ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

SEROPREVALENCE AND RISK FACTORS OF SMALL RUMINANT BRUCELLOSIS IN SELECTED DISTRICTS OF ARSI AND EAST SHOA ZONES, OROMIA REGION, ETHIOPIA

BY
ABIOT DEDDEFO

June, 2012
Debre-Zeit, Ethiopia

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A thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Master of Science in Veterinary Public Health

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BY

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ABBREVIATIONS

ELISA	Enzyme Linked Immunosorbent Assay
mRBT	Modified Rose Bengal Test
CMI	Cell Mediated Immunity
S-LPS	Smooth Lipopolysacchride
OIE	Office International des Epizooties
WHO	World Health Organization
CFT	Complement Fixation Test
SAT	Serum Agglutination Test
MRT	Milk Ring Test
iELISA	Indirect Enzyme Linked Immunosrbent Assay
CFSPH	Center for Food Security and Public Health
OPS	O-polysachride
R-LPS	Rough Lipopolysacchride
PCR	Polymerase Chain Reaction
FPA	Fluorescence Polarization Asssay
UK	United Kingdom
USA	United States of America
Ab	Antibody
PA	Peasant Association
OCB	Ovine Caprine Bovine
CMI	Cell Mediated Immunity
CFU	Colony Forming Unit
CSA	Central Statistical Agency
ECHCPD	European Commission Health and Consumer Protection Directorate General
UAE	United Arab Emirates
cELISA	Competitive Indirect Enzyme Linked Immunosrbent Assay
FAO	Food and Agriculture Organization
NAHDIC	National Animal Health Diagnostic and Investigation Center
2-MET	2-Marcaptoethanol Test
OFEDB	Oromia Finance and Economic Development Bureau

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ACKNOWLEDGEMENTS

First of all, I would like to express my heartfelt thanks to my Advisor Dr. Tesfaye Sisay for his valuable suggestions, guidance and encouragingly responsive attitude and support he given me while conducting the research and towards shaping this thesis. Without his assistance, the completion of this thesis would have been hardily possible.

Secondly, I forward my great appreciation and thanks to the management body and workers of Sebeta National Animal Health Diagnostic and Investigation Center for providing me all the equipments for blood collection and allowing me all the facilities for mRBT and ELISA tests. Especially, I never forget the help given to me by Dr. Getachew Tuli (my co-advisor), Dr. Mesfin Sahile (Director), Dr. Getnet Abie, Belachew (lab technician), and Huluagrish (lab technician).
Asella Regional Animal Health diagnostic and Investigation Laboratory staffs deserve deep hearted thank for allowing me all the necessary laboratory equipments during serum separation.

My institute head, Dr. Hunduma Dinka deserves a special thank for giving me permission and encouragement on my work.

This research will not be accomplished successfully without the help of my family. The motherly love and encouragement of my mom, Desu, gave me strength to finish this work. Thank you Mom! My heartfelt thanks also go to my brother, Gemedo for helping me financially during my research work. My wife, father, brothers and sisters deserve great appreciation and thanks for providing me constructive ideas. I thank my uncles and other relatives for their constructive ideas and encouragements they have given me.

Lastly, I acknowledge all the staff of livestock health and development agency, development agents and livestock owners of Tiyo, Dodota sire, and Adami Tulu- Jido Kombolcha districts who assisted me during blood sample collection.

ABSTRACT

A cross-sectional study was conducted between October, 2011 and March, 2012 in Arsi (Tiyo and Dodota Sire districts) and East Shoa (Adami Tulu-Jido Kombolcha district) zones of Oromia region to determine the seroprevalence of brucellosis in small ruminants and to assess possible risk factors associated with the disease. Two stage cluster sampling was employed by which 9 peasant associations were identified as primary sampling units and 131 herds/flocks as secondary units. A total of 840 blood samples (409 sheep and 431goats) were collected from small ruminants of the three districts. All the sera samples were initially screened by modified Rose Bengal Test (mRBT) and all mRBT positive reactors were further tested by Indirect Enzyme Linked Immunosorbent Assay (iELISA) test for confirmation. All the 39(4.6%) sera samples that were positive by mRBT also tested positive for iELISA test. The individual animal and herd level seroprevalence of brucellosis in the study area were 4.6% and 26% respectively. There is a statistically significant difference in seropositivity to the disease between different age groups ($P<0.05$), pregnancy status ($P<0.05$) and parity number ($P<0.05$) in univariable logistic regression. Parity and pregnancy status showed a statistically significant difference ($P<0.05$) upon further analysis with multivariable logistic regression. The difference in seroprevalence between male (6.7%) and female (4.4%) sheep and goats was not statistically significant ($P>0.05$). Risk of seropositivity was also independent of herd/flock size ($P>0.05$) and occurrence of abortion ($P>0.05$). In addition, a total of 80 owners of small ruminants were selected randomly and interviewed using structured questionnaire. Analysis of the questionnaire survey suggested that small ruminant owners in the study area practiced improper handling and disposal of aborted material. Moreover, they lack awareness about the disease and some of them consume raw milk. These practices might greatly contribute to further spread of the disease in their livestock and expose the community to a public health hazard. From management risk factors, seasonal migration of herds was found to be significantly associated ($P<0.05$) with the prevalence of the disease. The results of this study demonstrated that small ruminant brucellosis is widely prevalent in the study areas. Hence, the study suggests the need for implementing control measures and raising public awareness on prevention methods of brucellosis.

Keywords: Brucellosis, Small Ruminant, Zoonosis, Risk Factors, Arsi Zone, East Shoa zone

1. INTRODUCTION

The small ruminant population of Ethiopia is estimated to be nearly 23.33 million goats and 23.62 million sheep playing an important role in the livelihood of resource poor farmers. They provide their owners with a vast range of products and services such as meat, milk, skin, hair, horns, manure and urine for cash, security, gifts, religious rituals etc. In the central highlands of Ethiopia, where mixed crop-livestock production is practiced, small ruminants account for 40% of cash income and 19% of the household meat consumption. Sheep and goats are highly adaptable to broad range of environmental conditions. Moreover, low cost of production, requirement of little land and higher prolificacy made them attractive asset for development. Investment in sheep and goats avoid losses due to high inflation rates that are found in unstable economies of many underdeveloped countries like Ethiopia. This is because sheep and goats provide rapid cash turn over. There is also a growing export market for sheep and goats meat in the Middle East Gulf states and some African countries (Yami and Merkel, 2008).

Despite all these, the country fails to optimally utilize this huge resource as disease take lion's share among several factors that hamper the productivity of the sector such as poor feeding, poor management and low genetic endowment (Yami and Merkel, 2008). One of such diseases that hamper the productivity of small ruminant is brucellosis. Brucellosis is an infectious bacterial disease caused by members of genus *Brucella*. It is a disease of worldwide importance and affects a number of animal species. The different *Brucella* species exhibit host preference and vary in severity of the disease they cause. Brucellosis in small ruminants is mainly caused by *Brucella melitensis,* and rarely by *B. abortus* or *B. suis* (Hirsh and Zee, 1999).

The disease in naturally infected sheep and goats is characterized by abortion, stillbirth, and birth of weak offspring in females and acute orchitis and epididymitis in males (Rodostits *et al.*, 2006). Chronic infections are common (Hirsh and Zee, 1999). The consequences of brucellosis

in small ruminants include infertility, high mortality rate in lambs and kids, mastitis and reduced milk production (Radostits *et al.*, 2006).

Brucellosis is also an important zoonosis causing chronic debilitating disease in humans (Radostitis *et al.*, 2006). Brucellosis is also known as undulant fever, Meditranean fever, and Malta fever. The disease remains a worldwide problem, predominantly in developing countries. In humans, ovine and caprine brucellosis caused by *B. melitensis* is by far the most important clinically apparent disease (CFSPH, 2009). Groups at high risk for Brucellosis are animal health workers, butchers, farmers, and those who are habitually consume raw milk and come in contact with animals. In man, transmission occurs as a result of ingestion of milk, contact via skin abrasion, mucous membranes and inhalation (Radostits *et al.*, 2006).

In Ethiopia, few studies have been done so far on small ruminant brucellosis (Tekelye and Kasali, 1990; Teshale *et al.* 2006; Ashenafi *et al.*, 2007; Ferede *et al.*, 2011; Bekele *et al.*, 2011). Particularly, there is no study done on Small ruminant brucellosis in Arsi zone and few were done in East Shoa zone. There is high population of sheep and goats in these zones. Arsi zone ranked first from all Oromia zones in its sheep population (CSA, 2012). Subsequently, the disease could impose a tremendous economic loss due to reproductive wastages such as infertility, abortion, stillbirth etc. On the other hand, due to their dietary habits and management practices, these communities seem to be at a stake of being infected by the disease. Therefore, this study was initiated to study small ruminant brucellosis in Arsi and East Shoa zones.

Therefore, the objectives of this study are:

- To determine the seroprevalence of small ruminant brucellosis in the study areas
- To assess possible risk factors for seroprevalence of small ruminant brucellosis in the study area

2. LITRATURE REVIEW

2.1. Etiology

2.1.1. Morphology

Brucella are coccobacilli or short rods 0.6-1.5μm long by 0.5-0.7μm in width. They are arranged singly and less frequently in pairs or small groups. After 3-5 days incubation on selective serum agar, colonies are pinpoint, smooth, glistening, bluish, translucent and about 3-4mm in diameter. The morphology of *Brucella* is fairly constant except in old cultures, where pleomorphic forms may be evident. *Brucella* are non-motile. They do not form spores, flagella, or pili. True capsules are not produced. *Brucella* are Gram-negative and usually do not show bipolar staining. They are not truly acid-fast but resist decoloration by weak acids, thus stain red by the Stamp's modification of Ziehl-Neelsen method, which is sometimes used for the microscopic diagnosis of brucellosis from smears of solid or liquid specimens (Quinn *et al.*, 1994; Hirsh and Zee, 1999; ECHCPD, 2001).

2.1.2. Culture and growth characteristics

The growth of *Brucella* is enhanced on enriched media but they are able to grow on nutrient agar. On initial isolation, colonies are not apparent until 3-5 days incubation. Most colonies are detected by 10-14 days, but in some cases incubation for up to 21 days is required. Growth is best in an aerobic environment at 37^0c but can occur between 20^0c and 40^0c. *Brucella abortus* requires supplementation of 5-10% CO_2 for growth, but *B. melitensis* does not require supplementation of CO_2. The optimum pH for growth varies from 6.6 to 7.4 and culture media should be adequately buffered near pH 6.8 for optimum growth. The colony morphology of *Brucella* varies from rough to smooth forms. *B. abortus*, *B. melitensis*, *B. suis* and *B. neotome* are typically isolated in smooth form. *B. ovis* and *B. canis* are always in a rough form (Hirsh and Zee, 1999) (Table 1).

The growth of most *Brucella* strains is inhibited on media containing bile salts, tellurite or selenite. Growth is usually poor in liquid media unless culture is vigorously agitated. Growth in static liquid media favours dissociation of smooth-phase cultures to non-smooth forms. In semisolid media, CO_2-independent *Brucella* strains produce a uniform turbidity from the surface down to a depth of a few millimetres, while cultures of CO_2-requiring strains produce a disk of growth a few milli meters below the surface of the medium. On suitable solid media, *brucella* colonies are visible after 2 days incubation. After 4 days incubation, *brucella* colonies are round, 1-2 mm in diameter, with smooth margins, translucent and a pale honey colour when plates are viewed in the daylight through a transparent medium. When viewed from above, colonies appear convex and pearly white. Later, colonies become larger and slightly darker. Smooth *Brucella* cultures, especially *B. melitensis* cultures, have a tendency to undergo variation during growth, especially with subcultures, and dissociate to rough forms, and sometimes mucoid forms. Changes in the colonial morphology are generally associated with changes in virulence, serological properties and phage sensitivity. Brucella do not grow on MacConkey agar (Quinn *et al.*, 1994; ECHCPD, 2001).

2.1.3. Biochemistry

Brucella is catalase and oxidase positive (*B. ovis* and *B. neotomae* are oxidase negative) and they reduce nitrate to nitrite except *B. ovis*. Urease activity varies from rapid to slow. *Brucella* does not cause haemolysis on blood agar, does not produces acid on agar containing glucose, and does not ferment lactose, usually grow in the presence of basic fuchsin and thionin at standard concentration (Quinn *et al.*, 1994; Hirsh and Zee, 1999; ECHCPD, 2001).

2.1.4. Antigenic characteristics

Brucella species, except for *B. ovis* and *B. canis*, contain smooth lipopolysaccharide (S-LPS) in their outer cell wall. Smooth lipopolysaccharide contains an immunodominant O-polysaccharide (OPS). *Brucella ovis* and *B. canis* lack the OPS component and as a result, their outer surface

contains only rough lipopolysaccharide (R-LPS) and protein antigens. Because all smooth species share common epitopes in the OPS, virtually all serological tests for antibody to these bacteria use *B. abortus* antigen while R-LPS is commonly used as the main antigen for detection of antibody to *B. ovis* and *B. canis* (Poester *et al.*, 2010). Serological cross-reactions have been reported between smooth *Brucella* and various other Gram negative bacteria, eg. *Escherichia coli* O: 116 and O: 157, *Salmonella* group N (O: 30), *Pseudomonas multophilia*, *Vibrio cholarae* and especially *Yersinia enterocolitica* O: 9. These organisms can induce significant levels of antibodies which cross react with S-LPS *Brucella* antigens in diagnostic tests (Radostits *et al.*, 2006; ECHCPD, 2001).

2.1.5. Susceptibility to phages

Over 40 *Brucella* phages have been reported to be lytic for *Brucella* members. All phages are specific for the genus *Brucella*, and are not known to be active against any other bacteria that have been tested. Thus, lysis by *Brucella* phages is a useful test to confirm the identity of *Brucella* spp. and for speciation within the genus. The *Brucella* phages currently used for *brucella* typing are: Tbilisi (Tb), Weybridge (Wb), Izatnagar1 (Iz1) and R/C. The three former phages are used for differentiation of smooth *Brucella* species. R/C is lytic for *B. ovis* and *B. canis* (Radostits *et al.*, 2006; ECHCPD, 2001).

2.1.6. Susceptibility to dyes and antibiotics

Susceptibility to the dyes, thionin and basic fuchsin (20 µg/ml), which varies between biovars, is one of the routine typing tests of *Brucella*. *B. melitensis* grows in the presence of both dyes. On primary isolation, *Brucella* are usually susceptible *in vitro* to gentamicin, tetracyclines and rifampicine. Most strains are also susceptible to the following antibiotics: ampicillin, chloramphenicol, cotrimoxazole, erythromycin, kanamycin, novobiocin, spectinomycin and streptomycin, but variation in susceptibility may occur between species, biovars and strains. Most strains are resistant to ß-lactamins, cephalosporins, polymyxin, nalidixic acid, amphotericin

B, bacitracin, cycloheximide, clindamycin, lincomycin, nystatin and vancomycin at therapeutic concentrations (Hirsh and Zee, 1999; ECHCPD, 2001).

2.1.7. Taxonomy of *Brucella* species and biovars

Considering their high degree of DNA homology (> 90 % for all species), *Brucella* have been proposed as a monospecific genus in which all types should be regarded as biovars of *B. melitensis*. This proposal has not yet met with complete agreement and the old classification of the genus is used world-wide. Nine *Brucella* species are currently recognized, seven of them that affect terrestrial animals are: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, and *B. microti* and two that affect marine mammals are: *B. ceti* and *B. pinnipedialis* (Seleem *et al.*, 2010). Recently described *Brucella, Brucella pinnipedialis* affect seals, sea lions, and walruses; whereas, *Brucella ceti*, are predominantly isolated from whales, dolphins and porpoises. This classification is based mainly on differences in pathogenicity and host preference (Godfroid *et al.*, 2010; Glynn et al., 2008). For *B. melitensis, B. abortus* and *B. suis*, the identification at the biovar level is currently performed by four main tests; carbon dioxide (CO_2) requirement, production of hydrogen sulphide (H_2S), dye (thionin and basic fuchsin) sensitivity, and agglutination with monospecific A and M anti-sera. Several methods, mainly PCR, have been employed to find DNA polymorphism which would enable the molecular identification and typing of the *brucella* species and their biovars (Godfroid *et al.*, 2010; ECHCPD, 2001).

In sheep and goats, brucellosis is mainly caused by *Brucella melitensis. B. melitensis* contains three biovars (biovars 1, 2 and 3). All three biovars cause disease in small ruminants, but their geographic distribution varies. *B. melitensis* infections are also been reported occasionally in cattle, camels and dogs, and rarely in horses and pigs. Infections in sheep and goats can spill over into wild ruminants; *B. melitensis* infections have been reported in alpine ibex in Italy and chamois in the French Alps (CFSPH, 2009).

Table 1: *Brucella* species and biovars, preferential hosts and pathogenecity for humans

Species	Biovars	Colony morphology	Preferencial host(s)	Pathogenesity in man
B. melitensis	1-3	smooth	sheep, goat	High
B. abortus	1-6,9	smooth	cattle	High
B. suis	1,3	smooth	Pig	High
	2	smooth	wild boar, hare	Low
	4	smooth	reindeer, caribou	High
	5	smooth	rodent	No
B. neotome		smooth	desert rat	Moderate
B. ovis		rough	ram	No
B. canis		rough	dog	Moderate
B. pinnipedialis		smooth	seal	Mild
B. ceti		smooth	cetacean	Mild
B. microti		smooth	vole, fox	No

Source: Godfroid *et al.* (2010); Atluri *et al.* (2011)

2.2. Pathogenesis

Following exposure, *Brucella* penetrates intact mucosal surfaces. In the alimentary tract, the epithelium covering the ileal payer's patches are a preferred site for entry. The major route of infection appears to be through the mucous membranes of the oropharynx and upper respiratory tract or the conjunctiva. Other potential routes of infection are through the mucous membranes of the male or female genital tract (Hirsh and Zee, 1999).

Soon after entry into the host, the *Brucella* are engulfed by phagocytic cells in which they survive, multiply and transported to the regional lymph nodes. The fate of invading bacteria is

mainly determined by the cellular defenses of the host, chiefly macrophages and T- lymphocytes, though specific antibody undoubtedly plays a part. The organisms may be engulfed by phagocytic cells. Specific receptors on macrophages appear to mediate attachment and uptake of *Brucella*. Various mechanisms are employed by *Brucella* to allow survival inside phagocytic cells. They are capable of surviving and multiplying inside macrophages by inhibiting phagolysosome fusion. Brucella posses an endotoxin that contribute to the pathogenesis (Quinn *et al.*, 19994; Hirsh and Zee, 1999).

Following entry into the host, *Brucella* organisms, either free in the extracellular environment or in phagocytic cells, localize to regional lymph nodes. There they proliferate and infect other cells or are killed and infection is terminated. From regional lymph nodes, *Brucella* disseminates hamatogenously and localize in the reticuloendothelial system and reproductive tract. Brucellosis is essentially a disease of sexually mature animal, the predilection site being the reproductive tracts of male and female, especially the pregnant uterus. Unknown factors in the gravid uterus, collectively known as allantoic fluid factors, stimulate the growth of *Brucella.* The factors include Erythritol, possibly steroid hormones and other substances. Erythritol is present in the placenta and genital tract of cattle, sheep, goats, and pigs but not humans. A pyogranulomatous reaction occurs in affected placenta and abortion occurs from mid-gestation onwards. The virulence of *Brucella* varies considerably according to species, strain and the size of infecting inoculum. Host susceptibility is also variable and is associated with the reproductive status (Quinn *et al.*, 1994; Hirsh and Zee, 1999).

During this first stage of infection, the major clinical sign is abortion but other signs due to a localisation of *Brucella* may be observed. However, numerous animals develop self-limiting infections or they become asymptomatic latent carriers and potential excretors. Females generally abort once, after which a degree of immunity develops, and the animals remain infected and large numbers of *Brucella* can be excreted in foetal fluids at subsequent parturitions. The second stage is characterized by either elimination of *Brucella* or, more frequently, by a persistent infection of mammary glands and supramammary and genital lymph nodes with

constant or intermittent shedding of the organisms in the milk and genital secretions (Quinn *et al.*, 1994; Radostits *et al.*, 2006).

2.3. Immunity

Infection with *Brucella* usually results in the induction of both humoral and cell-mediated immune responses, but the magnitude and duration of these responses is affected by various factors including the virulence of the infecting strain, the size of infecting inoculum, pregnancy, sexual and immune status of the host (Poester *et al.*, 2010).

2.3.1. Humoral immunity

Following infection by natural exposure, a serological response can be expected within 2 to 4 weeks, but the response is variable and may be absent altogether. Invasion of the pregnant uterus can be expected to produce a large and persistent rise of antibodies, but this may be delayed until after abortion or parturition at the normal time. Invasion of the lactating udder causes a lesser serological response, and localization confined to a small number of lymph nodes may fail to stimulate any response at all, or only a minimal one. The serological response is transient and sometimes missing in young sexually immature animals (Radostits *et al.*, 2006; Poester *et al.*, 2010).

The pattern of the serological response in terms of immunoglobulin production has not been extensively studied in sheep and goats, but available information suggests close similarity to that in cattle. The antibody response to *Brucella* in animal consists of an early IgM isotype, the timing of which depends on route of exposure, the dose of bacteria and the health status of the animal. The IgM response is followed almost immediately by the production of IgG1 antibody, later by small amount of IgG2 and IgA. Most cross reacting antibody, which is antibody resulting from exposure to microorganisms other than *Brucella* species or environmental antigen consist mainly IgM. Serological test that measure IgM are therefore not desirable as false

positive results occur, leading to low assay specificity. IgG2 and IgA antibodies accumulate later after exposure and are usually present in small and inconsistent amounts. Therefore, assays that predominantly measure IgG1, are the most useful. Many serological diagnostic tests have been developed for brucellosis with the purpose of screening, confirmation and assays that also distinguish vaccinal antibody (Poester *et al.*, 2010).

B. melitensis Rev.1 vaccine strain when applied under standard conditions (full dose via the subcutaneous route in young replacement animals) may induce a long lasting serological response to the agglutination tests that seriously interferes with serological screening for infected animals (Seleem *et al.*, 2010).

2.3.2. Cell mediated immunity (CMI)

Immunity against brucellosis is principally mediated by cellular immune responses since it is an intracellular pathogen (Radostits *et al.*, 2006). After gaining entrance into the body, the organisms encounter the cellular defenses of the host and the fate of invading bacteria is mainly determined by the cellular defenses of the host, in particular macrophages and T-lymphocytes. Specifically sensitized T-lymphocytes release cytokines that activate macrophages which in turn control *Brucella* by reactive oxygen intermediates. Like the humoral response and depending on the method used to measure the CMI, the response can be expected as rapidly as a few weeks, but the response is variable and may not be detected. A more effective immunity develops when animals are infected prior to sexual maturity (Hirsh and Zee, 1999).

2.4. Clinical findings and lesions

2.4.1. Acute brucellosis

The predominant clinical manifestations of brucellosis in naturally infected sheep and goats are, as in all female ruminants, reproductive failure i.e. abortion, stillbirths and birth of weak

offspring. There may be a 'storm' of abortions when the disease is introduced in a naïve herd or flock (Radostits *et al.*, 2006). In general, animals do not exhibit overt systemic illness. Abortion generally occurs during the last 2 months of pregnancy and retention of foetal membranes is a possible sequela. Females generally abort once, although reinvasion of the uterus occurs in subsequent pregnancies and *Brucella* organisms are shed with the membranes and fluids. In the male, epididymitis and orchitis are the most common presenting signs and bacteria may be shed in the semen. Arthritis is also observed occasionally in both sexes. There is no evidence that the clinical features of *B. melitensis* infection in sheep and goats vary according to the biovar involved. In goats, *B. melitensis* infection results in acute mastitis with palpable nodules in the udder and watery milk (Hirsh and Zee, 1999).

2.4.2. Chronic brucellosis

Non-pregnant animals exposed to small numbers of organisms may develop self-limiting, immunizing infections or they may become latent carriers. Persistent infection of the mammary glands and supramammary lymph nodes is common in goats with constant or intermittent shedding of the organisms in the milk in succeeding lactations, while the self-limiting nature of the disease in sheep, which is seldom accompanied by prolonged excretion of the bacteria, has been observed. The inflammatory changes in the infected mammary gland reduce milk production by an estimated minimum of 10% (Radostits *et al.*, 2006).

2.4.3. Macroscopic and microscopic lesions

There are grossly visible lesions in the placenta associated with brucella abortions. The cotyledons are frequently necrotic, thickened, yellow gray in color, and covered with thick brown exudates. The degree of necrosis varies among *Brucella* species with *B.melitensis* infection in goats most severe. The aborted fetus is frequently edematous. The most common histological findings in the fetus are bronchitis and bronchopneumonia. *Brucella* infected animals generally develop granulomatous inflammatory lesions which frequently are found in lymphoid tissues and organs such as reproductive organs, udder and supramammary lymph

nodes and sometimes joints and synovial membranes. The lesions when present are not pathognomonic. The following could be observed: necrotising placentitis, palpable testicular alterations, necrotising orchitis and epididymitis with subsequent granuloma, necrotising seminal vesiculitis and prostatitis. In males palpable enlargement of epididymis, especially involving the tail region, is common. Epididymal lesions are characterized by hyperplasia and hydropic degeneration of the tubular epithelium. The testicular parenchyma becomes necrotic and is sometimes replaced by pus. Acute mastitis with palpable nodules and the production of clotted and watery milk may occur (Hirsh and Zee, 1999).

2.5. Epidemiology

The *Brucella* are somewhat host-specific but cross-species infections occur, especially with *B. melitensis* (WHO, 2006) (Table 1 and 2). Goats and sheep are highly susceptible to *B. melitensis* infection. Susceptibility in sheep varies with the breed. The organism is capable of causing disease in cattle and has been isolated from pigs. The prevalence of infection varies between countries and regions but in many countries prevalence has declined in the past decade in association with mandatory vaccination policies (Radostits *et.al.*, 2006).

Table 2: Animals affected by *Brucella* species

Host	*B. abortus*	*B. melitensis*	*B. suis*	*B. canis*	*B. ovis*
Cattle	+	+	+(rare)	_	_
Sheep	+(rare)	+	+(possible)	_	+
Goats	+(rare)	+	_	_	_
Camels	+(rare)	+	_	_	_
Dog	+	+	+(rare)	+	_
Swine	+(rare)	+(rare)	+	_	_
Horse	+	+(rare)	+(rare)	_	_
Buffalo	+	+	_	_	_
Carribou/ Reindeer	_	_	+(biovar 4)	_	_
Elk	+	_	_	_	_
Bison	+	_	_	_	_
Rodents	+(rare)	+(rare)	+(biovar 5)	_	_

Source: WHO (2006)

2.5.1. Global distribution

While brucellosis has been eradicated in most industrialized regions, its occurrence is increasing in developing countries. *B. melitensis* is particularly common in the Mediterranean. It also occurs in the Middle East, Central Asia, around the Arabian Gulf, and in some countries of Central and Latin America. The disease also occurs in Africa and India. Prevalence rate shows variation from place to place, which could be due to different factors such as herd size, management, and biology of the disease (Radostits *et.al.* 2006). However, North America (except Mexico) is believed to be free, as are Northern Europe (except for sporadic incursions from the south), Southeast Asia, Australia and New Zealand. Of the three different biovars of *B. melitensis* biovar 3 predominates almost exclusively in Mediterranean countries and Middle East, while biovar 1

seems to predominate in Latin America. The biovars 1 and 2 have also been reported in some southern European countries (ECHCPD, 2001).

The seroprevalence carried out in different African and Middle East countries revealed prevalence rates ranging from 0.3 to 21% in goats and 0.6% to 22.8 in sheep, of which, most prevalence rates fall less than 10% at individual animal level. The outbreaks occurred in Algeria in 1989 showed seropositivity of 2.18% in sheep and 12% in goats, and at the herd level the prevalence has 43.5% and 42%, respectively, while in Tunisia in 1990 in a place called Gafse, a herd prevalence rate of 61% in goats and 30% in sheep are recorded. In Morocco, in 1996 an outbreak occurred showing flock prevalence of 12.1% and 2.4%, respectively, in sheep and goats. In Jordan, *B. melitensis* infection is probably the most serious zoonosis. The prevalence of brucellosis in small ruminants in Jordan for 1992-1995 in sheep was 8.2% on individual level and 47.7% on flock level; in goats, the prevalence was 3.7% on individual level and 25.2% on herd level. The incidence of brucellosis in Saudi Arabia increased in the years 1986-1988 from 5.7 to 26.0% in sheep and goats (Refai, 2002).

Table 3: The prevalence of small ruminant brucellosis in different countries

Country	Sheep (%)	Goats (%)	Year
Morocco(East)	1.6	4.1	1997
Tunisia(Nation wide)	4.0	18.0	1992
Israel(Intensive)	8.2		1993
Egypt	2.4	8.2	1998
Iran	3.0	3.0	1998
Khartoum, Sudan	14.2	16.7	recent
Algeria		1.5	1998
syria	1.8		1988
UAE	2	3.4	1990
Yemen	0.6	0.4	1985
Nigeria(plateau state)	14.5	16.1	2010

Source: Benkirane (2006); Refai (2000)

2.5.2. Distribution in Ethiopia

In Ethiopia, few studies have been conducted on brucellosis in small ruminants. Tekelye and Kasali (1990) reported prevalence proportions of 1.5% in sheep and 1.3% in goats in the central highlands of Ethiopia. Yibeltal (2005) recorded prevalence proportions of 15% in sheep and 16.5% in goats in the Afar region and 1.6% in sheep and 1.7% in goats in the Somali region. Ashenafi *et al.* (2007) reported the prevalence rate of 5.8% and 3.2% in goat and sheep respectively in pastoral regions of Afar. Ferede *et al* (2011) reported the prevalence rate of 0.87% and 0% in and around Bahir Dar in goat and sheep respectively. Ashagre *et al.* (2011) reported prevalence rate of 4.2% brucellosis in goat in South Omo zone. However, in general, the status of small-ruminant brucellosis in Ethiopia is not well studied.

Table 4: Seroprevalence of brucellosis in different parts of Ethiopia and East African counties

Country/region	Sheep (%)	Goats (%)	References
Central Ethiopia	1.5	1.3	Teklaye and Kasali (1990)
Ethiopia (Afar region)	15	16.2	Yibeltal (2005)
Somali region	1.6	1.7	Yibeltal (2005)
Eritrea	1.4	3.8	Omer *et al* (2000)
Kenya	6.01		Waghela (1976)
Somalia	7.2	5.29	Falade and Husein (1997)
Bahir Dar (In and around)	0	0.87	Ferede *et al.* (2011)
Omo zone	4.2		Ashagrie *et al.* (2011)
Pastoral region of Afar	3.2	5.8	Ashenafi *et al.* (2007)
Jijiga	3.2	5.8	Bekele *et al.* (2011)
South Wollo	1.5		Yesuf *et al.* (2010)

2.5.3. Risk factors

2.5.3.1. Management risk factors

Management practices directed at eliminating infected animals and minimizing exposure to aborted tissues greatly reduce the incidence of the disease in commercial livestock operations. Management risk factors for high occurrence of infection are high stocking densities, poor housing and lambing/kidding, lack of sanitary measures (cleaning and disinfection), large herd sizes, communal grazing areas and water sources. The unrestricted movement of animals and personnel can facilitate the transmission of brucellosis between herds. In several countries there is a strong correlation between the prevalence of brucellosis in small ruminants and the practice of transhumance (Kusiluka and Kambarage, 1996; Radostits *et al.*, 2006).

2.5.3.2. Host risk factors

Susceptibility to infection depends on age, sex, breed and pregnancy status of the animals. Younger animals tend to be more resistant to infection and frequently clear infections although latent infections do occur. However, susceptibility increases after sexual maturity and especially with pregnancy. Only 2.6% of animals infected at birth remain infected as adults. Most animals infected as adults remain infected for life (Hirsh and Zee, 1999). No control studies have been conducted on the relative susceptibility of female and male sheep to brucellosis, nonetheless, based on the reactor rates it is probable that rams are more resistant than sexually mature female sheep, and less resistant than sexually immature lambs (Nicoletti, 1980; Radostits *et al.*, 2006). Breed may also affect susceptibility, particularly in sheep. The milking breeds seem to be the most susceptible to *B. melitensis.* Goats are highly susceptible to *B. melitensis* infection as compared to sheep (WHO, 2006).

2.5.3.3. Pathogen risk factors

B. abortus is a facultative intracellular parasite capable of multiplication and survival within host phagocytes. The organisms are phagocytosed by polymorphonuclear leukocytes in which some survive and multiply. These are then transported to lymphoid tissues and fetal placenta. The inability of the leukocytes to effectively kill virulent *Brucella* at the primary site of infection is a key factor in the dissemination to regional lymph nodes, reticuloendothelial system, and organs such as the uterus and udder. The organism is able to survive within macrophages because it has the ability to survive phagolysosome. The bacterium possesses an unconventional non-endotoxic lipopolysaccharide, which confers resistance to antimicrobial attacks and modulates the host immune response. These properties make lipopolysaccharide an important virulence factor for *Brucella* survival and replication in the host. *Brucella*s are able to survive within host leukocytes and may utilize both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanisms during the periods of hematogenous spread (Hirsh and Zee, 1999; Radostits *et al.*, 2006).

Brucella survives freezing and thawing. Under proper environmental conditions, they survive for up to 4 months in the milk, urine, water and damp soil (Hirsh and Zee, 1999). In liquid manure, the organism survives for months, in humid faeces for 22 weeks, in aborted fetus up to 4 months and in the dust for 44 days, in tap water for 30 days, in sterile water for 51 days, in desert soil up to 2 months, and in frozen soil for two years. Contaminated straw remains infectious for longer than a month. *Brucella* organisms do not multiply in the environment, but they persist and their survival is influenced by the environmental factors. The resistance of *Brucella* species to the environmental factors depends on whether they are protected against the sunlight and high temperature. Normal soil pH and moist environments, which are rich in organic materials, are favorable for the survival of the organism (Radostits *et al.*, 2006).

2.6. Transmission and source of infection

2.6.1. Routes of excretion and source of infection

The source of infection is the infected carrier animal. Materials excreted from the female genital tract forms the main supply of organisms for transmission to other animals and man. Therefore, in most circumstances, the primary route of dissemination of *Brucella* is the placenta, foetal fluids and vaginal discharges expelled by infected ewes/does after abortion or full-term parturition. In goats, excretion of the organisms from the vagina is prolonged and copious (2 to 3 months generally). In sheep excretion is generally less prolonged, usually ceasing within 3 weeks after abortion or full-term parturition. Shedding of *Brucella* is also common in milk and semen which can be prolonged or lifelong. The persistent infection of the mammary glands and supramammary lymph nodes leads to a constant or intermittent shedding of the organisms in the milk in succeeding lactations. It provides an important source of infection for man and young animals. Kids and lambs that nurse from infected dams may shed *B. melitensis* in the feces (Hirsh and Zee, 1999; CFSPH, 2009).

2.6.2. Modes of infection

Routes of infection for both adults and young are via ingestion, by nasal or conjunctival infection, and through skin abrasions, with infected placenta and uterine discharge as a major source (Radostits *et al.*, 2006). *Brucella* is disseminated by direct or indirect contact with infected animal. Animals become infected directly by infected aerosols or by uptake of infected material. Another mode of infection is grazing pastures where infected animals mix with brucellosis-free animals or get in touch with contaminated premises, manure, materials etc. In the male, localisation in the reproductive organs generally results in the shedding of *Brucella* in the semen. Venereal transmission is possible, but seems to be uncommon during natural mating. Experimental work with cattle has demonstrated that embryo transfer is unlikely to be a method of transmission when embryo washing and other hygienic measures are taken. Embryos can be

moved from infected donors to healthy recipients with minimal risk. Dogs have been shown to be mechanical and biological vectors of brucellosis (Hirsh and Zee, 1999; CFSPH, 2009).

Human brucellosis is transmitted by inhalation, animal contact and consumption of unpasteurized dairy products. Organisms can be found in meat but at lower concentrations, and meat contamination is rarely a public health risk when meat products are properly handled and cooked (Gwida *et al.*, 2010). Other routes, including *in utero* transmission, person-to-person transmission, and tissue transplantation associated transmission have been identified or suggested but are much less common. Brucellosis can develop after accidental injection with live *Brucella* vaccines and is thus an occupational hazard for veterinarians. In addition, brucellosis is one of the most commonly acquired bacterial laboratory infections worldwide, in part because of its low infectious dose and ease of aerosolization, which is exacerbated by outdated laboratory practices such as plate sniffing (Glynn *et al.*, 2008).

2.6.3. Vertical transmission

B. melitensis can be transmitted from the dams to lambs or kids. A small proportion of lambs or kids can be infected *in utero* but the majority of infections are probably acquired by consumption of colostrum or milk. It is also probable that a self-cure mechanism similar to that suggested in cattle takes effect in most of the infected lambs. In that case, lambs and kids remain fully susceptible when they reach sexual maturity (Radostits *et al.*, 2006).

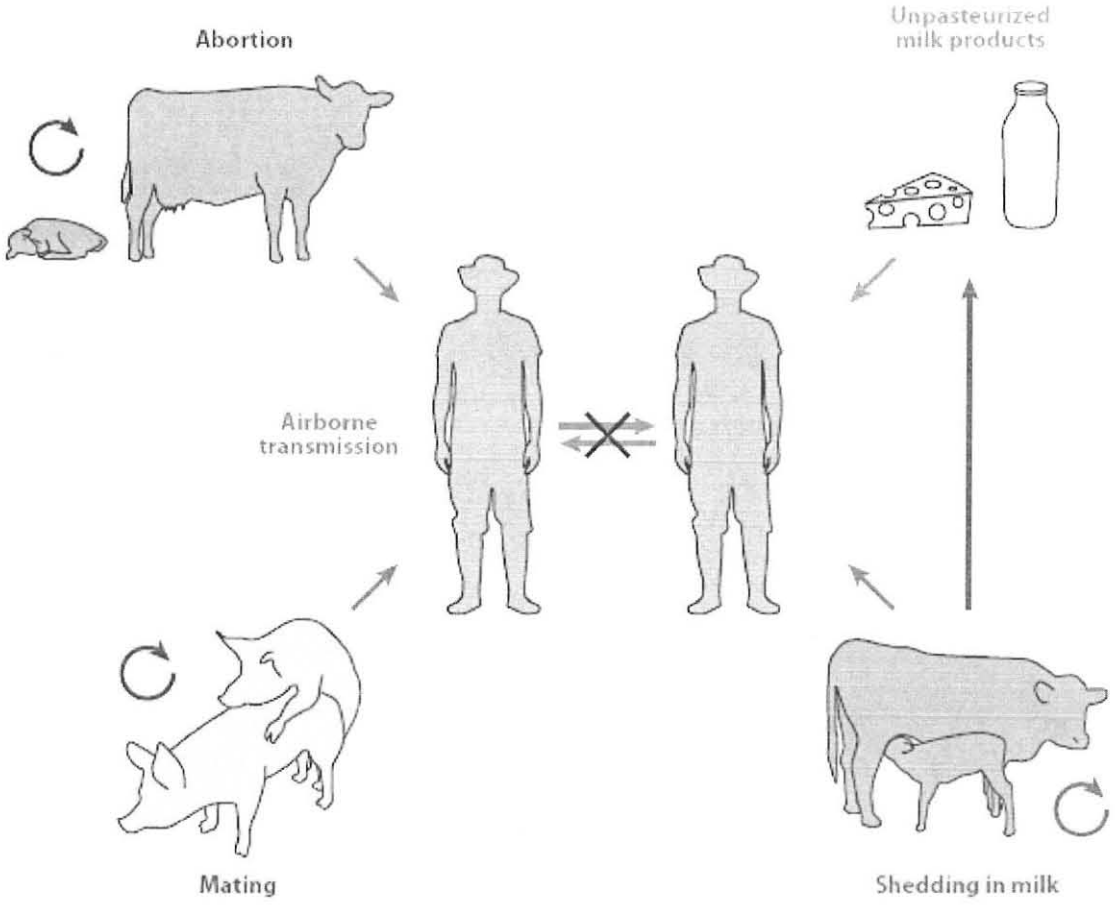
2.6.4. Survival of *B. melitensis* in the environment

The ability of *Brucella* to persist outside mammalian hosts is relatively high compared with most other non-sporing pathogenic bacteria, under suitable conditions. Thus, when pH, temperature and light conditions are favourable, *i.e* pH>4, high humidity, low temperature and absence of direct sunlight, *Brucella* may retain infectivity for several months in water, aborted foetuses and foetal membranes, faeces and liquid manure, wool, hay, on buildings, equipment and clothes. *Brucella* are able to withstand drying particularly in the presence of extraneous organic material

and will remain viable in dust and soil. Survival is prolonged at low temperatures, especially below 0°C. Contaminated equipment can be sterilised by autoclaving (121°C). Chemical treatment is recommended to destroy *Brucella* in contaminated premises. Xylene (1ml/litre) and calcium cyanamide (20 kg/m^3) have been found to be effective in liquid manure after 2-4 weeks. One hour treatment with 2.5% sodium hypochlorite, 2-3% caustic soda, 20% freshly slaked lime suspension, or 2% formaldehyde solution will suffice to destroy *Brucella* on contaminated surfaces (Quinn *et al.*, 1994; Radostits *et al.*, 2006).

The survival of *brucella* in milk and dairy products is related to a variety of factors including the type and age of product, humidity level, temperature, changes in pH, moisture content, biological action of other bacteria present and conditions of storage. At low concentration in liquid media, *Brucella* are fairly heat-sensitive. Thus, dilute suspensions in milk are readily inactivated by pasteurisation or by prolonged boiling (10 min). Previous pasteurisation of milk or cream is the only means to ensure safety of these products. *Brucella* are fairly sensitive to sterilizing doses of gamma-rays. In contrast to dairy products, the survival time of *brucella* in meat seems extremely short, except in frozen carcasses where the organism can survive for years. The number of organisms per gram of muscle is small and rapidly decreases with the pH drop of the meat (Quinn *et al.*, 1994; Radostits *et al.*, 2006).

Fig. 1: Transmission ways of brucellosis



Source: Atluri *et al.* (2011)

2.7. Diagnosis

Diagnosis of brucellosis is made possible by direct demonstration of the causal organism using staining, immunofluorescent antibody, culture, animal inoculation and polymerase chain reaction (PCR) and indirectly by demonstration of antibodies using serological techniques (Alton *et al.*, 1975).

2.7.1. Microscopic examination

The presumptive bacteriological diagnosis of *B. melitensis* can be made by means of the microscopic examination of smears from vaginal swabs, placentas or aborted foetuses stained with the Stamp's modification of the Ziehl-Neelsen method. However, abortive agents such as *B. ovis, chylamydia psittaci* or *Coxiella brunetti* are also stained red (Poester *et al.*, 2010; Godfroid *et al.*, 2010). Accordingly, the isolation of *B. melitensis* on appropriate culture media is recommended for an accurate diagnosis (Hirsh and Zee, 1999; Gwida *et al.*, 2010).

In the modified Ziehl-Neelsen method, the smear is dried and fixed over a flame, stained with carbol fuchsin for 10 minutes, washed in tap water, flooded with 0.5 % acetic acid for 30 seconds, washed thoroughly and counter stained using 1% methylene blue for 20 seconds. *Brucella* stained red with a blue background (Alton *et al.*, 1975).

Brucella spp. can be isolated on a variety of plain media, or selective media such as Farrell's medium or Thayer Martin's modified medium. Enrichment techniques can also be used (CFSPH, 2009).

2.7.2. Immunofluorescent Antibody Method

An anti-*Brucella* serum conjugated with two-resicin isothiocyanate fluorescent antibody is used. This is then applied on a smear on a thin microscope slide, which is fixed by acetone for 10 minutes. The preparation is allowed to react for 30 minutes to 1 hour at 37 ^{o}C in closed humidity container. If *Brucella* is present in the smear a green-yellow fluorescent appears (Alton *et al.*, 1975).

2.7.3. Animal Inoculation

Animal inoculation is the most sensitive method for detection of Brucella and is sometimes necessary when very low number of organisms is present. Guinea pigs are the most sensitive

laboratory animals. Two guinea pigs are inoculated intramuscular 0.5-1.0 ml of suspected tissue homogenate and are sacrificed at 3 and 6 weeks post-inoculation and serum is taken along with spleen and other abnormal tissue for serology and bacteriological examination, respectively (Hirsh and Zee, 1999).

2.7.4. Bacterial culture and biochemical method

The "gold standard" in the diagnosis of brucellosis is bacterial isolation, which requires long cultivation periods and is often unsuccessful. Isolation of the agent from a single animal is sufficient evidence to establish the infection status of a herd (Alton *et al.,* 1975; Quinn *et al.,* 1994; Gwida *et al.*, 2010). Isolation and identification of the *Brucella* is the only diagnostic procedure not subjected to equivocation, however, negative culture result is not sufficient to rule out infection (Weidman, 1991).

Brucella is pathogenic to man and great care should be exercised in handling materials suspected, if possible examination of culture especially of fluid media should be carried out in safety cabinet. Culture of placenta, cotyledon, vaginal discharge, fetal materials, lymph nodes and other should be made on solid medium since it facilitates recognition and isolation of developing colonies and limit the establishment of non-smooth mutants. *Brucella* grow well on 5-10% blood agar; however the specimens are likely to contain many contaminating bacteria and fungi, so selective media are usually required. The selective media contain a nutritive blood agar base with 5% sterile seronegative equine or bovine serum and an antibiotic supplement. There are many varieties of suitable media. Some of these are serum dextrose agar (SDA), tryptose soy agar (TSA), liver infusion serum agar and special media containing antibiotics to inhibit the growth of other bacteria present in samples. *Brucella* also grows on blood agar (WHO, 1971; Godfroid *et al.*, 2010).

The materials of choice for sampling from animals include stomach contents, spleen and lung from aborted fetuses, placentomes, fetal membranes, vaginal swabs, milk, semen and arthritis or hygroma fluids from adult animals. From animal carcasses, the preferred tissues for culture are the mammary, medial and internal iliac, retropharyngeal, parotid and prescapular lymph nodes

and spleen. All specimens must be packed separately and transported immediately to the laboratory cooled or preferably frozen in leak proof containers. For humans, blood for culture is the material of choice (Poester *et al.*, 2010).

2.7.5. Serological tests

The diagnostic method known to produce the best results in terms of specificity is the isolation of *Brucella* organisms from the suspected animal. However, this method has a limited sensitivity, is expensive and cumbersome and has the added difficulty of being practical to apply at a large scale in control campaigns. Prolonged incubation during isolation is also a limiting factor to examine a large population as in surveys. Hence, the indirect diagnosis through detection of *Brucella* antibodies in the serum and other body fluid is simpler and indicative that the animal has been in contact with the bacteria and may thus be infected (Ferreira *et al.*, 2003). Conventional serological tests will not differentiate infection with different species of *Brucella* nor will they differentiate infection associated with *Y. enterocolitica* O: 9 (Radostits *et al.*, 2006).

The antigen used in *Brucella* serological diagnosis is the O-polysaccharide (OPS) on the cell surface. The whole antigen or smooth lipo-polysaccharide (S-LPS) prepared by chemical extract is employed in serological tests. Because common epitopes (S-LPS) are present in *B. abortus, B. melitensis* and *B. suis*, virtually all serological tests in all these bacteria utilize *B. abortus* antigen. *B. ovis* and *B. canis* are diagnosed by use of their rough lipopolysaccharide (R-LPS) or protein antigen (Poester *et al.*, 2010).

No single serological test and antigen combination showed 100% sensitivity and specificity simultaneously. A battery of serological tests has been developed for the diagnosis of *Brucella*. Combination of tests shows a degree of sensitivity, which appear to be sufficient to detect infected animals. Frequently, highly sensitive but less specific tests are used for screening proposes, and this then are followed by more specific test for conformation (Walker, 1999; Hirsh and Zee, 1999; Poester *et al.*, 2010). The commonly used serological tests are the RBPT, SAT, CFT, ELISA (Quinn *et al.*, 1994).

The standard Rose Bengal (RB) and Complement Fixation (CFT) tests are the main serological tests used to detect antibodies against *B. abortus* and *B. melitensis* infections. Both tests have been used for several decades; nevertheless, there is evidence that both tests are significantly less effective for the diagnosis of brucellosis in sheep and goats than in cattle. To improve the sensitivity of the RB test a simple modification consisting in increasing the volume of sera to be tested has been recommended. During recent years, different indirect Enzyme Linked Immunosorbent Assays (iELISAs) have been developed using more or less purified S-LPS as the antigen and have been reported to be at least as sensitive and specific as the combination of both RB and CF tests for the diagnosis of brucellosis in ruminants. In fact, an iELISA with polyclonal conjugate (anti-IgG) has been reported to be effective enough for the diagnosis of the infection in sheep and goats (Ferreira *et al.*, 2003; FAO, 2010).

Blasco *et al.* (1994), Ferreira *et al.* (2003) and Godfroid *et al.* (2010) reported that mRT and iELISA are more sensitive than standard Ros Bengal test and CFT. A scientific research finding done by Ferreira *et al.* (2003) showed that the sensitivity of mRT, iELISA, RBT, CFT were 98.6%, 96.8%, 95% and 92.7% respectively on known positive animals. However, all the tests showed 100% specificity on known negative animals. In countries in which no vaccination programmes are being used, the CF test could be replaced by either the mRB or iELISA to increase diagnostic sensitivity (Ferreira *et al.*, 2003). FAO, 2010 also reported that under field conditions the sensitivity of CFT was lower than RBT and iELISA. Higher sensitivity of iELSA was also described by Godfroid *et al.* (2010) (Table 5).

Gall & Nielsen (2004) reviewed over 50 publications in which the sensitivity and specificity values of assays used for the detection of exposure to *Brucella abortus* had been examined.

They showed that primary binding assays, including the Fluorescence Polarisation Assay (FPA), the indirect Enzyme-Linked Immunosorbent Assay (iELISA) and the competitive Enzyme-Linked Immunosorbent Assay were more sensitive and specific than the conventional tests Compliment Fixation Test (CFT) and Rose Bengal Test (RBT). According to the review, the iELISA was less variable than the CFT and the RBT.

Table 5: Sensitivity and specificity of indirect tests for the diagnosis of cattle brucellosis

Tests	Sensitivity (%)	Specificity (%)
SAT	81.5	98.9
CFT	90-91.8	99.7-99.9
BAT	87	97.8
iELISA	97.2	97.1-99.8
cELISA	95.2	99.7
FPA	96.6	99.1
Skin test	78-93	99.8

Source: Godfroid *et al.* (2010)

2.7.5.1. Modified Rose Bengal Test (mRBT)

The Rose Bengal plate test is a spot agglutination technique, because the test does not need special laboratory facilities and it is simple and easy to perform, it is used to screen sera for *Brucella* antibodies. The test detect specific antibodies of the IgM and IgG types and is more effective in detecting antibodies of the IgG types than IgM and IgG2 types (Poester *et al.*, 2010).

The Rose Bengal plate test is very sensitive test; however, like all other serological tests, it could sometimes give a positive result due to S19 and or Rev-1 vaccination or due to false positive serological reactions. Therefore, positive reaction should be investigated using suitable confirmatory strategic and epidemiological investigation (Poester *et al.*, 2010).

The antigen consists of *Brucella* stained with Rose Bengal and suspended in buffer at pH 3.65$\pm$ 0.05. The test is conducted on ruled enamel strips, on a glass or ceramic tile or WHO hemagglutination plate. 75µl serum and 30µl antigen are put on white enamel or plastic trays, mixed, and read after 4 minute rocking. The test is usually interpreted as positive if any agglutination is apparent, and the reaction may be graded according to its rapidity and

appearance. The test is economical, simple and rapid, and gives few false negative and false positive results (Alton *et al.,* 1975; Blasco *et al.*, 1994; Ferreira *et al.*, 2003).

2.7.5.2. Serum Agglutination Test (SAT)

Serum agglutination test which is the ancestor of all serological tests is still widely used often in conjunction with the CFT. It has been used for many years in surveillance and control programs for animal brucellosis. The antigen represents a bacterial suspension in phenol saline (Nacl 0.85% [w/v] and phenol at 0.5% [v/v]. The pH of 7.2 must be readjusted in the antigen. It is conducted by making doubling dilution of the serum in phenol saline in a round bottomed tube and adding an equal amount of (volume) of standard antigen. After mixing the tube are incubated overnight at 37°C, and the degree of agglutination is read by comparing the opacity against standards representing various degrees of agglutination. The mixture of antigen and serum dilutions should be incubated for 16-24 hours at 37°C (OIE, 2008).

Although SAT is widely used, it has the following limitations: detects non specific antibodies, inability to detect chronic stage of the disease and inability to differentiate antibodies resulting from natural infection and vaccination. SAT detects antibodies of the classes IgG2 and IgM. It should be stressed that the serum agglutination test (SAT) is generally, regarded as being unsatisfactory for the purpose of international trade (Alton *et al.*, 1975; FAO, 2010).

2.7.5.3. Complement Fixation Test (CFT)

The compliment fixation test detects specific antibodies of the IgM and IgG1 type that fixes compliment. The CFT is highly specific, but it is laborious and requires highly trained personnel as well as suitable laboratory facilities. This makes CFT less suitable for use in developing countries. Although its specificity is very important for the control and eradication of brucellosis, it may test false negative when antibodies of the IgG2 type hinder compliment fixation (MacMillan and Cockrem, 1985). The CFT measures more antibodies of the IgG1 type than antibodies of the IgM type, as the later are partially destroyed during inactivation. Since

antibodies of the IgG1 type usually appear after antibodies of the IgM type, control and surveillance for brucellosis is best done with SAT and CFT (Olsen, 2002).

2.7.5.4. Enzyme Linked Immunosorbent Assay (ELISA)

ELISAs are methods that involve immobilization of one of the active components on a solid phase, and iELISAs are those in which the antigen is bound to a solid phase, usually a polystyrene microtitre plate so that antibody, if present in a sample, binds to the immobilized antigen and may be detected by an appropriate anti-globulin-enzyme conjugate which in combination with a chromogenic substrate gives a coloured reaction indicative of the presence of antibody in the sample. It is this method that is now familiar to most diagnosticians (Godfroid *et al.*, 2010).

The ELISA tests offer excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form. They are more suitable than the CFT for use in smaller laboratories and ELISA technology is now used for diagnosis of a wide range of animal and human diseases (WHO, 2006).

The test can be used for screening and confirmation of brucellosis in both milk and serum. The assay is very costly for use in individual animal tasting but it is an ideal test for screening suspected herds/flock. Most iELISAs use purified smooth LPS as antigen but a good deal of variation exists in the anti-bovine Ig conjugate used. Most iELISAs detect mainly IgGs or IgG sub-classes. Their main quality is their high sensitivity but they are also more vulnerable to non-specific reactions. These cross-reactions seen in iELISAs motivated the development of cELISAs. The O-chain of the smooth LPS of *Brucella* contains specific epitopes that are not shared with the LPS of *Yersinia enterocolitica* O: 9. Therefore, by using monoclonal antibodies directed against specific epitopes of the *Brucella* LPS, the development of more specific cELISAs has been possible (Godfroid *et al.*, 2010).

These ELISAs provide similar or better sensitivity than both the RB and CF tests but, like the classical tests, are unable to differentiate infected animals from recently vaccinated animals or other causes of false-positive serological responses (FAO, 2010). An ELISA test using purified antigen is described as being able to differentiate the seropositivity of *B. melitensis* from that of *B. ovis* (Radostits *et al.*, 2006). An OIE goat international standard will be available soon to allow the standardization of ELISAs in small ruminants (FAO, 2010).

2.7.6. Allergic Test (Brucellin Test)

The skin or Brucellin test is an allergic test that detects the specific cellular immune response induced by *Brucella* spp. infection. The injection of brucellergene, a protein extract of a rough strain of *Brucella spp.*, is followed by a local inflammatory response in a sensitized animal. The technique involves inoculation of 0.1 or 0.2 ml of *Brucella* allergen into the caudal fold or dorsal surface of the ear of goat intradermally. It can also be given at a dose of 0.5 ml subcutaneous or 0.1 ml intradermally in to the upper or lower eyelid (Alton *et al*, 1975). This delayed type hypersensitivity reaction is measured by the increase in skin thickness at the site of inoculation. This test is highly efficient in discriminating between true brucellosis cases and false positive serological reactions. The skin test is highly specific but its weak sensitivity makes it a good test for herds but not for individual certification (Godfroid *et al.*, 2010). This test can be used for screening unvaccinated herds, provided that a purified (free of SLPS) and standardized antigen preparation is used (Radostits *et al.*, 2006). The brucellin skin test has a very high specificity and results of this test may aid the interpretation of serological reactions thought to be false positive reactions due to infection with cross reacting bacteria. However, not all infected animals react; therefore this test alone cannot be recommended as the sole diagnostic test or for the purposes of international trade. It is essential to use a standardized defined brucellin preparation that does not contain S-LPS antigen, as this may provoke nonspecific inflammatory reactions or interfere with subsequent serological test. One such preparation is brucellin INRA prepared from a rough strain of *B. melitensis* that is commercially available. The test is well suited to large scale testing and avoids collection of numerous blood samples (Alton *et al*, 1975; Radostits *et al.*, 2006; Godfroid *et al.*, 2010).

The drawbacks of allergic test is that it does not differentiate infection from vaccination; animals recovered from infection may be detected as reactor, animals shedding the organism in the milk or genital secretions may be negative, hence less sensitive (Alton *et al.*, 1975).

2.7.7. Polymerase Chain Reaction (PCR)

In molecular technology, polymerase chain reaction (PCR) is a new approach and applied in many diagnostic works to overcome limitations and difficulties of bacterial culture and serological assays. In many works carried out PCR show high sensitivity, specificity and overcome the extraneous intervention of mimicry antibodies from sources of other than actual infection (Pouillot *et al.,* 1997; Radostits *et al.*, 2006).

2.8. Treatment

As a general rule, treatment of infected livestock with antibiotics is not recommended because of the high treatment failure rate, cost, potential risk of maintaining infected animals, and antibiotic residues in cheese production. However, combination of tetracycline and dihydrostreptomycin for 2-4 week period is commonly used (Hirsh and Zee, 1999).

2.9. Public health importance

Five out of the nine known *Brucella* species can infect humans and the most pathogenic and invasive species for human is *B. melitensis*, followed in descending order by *B. suis*, *B. abortus* and *B. canis*. The zoonotic nature of the marine brucellae, *B. ceti*, has been documented (Seleem *et al.*, 2010).

Common sources for infection are aborted fetuses, placentas, post abortion uterine fluids and raw milk or milk products. The live *B. melitensis* Rev-1 vaccine is also pathogenic for humans and

must be handled with caution to avoid accidental self injection or contamination of mucous membranes or abraded skin. Veterinarians, ranchers, herders, farmers, laboratory workers, and slaughterhouse workers are particularly at risk of acquiring infections (Hirsh and Zee, 1999; CFSPH, 2009).

In Human, brucellosis begins as an acute febrile illness with nonspecific flu-like signs such as undulating fever, headache, malaise, back pain, myalgia, generalized aches and drenching night sweats, fatigue and muscle and joint pains. Osteomyelitis is the most common complication. Some patients recover spontaneously, while others develop persistent symptoms that typically wax and wane. Occasionally seen complications include arthritis, spondylitis, and epididymo-orchitis. The mortality rate is low; in untreated persons, estimates of the case fatality rate vary from less than 2% to 5%. Deaths are usually caused by endocarditis or meningitis (Quinn *et al.*, 1994; CFSPH, 2009).

The epidemiology of human brucellosis has drastically changed over the past few years because of various sanitary, socioeconomic, and political reasons, together with increased international travel. New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening (Seleem *et al.*, 2010). Although in most countries brucellosis is a nationally notifiable disease and reportable to the local health authority, it is under reported and official numbers constitute only a fraction of true incidence of the disease. Thus the true incidence of human brucellosis is unknown and the estimated burden of the disease varies widely from <0.03 to >160 per 100,000 population (Seleem *et al.*, 2010). More than 500,000 new cases are reported each year, and according to the World Health Organization, (1997) this figure underestimates the magnitude of the problem. Thus, the impact of brucellosis is particularly acute in developing countries, where WHO has classified it as a neglected zoonosis (Atluri *et al.*, 2011).

B. melitensis, *B. suis* and *B. abortus* are listed as potential bio-weapons by the Center for Disease Control and Prevention in the USA. This is due to the highly infectious nature of these species, as they can be readily aerosolized. It is believed that fewer than 10 cfu are capable of infecting

humans and infection can occur from aerosol. Moreover, an outbreak of brucellosis would be difficult to detect because the initial symptoms are easily confused with those of influenza (Radostits *et al.*, 2006; Seleem *et al.*, 2010).

B. abortus and *B. suis* infections usually affect occupational groups, while *B. melitensis* infections occur more frequently than the other *Brucella* species in the general population. In Italy, 99% of human brucellosis is caused by *B. melitensis* (Seleem *et al.*, 2010).

Table 6: Human sero-reactors to *Brucella* antibody in Africa

Country	Number tested	Prevalence%	Serology	Author and year
Djibouti	108	6.5	CFT	Chantal *et al.*, 1994
Eritrea	130	7.1	CFT	Omer *et al.*, 2000
Eritrea	21	4.6	CFT	Omer *et al.*, 2000
Eritrea	105	3	CFT	Omer *et al.*, 2000
Nigeria	13,999	7.6 – 29.8	SAT	Chukwu, 1985
Nigeria	738	5.55	SAT	Chukwu, 1985
Somalia	353	0.6	SAT	Hussein *et al.*, 1987
Tanzania	540	22.6	SAT	Chukwu, 1985
Uganda	3,164	6.4	SAT	Chukwu, 1985

Table 7: Human *Brucella* antibody sero-reactors in Ethiopia

Author and year	Total tested	Number positive	Prevalence%	Serology
Taye, 1991	8	1	12.5	RBPT,CFT
Kassahun *et al.*, 2006	336	16	4.8	RBPT, 2MET
Tadele, 2004	126	3	3.4	RBPT, CFT
Kassahun, 2004	38	2	5.8	RBPT, CFT
Mussie, 2005	238	9	3.78	RBPT, CFT

Humans are accidental and almost always dead end hosts of *Brucella* infections; human-to-human transmission is very rare and no human to lower animal transmission has been reported (Nicoletti, 1980).

Due to intracellular localization of *Brucella* and its ability to adapt to the environmental conditions, treatment failure and relapse rates are high and depend on the drug combination and patient compliance. The best drug recommended by WHO is rifampicin at a dosage of 600-900mg combined with doxycycline at 200mg, and relapse is unusual after a course of treatment continued for at least 6 weeks. However recent studies show that the combination of doxycycline with streptomycin is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis (Seleem *et al.*, 2010).

2.10. Economic importance

Although estimates of the costs associated with brucellosis infections remain limited to specific countries, all data suggest that worldwide economic losses due to brucellosis are extensive not only in animal production (reduced milk, abortion and delayed conception), but also in public health (cost of treatment and productivity loss) as well as lost trade opportunities. The annual loss due to bovine brucellosis in Latin America is approximated to be $600 million. Although brucellosis eradication programs can be very expensive, they are estimated to save $7 for each $1 spent on eradication. The USA national brucellosis eradication program, while costing $3.5 billion between 1934 and 1997, the cost of reduced milk production and abortion in 1952 alone was $400 million (FAO, 2010; Seleem *et al.*, 2010).

2.11. Control and prevention

Over the last two decades, a number of developed countries have eradicated, or significantly reduced the prevalence of *B. abortus* infections. However, with the exception of some Western European countries, few countries have successfully eradicated *B. melitensis* infections. *B. melitensis* is much more common than *B. abortus* in many countries in Eastern Europe, Central Asia and the Middle East (FAO, 2010).

Regular tasting of animals, restriction of movement of animals, and personnel between herds, and purchase of animals with known health and reproductive records can prevent introduction and reduce the spread of the disease (Kusiluka and Kambarage, 1996).

Control measures must include hygiene at kidding or lambing, disposal of infected or reactor animals, separate pens for kidding does or ewes that can be cleaned and disinfected, early weaning and vaccination. In endemic areas all placentas and dead fetuses should be buried as a routine practice (Radostits *et al.*, 2006).

The control and prevention measures to be adopted on brucellosis should be realistically based on thorough understanding of local and regional variations in animal husbandry practices, social customs, infrastructure and epidemiological patterns of the disease (Radostits *et al.*, 2006). Approaches used to control brucellosis include:

2.11.1. Surveillance

Surveillance consists of the systematic collection, analysis, interpretation and prompt dissemination of data on specific diseases or syndromes to those who need to know, for relevant action to be taken. Surveillance of brucellosis is achieved through herd screening using RBPT or card test, allergic skin test and confirmation using more specific tests such as CFT and ELISA (WHO, 2006).

2.11.2. Immunization

B. melitensis strain Rev1 although highly infectious to human, is considered as the best vaccine available for the control of ovine and caprine brucellosis. It also protect against ram infection with *B. ovis*. However, the Rev1 vaccine shows a considerable degree of virulence and induces abortions when administered during pregnancy. This vaccine also interferes with serological tests, particularly when it is injected subcutaneously, but conjunctival administration to lambs and kids between the ages of 3 and 6 months minimizes this problem (Radostits *et al.*, 2006; Seleem *et al.*, 2010). Vaccination may be the only practical method of control in areas where there is a high prevalence of the disease, extensive management systems, communal grazing and a low socioeconomic level. Vaccination of animals 3-8 months of age confers a high degree of immunity that lasts for more than 4 years in goats and 2.5 years in sheep. The general approach in endemically infected countries is to institute a whole flock vaccination scheme followed by a young-stock vaccination scheme until the prevalence of the disease is reduced, at which time test and slaughter can be implemented (Radostits *et al.*, 2006).

2.11.3. Test and slaughter of positive reactors

It is usually accepted that a programme of eliminating brucellosis by test and slaughter policy is justified on economic grounds only when the prevalence of infected animals in an area is about 2% or less. Animals, which are positive to both RBPT and CFT or iELISA are slaughtered (WHO, 2006).

2.11.4. Management and hygienic measures

This include safe disposal of aborted fetus, disinfection of contaminated areas, isolation of purchased replacements for at least 30 days and serological test prior to commingling, prevention of contacts and commingling with herds of unknown status or those with brucellosis, proper disposal of placentas and non-viable fetuses and education of the public not to drink raw milk or eat products made from unpasteurized or otherwise untreated milk (WHO, 1997).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in Arsi (Tiyo and Dodota Sire districts) and West Shoa (Adami Tullu-Jido Kombolcha district) zones of oromia region.

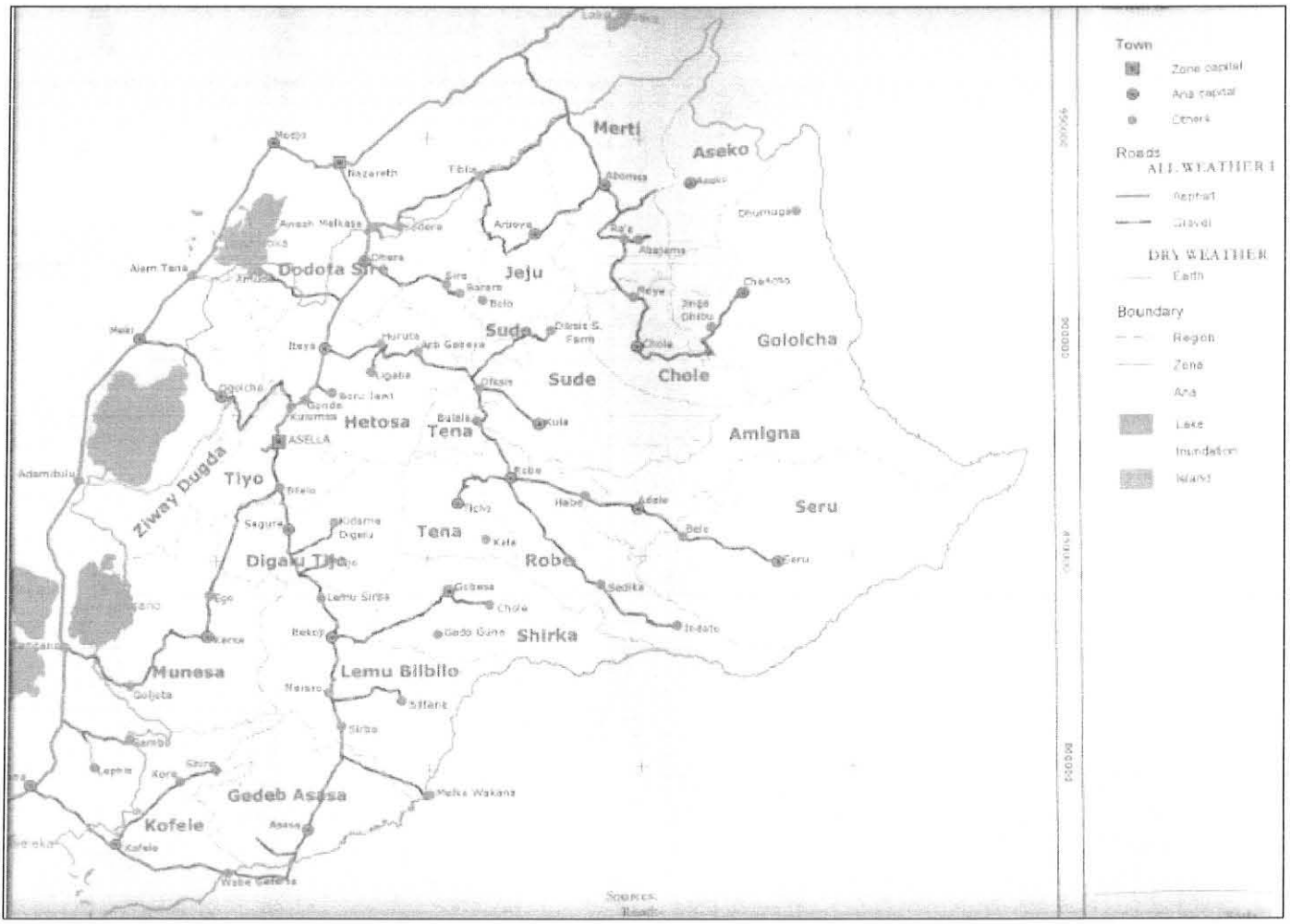
Arsi Zone is found in the central part of the Oromiya National Regional State. The zone lies between 6^0 45' N to 8^0 58'N and 38^0 32' E to 40^0 50' E. It shares borderlines with the Regional State of Nations, Nationalities and Peoples of Southern Ethiopia (SNNPS) and East Shewa, Bale and West Hararge Zones. Asella, the capital of Arsi zone is found at 175 km Southeast of Addis Ababa. Arsi zone has a total area of 23881 Km^2. The zone has 22 districts.

Tiyo district is one of the administrative units located in Northwestern central part of Arsi zone. The total area of the district is 665 km^2 and it has 21 administrative units of which 18 are Peasant associations and 3 urban administrative units. Undulating plain, hills, rift valley escarpment, Welkesa valley and mountain peak of Chilalo characterize the topography of the district. The altitude of the district ranges from 1500-4105 m. The highest peak in the district is Chilalo Mountain at an elevation of 4005m above sea level. The elevation of the district gradually reduces from Chilalo mountain peak toward the West low land, to Rift Valley. The district has a tropical high land climate characterized by heavy and erosive rainfall with long wet season. The district is divided into four ecological zones, namely high land (31.7%), Mid-altitude (42.5%), the temperate highland (20.1%) and the low land (5.7%). The annual mean temperature ranges from 15-22^0c and the mean annual rainfall ranges from 900-1100mm (OFEDB, 2007a). According to Arsi zone livestock development and health agency, the current animal population of the district is: 70, 967 cattle; 55,237 sheep, 14,157 goats, 8884 horses, 15,730 donkeys and 303 camels.

Dodota Sire is one of the administrative units of Arsi zone located in the North eastern part of the zone. It has total area of 986km^2. Dodota Sire district has 27 peasant associations and 4 urban administrative units. Dera Town is the capital of the district, located at 125km from Addis Ababa and 50km from zonal capital, Asella town. Rift Valley dominates the topography of the district. The altitude of the district ranges from 1400-2500 meters with undulating plain, hills, mountains and degraded land areas. The district has no known mountain peaks. All parts of the district are found within Awash drainage basin. Dodota Sire is divided into three agro-climatic zones; namely Dega /Temperate (14.5%), weina Dega /Sub-Tropical (24.5%) and Kola / Tropical (61%). The annual rainfall ranges from 800- 1000mm. The district is dominantly characterized by cool (10-15^{0}c), moderately cool (15-20^{0}c) and moderately warm (20-25^{0}c) annual temperatures (OFEDB, 2007b). According to Arsi zone livestock development and health agency, the current animal population of the district is: 37,325 cattle, 21,859 sheep, 22,561goats, 1157 horses, 8822 donkeys and 105 camels.

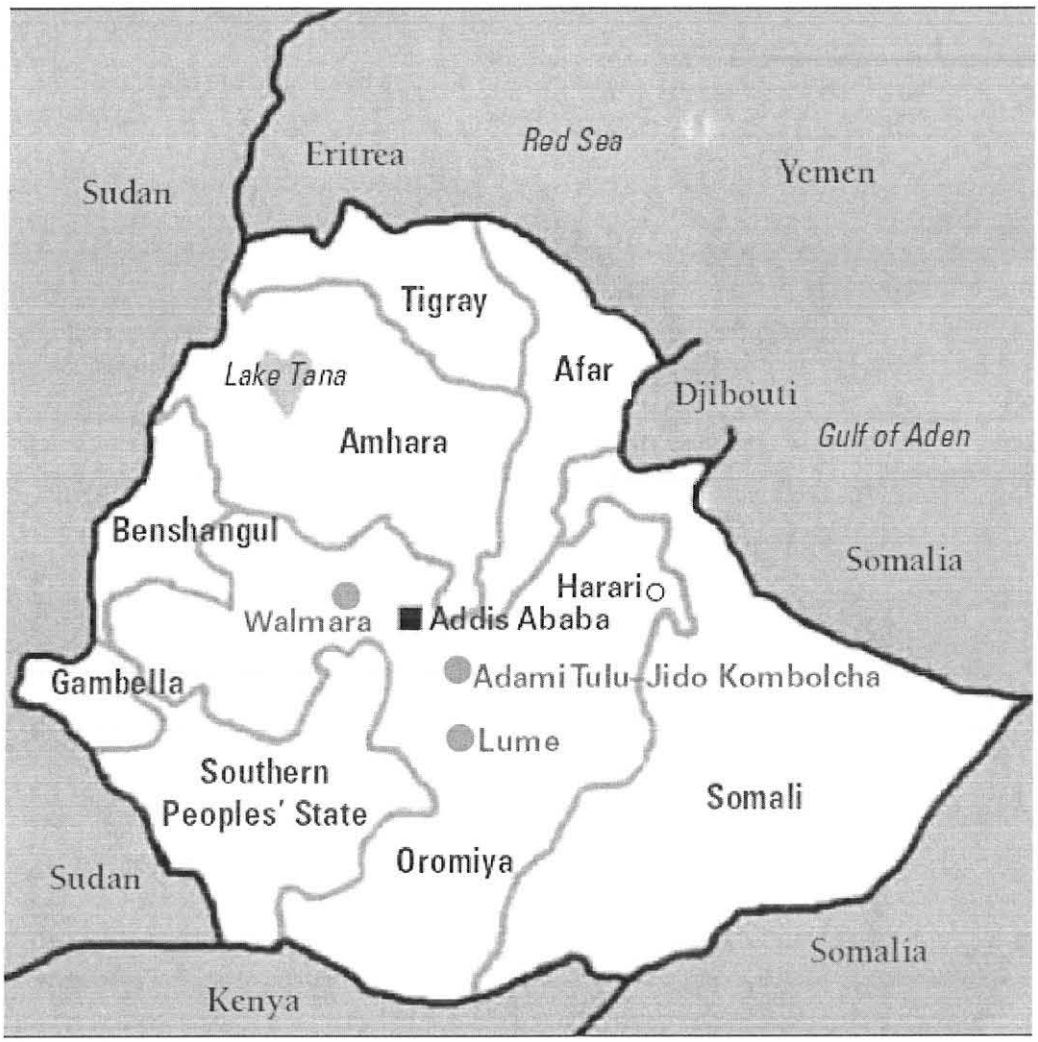
Adami Tulu-Jido Kombolcha district is situated in the mid-rift valley, East Showa zone of Oromiya regional state, 167 kms South of Addis Ababa. It has an altitude of 1650 meters above sea level with a bimodal unevenly distributed rainfall pattern. The average annual rainfall for the last 10 years was 760.9 mm and consisted of short rainy season (February to April) and long rainy season (July to September). AdamiTulu-Jido Kombolcha district has a minimum mean temperature of 12.7^{0}c with a relative humidity of 760. The predominant land around the area is suitable for livestock production, goats being the most selected for breeding purpose next to cattle. Goat milk is widely consumed in the area. According to district's livestock development and health agency, there are about 189,870 cattle, 85,365 goats, 11,330 sheep, 17,356 donkey, 256 mule, 1442 horses and 80,270 poultry in the district.

Fig. 2: Map showing different districts of Arsi zone including study districts



Source: Arsi Zone Livestock Development and Health Agency

Fig. 3: Map showing Adami Tulu- Jido Kombolcha district



Source: Jergefa *et al.* (2009)

3.2. Study animals

Arsi and East Shoa zones have high potentialities for livestock rearing. According to CSA (2012) the animal population of East Shoa is: 973,563 cattle, 299,284 sheep, 488,512 goats, 13,000 horses, 247,399 donkeys, 7,087 mules and 926,465 poultry. The animal population of

Arsi zone is: 2,295,138 cattle, 1,207,182 sheep, 653,327 goats, 202,467 horses, 369,218 donkeys, 21,587 mule and 1,449,583 poultry. Arsi zone ranked first in its sheep population (CSA, 2012). However, the society gives more emphasis to quantity than quality due to lack of awareness and consequently, the economic gain from this sector is low. The predominant sheep and goat breeds in the study area are Arsi-Bale breeds, which are managed under extensive system. Traditional housing, feeding and milking procedures are generally practiced. Farmers who own more than five sheep and goats keep them in barns while those who own less than five keep them in their own houses. The source of feed is traditional grazing and browsing.

3.3. Study design

A cross-sectional study was conducted from October, 2011 to March, 2012 to study the sero-prevalence of brucellosis and the association of risk factors with seroprevalence of the disease. In order to address these objectives questionnaire survey, blood sample collection and laboratory tests were employed.

3.3.1. Sampling Method and Sample size determination

To determine sampling unit, the sampling method employed was two stage cluster sampling. Peasant Associations (PAs) and individual animal owners or herds were used as the primary and secondary sampling units, respectively. The zones and districts were selected purposively based on their small ruminant population and accessibility. Simple random sampling technique was used to select peasant associations and herds/flocks. Inaccessible herds were replaced by accessible ones. Flocks/herds were considered as cluster units. All the sheep and goats above 6 months of age in the randomly selected herds/flocks were sampled. Three (3) PAs/Kebeles were selected randomly from each district. The number of animals included in the study was distributed proportionally over the PAs. As there was no study conducted on small ruminant brucellosis in the study area, 50% expected prevalence was considered in sample size

determination. The other determinants considered in sample size determination were 95% confidence interval and 5% desired absolute precision.

Hence: $n = \frac{(CI)^2 Pexp (1-Pexp)}{d^2}$ (Thrusfield, 1995)

Where;

n= the required sample size

Pexp = the expected prevalence rate (50%)

CI = the value of the required confidence interval (1.96)

D = desired absolute precision (5%)

Hence, the sample size required as per the above formula is 384 heads of each study species. However, a total of 840 blood samples (409 sheep and 431goats) were collected to increase precision.

3.3.2. Questionnaire survey

Eighty (80) randomly selected small ruminant owners from the three districts were interviewed by using structured questionnaire. By doing so, management practices that may predispose the public to brucellosis were accessed. The questionnaire focused on disposal and handling practices of aborted material, knowledge about zoonotic importance of the disease, habit of animal product consumption and presence of reproductive disorders.

3.3.3. Blood sample collection

About 7-10 ml of blood samples were collected from jugular vein of each animal using plain vacutainer tubes, which were properly labeled and be allowed to stand tilted overnight at room temperature. Then, serum was separated from clotted blood by either direct decanting or centrifuging and transferring to cryogenic vials. Separated sera were stored at -20^0C at Asella Regional Animal Health Diagnostic and Investigation Laboratory, until being transported to Sebeta National Animal Health Diagnostic and Investigation Center (NAHDIC) where modified

Rose Bengal Test (mRBT) and indirect Enzyme Linked Immunosorbent Assay (iELISA) tests were done. Moreover, relevant data on the study animals was recorded along with blood specimen collection. The individual animal details such as the species of the animal, sex, age, flock size, source of animal and history of abortion were recorded.

3.3.4. Laboratory tests

3.3.4.1. Modified Rose Bengal Test (mRBPT)

Modified Rose Bengal test (mRBT) was used to screen the sera samples. The sera antigen was removed from the refrigerator and left at room temperature for at least 30 minutes before the test was performed. All sera samples collected were initially screened by RBPT antigen (Lillidale diagnostics, Badbury view, Bothenwood, Wimborne, Dorset, BH21 4HU, UK) at Sebeta National Animal Health Diagnostic and Investigation Center (NAHDIC) according to the modified procedure of Blasco *et al.* (1994), mixing 75µl of sera and 25µl of antigen. Seventy five (75) µl of serum was taken from each sample and put in one side of each micro-plate well. Commercially prepared purified antigen of 25µl was put on the other side of the same well, mixed thoroughly and left for agglutination. After 4 minutes, any visible agglutination reaction was considered positive for the screening test. The interpretation of the results was done according to the degree of agglutination (Nielsen and Dunkan, 1990). Agglutinations were recorded as 0, +, ++ and +++. A score of 0 indicates the absence of agglutination; + indicates barely visible agglutinations; ++ indicates fine agglutination, and +++ indicates coarse clumping. Those samples with no agglutination (0) were recorded as negative while others were recorded as positive. Sera samples proved positive by the screening test were kept at -20^0c for confirmation by iELISA.

3.3.4.2. Indirect Enzyme Linked Immunosorbent Assay (iELISA)

Confirmation of mRBT positive sera was done by iELISA (SERELISA® *Brucella* OCB Ab Mono Indirect kit) (SYNBIOTICS EUROPE SAS, France) at Sebeta National Animal Health Diagnostic and Investigation Center (NAHDIC). This kit detects Anti *Brucella* lipopolysacchride antibodies in bovine sera (individual and pool) and ovine and caprine sera (individual). Indirect Enzyme Linked Immunosorbent Assay (iELISA) test was done according to manufacturer's manual. Preparation of the sample was done by diluting the sample at 1:10 in sample diluent in a dilution plate. Ten (10) µl of already diluted sample was dispensed in to 90 µl of sample diluent in the well. After the wells were covered with adhesive film, the plate was incubated at 37^0c for 1 hour. The adhesive film was carefully removed and the plates were washed four times with washing solution. One hundred (100) µl of the diluted conjugate (1:200 in conjugate diluent) was added to all wells; covered with new piece of adhesive film, and then incubated at 37^0c for 30 minutes. The adhesive film was removed again and the plates were washed four times. One hundred (100) µl of buffered peroxidase substrate was added into each well and mixed by plate agitator to ensure correct homogenization. After incubation at room temperature, shielded from light, fifty (50) µl of stop solution was added and mixed with plate agitator. The sample was then put into the ELISA reader and the result was obtained by printing from the computer connected to the ELISA reader.

3.4. Data analysis

Data obtained from field sampling, serological tests and questionnaires were stored in Microsoft Excel® spreadsheet. The individual animal level sero-prevalence was calculated by dividing RBPT and iELISA positive results by total number of animals. Similarly, herd level sero-prevalence was computed as the number of herds with at least one positive animal divided by the total number of herds tested. The analytical statistics employed were Chi-square test (χ^2), univarible logistic regression and multivariable logistic regression analysis using SPSS 16 for windows. Differences between factors were considered significant at $P<0.05$. The degree of

association between potential risk factors and seroprevalence was computed using Odd's Ratio (OR) at 95% confidence interval. All risk factors that had P-value <0.20 in the univariate logistic regression analysis were subjected to multivariate logistic regression analysis.

4. RESULTS

4.1. Overall seroprevalence

A total of 840 sera samples from sheep and goats were collected from Tiyo, Dodota Sire and Adami Tulu-Jido Kombolcha districts and 39(4.6 %) were positive for mRBT. Upon further testing of the 39 mRBT positive samples for confirmation, all were found to be iELISA positive. From 409 sheep sera samples, 18 (4.4%) were positive for brucellosis by mRBT and iELISA. Out of the 431 goats sera tested by mRBT and iELISA, 21(4.9%) were positive. The individual animal and herd level seroprevalence of the disease in the study area were 4.6% and 26% respectively (Table 8 and 13).

Sero-prevalence of brucellosis in the two zones and the three study districts is shown in Table 12, 8 and Fig. 4. The individual animal seroprevalence of the disease was higher in East Shoa zone (6.2%) than Arsi zone (4.1%). The individual animal level seroprevalence of the disease was found to be highest in Adami Tulu-Jido Kombolcha district (6.2%) followed by Tiyo (5.2%). Dodota Sire (2.8%) recorded the lowest seroprevalence of the disease.

The seroprevalence distribution of brucellosis in sheep and goats in the nine studied PAs is shown in Table 9, 10 and 11. Out of the 9 peasant associations studied, Anano (11.9%) was with the highest disease prevalence followed by Abosera (9%). Warja washgula recorded the least prevalence (1.6%).

The herd level seroprevalence of small ruminant brucellosis was higher in Adami Tulu-Jido Kombolcha district (34.2%) followed by Tiyo (29.5%). The herd level seroprevalence of the disease was lowest in Dodota Sire district (16.3%) (Table 8).

Table 8: Overall individual animal and herd level seroprevalence of small ruminat brucellosis in Tiyo, Dodota Sire and Adami Tulu-Jido Kombolcha districts

Individual seroprevalence				Herd level seroprevalence		
District	No examined	mRBT/ iELISA+ (%)	P-value	No of examined herds	mRBT/ iELISA+ (%)	P-value
Tiyo	326	17(5.2)	0.17	44	13(29.5)	0.144
Dodota Sire	287	8(2.8)	0.14	49	8(16.3)	0.132
Adami Tulu-Jido kombolcha	227	14(6.2)	0.63	38	13(34.2)	0.651
Total	840	39(4.6)		131	34(26)	

+=positive

Fig. 4: Individual animal seroprevalence of small ruminant brucellosis in the three districts

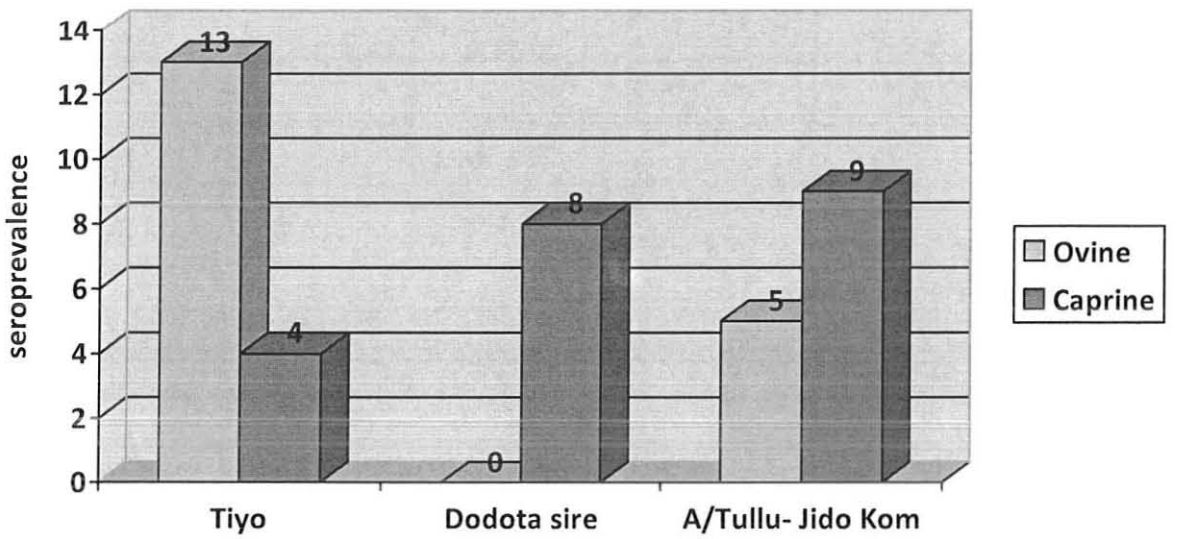


Table 9: Seroprevalence distribution of small ruminant brucellosis in Tiyo district of Arsi zone

PA	Species	Sex	Number tested	mRBPT positives No (%)	iELISA positive No (%)
Waji chilalo	Ovine	Male	14	0(0)	0(0)
		Female	12`6	5(4)	5(4)
	Caprine	Male	6	0(0)	0(0)
		Female	13	0(0)	0(0)
Sub total			159	5(3.1)	5(3.1)
Murkicha	Ovine	Male	2	0(0)	0(0)
		Female	33	1(3)	1(3)
	Caprine	Male	3	0(0)	0(0)
		Female	29	2(6.9)	2(6.9)
Sub total			67	3(4.5)	3(4.5)
Abosera	Ovine	Male	15	2(13.3)	2(13)
		Female	69	5(7.2)	5(7.2)
	Caprine	Male	6	0(0)	0(0)
		Female	10	2(20)	2(20)
Sub total			100	9(9)	9(9)
Total	Ovine		259	13(5)	15(5)
	Caprine		67	4(5.97)	4(5.97)
Over all seroprevalence			326	17(5.2)	17(5.2)

Table 10: Seroprevalence distribution of small ruminant brucellosis in Dodota Sire district of Arsi zone

PA	Species	Sex	Number tested	mRBPT positives No (%)	iELISA positive No (%)
Awash Malkasa	Ovine	Male	3	0(0)	0(0)
		Female	7	0(0)	0(0)
	Caprine	Male	16	1(6.3)	1(6.3)
		Female	63	2(3.2)	2(3.2)
Sub total			89	3(3.4)	3(3.4)
Dodota Alem	Ovine	Male	0	0(0)	0(0)
		Female	20	0(0)	0(0)
	Caprine	Male	2	0(0)	0(0)
		Female	86	3(3.5)	3(3.5)
Sub total			108	3(2.8)	3(2.8)
Awash Bishola	Ovine	Male	1	0(0)	0(0)
		Female	29	0(0)	0(0)
	Caprine	Male	5	0(0)	0(0)
		Female	55	2(3.6)	2(3.6)
Sub total			90	2(2.2)	2(2.2)
Total	Ovine		60	0(0)	0(0)
	Caprine		227	8(3.5)	42(3.5)
Over all seroprevalence			287	8(2.8)	8(2.8)

Table 11: Seroprevalence distribution of small ruminant brucellosis in Adami Tulu- Jido Kombolcha district of East Shoa zone

PA	Species	Sex	Number tested	mRBPT positives No (%)	iELIA positive No (%)
Warja Washgulla	Ovine	Male	5	0(0)	0(0)
		Female	39	1(2.6)	1(2.6)
	Caprine	Male	2	0(0)	0(0)
		Female	18	0(0)	0(0)
Sub total			64	1(1.6)	1(1.6)
Bochessa	Ovine	Male	5	1(20)	1(20)
		Female	31	2(6.5)	2(6.5)
	Caprine	Male	4	0(0)	0(0)
		Female	64	3(4.7)	3(4.7)
Sub total			104	6(5.8)	6(5.8)
Anano	Ovine	Male	1	0(0)	0(0)
		Female	9	1(11.1)	1(11.1)
	Caprine	Male	4	1(25)	1(25)
		Female	45	5(11.1)	5(11.1)
Sub total			59	7(11.9)	7(11.9)
Total	Ovine		90	5(5.6)	5(5.6)
	Caprine		137	9(6.7)	42(6.7)
Over all seroprevalence			227	14(6.2)	52(6.2)

4.2. Association of risk factors with seroprevalence of brucellosis

Administrative Zones

Individual animal seroprevalence of brucellosis was found to be higher in East Shoa zone (6.2%) than Arsi zone (4.1%). However, there was no statistically significant difference (P>0.05) in seropositivity to the disease at individual animal level between the two zones (Table 12).

Districts

Among districts, the prevalence of the disease at individual animal level was found to be highest in Adami Tulu- Jido Kombolcha (6.2%) followed by Tiyo(5.2%) and Dodota Sire (2.8%) recorded the lowest seropositivity. The prevalence of the disease at herd level was 34.2%, 29.5%, and 16.3% for Adami Tulu-Jido Kombolcha, Tiyo and Dodota Sire districts respectively. However, there was no statistically significant difference (P>0.05) in seropositivity to the disease both at individual animal level and herd level among the three study districts (Table 12).

Peasant associations

The seroprevalence of brucellosis was found to be highest in Anano PA (11.9%) of East Shoa zone followed by Abosera (9%) and Bochessa (5.8%). The lowest prevalence of the disease was recorded in Warja Washgula (1.6%). Univariate logistic regression analysis of the association of the prevalence of the disease with these PAs showed the absence of statistically significant difference (P>0.05) among them (Table 12).

Table 12: Univariable logistic regression analysis of location potential risk factors for seropositivity to *Brucella* antibodies in small ruminant.

Risk factor	No tasted	mRBT/iELISA +	OR(95%CI)	P-value
Zones				
Arsi	613	25(4.1%)		
East Shoa	227	14(6.2%)	1.55(0.79-3.03)	0.204
Districts				
Tiyo	326	17(5.2%)		0.17
Dodota Sire	287	8(2.8%)	0.52(0.22-1.23)	0.14
A/Tullu Jido Kom	227	14(6.2%)	1.2(0.58-2.48)	0.63
Peasant associations				
Waji Chilalo	159	5(3.1%)		0.08
Murkicha	67	3(4.5%)	1.44(0.34-6.22)	0.62
Abosera	100	9(9%)	3.05(0.99-9.37)	0.052
Awash Malkasa	89	3(3.4%)	1.07(0.25-4.61)	0.92
Dodota Alem	108	3(2.8%)	0.70(0.13-3.68)	0.67
Awash Bishola	90	2(2.2%)	0.88(0.21-3.76)	0.86
Warja Washgula	64	1(1.6%)	0.49(0.06-4.27)	0.52
Bochessa	104	6(5.8%)	1.89((0.56-6.35)	0.31
Anano	59	7(11.9%)	4.15(1.26-13.63)	0.02

*+ =positive

Species

The prevalence of *Brucella* antibodies in goats was 4.9% while it was 4.4% in sheep. There was no statistically significant difference (P=0.746) observed between the two species in the study area (Table 13).

Sex

The prevalence of small ruminant brucellosis was higher in male (6.7%) than females (4.4%). However, there was no statistically significant difference (P=0.625) in the seroprevalence of the disease among the two sexes (Table 13).

Age

There was higher rate of infection with brucellosis in adult age group (5.4%) than young age group(1.3%). The seroprevalence of small ruminant brucellosis was significantly associated with age groups in univariable logistic regression analysis (P=0.038). In univariable logistic regression, older sheep and goats were 4.55 times more likely to be affected by brucellosis than younger ones. Age was confounded with parity and it was removed from multivariable regression (Table 13 and 14).

Flock/herd size

Seroprevalence of 4.1%, 4.8% and 6.6% were found for herd sizes of [0-10], [11-20] and >20 respectively. However, this difference was not statistically significant (P>0.05) (Table 13).

Pregnancy status

The seroprevalence of small ruminant brucellosis in pregnant and non-pregnant sheep and goats was 6.9% and 2.3% respectively. There was a statistically significant difference (OR=3.14; P=0.004) in seropositivity to brucellosis infection between pregnant and non pregnant sheep and goats in univariable logistic regression analysis. Further analysis with multivariable logistic regression analysis also showed a significant difference (OR=3.28; P=0.003) between the two groups. The odds ratio indicated that pregnant sheep and goats were 3.28 times more likely to be infected with brucellosis than the non pregnant ones (Table 13 and 14).

Parity

The prevalence of brucellosis in sheep and goats of [0-1] parity, [2-4] parity and >4 parity were 1.46%, 5.59%, 8.4% respectively. There is a statistically significant difference in seropositivity to brucellosis among the different parity groups ($P<0.05$). Further analysis of association of this risk factor with the prevalence of small ruminant brucellosis using multivariable logistic regression showed that animals with parity of more than four (OR=6.58, $P<0.05$) were at higher risk of infection with the disease than other animals of lower parity. The risk of seropositivity was 6.19 and 3.89 times higher in >4 and [2-4] parity groups respectively, in comparison to [0-1] parity group in multivariable logistic regression (Table 13 and 14).

Reproductive problems

There prevalence of the disease was 4.58% and 3.7% in sheep and goats without previous abortion and in those with previous abortion respectively. However, this difference was not statistically significant ($P=0.77$) (Table 13).

Table 13: Univariable logistic regression analysis of potential risk factors for seropositivity to *Brucella* antibodies in small ruminant.

Risk factor	Category	Animals tested	No.of positives	Prevalence (%)	P-value	OR (95% CI)
Species	Goat	431	21	4.9	0.746	1.11(0.584-2.12)
	Sheep	409	18	4.4		
Sex	Male	88	5	5.68	0.625	1.27(0.48-3.34)
	Female	752	34	4.5		
Age	(0.6-1year)	160	2	1.3		
	(>1year)	680	37	5.4	0.038	4.55(1.08-19.06)
Flock size	[1-10]	438	18	4.1	0.59	
	[11-20]	311	15	4.8	0.64	1.18(0.59-2.38)
	>20	91	6	6.6	0.31	1.65(0.64-4.27)
Pregnancy	Pregnant	362	25	6.9	0.004	3.14(1.45-6.82)
	Non-pregnant	390	9	2.3		
Parity	0-1parity	274	4	1.46	0.01	
	2-4parity	358	20	5.59	0.013	3.98(1.35-11.79)
	>4 parity	119	10	8.4	0.002	6.19(1.9-20.20
Abortion	No	698	32	4.58	0.77	1.25(0.3-5.4)
	Yes	54	2	3.7		

*Male goats and sheep aged less than or equal to one year and female animals that had not yet given birth were included in the younger age group

Table 14: Multivariable logistic regression analysis of risk factors for *Brucella* seropositivity in small ruminants

Risk factor	level	OR(95%CI for OR)	P-value
Pregnancy	Non pregnant		
	Pregnant	3.28(1.5-7.2)	0.003
Parity	[0-1]		0.008
	[2-4]	3.89(1.31-11.55)	0.015
	>4	6.58(2.01-21.57)	0.002

4.3. Questionnaire survey

Structured questionnaire was presented to eighty (80) herd owners from whom animal the blood sample was taken. The responses of the herd owners on potential risk factors that would expose them to small ruminant brucellosis and their perception about the zoonotic importance of the disease is indicated on Table 15. Out of the interviewed small ruminant owners, 15 (18.75%) were found to have at least one seropositive sheep or goat in their herd. 72.5% of interviewed small ruminant owners responded that abortion had occurred at least once in their herd. Out of the interviewed owners, 41.25% had once assisted delivery while 58.75% did not. Most of the owners (77.5%) practiced selling of frequently aborting sheep or goat. Raw milk consumption was practiced by 17.5% of the herd owners interviewed. 27.5% of the owners responded that they practiced migrating their herds during wet season of the year. All the interviewed owners claimed that they do not have any knowledge of the disease neither do they practice safe disposal and handling of the aborted materials.

Table 15: Univariable logistic regression analysis of management risk factors obtained from questionnaire survey

Risk factor	Category	Herd owner response (%)	No.of positive herd	Prevalence in herd	P-value	OR (95% CI)
Migration of the herd	Yes	20(25)	7	35	0.038	3.5(1.1-11.4)
	No	60(75)	8	13.3		
Decision on frequently aborting animal	Sell	62(77.5)	12	19.5	0.8	1.2(0.3-4.8)
	Keep	18(22.5)	3	16.7		
Abortion encountered	Yes	58(72.5)	14	24.13	0.075	6.7(0.8-54.3)
	No	22(27.5)	1	4.5		
Delivery assisted	Yes	33(41.25)	5	15.2		
	No	47(58.75)	10	21.3	0.49	0.66(0.2-2.2)
Knowledge of the disease	Yes	0(0)	0	0		
	No	80(100)	15	18.75		
Aborted material disposal	Throw into field	58(100)	14	24.13		
	Burning/burying	0(0)	0	0		
Handling of aborted fetus and membrane	Bare hand	58(100)	14	24.13		
	Protective material	0	0	0		
Raw milk consumption	Yes	14(17.5)	5			
	No	66(82.5)	10		0.075	6.68(0.8-54)

Migration of the herd

Seasonal migration of herds was practiced by 25% of the small ruminant owners which participated in the questionnaire survey. The result of the questionnaire survey showed that the prevalence of the disease was found to be higher in animals which migrate seasonally (35%) than those which do not migrate (13.3%). There was a statistically significant difference (P=0.038) between these two groups in univariate logistic regression (Table 15). However, there was no

statistically significant difference in seropositivity to the disease in multivariable logistic regression.

Decision on frequently aborting sheep and goats

Even though there was a higher prevalence of the disease in those herds that the owners practice selling of frequently aborting animal (19.5%) than in those which the owners keep (16.7%), the difference is not statistically significant (Table 15).

Abortion encountered in the herd

The prevalence of the disease was higher in those herds which encountered abortion (24.13%) than those which do not (4.5%). However, there is no statistically significant difference between these two groups (Table 15).

Delivery assisted

There prevalence of the disease was higher in herds that the owners did not assist delivery problem (21.3%) than those whose owners assisted delivery (15.2%). However the difference was not statistically significant (Table 15).

Knowledge of the disease and disposal and handling of aborted material

All of the respondents claimed that they do not have any knowledge about the disease. All of them did not use any protective material when handling aborted fetus or fetal membranes. Moreover, none of them practiced any safe disposal of aborted material (Table 15).

5. DISCUSSION

In the present study, the occurrence of *brucella* sero-reactor sheep and goats in Arsi and East Shoa zones was investigated through serological analysis and questionnaire survey. The seroprevalence of small ruminant brucellosis of the present study was reported based on the result of modified Rose Bengal test (mRBT) and indirect Enzyme Linked Immunosorbant Assay (iELISA). A total of 840 sera were tested for the presence and absence of serum antibodies against *Brucella* infection. Thirty nine (39) sera were found to be positive for both modified Rose Bengal Test and indirect Enzyme Linked Immunosorbent Assay tests. The overall individual animal and herd level seroprevalence of small ruminant brucellosis in the study area was 4.6% and 26% respectively.

The prevalence of the small ruminant brucellosis in this finding in goats and sheep was (4.9%) and 4.4% respectively. The seroprevalence in the present study was higher than the report of Tekelye and Kasali (1990) who reported prevalence rates of 1.5% in sheep and 1.3% in goats in Central Ethiopia. It was also higher than that reported by Fercde *et al.* (2011) who reported prevalence proportions of 0.87% in goats and 0% in sheep in and around Bahir Dar. Ashagre *et al.* (2011) reported prevalence rate of 4.2% in goats in South Omo zone which is comparable to the present study. Ashenafi *et al.* (2007) reported the prevalence rate of 5.8% and 3.2% in goat and sheep respectively in pastoral regions of Afar with fair agreement with this finding. The report of Bekele *et al.* (2011) who recorded the prevalence rates of 1.2% in sheep and 1.9% in goats in Jijiga area was lower than the current study. The difference in the prevalence of brucellosis between the current and previous studies might be attributed to the differences in geographical location, sample size, management and production systems, and serological tests employed. Considering the small herd size of animals kept in the study area in comparison with large herd sizes kept in Somali and Afar areas at where two of the above mentioned studies are done, this finding showed higher prevalence. This could be attributed to the lower sensitivity and specificity of serological tests used by the above mentioned authors. All the above mentioned authors used standard Rose Bengal Plate Test for screening and Compliment Fixation Test to

confirm RBPT positive sera samples. But in this study modified Rose Bengal Test and Indirect Enzyme Linked Immunosorbent Assay (iELISA) were used for screening and confirmation respectively. Ferreira *et al.* (2003), Blasco *et al.* (1994); Godfroid *et al.* (2010) and FAO (2010) reported that mRT and iELISA are more sensitive than standard Rose Bengal test and CFT.

The higher prevalence rate recorded by Bertu *et al.* (2010), Falade and Hussein (1997) and Waghela (1967) in Nigeria, Somalia and Kenya respectively compared to the present study could be due to differences in agro-ecology and animal husbandry system.

However, the result in this study was lower than the prevalence reported by Yibeltal (2005) in Afar region who reported prevalence rates of 15% in sheep and 16.5% in goats. The difference could be due to the difference in herd size and management system. This study is done in Arsi and East Shoa zones which are characterized by mixed farming, in which fewer animals are raised in separate herds; whereas in the Afar region and Somali region, large numbers of different species of animals are raised and communal pastures and commingling of large number of animals at watering areas are practiced.

Small-ruminant brucellosis was found to be distributed in all nine peasant associations in the study area, and there was no statistically significant difference among the PAs. Similarly, the analysis of the prevalence of the disease in the two zones and the three districts of this study showed the absence of statistically significant difference in seropositivity to the disease. Unrestricted animal movement and mixing at pasture, watering place, and market may have contributed to the universal spread of infection. Similarities in agro-ecological conditions and animal husbandry systems among the studied areas might have also contributed to this absence of significant difference in seropositivity to disease between different location risk factors.

The prevalence of small ruminant brucellosis in the current study in goats (4.9%) was comparable to sheep (4.4%). There was no statistically significant difference (P=0.746) between these species in seropositivity to the disease in the study area which agrees with the findings of Bekele *et al.* (2011) in Jijiga district, Tekleye and Kasali, 1999 in central Ethiopia and Bertu *et al.*(2010) in plateau state in Nigeria. However, Omer *et al.* (2000), Teshale *et al.* (2006),

Ashenafi *et al.* (2007) and Bekele *et al.* (2011) reported higher prevalence in goats than in sheep. According to Radostitis *et al.* (1994), greater susceptibility of goats to *Brucella* infection than sheep is due to the fact that sheep unlike goats do not excrete the *Brucella* organism for longer periods of time. The difference of the current study with these authors could be due to higher number of sheep than goats in the study area which increased the degree of transmission in these species.

In this finding, no significant difference (P=0.625) was observed in the prevalence of brucellosis between male and females. Hirsh and Zee (1999) have reported that male animals are less susceptible to *Brucella* infection, due to the absence of erythritol. However, in support of the present finding, Teshale *et al.* (2006), Ashenafi *et al.* (2007), Hirut (2011), Ashagrie *et al.* (2011) and Bekele *et al.* (2011) have also reported the absence of statistically significant difference in the seroprevalence of brucellosis between male and female sheep and goats. The lack of statistically significant difference between the two sexes observed in this study might be due to the smaller sample size of males.

The result of the present study showed that sexually mature and pregnant sheep and goats have significantly higher odds of infection by the disease than sexually immature and non-pregnant animals respectively, which were statistically significant (P<0.05) in univariable logistic regression. Further analysis with multivariable logistic regression also showed that pregnant animals were significantly at a higher risk of infection with the disease (OR=3.14, P=0.003) than non pregnant animals. The odds ratio indicated that pregnant sheep and goats were 3.28 times more likely to be infected with brucellosis than the non pregnant ones. Age was removed from multivariable logistic regression due to confounding with parity. According to Radostits *et al.* (2006), sexually mature and pregnant animals are more susceptible to infection with the organism than sexually immature animals of either of sex, which is due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organism, tend to increase in concentration with age and sexual maturity. Similarly, Walker (1999) also indicated that younger animals tend to be more resistant to infection and frequently clear the established infections, although latent infection could occur. Yibeltal (2005) and Ashenafi *et al.*

(2007) also reported higher prevalence in adult than in younger ones, which agrees with this finding. The increase in prevalence of the disease with age of the animal was also reported by Tekelaye and Kasali (1990). Seroprevalence may increase with age as the result of prolonged exposure to pathogen, particularly in traditional husbandry practice where females are maintained in herds over long period of time.

Analysis of association between parity number and seropositivity to brucellosis with univariable and multivariable logistic regression revealed that animals with parity of more than four (>4) were at a significantly higher risk (P>0.05) of being infected by the disease compared to animals of lower parity. The risk of seropositivity was 6.19 times higher in >4 parity group in comparison to [0-1] parity group in multivariable logistic regression. The significant association between parity number and seroprevalence of caprine brucellosis was reported by Ashagrie *et al.* (2011) which agrees with the current finding.

It was found out in this study that flock size did not have statistically significant difference on the prevalence of the disease in the area. Similarly, Ashagrie *et al.* (2011) also reported the absence of statistically significant association between flock size and seroprevalence of the disease. This might be attributed to small difference in flock size between herds and due to uncontrolled herd mixing that could create equal chance for transmission of *Brucella* among different flocks of goats and sheep. Similarly, there was no significant difference in seropositivity to the disease between animals which had experienced abortion previously and those which did not; which might be due to poor knowledge of owners whether an animal had aborted or not.

Statistical analysis of different management risk factors obtained from questionnaire survey showed the absence of statistically significant association with seropositivity to the disease at herd level except in seasonal migration of the herd. Seropositivity of brucellosis was significantly higher (P=0.038) in seasonally migrating herds than those which do not in univariable logistic regression. However, multivariable logistic regression showed the absence of statistically significant difference in seropositivity between seasonally migrating and non-migrating herds. Berhe *et al.* (2007) indicated significantly higher prevalence of brucellosis in

transhumance system compared to sedentary system in cattle. Radostits *et al.* (2006) indicated that in several countries there is a strong correlation between the prevalence of brucellosis in small ruminants and the practice of transhumance which agrees with the current finding. This might be due to the fact that mobile herds have greater opportunity to come in contact with other infected herds.

According to the result of questionnaire survey, the owners practiced selling of sheep and goats which frequently abort which may favor transmission of the disease to other herd and areas. The result of the survey also showed that all the respondents neither have any knowledge about the disease nor did they practice proper disposal of the aborted material. They throwed the aborted fetus and fetal membrane into the field and this practice may increase the transmission of the disease. All of the respondents who assisted delivery did not use any protective material to avoid infection by the disease. The habit of consumption of raw milk by some of the respondents may also predispose them to zoonotic diseases such as brucellosis.

6. CONCLUSION AND RECOMMENDATIONS

The current study showed that brucellosis is a well-established infection among goats and sheep across all studied peasant associations and districts with individual and herd seroprevalences of 4.6% and 25.2% respectively. The absence and presence of pregnancy, and parity number were found to be most important risk factors associated with seroprevalence of brucellosis showing statistically significant difference ($P<0.05$) both in multivariate and univariate logistic regression analysis. There was a statistically significant difference between age groups in seropositivity to brucellosis in univariate logistic regression. However, age was not associated with the prevalence of the disease in multivariable logistic regression. Seasonal migration of herds showed a statistically significant association with seropositiovity to the disease with migrating animals being at higher risk of infection in univariable logistic regression. Questionnaire survey result showed that the owners of small ruminants in the studied areas did not have any knowledge about brucellosis. Subsequently, they neither use any protective material while handling aborted material nor do they practice any safe disposal of aborted material. Moreover, some of the owners practiced the habit of drinking raw milk. Generally, this finding revealed high risk of transmission of the disease in the small ruminants in the studied area. The questionnaire survey also showed high risk of exposure of people of the studied area to the disease.

Hence, the following recommendations are forwarded:

- The species and biotypes of *Brucella* involved in small ruminants under Ethiopian conditions should be identified since such investigations have important implications for the type of vaccine that should be used and when monitoring the efficacy of control programmes.
- The public should be informed about the risk of brucellosis and the way of transmission among small ruminants and from animal to human.
- In order to mount more specific intervention efforts, knowing the exact status of brucellosis in the area, and conducting detailed and continuous studies are recommended.

- Strict animal movement control should be practiced in order to prevent the spread and transmission of the disease from infected sheep and goats to the non-infected ones.
- Proper hygienic practices and good husbandry management should be exercised to minimize the spread of disease in the flock.
- Brucellosis should be sought as a disease of public health importance and hence, collaboration between veterinary and human health personnel is utmost important.

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8. ANNEXES

Annex 1: Questionnaire survey presented to individual small ruminant owners

Date_____

1. Name of the respondent_____ sex_____Age____

2. Address: Wereda/district) ---------- PA/kebele---------- Village----------

3. Occupation: 1) Farmer 2) Civil servant 3) Others_____

4. Level of education of the respondent 1) No formal education 2) Primary school
   3) Secondary school 4) College/ University graduate

5. Family status of the respondent

   Married_____Unmarried_____Sons_____Daughters_____

6. Labor distribution in small ruminant production:

Responsible family member	Feeding	Milking	Herding	Housing
Husband				
Wife				
Sons				
Daughters				
Laborer				

7. What is the purpose of your small ruminant farm? 1. Produce milk for home consumption, 2. Produce milk for market, 3. To sell 4. For house consumption of meat 5. Others (specify)_____

8. Which species of domestic animals do you own?

   1) Cattle 2) sheep 3) goats 4) Horses /mules / donkeys 5) Poultry

9. Under which scale is the farm categorized? 1. Small scale farm 2.Large scale farm
   3. Small holder mixed farming 4. Others (specify) _____

10. How is the farm management practiced? 1. Intensive 2.Semi-intensive 3. Extensive

11. Does your sheep and goat mix with other cattle? 1. Yes 2. No 12. If the answer for No 11 is yes, where? a) Watering points b) Grazing fields c) Market
13. What is the housing system of the farm? 1. Indoor (separate house) 2.Outdoor (tether,field) 3. Fenced enclosure (barn) 4. Share the same house with their owners
14. What is the herd/flock size? a) Sheep: 1. 1-10 2. 10-20 3. >20.
b) Goat: 1. 1-10 2. 10-20 3. >20
15. What is the sanitary condition of the barn or house: 1.Poor 2. Medium (satisfactory) 3.Excellent
16. How do you manage sheep and goats? A) Free grazing b) Stall feeding
17. Do you share the same house with small ruminants? a) Yes b) No
18. Do you use the same watering point with your animals? a) Yes b) No
19. What is the Grazing type of your herd? 1. Communal, 2.zero grazing, 3.tethering, 4.Fencing/paddocking
20. Do you migrate your small ruminant? If yes, in what season?_____
21. What are the major health problems common in sheep and goats in your area? Which are the most important ones? /Rank them. 1. Brucellosis/abortion 2.Tuberculosis 3.Internal parasite 4. External parasite 5.Anthrax 6.pasteurollosis 7.Others_____
22. Did you encounter abortion in your sheep and goat? 1. Yes 2. No
23. Do you know any diseases that cause abortion in sheep/goat? 1. Yes 2. No If yes, what is the local name?_____
24. At what stage of pregnancy do you think the problem of this abortion is more common?
1. First trimester 2. Second trimester 3. Third trimester 4. Any stage
25. At what stage of parity do you think the problem of this abortion is more common?
1. 1st parity 2. 2nd parity 3. 3rd and above stage of parity 4. At any stage
26. Which age group is mostly affected? 1. Young 2.old
27. How do you dispose off fetal membranes and aborted fetus? 1. Burying 2.Offering to dogs 3. Leaving in the field 4.Others specify_____
28. Have you ever handled the aborted fetus or membrane? 1. Yes 2. No
29. If yes, did you use any protective material on your hand? 1. Yes 2. No
30. What do you do with sheep/goat that frequently aborts? 1. Sell 2. Slaughter

3. Keep 4. Other_____

31. Do you have awareness on brucellosis that it can be transmitted to human through raw milk & milk products consumption from infected animal? 1. Yes 2. No
32. Do you know the control method of brucellosis? 1. Yes 2.No
33. Do you have awareness on brucellosis that it can also be transmitted to human through handling aborted fetus and other reproductive organs and discharges from infected animal? 1. Yes 2. No
34. What is the small ruminant milk & milk product consumption habit of your family?
    a) Sheep 1. Raw 2. Boiled 3.Traditional processing 4. Others_____
    b) Goat 1. Raw 2.Boiled 3.Traditional processing 4. Others_____
35. Why do you boil milk? 1. Fear of disease transmission 2. Culture
    3. Dislike/psychological 4. To increase taste/palatability 5. No reason
36. How frequently do you drink raw milk/its products? 1) Never 2) Rarely 3) Frequently
37. Do you separate ewes/does during parturition? 1. Yes 2.No
38. What is the meat consumption habit of the family? 1) Raw 2) boiled 3) roasted 4)kitfo 5)others____
39. Have you ever done delivery/parturition assistance in small ruminant? 1. Yes 2. No
40. If the answer for question 39 is yes, how? 1. Bare hand pulling 2.Other means_____

Annex 2: Questionnaire format for blood sampling from individual animal

No	Animal ID	species	sex	Age	Flock size	Pregnancy	Parity No	History of abortion, stillbirth, weak new born			
								Yes /No	Month	parity	frequency

Annex 3: Procedures of iELISA test

For confirmation of mRBT positive sera, SERELISA *Brucella* OCB Ab Mono Indirect kit is used.

Principle of the test:

The SERELISA *Brucella* OCB Ab Mono Indirect kit uses an indirect immunoenzymatic technique allowing the detection of *Brucella* lipopolysacchride (LPS) antibodies in individual bovine, ovine and caprine serum samples or pools of 10 bovine sera.

The reaction is composed of three steps:

1. Each individual or pooled serum sample is placed in a well sensitised with *Brucella* LPS. Antibodies present in sample bind to the bacterial antigen coated to the wells.
2. After a wash step, a peroxide conjugate is added. It fixes to the immunoglobulins (antibodies) previously captured, forming a complex.
3. Excess conjugate is eliminated by a wash step. The enzyme linked to the complex is revealed by addition of a substrate, which is transformed into a coloured product. After stopping the reaction, the optical densities are measured. The presence or absence of antibodies is determined using threshold values obtained from the positive control.

1. Materials and reagents required

- Distilled or demineralised water
- Adjustable or set pipettes to measure and deliver between 0 to 1000µl.
- Graduated cylinders(100-1000 ml)
- Manual, automatic or semi-automatic washing device for micro titration plates.
- Micro plate reader, fitted with filters for biochromatic reading at at 450 and 630nm.
- Incubator

2. Procedure

A. Preliminary steps

- Prepare samples to be tasted
- Dilute the sample at 1:10 in sample diluent in test tube or in a dilution plate
- Dilute again at 1:10 in a test tube or directly into the wells.
- Dispense 10 µl of already diluted sample in 90 µl of sample diluent

B. Test Procedure

I. Control and sample distribution

1. Control distribution:

- The controls are diluted at 1:10 in sample diluent in a test tube or into a dilution plate, then dilute again at 1:10 in a test tube.
- Dispense 10 µl of already diluted control in 90µl of sample diluent.
- 100 µl of the diluted negative control is distributed in wells A1 and A2, 100 µl of the diluted positive control in wells B1 and B2.

2. Sample distribution

- The samples can be tested individually or in duplicate. Distribute 100 µl in each well.
- Strips should always be placed on the frame so that both washer and reader can be used.
- Cover the wells with adhesive film, cut to the necessary length by the number of strips used
- Mix by gentle shaking the plate manually or by using a plate agitator.

3. Incubation of the plate

- 1hour (±5 min) at 3700c (±3^0c)

Washing

- Wash buffer: dilute the concentrated washing solution 1:10 in distilled or demineralized water.
- Carefully remove the adhesive film and wash 4 times

II. Addition of conjugate

1. Preparation of conjugate

- Dilute the concentrate conjugate 1:200 in conjugate diluent . 2 ml are needed for one strip, meaning 10 µl of conjugate in 1.98 ml of conjugate diluents

2. Distribution of conjugate

- Add 100 µl of diluted conjugate to all the wells and cover with a new piece of adhesive film.

3. Incubation of conjugate

- Incubate for 30 minutes (±5 min) at 37^0c (±3^0c)

Washing: Carefully remove the adhesive film and wash 4 times

III. Revelation

1. Addition of the substrate:
   - Add 100 µl of the buffered peroxidase substrate per well. Do not cover with adhesive film at this stage.
   - Mix by gentle shaking of the plate manually or use a plate agitator to ensure correct homogenization
2. Incubation of substrate
   - Incubate for 30 minutes (±5 min) at laboratory temperature (20^0c $+3^0$c), shielded from light
3. Addition of stop solution
   - Add 50 µl of stop solution per well.
   - Mix by gentle shaking the plate manually or by using a plate agitator. Make sure that no bubbles occur in the wells
4. Measure the optical density
   - Measure the optical density (OD) bichromatically at 450 and 630 nm or monochromatically at 450 nm (in the yellow band).
   - Reading bichromatically is strongly recommended. Should a monochromatic reader be used, ensure the cleanliness of the bottom of the wells prior to reading.

III. Expression and Interpretation of the results

The presence or absence of antibodies against LPS of *Brucella* is determined by comparing the optical densities to the threshold values obtained from the positive control.

9. CURRICULUM VITAE

1. Personal information

Name: Abiot Deddefo
Birth Date: March 23, 1980 G.C
Birth Place: Munessa, Arsi zone, Ethiopia
Sex: Male

2. Work Experience

i. For 1 year and 5 ½ months as junior instructor at Alagae ATVET College (starting from January, 2005)
ii. 4 year and 7 months as senior instructor and Academic Vice Dean at Kombolcha ATVET College
iii. Starting from 9/3/2011 up to now Artificial Insemination officer at Adama Science and Technology University

3. Seminar Titles:

i) The Role of immunoglobulins in reducing neonatal mortality (under graduate- 6th year)
ii) The Impact of climate change on Food safety (Seminar on 3rd year MSC programme)

4. Thesis Title (DVM):

Bacteriological quality of raw milk collected from milk units in Selalle, North Shoa zone, Ethiopia.

5. Trainings participated:

i. Veterinary public Health, at Addis Ababa University Faculty of Veterinary Medicine
ii. Agricultural occupations standard setting workshop
iii. Pedagogy training at Kotebe teachers training
iv. Agricultural product value chain, Organized by Oromia TVET Agency at Asella
v. Different trainings on Business plan Reengineering (BPR) at different areas

6. Educational Background

1995-98: Holeta Comprehensive secondary school (9^{th}-12^{th} grades)
1999-2004: Addis Ababa University, Faculty of Veterinary Medicine

7. Degree awarded

Doctor of Veterinary Medicine from Addis Ababa University, Faculty of Veterinary Medicine

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