygienic practices and lack of good husbandry management play a great role in increments of the prevalence of brucellosis. The source of replacement stock was found to affect the prevalence of brucellosis as a matter of a fact that the reproductive and health status of these replacement animals may be under the risk of *brucellosis.* The main risk for introducing the disease into a previously non-infected area is by purchase of infected animals (Tigist *et al.*, 2011).

2.5. Pathogenesis

The initiation of*Bruicella* infection depends on exposure dose, virulence of*Brucella*species and the natural resistance of the animal to the organism (Radostits *et al.*, 2007; Sharifi *et al.*, 2015). Resistance to infection is on the basis of host's ability to prevent the establishment of infection by the distraction of the invading organism. Invading *brucellosis* usually localize in the lymph nodes, draining the invasion site, resulting in hyperplasia of lymphoid and reticulo-endothelial tissue and the infiltration of inflammatory cells. Survival of the first line of defense by the bacteria results in local infection and the escape of*Brucella*from the lymph nodes into the blood (Tadeg *et al.*, 2015). During the bacteremic case, which may last 2-8 weeks, bones, joints, eyes, and brain can be infected, but the bacteria are most frequently isolated from superamammary lymph nodes, milk, iliac lymph nodes, spleen and uterus (Radostits *et al.*, 2007).

There is preferential localization to the reproductive tract of the pregnant animals. Unknown factors in the gravid uterus collectively referred to as allantoic fluid factors, stimulate the growth of *Briucella*. Erythritol, a four-carbon alcohol, is considered to be one of these factors. Abortion is associated with the extensive replication of the*Brucella* within the chorioallantoic trophoblasts that form a vital component of the placenta. This massive intracellular replication ruptures the infected trophoblasts and allows the bacteria direct access to the fetus. The resulting loss of placental integrity and fetal infection lead to termination of the pregnancy or the premature birth of a weak and infected calf (Hotez *et al.*, 2012).Localization in the placenta leads to the development of placentitis with subsequent abortion. After an abortion, the uterine infection persists for up to 5 months, and mammary gland may remain infected first years (Radostits *et al*., 2007, Saxena *et al.*, 2018).There is initial bacteremia, often with a mild systemic reaction, and the organism can be isolated from the internal organs of animals slaughtered after experimental infection. However, systemic disease is not a feature of the natural disease, and clinical disease results from localization in this area results in sperm stasis and extravasations with a subsequent immunological reaction which is usually in the tail and unilateral, causing a spermatocyte and therefore reduced fertility. Not all infected rams have palpable lesions in the epididymis and infection can also establish in the seminal vesicles. In either case, it is shed in the ejaculate.Testicular and epididymis lesions can be palpated at about nine weeks after infection

but may occur earlier in some rams. A significant proportion of infected rams have no palpable lesions but still excrete the organism (Radostits *et al.,* 2007). This disease is well described by its original name undulant fever. The disease does not have precise symptoms besides general malaise, making it difficult to diagnose clinically.

2.6. Clinical Signs

Brucellosis affects many animal species but can cause several problems for livestock holders when the disease occurs among food-producing animals. Important clinical signs are; abortions usually during the last third of pregnancy, premature births, retained placenta, reduced fertility and lowered milk production. Epididymitis and orchitis in males are two important clinical signs. The mortality rate is relatively low, especially when the patient is treated with adequate antibiotics; however this is not the case for everyone in low income countries. The agent erythritol (polyhydric alcohol) is found in animal placental tissue but worth mentioning not in human placental tissue. Erythritol acts as a growth factor for Brucella *species* and promotes infection in placenta and fetus and often followed by abortion. The same agent can also be found in mammary glands and epididymis (Quinn *et al.,* 2002). The disease manifests with continued, intermittent or irregular fever (hence the name undulant fever), headache, weakness, profuse sweating, chills, arthralgia, depression, weight loss, hepatomegaly, splenomegaly and generalized aching. Cases of arthritis, spondylitis, osteomyelitis, epididymitis, orchitis and in severe cases neuro-brucellosis, liver abscesses, and endocarditis with infection of the aortic valves and other multiple valves with*Brucella*has been reported in human(Franco *et al*., 2007) .

2.7. Diagnostic Techniques of Brucellosis

The most reliable and the only unique method for diagnosing animal brucellosis is isolation of Brucella species (Alton *et al.,* 1988). In the history of microbiology, very few diseases have more diagnostic tests than brucellosis. Diagnostic tests are applied for the following purposes: confirmatory diagnosis, screening or prevalence studies, certification, and, surveillance in order to avoid the reintroduction of brucellosis (in countries where brucellosis is eradicated) through importation of infected animals or animal products (Godfroid *et al.,* 2010). The diagnostic methods include direct tests, involving isolation of organism or DNA detection by polymerase

chain reaction (PCR)-based methods and indirect tests, which are applied either in vitro (mainly to milk or blood) or in vivo (allergic test). Isolation of*Brucella* species or detection of*Brucella species* DNA by PCR is the only method that allows certainty of diagnosis. Definitive diagnosis of brucellosis is based on culture, serologic techniques or both. Presumptive evidence of brucellosis is provided by the demonstration by modified acid-fast staining of organisms of*Brucella*in abortion material or vaginal discharge, especially if supported by serological tests. Whenever possible,*Brucella species* should be isolated using plain or selective media by culture from uterine discharges, aborted fetuses, udder secretions or selected tissues, such as lymph nodes and male and female reproductive organs. Species and biovars should be identified by phagelysis, and by cultural, biochemical and serological criteria. Polymerase chain reaction (PCR) can provide both a complementary and bio-typing method based on specific genomic sequences (Alton *et al.*, 1988; OIE, 2009).

2.7.1 Serological Diagnosis

Despite the development of numerous serological tests, no single test identifies all infected animals and a wide variation exists in estimates of their diagnostic accuracy (Adone and Pasquali, 2013; Abernethy *et al.*, 2012). The current serological tests used for the diagnosis of *B.melitensis* and *B. ovis* in sheep and goats were initially developed for the diagnosis of B. *abortus* in cattle (OIE, 2012). Although not formally validated for use in sheep and goats, these tests, especially RBPT, CFT and ELISA, have been widely used for the serological diagnosis of brucellosis in sheep and goats (Macmillan, 1990; Farina, 1985).They are also the official tests for international trade (European Commission, 2001; OIE Collective Manual, 2004). Serological tests can not differentiate between*Brucella*species and cannot therefore identify which species has induced host antibodies. Therefore, only isolation of the species or specific DNA detection by polymerase chain reaction (PCR), allows identification of the infecting strain (Godfroid *et al.*, 2010; Plumb *et al.*, 2013).

Rose Bengal plate test (RBPT)

This test was developed by Rose and Roekpe in 1957 for the diagnosis of bovine brucellosis to differentiate specific*Brucella*agglutinins from non-specific factors. When the antigen was buffered at pH 4.0 they observed that agglutination of *B. abortus* cells by non-specific

agglutinins of bovine serum was inhibited whereas the activity of specific*Brucella*antibodies was not affected. Despite the scanty and sometimes conflicting information available (Alton, 1990), this test is internationally acknowledged as the test of choice for the screening of brucellosis in cattle as well as in small ruminants (Garin and Blasco, 2004; WHO, 2006). However, the standardization conditions suitable for diagnosing cattle infection (European Commission, 2001; Garin and Blasco, 2004) are not adequate in sheep and goats and account for the low sensitivity of RBPT in small ruminants. If the antigen is standardized differently, to give a higher analytical sensitivity, the diagnostic sensitivity to B. *melitensis* infection will be improved (Macmillan, 1990). The RBPT 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. Also the low pH (3.65) of the antigen enhances the specificity of the test by inhibiting non-specific agglutinins.

The temperature of the antigen and the ambient temperature at which the reaction takes place may influence sensitivity and specificity (Macmillan, 1990). The RBPT could be modified for testing of sera in endemic, low prevalence areas to increase the sensitivity of the test. This simple modification is achieved by increasing slightly the amount of sera for the test dose from 25 µl to 75 µl, at the same time maintaining the antigen volume at 25 µl. This results in significantly increase in the sensitivity of the test without affecting the specificity (Blasco *et al.,* 1994; Ferreira *et al.,* 2003).

Complement fixation test (CFT)

Complement fixation test is the most widely used confirmatory test and recommended by OIE (Garin *et al.,* 2006). As in bovine brucellosis, there is agreement that this test is effective for the serological diagnosis of brucellosis in sheep and goats despite the complexity and the heterogeneity of the techniques used in different countries. The CFT is based on the detection of specific antibodies of the IgM and IgG1 that fix complement. It is highly specific but laborious and requires highly trained personnel as well as suitable laboratory facilities. Its specificity is very important for the control and eradication of brucellosis but may test negative when antibodies of the IgG2 type hinder complement fixation (Farina, 1985; Alton, 1990; Macmillan, 1990).

Enzyme linked immune sorbent assay (ELISA)

The ELISA tests offer excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form. They are more suitable than the CFT for use in smaller laboratories and ELISA technology is now used for diagnosis of a wide range of animal and human diseases. Although in principle ELISAs can be used for the tests of serum from all species of animal and man, results may vary between laboratories depending on the exact methodology used. Not all standardization issues have yet been fully addressed. For screening, the test is generally carried out at a single dilution. It should be noted, however, that although the ELISAs are more sensitive than the RBT, sometimes they do not detect infected animals which are RBT positive. It is also important to note that ELISAs are only marginally more specific than RBT or CFT (WHO, 2006).

2.7.2 Microscopic examination of stained smears

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) method. The presence of large aggregates of intracellular, coccobacillus red organisms is presumptive evidence of brucellosis. It is still often used, even though this technique is not specific as other abortive agents such as *Chlamydophila abortus* or *Coxiella burnetii* are also stained red (Alton *et al.,* 1988; FAO, 2006).

2.7.3 Cultural isolation

The only 'gold standard' method for the diagnosis of brucellosis is the cultural isolation or detection of*Brucella* organisms from the infected host (Alton *et al.,* 1988; OIE, 2009; Smirnova*et al.,* 2013). This can be made by means of microscopic examination of smears stained with the modified Ziehl-Neelsen method from vaginal swabs, placenta or aborted fetuses (Stamp, 1950). However, morphologically related microorganisms such as *Chlamydia psittaci* and *C.burnetii* can mislead one in the diagnosis (Garin, 2006; Radostits *et al.,* 2007). So bacterial culture plays an important role in confirming the presence of disease and it is essential for antimicrobial susceptibility, biotyping and molecular characterization which provide valuable epidemiological information to know the sources of infection in outbreak scenarios and the strain diversity in endemic regions (Kattar *et al.,* 2008).

Important clinical samples include aborted fetuses (stomach, spleen, and lung), fetal membranes, vaginal secretions, colostrum, milk, sperm and hygroma fluid. Brucella may also be isolated post-mortem from supra-mammary, internal iliac and retropharyngeal nodes, spleen, udder tissue, testes and gravid uterus. Care should be taken to minimize the fecal and environmental contamination of the material to give the greatest chance of successfully isolating Brucella . However, vaginal swabs and milk from aborted animals are the best materials/samples for the isolation of*Brucella* species, while spleen and lymph nodes (iliac, mammary and prefemoral) are the most reliable samples for isolation purposes in necropsy animals (Marin *et al.,* 1996).For the isolation of *Brucella* species the most commonly used media is Brucella Selective Media with sterile inactivated horse serum, which contains antibiotics able to inhibit the growth of other bacteria present in clinical samples.

2.7.4 Biotyping

The identification of*Brucella*involves Stamps modified Ziehl-Neelson's Gram's reaction, colonial and cellular morphology and routine biochemical tests (Corbel *et al.*, 2006). Species are distinguished on the basis of lysis by bacteriophages and oxidative reactions on amino acids and carbohydrate substrates. Biotyping of*Brucella* species is performed using different tests, like agglutination tests with antibodies against rough (R antigen) or smooth LPS (against the A or M antigens); lysis by phages, dependence on CO2 for growth; production of H2S; production of urease; growth in the presence of basal fuchsine or thionine; and the crystal violet or acriflavine tests (Alton *et al.*, 1988). These techniques must be carried out using standardized procedures by experienced personnel and usually performed only in reference laboratories.

2.7.5 Molecular typing

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 (OIE, 2009).*Brucella*biotyping and distinguishing vaccine strains by PCR can be accomplished satisfactorily but there has been limited validation of the PCR for primary diagnosis. The first species-specific multiplex PCR assay for the differentiation of*Brucella* was described by Bricker & Halling. The assay, named AMOS-PCR, was based on

the polymorphism arising from species-specific localisation of the insertion sequence IS711 in the *Brucella*chromosome and comprised five oligonucleotide primers that can identify without differentiating B. *abortus*, biovars 1, 2 and 4 but could not identify biovars 3, 5, 6 and 9. Modifications to the assay have been introduced over time to improve performance and additional strain-specific primers were incorporated for identification of the B. *abortus* vaccine strains and other biovars and species (OIE, 2009).

A new multiplex PCR assay (Bruce-ladder) has been proposed for rapid and simple one-step identification of*Brucella* . The major advantage of this assay over previously described PCRs is that it can identify and differentiate in a single step most*Brucella*species as well as the vaccine strains *B. abortus* S19, *B. abortus* RB51 and *B melitensis* Rev.1. In contrast to other PCRs, Bruce-ladder is able to detect also DNA from B. neotomae, *B. pinnipedialis* and *B ceti*. In addition, *B abortus* biovars 3, 5, 6, 7, 9, and B. suis biovars 2, 3, 4, 5 can be identified by this new multiplex PCR. The only minor inconvenience of the Bruce-ladder is that some B *canis* strains can be identified erroneously as B. *suis* (López *et al.,* 2011).

2.8. Significance of the Disease

2.8.1. Economic Significance

Endemic brucellosis in low-income countries of sub-Saharan Africa and South Asia has multiple economic implications across agriculture and public health and broader socio-economic development sectors. Efforts to control the disease in low-income countries must take a different approach. Simply replicating past successes in brucellosis control and eradication in high-income countries will not work. Low-income countries have at least a ten-fold higher burden of infectious disease from a wide variety of pathogens (Mc Dermott and Grace, 2013). The assessment of the economic aspects of brucellosis, with emphasis on the low-income countries of Africa and Asia, is structured in three main parts. The first describes an overall framework for economic assessment of disease burdens and the impacts of potential control programs. The second part systematically reviews available animal, human and joint burden estimates from studies conducted in these regions. The third section provides estimates, when available, of different costs associated with brucellosis illness and its control. This section also comments on tools and approaches for assessing control programs that are of relevance to low and middle-

income counters (Zamri-saad and Kamarudin, 2016). When brucellosis is detected in a herd, region or country, international veterinary regulations impose restrictions on animal movements and trade; which result in huge economic losses. The economic losses as well as its zoonotic importance are the reasons why programs to control or eradicate brucellosis in animals is necessary (OIE, 2008).

2.8.2. Public Health Significance

Briucellaabortus, B. melitensis and *B. suis* are highly pathogenic for humans (OIE, 2009). Brucellosis remains the most common zoonotic disease in the world with more than 500,000 new cases reported annually (Godfroid *et al.*,2013); the actual number of cases, including undetected and unreported cases, is believed to be considerably higher (Dahouk *et al*., 2013). Brucellosis is often a neglected disease despite being endemic with high zoonotic potential in many countries (Poester *et al.*, 2013). The prevalence of human brucellosis differs between areas and has been reported to vary with standards of personal and environmental hygiene, animal husbandry practices and species of the causative agent and local methods of food processing (Chugh, 2008).The Brucellosis 2003 International Research Conference estimated that 500,000 human infections occur per year worldwide, with incidences ranging from less than one case per 100,000 populations in UK, USA and Australia, through 20 to 30 cases per 100,000 in southern European countries such as Greece and Spain, to more than 70 cases per 100,000 in Middle Eastern States such as Kuwait and Saudi Arabia (Cutler and Whatmore 2003).

The majority of reported human brucellosis cases are caused by *B melitensis*, *B abortus*, and *B suis*, in occurrence order, novel and atypical*Brucella* are also being investigated (Dahouk *et al*., 2013). As compared to study of animal brucellosis, study of human brucellosis in Ethiopia is sparse with even less information on risk factors for human infection. For instance, out of 56 cases with fever of unknown origin, two (3.6%) were reported to be positive for *B. abortus* antibodies by RBPT and CFT (Jergafa *et al*., 2009). A study conducted in traditional pastoral communities by Ragassa and others (Regassa *et al*., 2007) using *B. abortus* antigen revealed that 34.1% patients with febrile illness from Borena, 29.4% patients from Hammer, and 3% patients from Metema areas were tested positive using Brucella IgM/IgG lateral flow assay. Studies conducted in high risk group such as farmers, veterinary professionals, meat inspectors and

artificial insemination technicians in Amhara Regional State (Mussie *et al*., 2007), Sidama Zone of Southern People Nations and Nationalities Sate (Kassahun *et al.*, 2007), and Addis Ababa (Kassahun *et al*., 2006).Found a sero-prevalence of 5.3%, 3.78% and 4.8% by screening sera from 238, 38 and 336 individuals respectively. The discrepancy between and others might be due to difference in milk consumption habits and sensitivity of test methods used (Ferede *et al.*, 2011). Humans may become infected by ingestion of unpasteurized cheese or milk, by direct transmission through contact with infected animals or by handling specimens containing Brucella spp. in laboratory. It also transmitted to human by the consumption of raw dairy products and by direct contact with the skin or mucosa during parturition and abortion (Ferede *et al.*, 2011).

In South Sudan a fraught with several potential risk factors could fuel the dissemination of brucellosis to livestock and humans (Lado *et al.*, 2012). The traditional pastoralist's practice of assembling several herds into cattle camps with close livestock-human interactions is one of the key milestones. Moreover, poor awareness is a risk milestone to occurrence and perpetuation of brucellosis in livestock which could create human health hazards (Ibrahim, 1990).

2.9. Treatment, Prevention and Control

Treatment regimens for human brucellosis require combination of antibiotics like rifampicin or gentamicin and doxycycline twice daily is the combination most often used and appears to be efficacious (Yohannes *et al.*, 2013).The combination of doxycycline with streptomycin is the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis (Seleem *et al.*, 2010).

One of the most successful methods for prevention and control of livestock brucellosis is through vaccination. In different parts of the world both live vaccines, such as *B. abortus* S19, *B. melitensis* Rev1, *B. suis* S-2, rough *B. melitensis* strain M111 and *B. abortus* strain RB51 and killed vaccines, such as *B. abortus* 45/20 and B. melitensis H.38 are available. Use of the RB51 attenuated live vaccine has gained popularity for control of brucellosis in cattle (Cheville *et al.*, 1996). Hitherto, no vaccine has been approved for the prevention of human brucellosis. Therefore, human brucellosis is usually prevented by controlling the infection in animals. Pasteurization of dairy products is an important safety measure where this disease is endemic.

Implementation of measures to reduce the risk of infection through personal hygiene, adoption of safe working practices, protection of the environment and food hygiene should minimize risks of further infection in nomadic populations where people travel in search of green pasture and water, the proper handling and burying of abortion materials to prevent contamination of water sources and pasture is of paramount importance. Furthermore, the common practice of feeding abortion materials to dogs should be avoided as this increases the risk of transmission to other animals. It is imperative to educate on risks for infection to populations in order to influence behavioral practices that will reduce risks of transmission (Yohannes *et al.,* 2013).

The development of a national veterinary extension services in the country, is essential to promote awareness about brucellosis, its impact on livestock production and zoonotic risks, would provide a valuable prevention measure. This would help to unify both community/dairy cattle producers to control and eliminate brucellosis. Currently, many dairy cattle producers hide or dispose of animals with a history of abortion, potentially facilitating disease transmission between farms and regions. This seriously undermines efforts of controlling and preventing the disease (Yohannes *et al.,* 2013).

2.10. Status of Small Ruminant Brucellosis in Ethiopia

Ethiopia located in Eastern Africa, is predominantly an agrarian country with over 85% of its population engaged in agricultural activity. The country has diverse agro ecological zones, which have contributed to evolution of different agricultural production systems. Animal husbandry forms an integral part of agricultural production in almost all ecological zones of country (Haileselasie *et al.,* 2010). Studies conducted on small-ruminant brucellosis in Ethiopiahave indicated that sero-prevalence of the disease is varied from place to place (Ashagirie *et al.,* 2011; Bekele *et al.,* 2011). This might be due to the differences in animal production and management systems as well as reasonably difference in agro-ecological conditions of the study area (Table 1). Reports indicated that the prevalence of small-ruminant brucellosis was much higher in area where farmers practice the communal use of grazing land than in clan-based flock/herd segregation areas (Yibeltal, 2005). This might be due to mixing animals from various areas in communal grazing system and watering points. reported prevalence proportion of 1.5% in sheep and 1.3% in goats in the central highlands, 15% in sheep and 16.5% in goats in the Afar region,

1.6% in sheep and 1.7% in goats in the Somali region (Yibeltal, 2005) and 1.6% in sheep and 1.7% in goats in Somali region (Teshale *et al.*, 2006).

Table 1:Prevalence of small ruminant brucellosis in different regions of Ethiopia

Region	Prevalence %				Source
	Sheep		Goat		
	NOof tested	Percentage %	NOof tested	Percentage %	
Afar		15%		16%	Yibelta ,2005
		3.2%		5.8%	Ashenafi*etal.*,2007
Somali		1.64%		1.51%	Mohammed, 2009
Oromia		1.9%		4.8%	Haileleul, 2012
SNNP		1.6%		3.2%	Mengistu, 2007
Tigray		1.4%		5.5%	Teshale *et al.*,2013
Yabello					Dabassa *et al*. 2013
Amhara					Shimeles, 2008

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The present study was conducted in Berbere district. Berbere is one of the districts in Bale Zone of Oromia Region, Ethiopia.Bale zone is found at 6°.44′ to 59°.99′ latitude and 40°14′ to 60°.00′ longitudeBerbere is bounded on the south by Mennaa, on the northwest by Goba, on the north by Sinana, on the northeast by Goro, on the East by Guradhamole and Somali regional state. The administrative center of the Woreda is Haro Dumal which is located at a 530 km south east of Addis Ababa, the capital city of Ethiopia. The annual average temperature of the district is 27^{o}C whereas the minimum and maximum temperature is 16^{o}C and 38^{o}C, respectively. The annual average rainfall is 730mm whereas the minimum and maximum rainfall is 600 and 855mm, respectively. The study was carried out in five randomly selected peasant association of Berbere District namely Sirima, Walta'i Darasa, Galma, Haro Dumal and Gabe. Livestock rearingplay an important role in the life the population in the districtespecially in the rural and lowland areas of the district, rearing and breeding is the main stay of the people. There are about 311,881 Bovines, 14,931 Sheep, 155,265 Goats, 46,011 Equines and 132,755 Chickens (BDAO, 2015).

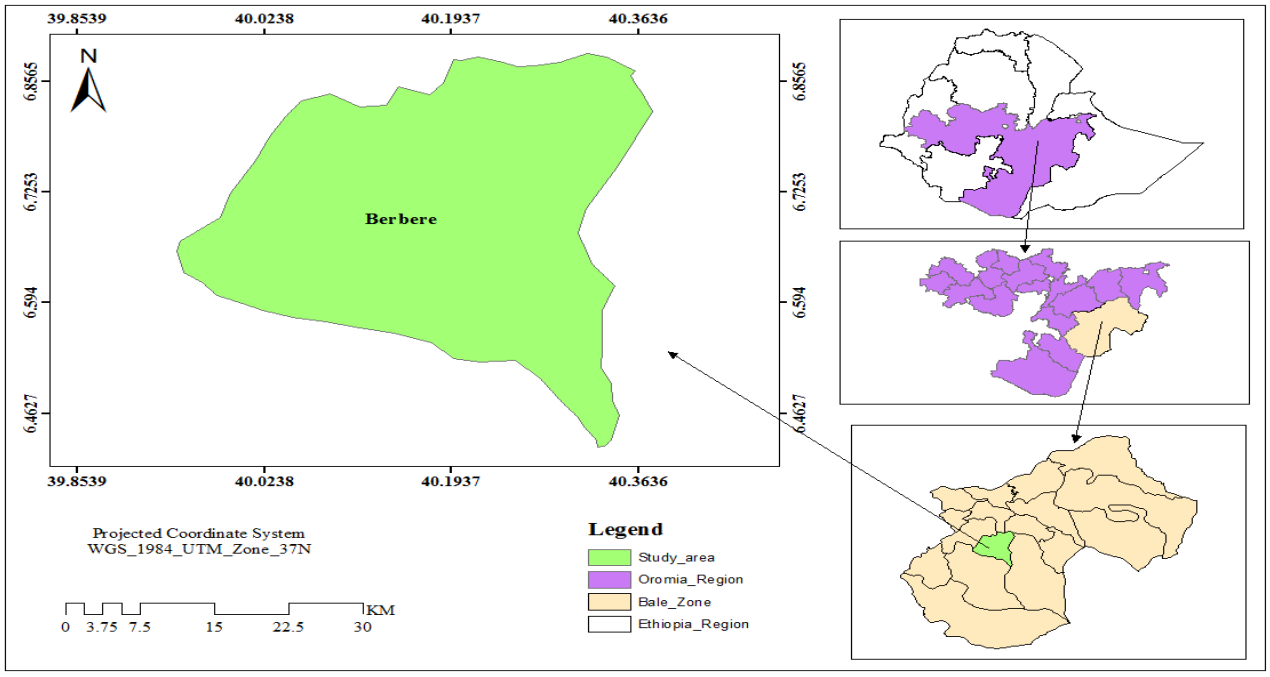

Figure 1: Map of Study District

3.2. Study Design

A cross-sectional study was conducted from November 2018 to November 2019 in small ruminantsunder extensive production system to estimate the overall prevalence and flock prevalence of brucellosis and structured questionnaire survey was conducted to collect data on factors believed to influence the spread and dissemination of brucellosis.

3.3. Study Animal

The study population is small ruminants in study area kept under extensive management system above 6 months. Study animal are those individual selected sheep and goat above 6 month from 5 Peasant Association. Those factors conceded as risk factor for brucellosis was collected before the blood sample was collected.These includepeasant association, species, sex, age, body condition, reproductive status, parity, history of abortion, stage of abortion and retained fetal membrane.For the questionnaire survey heads of household or any individual from the family member whose age is >18 were considered.

3.4. Sample Size Determination

The sample size for this study was determined as described by Thrusfield (2007) as follows.

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

Where:

n = required sample size

Pexp= expected prevalence.

d = desired absolute precision

There is no report on prevalence of brucellosis in small ruminant in Bale Zone. Therefore, the average expected prevalence was assumed to be 50% for the area within 95% confidence interval (CI) at 5% desired precision. According to above formula the minimum sample size was 384,

however total of 470 serum sampleswere collected to increase the precision (306 goats and 164 sheep) of both male and female small ruminants.A questionnaire survey was administered to 80 animal owners/attendant respondents whose animals were included in the study by usinglocal language (Afaan Oromo).

3.5. Sample and Data Collection

Berbere district was selected purposively due to absence research done to quantify and document the actual prevalence of brucellosis in small ruminant. Study animals were selected by a simple random sampling method. A structured questionnaire was distributed to 80 small ruminant owners/ attendant and to gather data about risk factor, socio-demographic, herd characteristic Data regarding knowledge, attitude and practice about brucellosis were also recorded. Samples were collected after informed and consent is made with selected participants (Appendix 5)

3.5.1. Blood Sample Collection

Sheep and goats selected for sample collection were individually restrained and approximately 5ml of blood was collected from the jugular vein following standard procedures by using plain vacutainer tubes. Identification of each animal was labeled on the corresponding vacutainer tube. The collected blood sample allowed to stand overnight in order to get the serum. Serum was collected from the vacutainer using a disposable plastic Pasteur pipette dispensed to cryovial tube and stored in the freezer at -20°C until used for serological testing.

3.5.2. Serological Tests

Rose Bengal plate test (RBPT)

The test procedures were done at the regional veterinary laboratory (RVL) in Asela. The protocol of RBPT as recommended by OIE is used as screening test for the presence of Brucella antibody in the sampled sera (Appendix 3). This test is generally considered to be as a sensitive test which reported as 97.9% sensitive for RBPT (Dohoo *et al*., 1986). The test is performed according to manufacturer's manual. Before performing test, antigen and sera are brought to room temperature. 30µl of serum was mixed with an equal volume of antigen suspension on a glass

plate. After four minutes of rocking, any visible agglutination was considered a positive result (Appendix 3).The screened positive sample and the sera were preserved at -20^{o}c until CFT test.

Complement fixation test (CFT)

All sera which tested positive to the RBPT were further tested using CFT for confirmation. The CFT was performed at the National Veterinary Institute, Bishoftu, Ethiopia. For confirmation using standard *B. abortus* antigen S99(Veterinary Laboratories Agency, New Haw, Addle stone, Surrey KT15 3NB, United Kingdom), preparation of the reagent is evaluated by titration and performed according to protocols recommended by World Organization for Animal Health (OIE, 2009) (Appendix 4). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above is classified as positive and lack of fixation/complete hemolysis is considered as negative. An animal was considered positive if the serum specimen tested positive on both RBPT and CFT whereas a herd was considered positive if at least a single serum specimen from an animal within the herd tested positive on both RBPT and CFT.

3.5.3. Questionnaire

A questionnaire was conducted to 80 small ruminant owner or attendants to collect information about potential risk factors associated with brucellosis in individual and herd sero-positivity and to gather data on knowledge, attitude and practices of pastoralists towards brucellosis. The following data were collected on individual animal and herd attributes: peasant association, species, sex, age, body condition, reproductive status, parity, history of abortion, stage of abortion and retained fetal membrane. Some of the questions to assess knowledge, attitude and practices towards Brucellosis included what is Brucellosis, causes,transmission, symptoms and signsin both humans and animal attitude of community towards handling aborted fetus, retained fetal membranes and drinking raw milk and practices such like assisting animals during parturition or during abortion and methods of disposal of aborted fetuses and placenta knowledge about zoonotic importance of brucellosis.

3.6. Data management and statistical analysis

The data were entered into a computer on a Microsoft Excel spreadsheet and Statistical analysis was done using SPSS 20 window version. All analyses were based on the CFT serological test results. Two epidemiological parameters were generated namely individual animal and herd level prevalence. Individual animal prevalence was computed by dividing the numberof test positives by the total number examined multiplied by 100.In the same wayherd level prevalence was also calculated by dividing the number of herds havingat least one brucella positive animal by the total number of examined herdsmultiplied by 100. In these study a herd, defined as the total number of small ruminant belonging to the same household. Univariable logistic regression was used to test the significance of the effect of different risk factors on sero-prevalence of brucellosis. All risk factors that had non-collinear effect and p-value ≤ 0.25 in the univariable logistic regression analysis were subjected to multivariable logistic regression analysis. The multiple effect between predictor variables and outcome variable was assessed by Odds ratio (OR) and 95% CI values in logistic regression model. In all the analyses, a 95% confidence interval and P-value ($P<0.05$) was set for significance of statistical associations between the dependent and independent variables.

4. RESULTS

4. 1. Seroprevalence of Brucellosis

In the present study, a total of 470 small ruminants (306 goats and 164 sheep) sera were collected out of those 17(3.61%) were positive in a RBPT and 14 (2.97%) of them were confirmed to be seropositive for brucellosis using CFT, an overall animal level seroprevalence of 2.97 % and 17.5 % herd level seroprevalence were recorded.

Table 2: Overall individual animal and herd level brucellosis seroprevalence based on RBPT and CFT

Test assay	Classification	No of Individual Animal	Prevalence % for Individual Animal	No of Herd	Prevalence % for Herd
RBPT	Negative	453		64	
	Positive	17	3.61	16	20
CFT	Negative	3		2	
	Positive	14	21.42	14	14.28
Total		470		80	

No = Number

At individual animal level the prevalence of small ruminant brucellosis was significantly higher in large herd size (p = 0.031) and not significantly different when compared animals from household introduction new animal and those who do not (P >0.05). However higher proportion of seropositivity was observed in those introduced new animal in the herd (4.12%) when compared to those not introduced (1.98%). Similarly, the study failed to detect a significant variation a seroprevalence between the different age group (Table 3). Sex was found to be insignificant factor of brucellosis infection in study area (P = .068) despite females having a slightly higher proportion of infection 4.12% (n=291) compared to males 1.11% (n=179). Among 291 females' small ruminant 63(21.64%) showed retained fetal membrane 89 (30. 85%) with history of abortion, among those 89 have history of abortion 47 (52.8%) were aborted <3-month fetus and 42 (47.1%) aborted >3-month fetus, based on reproductive status 28.26% were

pregnant, 20% lactating, 19.31 % dry and 32.06% were lamb/kid. Parity were a significant factor of brucellosis infection in study area, (P = .033) despite > 3 parity having a higher proportion of infection 41.37% (n=291) compared to null parity and 1-3 parity 24.8% (n=290).Small ruminant herd size, age, parity and history of retained fetal membrane were having a significance effect on seroposativity of small ruminant brucellosis in the study area and introduction of new animal, sex, reproduction status, abortion and gestation of abortion are those variables were not significantly associate with animal level seropositivity asit is indicated in Table 2 and 3.

4.1.1. Animal level risk factors analysis

In table 2, the results of the univariable risk factor analysis for brucellosis in small ruminant indicated that herd size (small, medium vs. large), introduction animal (introduced vs. not introduced), age (young, adult vs. Old), sex (male vs. female), parity (null parity, 1 -3 parity vs. More than three parity), states of production (pregnant, lactating, dry vs. lamb/kid) abortion history (absent vs. present) a stage gestation while abortion (<3 month vs.> 3 month) and history of retained fetal membrane (present vs. absent) were significantly associated with seropositivity at the animal level ($p < 0.05$) (Table 2). In addition, the animal level factor i.e.peasant association,species and body condition score was not significant at 5%. All variable which have p values ≤0.25 in (Tables 2) were subjected to the multivariable logistic regression analysis.

Table 3 : Influence of common risk factors on sero-prevalence of small ruminant brucellosis at individual animal level in study areas (Univariable regression analysis)

Factors	**Category**	**No testedanimal**	**CFT positive (%)**	**p-value**
Peasant Association	Haro Dumal	85	2(2.35)	
	Gabe	85	3(3.52)	
	Galma	100	3(3)	
	Walta'I Darasa	100	3(3)	
	Sirima	100	3(3)	0.312
Herd Size	Small	78	-	
	Medium	203	4(1.97)	
	large Herd Size	189	10(5.29)	0.037
Introduction of New	No	252	5(1.98)	
	Yes	218	9(4.12)	0.173
Age	Young	153	-	
	Adult	219	4(1.82)	
	Old	98	10(10.2)	0.001
Sex	Male	179	2(1.11)	
	Female	291	12(4.12)	0.063
Species	Ovine	164	4(2.43)	
	Carnie	306	10(3.26)	0.614
Body Condition	Good	156	7(4.48)	
	Moderate	211	6(2.54)	
	Thin	103	1(0.97)	0.262
Parity	Null parity	98	-	
	1-3 parity	72	1(1.38)	
	>3 parity	121	11(9.09)	.003
Reproduction status	Heifer	93	-	
	Lactating	84	5(5.95)	
	Pregnant	114	7(6.14)	0.121
Abortion	No	201	3(1.49)	
	Yes	89	9(10.11)	0.006
GS abortion	< 3 month	48	4(8.33)	
	>3 month	41	5(12.19)	0.003
history of RP	No	227	2(0.88)	
	Yes	64	10(15.62)	0.001

GS = Gestation No = number RP = Retention of Placenta.

In Table 3, the results of Multivariable logistic regression analysis showing important risk factors for individual animal Brucella seropositivity recorded. Small ruminant in herd size, were large herd size (5.29%) revealed a statistically significant variation($p<0.05$) with the odds of seropositivity being at least 3.8 times more likely to be infected with Brucella organisms than shoat have small herd size.Accordingly,the odds of brucellosis seropositivity were found to be 8.3 times higher among older shoat compared to those of the younger one.Correspondingly, parity and history of retuned fetal membrane status in females were to be significantly associated with seropositivity. Brucellosis was significantly ($p=.033$) higher in small ruminant with more than three parities with 8.4 times more likely to be seropositive than animals with null parity. There was a significantly high sero-prevalence (P= 002) of small ruminant brucellosis in those have a history of retuned fetal membrane when compared to small ruminant not have history of retuned fetal membrane. Accordingly,the odds of brucellosis seropositivity were found more than 12.8times higher among those have a history of retuned fetal membrane from those not have history of retuned fetal membrane. The rest risk factor showed no statistically significant associations regardless of the seropositivity recorded (Table 3).

Table 4 : Potential risk factors of brucellosis at animal level based on multivariable logistic regression

Factors	Category	CI 95%	OR	P-value
Herd Size	Small			
	Medium	0.13 - 39.3	2.28	
	large	1.287 - 11.401	3.830	0.016
Introduction	No			
	Yes	0.427 - 4.617	1.404	0.577
Age	Young			
	Adult	0.73- 40.78	5.47	
	Old	2.786 - 25.170	8.374	0.000
Sex	Male			
	Female	0. 900 - 20.149	4.258	0.068
Parity	Null parity			
	1-3 parity	0.032 - 43.1	6.122	
	>3 parity	1.187 - 60.880	8.499	0.033
Reproduction status	Lamb/kids			
	Lactation	0.338 - 26.4	0.562	
	Pregnant	0.301-1.424	6.55	0.285
Abortion	No			
	Yes	.018 - 13.033	0. 490	0.670
GS abortion	<3 month			
	>3 month	0.984-4.541	2.113	0.055
history of RP	No			
	Yes	2.575-64.585	12.896	0.002

No = Number OR = Odds Ratio, CI = Confidence Interval

4.1.2. Herd level risk factors analysis

Out of 80 herds studied, 14 (17.5%) were positive using CFT. The herd level univariable regression analysis revealed that herd size, abortion in heard and placenta retention in herd were found to be strongly associated with herd seropositivity to Brucella (p-value ≤ 0.25).The herd level Multivariables logistic regression analysis revealed that herd size, history of abortion and retention fetal membrane in herd was found to be strongly associated with herd seropositivity to Brucella (p-value < 0.05) in (Table 5).

Table 5: Potential risk factors of brucellosis seropositivity in herd level based on univariable logistic regression

Factors	**Category**	**Number of Herd**	**CFT (%)**	**p-value**
Herd Size	Small	35	-	
	Medium	23	4(17.4)	
	Large	22	10(45.45)	0.000
Abortion in Heard	No	50	3(6)	
	Yes	30	11(36.6)	0.000
Placenta Retention	No	60	6(10)	
	Yes	20	8(40)	0.002

In Table 5, the results of Multivariable logistic regression analysis showing important risk factors for Brucella seropositivity of herds. Therefore, herd size, abortion and retained fetal membrane was fitted for multivariable logistic regression model and all of them namely: herd size, abortion and retained fetal membrane were significantly associated with herd level Brucella seropositivity ($p<0.05$) Multivariable logistic regression analysis depicts that large herd size were more than 11 times more likely to become Brucella positive compared to that small herd size.

Table 6: Potential risk factors of brucellosis at herd level seropositivity based on multivariable logistic regression

Factors	Category	CI 95%	OR	P-value
Herd Size	Small			
	Medium	1.985 – 23.102	5.102	
	Large	2.582 - 47.023	11.018	.001
Abortion	No			
	Yes	.017 - 0.627	.102	.014
Placenta Retention	No			
	Yes	.021 - 0.759	.127	.024

OR = Odds Ratio, CI = Confidence Interval

4.2. QuestionnaireSurvey

4.2.1. Socio-demographic characteristics of the respondents

The socio-demographic characteristics of the respondents are presented in Table 7. The age of most of respondents was between 41-50 years old. Majority of the respondents in the study areas were Male (68.8%). Significant number of the community are Illiterate (41.3%) and 6.3% of them are college Graduate.

Table 7: Socio-demographic composition of study population (n=80)

Parameter	Category	Number of respondents	Percentage
Age (years)	18-30 Years	7	7.75
	31-40 Years	24	30.00
	41-50 Years	32	40.00
	Above 51 Years	17	21.25
Sex	F	25	3125
	M	55	68.75
Education level	Illiterate	33	41. 25
	Primary	25	31. 25
	Secondary	17	21. 25
	College Graduate	5	6. 25

4.2.2. Knowledge, Attitude and practice of community about brucellosis

Knowledge, attitude and practice of community about brucellosis of the studied population are presented in Table 7. Livestock reared in the area was camel, cattle, goat, sheep, chicken, and donkey. All of studied households were rearing small ruminants. 46.2%, 27.5% and 26.3% of them holds small (1-29), medium (30-50) and large (> 51) herd size respectively. Livestock are retained inherited generation to generation; however, herd size increases naturally and through selling of old animals and the practice of buying younger ones was observed in 48.8% of the households. Thirty-seven point five (37.5%) of heard have abortion history and 94.4% of study population were not support during abortion. Majority of community (82.5%) not using protective glove when assisting of animal during calving, working with abortion animaland retention placenta. 77.1% of community in study area is never know prevention and control method of brucellosis in animal and human. The three main practices for management of aborted material and fetus in the study area were giving to dogs, dispose it in the ground and burying in 39.6%, 33.3% and 27% of the cases respectively. Furthermore 55% of the respondents explained that they were in contacts with fetal membrane and/or fetal fluids in one way another. Only 33.8% and 16.3% of them was wash their hands after contact with animal and animal products respectively. 76.3% of respondent explained they consume raw milk and milk by product. 56.3% of the respondents participated in this study had never heard of a disease known as brucellosis (Table 7).

Table 8: Knowledge, attitude and practice of community about brucellosis (n=80).

Parameter	Category	Number of respondents	Percentage (%)
Rearing of sheep and goat	Yes	80	100
	No	0	
Herd Size	Small	35	43.75
	Medium	23	28.75
	Large	22	27.5
Introduction of new animal	Yes	39	48.80
	No	41	51.20
Abortion History of herd	Yes	30	37.50
	No	50	62. 50
Assisting during abortion(30)	Yes	13	43.33
	No	17	56.6
Who is assist (13)	Veterinary professionals	3	23.07
	Traditional healers	6	46.15
	By owner	4	30.76
Do you use gloves while assisting (13)	Yes	6	46.15
	No	7	53.84
Have a contacts with animal product	Yes	80	100.00
	No	-	-
Hands wash after contact with animal	Yes	27	33.80
	No	53	66.30
Hands wash after contact animal products.	Yes	13	16.30
	No	67	83.80
Do you consume raw milk and/or milk by products	Yes	61	76.30
	No	19	23.80
Have you heard of brucellosis	Yes	35	43.80
	No	45	56.30
Which animals affected by Brucellosis(35)	Shoat	6	17.14
	Wild animal	10	28.50
	Human	3	8.57
	I don't know	16	45.70

5. DISCUSSION

The present study revealed the overall prevalence of small ruminant brucellosis is 2.97% in the Berbere district of Bale Zone South East Ethiopia. The prevalence in this study was closely in agreement with the findings of 2.7% (Nigatu *et al.,* 2014) in Selected Export Abattoirs Addis Ababa. 2.25 % (Bezabih and Bulto 2015) in Werer Agricultural Research Center Afar, 3.5% (Teklue *et al.,* 2013), Southern Zone of Tigray. (3.7%) (Melese 2016) in Arba Minch zuria and Mirab Abaya districts of Gamo Gofa Southern Ethiopia. It is lower than the previous reports of seroprevalences of small ruminant brucellosis reported elsewhere in Ethiopia including 12.35% reported in Afar region (Anteneh *et al.*,2014), 9.6% in Yabello pastoral Area (Yohannes *et al.,* 2013) and 9.11% in Dire Dawa (Negash *et al.,* 2012).

However, the prevalence obtained in this study is higher than the prevalence of (Teshale *et al.,* 2006) also reported a seroprepvalence of 1.7% in Goat and 1.6% reported from sheep in Somali Pastoral Area Other studies revealed seroprevalence of 1.3% in goats and 1.5% in sheep (Teklay and Kasali, 1990) in central highlands of Ethiopia. These differences could due to variation in sensitivity and specificity of the various tests, agro-ecological location and amount of sampled study population, management, production systems and husbandry condition in the study areas Those conditions could facilitate the rate of transmission of the disease (Radostits *et al.,* 2000). In the present study herd size had significant effect in small ruminant seropositivity The chance of being seropositive was approximately more than three times higher in large flock than small and medium (3.8, CI: 1.287 - 11.401) was agree with the study report (2.7, CI: 1.4, 5.1) by (Asmare *et al.,* 2013) and (3.45, CI: 1.12, 10.27) by (Melese, 2016).This difference could be due to poor flock management.

Age is supposed to have association with occurrence of brucellosis, because sexual maturity is very important for the rapid multiplication of Brucella organism (Mohammed, 2009). In this finding old age (above three years) category were eight times more likely to be seropositive than young animals (less than one year of age) (OR=8.374; 95% CI: 2.786 - 25.170) and in agreement with report from Afar (Ashenafi *et al.,* 2007), Borana (Megersa *et al.,* 2011), South omo (Ashagrie *et al.,* 2011), Jigjiga (Mihretab *et al.,* 2011) South region and (Asmare *et al.,* 2013) Oromia region.

This increased susceptibility with increased sexual maturity is due to the influence of sex hormones and erythritol on the pathogenesis of Brucellosis (Radostits *et al.,* 2000). Similarly, multivariable logistic regression revealed the risk of seropositivity was more than eight times higher in (>3) parity compared to (1-2) and null parity group. Higher parity was also significantly associated with the disease which agrees with the finding of (Ashagrie *et al.* 2011; Asmare *et al.,* 2013). In this study, statistically high significance difference (P=0.000) was recorded with high sero-prevalence of brucellosis in small ruminants having history of retained fetal membrane than those without these problem (Radostits *et al.,* 2000; Swell and Brocklesby, 2002).

In the present study there is not significant association between male and female however the smaller number reactors was recorded in male than female. Some studies reported that serological response of male animals is limited and thus infected animals are usually observed to be non-reactors or show low antibody titer (FAO/WHO, 1989). Furthermore, male animals are known to be less susceptible to Brucella infection due to the less amount of carbon 4-sugar erythritol (Hirsh and Zee, 1999). In addition to that in the present study the sample size for male is more than female. History abortion and gestation of abortion was also no significant at individual animal level in the present study. This finding was disagreeing with finding of (Muluken Tekle 2016).

This result duo to difference in sample number of collected from that animal has history abortion and gestation of abortion number of sampled from small ruminant those have history of abortion was (89) and (201) from those not have history of abortion. However high seroprevalence were recorded in those animal have history abortion than those not have abortion history and more than two-time high prevalence were recorded in those have history of abortion in late stage of gestation than in early stage. This could be explained by the presence of higher concentration erythritol (2R, 3S) - butane- 1, 2, 3, 4, tetraol, a low calorie sugar alcohol produced naturally by the developing fetus may favors multiplication of Brucella where it causes degeneration and necrosis of the cotyledons leading to abortion from about the last months of gestation (Smith *et al.,* 1972; Coetzer and Tustin, 2004; Radostits *et al.,* 2007).

There was no significantly association observed in seropositivity in small ruminant body condition score. Nutrition plays great role in immunity against various infectious diseases. Underfed animals are expected to have a decreased immunity that is manifested by poor body condition (Faye and Bengoumi, 2006; Radostits *et al.,* 2007).

The overall herd level seroprevalence of small ruminant brucellosis was 17.5% which is comparable to herd level seroprevalence report of 5.8% (Yohannes *et al.,* 2017), 4.9% (Adugna *et al.,* 2013) and 7.3% (Tsegaye *et al.,* 2016) under extensive management systems. Nevertheless, higher herd level seroprevalences have been reported in other parts of Ethiopia in herds under extensive production systems (Berhe *et al.*,2007; Kebede *et al.,* 2008; Tolosa *et al.*, 2008; Dinka & Chala 2009; Jergefa *et al.,* 2009; Asmare *et al.*,2010; Ibrahim *et al.,* 2010; Asgedom *et al.,* 2016).

Small ruminants with a history of abortion were significantly affects herd seropositivity. The herd seroprevalence of brucellosis was higher in herds that had a history of abortion compared with no history of abortion. This could be explained by the fact that abortion is typical outcomes of brucellosis. The present study showed that participants recruited in this study had poor information of brucellosis. In study area brucellosis is known through "Gatachisa" in Afan Oromo which means, a disease destructing pregnancies or cause abortions. The finding that the most of the respondents had never heard of the disease brucellosis similar to studies in Kenya and Tajisktan (Kang'ethe *et al.*,2008; Lindahl *et al.,* 2015) but in contrast to studies carried out in Egypt and Jordan which showed a high awareness of the disease (Holt *et al.,* 2011; Musallam, *et al.*,2015).

The authors of those studies explained this high awareness by an endemic situation of brucellosis in the study area. The low awareness in this study could therefore in part be explained by a lower herd seroprevalence compared to Egypt and Jordan. Of the participants who had heard of the disease, knowledge about the cause, transmission routes controlling and prevention was still poor, among participants heard brucellosis about half was not knew even if which animals affected by brucellosis. Rearing of Small ruminant is common in study area even all of my respondent were rear Small ruminant. Livestock are inherited generation to generation; however, herd size increases naturally and through selling of old animals and buying younger one.Direct contact with animals and their secretions are miss practice on the study area.A community

observed in the current study was assisting animals during normal delivery or abortions they touch the animal with bare hands and there after they wash their hands with water and soap. (Lim *et al.* 2005) reported a similar case, where touching calves and/or placenta of infected animal was a risk factor for brucellosis transmission (Mishal *et al.,* 1999).

Regarding zoonotic disease risks, majority of people were not aware that humans could become infected with brucellosis from animalsthose heard brucellosis two third of them have no information (knowledge) about prevention and control methods of brucellosis in animals and humans. The majority was aware of the risks through raw milk however 76% of them were consuming raw milk. Unsurprisingly, this study found that all farmers were engaged in at least one risky practice conducive to transmission ofBrucella to other animals and humans. Knowledge about the disease and preventive herd management practices have previously been identified as the most important factors needed for minimizing the disease risk in animals (Díez and Coelho, 2013). Infected female animals excrete high concentrations of organism in their milk, placental membranes and aborted fetus (Radostits *et al.*, 2006).

Most respondents did not wash their hands with soap after dealing with aborted material, but only one third of them reported. The practice of study cleaning the area with just a brush leaves a very high risk of contamination and bacteria could easily survive in the environment leading to transmission to other animals or humans. Brucella in aqueous suspensions are readily killed by most disinfectants (The Center for Food Security and Public Health, 2009), so use of disinfectants and protective gloves should be considered as part of a future control program by encourage farmers to use commonly. Only 27% farmers in this study reported disposing of placental membranes by burying, which is one of the most effective methods of reducing disease risks and with most reporting to discard them into the open environment, outside the boundaries of their home or even feed them directly to dogs. The pathogen has been recovered from fetuses that have remained in a cool environment for over 2 months; this also could present a transmission risk to both animals and humans in the area (Kahn and Line, 2010).

Similar results were found in Jordan and Pakistan, but in contrast, a study in Tajikistan found 94% or respondents would bury the placenta and aborted materials (Lindahl *et al.*, 2015; Musallam*et al.*,2015; Arif *et al.*, 2017). It is interesting to note that often the placenta and

aborted fetus are not disposed of in the same way; among farmers who commonly bury the placenta, many would still discard aborted material either to dogs or into the open environment rather than bury. This is perhaps because of the larger size of fetuses making them more difficult to bury and suggests that those who bury the placenta may not be doing it due to an awareness of disease transmission risks but rather for other reasons such as practicality. Direct contact with placental membranes and aborted fetuses is a major route of human infection (Corbel, 2006). This lack of knowledge could explain the fact that the majority did not use protective gloves when assisting with kidding/lambing when caring withaborted animal or aborted materials. This could also in part be due to lack of access to protective gloves, which would have to be bought at the farmer's expense. Similar results have been reported from Tajikistan, Egypt and Jordan, suggesting that the use of gloves is not common practice in many lower income countries (Holt *et al.,* 2011; Lindahl *et al.,* 2015; Musallam*et al.,* 2015).

6. CONCLUSION AND RECOMMENDATIONS

This study revealed that small ruminant brucellosis was found to be 2.97% and 17.5% at animal and herd level respectively. This show small ruminant brucellosis was prevalent in Berbere District of Bale Zone of Oromia Region, South East Ethiopia. Thus, the herd size, age, parity status and history of retained fetal membranes of the animal are found to be significantly associated with seropositivity at animal level, herd size, abortion and retention placenta was also found to be significantly associated with herd level prevalence in study area. The low awareness of livestock owners on zoonotic importance of brucellosis and habit of consumption of raw milk, assisting parturition and handling of aborted materials are factors contributing for human brucellosis. This emphasizes impact of brucellosis in animals need to control and prevent brucellosis in the study areas.

Based on the above-mentioned conclusions, the following recommendations are forwarded to minimize further spread of the disease in both animal and human populations

- ⇒ The government should be preparing a strategy to regulate the control mechanism of brucellosis in small ruminants at national level.
- ⇒ Interdisciplinary collaboration and joint efforts among veterinary and public health professionals should be encouraged to prevent and control this disease.
- ⇒ The awareness should have been creating for pastoralists, farmer and other stakeholders about transmission, economic and public health importance of Brucellosis in the study area.
- ⇒ Further research on the isolation and molecular characterization of circulating Brucella species in livestock (small ruminants, cattle, camel and dog) and human in study area.

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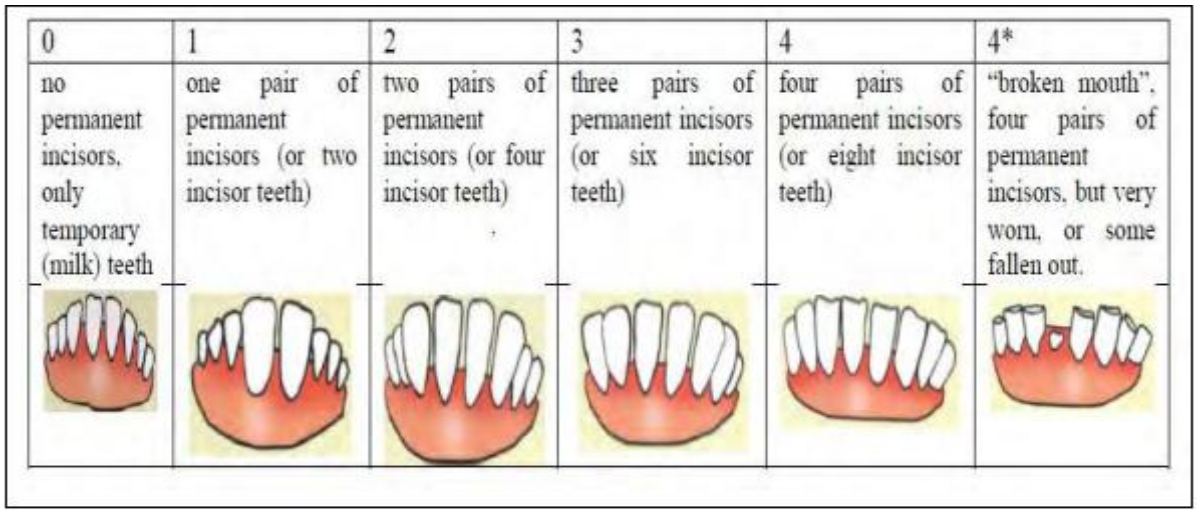
8. APPENDIXS

Appendix 1:Age and Dentition

Number of pairs of permanent incisors	Sheep	Goat
0	Less than one year	Less than one year
1	1 to 1.5 years	1 to 2 years
2	1.5 to 2 years	2 to 3 years
3	2.5 to 3 years	3 to 4 years
4	More than 3 years	More than 3 years
Broken mouth (teeth missing or worn down)	Aged	Aged

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

Appendix 2: Age determination with figure



Source: AU-IBAR-STSD and VS

Appendix 3: Rose Bengal Plate Test Procedure (RBPT) reagents material and equipment and procedure.

Rose Bengal Plate Test Procedure

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

1. Serum was mixed with antigen 1:3 (25 µl Ag and 75 µl serum) volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter.

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

3. Rock plate by hand for 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 recorded as positive.

Appendix 4: Complement Fixation Test Procedure

1. Test sera and appropriate working standards are diluted with an equal volume of veronal buffered saline in small tubes and incubated at 58°C for 50 minutes in order to inactivate the native complement.
2. Using standard 96-well U-bottom microtitre plates, 25 µl volumes of diluted test serum are placed in the wells of the first and second rows, and 25 µl volumes of veronal buffered saline are added to all wells except those of the first row.
3. Serial doubling dilutions are then made by transferring 25 µl volumes of serum from the second row onwards continuing for at least four dilutions.
4. Repeat steps ii and iii above for each serum to act as ant complementary serum controls (see below).
5. Volumes (25 µl) of complement at 1.25 MHD are added to each well and 25 µl of antigen, diluted to working strength, are added to all wells excluding those of the anti-complementary controls. These latter wells receive 25 µl of veronal buffered saline instead.
6. Control wells containing: diluent only, negative serum + complement + diluent, antigen + complement + diluent, and complement + diluent, are set up to contain 75 µl total volume in each case.
7. The plates are incubated at 37°C for 30 minutes with agitation at least for the initial 10 minutes, or at 4°C for 14- 18 hours.
8. Volumes (25 µl) of sensitized SRBC suspension are added to each well, and the plates are re incubated at 37°C for 30 minutes with agitation at least for the first 10 minutes.
9. The results are read after the plates have been left to stand at 4°C for up to 1 hour to allow unlysed cells to settle.

Interpretation

Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above will be classified as positive and lack of fixation/complete hemolysis will be considered as negative.

Appendix 5: Questionnaires survey use for assessment of risk factor of brucellosis in small ruminant and KAP

Please Mark your answer with circling in a given later.

1. Questionnaire number_____; Date____/___/2019; Zone _____; PAs _____;
2. Identification

A) Name: _____

C) Sex M _____ F_____

D) How you old are you _____

E. Occupation_____

3. Educational level A) Illiterate ____ B) Primary _____C) Secondary____

D) Colleges graduate_____ E) others _____

4. Do you rear or keep small ruminant? A) Yes _____ B) No_____

5. Have you had any contacts with small ruminant? A) Yes _____ B No_____

6. How much sheep and goat you have?

7. Have you ever had introduced new small ruminant to your herd? A) Yes B) No

8. Have you had encountered with any abortion in this heard?

If your answer is NO, please go to question number 13. A) Yes B) No

9. If yes, at what months of pregnancy? A) 1–3 month B) 4–6 moth

10. Have you had any assistance during that abortion? A) Yes B) No

11. If yes, who assisted? A) Veterinary professionals B) Traditional healers

C) Yourself

12. How did you manage the aborted fetus?

A) Bury B) Give to dogs C) Dispose on the ground

13. Have you ever had a retained placenta problem in your sheep/goat? A) Yes B) No

14. If yes, who managed that situation? A) Veterinarians B) Traditional healers C) yourself

15. During delivery time do you assist your small ruminant to deliver? A) Yes B) No

If NO proceed to question 18.

16. If yes, who managed that situation? A) Veterinarians B) Traditional healers C) yourself

17. Do you use gloves while assisting birth? A) Yes B) No

18. Have you ever had any contacts with aborted fetuses? A) Yes _____ B) No_____

19. Have you ever had any contacts with fetal membrane and/or fetal fluids? A) Yes__ B) No___

20. Have you had any contacts with animal products? A) Yes _____ B) No_____

21. Do you wash your hands appropriately at any contact with animal? A) Yes ____B) No_____

22. Do you wash your hands appropriately at any contact with animal products? A) Yes___
B) No_____

23. In your small ruminant are you using the same milking equipment for all of them?

A) Yes B) No

24. When you milking the small ruminant, do you wash your hand appropriately before you go to the next animal? A) Yes B) No

25. Do you consume small ruminant raw milk and/or milk products? A) Yes_____ B) No____

26. Do you Eat Raw Meat? A) Yes _____ B) No_____

27. Have you had irregular fever, chronic back and joint pain? A) Yes ____ B) No ____

If your answer is no proceed to question number 30

28. If yes, how long or since when? A) 1-6 month B) 7-12 month C) 1-3 year D) >3 year

29. Did you access to health service providers and consult to solve your problem?

A) Yes ___B) No____

30. Did you use any traditional drugs to solve your health problem? A) Yes ____ B) No ____

31. Do you know a disease called brucellosis? A) Yes ____ B) No____

32. Which animals affected by Brucellosis? A) Cattle Only B) small ruminantOnly

C) Human Only D) ALL E) don't know

35. Which one of the following you expect as means of Brucellosis transmission from animal to animal? A) Contact with infected domestic and wild animals B) by inhalation of aerosol /coughing C) contaminated feed D) Coitus E) other mention _____

36. Which one of the following you expect as means of Brucellosis transmission from animal to human? A) Eating raw meat B) drinking raw milk C) by inhalation of aerosol during coughing D) sharing the same house with infected animal/human

37. Do you know prevention and control measures? A) Yes ____ B) No ____

38. If yes, mention how to prevent and control?

A) Avoid sharining male B) Proper hygiene C) Avoid with domestic and wild animals

THANK YOU!!!!

Appendix 6: Data recording format for blood sample

Peasant Association/ Town_____ village _____

Number	Farmer name	Herd Size	Introduction new animal	Blood Sample	Code	Age	Sex	Breed	BCS	Parity	RP status	History of maternal	Frequency of abortion	Gestational stage of abortion	History of RP, TS,	Lab. result (+/-)	
																RBP	CFT
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	

BCS - Body condition score (1. Good 2. Moderate 3. Thin); PR-Retained placenta (Yes/No); RP Reproduction status (1. Pregnant 2. Lactation 3. Lamb/kids); Introduction of new animals (Yes/No). Herd Size (1. small 2. medium 3. Large)