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SEROPREVALENCE OF BOVINE BRUCELLOSIS UNDER EXTENSIVE PRODUCTION SYSTEM IN WOLAITA ZONE, SOUTHERN ETHIOPIA

MVSc THESIS

BY

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BISHOFTU, ETHIOPIA

SEROPREVALENCE OF BOVINE BRUCELLOSIS UNDER EXTENSIVE PRODUCTION SYSTEM IN WOLAITA ZONE, SOUTHERN ETHIOPIA

A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Clinical Studies

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Yohannes H/Michael Jatana

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STATEMENT OF THE AUTHOR

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

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LIST OF ABBREVIATIONS

AMOS PCR	Abortus-Melitensis-Ovis-Suis Polymerase Chain Reaction
BSL3	Biosafety Level three
C-ELISA	Competitive Enzyme Linked Immunosorbent Assay
CFT	Complement Fixation Test
CSA	Central Statistical Agency
CVMA	College of Veterinary Medicine Agriculture
DNA	Deoxyribo Nucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FPA	Fluorescent Polarization Assay
I-ELISA	Indirect Enzyme Linked Immunosorbent Assay
IFPRI	International Food Policy Research Institute
MZN	Modified Ziehl–Neelsen
OIE	Office International des Epizooties
PAs	Peasant Associations
PCR	Polymerase Chain Reaction
RAPD-PCR	Random Amplified Polymorphic Polymerase chain reaction
RBPT	Rose Bengal Plate Test
RFM	Retained Fetal Membrane
RLPS	Rough Lipopolysaccharide
SNNP	Southern Nation Nationalities and People
SLPS	Smooth Lipopolysaccharide
SRBC	Sheep Red Blood Cells
WHO	World Health Organization

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ABSTRACT

A cross-sectional epidemiological study was carried out in Sodo Zuria and Humbo districts of Wolaita zone southern Ethiopia from November 2016 to April 2017 to determine the seroprevalence and potential risk factors for bovine brucellosis in cattle under extensive production systems. The study populations comprised both indigenous and cross breed cattle were kept with other species such as sheep and goats. Serum samples were collected from 462 extensively managed cattle at least one year of ageby using multistage sampling technique. All serum were screened for *Brucella* antibodies by the Rose Bengal Plate Test and reactor sera were further tested by the Complement Fixation Test. Moreover, information was gathered on individual animal and herd level risk factors using a structured questionnaire survey. The overall seroprevalence of brucellosis was 1.3% (95% CI: 0.5-3) and 5.8% (95% CI:2-12) at both animal and herd level respectively. The results of univariate logistic regression analysis revealed that seropositivity to brucellosis was significantly ($p < 0.05$) higher in herd size > 10compared to those herd size <6. The result also indicated that there was a statistically significant increase in seroprevalence of brucellosis in cow with history of abortion and retained placenta. Nevertheless, in the multivariable logistic regression analysis, herd size ($p = 0.02$, OR=13.7, CI:1.4 -29.7)and abortion ($p = 0.01$, OR=9.8, CI:1.5 - 64.4) were statistically significant risk factors for individual animal seroprevalence. A total of 80 owners and/or attendants of cattle were interviewed parallel to blood sample collection, using structured questionnaire. Themajority of the respondents (90%) have lowawarenessregarding brucellosis and its risk foctors. Thus in the area people usually consume raw animal products, as result there is increased risk of acquiring infection.Although the overall prevalence of brucellosis was low, it could serve as source of infection to different herds as there is free movement of animals between herds.Therefore, control measures such as culling of aborted animal, proper disposal of aborted fetus, pasteurization or boiling of milk before consumption should be carried out to reduce risk of infection and transmission of the disease in livestock and human in the study area.Furtherbiochemical and molecular investigations of brucellosis need to be conducted to identify the specific species prevailing in the study area to promote effectivecontrol strategies.

***Key words*:** Bovine brucellosis —Ethiopia — Risk factors—Seroprevalence —Wolaita

1. INTRODUCTION

Ethiopia maintains huge number of livestock population ranking first in Africa. A huge and diverse livestock species of Ethiopia is maintained under different agro-ecological zones, management, migration and animal health care system. Livestock represents a major national resource and form an integral part of the agricultural production system(IFPRI, 2006; Lobago *et al*., 2006).

Comparatively huge livestock resources of the country and the economic return gained from this subsector do not coincide. The main technical limitations on livestock development and that determine the biological efficiency of production in Ethiopia are inadequate feeding, poor animal health, low potential of the genotypes used for yield traits and the traditional low input livestock management practices (Shiferaw *etal*., 2003). Bovine brucellosis is one of theselimiting factor and has been reported from several parts of the country (Bekele *et al*., 2000; Tolosa *et al*., 2008; Kebede *et al*., 2008; Asmare *et al*.,2010; Megersa *etal*., 2011; Adugna *et al*.,2013, Alemu*et al*., 2014, Bashitu*et al*., 2015; Asegdom *et al*., 2016).

Brucellosis is an infectious contagious bacterial disease usually caused by *Brucella abortus* in cattle,*B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs. It is slow-growing, Gram negative, small cocobacilli and intracellular bacteria that is capable to survive and multiply within epithelial cells, placental trophoblasts, dendritic cells and macrophages (Gorvel, 2008).

The disease is an important zoonosis that exists worldwide and is more or less endemic in most African countries (John *et al*., 2002). It causes significant reproductive losses in animals. Abortions, placentitis, stillbirth and birth of weak offspring in female and epididymitis and orchitis in male are the most common consequences (OIE, 2009). Bovine brucellosis is an infectious and contagious disease known for its impact on reproductive performance of cattle in Africa (McDermott and Arimi, 2002). The disease is primarily caused by*Brucella abortus* and occasionally by *Brucella melitensis* where cattleare kept together with infected sheep or goats (OIE, 2009). Bovine brucellosis has been eradicated in most developed countries that have implemented a tight eradication program (Makita *et al*., 2008). Yet, it is prevalent in the Mediterranean basin, Middle East, Western and Central Asia, Latin America, Africa and India.

The diseasehas a considerable impact on animal and human health, as well as wide socio economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products (Roth *et al*., 2003).It poses a barrier to trade of animals and animal products, an impediment to free animal movement. The economic and public health impact of brucellosis remains of concern in developing countries (Roth *et al*., 2003).

The epidemiology of brucellosis in livestock and cost-effective prevention measures are not well understood and available data are limited particularly in sub-Saharan countries (McDermott and Arimi, 2002). Hence, brucellosis remains widespread in livestock population and presents enormous economic and public health problems. It also causes losses due to abortion or breeding failure in the affected animal population, diminished milk production and causing reduced work capacity through sickness of the affected human (FAO, 2003).

Brucellosis is endemic in Ethiopia since 1970. Since then, few fragmented studies have demonstrated thepresence of antibodies against Brucella in animals and humans in different parts of the country. The prevalenceof brucellosis has been found to range from 0.2% to 38%in cattle (Bekele *et al*., 2010; Ibrahim *et al*., 2011).

Though various prevalence studies of brucellosis were carried out in different agro-ecological zones of country, there is limited information on the status of bovine brucellosis in Wolaita zone of SNNP region.

Theobjective of the current study is:
General objective

- To determine the seroprevalence of bovine brucellosis and its associated risk factors in cattle under traditional extensive husbandry in Wolaita zone, Southern Ethiopia.

Specific objectives

- To determine the seroprevalenceof brucellosis in cattle under extensive production system.
- To determine potential risk factors and theirassociation with*Brucella*seropositivity.

2. LITERATURE REVIEW

2.1. Etiology

Brucella abortus mainly infective for cattle, but occasionally other species of animals such as sheep, swine, dogs, horses and some wild animals may be infected. In horses, *B. abortus* together with *Actinomyces bovis* is commonly present in poll evil and fistulous withers (Radostits *et al*., 2000).*Brucella* species are facultative intracellular gram-negative, cocco-bacilli, non-spore forming and non-capsulated comprised of species based upon biochemical features and their correlation with preferred host speciesOIE (2000).

Currently ten species are recognized including the better known six classical species comprised of *B. abortus* (cattle, biovars 1-6, and 9), *B. melitensis* (goats, sheep, biovars 1-3), *B. suis* (pigs, reindeer and hares, biovars 1-5), *B. ovis* (sheep), *B. canis* (dogs) and *B. neotomae* (desert wood rats). More recently, new members to the genus include *B. ceti* and *B. pinnipedialis* (dolphins/porpoises and seals respectively), *B. microti* (voles) and *B. inopinata* (reservoir undetermined) (Godfroid *et al*., 2011).

2.2.Morphology

*Brucella*are Gram-negativecoccobacilli or short rods measuring from 0.6 to 1.5 μm long and from 0.5 to 0.7 μmwide, non-motile, non-spore forming, non-capsulated, non-flagellated, aerobic, facultativeintracellular bacteria capable of invading, survive and multiply within epithelial cells, placentaltrophoblasts, 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. The *Brucella* have no classic virulence genes encoding capsules, plasmids, pili or exotoxins and compared to other bacterial pathogen relatively little is known about the factors contributing to the persistence in the host and multiplication within phagocytic cells. Also, many aspects of interaction between *Brucella* and its host remain unclear (Seleem *et al*., 2008; Sriranganathan *et al.*, 2010).

The *Brucella*are not truly acid-fast, but are resistant to decolonization by weak acids and thusstain 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 anda pale honey color when plates are viewed in the daylight through a transparent medium. Whenviewed from above, colonies appear convex and pearly white. Later, colonies become larger andslightly 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 lipopoly-saccharide (RLPS) and protein antigens (Blasco *et al*.,1994).

2.3.Epidemiology

The epidemiology of cattle brucellosis is influenced by several factors including factors associated with disease transmission between herds, factors influencing the maintenance and spread of infection within herds (Crawford *et al*. 1990). Understanding the epidemiology of brucellosis is therefore, vital for strategizing evidence based disease control measures. However, such information is inadequate in sub-Saharan Africa. Consequently, appropriate preventive measures have not been undertaken in this part of the world (McDermott and Arimi 2002).

2.3.1. Worlddistribution

Brucellosis is a disease of worldwide distribution occurring in domestic as well as wild animals. It has been reported wherever animals are raised all over the world (Seifert, 1996). Although some of the industrialized countries including Australia, Canada, Cyprus, Denmark, Finland, The Netherlands, New Zealand, Norway, Sweden and the United Kingdom eradicted the disease,it is still a serious problem in developing countries (Warner, 2001; Ragan, 2002). The continues to be a major public and animal health problem in many regions of the world, particularly where livestock are a major source of food and income. There are many reasons why brucellosis remains endemic. These include expansion of livestock herds and flocks, with associated uncontrolled movements; lack of veterinary support services and vaccines and husbandry practices (McDermot and Arimi, 2002).

2.3.2. Distribution in Africa

In Africa, bovine brucellosis was first recorded in Zimbabwe (1906), Kenya (1914) and in Orange free state of South Africa in the year 1915 (Chukwu, 1985). However**,** still the

epidemiology of the disease in livestock and humans as well as appropriate preventive measures are not well understood and such information is inadequate particularly in sub Saharan Africa. The importance of brucellosis reflects its widespread distribution and its impacts on multiple animal species, including cattle, sheep, goats, pigs and humans. While the importance of brucellosis is widely assumed, the benefits of programs to control it, relative to their costs, need to be assessed. (Mc Dermot *et al*., 2002).Some countries in Africa where seroprevalence of brucellosis had been reported to be less than 10% were Benin 4.3%, Ethiopia 4.2%, and Ghana 6.6% (Kubuafor *et al*., 2000; Megersa *et al*., 2011).

Table 1: Distribution of bovine brucellosis in some African countries.

Country	Host	No. tested	Prevalence (%)	Tests used	References
Eretria	Cattle	15049	2.77	CFT	Scacchia *et al*., 2013
Zambia	Cattle	395	20.7	c-ELISA	Muma *et al*., 2013
Sudan	Cattle	250	2	ELISA	Senein and Abdelkadir, 2012
Kenya	Cattle	393	1	c-ELISA	Kang'ethe *et al*.,2007
Zimbabwe	Cattle	1291	5.5	c-ELISA	Matope *et al*., 2010
Somaliland	Cattle	153	1.96	RBPT	Ahmed, 2009
Nigeria	Cattle	220	5.45	RBPT	Bwala *et al*., 2015
Tanzania	Cattle	655	5.3	RBPT	Swai and Schoonman, 2010
Uganda	Cattle	423	5	c-ELISA	Makita *et al*., 2011
Gambia	Cattle	465	1.1	CFT	Unger *et al*., 2003
Senegal	Cattle	479	0.63	CFT	Unger *et al*., 2003
Ghana	Cattle	444	2.93	RBPT	Folitse, 2014
Cameroon	Cattle	840	9.64	i-ELISA	Shey –Njila, 2005

2.3.3. Status of bovine brucellosis in Ethiopia

Eventhough, several serological surveys have showed bovine brucellosis is an endemic and widespread disease in Ethiopia, most of the studies on cattle brucellosis have been carried out in central and northern Ethiopia and do not provide an adequate epidemiological picture of the disease in different agro-ecological zones and livestock production systems of the country (Dinka and Chala, 2009; Megersa *et al.*, 2011).

The evidences of brucellosis in Ethiopian cattle have been serologically demonstrated by different authors. Most of the studies suggested a low seroprevalence (below 5%) in cattle

under crop-livestock mixed farming (Berhe *et al*., 2007; Ibrahim *et al*., 2010; Adugna *et al*., 2013). The evidences of *Brucella* infections in Ethiopian cattle have been serologically evaluated in different parts of the country by different authors as indicated in (Table 2).

According to some reports, *Brucella* seroprevalence is higher in intensive farming system than within extensive cattle rearing systems. In Borena zone of Oromia region, the highest seroprevalence (50%) was documented using ELISA in Dididtuyura Ranch (Alem and Solomon, 2002). Tolosa *et al*. 2008 reported overall individual animal prevalence and herd prevalence of 0.77 and 2.9%, respectively in Jimma Zone. Reportsfrom North West, Tigray region (Haileselassie *et al*., 2010) and Southern Sidama zone (Asmare *et al*., 2010), recorded an overall prevalence of 1.2 and 1.66% following screening 848 and 1627 cattle from intensive and extensive system, respectively. Another study conducted on cattle brucellosis in traditional husbandry practice from 1623 cattle sera insouthern and eastern Ethiopia showed that 3.5% of the animals and 26.1% of the herds were tested positive (Megersa *et al*., 2011).

Table 2:Seroprevalence of bovine brucellosis in different parts of Ethiopia.

Breed	Location	No. tested	Prevalence (%)	Tests used	References
Local	South east	180	1.4	RBPT	Donde, 2013
Local	West	1152	1	CFT	Adugna *et al*., 2013
Local	North	1968	4.9	CFT	Haileselassie *et al*., 2010
Mixed	Assela	304	14.14	RBPT	Deselgn & Gangwar, 2011
Mixed	Central	1238	2.9	CFT	Jergefa *et al*., 2009
Cross	Ambo	169	0	CFT	Bashitu *et al*., 2015
Cross	Derebrhan	246	0.2	CFT	Bashitu *et al*., 2015
Local	South east	862	1.4	CFT	Gumi *et al*., 2013
Mixed	Southern	811	1.66	CFT	Asmare *et al*., 2007
Local	East Showa	1106	11.2	RBPT	Dinka and Chala, 2009
Local	Eastern	435	1.38	CFT	Degefu *et al*., 2011
Mixed	Wollega	406	1.97	CFT	Moti *et al*., 2012
Local	Arsi zone	370	0.05	CFT	Degefa *et al*., 2011
Mixed	Debrezeit	300	2	CFT	Alemu *et al*., 2014
Mixed	Alage	804	2.4	ELISA	Asgedom *et al*., 2016
Mixed	Asela	756	2.9	CFT	Tsegaye *et al*., 2016

2.3.4. Host range and Brucella diversity

Hosts for *Brucella abortus* include cattle, bison (*Bison* spp.), water buffalo (*Bubalus bubalus*), African buffalo (*Syncerus caffer*). Cattle can also become transiently infected by *B. suis* and more commonly by *B. melitensis* when they share pasture or facilities with infected pigs, goats and sheep. *B. melitensis* and *B. suis* can be transmitted by cow's milk and cause a serious public health threat. A feral pig population has recently been reported to maintain *B. abortus*. (Acha *et al*., 2003; Sriranganathan *et al.*, 2010).

The main etiologic agent of brucellosis in goats is *B. melitensis*. However, in certain countries like Brazil where there is no *B. melitensis*, goats can get infected with *B. abortus* (Lilenbaum *et al*., 2007). Camels can be infected by *B. abortus* and *B. melitensis* when they are pastured together with infected sheep,goats and cattle. Milk from infected camels represent a major source of infection that is underestimated in the Middle East (Musa *et al*., 2008). The main etiologic agent for dog brucellosis is *B. canis*, but sporadic cases of brucellosis in dogs caused by *B.abortus*, *B. suis* and *B. melitensis* have been reported (Acha and Szyfres, 2003).

2.3.5. Possible risk factors

The prevalence of brucellosis is influenced by a number of risk factors related to productionsystems, biology of the individual host and environmental factors. These include age, herd sizeand composition, hygienic status of the farm, rate of contact between infected and susceptibleanimals, farm biosecurity and climate (McDermott and Arimi, 2002; Radostits *et al*., 2007).

Susceptibility of cattle to *B. abortus* infection is influenced by the age, sex and reproductive status of the individual animal. Sexually mature pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex. Susceptibility increases as stage of gestation increases (Colibaliy *et al*., 2005).Thebacterialocalizing mainly in the reproductive tract especially in pregnant animals; there is also evidencethat mammary gland may be even more favored for localization than the reproductive tract(Anonymous, 2007).

Brucella abortus is a facultative intracellular organism capable of multiplication and survival within the host phagosome. The organisms are phagocytized by polymorpho nuclear leucocytes in which some survive and multiply. The organism is able survive with in

macrophages because; it has the ability to survive phagolysosome. The bacterium possesses an unconventional non-endotoxin lipopolysacharide which confers resistance to antimicrobial attacks and modulates the host immune response. These properties make lipopolysacharide an important virulence factor for *Brucella* survival and replication (Colibaliy *et al*., 2005).

Risk factors for human brucellosis include the handling of infected animals, ingestion of contaminated animal products such as unpasteurized milk and milk products (including cow, goats and camel milk), meat and improper handling of cultures of *Brucella* species in laboratories. Laboratory workers handling *Brucella* cultures are at high risk of acquiring brucellosis through accidents, aerosolizing and/or inadequate laboratory procedures. In addition to this, abattoir workers, farmers and veterinarians are at high risk of acquiring the infection (Chain *et al*., 2005). In the rural parts of Ethiopia, for instance, human life ishighly associated with livestock population in the different livestock production systems. In bothpastoral and mixed livestock production systems people live very closely with livestock having ahigh incidence of brucellosis and thus, are at higher risk of acquiring the infection (Gebretsadik *etal*., 2007).

2.3.6. Source of infection andmode of transmission

The transmission of *Brucella* is facilitated by coming of flocks and herds belonging to different owners and by purchasing animals from unscreened sources. The sharing of male breeding stock for artificial insemination also promotes transfer of infection between farms. Transhumance of seasonal grazing is a significant promoting factor in some areas as is the mingling of animals at markets or fairs. In cold climates, it can be the custom to house animals in close space and this also facilitates transmission of infection (Habtamu *et al*., 2015).

The most significant feature of bovine brucellosis epidemiology is the shedding of large numbers of organisms during the 10 days after abortion or calving of infected cows and the consequent contamination of the environment. The movement of infected cattle into a herd can result in transfer of the disease when cattle ingest the bacteria from aborted fetuses, placenta, and discharges from cows that have aborted or contaminated pasture or water (Park *et al*., 2005).In cattle and other *Bovidae*, *Brucella* is usually transmitted from animal to animal by contact following an abortion. Infected animals after abortion or full-term parturition could be infectious for the other healthy animals. *B. abortus* may also be present in the milk, urine,

semen, feces and hygroma fluids. Shedding in milk may be prolonged or lifelong, and can be intermittent. Many infected cattle can become chronic carriers and can shed lower numbers of organisms via milk and reproductive tract discharges, and also vertically transmit infection to subsequently born calves, and maintain disease transmission (McDermott and Arimi, 2002). Infection with *B. abortus* can also be transmitted by ingestion or through mucous membranes or through broken skin. Mammary gland is usually colonized during the course of an infection and may be infected by direct contact because of subsequent shedding of the organisms in the milk (CFSPH, 2009).

Pasture or animal barn may be contaminated and the organisms are probably most frequentlyacquired by ingestion and inhalationand skin contamination are other possibilities. The use of pooled colostrum for feeding newborn calves may also transmit infection. The disease also spreads by infected animals that shed *Brucella* through vaginal discharge, milk, semen from males, artificial insemination (Olsen & Tatum, 2010).*B. abortus* achieves its greatest concentration in the contents of the pregnant uterus, the fetus and the fetal membranes after birth (Radostits *et al*., 2000). The risk associated with exposure of susceptible animals to the disease following parturition or abortion of infected cattle depends on the number of organisms excreted, the survival of these organisms under the existing environmental condition and the probability of susceptible animals being exposed to enough organisms to establish infection.

Sexual transmission usually plays little role in the epidemiology of bovine brucellosis. However, artificial insemination can transmit the disease and semen must only be collected from animals known to be free of infection (Robinson *et al*., 2003).*B. abortus* can be spread by contaminated semen during artificial insemination when semen is deposited in the uterus but not in the mid cervix. In conditions of high humidity, low temperatures, and no sunlight, the *Brucella* organisms can survive in water, aborted fetuses, manure, wool, hay, equipment and clothes for several months. *Brucella* species can resist drying and survive in dust and soil if there is an organic material in the environment. Survival rate of *Brucella* organism is longer in low temperatures especially in deep freezing (CFSPH, 2009).

Brucellosis in human also known as “undulant fever”, “Mediterranean fever” or “Malta fever” is a zoonosis and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. It affects people of all age groups but those less than 14 ages are less susceptible and of both sexes (Corbel, 2006). Human brucellosis is caused

mainly by *B. abortus*, *B. melitensis* and *B. suis*, also the main causes of brucellosis in cattle, goats/sheep and pigs respectively (WHO, 2006; Makita *et al*., 2011). Wildlife animals are also equally affected and these may act as reservoirs to both domestic animals and humans (Godfroid *et al*., 2011).Human also acquire infection from a contaminated environment, occupational exposure usually resulting from direct contact with infected animals, and food-borne transmission. More potential significance is transmission through blood donation or tissue transplantation. Certain occupations are associated with a high risk of infection with brucellosis (WHO, 2006).

Brucellosis is not only a major zoonotic problem but is also linked with bioterrorism and belongs to category B (Anonymous, 2000). The severity of this disease, lack of vaccines suitable for use in man and frequent failure of clinical laboratories to correctly identify isolates led to the investigation of *Brucella*as an agent for bioterrorism.

2.4.Clinical Manifestation

Brucellosis could be suspected in any herd with history of abortion during the last stage of pregnancy (Poester *et al*., 2010). The major clinical sign in the first stage of the disease is abortion, but other signs due to localization of the organism may be observed. These signs include orchitis, epididymitis, hygroma, arthritis, metritis and subclinical mastitis among others (Radostits *et al*., 2007). However, numerous animals develop self-limiting infection or they may become asymptomatic latent carriers and potential execrators (WHO, 2003).

Females that are born into an infected area and get infected generally abort less than others. This explains the high level of abortions in newly infected herds and their relatively low frequency in herds where infection is enzootic. The udder is a very important predilection site for *Brucella* organisms. Infection in lactating, nonpregnant animal is likely to lead to colonization of the udder with excretion of *Brucella* organisms in the milk (Radostits *et al*., 2007). Retention of placenta and metritis are common sequels to abortion. Females usually abort only once, presumably due to acquired immunity. In general, abortion with retention of the placenta and the resultant metritis may cause prolonged calving interval and permanent infertility (Walker, 1999). Sexually immature may remain sub-clinically infected until maturity and pregnancy without showing any sign of the disease (AHA, 2005).

Infection in males may result in either temporary or permanent infertility, depending on the intensity of the lesions (Megid *et al*., 2010). Orchitis is occasionally manifested, that is often associated with a vesiculitis and epididymitis and is usually unilateral, but both testicles may be affected. Scattered foci of necrosis coalesce to produce total testicular necrosis (Foster and Ladds, 2007). Hygromas, usually involving leg joints, are common manifestations of brucellosis in some tropical countries and may be the only obvious indicator of infection (OIE, 2009).

Human brucellosis has a wide clinical spectrum, presenting various diagnostic difficulties because it mimics many other diseases for example malaria, typhoid, rheumatic fever, joint diseases and other conditions causing pyrexia (Kunda *et al*., 2007). The disease manifests with continued, intermittent or irregular fever (hence the name undulant fever), headache, weakness, profuse sweating, chills, arthralgia, depression, weight loss, hepatomegaly, and splenomegaly and generalized aching. Cases of arthritis, spondylitis, osteomyelitis, epididymitis, orchitis, and in severe cases neurobrucellosis, liver abscesses and endocarditis with infection of the aortic valves and other multiple valves with *Brucella* has been reported in human (Maríapía *et al*., 2007).

2.5.Pathogenesis

The major route of infection is through mucous membranes of the oropharynx and upper respiratory tract or conjunctiva (Tabak *et al*., 2008). Another route is through the mucous membrane of the male and female genital tract. On entering into the body of the host, the organism encounters the cellular defenses of the host but generally succeed in arriving via the lymph vessels at the nearest lymph node after escaping the cellular defenses (Kho and Splitter, 2003). The fate of the invading bacteria is mainly determined by cellular defenses of the host chiefly macrophage and T-lymphocytes though specific antibody also plays apart (Radostits *et al*., 2007).

In contrast to other pathogenic bacteria,*Brucella* lack classical virulence factors, such as exotoxins, cytolysins, capsules, fimbria, plasmids, lysogenic phages, drug resistant forms, antigenic variation,but possibility that they might have unique and subtle mechanisms to penetrate host cells, elude host defenses, alter intracellular trafficking to avoid degradation and killing in lysosomes and modulate the intracellular environment to allow long-term intracellular survival and replication (Moreno and Moriyon, 2002; Delrue *et al*., 2004).

Brucella uses a number of mechanisms for avoiding or suppressing bactericidal responses inside macrophages. The smooth lipopolysaccharides that cover the bacterium and proteins involved in signaling, gene regulation, and trans-membrane transportation are among the factors suspected to be involved in the virulence of *Brucella* (Lapaque *et al*., 2005).When the bacteria prevail over the host's defenses, a bacteremia is generally established. The bacteremia is always detected after 10 to 20 days and persists from 30 days to more than two months. If the animal is pregnant, bacteraemia often leads to the invasion of the uterus (Olsen& Tatum, 2010). At the same time, infection becomes established in various lymph nodes and organs, often in the udder and sometimes in the spleen (WHO, 2006).

The main lesions which appear in the male animals are orchitis and epididymitis, as well as inflammation of the joints and bursa. Abortion may also occur in the females presenting the typical yellowish sticky layers on the placenta. The consequences of brucellosis in small ruminants are infertility, high mortality rate in calves, mastitis and reduced milk production (Oyedipe *et al*., 1981; Radostits *et al*., 2007).

2.6.Diagnosis

The development of a definitive diagnostic test for brucellosis remains an elusive target. The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis. In the history of microbiology, very few diseases have more diagnostic tests than brucellosis. Diagnostic tests are applied for the 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).

2.6.1. Bacteriological method of diagnosis

Stained smears

Marin *et al*. (1996) reported that a presumptive bacteriological diagnosis of *Brucella* can be made by means of the microscopic examination of smears from vaginal swabs, placentas or aborted foetuses, stained with the Stamp modification of the Ziehl-Neelsen staining method. However, morphologically-related micro-orga-nisms, such as *Chlamydophila abortus, Chlamydia psittaci* and *Coxiella burnetti* can mislead the diagnosis because of their superficial similarity (Marin *et al*., 1996; Poiester *et al*., 2010). Accordingly, the isolation of

B. melitensis on appropriate culture media such as Farrell's selective media is recommended for an accurate diagnosis (Farrell, 1974). Vaginal swabs and milk samples are the best samples to use in isolating *B. melitensis* from sheep and goats (Marin *et al*., 1996).

Cultural isolation

Definitive diagnosis of brucellosis is based on culture, serologic techniques or both. Isolation of the organism is considered the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, which is relevant under an epidemiological point of view (Bricker, 2002; Al Dahouk, 2003). However, in spite of its high specificity, culture of *Brucella* spp. is challenging. *Brucella* spp. is a fastidious bacterium and requires rich media for primary cultures. Furthermore, its isolation requires a largenumber of viable bacteria in clinical samples, proper storage and quickdelivery to the diagnostic laboratory (Seleem *et al*., 2010;Hadush *etal*., 2013).

In addition, fetal organs such as the lungs, bronchial lymph nodes, spleen and liver, as well as fetal gastric contents, milk, vaginal secretions and semen are samples of choice for isolation (Poester *et al*., 2006;Lage *et al*., 2008). Milk samples should be a pool from all four mammary glands. Non- pasteurized dairy products can also be sampled for isolation (Lage *et al*., 2008;Poester *et al*., 2010).

Reliable samples for isolation purposes in necropsied animals include mammary, iliac, pharyngeal, parotids and cervical lymph nodes, and spleen. Samples must be immediately sent to the laboratory, preferentially frozen at -20°C, and they must be identified as suspect of *Brucella* spp. infection (Poester *et al*., 2010).

Contamination of samples is complicating factor for*Brucella* spp. isolation. Therefore, the use of nutrient-rich mediasupplemented with antibiotics is used to inhibit growth ofcontaminants that may prevent isolation of *Brucella* spp. (De Miguel *et al*., 2011).Another limiting factor for culturing *Brucella* spp. is therequirement for appropriate laboratory conditions and personneltraining so the procedure can be performed safely (Nielsen & Ewalt, 2004). *Brucella* spp. isclassified as a Biosafety level 3 organism, whose manipulation shouldbe performed in biosafety level-3 laboratories (Lage *et al*., 2008).

Usually, solid media such as dextrose agar, tryptose agar, and trypticase soy agar, are recommended for primary isolation of *Brucella*, but some species, i.e., *B. ovis* and *B.*

*canis*require addition of 5-10% of sterile bovine or equine serum to the culture media. In the case of blood or milk, biphasic media such as Castañeda's medium is recommended for improving sensitivity (Poester *et al*.,2010).

2.6.2. Serological diagnosis of brucellosis

Serological tests are relatively easy to perform and provide a practical advantage in detecting the prevalence of *Brucella* infection1. The tests are crucial for laboratory diagnosis of brucellosissince most of control and eradication programs rely on these methods. 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 (Abernethy *et al*., 2012; Adone and Pasquali, 2013). The serological tests are presumptive diagnosis for brucellosis in animals as well as human (OIE, 2012).

However Serological tests cannot differentiate between *Brucella*species and cannot identify which species has induced host antibodies. Inactivated whole bacteria or purified fractions (i.e.,lipopolysaccharide or membrane proteins) are used as antigens fordetecting antibodies generated by the host during the infection.Antibodies against smooth *Brucella* species (e.g., *B. abortus,B.melitensis*, and *B. suis*) cross react with antigen preparations from *B.abortus*, whereas antibodies against rough *Brucella* species (e.g., *B. ovis*and *B. canis*) cross react with antigen preparations from *B. ovis*(Nielsen, 2002).

Several serological tests are used today, but most commonly used serological tests are screening tests (e.g., buffered antigen plateagglutination-BPATor RBPT), monitoring or epidemiological surveillance tests(e.g., milk ring test), and complementary or confirmatory tests (complement fixation, ELISAs,). Selection of a given test should take into accountthe species affected as well as local regulations (Nielsen, 2002; Poester *et al*.,2010).

Rose bengal plate test

This test was developed by Rose and Roekpe (1957) for the diagnosis of bovine brucellosis to differentiate specific *Brucella* agglutinins from non-specific factors. It is a rapid, slide-type agglutination assay performed with a stained *B. abortus* suspension at pH of 3.6-3.7 and plain serum. Its simplicity made it an ideal screening test for small laboratories with limited resources. It does not differentiate between field and S19 vaccine strain reactions and low sensitivity particularly in chronic cases (Díaz *et al*., 2011). The overall sensitivity is 92.9%, so

the use of RBT should be considered carefully in endemic areas, particularly inindividuals exposed to brucellosis and those having history of *Brucella* infection (Ruiz-Mesa *et al*., 2005).

As sensitivity is high, false negative results are rarely encountered. To increase specificity, the test may be applied to a serial dilution (1:2 through 1:64) of the serum samples (Christopher *et al*., 2010). The present World Health Organization (WHO) guidelines recommend the confirmation of the RBT by other assays (Christopher *et al*., 2010; Araj,2010).

Complement fixation test

Due to its high accuracy, complement fixation is used asconfirmatory test for *B. abortus, B. melitensis,* and *B. ovis* infectionsand it is the reference test recommended by the OIE for internationaltransit of animals (Gall *et al*., 2001; OIE, 2009). In most cases, the CFT is used on RBPT positive sera, but like the RBPT.The test hasdisadvantages such as high cost, complexity for execution, andrequirement for special equipment and trained laboratory personnel. Sensitivity of complement fixation ranges from 77.1 to 100% and its specificity from 65 to 100% (Gall *et al*., 2001; Perrett *et al*., 2010). The reagents include *B. abortus* CFT antigen, complement, amboceptor (haemolysin), ovine erythrocytes and test serum with Veronal buffer as the diluents (WHO, 2006; IBM, 2013).

Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assay (ELISA) has become popularas a standard assay for the diagnosis of brucellosis serologically. Itmeasures IgG, IgA and IgM antibodies and this allows a betterinterpretation of the clinical situation. The diagnosis of brucellosis isbased on the detection of antibodies against the smooth LPS.Detection of IgG antibodies is more sensitive than detection of IgMantibodies for diagnosing cases of brucellosis but specificity iscomparable (Araj, 2010; Sathyanarayan *et al*.,2011; Agasthya *et al*., 2012).

Compared to the conventional agglutinationmethods, ELISA is more sensitive in acute and chronic cases ofbrucellosis and it offers a significant diagnostic advantage in thediagnosis of brucellosis in endemic areas. This test is an excellentmethod for screening large populations for *Brucella* antibodies and fordifferentiation between acute and chronic phases of the disease (Gall *et al*., 2003). Itis the test of choice for complicated, local or chronic cases particularlywhen other tests are negative while the case is under high clinicalsuspicion. It can

reveal total and individual specific immunoglobulins(IgG, IgA and IgM) within 4-6 hours with high sensitivity andspecificity. In addition to the detection of immunoglobulin classes,ELISA can also detect *Brucella*-specific IgG subclasses and other*Brucella* immunoglobulins such as IgE (Araj, 2010).

The indirect ELISA (i-ELISA) has been used for serologic diagnosisof brucellosis in sheep, goats and pigs. It has also been used fordiagnosis using serum or milk from cattle (Gall *et al*., 2003; Di Febo *et al*., 2012). O ELISA-i has beenusually used for smooth LPS *Brucella* spp., and it is sensitive andspecific for *B. abortus* or *B. melitensis*, but it is not capable ofdifferentiating antibodies induced by the vaccine strains S19 or Rev1(Lim *et al*.,2004; Ko KY *et al*., 2012). Sensitivity of i-ELISA varies from 96 to 100% and itsspecificity from 93.8% and 100% (Gall *et al*., 2001; Gall & Nielsen, 2004). On the other handcompetitive enzyme immunoassays were developed in order to eliminate some, but not all of the problems arising from residual vaccinal antibody, and from cross-reacting antibodies.The assays are carried out by selecting a monoclonal antibody with slightly higher affinity for the antigen than most of the vaccinal/cross-reacting antibody, but with lower affinity than antibody arising from infection (Munoz *et al*., 2005; OIE, 2009; Poiester *et al*., 2010). The specificity of the competitive enzyme immunoassay is very high and is able to detect all antibody isotypes (IgM, IgG1, and IgG2 and IgA) (Nielsen, 2002). However, it is slightly less sensitive than the indirect enzyme immunoassay. This assay is an outstanding confirmatory assay for the diagnosis of brucellosis in most mammalian species.

Fluorescence polarization assay (FPA)

It is based on the physical principle of the mass-dependent change of the molecules rotation speed in a liquid medium. The smaller the molecule, the faster it rotates and the depolarization of a polarized beam of light occurs. In FPA the serum sample is incubated with a specific *Brucella* antigen, conjugated with a fluorescent label. In case there are anti-*Brucella* antibodies in the serum, large fluorescently labeled antigen-antibody complex is formed, which can easily be distinguished from the unbound antigen negative control. FPA method has a high specificity but less sensitivity than i-ELISA (McGiven *et al*., 2003). In Europe and the USA FPA method is used in programs to monitor and control the spread of brucellosis, but it requires special equipment and it is not suitable for rapid and easy testing.

Milk ring test

The milk ring test is based on agglutination of antibodies secreted into the milk. This test allows screening of large number of cattle by using milk samples from tanks or pools from several cows. This test is useful for monitoring cattle herds or areas free of brucellosis so it is classified as surveillance or monitoring test (OIE, 2009). Importantly, the number of false positive results is proportional to the number of cows secreting acidic milk due to colostrums or mastitis (OIE, 2009). A positive result indicates the presence of infected cattle in the herd so the test should be followed by individual serological test in the entire herd.

2.6.3. Molecular methods for Brucella species genotyping

Molecular techniques are important tools for diagnosis andepidemiologic studies, providing relevant information foridentification of species and biotypes of *Brucella* spp., allowingdifferentiation between virulent and vaccine strains (Le Flèche *et al*., 2006; López-Goñi *et al*., 2008). Moleculardetection of*Brucella*spp. can be done directly on clinical sampleswithout previous isolation of the organism. In addition, thesetechniques can be used to complement results obtained fromphenotypic tests (Bricker, 2002). Despite the high degree of DNA homology within the genus *Brucella*, several molecular methods, including PCR, have been developed that allow, to a certain extent, differentiation between *Brucella* species and some of their biovars (OIE, 2009).

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is the most broadly used moleculartechnique for brucellosis diagnosis (Bricker,2002). The technique is chosen basedon the type of biological sample and the goal, i.e., diagnosis ormolecular characterization or epidemiological survey. Most of themolecular diagnostic methods for brucellosis have sensitivity rangingfrom 50% to 100% and specificity between 60% and 98%. The DNAextraction protocol, type of clinical sample, and detection limits ofeach protocol, are factors that can influence the efficiency of thetechnique (Mitka *et al*.,2007).

Since the routine identification and differentiation of brucellosis suspected specimens, based on culture isolation and phenotypiccharacterization, requires Biosafety level-3 (BSL-3) protocols for thehigh risk of laboratory-acquired infections (Boschiroli *et al*., 2001), molecular methodshave been explored in order to overcome these difficulties.Furthermore,

the PCR-based assays have shown a higher sensitivitywith respect to the standard microbiological assay for the diagnosis ofbrucellosis (Hoover & Friedlander,1997).

Multiplex polymerase chain reaction typing

Several multiplex PCRs which identify the genus *Brucella* at the species level and partly at the biovar level using different primer combinations have been reported. The first multiplex PCR, called AMOS PCR assay (AMOS is an acronym from ‘‘*abortus-melitensisovis- suis*’’), comprised five oligonucleotide primers for the identification of selected biovars of four species of *Brucella*. 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).

Real-time PCR

Real-time PCR is more rapid and more sensitive than conventionalPCR. It does not require post amplification handling of PCR products,thereby reducing the risk of laboratory contamination and false positiveresults. Real-time PCR assays have been recently described inorder to test *Brucella* cells in urine, blood, and paraffin-embeddedtissues (Redkar *et al*.,2001; Kattar *et al*., 2007).

2.7.Prevention and Control

Compatible relationships of *Brucella* species with the hosts including variable incubation periods, long survival time in both extracellular and intracellular environments, asymptomatic carrier stages and resistance to treatment are the major problems. These and animal husbandry factors such as nomadism, co-mingling, and increasing population sizes assure difficulties in control of disease (Rahman *et al*., 2006).

Bovine brucellosis is usually introduced into a herd in an infected cow, but it can also enter in semen from infected bulls and on fomites. As the disease often goes undetected theidentification of infected herds and animals is of primeimportance (Aulakh *et al*. 2008). The treatment of brucellosis in the cow has generally been unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs (Radostits *et al*., 2000; Tolosa *et al*., 2004).

One of the most successful methods for prevention and control brucellosis isthrough vaccination. In different parts of the world both live vaccines, such as *B. abortus* S-19, *B. melitensis* Rev1, *B. suis* S-2, rough *B. melitensis* strain M111 and *B. abortus* strainRB51 and killed vaccines, such as *B. abortus* 45/20 and *B. melitensis* H.38 are available. Useof the RB51 attenuated live vaccine has recently gained popularity for control of brucellosisin cattle (Cheville,*et al*., 1996).

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 vital to education on risks for infection to these populations in order to influence behavioral practices that will reduce risks of transmission (Moti*et al*., 2013).

Eradication can be accomplished by quarantine of infected herds, vaccination, test-and-slaughter techniques, various forms of surveillance and trace backs. *Brucella* species are readily killed by most commonly available disinfectants including hypochlorite solutions, 70% ethanol, isopropanol, iodophores, phenolic disinfectants, formaldehyde, glutaraldehyde and xylene; however, organic matter and low temperatures decrease the efficacy of disinfectants.

The development of a national veterinary extension services in the country, is essential to promote control strategies in both animals and human. 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 (Moti *et al*., 2013).

No vaccine has been approved for the prevention of human brucellosis. Therefore, human brucellosis is usually prevented by controlling the infection in animals. 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 human. Pasteurization of dairy products is an important safety measure where this disease is endemic. 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 (Moti*et al*., 2013). The combination of doxycycline with streptomycin isthe best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis (Seleem *et al*., 2010).

3. MATERIALS AND METHODS

3.1.Study Area Description

The present study was conducted in Sodo Zuria and Humbo districts of Wolaita zone southern Ethiopia. The study areas situated at6º35' N 37º 50' E to 6º53 N 37º 49' E. Wolaita zone is one of the thirteen zones of the SNNPR region covering an area of 4471.3 km^2. It is located at a distance of 332 km. south of Addis Ababa and 157 km away from Hawassa town. It is one of the Omotic speaking people inhabiting the basins of Omo river and lake Abaya. For administrative purpose the zone is divided in to twelve woredas or districts. Topographically it lies on an elevation ranging from 1200 to 2950 meters above sea level. The rainfall pattern is bimodal, a short rainy season runs from March to May and long rainy season runs from June to September. The mean annual temperature of the zone is about 19°C being maximum in February which is 29°C and minimum in August which is 15°C. Regarding the land utilization data, 261,000 hectares (ha) is used for cultivation, 5318 ha for grazing, 8261 ha for Bush- land and the remaining 35382.5 ha is a cultivable land. Wolaita zone is bounded by Hadiya and Kanbata Timbaro zone in the north, Gamo Gofa zone in the south, Sidama zone in the East and Dwuro zone in the west. The farming system of the study area is largely characterized by mixed crop-livestock production system. Considerably, variable number and diversity of animal species are maintained under traditional extensive management system. Livestock production system is generally predominated by extensive system in which animals are allowed to forage freely during day time and kept in house during the night. According to data from districts livestock and fishery office, the total number of cattle in Humbo and Sodo Zuria districts was 34, 446 and 21, 243 respectively. Cattle are the mainstay of the household economy as they provide draught power for tillage for crop production, are the main sources of meat and milk, and provide income through the live animal market. The Humbo and Sodo Zuria districts were selected purposively due to high cattle population in relation to other districts in the zone and the highest suppliers of animal products to Wolaita Sodo town. Stock composition varies between keeping cattle as major stock with small ruminants and equines (WZFEDD, 2013; CSA, 2008).

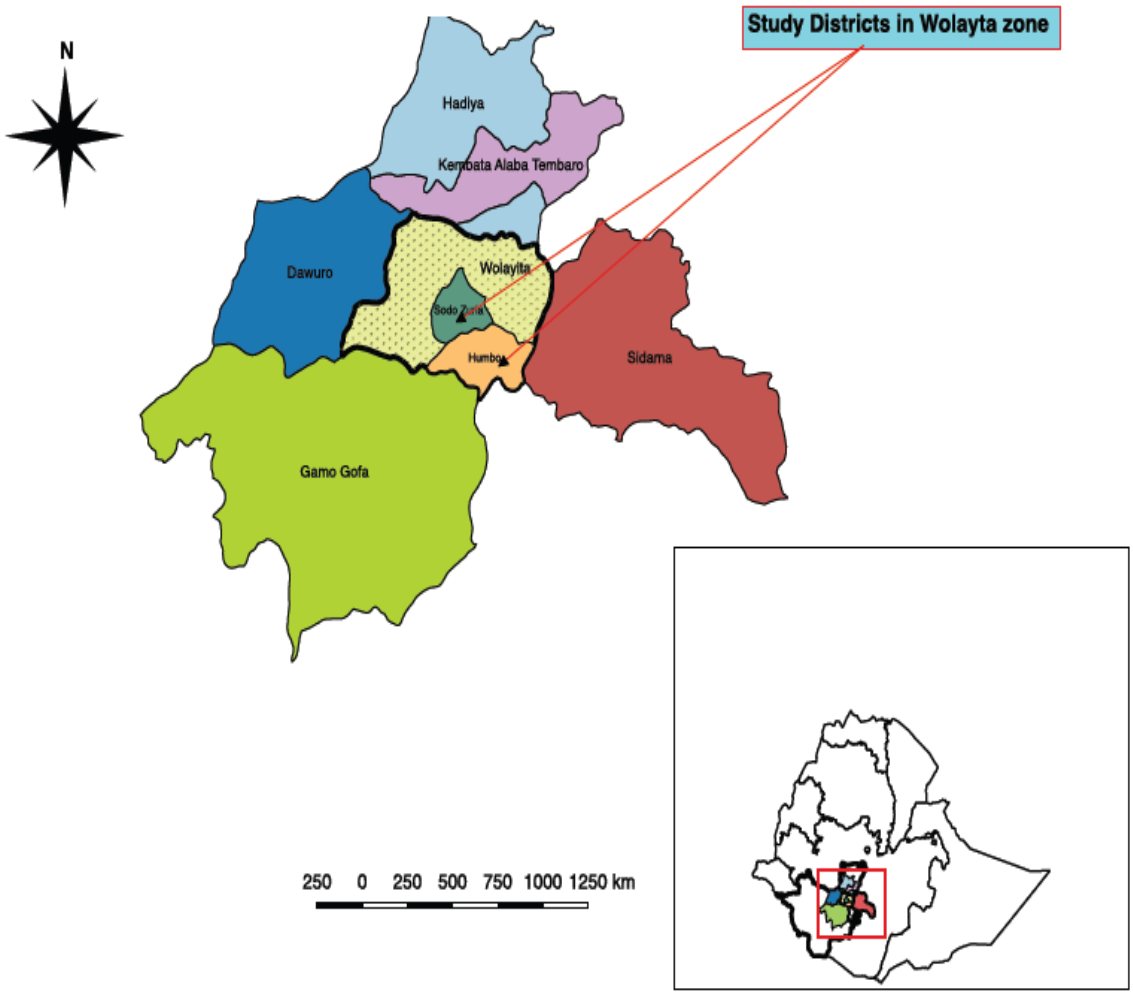

Figure **1**:Map of the study area.

Source: Supported by QGIS version 2.14.10-Essen

3.2.Study Design

A cross-sectional epidemiological study was carried out on both indigenous and cross breed cattle to determine seroprevalance of brucellosis and their association with different risk factors using two serological tests Rose Bengal Plate Test (RBPT)and Complement Fixation Test (CFT) and structured questionnaire survey from November 2016 to April 2017 in Wolaita zone, Southern Ethiopia.

3.3.Sample Size Determination

In order to determine the desired sample size, there were no previous reports of prevalence of brucellosis in the present study area. Therefore, the average expected prevalence rate was assumed to be 50% for the area within 95% confidence interval (CI) at 5% desired precision

as stated by Thrusfield (2007). Hence, using the formula, calculated sample for the current study becomes 384 heads of cattle; however, a total of 462 serum samples (234 from Humbo and 228 from Sodo Zuria districts) of both sexes having different ages were sampled to increase the precision of the result.

$$N = \frac{1.96^2 (P)(1-P)}{d^2}$$

Where

N= Total calculated sample size

P= expected prevalence

d= absolute precision

3.4.Study Animals and their Management

The study animals were both indigenous and cross breeds dairy cattle with no history of vaccination against brucellosis,reared under traditional extensive production system and were kept with other species such as sheep and goats.Cattle above 1 year of age including milking, none milking, replacement heifers and bulls were included.

3.5.Sampling Procedure

Multistagesampling method was used in the study. Peasant association, village(*Ketena*) and herd were the primary, secondary and third units respectively. At each stage, sampling units were selected randomly (Dohoo *et al*., 2003). According to data from districts livestock and fishery office, the totalnumber of cattle in Humbo and Sodo Zuria districts is 55, 689.From two districts 10peasant association were sampled from a total of 69 in these two districts and from each PAs three villages (*Ketenas*) wererandomly selected. This was followed by random sampling ofherds (house holds) in the selected *Ketena*.In the community, *Ketena* entails a group of often related households that pursue similar socio-economic activities such as search for pasture, water and others. *Ketena* is, therefore, more or less synonymous to a village herds.According to information from local animal health office, the number of *Ketena* in each PAs varies from 3 to 5. Also there were 70-100 households within each *Ketena*. The sampling unit was an individual animal of different age groups belonging to herds (households) in the *Ketena*. In each study area, the numbers of herds were listed with the assistance of local animal health technician. A total of 104 herds(households) with an average herd size of 5 (ranging from 3 to 18), were randomly sampled. Therefore five animals were

selected using systemic random sampling from the selected herds and all animals were sampled fromthose herd size less than five animals. An average of 10 herds from each peasant association were sampled including cattle of both sexes and at least one year of age. Information pertaining to individual animals such as age, sex, breed, parity, mating system, reproductive status and history of reproductive problem (abortion and retained placenta) was recorded. A total of 462 cattle (234from Humbo and 228 from Sodo Zuria districts) were sampled.

3.5.1. Blood sample collection

Approximately 7-10 ml of blood was collected from the jugular vein of each selected animal using plain vacatainer tubes and needle. During the sampling, animals were restrained and the area was first disinfected by using 70% alcohol before puncturing. Identification of each animal was labeled on corresponding vacutainer tubes and kept overnight at room temperature to allow clotting. At the next morning sera were collected from the clot by siphoning in to the sterile cryovial tube (2ml), to which animals /identification was coincided. Sera were kept at − 20 °C in Wolaita Sodo regional veterinary laboratory until serological tests were conducted. All serum samples were screened by Rose Bengal Plate Test (RBPT) at Wolaita Sodo regional laboratory. The sera that tested positive to the RBPT were further subjected to the Complement Fixation Test (CFT) for confirmation at National Veterinary Institute(NVI) at Bishoftu.

3.5.2. Questionnaire survey

Verbal consent was obtained from the respondents and the objective of the survey explained to them before start of the interview. A total of 80 owners and/or attendants of cattle were interviewed parallel to blood collection using structured questionnaire which covers demographic data (including age, educational level and location), consumption habit (milk,and other animal products), knowledge of transmission of brucellosis, zoonotic risk of milk, method of consumption of raw milk,close occupational contact with animals and mode of contact (delivery or handling and disposal practice of abortus). Similarly, information related to the animals like herd size, mating system, history of abortion, placenta retention and others were also collected using a separate questionnaire format prepared for this purpose.

3.5.3. Serologicalsurvey

Rose Bengal Plate Test (RBPT)

For the RBPT the procedure described by Staak *et al.* (OIE,2012) was followed. Briefly, 30µlof the sera samples were dispensed onto the plate and 30µl of RBPT antigen was dropped alongside the sera. The plate was rocked by hand for 4 min and the test was read by comparing with the positive and negative control sera by examining for agglutination in natural light. Results of RBPT were interpreted as 0, +, ++ and +++ as described by Staak *et al.* (OIE,2012). 0 = no agglutination; + = barely visible agglutination (seen by using magnifying glass); ++ = fine agglutination and +++ = coarse agglutination. Samples with no agglutination (0) were recorded as negative while those with +, ++ and +++ were recorded as positive.

Complement Fixation Test (CFT)

The CFT procedure was undertaken in the National Veterinary Institute, at Bishoftu, Ethiopia. Preparation of the reagents was performed according to OIE protocols. A titration of hemolysin and antigen was performed before the test. The minimum hemolytic dose was also estimated for each run. As for the interpretation of test results, positive reactions were indicated by sedimentation of Sheep Red Blood Cells (SRBC) and absence of hemolysis. Negative reactions were revealed by hemolysis of SRBC. According to OIE sera with strong reaction, more than 75% fixation of complement at a dilution of 1:10 and at least with 50% fixation of complement at a working dilution (1:5) was classified as positive.

3.6.Data Analysis

Data was collected and stored in Microsoft (MS) Excel Spread Sheet program and Categorical variables were summarized as frequency and percentages while continuous variables were summarized as mean ± standard deviation (SD). Descriptive statistical analysis of various risk factors and dependent variables were done using (STATA software version 13). The Fisher's exact test was used to test *Brucella* seroprevalence association with incriminated categorical risk factors. The total prevalence was calculated by dividingthe number of RBPT- and CFT-positiveanimals by the total number of animalstested. Herd prevalence was calculated bydividing the total number of herds with at leastone reactor in RBPT and CFT by the numberof all herds tested. In these study a herd, defined as the total number of cattle

belonging to the same household. Univariate logistic regression was used to test the significance of the effect of different risk factors on sero-prevalence of brucellosis. Odds ratio (OR) was utilized to measurethe degree of association between riskfactors and *Brucella* seropositivity. All risk factors that hadnon-collinear effect andp-value < 0.25 in the univariable logistic regression analysis were subjected to multivariable logistic regression analysis.Age of animals were categorized into <3, 3–6 and >6 years; herd size was categorized into<6, 6–10 and >10 heads of cattle and parity number 0,1 and >1was categorized as nullparous, monoparous and multiparous.

3.7.Ethical Consideration

Before any attempt to collect sample the protocol was approved by Addis Ababa university, college of veterinary medicine animal research ethical committee with reference number VM/ERC/17/06/09/2017. Official permisioin was also obtained from Agricaltural administration office of the zone and districts as well as animal owners. Moreover the guideline was also used.

4. RESULTS

4.1.Seroprevalence of Bovine Brucellosis

A total of 462 animals, 105(22.7%) male and 357(77.3%) female animalsabove 1 year of age were sampled andtested for *B. abortus* antibodies. Of which 10 (2.2%) (95% CI: 1-4) animals tested positive by RBT and 6 animals were confirmed positive by CFT, giving seroprevalence of 1.3% (95% CI:0.5- 3)(Table3). Theprevalence of bovine brucellosis was significantly higher in animals included in herd size greater than 10 (p = 0.009) and did notsignificant differaccording to study districts (P > 0.05). However relatively higher proportion of seropositivity was observed in Humbo district (2.13%) when compared to Sodo Zuria district.Regarding age category prevalence of brucellosis was higher in those cattle with age 3 years and above compared to the lower age groups with no statistical variation among the age groups (P>0.05).seroprevalence rate of 2.03% was observed in older animals (>6 years) and 1.23% in animals within 3-6 years old. No animal less than 3 years old was found to be seroreactive (Table 3).

All seropositive animals were females and were either pregnant or lactating. Among 357 female animals 19(5.3%) showed history of abortion, 32 (8.9%) with history of retained fetal membrane and 38% were lactating. Except for the cow with history of abortion and RFM, other variables did not significantly associate with animal level seropositivity in female animals. The seroprevalences of brucellosis were significantly associatedwith aborting cows (p=0.035) and cows with a history of RFM (p=0.011)(Table 3).

Table 3: Association of risk factors withbrucellosis seropositivity at individual animal level.

Risk factors	No. Tested	RBPT positive No. (%)	CFT positive No. (%)	fisher's exact test p-value
Study districts				0.216
Humbo	234	7 (2.99%)	5 (2.13%)	
Sodo Zuria	228	3(1.3%)	1 (0.44%)	
Age				0.438
<3 years	103	0 (0%)	0 (0%)	
3-6 years	162	2(1.23%)	2 (1.23%)	
>6years	197	8(4.06%)	4 (2.03%)	
Sex				0.345
Female	357	10(2.8%)	6 (1.68%)	
Male	105	0(0%)	0 (0%)	
Breed				1.00
Local	364	9(2.74%)	5 (1.37%)	
Cross	98	1(1.02%)	1 (1.02%)	
Herd size				0.009
<6	221	4(1.8%)	1 (0.45%)	
6-10	168	2 (1.19%)	1 (0.59%)	
>10	73	4(5.48%)	4 (5.48%)	
Repro. Status				0.530
Heifer	80	0(0%)	0 (0%)	
Pregnant	111	4(3.6%)	2 (1.8%)	
Lactation	140	5 (3.57%)	4 (2.85%)	
Dry	26	1(3.85%)	0(0%)	
Mating system				0.172
Non serviced	79	0(0%)	0(0%)	
Natural	190	6(3.57%)	3(1.57%)	
AI	51	1(1.96%)	1(1.96%)	
Mixed	37	3(8.1%)	2(5.4%)	
Parity				0.061
Null parous	115	0(0%)	0(0%)	
Mono parous	70	0(0%)	0(0%)	
Multiparous	172	10(5.8%)	6(3.5%)	
Abortion				0.035
Absent	338	8(2.37%)	4(1.18%)	
Present	19	2(10.5%)	2(10.5%)	
RFM				0.011
Absent	325	5(1.54%)	3(0.9%)	
Present	32	5(15.6%)	5. (9.37%)	

4.1.1. Animal level risk factors analysis

The results of univariable analysis showing the association of predictor variable and *Brucella* seropositivity (Table 4). Seroprevalence, recorded for cattle, in herd size greater than 10(5.48%) revealed a statistically significant variation($p<0.05$)with the odds of seropositivity being at least 13times more likely to be infected with *Brucella* organisms than animals included herd size less than <6 animals. Similarly, History of abortion and placenta retention in females were found to be significantly associated with seropositivity. Seropositivity to brucellosiswas significantly ($p=0.01$) higher (10.5%) in cattle with a history of abortion with 10 times more likely to be seropositive than animals with no history of abortion. The prevalence of *Brucella* seropositivity significantly higher (9.37%) in cattle having a history of placenta retention (Table 4). The rest was showed no statistically significant associations regardless of the seropositivity recorded.

Table 4: Univariable logistic regression analysis based on individual animal level seroprevalence of brucellosis and associated risk factors

Risk factors	No. Tested	CFT positive (%)	95%CI	OR	P-value
Study districts					
Humbo	234	5 (1.86%)			
Sodo Zuruia	228	1 (0.44%)	0.02, 1.74	0.20	0.14
Breed					
Local	364	5(1.37%)			
Cross	98	1(1.02%)	0.1, 6.4	0.74	0.78
Herd size					
<6	221	1(0.45%)			
6-10	168	1(0.59%)	0.1, 21.2	1.32	0.85
>10	73	4 (5.48%)	1.4, 26.0	12.75	0.02
Abortion (n=357)					
Absent	338	4(1.18%)			
Present	19	2(10.5%)	1.7, 57.4	9.82	0.01
RFM (n=357)					
Absent	325	3(0.9%)			
Present	32	3(9.37%)	2.14, 57.52	11.10	0.00

4.1.2. Herd level risk factors analysis

Out of 104 herds studied, 6 (5.8%) were positive using CFT. The herd level binary logistic regression analysis revealed that history of placenta retention was found to be strongly associated with herd seropositivity to *Brucella* (p-value ≤ 0.05). Herds with pervious history placenta retention showed at least 15times more likely to be seropositive to the disease than those herds with no history of placenta retention with statistical (p = 0.01) significance level (Table 5).

Table 5: Univariable logistic regression analysis based on herd level seroprevalence of brucellosis and associated risk factors

Variables	No. herd tested	CFT Positive No. (%)	95% CI	OR	p- value
Study Districts					
Humbo	50	5 (10%)			
Sodo Zuria	54	1 (1.85%)	0.02, 1.5	0.17	0.11
Herd size					
<6	55	2 (3.6%)			
6-10	35	3 (8.6%)	0.4, 15.7	2.48	0.33
>10	14	1 (7.1%)	0.2, 24. 2	2.04	0.57
Abortion					
Present	19	3 (15.8%)	0.9, 27.7	5.12	0.06
Absent	85	3 (3.5%)			
RFM					
Present	29	5(17.2%)	1.7, 138.6	15.4	0.01
Absent	75	1(1.3%)			

In Table 6,the results of multivariable logistic regression analysis showing important risk factors for *Brucella* seropositivity.Accordingly, risk factors with p-value ≤0.25 in the univariate logistic regression model where included in the separate multivariable logistic regression model fitted. Therefore,study districts,herd size and history of abortion were included in the final logistic regression model. However, herd size and history of abortion were significantly associated with *Brucella* seropositivity. RFM were not included in the multivariable regression because of their multicollinearity with abortion. The rest of the variables were not included in the final model. Thus multivariable logistic regression analysis depicts that animals involved in herd size greater than 10 are more likely to be at higher risk

for *Brucella* infection than animals in herd size less than6 (OR=13.7). Similarly, the multivariate analysis revealed that cattle with history of abortion are 9.8 times more likely to be seropositive to *Brucella* (OR= 9.8)(Table 6).

Table 6: Multivariable logistic regression analyses identifying the association of potential risk factors to *Brucella*seropositivity in cattle.

Risk factors	No. Tested	CFT Positive No. (%)	95%CI	OR	P-value
Herd size					
<6	221	1 (0.45%)			
6-10	168	1 (0.59%)	3.9, 26.2	1.6	0.74
>10	73	4 (5.48%)	1.4, 29.7	13.7	0.02
Abortion					
Absent	338	4 (1.18%)			
Present	19	2 (10.5%)	1.5, 64.4	9.8	0.01

4.2. Questionnaire Survey

The questionnaire result indicated that respondents having different age groups with the maximum age of 70 and minimum age of 28 with average mean age of 44.5 $\pm$ 1.2 years were included. Out of the respondents 77.5% of them were males and 22.5% females and of the total households interviewed, 72.5% were located in rural area and 27.5% were peri-urbun. With regard to educational status, among the respondents 33.75% had not received education. (Table 7).

Table 7: Socio-economic characteristics of respondents

Parameters	Number of respondents
Location	
Humbo	43 (53.75%)
Sodo Zuria	37 (46.25%)
House hold classification	
Rural	58 (72.5%)
Peri-urban	22 (27.5)
Education level	
Illiterate	27 (33.75%)
Lower grade	49 (61.25%)
College	4 (5%)
Sex	
Male	62 (77.5%)
Female	18 (22.5%)

Out of the total households interviewed, only 18.75% were depending on livestock production, the remaining perform crop-livestock (mixed) production system. According to the survey result majority of respondents keeping cattle for mixed purpose (44%). Others, 11% for meat 20% milk and 5% for draft purpose and most of the respondents (46.25%) reported herd trend decrement (Table 8).

Table 8: Response of the interviewed respondents on farming activities, purpose of keeping cattle and herd trend

Parameter	Number of respondents
Farming activities	
Livestock production	15 (18.75%)
Crop-livestock production	65 (81.25%)
Purpose of keeping cattle	
Meat	11 (13.75%)
Milk	20 (25%)
Draft	5 (6.25%)
Mixed	44 (55%)
Herd trend	
Increasing	35 (43.75%)
Decreasing	37 (46.25%)
Stable	8 (10%)

Most of the respondents (37.5%) breed their animals using both hand mating and artificial insemination (AI), while 25%, 21.25%, and 16.25% were used uncontrolled mating, hand mating and AI respectively. Majority of the interviewed respondents (92.5%) were mentioned their access to government veterinary clinics (Table 9).

Table 9: The Response of respondents on mating system and access to veterinary service

Parameter	Number of respondents
Mating system	
Uncontrolled	20 (25%)
Controlled mating	17 (21.25%)
AI	13 (16.25%)
Controlled mating & AI	30 (37.5%)
Access to veterinary service	
Government clinic	74 (92.5%)
Private veterinary clinic	6 (7.5%)

Respondents were also interviewed to describe the occurrence of some reproductive problems and indicated 17.5% abortion, 12.5% retained fetal membranes and 8.75% testicular swelling. Most of the farmers (71.5%) had no knowledge on cause of abortion in cattle and (85.7%) of them had not isolating aborted animal from others. The majority of the respondents consume raw milk (95%) and raw meat (82.5%). Similarly, most of the farmers (86.25%) have habit of assisting cows during parturition, of which only few (2.9%) of them use protective glove (Table 10).

Table 10: Response of farmers about knowledge of brucellosis and risk factors.

Parameter	number of respondent	
	Yes (%)	No (%)
Abortion	14 (17.5%)	66 (82.5%)
Retention of placenta after birth	10 (12.5%)	70 (87.5%)
Separation of aborted animal	2(14.3%)	12(85.7%)
Perception on cause of abortion	4 (28.5%)	10 (71.5%)
Raw milk consumption	76 (95%)	4 (5%)
Raw meat consumption	66 (82.5%)	14 (17.5%)
Testicular swelling	7 (8.75%)	73 (91.25%)
Assisting cow during parturition	69 (86.25%)	11(13.75%)
Using protective glove during assisting	2(2.9%)	69 (97.1%)

5. DISCUSSION

Cross-sectionalserological study, attempted to look the status of bovine brucellosis in two districtsWolaita zone, southern Ethiopia. The study reveals that, the animal level prevalence of bovine brucellosis in extensive management system was found to be (1.3%). This relatively low prevalence might be attributable to extensive grazing conditions; these could reduce both animal to animal contact and the contamination of pastures under dry climatic conditions (Crawford *et al*., 1990; Adugna *et al*., 2013). Another explanation could be that, in the area studied, most of the farmers partly practice alternative farm products such ascash crops.Therefore, in the area small numbers ofanimals(on average five animals) are kept separately and free movement of animals were restrictedandaretied around farmland specially during crop harvestingin order to feed on byproducts(post-harvest products)of the farms as reported by (Megersa*et al*., 2011). In the present study area, the majority of farmers replace their animals from their own stock instead of buying animals from markets.

Corresponding present study the low prevalence of bovine brucellosis has been reported in other studies on cattle under similar production systems in different parts of Ethiopia; 1.66% prevalence reported from Sidama zone(Asmare *et al*., 2010), 1% from Benshangul Gumuz (Adugna *et al*., 2013), 1.97% from East Wollega(Moti*et al*., 2012), 1.2% from Western Tigray (Haileselassie*et al*., 2010), 1.7% from Arsi Zone (Tsegaye*et al*., 2016) and3.3% from Alage district (Asgedom *et al*.,2016). It also agrees with 2% from Sudan (Senein and Abdelgadir, 2012), 2.77% from Eritrea (Scacchia *et al*., 2013). lower prevalence of brucellosis also has been reported in intensive farms (Bashitu*et al*., 2015; Asgedom *et al*., 2016). On the contrary higherprevalence has been reported from the highland areas of Ethiopia among cattle in smallholder production systems based on the same diagnostic tests (Kebede *et al*., 2008). This variation is merely due to differences in cattle production systems (Mohan *et al*., 1996). Based on the same test, a higher prevalence was also reported in pastoral areas, compared with an extensive cattle production system (Dinka &Chala 2009; Tibesso *et al*., 2014).

The present study showed that there is non-significant difference in seroprevalence of brucellosis between the twodistricts (Sodo Zuria and Humbo). This finding is in agreement with the report of (Berhe *etal*., 2007; Ibrahim *etal*., 2009; Adugna *et al*., 2013).This could be due to similarity among traditional management systems in the two districts and where

sedentary livestock raising is predominant. The study also revealed that all seropositive animals were females. This finding agrees with the reports by (; Kebede *et al*., 2008; Tolosa *et al*., 2008; Dinka & Chala 2009; Adugna *et al*., 2013). However, Hailemelekot *etal*., (2007)reported 2.11% seroprevalenceof brucellosis in maleunder extensive management system.Female animals are maintained in herds over extended time period thus, have ample time for exposure to the pathogen and being source of infection for other animals(Megersa *et al*., 2011; Adugna *et al*., 2013). Other explanation for this finding could be that the number of male animals in each herd was low and weremostly reared separately, thus the chance of exposure is lower for males.

Breed of animal was not significantly associated with brucellosis in this study.Breed differences in susceptibility have not been clearly documented in cattle, although genetically determined differences in susceptibility of individual animals have been demonstrated (Corbel, 2006). There is still argument among different authors on the issue of breed susceptibility to brucellosis. In this study, the seroprevalence was found to be higher in local breed animals (1.37%) than cross breed (1.02%). However, this difference was not statistically significant which is in agreement with the report of (Lidia, 2008) and (Moti*et al*., 2012) in central highland and East Wollega zone of Ethiopia respectively. This could be due to, limited number of cross breed animals in this study because of their low number inextensive production system. On the contrary, (Jergefa *et al*.,2009) in their study found that breed of cattle has significant effect on the sero prevalence of brucellosis and is higher in crossbreed than in indigenous ones. This is due to the compounded effect of management systems in cross-breed and also the farmers who owned cross-bred tend to followintensive management.

The presentstudy also revealed that the seroprevalence of bovine brucellosis was not significantly associated with the age of the cattle. Brucellosis appears to be more associated with sexual maturity (Radostits *et al*., 2007), and higher seroprevalence is repeatedly reported in sexually matured animals. In this study, seropositive to brucellosis were insignificantly higher in age greater than three years including males. This agrees with the report of (Asfaw *et al*., 1998; Bekele *et al*., 2000; Omer *et al*., 2000; Jergefa *et al*., 2009; Asmare *et al*., 2010; Adugna *et al*., 2013). In this study, seropositivity occurred only in cow having at least one parity. Similarly, higher seropositivity has been reported in other studies in animals older than five years, when compared with younger animals (Berhe *et al*., 2007; Dinka & Chala, 2009;

Adugna *et al*., 2013).Seroprevalence may increase with age as a result of acquired immunity in infected animals and prolonged exposure to pathogen.

Herdsize remained independently and significantly associated with theanimal level seropositivity to brucellosis in this study. Thisfinding is in agreement with the reports (Asfaw *et al*., 1998;Tolosa *et al*., 2008; Asmare *et al*., 2010, Haileselassie*et al*., 2010; Ibrahim *et al*., 2010; Adugna *et al*., 2013;). An increase in herd size is usually accompanied by an increase in stocking density, as well as an increase in the risk of exposure to infection. Stocking density is an important determinant of the potential for transmission between susceptible and infected animals (Crawford 1990; Omer *et al*., 2000). In this study the number of animals per herd was generally low, with a maximum herd size of 18 animals, which is typical of mixed livestock and crop production. This would suggest that the risk of brucellosis increases with herd size. Similarly, the increased herd seropositivity has been reported in Zimbabwe (Matope *et al*., 2010). The result of present study indicates that bovine brucellosis should be considered in extensive productionsystem as in intensive production in Ethiopia.

Therewas no seropositive reactor in nulliparous, monoparous as well asin animals less than 3 years of age. This finding in accordance with the prevalencereport of 0.0% by(Ibrahim *et al*., 2009), 0.69%(Berhe *et al*., 2007),1.4%(Kebede *et al*., 2008) for the same group of animals. This shows thatbrucellosis is highly related withage and sexualmaturity of animals. The reproductive status did not significantly determine seropositivity in the present study. However, all seropositive animals were either pregnant or lactating. This agrees with the report of (Omer *et al*., 2000; Tolosa *et al*., 2008;Adugna *et al*., 2013). Sexually mature and pregnant cows are more susceptible to infection with *Brucella* than sexually immature cattle of either sex. This has been attributed to the affinity of these bacteria to the pregnant uterus and to erythritol in fetal tissue, possibly also to steroid hormones (Radostits *et al*., 2000).

Seroprevalence of brucellosis was significantly associated in cow with history of abortion and placenta retention in the current study. Thus the history of abortion and placenta retention were found to be 10 and 11times more likely to be seropositive when compared to no history of abortion and RFM respectively. Association between brucellosis seroprevalence and occurrence of abortion and placenta retention also reported (Berhe*et al*., 2007; Tolosa *et al*., 2008; Ibrahim *et al*., 2010; Adugna *et al*., 2013; Tsegaye *et al*.,2016). Due to its collinearity with history ofabortion, history RFM was not included in the multivariable analysis. Thereason isthat, in most casethe effects of abortionleads to placenta retention. This could be

explained probably by the fact that abortion is the typical outcome of brucellosis infections (Schelling *et al*. 2003).

The overall herd level seroprevalence of bovine brucellosis was 5.8%, which is comparable to herd level seroprevalencereport of 3.3%(Haileselassie*et al*., 2010), 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). Similarly higher herd-level prevalence has also been reported in dairy cattle in other African countries (Matope *et al*., 2010). This inconsistency could be due to relatively larger herd sizes compared with herds in the this study and different in management.

Cowwith a history of RFM was significantly affects herd seropositivity. The herd seroprevalence of brucellosiswas higher in herds that had a history of RFM (17.2%), compared with no history of RFM (1.3%).This could beexplained by the fact that retained placenta is typical outcomesof brucellosis. On the contrary the presence of a cow with a history of abortion did not significantly affect herd seropositivity. However, the herd seroprevalence of brucellosis was higher in herds that had history of abortion (15.8%) compared with non-aborted (3.5%).This could be due to the presence of other causes of abortionin herd. This finding is in agreement with aprevious reports (Kebede *et al*., 2008; Adugna *et al*., 2013).

A total of 80 cattle owners and attendants were interviewed to assess their awareness levels regarding animal management, brucellosis and occupational risks using structured questionnaire. Knowledge of diseases is a crucial step in the development of prevention and control measures (Prilutski, 2010). Despite huge efforts of the government and non-government institutions to improve animal production in the areas, general knowledge of brucellosis among the farmers was still poor.The educational status attained by majority of the respondents was low which falls between illiterate and lower grades. This low level of educational status may lead to reduced production of dairy farms because of low use of dairy innovations such as cultivation of improved forages, breeding techniques and use of modern dairy farming. In addition to this, personal hygiene, proper disposal of aborted materials and the use of a separate parturition pen were not under consideration. These could have led high

risks of transmitting the disease within and between the herds and human. This is in agreement with previous studies in extensive livestock production system (Ragassa *et al*. 2009; Megersa *et al*. 2011; Adugna *et al*., 2013). Likewise, mixing of different animal species although having its own economic importance also increases the chances of transmission of brucellosis to the cattle.

The occurrence of brucellosis in humans is associated with contact with domestic animals (Alballa, 1995), exposure to aborted animals and assisting animal parturition (Cooper 1992; Kozukeev *et al*. 2006). In this study, the majority of the farmers have the habit of drinking raw milk and assisting parturition. This implies that little attention has been given to preventing brucellosis and that this, in turn, contributes to the spread and transmission of the infection to human in the area.

Limitations of this research

The limitations of this study were due to financial constraints, wide area was not covered and only serological tests were conducted, not supported with biochemical and molecular tests in order to isolate species and biovariant. Another limitation was, during the study,small number of animals per herd and per village were sampled, positive animals might be missed, and village or herd might be considered as negative. There is a need to include large study population,area coverageand supported by molecular diagnosis.

6. CONCLUSION AND RECOMMENDATIONS

The present study has established that the bovine brucellosis persists at a low seroprevalence in Wolaita zone southern Ethiopia.The seroprevalence of bovine brucellosis was found to be 1.3% and 5.8% at animal level and herd level respectively. At the same time lowprevalence of the disease was observed in different sex and young age groups.Although it was observed that cattle herds in extensive production system were small in size and with little possibility of contact with other infected herds,large herd size is at increased risk of acquiring *Brucella* infection. thus, the herd sizewas found to be significantly associated with seropositivity.The infection is also manifested by abortion and retained placenta in infected cows. The study revealed that, the seroprevalence of brucellosis was significantly associated in cow with history of abortion and placenta retention.Thelow awareness of livetock owners on zoonotic importance of brucellosisand custom of consumption of raw milk, assisting parturition and handling of aborted materials were to be risk factors for human brucellosis. Therefore, the low prevalence of brucellosis in the present study area could serve as source of infection to other cattle of the different herd as there is free movement of animals between herds.

- Community educational program should be carried out targeting brucellosis in the areas to aware livestock owners as well as general public inorder to avoid direct or in direct contact with infected animals and their products.
- Control measures such as isolation or culling of aborted animal, proper disposal of aborted fetus, pasteurization or boiling of milk before consumption should be carried out to reduce risk of infection and transmission of the disease in livestock and human in the study area.
- Further isolation and identification based on biochemical and molecular techniques should be carried out to isolate and identify*Brucella* species and its biovariant dominant in the study area in order to design evidence based disease control measures.

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WZFEDD (Woliata Zone Finance and Economic Development department), (2013): Socioeconomic profile, 2012/13.Woliata Sodo, Ethiopia.

8. ANNEXES

Annex 1: Data recording format for blood sampling

NO	District	PAS	Agro-ecology	HHDS	Herd Size	Breed	Sex	Age	Parity	Mating	R. Status	Abortion	Rfm
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													

Annex 2: Questionnaire survey for the assessment of brucellosis and associated risk factors.

A. Owners house location, Educational Status, Sex and Farming type.

1. Personal Address

Name of Respondent_____ Age____ sex__ Educational level_____ District_____ kebele_____Peasant Association_____

2. Farm type

a. Communal b. Small-scale Commercial

3. Agro ecology

a. Highland b. Midland c. Lowland

4. Household Classification

a. Rural b. Urban c. Peri-urban

5. Number of People Residing in Household

a. Males____ b. Females_____ c. Children <15yrs____

6. Educational Level

a. Illiterate b. grade < 8 c. grade 8-12 d. grade >12

B. Information on production system and management activities and herd trend

1. Major Farming activity

a. Livestock Production b. Crop Production c. Mixed type Production

2. System of Production

a. Intensive b. Semi intensive c. Extensive

3. Purpose of Keeping Cattle

a. Milk b. Meat c. Work (draft) d. Cash from Sells e. Manure f. mixed

4. Is there frequent contact between your animals with other herds?

a. Yes b. No

5. Trend within herd

a. Increasing b. Decreasing c. Stable

6. Housing system

a. Kraal b. Stall (shed) c. Yard d. Others (specify)_____

7. Are calves housed together with adults?

a. Yes b. No

8. Materials used for housing

a. Untreated Wood (bush) b. Treated Wood c. Iron sheets e. Mad f. others

9. Form of housing.

a. Roof b. Solid Well c. Concrete Floor d. Wooden floor

10. Supplementation

a. Roughage (crop residue) b. Minerals (salts)Vitamins c. Bought in Feed d. Others

11. How cattle were watered?

a. Animals go to water b. Water is fetched (provided) c. Both

12. Source of water.

a. Bore hole b. Dam(pond) c. River d. Water well e. Spring f. Municipal

13. Water quality

a. Good (clear) b. Muddy c. Salty d. Smelly

C. information on mating and access to veterinary clinic.

1. Mating system

a. Uncontrolled b. Hand mating c. A.I d. hand mating and AI

2. Access to veterinary services

a. Government clinic b. Private vet c. Extension

D. information on brucellosis and other reproductive disease

1. Have you ever seen reproductive problem in your farm? a. Yes b. No

2. Did you see any abortion in flock of cattle? a. Yes b. No

3. How many animals did you see with abortion at any time within 5 years? ____

4. At what stage pregnancy do you face abortion? _____

5. In which stage of parity abortion is observed? _____

6. Have you seen any retention of placenta after birth? a. Yes b. No

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

8. Is there any vaccination given for abortion? a. Yes b. No

9. Where do you dispense placentas, dead fetus and aborted fetuses?

a. Water canals b. Throwing on field c. Burying d. feed dog

10. What do you do when your animal is infected with such reproductive disease?

a. Separate the infected animal b. Sell to neighbor c. Sell to market d. take to the local veterinarian clinic

11. Do you use separate housing for your cattle? a. Yes b. No

12. Do you separate aborted animal from other? a. Yes b. No

13. Who is responsible in milking? a. Woman b. Man c. Child

14.What do you do the milk produced from your farm?

a. Sell raw milk b. cheese c. butter

15. Do you consume milk? a. Yes b. No

16. Do you boilmilk? a. Yes b. No

17. If yes a. Before consumption b. Boiled before processing

18. Do you keep your animal separated from other flocks during grazing and watering?

a. Yes b. No

19. Do you use mixed grazing and watering with other animals? a. Yes b. No

20. Do you migrate your animals to other area? a. Yes b. No

If yes:

a. To where _____

b. In which season _____

21. Have you seen any lameness in your cattle? a. Yes b. No

22. Do you consume raw meat of cattle? a. Yes b. No

23. Did you see any testicular swelling? a. Yes b. No

E. Information on Knowledge, Attitude and Practices farmers towards brucellosis

1. Have you ever heard about brucellosis ("*Wurja Beshita*" in Amharic)? a. Yes b. No

2. If "yes" from whom?

a. Friend(s) b. Health workers/veterinarians c. Media d. Patient of brucellosis e. family member/people from the village

3. Do you think brucellosis a zoonotic disease? a. Yes b. No

4. Does brucellosis present like other illnesses? a. Yes b. No

5. Is brucellosis a health problem in this area? a. Yes b. No

6. If "yes" since when? a. Recent b. I cannot remember

7. Do you know household member suffered from brucellosis?

a. Yes b. No c. I don't remember

8. If "yes" most affected (sex)

a. Men b. Women c. Both sexes d. I don't know

9. Is brucellosis seasonal?

a. Yes b. No c. I don't know

10. What do you think factors for the exposure or transmission of brucellosis?

a. close contact with infected animal b. consuming raw milk/milk products

c. consumption of raw meat d. contact with fetuses or fetal materials of infected animals e. assisting animal during parturition/abortion f. contact with infected people

11. Which other illnesses?

a. Malaria b. Typhoid c. Tuberculosis d. I do not know

12. Is the brucellosis treatable in humans? a. Yes b. No

13. What do you do an animal suspected of brucellosis? a. selling b. giving medication c. calling veterinarian d. separating e. slaughtering in the house

15. Do you assist the dairy cows during parturition? a. Yes b. No

16. If yes do you use protective gloves(masks) when assisting with the parturition (abortion of animals) hand ling placentas and aborted fetuses? a. Yes b. No

17. What is your practice when an animal aborts?

a. Feeding aborted fetus to dogs b. Giving medications to aborted animal c. Throwing aborted fetus in water canals d. Throwing aborted fetus in streets e. Selling aborted animal in the market f. Slaughtering aborted animal the house g. Selling aborted animal to neighbors h. Calling the local veterinarian i. Selling aborted animal to the butcher j. Separating aborted animal from other animals k. Burning aborted fetus

l. Burying aborted fetus m. Wearing protective gloves when disposing aborted fetus

n. Wearing protective mask when disposing aborted fetus.

Annex 3: Rose Bengal Plate Test (RBPT)principle, material and equipment and procedure

The Rose Bengal Test is one of the buffered *Brucella* antigen tests. It is a rapid agglutination test. The reaction mixture consists of 50 % serum and 50 % antigen (0.5 % phenol-saline suspension of *Brucella abortus* biovar 1, strain 99, inactivated, stained with Rose Bengal and buffered to pH = 3.65 ± 0.05).

Material and equipment

Applicator

Micropipette

Micropipette tips

Plates

Reagent:

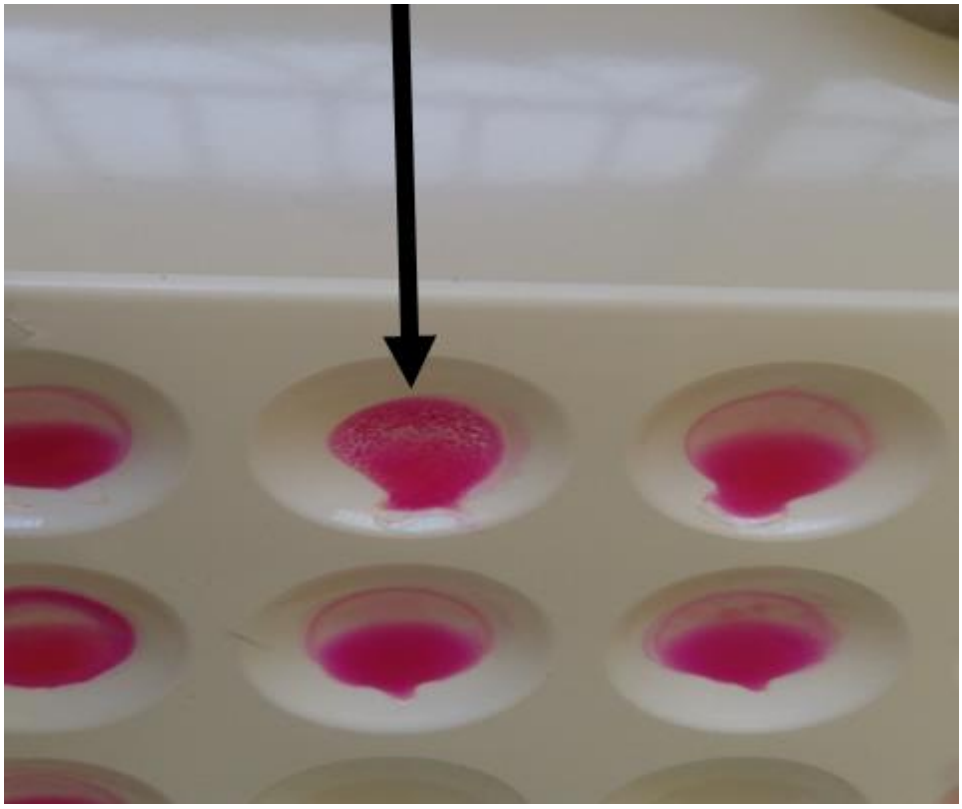
Rose bengal stained antigen

Known positive and negative sera

Procedure

For the RBPT, the procedure described by OIE (2009) was followed. Both serum and rose bengal antigen was removed from the refrigerator and left at room temperature for at least 30 min before the test was performed.*Brucella abortus* antigen Strain 99 was used manufactured by Institut Pourquier, rue de la Galera 34097 Montpellier, France and positive as well as negative control sera from Wolaita Sodo regional Laboratory. Antigen bottle was shaked gently before use. Briefly, 30 µl of sera samples were dispensed on to the plate, and 30 µl of RBPT antigen was droppedalongside the sera. The antigen and serum were mixed thoroughly using an applicator stick (a stick being used used only once). Examine for agglutination in a good light and use magnifying glass when micro agglutination suspected.

Positive and negative controls were employed forinterpretation of the results. Reading is made immediately, with the naked eye and magnifying glass. Agglutinates revealed after 4 minutes. Results of RBPT were interpreted as 0, +, ++, and +++ as hasbeen described by OIE (2009) with 0=no agglutination; +=barely visible agglutination(seen using magnifying glasses); ++=fine agglutination; and +++=coarse agglutination.Those samples with no agglutination (0) were recorded as negative while those with +,++, and +++ were recorded as positive.



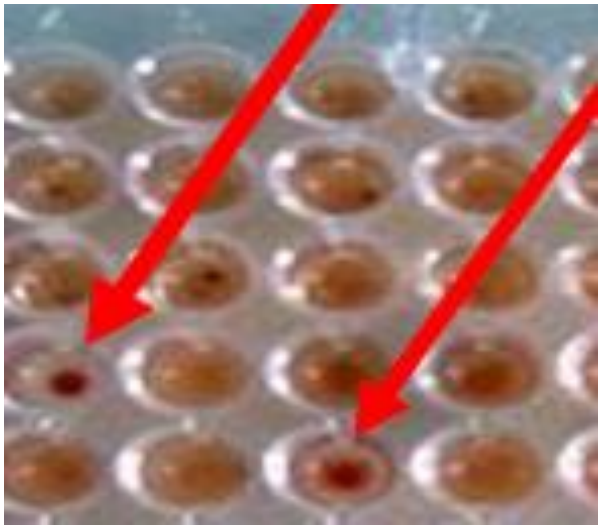
Arrows indicates RBT positive reaction

Annex 4: Complement Fixation Test procedure (CFT)

Procedure

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

thenative complement.Using standard 96-well U-bottom microtitre plates, 25 µl volumes of diluted test serum areplaced in the wells of the first and second rows, and 25 µl volumes of veronal buffered salineare added to all wells except those of the first row.Serial doubling dilutions are then made by transferring 25 µl volumes of serum from thesecond row onwards continuing for at least four dilutions.Repeat steps ii and iii above for each serum to act as anticomplementary serum controls. 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-complementarycontrols. These latter wells receive 25 µl of veronal buffered saline instead.Control wells containing: diluent only, negative serum + complement + diluent, antigen +complement + diluent, and complement + diluent, are set up to contain 75 µl total volume ineach case.The plates are incubated at 37°C for 30 minutes with agitation at least for the initial 10minutes, or at 4°C for 14- 18 hours.Volumes (25 µl) of sensitised SRBC suspension are added to each well, and the plates arereincubated at 37°C for 30 minutes with agitation at least for the first 10 minutes.The results are read after the plates have been left to stand at 4°C for up to 1 hour to allowcells to settle.For interpretation: Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 orat least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classifiedas positive and lack of fixation/complete hemolysis was considered as negative.



Arrows indicates sera with positive reaction

Annex 5: Age determination in cattle based on teeth eruption.

No	Teeth	Ages
1	I_1 erupts	$1_{1/2}$-2 years
2	I_2 erupts	2-$2_{1/2}$ years
3	I_3 erupts	3 years
4	C erupts	$3_{1/2}$-4 years
5	All incisors are wear	5 years
6	I_1 is level and the neck has emerged from the gum	6 years
7	I_2 is level and the neck is visible	7 years
8	I_3 is level the neck is visible	8 years
9	C is level and the neck is visible	9 years
10	The teeth that have not fallen out are reduced to small round pegs	15 years

Source: (De-Lahunta and Habel, 1986).