Thesis Ref NO._____

SEROPREVALENCE OF BOVINE BRUCELLOSIS AND ITS PUBLIC HEALTH SIGNIFICANCE IN HOLETA TOWN, WOLMERA DISTRICT AND ADDEA BERGA ETHIOPIAN INSTITUTE OF AGRICULTURAL RESEARCH DAIRY FARM, OROMIA REGIONAL STATE, ETHIOPIA

MVSc THESIS IN VETERINARY PUBLIC HEALTH

By:

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JUNE 2020

BISHOFTU, ETHIOPIA

SEROPREVALENCE OF BOVINE BRUCELLOSIS AND ITS PUBLIC HEALTH SIGNIFICANCE IN HOLETA TOWN, WOLMERA DISTRICT AND ADDEA BERGA ETHIOPIAN INSTITUTE OF AGRICULTURAL RESEARCH DAIRY FARM, OROMIA REGIONAL STATE, ETHIOPIA

By:

Temesgen Kassa Getahun

A Thesis submitted to Addis Ababa University College of Veterinary Medicine and Agriculture in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Public Health.

June 2020

Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology and Public health

As MVSc research advisors, I here certify that I have read and evaluated the Thesis prepared under my guidance by **Temesgen Kassa entitled: "Seroprevalence of Bovine Brucellosis and its Public Health Significance in Holeta Town, Wolmera District and Addea Berga Ethiopian Institute of Agricultural Research Dairy Farm, Oromia Regional State, Ethiopia**"; I recommend that it can be submitted as fulfilling the MVSc Thesis requirement.

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Date of Submission: 08/06/2020GC

ACKNOWLEDGEMENTS

First of all I would like to thank the Almighty GOD for all his glorious and merciful that always keeps my life confident and peace.

I would like to express my deepest and sincere gratitude to my academic advisor Dr. Gezahegn Mamo (Associate Professor) Addis Ababa University (AAU), College of Veterinary Medicine and Agriculture (CVMA) and his overall research guidance and taking his time to correct this manuscript.

I express my heartfelt gratitude to Dr. Beksisa Urge for his support in collecting field data and Dr. Samson Leta for his continued support in data analysis.

My special thanks goes to Ethiopian Institute of Agricultural Research Holeta Agricultural Research Center Animal Health Research Program staff members, and Addis Ababa University, College of Veterinary Medicine and Agriculture (AAU, CVMA), Bishoftu, Ethiopia, academic and support staff members of the college, for their positive cooperation during my research work.

My final gratitude is reserved to my wife Gete Fikadu, and the rest of all my beloved families for their invaluable material and moral support in all facets of life and their constant encouragement to prepare this paper.

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LIST OF ABBREVIATIONS

AHO	American Health Organization
AMOS	Abortus-melitensis-ovis-suis
AST	Allergic Skin Test
BPAT	Buffered Plate Agglutination Test
EIAR	Ethiopian institute of agricultural research center
FPA	Fluorescence Polarization Assay
HARC	Holeta agricultural research center
kD	Kilo Dalton
LPS	Lipopolysaccharide
MBM	Malachite *Brucella* Medium
Mbp	Mega base pair
MIVP	Microbiology, Immunology and Veterinary Public Health
MLVA	Multiple locus variable analysis
MZN	Modified Ziehl–Neelsen
NK	Natural Killer
OMP	Outer Membrane Protein
OPS	Polysaccharides
PFGE	Pulsed-Field Gel Electrophoresis
RER	Rough Endoplasmic Reticulum
RLPS	Rough Lipopolysaccharide
SAT	Serum Agglutination Test
SDA	Serum Dextrose Agar
SLPS	Smooth Lipopolysaccharide
SNPs	Single Nucleotide Polymorphisms
TSA	Tryptose Soya Agar
VNTR	Variable Number of Tandem Repeats
RFM	Retained Fetal Membrane

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ABSTRACT

A cross-sectional study was conducted from December, 2019 to May, 2020 with the aim to determine seroprevalence and identify the potential risk factors of brucellosis in dairy cows with recent case abortion and their owners and farm workers, and to assess knowledge, attitude and practices of the owners and farm workers toward the disease in Holeta Town, Wolmera District and Addea Berga Ethiopian Institute of Agricultural Research dairy farm Oromia regional state, Ethiopia. Purposive sampling was performed in the respective farms and kebeles to screen recent cases of abortion from dairy cows. A total of 352 blood samples from dairy cattle, 149 blood samples from animal owners and 17 from farm workers were collected for serological test. The serum samples collected were initially screened using Rose Bengal Plate test and Complement Fixation test was used as confirmatory test. The overall seroprevalence of bovine brucellosis were 1.2% (95%CI: 0.0047-0.0297) by RBPT alone and 0.6% (95%CI: 0.0016-0.0209) using combined RBPT and CFT tests. In human, the overall seroprevalence of zoonotic brucellosis was 4.2% (95%CI: 0.0204-0.0842) by RBPT and 1.2% (95%CI: 0.0032-0.0427) using combined RBPT and CFT tests. Statistical analysis of risk factors by univariable firth's bias reduced logistic regression indicates that late stage of abortion (OR=14.74, p=0.0002), retained fetal membrane (OR=32.74, p=0.006), market based stock replacement (OR=16.55, p=0.002), natural breeding method (OR=7.58, p=0.05) and presence of parturition pen (OR=11.511, p=0.027) were found to be significantly associated with the seropositivity for *Brucella* infection in dairy cattle. The seropositivity of human brucellosis was significantly influenced by human housing (OR=1.8, p=0.002), contact with aborted fetus (OR=21.19, p=0.017), drinking raw milk from non-aborted (OR=24.99, p=0.012), aborted (OR=5.72, 0.019) and retained fetal membrane (OR=4.22, p=0.029) cows. A structured interview question was administered to 284 respondents. Accordingly, most respondents had no knowledge on brucellosis (93.3%) and other zoonotic diseases transmitted by handling animal delivery (88%) and consuming raw milk and other animal products (90.0%). In conclusion, the present seroprevalence study revealed that brucellosis is prevalent at lower rate among dairy cattle and in exposed individuals in the study areas. However, since there were no control strategies implemented in the area, there is a potential risk for transmission of brucellosis in dairy cattle and exposed human population of study areas. With the low prevalence of bovine brucellosis in government owned and small holder farms, implementation of test and slaughter strategy with compensation to farmers is recommended; while in case of human brucellosis, continuous social training with feedback assessments, implementing one health approach framework and since its presence is confirmed and the risk factors are identified, the medical personnel should give attention as to differential diagnosis of the disease which has been overlooked so far.

Keywords: *Abortion, Bovine Brucellosis, Human Brucellosis, Risk factors, Seroprevalence.*

1. INTRODUCTION

Brucellosis is a contagious and economically important bacterial disease of animals worldwide and it is considered as one of the neglected zoonoses in the world. It has been virtually eliminated from the majority of the developed countries, but it is still endemic in Africa, the Middle East, Central and Southeast Asia, Central and South America and in most of the Southern European countries. Despite being endemic in many developing countries (Donev *et al*., 2010), brucellosis remains under diagnosed and under reported. It is an important disease among livestock and people in sub-Saharan Africa (Smits *et al*., 2007).

The genus *Brucella* consists of commonly known six classically recognized species based on antigenic/biochemical characteristics and primary host species: *B.abortus* (cattle), *B.melitensis* (sheep and goats), *B.suis* (swine, cattle, rodents, wild ungulates), *B.ovis* (sheep), *B.canis* from dogs and *B.neotomae* of rodents (Tiller *et al*., 2010). *Brucella* bacterium is 0.5-0.7μm in diameter and 0.6-1.5μm in length. They are oxidase, catalase and urease positive. *Brucella* species are facultative intracellular, Gram negative, non-spore-forming and partially acid-fast coccobacilli that lack capsules, endospores or native plasmids. They survive freezing and thawing but most disinfectants active against *Brucella*. Pasteurization effectively kills *Brucella* in milk (Fretin *et al*., 2005).

Bovine brucellosis is an infectious and contagious disease and is predominantly a disease of sexually mature animals which usually caused by *B.abortus*; occasionally by *B.melitensis* and *B.suis*. It is of major economic importance in most countries of the world. It affects approximately 5% of the livestock population worldwide and continues to increase in distribution. The disease poses a barrier to trade of animals and animal products, represents a public health hazard, and is an impediment to free animal movement. Economic loss due to delayed heat, loss of calves, reduced milk production, culling and economic losses from international trade bans in tropics and subtropics (WHO, 2001).

In cattle the mode of transmission is usually from animal to animal by contact following an abortion and retained placenta. Pasture or animal barn may be contaminated and the organisms are most frequently acquired by ingestion but also inhalation and conjunctival inoculation are other possibilities. The use of pooled colostrum for feeding newborn calves may also transmit infection. Sexual transmission usually plays little role in the epidemiology of bovine brucellosis. However, artificial insemination can transmit the disease and semen must only be collected from animals known to be free of infection (WHO, 2001).

Brucellosis in bovine is characterized primarily by abortion late in pregnancy, frequently followed by fetal membrane retention and endometritis which may be the cause of infertility in subsequent pregnancies. In fully susceptible herds, abortion rates may vary from 30-80% (WHO, 2001, Yilma *et al*., 2016). Since the first report of brucellosis in the 1970s in Ethiopia, the disease has been noted as one of the important livestock diseases in the country (Geresu *et al*., 2016).

Brucellosis is considered as one of the most widespread but neglected zoonoses in the world. It was the second most important zoonotic disease in the world after rabies (WHO, 2006). It is also most important zoonotic disease in most developing countries, which have no national brucellosis control and eradication program (Donev *et al*., 2010). Brucellosis in human known as "undulant fever", "Mediterranean fever" or "Malta fever" and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. *B.melitensis*, *B.suis*, *B.abortus* and *B.canis* are zoonotic pathogenic species of *Brucella* (WHO, 2006, OIE, 2009).

It affects people of all age groups and of both sexes. The brucellosis 2003 International Research Conference estimated that 500,000 human infections occur per year worldwide, with incidences ranging from less than one case per 100,000 population in UK, USA and Australia, through 20 to 30 cases per 100,000 in southern European countries such as Greece and Spain, to more than 70 cases per 100,000 in Middle Eastern States such as Kuwait and Saudi Arabia (Pappas *et al*., 2006; Cutler *et al*., 2005). In some African countries human brucellosis was expected to be possibly endemic (Pappas *et al*., 2006).

Human clinical disease is characterized by severe flu-like illness, with a high fever that comes and goes (hence the name "undulating fever"), which may progress to a more chronic form with serious complications in joints (arthritis) or internal organs (heart failure). In this chronic, recurring form, humans can be so debilitated that they are no longer able to work and they become a health care burden on their families (Pappas *et al*., 2006).

Human can acquire brucellosis by contact with infected tissues, urine, vaginal discharges, aborted animal fetuses and especially placentae. It can also be transmitted by inhalation of aerosols which may occur in animal pens and stables, abattoirs and laboratories. A small number of cases have occurred following accidental self-inoculation of the strain 19 animal *Brucella* vaccine (WHO, 2001).

In Ethiopia there is no documented information on how and when bovine brucellosis was introduced and established. However, in the last two decades several serological surveys have showed that it is endemic and widespread (Berhe *et al*., 2007; Ibrahim *et al*., 2010). The disease is prevalent in cattle in high land and lowland areas (Eshetu *et al.*, 2008; Kebede *et al*., 2017; Edao *et al*., 2018). Even though a large number of studies on bovine brucellosis have been reported in different part of the country, studies conducted on the role of bovine brucellosis in relation to public health significance in occupational exposed individuals are limited in intensive and extensive production systems (Tolosa *et al*., 2010; Megersa *et al*., 2011).

Though there is limited information on the seroprevalence of bovine brucellosis in some farms in Holeta Town, no previous seroprevalence report of brucellosis in small holder dairy cattle and in exposed farm owner and farm employees found in smallholder and government owned farm in Holeta Town, Wolmera District and Adda Berga Ethiopian Institute of Agricultural Research (EIAR) dairy farm which are located in the milk-shed areas for Addis Ababa and its surrounding. In addition, assessment of the status of the disease and understanding of the awareness among the community has paramount importance in order to identify the risk factors for infection and zoonotic transmission to design appropriate measures to reduce the public health significance of brucellosis. Therefore, this study was carried out with the following objectives:

1.1. General Objective

- ✓ To determine the seroprevalence of brucellosis in recently aborted dairy cows, and their owners and farm workers, and assess its public health importance in Holeta town, Wolmera District and Adea Berga Ethiopian Institute of Agricultural Research dairy farm, Oromia Regional State, Ethiopia.

1.2. Specific Objectives

- ✓ To determine the current seroprevalence status of brucellosis in bovine with recent history of abortion in the study area.
- ✓ To determine the seroprevalence of brucellosis in animal owners and government farm employees.
- ✓ To assess the associated risk factors of bovine and human brucellosis in the study area.
- ✓ To assess knowledge, attitude and practice of animal owners and government farm employees in relation to zoonotic brucellosis.

2. LITERATURE REVIEW

2.1. Etiology

2.1.1. Taxonomy

The genus *Brucella* resides within the family *Brucellaceae* (familyIII) with *Mycoplana* and *Ochrobactrum*, of the order *Rhizobiales* in the class *Alphaproteobacteria* of the phylum *Proteobacteria*. Currently ten species are recognized including the better known six classical species comprised of *B.abortus, B.melitensis, B.suis, B.ovis, B.canis* and *B.neotomae*. More recently, new members to the genus include *B.ceti*, *B.pinnipedialis*, *B.microti* and *B.inopinata* (Godfroid *et al*., 2005) and about 25 additional *Brucella* strains/species are being sequenced (Banai and Corbel, 2010).

2.1.2. Host preferences

Brucella has definite host preferences. Secondary hosts play a minor role in the maintenance and spread of a particular *Brucella* species. *B.abortus* mainly infects cattle and is the main cause of contagious abortion. However, sheep, goats, dogs, camels, buffaloes as well as feral animals may also contract *B.abortus* infections (Radostits *et al*., 2007). The species of *Brucella* based on preferential host specificity are *B.abortus* (cattle), *B.suis* (swine), *B.canis* (dogs), *B.ovis* (sheep), *B.neotomae* (desert wood rats), *B.cetacea* (cetacean), *B.pinnipedia* (seal), *B.microti* (voles), and *B.inopinata* (unknown) (Godfroidah` *et al*., 2005) (Table 1).

Table 1: *Brucella* species and their host preferences

Species	Zoonotic importance	Host preference
B abortus	Moderate	Cattle*, sheep, goat, pig,, horse
B.melitensis	High	Sheep*, Goat, cattle
B.suis	Moderate	Pig*
B.canis	Mild	Dog*
B.ovis	Absent	Sheep*
B.neotomae	Absent	Deseret wood rat*
B.ceti	Mild	Ceteceans*
B.Pinnipedials	Mild	Seals*
B.microt	Absent	Common Voles*
B.inopinata	Mild	Undetermined host*

*- Represent natural host

Source: (Godfroida *et al*., 2005)

B.melitensis, B.abortus, B.suis, and *B.canis* are known to cause human disease. *B.neotomae* and *B.ovis* are not pathogenic to humans. The majority of human cases worldwide are attributed to *B.melitensis*. Some *Brucella* specie like *B.abortus, B.melitensis, B.suis* and *B.canis* can affect a ranges of hosts in addition to their natural hosts resulting hazards on the health of animals including humans; due to this, infected countries are challenged and have been under difficulties to overcome or control brucellosis effectively (Pappas *et al*., 2006).

2.1.3. Genome and its characteristics of Brucella organism

In 1985, it was proposed that the six *Brucella* species should be grouped as biovars of a single species based on DNA-DNA hybridization studies. The genomes sequenced from genus *Brucella* are also known to be very similar in terms of both base composition and genome size. All sequenced species have a GC content of approximately 57%, and most genomes consist of approximately 3.3Mbp divided on two chromosomes. None of the sequenced members of the *Brucella* genus have any plasmids reported. The first *Brucella* species to be sequenced was

B.melitensis 16M (biovar 1) followed closely by *B.suis* (biovar 1). Analysis of 16S rRNA sequences places *Brucella* species as members of the alpha-2 *Proteobacteria* (Bohlin *et al.*, 2010).

Pulsed-field gel electrophoresis (PFGE) maps of the classical *Brucella* species genomes are composed of two circular chromosomes of approximately 2.1 and 1.2Mbp, with the exception of *B.suis* biovar 3, which has a single chromosome of 3.1Mbp. PFGE studies revealed other differences, including a 640-kb inversion in the small chromosome of *B.abortus* and a deletion in the small chromosome of *B.ovis*. The two chromosomes of *Brucella* differ in important ways. The origin of replication of the large chromosome (Chr I) is typical of bacterial chromosomes, while that of the small chromosome (Chr II) is plasmid like. Further, most of the essential genes are located on Chr I. The GC content of the two chromosomes is nearly identical, consistent with the assertion that the assimilation and stabilization of a plasmid was an ancient event in *Brucella* (Paulsen *et al*., 2002).

The genome sequences of *B.melitensis* and *B.suis* have been determined. Comparative analyses revealed both that the two genomes are extremely similar and that they have many similarities to both bacterial plant and animal pathogens and symbionts (Halling *et al*., 2005). The sequence identity for most open reading frames (ORFs) was 99% or higher. Nevertheless, unique fragments were reported to exist between these two genomes. Prior to sequencing the *B.abortus* genome, a large number of short sequences were available in gene bank. Many of these sequences were derived from analyses of plasmids estimated to cover 20% of the genome from a random shotgun library of *B.abortus* S2308 (Bohlin *et al.*, 2010).

The *Brucella* cell envelope is a three-layered structures namely an inner or cytoplasmic membrane, a periplasmic space and an outer membrane can be differentiated (Lapaque *et al*., 2005). The outer cell membrane closely resembles that of other Gram-negative bacilli with a dominant lipopolysaccharide (LPS) component and three main groups of proteins. It contains lipopolysaccharide (LPS), proteins, and phospholipids. The major *Brucella* outer membrane proteins (OMPs) are group 2 porin proteins having 36-38kDa; group 3 proteins contain 25-27 kD

molecular mass and a lipoprotein covalently linked to peptidoglycan (Paulsen *et al*., 2002). Group 1 minor proteins have a molecular mass of 88 to 94 kDa (Delvecchio *et al*., 2002).

2.1.4. Antigenic characteristics

Brucella has lipopolysaccharide (LPS) as a major component of their outer membrane and an important virulence factor like other Gram-negative bacteria. Thus, their colonial morphology is termed as either smooth or rough depending on the LPS structure. Structural variation in the LPS of smooth strains also defines the so called A and M antigens that have some significant role in typing (Godfroid *et al*., 2010). These antigens reflect differential O-side chain which is linked to α-1, 2 in A dominant strains but with every fifth residue linked α-1, 3 in M dominant strains. The O-polysaccharide (O-PS) is involved in bacterial virulence. It contributes in complement resistance and more importantly critically modulates bacterial entry into cells so that its removal causes attenuation (Godfroid1 *et al*., 2010).

All smooth *Brucella* cross-react with one another in agglutination tests. This cross-reaction does not occur with non-smooth or the rough *Brucella* strains. Lipopolysaccharide (LPS) comprises the major surface antigens of the corresponding colonial phase involved in agglutination. The (S-LPS) molecules carry the A and M antigens, which have different quantitative distribution among the smooth *Brucella* strains. This is of value in differentiating biovars of the major species using absorbed monospecific A and M antisera (European Commission, 2001). Serological cross-reaction has been reported between the smooth *Brucella* and various other Gram negative bacteria like, *E. coli* (European Commission, 2001; Corbel, 2006).

2.1.5. Morphology and staining

Brucella species are slow-growing, Gram negative coccobacilli or short rods measuring from 0.6 to 1.5μm long and from 0.5 to 0.7μm wide, non-motile, non-spore forming, non-capsulated, non-flagellated, aerobic, facultative intracellular bacteria capable of invading, survive and multiply within epithelial cells, placental trophoblasts, dendritic cells and macrophages. They are usually arranged singly, and less frequently in pairs or small groups. The morphology of *Brucella* is

constant, except in old cultures where pleomorphic forms may be evident. Usually do not show bipolar staining, not truly acid-fast, but are resistant to decolorization by weak acids and thus stain red by the Stamp's modification of the Ziehl–Neelsen's method (Gorvel, 2008) (Figure 1). The presence of intracellular, weakly acid-fast organisms of *Brucella* morphology or immuno-specifically stained organisms is presumptive evidence of brucellosis. However, these methods have a low sensitivity in milk and dairy products where *Brucella* is often present in small numbers, and interpretation is frequently impeded by the presence of fat globules (OIE, 2012).

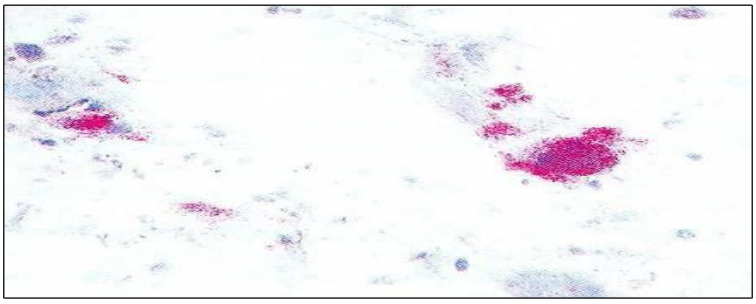

Figure 1: *B.bortus* in an MZN-stained smear of a cotyledon from a case of bovine abortion
Source: (Quinn *et al*., 2013).

2.1.6. Growth requirement and cultural characteristics

Selective media

Brucella species are slow growing and the use of enriched selective media is recommended for primary isolation from most clinical specimens because of the high numbers of over growing contaminants may inhibit the isolation. Such selective media are prepared by incorporating antibiotics and bacteriostatic dyes onto basic enriched media such as *Brucella* selective medium base. Prepared by adding six antibiotics; bacitracin, vancomycin, nalidixic acid, polymixin B, nystatin and cycloheximide onto sucrose dextrose agar for the isolation of *Brucella* species from the clinical sample (Mari´n *et al*., 1996).

These antibiotic supplements of the Farrell's medium are commonly used, in different combinations and proportions onto any one of the basal media such as *Brucella* medium base (Oxoid), Tryptone soya agar (Oxoid), Serum dextrose agar (Oxoid), Columbia blood agar (Bio

Merieux) and other medium bases, for the formulation of selective media for isolation of *Brucella* species. Moyer and Holocomb, (2005) reported the use of chocolate agar containing selective supplements for the isolation of *Brucella* spp. Similarly, the use of new media such as rifampin *Brucella* medium and malachite *Brucella* medium (MBM), together with Tryptose Soya Agar (TSA), was found to enhance the recovery of *B.abortus* RB 51 (Hornsby *et al*., 2000). For the isolation of *Brucella* species from milk samples although solid media have been used successfully, the use of enrichment media such as serum dextrose, tryptone soya or *Brucella* broth containing selective supplements of at least amphotericin B and vancomycin should be used because the microorganisms are usually present in too low numbers to be detected on solid media (OIE, 2004).

Growth temperature

The optimum growth temperature for *Brucella* organisms is 36-38^{o}C, but most strains can grow between 20^{o}C and 40^{o}C (European Commission, 2001).

Colonial behavior

Brucella requires biotin, thiamin and nicotinamide and the growth are improved by serum or blood. On suitable solid media *Brucella* colonies are visible after 3 days incubation. After four days, *Brucella* colonies become round, 1-2mm in diameter, with smooth (S) margins, transparent and pale honey color when plates are viewed in transmitted light and have a bluish translucent appearance in reflected light. When viewed from above, colonies appear convex and fairly white. Later, colonies become larger and slightly darker. Rough *Brucella* isolates produce similar colony size and shape but are more opaque off-white in color with a rather granular surface (Alton *et al*., 1988). Growth on blood agar is slower than on Serum Dextrose Agar (SDA) with the production of non haemolytic, greyish-white glistening colonies after 72 hours incubation. Growth in liquid media is usually poor unless the culture is vigorously shaken. On semi-solid media, CO2-independent *Brucella* strains produce uniform turbidity from surface down to 3mm depth while CO2-dependent strains produce a disk of growth 2mm below the surface of the medium. Little or slow growth is produced by many *Brucella* strains on MacConkey agar, even after five days at

37^0C. The growth of most *Brucella* strains is inhibited by media containing bile salts, tellurite or selenite and does not require haeme (V-factor) and nicotinamide-adeninedinucleotide (X-factor) (Corbel, 2006).

Smooth *Brucella* cultures, especially *B.melitensis* cultures, have a tendency to undergo variation during growth, especially with subcultures, and dissociate to rough (R) forms, and sometimes mucoid (M) forms. Colonies are then much less transparent with more granular, dull surface (R) or a sticky gelatinous texture (M), and range in colour from matt white to brown in reflected or transmitted light. Intermediate (I) forms between S, R and M forms may occur in cultures undergoing dissociation to the non-smooth state. Checking for dissociation is easily tested by crystal violet staining: rough colonies stain red/violet and smooth colonies do not uptake dye or stain pale yellow. Changes in the colonial morphology are generally associated with changes in virulence, serological properties and phage sensitivity (OIE, 2009).

Carbon dioxide and pH requirements

B.abortus does require 5 to 10% CO_2 and can be isolated on containing *Brucella* selective supplement solid media under aerobic and anaerobic conditions at 37^oC. The optimum pH for the growth of *Brucella* species is from 6.6-7.4 and culture media should be adequately buffered near pH 6.8 for optimum growth (Alton *et al*., 1988).

2.1.7. Biochemical characteristics

Brucella metabolism is oxidative and cultures show no ability to acidify carbohydrate media in conventional tests. *Brucella* species are usually catalyzed and oxidase positive and they reduce nitrate to nitrite except *B.canis* strains. The production of H_2S from Sulphur containing amino acid varies (European Commission, 2001). Urease activity of *Brucella* species varies from fast to very slow. Indole and acetyl methyl carbinol are not produced from tryptophan and glucose respectively. Methyl red and Voges-Proskauer tests are negative and *Brucella* neither liquefies gelatine nor lyses red blood cells (European Commission, 2001).

A summary of the differential characteristics and biochemical tests used to identify *Brucella* species from other bacteria is given in Tables 2 and 3.

Table 2: Differential characteristics of *Brucella* species

Test	***Brucella***	***Bordetella Bronchoseptica***	***Campylobater fetus***	***Moraxella Species***	***Acinetobacter species***	***Yersinia Enterocolitica***
Morphology	S.coccobacilli	Small coccobacilli	Coma shaped	Diplococcoid	Diplococcoid	Rods
Motility at 37^{o}C	-	+	+	-	-	-
Motility at 20^{o}C	-	-	-	-	-	+
Lactose fer.	-	-	-	V	V	-
Acid production	-b	-	-	-	V	+
Haemolysis on Blood Agar	-	+	-	V	V	-
Catalase	+	+	+	V	-	-
Oxidase	+c	+	+	+	-	-
Urease	+d	+	-	V	V	+
Nitrate reduction	+e	+	+	V	-	+
Citrate utilization	-	+	-	-	**V**	-

V= variable

Source: (Alton *et al*., 1988)

Table 3: Differentiation of the species and biovars of the genus *Brucella*

Characterstic	***B.melitensis* biovars**	***B. abortus* biovars**	***B.suis* biovars**	***B.ovis***
	1 2 3	1 2 3 4 5 6 7 9	1 2 3 4 5	
Catalase	+ + +	+ + + + + + + +	+ + + + +	+
Oxidase	+ + +	+ + + + + + + +	+e + + + +	–
Urease	+ + +	+ + +f + + + + +	+ + + + +	–
CO_2 req.	– – –	[+] [+] [+] [+] – – – –	– – – – –	+
H_2S prod.	– – –	+ + + + – [–] [+] +	+ – – – –	–
Growth on Media containing Dyes				
Thionin	+ + +	– – + – + + + +	+ + + + +	+
Basic fuchsin	+ + +	+ – + + + + + +	[–] – + [–] –	[–]
Agglutination With monospecific Anti-sera				
A	– + +	+ + + – – + + –	+ + + + –	–
M	+ – +	– – – + + – + +	– – – + +	–
R	– – –	– – – – – – – –	– – – – –	+

(a), Symbols: +, positive; [+], positive for most strains, [-], negative for most strains, -, negative, (b), For more certain differentiation of biovar 3 and 6, thionine at 1:25, 000 (w/v) is used; biovar 3 gives a positive growth response, biovar 6, (c),Dye concentration, 1:50, 000 (w/v). (d), Growth will occur in the presence of thionine at a concentration of 1:150, 000 (w/v). (e), Rapid reaction, most strains of *B. suis* test positive within 5 minutes. (f), Some field strains of *B. abortus* may be negative.

Source: (OIE, 2004; Garritty *et al*., 2005).

Susceptibility to dyes

The effect of the dyes thionin and basic fuchsin on various *Brucella* species and biovars varies (European Commission, 2001)). *Brucella abortus* grows in presence of basic fuchsin but does not grow in presence of thionin at the mentioned concentrations (Alton *et al*., 1988).

Susceptibility to antibiotics

Brucella species are sensitive to a wide range of antibiotics. Penicillin is used for the routine differentiation of the vaccinal strain of *B.abortus* species biovar 1 strain 19, used for the immunization of cattle from its respective field strain. This is because the S19 vaccine strain is sensitive to penicillin while the field virulent strain is resistant. Rev.1 vaccine is sensitive to streptomycin while the field virulent strain is resistant to streptomycin (Alton *et al*., 1988). On primary isolation, *Brucellae* are usually susceptible *in vitro* to gentamicin, tetracycline and rifampicin. Most strains are also susceptible to ampicillin, chloramphenicol, cotrimoxazole, erythromycin, spectinomycin and streptomycin. Most strains of *Brucella* are resistant to β lactans, cephalosporins, polymixin B, bacitracin, cycloheximide, clindamycin, linomycin, nystatin and vancomycin at therapeutic concentrations (European Commission, 2001).

2.2. Epidemiology of Brucellosis

The epidemiology of brucellosis is complex and it changes from time to time. Wide host range and resistance of *Brucella* to environment and host immune system facilitate its survival in the populations. Since cattle are found throughout the world, prevalence of brucellosis in cattle has been reported from a wide range of countries.

2.2.1. World distribution

The disease occurs worldwide, except in those countries where bovine brucellosis has been eradicated. These countries include Australia, Canada, Cyprus, Denmark, Finland, Netherlands,

New Zealand, Norway, Sweden and the United Kingdom. It remains endemic among Mediterranean countries of Europe, Northern and Eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America (FAO, 2003) (Figure 2). While *B.melitensis* has never been detected in some countries, there are no reliable reports that it has ever been eradicated from small ruminants in any country (Robinson, 2003). Although in most countries brucellosis is a nationally notifiable disease and reportable to the local health authority, it is under reported. Furthermore, brucellosis is also considered as a re-emerging problem in many countries (Cutler *et al*., 2005).

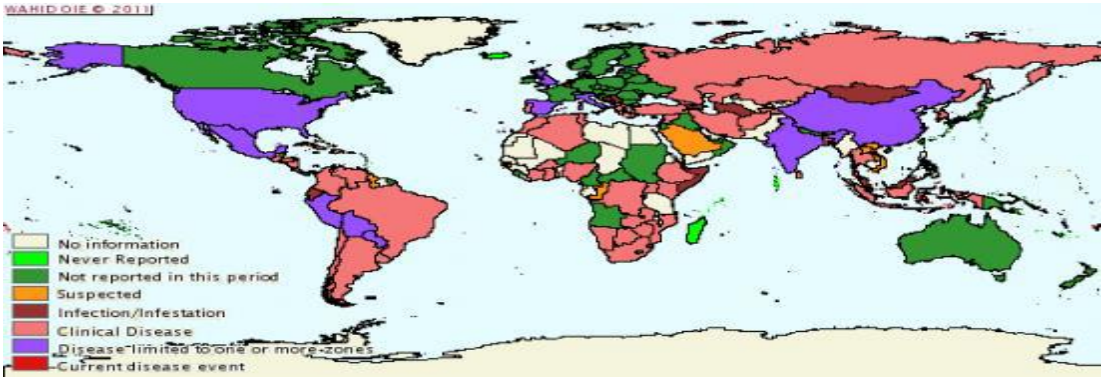

Figure 2: Worldwide distribution of bovine brucellosis (*B. abortus*) report
Source: (OIE, 2011)

2.2.2. Distribution in Africa

Brucellosis is a disease that has been known in Africa for a very long time, in both animals and humans. Bovine brucellosis was first recorded in Zimbabwe in 1906, Kenya in 1914 and in Orange Free State of South Africa in 1915 (Chukwu, 1985). The surveillance and control of brucellosis in this region was rarely implemented outside South Africa. In dairy production, the disease is a major obstacle to the importation of high yielding breeds and represents a significant constraint to the improvement of milk production through cross breeding (Mustafa and Nicoletti, 1995). Here is the summary of the seroprevalence of bovine brucellosis in some African countries in (Table 4).

Table 4: Seroprevalence of bovine brucellosis in some African countries

Country	No of cattle tested	Prevalence (%)	Test applied	Reference
Zambia	1245	14.1	RBPT, c-ELISA	(Muma *et al*., 2006)
Kenya	393	1	c-ELISA,CFT	(Kang'Ethe *et al*., 2007)
Sudan	574	24.5	c-ELISA	(Angara *et al*.,2004)
Ghana	444	2.9	RBPT	(Folitse *et al*., 2014)
Nigeria	-	24.0	RBT+ELI	(Mai *et al*. 2012)
Uganda	-	14	SA RBPT	(Miller *et al*., 2016)
Uganda	-	5	ELISA	(Bertu *et al*., 2010)
Zimbabewe	1291	5.5	RBPT, c- ELISA	(Matope *et al*., 2011)
Algeria	1032	9.7	BPAT	(Gwida, 2010)
Egypt	1966	5.4	BPAT	(Samah *et al*. 2008)
South Africa	5 059	1.5	RBPT, CFT	(Bishop *et al*., 1994)
Eritrea	1294	8.5	RBPT, CFT	(Omer *et al*., 2000)
Ghana	183	6.6	RBPT	(Kubuafor *et al*., 2000)

2.2.3. Status of brucellosis in Ethiopia

In Ethiopia, brucellosis in animals and humans has been reported from different localities of the country, particularly associated with cattle in both intensive and extensive management systems (Jilo, 2017). These prevalence studies in animals and human were largely confined to serological surveys and commonly targeted bovine brucellosis. Higher individual bovine brucellosis seroprevalence has been recorded in intensively managed cattle herds as compared to those in the extensive management system. These studies were conducted in local, pure and cross breed of cattle. In these studies, seroprevalence of brucellosis in cattle ranging from 0.06-11.2% were reported by CFT as depicted in Table 5.

Table 5: Seroprevalence of brucellosis in cattle in some part of Ethiopia

Location	Prevalence			Reference
	RBPT (%)	CFT (%)	c-ELISA(%)	
Addis Ababa	2.77	-	0.06	(Edao *et al*., 2018)
Tigray Region	3.3	3.19		(Berhe *et al*., 2007)
Sidama Zone	-	1.66		(Asmare *et al*., 2010)
West Tigray	-	4.9		(Haileselassie *et al*., 2010)
Jimma zone	-	0.77		(Tolosa *et al*., 2008)
Debrebirhan and Ambo	0.7	0.2		(Bashitu *et al*., 2015)
Adami Tulu	4.5	4.3		(Gebawo et al., 2014)
Debre-Zeit	3.3	2		(Alemu *et al*., 2014)
Somali and Oromia	-	0.9		(Gumi *et al*., 2013)
Benishangul Gumuz	1.2	1		(Adugna *et al*., 2013)
East Wollega Zone	2.96	1.97		(Yohannes *et al*., 2012)
East Showa Zone	11.2	-		(Dinka and Chala, 2009)
Wuchale-Jida district	12.5	11.0		(Kebede *et al*., 2008)
Central Oromia	4.9	2.9		(Jergefa *et al*., 2009)
Arsi Negele District	2.6	-		(Amenu *et al*., 2010)
Jimma zone	-	3.1		(Ibrahim *et al*., 2010)
Jijjiga zone	1.84	1.38		(Degefu *et al*., 2011)
WesternTigray	-	6.1		(Haileselassie *et al*., 2011)
Alage	2.28	2.4		(Asgedom *et al*.,2016)
Central Ethiopia	-	1.40		(Geresu *et al*., 2016)

2.2.4. Sources of infection

The risk associated with exposure of susceptible animals to the disease following parturition or abortion of infected cattle depends on three factors:- the number of organisms excreted, the survival of these organisms under the existing environmental condition and the probability of susceptible animals being exposed to enough organisms to establish infection. *B.abortus* achieves

its greatest concentration in the contents of the pregnant uterus, the fetus and the fetal membranes after birth (Radostits *et al*., 2006). In addition, vaginal discharge and to a lesser extent, farm areas contaminated by fecal matter of calves fed on contaminated milk could be considered as main source of infection. Infected animals also shed organisms in the milk. Therefore, raw milk or raw milk products of bovine origin are ready sources for infections in humans. There can be also accidental self-inoculation with live Brucella vaccine strains that result in the disease (PAHO/ WHO, 2001).

2.2.5. Mode of transmission and route of infection

The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water (Figure 3). Moreover, cows customarily lick after birth, fetuses, and newborn calves, all of which may contain a large number of the organisms and constitutes a very important source of infection. Bulls do not usually transmit infection from infected cows to non-infected mechanically. The use of infected bulls for AI constitutes an important risk, since the infection can be spread to many herds (PAHO/ WHO, 2001).

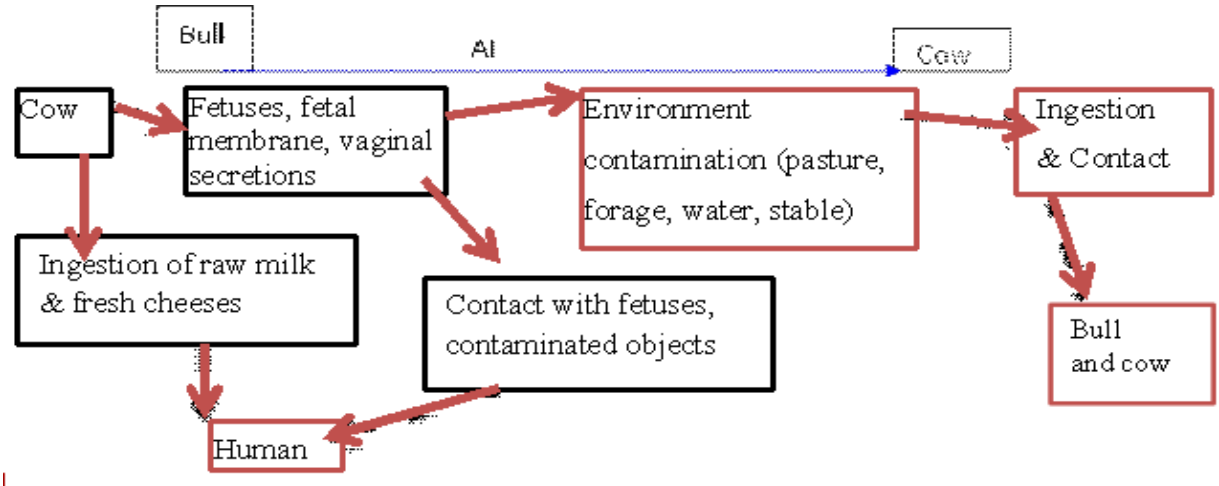

Figure 3: Bovine brucellosis mode of transmission

Source:(PAHO/WHO, 2001)

2.2.6. Reservoirs/ Carriers of Brucella species

Domestic animals

Domestic animals such as cattle, goats, sheep, pigs, camel, buffalo and dogs serve as a reservoir hosts (Moreno *et al*., 2002). *Brucella* species can survive in proper environmental condition, damp soil and seawater and can be a source of infection. Notably abortion materials such as fetal parts, and fetal membranes, amniotic fluid and vaginal discharges of infected animals may contain high amounts of the bacterium and act as source of brucellosis (Henk and Kadri, 2005).

Wild animals

Brucella abortus and *B.suis* have been isolated from a great variety of wildlife species (Godfroid, 2002). Wild ruminants have been suggested as brucellosis carriers, but they are probably not true reservoirs (Godfroid *et al*., 2013). Other works showed that wild ruminants do not play a relevant role in the maintenance of *B.abortus* and *B.melitensis* infections since limited cases of brucellosis have been reported in wild ruminants (Godfroidah` *et al*., 2005). However, a potential risk for brucellosis infection of livestock by wild animals could be associated when artificial management such as winter feeding increases aggregation (Godfroid, 2002; Gortázar *et al*., 2007).

2.2.7. Risk factors for infection

The risk factors that influence the initiation, spread, maintenance and/or control of bovine brucellosis are related to the animal population, management and to biology of the disease (Radostits *et al*., 2006).

Agent risk factors

Brucella species are a facultative intracellular pathogen, which is capable of multiplication and survival within host phagocytes. The organisms are phagocytosed by polymorph nuclear 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 and other sites such as the reticuloendothelial system and organs such as the uterus and udder. The organism is also able to survive within macrophages because it has the ability to survive phagolysosome. *Brucella* are able to survive within host leukocytes and may utilize both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanism during the period of haematogenous spread (Radostits *et al*., 2006).

Risk factors associated with host

Age has been referred to as one of the intrinsic factors associated with brucellosis. Higher seroprevalence of brucellosis has been observed in older animals. Brucellosis has traditionally been considered a disease of adult animals since susceptibility increases after sexual maturity and pregnancy. *Brucella species* presented a tropism to the reproductive tract due to the production of erythritol, a 4-carbon sugar produced in the foetal tissues of ruminants that stimulates the growth of *Brucella*. Thus, it may also explain the higher prevalence in adult animals than in young (Bekele *et al*., 2011).

Female ruminants presented a higher odd of brucellosis infection. It could be associated with the intrinsic biology of the microorganisms and its tropism to the foetal tissue. The prevalence in males could be lower than females because they may be culled faster. On the other hand, the absence of clinical signs such as abortion or metritis in non-pregnant infected females or the absence of observation/identification/ of abortions in extensive herds may also explain the higher prevalence in females (Coelho *et al*., 2013).

Management risk factors

The spread of the disease from one herd to another and from one area to another is usually due to the movement of an infected animal from an infected herd into a non-exposed herd. Whether a herd raises its own replacement animals or purchases replacement animals affects the potential for introduction into the herd. The unregulated movement of cattle from infected herds or areas to

brucellosis free herds or areas is the major cause of breakdowns in brucellosis eradication programs. Once the herds are infected, the time required to become free of brucellosis is increased by large herd size, by active abortion, and by loose housing (Radostits *et al*., 2006). A contaminated environment or equipment used for milking or artificial insemination is further sources of infection. Permanent calving camps and lush pastures, particularly if they are wet and muddy, may play a very important role in the spread of the disease (Bishop *et al*., 1994).

2.3. Pathogenesis

Brucella may enter the host via ingestion or inhalation, or through conjunctiva or skin abrasions. *Brucella* specie can invade epithelial cells of the host, allowing infection through mucosal surfaces: M cells in the intestine have been identified as a portal of entry for *Brucella* species (Köhler *et al*., 2002). Once *Brucella* species has invaded, usually through the digestive or respiratory tract, they are capable of surviving intracellularly within phagocytic or non-phagocytic host cells (Pizarro-Cerdá *et al*., 2000). *Brucella* has the ability to interfere with intracellular trafficking, preventing fusion of the *Brucella*-containing vacuole (BCV) with lysosome markers, and directing the vacuole towards a compartment that has rough endoplasmic reticulum (RER), which is highly permissive to intracellular replication of *Brucella* (Pizarro-Cerdá *et al*., 2000).

Invading *Brucella* usually localize in the lymph nodes, draining the invasion site, resulting in hyperplasia of lymphoid and reticulo-endothelial tissue and the infiltration of inflammatory cells. Survival of the first-line of defense by the bacteria results in local infection and the escape of *Brucella* from the lymph nodes into the blood. Smooth *Brucella* inhibit host cell apoptosis, favoring bacterial intracellular survival by escaping host immune surveillance, while rough *Brucella* mutants (*B.canis* and *B.ovis* are two exceptions) induce necrosis in macrophage. However, the mechanisms and virulence factors that mediate macrophage cell death have not been identified (Pei *et al*., 2006).

In contrast to other pathogenic bacteria, no classical virulence factors, such as exotoxins, cytolysins, capsules, fimbria, plasmids, lysogenic phages, endotoxic lipopolysaccaride (LPS) have

been described in *Brucella* (Moreno and Moriyo, 2002). *Brucella* uses a number of mechanisms for avoiding or suppressing bactericidal responses inside macrophages. The smooth lipopolysaccharides that cover the bacterium and proteins involved in signaling, gene regulation, and transmembrane transportation are among the factors suspected to be involved in the virulence of *Brucella* (Lapaque *et al*., 2005). The smooth phenotype of *Brucella* is due to the presence in the outer cell membrane of a complete LPS, which is composed of lipid A, a core oligosaccharide, and an O side chain polysaccharide. Rough (vaccine) strains (i.e, strains with lipopolysaccharide lacking the O side chain) are less virulent because of their inability to overcome the host defense system (Lapaque *et al*., 2005).

Brucella display strong tissue tropism and replicate within vacuoles of macrophages, dendritic cells (DCs), and placental trophoblasts. However, the pathogen has the ability to replicate in a wide variety of mammalian cell types, including microglia, fibroblasts, epithelial cells, and endothelial cells. The intracellular lifestyle of *Brucella* limits exposure to the host innate and adaptive immune responses, sequesters the organism from the effects of some antibiotics, and drives the unique features of pathology in infected hosts, which is typically divided into three distinct phases: the incubation phase before clinical symptoms are evident, the acute phase during which time the pathogen invades and disseminates in host tissue, and the chronic phase that can eventually result in severe organ damage and death of the host organism. Chronic infection results from the ability of the organism to persist in the cells of the host in which *Brucella* are distributed by way of the lymphoreticular system to eventually cause cardiovascular, hepatic, lymphoreticular, neurologic, and osteoarticular disease (Baud and Greub, 2011).

2.3.1. Intracellular survival of Brucella

Brucella species are facultative intracellular bacteria and can become secluded within the endoplasmic reticulum of cells and thereby avoid lysosome fusion. By controlling the maturation of the brucellosome (*Brucella*-containing vacuole) at the onset of infection, unopsonized *Brucella* can enter, survive and replicate in a variety of cells, including dendritic cells and macrophages to evade the host innate immune response before activation of anti- *Brucella* mechanisms by adaptive immunity. To restrict long-term protective immunity, the organism first avoids the innate

immune response by stealthy entry into host cells. From there, the organism controls aspects of protein secretion, intracellular trafficking, and bacterial replication, ultimately altering the course of the innate and adaptive immune responses (Xavier *et al*., 2013).

The two-component BvrR/BvrS gene sensing system that also acts through a cascade of protein phosphorylation to modulate bacterial gene expression is thought to be one of the key factors involved in the modulation of cell binding and penetration. In *Brucella*, VirB is thought to be essential for intracellular survival. In these brucellosome, *Brucella* organisms are able to produce virulence genes (*VirB*) which promote multiplication of the organisms in such environments. The VirB pumping system is built from a series of proteins encoded by the VirB operon. Many attenuated *Brucella* strains show mutations within the VirB operon, indicating that an intact VirB is essential for virulence. VirB seems to have a role in adherence of the bacterium to the host cell, cell entry, and it modulates the intracellular trafficking and replication of the bacterium (Boschiroli *et al*., 2001) (Figure 4).

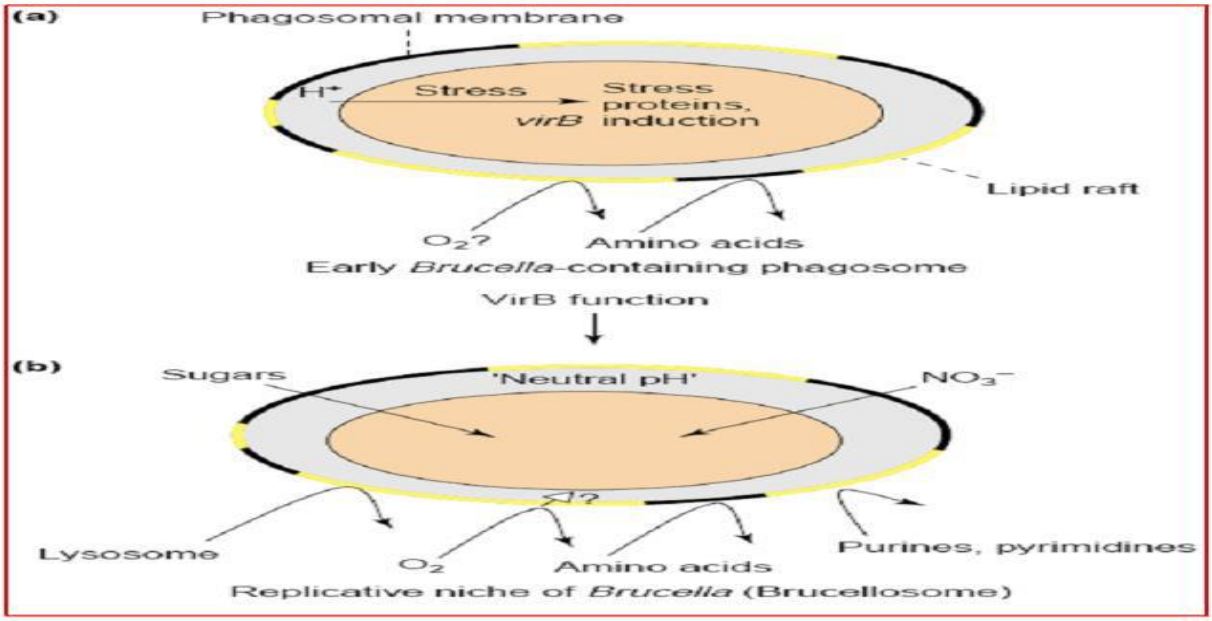

Figure 4: Characteristic properties of an early vacuole and the replicative niche of *Brucella* spp. Source: (Köhler *et al*., 2003).

2.4. Immunity Against *Brucella* Infection

2.4.1. Humoral immune response

Naturally infected animals and those vaccinated as adults with strain 19 remain positive to the serum and other agglutination tests for long periods. The serum of infected cattle contains high levels of IgG1, IgG2, IgM and IgA isotypes of antibody. Similar isotypes at different relative concentrations occur in milk, although most of the IgA is present in secretory form. The first isotype produced after an initial heavy infection or strain 19 immunization is IgM and is soon followed by IgG antibody. IgG1 immunoglobulin is the most abundant in serum and exceeds the concentration of IgG2. The magnitude and duration of the antibody response following immunization is directly related to the age at immunization and the number of organisms administered (Tegegne and Crawford, 2000).

Residual antibody if present, is usually predominantly of the IgM class. Following exposure to virulent *B.abortus*, antibody may appear in 4-10 weeks or longer, depending on the size and route of entry of the inoculums and the stage of pregnancy of the animal. Antibodies of IgM, IgG1 and IgG2 isotypes can all react in the tube agglutination but those of the IgM class are by far the most efficient (Tegegne and Crawford, 2000).

2.4.2. Cellular immune response

Brucella species are readily phagocytized by macrophages and polymorph nuclear leukocytes and, in the case of virulent strains, are capable of surviving within these cells and phagocytosis is promoted by antibody. However, since virulent *Brucella* can survive within normal macrophages for long periods, recovery from infection is likely to be dependent acquisition of increased bactericidal activity by phagocytic cells. Macrophage activation occurs when T-lymphocytes of the appropriate subset are stimulated to release lymphokines (Bekele *et al.*, 2011) (Figure 5).

The release of these activating factors is dependent upon recognition of the appropriate antigen by the T-lymphocyte and is subject to regulation through the major histocompatibility complex. Live

organisms capable of establishing persistent intracellular infection and certain types of antigen, with or without adjuvant, are the most effective inducers of cell-mediated immunity (Bekele *et al*., 2011).

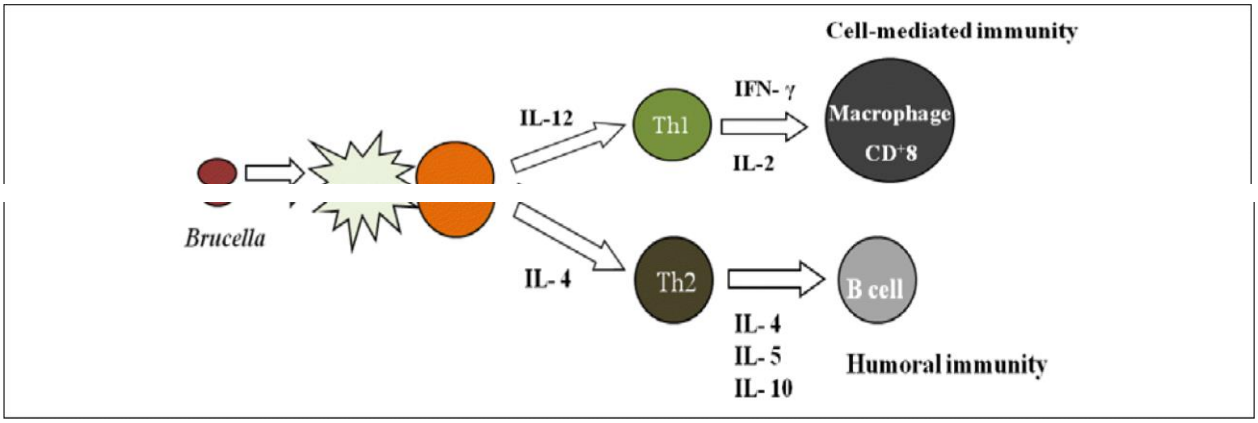

Figure 5: Humoral and cellular immune response of host body to *Brucellae*
Source: (Tegegne and Crawford, 2000)

2.5. Clinical Manifestation

The incubation period varies between 14 and 120 days. The major clinical sign in the first stage of the disease is abortion, but other signs due to localization of the organism may be observed. These signs include Orchitis, epididymitis, hygroma, arthritis, metritis and subclinical mastitis among others (Radostits *et al*., 2007) (Figure 6). However, numerous animals develop self-limiting infection or they may become asymptomatic latent carriers and potential excretors. The second stage is characterized by either elimination of *Brucella* or more frequently, by persistent inflammation of mammary gland and supra mammary and genital lymph nodes, with constant or intermittent shedding of the organisms in milk and genital secretions (Poester *et al*., 2010).

Abortion after the 5th month of pregnancy is cardinal feature of the disease in highly susceptible non-vaccinated pregnant cattle (Radostits *et al*., 2006) and other clinical signs are mainly the calving-associated problems and breeding-associated problems such as repeat breeding, a retained placenta and metritis (Acha and Szyfres, 2003). The infected cows usually abort only once after

which a degree of immunity develops and the animals remain infected. At subsequent calving, the previously infected cows excrete huge numbers of *Brucella* in the fetal fluids. Brucellosis does not usually result gross organic lesions, but sometimes a mild interstitial inflammatory reaction in the mammary gland may be observed, which is associated with elimination of bacteria in the milk (Xavier *et al*., 2013).

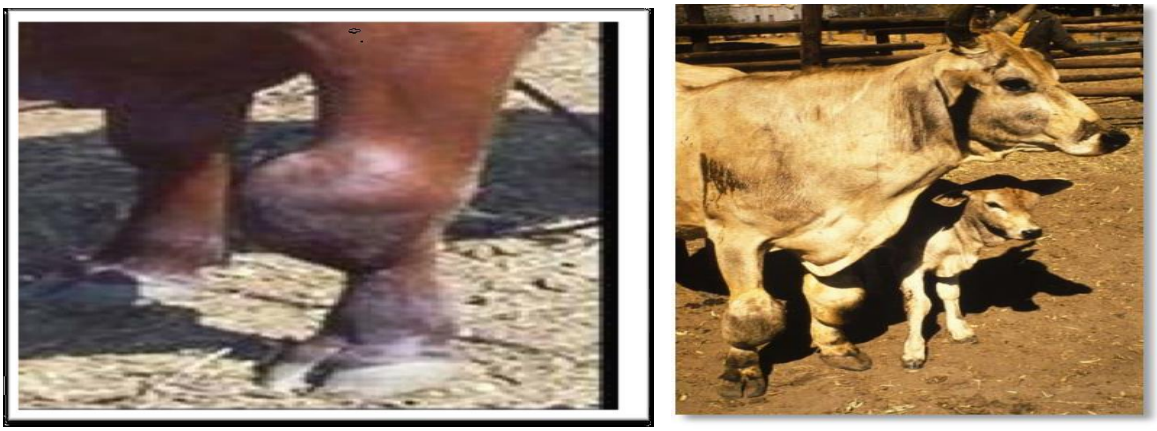

Figure 6: Unilateral and bilateral *Brucella abortus*-induced hygroma in cattle
Source: (Mantur *et al*., 2019)

2.6. Public Health Importance of Brucellosis

Brucellosis in humans is known as "undulant fever" or "Mediterranean fever", "Malta fever" or "Bangs disease" (Corbel, 2006). Brucellosis remains amongst the most normally disregarded zoonotic diseases worldwide. The true incidence of brucellosis in human and animals worldwide is obscure and the occurrence is expanding in low and middle income nations. The bacterial pathogen is considered by US Centers for Disease Control and Prevention (CDC) as a category (B) pathogen that has potential for improvement as a bio-terrorism weapon with a capability of airborne transmission (Sriranganathan *et al*., 2010).

Human brucellosis is caused by four species of *Brucella*, namely, *B.melitensis*, *B.abortus*, *B.suis*, and *B.canis*, with the majority of cases of the disease in humans being attributed to *B.melitensis*, although there is a possibility that human infection by the other three species is under appreciates.

B.melitensis is the type most frequently reported as a cause of human disease and the most frequently isolated from cases. It is the most virulent type and associated with severe acute disease. It is recorded as endemic in several countries and accounts for a disproportionate amount of human brucellosis. In humans, brucellosis is described as a chronic febrile debilitating disease with an incubation period of 2 to 24 weeks, which leads to significant socioeconomic losses owing to long-term treatment and inability of the affected individuals to provide for their families (Atluri *et al*., 2011).

2.6.1. Transmission of brucellosis to humans

The possible means of acquisition of brucellosis include: person-to-person transmission, infection from a contaminated environment, occupational exposure usually resulting from direct contact with infected animals, and foodborne transmission.

Person-to-person transmission

This is extremely rare. Occasional cases have been reported in which circumstantial evidence suggests close personal or sexual contact as the route of transmission. Of more potential significance is transmission through blood donation or tissue transplantation. Bone marrow transfer in particular carries a significant risk. It is advisable that blood and tissue donors to be screened for evidence of brucellosis and positive reactors with a history of recent infection be excluded. Transmission to attendants of brucellosis patients is most unlikely but basic precautions should be taken. Laboratory workers processing sample from *Brucella* patients have a much greater risk (WHO, 2004; WHO, 2006).

Infection from a contaminated environment

Infected animals passing through populated areas or kept in close proximity to housing may produce heavy contamination of streets, yards and market places, especially if abortions occur. Inhalation brucellosis may then result from exposure to contaminated dust, dried dung etc., (WHO, 2004). Contact infection may also result from contamination of skin or conjunctivae from

soiled surfaces. Water sources, such as wells, may also be contaminated by recently aborted animals or by run-off of rain water from contaminated areas. *Brucella* spp. can survive for long periods in dust, dung, water, slurry, aborted fetuses, soil, meat and dairy products (WHO, 2006).

Occupational exposure

Certain occupations are associated with a high risk of infection with brucellosis are people who work with farm animals, farmers, farm laborers, animal attendants, stockmen, shepherds, sheep shearers, goatherds, pig keepers, veterinarians and inseminators are at risk through direct contact with infected animals or through exposure to a heavily contaminated environment. The families of farmers and animal breeders may also be at risk as domestic exposure may be inseparable from occupational exposure when animals are kept in close proximity to living accommodation. Persons involved in the processing of animal products may be at high risk of exposure to brucellosis. These include slaughter men, butchers, meat packers, collectors of fetal calf serum, processors of hides, skins and wool, renderers and dairy workers (WHO, 2004). The preparation and use of live vaccines is also hazardous as strains such as *B.abortus* S19 and *B.melitensis* Rev 1 are not completely avirulent for humans. The rough vaccine strain *B.abortus* RB 51 appears to be of low pathogenicity but still presents a potential hazard through accidental injection and is rifampicin-resistant (WHO, 2006).

Food borne transmission

This is usually the main source of brucellosis for urban populations. Ingestion of fresh milk or dairy products prepared from unboiled milk is the main source of infection for most populations. Cow contaminated with *B.melitensis* is particularly hazardous as it is drunk in fairly large volume and may contain large numbers of organisms. Butter, cream or ice-cream prepared from such milk also presents a high risk (Young, 1990). The cheese-making process may actually concentrate the *Brucella* organisms, which can survive for up to several months in this type of product. Such cheeses should be stored in cool conditions for at least six months before consumption. Hard cheeses prepared by lactic and propionic fermentation presents a much smaller risk. Similarly, yoghurt and sour milk are less hazardous. *Brucella* dies off fairly rapidly when the acidity drops

below pH 4, and very rapidly below pH 3.5. Raw vegetables may be contaminated by infected animals and present a hazard. In endemic areas, tourists consuming “ethnic” food products may be particularly at risk (Young and Corbel, 1989).

Travel-acquired brucellosis

Tourists or business travelers to endemic areas may acquire brucellosis, usually by consumption of unpasteurized milk or other dairy products. Travelers may also import infected cheeses or other dairy products into their own countries and infect their families or social contacts by this means. Imported cases now account for most of the acute brucellosis cases seen in North America and Northern Europe (WHO, 2004).

2.6.2. Clinical manifestations in human

Brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia and prostration, and which, in the absence of specific treatment, may persist for weeks or months. Typically, few objective signs are apparent but enlargement of the liver, spleen and/or lymph nodes may occur, as many signs referable to almost any other organ system. The acute phase may progress to a chronic one with relapse, development of persistent localized infection or a non-specific syndrome (WHO, 2004; WHO, 2006).

Osteoarticular complications with bone and joint involvement are the most frequent complications of brucellosis, occurring in up to 40% of cases. A variety of syndromes have been reported, including sacroiliitis, spondylitis, peripheral arthritis, osteomyelitis, bursitis, and tenosynovitis. *Brucella* sacroiliitis is especially common. Patients present with fever and back pain, often radiating down the legs (sciatica). Children may refuse to walk and bear weight on an extremity (WHO, 2004).

Foodborne brucellosis resembles typhoid fever, in that systemic symptoms predominate over gastrointestinal complaints. Nevertheless, some patients with the disease experience nausea,

vomiting, and abdominal discomfort. Rare cases of ileitis, colitis and spontaneous bacterial peritonitis have been reported (Yong and Corbel, 1989).

Hepatobiliary complications often occur in brucellosis, although liver function tests can be normal or only mildly elevated. The histological changes in the liver are variable, but disease caused by *B.abortus* may show epithelioid granulomas that are indistinguishable from sarcoidosis lesions. A spectrum of hepatic lesions has been described in cases due to *B.melitensis,* including scattered small foci of inflammation resembling viral hepatitis. Occasionally larger aggregates of inflammatory cells are found within the liver parenchyma with areas of hepatocellular necrosis. In other cases, small, loosely formed epithelioid granulomas with giant cells can be found (WHO, 2004).

Aerosol inhalation of *Brucella* specie cause pulmonary complications, including hilar and paratracheal lymphadenopathy, interstitial pneumonitis, bronchopneumonia, lung nodules, pleural effusions, and empyema (WHO, 2006).

Orchitis and epididymitis are the most frequent genitourinary complications of brucellosis in men. Usually unilateral, *Brucella* Orchitis can mimic testicular cancer. Although *Brucella* organisms have been recovered from banked human spermatozoa, there have been a few reports implicating sexual transmission. In women, rare cases of pelvic abscesses and salpingitis have been reported (Young, 1990).

Brucellosis during the course of pregnancy carries the risk of spontaneous abortion or intrauterine transmission to the infant. Abortion is a frequent complication of brucellosis in animals, where placental localization is believed to be associated with erythritol, a growth stimulant for *B.abortus.* Although erythritol is not present in human placental tissue, *Brucella* bacteremia can result in abortion, especially during the early trimesters (WHO, 2004).

Cardiovascular complications with endocarditis are the most common cardiovascular manifestation, and it is said to be the most common cause of death from brucellosis. Endocarditis is reported in about 2% of cases, and can involve both native and prosthetic heart valves. The

aortic valve is involved more often than the mitral valve. Direct invasion of the central nervous system occurs in about 5% of cases of *B.melitensis* infection, and meningitis or meningoencephalitis is the most common manifestations (WHO, 2006).

Cutaneous complications with a variety of skin lesions have been reported in patients with brucellosis, including rashes, nodules, papules, erythema nodosum, petechiae, and purpura. Although uncommon, a variety of ocular lesions have been reported in patients with brucellosis (WHO, 2006).

2.6.3. Public health importance of brucellosis in Ethiopia

In Ethiopia, mixed cropping farmers and pastoral and agro pastoral peoples depends on domestic animals, milk and milk product to fulfill their dietary requirement which is the well-known transmission route of brucellosis from animals to human. On the other hand, traditional type of food animal slaughtering in non-hygienic methods is common practices which definitely downgrade the hygiene, safeness and wholesomeness of food of animal origin. Consumption of such contaminated food which may contain *Brucella* bacteria has the potential to cause an adverse health effect (Desta, 2016). Majority of mixed cropping farmers and pastoral and agro pastoral peoples do not use any protective materials during handling parturient animals, removing placenta and/or other aborted materials since most of the people had poor knowledge about brucellosis (Desta, 2016).

So, these practices could potentially facilitate the transmission of zoonotic *Brucella* pathogens from domestic animals to humans (Bekele *et al*., 2013). Generally, human brucellosis is increasing in Ethiopia like many other developing countries due to various sanitary and socioeconomic (Pappas *et al.,* 2006). Thus, collaborative work of different stakeholders to prevent and control the disease as well as to enhance public awareness level of livestock keepers is required (Catley *et al*., 2005) (Table 6).

Table 6: Seroprevalence of human brucellosis in some part of Ethiopia

District	N_o: examined	Sample taken	Test employed	prevalence	Reference
Fafan zone	211	serum	CFT	0.4%	(Lakew *et al*., 2019)
Afar	200	serum	RBPT	16%	(Zewolda and Wereta, 2012)
			CFT	15%	
	630	Serum	RBPT	12.7%	(Zerfu *et al*., 2018)
	80	Serum	CFT	35%	
Bishoftu	149	serum	RBPT	4.7%	(Tuli *et al*., 2017)
Modjo			CFT	1.3%	
Addis	360	serum	RBPT	-	(Kassahun *et al*., 2006)
Ababa			2-MET	4.8%	

2.7. Diagnostic Techniques of Brucellosis

Brucellosis signs are non-pathognomonic in livestock and human and definitive diagnosis depends on laboratory testing. Laboratory diagnosis includes indirect tests that can be applied to milk or blood, as well as direct tests (classical bacteriology and direct polymerase chain reaction or PCR based methods). The choice of a particular testing strategy depends on the prevailing epidemiological situation of brucellosis in susceptible animals (livestock and wildlife) within a country or region (Godfroid *et al*., 2013).

There is no single test by which a bacterium can be identified unequivocally as *Brucella*. Accordingly, for a definitive identification, a combination of growth characteristics, serological, bacteriological or molecular methods is required (Alton *et al*., 1988; FAO, 2003). The existence of different *Brucella* biotypes among the *Brucella* species and their identification is important to confirm the infection. Because of the complications involved in the diagnosis of the disease, including the difficulties in distinguishing between infected and vaccinated animals by conventional serological tests, bacteriological isolation and identification of biotypes of the

etiological agent are necessary steps in the design of epidemiological and eradication programs (Zinsstag *et al*., 2005).

2.7.1. Bacteriological diagnosis

The "gold standard" of the brucellosis diagnosis is the direct bacteriological testing: cultivation of *Brucella*, isolated from body fluids (blood, cerebrospinal fluid, urine and others) or tissues (Smirnova *et al*., 2013). It should be noted that all infected materials present a serious hazard, and they must be handled with adequate precautions during collection, transport and processing (Corbel, 2006). Isolation of the organism is considered the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, which is relevant under an epidemiological point of view. However, in spite of its high specificity, culture of *Brucella* species is challenging. *Brucella* species is a fastidious bacterium and requires rich media for primary cultures. Furthermore, its isolation requires a large number of viable bacteria in clinical samples, proper storage and quick delivery to the diagnostic laboratory and it requires biosafety level 3 facilities which are not available in most developing countries. However, the isolation and cultivation of bacteria are also necessary preliminary steps for staining and biotyping of *Brucella* species (Al Dahouk *et al*., 2013).

Staining

Stamp staining is still often used, even though this technique is not specific: other abortive agents such as *Chlamydophila abortus* (formerly *Chlamydia psittaci)* or *Coxiella burnetii* are also stained red (Corbel, 2006). They are not truly acid fast; however, they are resistant to decolonization by weak acids, and stain red against a blue background with the Stamp's modification of the Ziehl-Neelsen method. *Brucella* species is a coccobacillus measuring 0.6-1.5µm long and 0.5-0.7µm wide. They generally occur singly and are observed in clusters of two or more. Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) method. The presence of large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is presumptive evidence of brucellosis (Alton *et al*., 1988).

Culture

Bacterial isolation is always required for the biotyping of strains. For the definitive diagnosis of brucellosis, the choice of samples depends on the clinical signs observed. In the case of clinical brucellosis, valid samples include aborted fetuses (stomach, spleen, and lung), fetal membranes, vaginal secretions, colostrum, milk, sperm, and fluid collected from arthritis or hygroma. At slaughter, in order to confirm suspected cases of acute or chronic brucellosis, the preferred tissues are the genital and oropharyngeal lymph nodes, the spleen, and the mammary gland and associated lymph nodes (Corbel, 2006). Direct isolation and culture of *Brucella* are usually performed on solid media which is most satisfactory method as it enables the developing colonies to be isolated and recognized clearly. A wide range of commercial dehydrated basal media is available, such Tryptose Soya Agar (TSA), blood agar base (Oxoid), Columbia agar, serum dextrose agar (SDA) or glycerol dextrose agar can be used (Alton *et al*., 1988).

The most widely used selective medium is the Farrell's medium, which is prepared by the addition of antibiotics to a basal medium. Farrell's medium, have inhibitory effect on some *B.abortus* and *B.melitensis* strains. Therefore, the sensitivity of culture increases significantly by the simultaneous use of both Farrell's and the modified Thayer Martin medium (OIE, 2012). Some *Brucella* species, like *B.abortus* wild type (biovars 1-4), need CO 2 for growth, while others, like *B.abortus* wild type (biovars 5, 6, 9), *B.abortus* S19 vaccine strain, *B.melitensis*, and *B.suis*, do not (Alton *et al*., 1988).

For liquid samples (milk or blood), sensitivity is increased by the use of a biphasic medium like the Castaneda medium, originally described for use with human blood cultures. Growth may appear after 2-3 days, but cultures are usually considered negative after 2-3 weeks of incubation (Alton *et al*., 1988). The identification of *Brucella* species is based on morphology, staining and metabolic profile (catalase, oxidase, and urease) (Corbel, 2006).

All culture media should be subject to quality control and should support the growth of *Brucella* strains from small inocula or fastidious strains, such as *B.abortus* biovar 2. On suitable solid media, *Brucella* colonies can be visible after a 2–3 days incubation period. After 4 days

incubation, *Brucella* colonies are round, 1–2mm in diameter, with smooth margins. They are translucent and a pale honey color when plates are viewed in the daylight through a transparent medium (OIE, 2012).

Smooth (S) *Brucella* cultures have a tendency to undergo variation during growth, especially with subcultures, and to dissociate to rough (R) forms. Colonies are then much less transparent, have a more granular, dull surface, and range in color from matt white to brown in reflected or transmitted light. Checking for dissociation has been easily tested by crystal violet staining: rough colonies stain red/violet and smooth colonies do not uptake dye or stain pale yellow (OIE, 2012).

Biochemical test

Identification of *Brucella* strains using different biochemical tests like oxidase activity, urease activity, H2S production, Dye tolerance (basic fuchsin and thionin) and sero-agglutination. It has been also recommended that Gram stain morphology and modified ZN staining, coupled with the urease test, for rapid identification of *Brucella* to the level of genus where facilities for further identification are not available (Mantur *et al*., 2019).

2.7.2. Serological diagnosis

Serology is the mainstay of diagnosis for brucellosis because the diagnostic material is relatively easily accessible, and the tests are relatively cheap, available and sensitive. Therefore, Serological tests are crucial for laboratory diagnosis of brucellosis since most of control and eradication programs rely on these methods. Inactivated whole bacteria or purified fractions (i.e. lipopolysaccaride or membrane proteins) are used as antigens for detecting antibodies generated by the host during the infection. Antibodies against smooth *Brucella* species (e.g. *B. abortus, B. melitensis,* and *B. suis*) cross react with antigen preparations from *B. abortus*, whereas antibodies against rough *Brucella* species (e.g. *B. ovis* and *B. canis*) cross react with antigen preparations from *B. ovis* (Nielsen, 2002).

Although several serological methods are currently available, these tests can be classified as screening tests (e.g. buffered antigen plate agglutination - BPAT), monitoring or epidemiological surveillance tests (e.g. milk ring test), and complementary or confirmatory tests (e.g. 2-mercaptoethanol, complement fixation, ELISAs, and fluorescence polarization assay).

Rose Bengal Plate test (RBPT)

The RBPT is a rapid, slide-type agglutination assay performed with a stained *B.abortus* suspension at pH of 3.6-3.7 and plain serum. Its simplicity made it an ideal screening test for small laboratories with limited resources. The drawbacks of RBPT include: low sensitivity particularly in chronic cases, relatively low specificity in endemic areas and prozones make strongly positive sera appear negative in RBPT. The overall sensitivity is 92.9%, so the use of RPBT should be considered carefully in endemic areas, particularly in individuals exposed to brucellosis and those having history of *Brucella* infection (Ruiz-Mesa *et al*., 2005). RBPT is an agglutination test that is based on reactivity of antibodies against smooth lipopolysaccharide (LPS). As sensitivity is high, false negative results are rarely encountered. To increase specificity, the test may be applied to a serial dilution [1:2 through 1:64] of the serum samples. The present World Health Organization (WHO) guidelines recommend the confirmation of the RBPT by other assays such as serum agglutination tests (Ruiz-Mesa *et al*., 2005).

The RBPT is based on the detection of specific antibodies of the IgM and IgG types but more effective in detecting antibodies of the IgG1 type than the IgG2 and IgM types. Also the low pH (3.65) of the antigen enhances the specificity of the test by inhibiting nonspecific agglutinins. The temperature of the antigen and the ambient temperature at which the reaction takes place may influence sensitivity and specificity (Díaz *et al*., 2011).

Complement Fixation test (CFT)

Complement fixation test (CFT) detects specific antibodies of the IgM and IgG1 type that fix complement. The CFT is highly specific but it is laborious and requires highly trained personnel as well as suitable laboratory facilities that makes 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 complement fixation. The CFT measures more antibodies of the IgG1 than antibodies of the IgM type, as the latter 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 by CFT (Perrett *et al*., 2010).

Complement fixation test is used as confirmatory test for *B.abortus*, *B.melitensis*, and *B.ovis* infections due to its high accuracy, and it is the reference test recommended by the OIE for international transit of animals (OIE, 2009). However, this method has some disadvantages such as high cost, complexity for execution, and requirement for special equipment and trained laboratory personnel. In addition, the test presents limitations with hemolysed serum samples or serum with anti-complement activity of some sera, and the occurrence of prozone phenomena (OIE, 2009). Sensitivity of complement fixation ranges from 77.1 to 100% and its specificity from 65 to 100% (Perrett *et al*., 2010).

Serum Agglutination Test (SAT):

Serum agglutination test measures the total quantity of agglutinating antibodies IgM and IgG. The quantity of specific IgG is determined by treatment of the serum with 0.05M 2-mercaptoethanol (2ME), which inactivates the agglutinability of IgM. SAT titers above 1:160 have been considered diagnostic in conjunction with a compatible clinical presentation. However, in areas of endemic disease, using a titer of 1:320 as cut off may make the test more specific. The differentiation in the type of antibody is also important, as IgG antibodies are considered a better indicator of active infection than IgM and the rapid fall in the level of IgG antibodies is said to be prognostic of successful therapy (Buchanan and Faber, 1980).

Enzyme linked immune sorbent assays test (ELISA)

ELISAs are divided into two categories, the indirect ELISA (iELISA) and the competitive ELISA (c-ELISAs) (Saegerman *et al*., 2004). They are more suitable than the CFT for use in smaller laboratories and ELISA technology is now used for diagnosis of a wide range of animal and

human diseases. Although in principle ELISAs can be used for the tests of serum from all species of animal and man, results may vary between laboratories depending on the exact methodology used. Competitive ELISA (c-ELISA) and Indirect ELISA (iELISA) tests can be used as supplementary tests to CFT. Not all standardization issues have yet been fully addressed. For screening, the test is generally carried out at a single dilution. It should be noted, however, that although the ELISAs are more sensitive than the RBPT, sometimes they do not detect infected animals which are RBPT positive (McGiven, 2013).

i. Indirect ELISA (i-ELISA)

The method is based on the specific binding of antibodies present in the test sample with immobilized antigen. The binding event is visualized using chemically or enzymatically derived fluorescent, luminescent or colorimetric reaction. Many iELISA tests are available on the market (Poester *et al*., 2010). It has been used for diagnosis using serum or milk from cattle. i-ELISA has been usually used for smooth LPS *Brucella* species, and it is sensitive and specific for *B.abortus* or *B.melitensis*, but it is not capable of differentiating antibodies induced by the vaccine strains S19 or Rev1. Sensitivity of i-ELISA varies from 96 to 100%, and its specificity from 93.8% and 100% (Gall *et al*., 2001).

ii. Competitive ELISA (c-ELISA)

With smooth *Brucella* LPS as antigen is used for detection of anti-*Brucella* in serum samples from cattle, sheep, goats, and pigs. This test is capable of differentiating vaccine antibody response from actual infections, and its sensitivity varies from 92 to 100%, whereas the specificity ranges from 90 and 99% (Perrett *et al*., 2010). It can also be used both for screening and confirmatory tests (FAO, 2003). Antibodies against smooth LPS are used in all the above mentioned tests. They have a common significant disadvantage: O-polysaccharides of *Brucella* are similar to that of *Yersinia enterocolitica* and other bacteria. It leads to the false positive results and thus reduces the specificity of the test (Nielsen *et al*., 2004). Partly this problem is solved in the competitive ELISA (cELISA), where the specific epitopes of *Brucella* O-polysaccharides are used as antigens, but the sensitivity of c-ELISA is significantly lower than the iELISA.

2.7.3. Molecular methods

In order to avoid difficulties of bacteriological testing the molecular biological techniques, often based on the polymerase chain reaction (PCR) amplification, are successfully used for *Brucella* identification and typing (Smirnova *et al*., 2013). Molecular methods for *Brucella* species genotyping molecular techniques are important tools for diagnosis, providing relevant information for identification of species and biotypes of *Brucella* species, allowing differentiation between virulent and vaccine strains (Lopez-Goñi *et al*., 2008). Initially, PCR based identification has been developed for the determination of bacterial isolates but now these methods are also used for detection of *Brucella* species in clinical samples of human and animals without previous isolation of the organism (Smirnova *et al*., 2013). In addition, these techniques can be used to complement results obtained from phenotypic tests (Bricker, 2002).

PCR DNA-based methods such as gene probes and PCR utilize primers derived from different polymorphic regions in the genomes of *Brucella* species. Different PCR methods for the detection of *Brucella* species that utilize primers derived from different polymorphic regions in the genomes of *Brucella* species as i.e. (1) a gene encoding a 31kDa *B.abortus* antigen which is conserved in all *Brucella* species (primers B4/B5) (Baily *et al*., 1992), (2) a sequence +16S rRNA of *B.abortus* (primers F4/R2), (3). a gene encoding an outer membrane protein of 26kDa (omp-2) (primers JPF/JPR and primers P1/P2), (4) outer membrane proteins (omp 2b, omp2a and omp31), (5) proteins of the omp25/omp31 family of *Brucella* spp. (Vizcaíno *et al*., 2004), the entire bp26 gene of *B. melitensis* 16M, encoding the BP26 protein (omp 28) (primers 26A/26B) (Cloeckaert *et al*., 2000) were described.

Multiplex PCR typing

Multiplex PCR typing is more effective method of diagnosis and identification of *Brucella*. Several multiplex PCRs which identify the genus *Brucella* at the species level and partly at the biovar level using different primer combinations have been reported. It provides identification of all known *Brucella* species at the species or even biovars level by using certain combinations of primer pairs. The first multiplex PCR based test for *Brucella* detection was developed in 1994

(Bricker and Halling, 1994), it is also called AMOS PCR assay. It allowed identification of the four *Brucella* species (*Brucella abortus, Brucella melitensis, Brucella ovis* and *Brucella suis*) and was named AMOS PCR (AMOS is an acronym from abortus-melitensis-ovis-suis) for the first letters of species names. It comprised five oligonucleotide primers for the identification of selected biovars of four species of *Brucella*. The assay exploited the polymorphism arising from species-specific localization of the genetic element IS711 in the *Brucella* chromosome (Smirnova *et al*., 2013).

Real-Time PCR

It is more rapid and more sensitive than conventional PCR. It does not require post amplification handling of PCR products, thereby reducing the risk of laboratory contamination and false-positive results. Real-time PCR assays have been recently described in order to test *Brucella* cells (Redkar *et al*., 2001), urine (Queipo-Ortuño *et al.*, 2005), blood, paraffin-embedded tissues (Kattar *et al*., 2007), serum, and other tissues (Smirnova *et al*., 2013). Three separate real-time PCRs were developed to specifically identify seven biovars of *B. abortus*, three biovars of *B. melitensis* and biovar one of *B. suis* using fluorescence resonance energy transfer. The upstream primers used in these real-time PCRs derived from the insertion element, IS711 whereas the reverse primer is selected from unique species or biovar-specific chromosomal loci. Sensitivity of *B.abortus*-specific assay was as low as 0.25 pg DNA corresponding to 16-25 genome copies and similar detection levels were also observed *for B. melitensis* and *B. suis*-specific assays (Redkar *et al*., 2001).

2.8. Treatment, Prevention and Control

2.8.1. Treatment

Treatment of brucellosis in domestic animals is not indicated (Kassahun, 2003). In human, due to intracellular localization of *Brucella* and its ability to adapt to the environmental conditions encountered in its replicative niche e.g. macrophage (Sriranganathan *et al*., 2010), treatment

failure and relapse rates are high and depend on the drug combination and patient compliance. The optimal treatment for brucellosis is a combination regimen using two antibiotics since mono therapies with single antibiotics have been associated with high relapse rates *(*Sriranganathan *et al*., 2010). The combination of doxycycline with streptomycin (DS) is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis. Neither streptomycin nor doxycycline alone can prevent multiplication of intracellular *Brucella.* A combination of doxycycline treatment (6 weeks duration) with parenterally administered gentamicin (5mg/kg) for 7 days is considered an acceptable alternate regimen (Sriranganathan *et al*., 2010).

2.8.2. Prevention and control

Brucellosis is an infectious disease which has been controlled and eradicated in some countries in the world (Godfroid *et al*., 2005). In sub-Saharan Africa, animal health services delivered by the public sector have greatly decreased over the last 20 years due to various factors such as decreasing government budgets, particularly for operational costs of disease control. Thus, programs that require coordinated surveillance, information exchange and application of control measure are not implemented in many sub-Saharan countries (Mcdermott and Arimi, 2002). The general strategies proposed in FAO, (2003) by the WHO including Mediterranean Zoonoses Control Program to eradicate animal brucellosis were: prevention of spread between animals and monitoring of brucellosis-free herds and zones, elimination of infected animals by test and slaughter programs to obtain brucellosis-free herds and regions, and vaccination to reduce the prevalence (FAO, 2003).

Immunization

One of the most successful methods for prevention of livestock brucellosis is through vaccination. In different parts of the world both live vaccines, such as *B.abortus* S-19, *B.melitensis* Rev-1, *B.suis* S-2, rough *B.melitensis* strain M111 and *B.abortus* strain RB51 and killed vaccines, such as *B.abortus* 45/20 and *B.melitensis* H.38 are available (Kassahun, 2003).

Use of the RB51 attenuated live vaccine has recently gained popularity for control of brucellosis in cattle. But on a cautionary note, the failure of this strain to induce serological reactivity, coupled with its inherent resistance to rifampicin, might complicate detection and management of zoonotic infection spilling into humans with occupational risk factors for acquiring brucellosis. Currently, despite huge research efforts, no vaccine has been approved for the prevention of human brucellosis (Marzetti *et al*., 2013).

Application of farm Biosafety measures

Implementation of measures to reduce the risk of infection through personal hygiene, adoption of safe working practices, protection of the environment and food hygiene should minimize risks of further infection. Under appropriate conditions, *Brucella* organisms can survive in the environment for prolonged periods. Their ability to withstand inactivation under natural conditions is relatively high compared with most other groups of non-sporing pathogenic bacteria (WHO, 2006). *B.abortus* is inactivated by pasteurization and its survival outside the host is largely dependent on environmental conditions. The pathogen may survive in aborted fetus in the shade for up to eight months, for two to three months in wet soil, one to two months in dry soil, three to four months in faeces, and eight months in liquid manure tanks (OIE, 2004). For example, in nomadic populations where people travel in search of green pasture and water, the proper handling and burying of abortion materials to prevent contamination of water sources and pasture is of paramount importance (OIE, 2004). Brucellae in aqueous suspensions are readily killed by most disinfectants. A 10g/l solution of phenol will kill brucellae in water after less than 15 min exposure at 37°C. Formaldehyde solution is the most effective of the commonly available disinfectants, provided that the ambient temperature is above 15°C (WHO, 2006).

Application of veterinary extension

The development of a national veterinary extension services in the country, is essential to promote awareness about brucellosis, its impact on livestock production and zoonotic risks, would provide a valuable prevention measure. This would help to unify both community/dairy cattle producers to control and eliminate brucellosis. Currently, many dairy cattle producers hide or dispose of

animals with a history of abortion, potentially facilitating disease transmission between farms and regions. This seriously undermines efforts of controlling and preventing the disease (OIE, 2004).

2.9. Economic Impact

Food and Agriculture Organization of the United Nations (FAO) and the Organization of Animal Health (OIE) consider brucellosis as has not only direct public health implications, it also poses a barrier to trade of animals and animal products (Fitcht, 2003) and has a wide socioeconomic impacts especially in countries where people in rural areas rely to a large extent on livestock breeding and dairy products as a source of income (Zinsstag *et al*., 2005). Brucellosis is consistently ranked among the most economically important zoonoses globally. It is a multiple burdens disease with economic impacts attributable to human, livestock and wildlife disease. The epidemiology and economic impact of brucellosis vary by geography and livestock system. In many high-income countries, brucellosis has been successfully controlled or eliminated in livestock populations. Where it persists, wildlife populations have become the main reservoirs (for example, bison and elk in North America). In emerging middle-income countries, the brucellosis picture is much more variable. Middle-income countries tend to report the greatest number of outbreaks and animal losses (ILRI, 2012). Data on the yearly economic impact of brucellosis in the developing world associated with disease in livestock have generally been hard to assess, especially in Africa (Smits *et al*., 2007).

In countries such as Argentina and Mexico, which depend heavily on the sale of livestock products for both domestic and international markets, these annual costs for control are estimated to be US$60 million and $200 million, respectively. Studies done in developing countries by the United Nations highlight that the need for effective control programmes which have an obvious benefit to the health of both human beings and livestock. If the costs of control programs are shared between the public and private sectors and include international aid, they are likely to be profitable and cost effective (Smits *et al*., 2007). The economic impact in terms of human disease has been even harder to gauge (Smits *et al*., 2007).

The economic losses due to bovine brucellosis include: losses of calves due to abortion, reduced milk yield, culling and condemnation of valuable cows because of breeding failure, endangering animal export trading of a nation, loss of man power, medical costs and government cost for research and eradication programs (Chukwu, 1987). In pregnant, abortion occurs during the second half of the pregnancy, often with retention of the placenta and resultant metritis, which may cause permanent infertility. It is estimated that the infection causes a 20% to 25% loss in milk production as a result of interrupted lactation due to abortion and delayed conception (Mcdermott and Arimi, 2002).

3. MATERIAL AND METHODS

3.1. Description of Study Areas

The study was conducted in Holeta Town, Wolmera District and Adea Berga EIAR dairy farm, Oromia regional state, Ethiopia that are known for well-developed dairy production and constituting the major milk sheds of Addis Ababa. Holeta Town hosts Ethiopian Institute of Agricultural Research dairy farms (Figure 7).

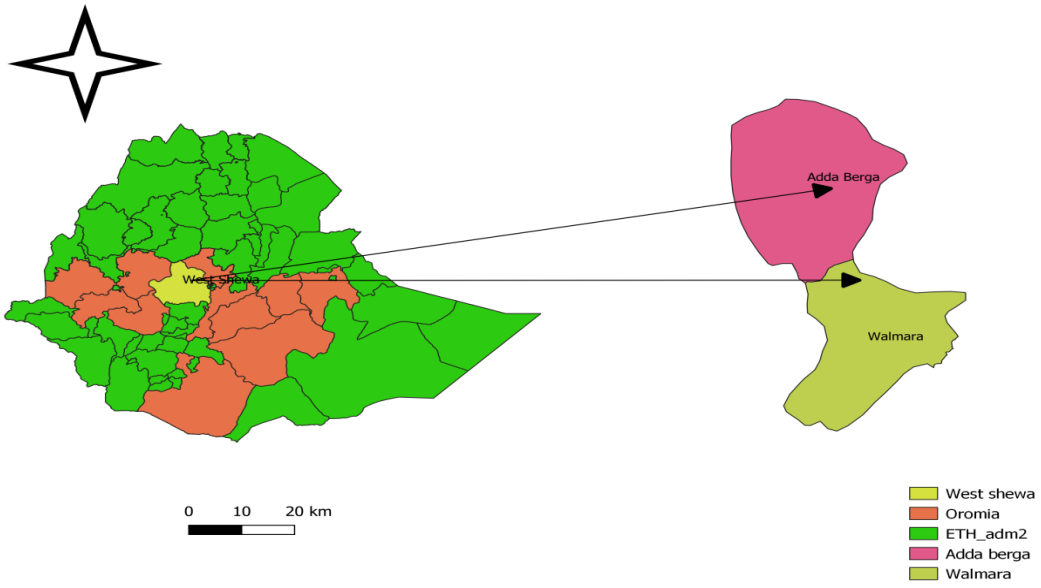

Figure 7: Wolmera and Addea Berga Districts Oromia regional state, Ethiopia
Source: Diva GIS (Data source) and projected by me using QGIS

Holeta a town of Wolmera District is situated in Oromia special zone surrounding the capital city Addis Ababa. The town is located 29 kilometers west of Addis Ababa at 9°30' N and 38°30' E with altitude ranging from 2300-3800m above sea level which is actually parts of central highlands of Ethiopia. The average annual minimum and maximum temperatures were 6°C and 22°C, respectively. The area is also characterized by occasional frost that occurs in the months of October to December, where temperatures below zero for few days during these months. The annual rainfall ranges from 900-1100 mm. According to the population and housing census of

2007 the population of the town is 23,296 (male=11512, female=11,784) (CSA, 2013). The major livestock production systems in the area include: mixed crop-livestock farming surrounding the towns, where animals are managed under extensive traditional grazing systems; market oriented, peri-urban dairy production and urban dairy production systems (CSA, 2016). The total cattle population of the study area is estimated to be 175,741, out of which 172,769 (98.3%) heads of cattle are local breeds and 2972 (1.7%) are cross kept under extensive and semi intensive management systems. Holeta town hosts 11 medium scale dairy farms (WoWAHA, 2015).

Adda Berga located at 9° 15′ 0″ N, 38° 25′ 0″ E is one of the woredas in the Oromia Region of Ethiopia. It hosts Ethiopian Institute of Agricultural Research dairy farm. The 2007 national census reported a total population for this woreda of 120,654, of whom 60,366 were men and 60,288 were women; 15,940 or 13.21% of its population were urban dwellers. Adea Berga dairy farm was established at Adea Berga wetland in 1986 for commercial milk production under government state farm by using introduced 400 pure Jersey pregnant heifers and 2 sires (foundation stock) from Denmark (Siyoum *et al.*, 2016). The farm had been engaged in the production and rearing of pure Jersey breed from the foundation stock for milk supply for dairy development enterprises and also serve as a bull dam station for the national artificial insemination center (NAIC). Then the farm was transferred to Holeta Agricultural Research Center for genetic improvement research program since 2007. Currently this research dairy farm had 350 pure Jersey, Boran, and Holstein Friesian and Jersey cross breeds kept under semi intensive rearing system.

3.2. Study Population

The target study populations were dairy cattle with recent case of abortion in Holeta Town, Wolmera District and large scale dairy farms of Ethiopian Institute of Agricultural Research located in Adea Berga. The occurrence of abortion cases in one month referred to as recent abortion was assessed in the respective site during the entire periods of this study. The dairy cows under study comprised pure Holstein Friesian and Jersey breeds, indigenous breed, and Boran Holstein Friesian and Boran Jersey cross breeds which have no history of vaccination. Study

animals related traits such as species, age, body condition score, lactation, reproductive status, parity number; period of abortion and history of abortion were collected and recorded at the time of sampling. Dairy cows were classified into three age groups, <4 years, 4-8years and >8 years as young, adult and old respectively based on Ibrahim *et al*., (2011). Body condition score (BCS) was subjectively estimated based on the guides published by Svendsen, (1997).

Employees of the Holeta and Adda Berga Agricultural Research Center dairy farm, dairy cow owners in Holeta Town and Wolmera District who have direct contact with dairy cows and who are willing to participate in the study and sign the informed consent at the age above 18 years old were included.

3.3. Study Design

A cross-sectional study was conducted from November 2019 to May 2020 to study brucellosis in dairy cows with recent history of abortion, and workers of the Holeta and Adda Berga Agricultural Research Center dairy farm and dairy cow owner in Holeta town and Wolmera District who had direct contact with dairy animals.

3.4. Sampling Technique and Sample Size Determination

Purposive sampling technique was applied to select medium, large and small scale farms. Accordingly, all eight kebeles, all eleven medium scale and one large scale farm of Ethiopian Institute of Agricultural Research of HARC from Holeta Town were included. On the other hand fourteen kebeles out of twenty three kebeles of Wolmera District were selected purposively based on accessibility and number of dairy cows. One large scale farm of Ethiopian Institute of Agricultural Research located in Adda Bberga District was also purposively included in the study.

The sample size for serological study of brucellosis in recently aborted dairy cows was estimated based on the previous study result by Shanko, (2017) in Holeta Town in dairy farms which were

0.92% seroprevalence. The sample size for the study was calculated using the formula described by Thrusfield, (2007) with defined precision of 5 % and 95% level of confidence interval.

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

Where, *n*=required sample size, *P*ex=expected prevalence, and *d*=desired absolute precision

Hence, based on the above formula and taking into account 50% prevalence, the minimum sample size is:

$$n=\frac{1.96^2 \text{ X } 0.0092 \text{ X } (1-0.0092)}{(0.05)2}$$

n= 14

However, in order to increase precision and reduce standard error, all recently aborted cows in the study area during the study duration were included. Therefore, a total of 352 recently aborted cows were sampled in the study duration. On the other hand 149 voluntary animal owners from Holeta Town and Wolmera District and 17 farm employees from Holeta and Adda Berga EIAR dairy farms were also included in the study.

3.5. Sample Collection

3.5.1. Blood sample collection

The dairy cows with history of recent abortion were isolated and restrained properly to avoid unexpected personal injury beside to minimizing unnecessary stress that might be happen to the animals. I have been collected blood samples of 7-10ml after disinfecting the site of jugular vein from each cattle and on the other hand, 5 mL of blood samples were collected from the cephalic vein of voluntary animal owner and farm workers by a registered nurse in sterile plain vacutainer tubes. The blood samples were kept in a slanting position overnight at room temperature to separate the serum according to OIE, (2009) manual. Then each serum was gently decanted into sterile screw cupped Eppendorf tubes (1.8ml), labeled and stored at -20^{o}C in Ethiopian Institute of

Agricultural Research (EIAR) Holeta Agricultural Research Center (HARC) Animal Health Microbiology laboratory until tested for antibodies against natural *Brucella* exposure analysis using RPBT and CFT for confirmation of the RBPT positive samples. All serum samples collected from animals were tested for RBPT and CFT in serology laboratory of NVI (National Veterinary Institute), Bishoftu. Serum sample collected from human were screed by RBPT in Holeta Town human Health clinic and confirmed for CFT.

3.5.2. Bacteriological sample collection

Vaginal swab samples were collected according to previous reported work of Geresu *et al*., (2016b) and the swab was collected with sterile applicator stick in Amies Transport Medium (HiMedia, Mumbai, India) and transported to HARC Microbiology laboratory in cold chain and stored at -20^{o}C until processed for culturing and isolation under Biosafety level III (BSL3) with personal protection (safety measures) according to OIE, (2009) in Addis Ababa University Pathobiology institute (Annex 3).

3.6. Laboratory Diagnosis

3.6.1. Rose Bengal Plate Test (RBPT)

All serum samples collected from bovine and human were screened using RBPT according to the procedures described by Alton *et al*., (1988), the World Organization for Animal Health (OIE, 2004) and manufacturer's instruction. The antigen used was Rose Bengal antigen, which constitutes a suspension of *Brucella (*Annex1). Briefly, for the method, 30µl of serum and 30µl of antigen was mixed on a test plate and rocked for 4 minutes. After four minutes of rocking, visible agglutination was considered as positive. Agglutinations was recorded as 0, +, ++ and +++, according to the degree of agglutination (Nielsen and Duncan, 1990). A score of 0 indicates the absence of agglutination; + indicates barely visible agglutination; ++ indicates fine agglutination, and +++ indicates coarse clumping. The presence of agglutination was considered positive reaction while the absence of agglutination was considered negative**.** *Brucella* positive and

negative control sera were also tested along with the test sera to guide in the reading of the results (Nielsen and Duncan, 1990). The results was recorded and stored in Microsoft Excel.

3.6.2. Complement Fixation Test (CFT)

Serum that positive for RBPT were further tested using CFT for confirmation using standard *Brucella* antigen. Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organization for Animal Health (OIE, 2009) (Annex 2). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above was considered as positive and lack of fixation/complete hemolysis was considered as negative.

3.6.3. Brucella Microbiological culture

Vaginal swab collected parallel to blood sampling were streaked directly from Amies transport medium to *Brucella* medium Agar base supplemented with horse serum with Selective Antibiotics supplement active ingredients (CMO 169 (OXOID), under Biosafety Level III (BSL3) facilities with proper personal protections (Alton *et al*., 1988; Lopez *et al*., 2006) (Annex 3).

The inoculated plates with specimens were incubated at 37^{o}C both in the absence and presence of 5% to 10% CO2 (using AnaeroGas Pack 1.5L) for up to two weeks. Colonies were checked every 24hrs for growth of *Brucella* species. *Brucella*-suspected colonies were characterized based on their typical round, glistening, pinpoint and honey drop-like appearance according to Alton *et al*. (1988) (Annex 3).

3.7. Questionnaire survey

A structured interview question was prepared and applied to all concerned workers of the farm and animal owners who have direct contact with animal and animal products in the study area. Information related to personal demography like age, sex, educational background and

knowledge, attitude and practice toward brucellosis, raw milk drinking practice, aborted fetus handling practice, history of abortion, chronic headache, Knee pain and testicular swelling were collected on format developed (Annex 4).

The presence of abortion, still births, retention of fetal membranes, separate parturition/maternity pen, and contact between animals with other herds was recorded. Breeding methods was characterized by service types (artificial insemination (AI), bull or both). The method of after birth disposal (placenta, aborted material and dead fetus) was also recorded as burying, ate to dog or thrown to open dump.

3.8. Data Management and Analysis

Data collected from field and serological test was coded and stored in Microsoft Office Excel spread sheet and transferred to R.software version 4.0 for statistical analysis. The seroprevalence for animal level was calculated on the basis of RBPT and CFT positivity dividing the number of *Brucella* reactors by total number of tested animals. The Chi-square ($\chi 2$) was used to analyze descriptive questioner results and Firth's Bias-Reduced Logistic Regression analysis was employed to identify association of seropositivity with the potential risk factors (Thrusfield, 2007).

3.9. Ethical Considerations

Ethical clearance certificate was obtained from the animal research ethical review committee of the College of Veterinary Medicine and Agriculture (Date 15/10/2019GC, Ref. No. VM/ER//10/01/12/2020) and Oromia Health Bureau (Date 24/9/2012EC, ReF. No. BEF/HBTFU/146/914) based on the assessment of the research proposal. The standard ethical principles and conducts was implemented both in animal and human study participants. Written and oral informed consents were obtained from human study participants and livestock owners.

4. RESULT

4.1. Seroprevalence of Brucellosis in Dairy Cattle

The present study revealed that out of 352 dairy cows with history of recently abortion tested (222 cross and 130 local breed), 4 (1.2%) (95%CI: 0.0047-0.0297) were tested positive by RBPT. The RBPT positive serum samples were further tested using CFT for confirmation. Only 2 samples ware confirmed seropositive for bovine brucellosis in the study area. The overall seroprevalence of bovine brucellosis were thus 0.6% (95%CI: 0.0016-0.0209) based on CFT tests in the study area (Figure 8).

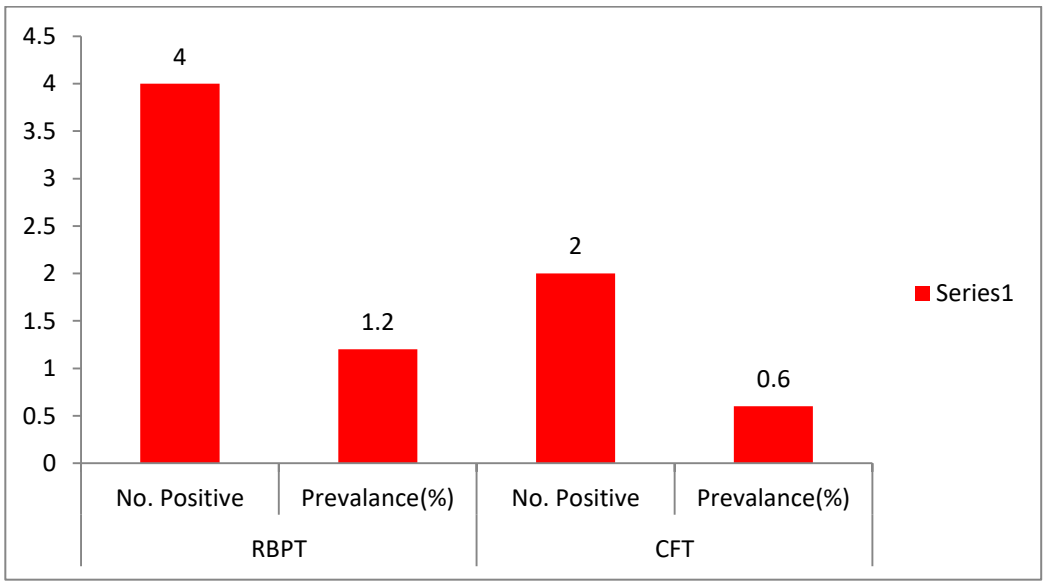

Figure 8: The overall seroprevalence of brucellosis in dairy cow with a history of recent abortion by RBPT and CFT diagnostic techniques

4.1.1. Association of putative risk factors with bovine brucellosis seropositivity

Univariable Firth's Bias-Reduced Logistic Regression analysis was computed to evaluate the association between brucellosis seropositivity and different risk factors. Out of 352 serologically screened dairy cows, 1 (0.3%) from Ethiopian Institute of Agricultural Research (EIAR) Holeta Agricultural Research Center (HARC) large scale dairy farm, 1(0.3%) from Fayitu medium scale

farm and 2(0.6%) from Burka Harbu kebele were positive by RBPT and 2(0.6%) from Burka Harbu kebele were further confirmed by CFT. The analysis indicates that there was no statistically significant association between animal origins and bovine brucellosis (P > 0.05) (Table 7).

The seroprevalence of bovine brucellosis in late stage of abortion (OR=14.76, p=0.0002), retained fetal membrane (OR=32.74, p=0.0064), market source of stock replacement (OR=16.548, p=0.0022), natural breeding method (OR=7.581, p=0.05) and parturition pen (OR=11.533, p=0.027) were statistically significant while other factors were not statistically significant (P > 0.05) (Table 7).

The brucellosis seroprevalence in local and cross breed cows was 0.3% for each and there were no statistically significant association between brucellosis seroprevalence and breeds (P>0.05). According to the result of this study age categories were not significantly associated with bovine brucellosis. Even though no statistically significant association with age groups, there were higher seroprevalence (0.6 %) of bovine brucellosis in cows with young age compared to animals with adult. Seropositivity in primipareous and pluripareous cow parity was 0.3% for each and there were no statistically significant association between parity status of the cows and brucellosis seropositivity (Table 7).

The serology result of the apparent study also reveals positive test result recorded in animals managed in semi-intensive (0.3%) and extensive production systems (0.3%) than in those of intensive systems. A higher seroprevalence of brucellosis was observed on small holder farmers than large scale and medium scale farms. All cases of positive animal were kept at poor hygiene barn. Most of the cows were separated during parturition from the herd even though most respondents have no parturition pen (Table 7).

Table 7: Univariable Firth's Bias-Reduced Logistic Regression analysis of risk factors associated with bovine brucellosis seropositivity with combined RBPT and CFT

Variables	No. of cows sampled	No. positive		OR(95%CI)	P-Value
		RBPT (%)	CFT (%)		
Origin/Site					
Adea Berga farm	30	0	0	1.0	
Holeta Town	163	4(1.2)	2(0.6)	1.944(0.0744-13.1495)	0.97
Wolmera	159	0	0	1.912(1.0271-35.596)	0.4334
Herd size					
Large scale	58	1(0.3)	0	1.0	
Medium scale	40	1(0.3)	0	1.44(0.7754-2.6907)	0.8552
Small holders	254	2(0.6)	2(0.6)	1.158(9.2643-60.6058)	0.923
Breed					
Cross	222	3(0.9)	1(0.3)	1.0	
Local	130	1(0.3)	1(0.3)	1.71 (0.137-21.224)	0.645
Age					
Young	78	1(0.3)	2(0.6)	1.0	
Adult	233	2(0.6)	0	3.356 (0.269-42.767)	0.312
Old	41	1(0.3)	0	9.245 (0.061-18.527)	0.274
Parity status					
Primipareous	57	1(0.3)	1(0.3)	1.0	
Pluripareous	295	3(0.9)	1(0.3)	5.212 (0.417-65.049)	0.176
Stage of abortion					
Late Sage	13	3(0.9)	2(0.6)	14.76(1.1211-2.042)	0.0002***
Early stage	339	1(0.3)	0	1.0	
Source of stock replacement					
Government	1	0	0	1.0	
Own source	198	3(0.9)	1(0.3)	0.431(2.326-7.974)	1.000
Market purchase	16	1(0.3)	1(0.3)	16.548(8.436-24.612)	0.0022**
Both	52	0	0	4.981(2.607-7.324)	0.143

….Continued

Variables	No. of cows sampled	No. positive RBPT (%)	CFT (%)	OR(95%CI)	P-Value
Hygiene of barn					
Poor	111	2(0.6)	2(0.6)	4.635 (3.713-64.2)	0.256
Medium	140	2(0.6)	0	0.722 (3.902-133.75)	0.871
Good	101	0	0	1.0	
Type of farming					
Intensive	32	0	0	1.0	
Extensive	164	1(0.3)	1(0.3)	1.564(8.272-22.933)	0.776
Semi-intensive	156	3(0.9)	1(0.3)	3.638(19.105-53.43)	0.394
RFM					
Yes	48	4(1.2)	2(0.6)	32.74(2.611-4.544)	0.0063**
No	304	0	0	1.0	
Mating practice					
AI	137	0	0	1.0	
Natural mating	80	4(1.2)	2(0.6)	7.581(0.609-1.049)	0.05*
Both	135	0	0	2.095(1.131-3.882)	0.715
Presence of parturition pen					
Yes	7	2(0.6)	0	1.0	
No	260	2(0.6)	2(0.6)	11.533(0.0604-0.22)	0.027*
Separation of cow during parturition					
yes	335	4(1.2)	2(0.6)	1.0	
No	17	0	0	0.262(0.02-26.72)	0.461
Cleaning of calving area after parturition					
No	280	3(0.9)	2(0.6)	0.768(0.554-9.57)	0.861
Yes	72	1(0.3)	0	1.0	

*=statistically significant, OR=Odds ratio, CI=Confidence interval

The results of multivariable Firth's Bias-Reduced Logistic Regression analysis showed the association of predictor variable with bovine brucellosis seropositivity. There was no

multicollinearity between variables. Accordingly the stepwise multivariable Firth's Bias-Reduced Logistic Regression analysis results were showed important risk factors for bovine brucellosis seropositivity. Therefore, stage of abortion, retained fetal membrane, source of animal and presence of parturition pen were included in the final model. However, stages of abortion, RFM and source of animal for replacement were significantly associated with brucellosis seropositivity (Table 8).

Thus the reduced model depicted that cows with late stage of abortion, retained fetal membrane, and market purchase herd replacement were 1.283, 1.046 and 1.0638 times more likely to be seropositive to *Brucella* infection than with early stage of abortion, without retained fetal membrane, and own and government source of herd replacement respectively.

Table 8: Multivariable Firth's Bias-Reduced Logistic Regression analysis of risk factors associated with dairy cow brucellosis seropositivity by combined RBPT and CFT

Variables	**No. of cow tested**	**CFT (%)**	**OR(95%CI)**	**P-Value**
Stage of abortion				
Early stage	339	0	1	
Late Sage	13	2(0.6)	1.283(1.215-1.3557)	0.000***
RFM				
No	304	0	1	
Yes	48	2(0.6)	1.046(1.0187-1.0754)	0.0014**
Source of stock replacement				
Government	1	0	1	
Own source	198	1(0.3)	6.549(30.066-76.357)	0.075
Market Purchase	16	1(0.3)	1.0638(1.026-1.1029)	0.0008***
Both	52	0		0.399
Presence of parturition pen				
Yes	7	0	1	
No	260	2(0.6)	11.53(0.0603-2.2055)	0.281

OR=Odds ratio, CI=Confidence interval

4.2. Seroprevalence of Brucellosis in Dairy Cattle Owners and Dairy Farm Workers

Owners of dairy cow with a history of recent abortion from twenty two kebeles (146 human), government workers from EIAR HARC Holeta and Adda Berga large scales dairy farms (17 human), and farm owners from three medium scale farm (3 human) were sampled for this study. The present result revealed that out of 166 human serum sample tested (109 males and 57 females), about 7 (4.2%) (95%CI: 0.02046-0.08425) were tested positive by RBPT. Sera screened positive for RBPT were further confirmed with CFT which showed 2 sera were confirmed positive, giving overall seroprevalence of human brucellosis among the blood samples tested was 1.2% (95%CI:0.003289-0.04278) in the study area (Figure 9).

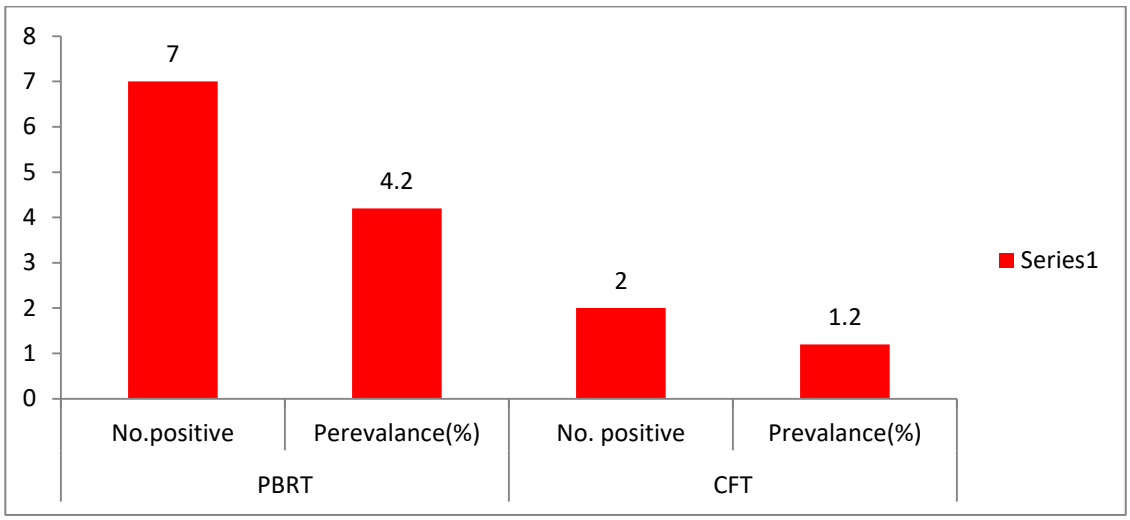

Figure 9: The overall seroprevalence of brucellosis in dairy cattle owners and dairy farm workers with combined RBPT and CFT diagnostic techniques

4.2.1. Association of putative risk factors with human brucellosis seropositivity

Univariable Firth's Bias-Reduced Logistic Regression analysis for association between human brucellosis and different risk factors using combined RBPT and CFT were carried out. Out of 166 serologically screened serum samples, 2 (1.2%) farm workers from EIAR Adaberga large scale dairy farm, 3(1.8%%) small holder farmers from Burka Harbu and 2(1.2%) from Goro Kerensa kebele of Holeta Town were positive by RBPT and 2(1.2%) from Burka Harbu kebele were

further confirmed by CFT. The finding also indicated that there was no statistically significant difference between brucellosis seropositivity and human origin (P > 0.05) (Table 9).

Demographic factors (age, sex and educational status), factors related with human animal contacts (human housing, contact with aborted fetus, way of contact with aborted material, drinking row milk, drinking raw milk from aborted cow, and drinking raw milk from cow with RFM), and symptoms from disease events experienced during the previous month (history of abortion, history of sterility, history of chronic headache, history of knee pain and history of Orchitis) were compared to the sero-status of participants.

The Risk factor analysis showed that there was statistically significant associations between human brucellosis and human housed with dairy animals (OR=1.8, p=0.002), contact with aborted fetus (OR=21.19, p=0.017), drinking row milk (OR=24.99, p=0.012), drinking raw milk from aborted animals (OR=5.72, p=0.019), drinking raw milk from cow with RFM (OR=4.217, p=0.029) and Orchitis (OR=230.71, p=0.000) (Table 9).

There were no statistically significant difference between males and females in seropositivity (P>0.05). Relatively high brucellosis seropositivity was observed in male respondents than in female. The difference in seropositivity among the different age groups was not statistically significant. However, the highest proportion of seropositive individuals was observed at age group between 31-45 years. In other way high seroprevalence were observed in respondents who are not educated or low grade educational level, even though there is no statistically significant association between educational status and human brucellosis (Table 9).

The most common symptoms of human brucellosis mentioned by respondents were abortion 9(5.42%), sterility 1(0.6%), chronic headache 66(39.8%), knee pain 68(40.9%), and testicular swelling 5(3.01%). Statistically significant association was observed in respondents with a history of testicular swelling (Table 9).

Table 9: Univariable Firth's Bias-Reduced Logistic Regression analysis of risk factors associated with human brucellosis seropositivity

Variables	No. sampled	No. positive (%)		OR(95%CI)	P-Value
		RBPT (%)	CFT (%)		
Origen/site					
HARC Berga farm	4	2(1.2)	0	1.0	
HARC Holeta farm	13	0	0	3.33(1.6771-66.0154)	0.603
Holeta Town	55	5(3.01)	2(1.2)	4.21(0.276-61.4554)	0.623
Walmera district	94	0	0	4.76(0.2433-9.2818)	0.191
Sex					
Male	109	4(2.4)	2(1.2)	2.67(2.1291-37.1307)	0.486
Female	57	3(1.8)	0	1.0	
Age					
<30 years	41	1(0.6)	0	1	
31-45 years	91	6(3.6)	2(1.2)	1.91(0.1498-2.6617)	0.658
46-60 years	30	0	0	0.66(0.0035-0.1238)	8.38
>60 years	4	0	0	6.99(0.0357-1.3771)	0.373
Educational status					
College	18	0	0	1.0	
Not read and write	34	1(0.6)	1(0.6)	1.66(0.0839-0.2468)	0.752
Non-academic	4	1(0.6)	0	4.11(0.0208-8.1315)	0.507
Informal	6	0		2.85(0.0146-55.2905)	0.616
Primary school	69	4(2.4)	1(0.6)	0.81(0.0414-120.241)	0.9004
Secondary school	28	1(0.6)	0	0.65(0.0034-1.2233)	0.831
University	7	0	0	2.467(0.0127-47.664)	0.663
Human housing					
Common housing with dairy animals	14	3(2)	2(1.2)	1.8(0.013-0.2426)	0.0022**
Separate housing	135	2(1.3)	0	1.0	
Contact with aborted fetus					
Yes	89	7(4.2)	2(1.2)	21.19(1.6708-2.9513)	0.017*
No	77	0	0	1.0	

….Continued

Variables	No. sampled	No. positive (%)		OR(95%CI)	P-Value
		RBPT (%)	CFT (%)		
Way of contact with aborted material					
Bare hand	89	7(4.2)	2(1.2)	1.0	
Wear glove	20	0	0	0.84(6.0283-10.9012)	0.912
Using plastic	55	0	0	0.31(0.02204-3.85402)	0.394
With other material	2	0	0	6.92(0.0455-12.58103)	0.331
Washing hands after handling aborted materials					
Yes	147	6(3.4)	2(1.2)	1.0	0.609
No	19	1(0.6)	0	0.67(0.0519-93.7542)	0. 81
Drinking row milk					
Yes	137	7(4.2)	2(1.2)	24.99(1.9644-3.4857)	0.012*
No	29	0	0		
Drinking habit of milk from aborted cow					
Boiling	47	1(0.6)	0	1.0	
Without boiling	43	6(3.5)	2(1.2)	5.72(44.893-79.8198)	0.019*
Not drinking	76	0	0	0.62(3.3394-11.5445)	0.813
Drinking habit of milk from RFM cow					
Boiling	75	0	0	1.0	
Without boiling	91	7(4.2)	2(1.2)	4.2178(3.3643-5.8527)	0.029*
History of abortion					
Yes	9	1(0.6)	0	3.27(0.023-44.2266)	0.51
No	157	6(3.6)	2(1.2)	1.0	
History of sterility					
Yes	1	0	0	21.80(0.1359-5.4571)	0.167
No	165	7(4.2)	2(1.2)	1.0	
History of chronic headache					
Yes	66	6(3.6)	2(1.2)	7.79(6.2095-10.8132)	0.117
No	100	1(0.6)	0	1.0	
History of knee pain					
Yes	68	4(2.4)	2(1.2)	7.59(6.0545-10.5418)	0.123
No	98	3(1.8)	0	1.0	
History of testicular swelling					
Yes	5	2(1.2)	2(1.2)	230.71(15.332-34.33)	0.000***
No	161	5(3)	0	1.0	

The results of multivariable Firth's Bias-Reduced Logistic Regression analysis showed the association of predictor variable with human brucellosis seropositivity. Generally there was no multicollinearity between variables. Accordingly the stepwise multivariable Firth's Bias-Reduced Logistic Regression analysis results were showed important risk factors for brucellosis seropositivity. Therefore, human housing, contact with aborted fetus, drinking raw milk and history of Orchitis were included in the final model. Thus the analysis depicted that human who had common housing with dairy animals, contact with aborted fetus with bare hand, drinking row milk, and had history of Orchitis were 1.947, 1.022, 1.019, and 1.919 times more likely to be seropositive to *Brucella* infection than human who had separate housing, use protective materials when contact with aborted fetus, boiling before drinking raw milk and did not had history of Orchitis respectively (Table 10).

Seroprevalence, recorded for human housed with dairy animals revealed a statistically significant variation with odds of being seropositive by 1.497 times more likely to be infected with *Brucella* organisms than human who had separate housing with animals. Similarly, contact with aborted fetus with bare hand and drinking raw milk without boiling were found to be statistically significantly associated with seropositivity. Seropositivity to human brucellosis was significantly higher in human those make a contact with aborted fetus with bare hand with 1.022 times more likely to be seropositive than humans those make a contact using protective materials.

Table 10: Multivariable Firth's Bias-Reduced Logistic Regression analysis of risk factors associated with human brucellosis seropositivity

Variables	No. Sampled	CFT (%)	OR (95% CI)	P-Value
Human housing				
With dairy animals	14	0	1	
Separate housing	135	2(1.2)	1.947(0.912-0.983)	0.0049**
Contact with aborted fetus				
No	77	0	1	
Yes	89	2(1.2)	1.022(0.989-1.057)	0.012*
Drinking row milk				
No	29	0		
Yes	137	2(1.2)	1.019(0.98491.055)	0.046*
History of Orchitis				
No	161	0		
Yes	5	2(1.2)	1.919(1.775-2.076)	0.000***

OR= Odds ratio, CI= Confidence interval

4.2. Questionnaire Surveys

4.2.1. Demographic characteristics of the respondents

A total of 284 participants were interviewed (213 male and 71 female) to assess their knowledge, attitude and practices towards brucellosis. From those respondents 2 (0.7%) were large scale farm managers, 11 (3.9%) medium scale farm owners, 254 (89.4%) small holder farmers and 17(5.9%) government farm employees. About 3.9% of respondents were university graduate, 11.7% collage graduate, 16.5% secondary education, 29.9% primary education, 20.4% not read and write and 13.7% informal education. Majority of respondent population of the participants (49.3%) were in 35-45 age groups. Also 242 (85.2%) of the participants were married and 42 (14.8%) were single (Table 11).

Table 11: Demographic characteristics of respondents

Demographic variables	No. Respondents	Percentage (%)
Gender		
Male	213	75
Female	71	25
Age categories		
<30 years	57	20.1
31-45 years	140	49.3
46-60 years	71	25
>60 years	16	5.6
Marital status		
Married	242	85.2
Single	42	14.8
Educational status		
Not read and write	58	20.4
Informal	39	13.7
Non academic	11	3.9
Primary school	85	29.9
Secondary school	47	16.5
College	33	11.7
University	11	3.9
Animal ownership status		
Farm employees	17	5.9
Small holder animal owners	254	89.4
Medium scale farm owners	11	3.9
Large scale farm owners	2	0.7

4.2.2. Farm characteristics of different scale farms

Management system of both two large scale farms have semi intensive, eight of medium scale farms have intensive and three of them have semi intensive management system while most small holder (164) farmers have extensive management system. It was also found that, 57(34.8%) of sampled cows from large scale were using AI breed system, while 107(97.3%) of small holder farmers were dependent on natural mating and also 76(97.4%) of small holder farmers were using both AI and natural mating (Table 12).

More than half of large scale, medium scale and small holder farmers were dependent on own sources for replacement stock. The practices of provision of separate parturition pens, separation of cows during parturition, and cleaning and disinfection of contaminated areas was done in a relatively better in the intensive management system. In addition, 43 small holder farmers encounter a problem of repeat breeding (Table 12).

Table 12: Farm characteristics of different scale farms

Variables	Proportion of Respondents		
	Large scale farm (%)	Medium scale farm (%)	Small holder farmer (%)
Type of farming			
Extensive	0	0	164(100)
Intensive	0	8(25)	24(75)
Semi intensive	2(2.8)	3(4.2)	66(93)
Source of stock replacement			
Own farm raised	55(19.4)	29(10.2)	200(70)
Market purchased	3(6.1)	10(20.4)	36(73.5)
Government gift	0	1(5.3)	18(94.7)
Service type			
AI	56(34.8)	33(22)	48(43.3)
Natural mating	1(0.9)	2(1.8)	107(97.3)
Both	0	2(1.6)	76(97.4)
Having parturition pen			
No	0	8(3.1)	251(96.9)
Yes	2(28.6)	2(28.6)	3(42.9)
Cleaning parturition area after birth			
Yes	2(0.75)	2(0.75)	4(1.49)
No	0	9(3.37)	250(93.63)
Hygiene of barn			
Good	30(29.7)	12(11.9)	59(59.4)
Medium	28(20)	23(16.4)	89(63.6)
Poor	0	5(4.3)	106(95.5)
Repeat breeding			
No	50(16.8)	37(12.4)	211(70.8)
Yes	8(14.8)	3(5.6)	43(79.6)

4.1.3. Knowledge, attitude and practices regarding brucellosis

In current study the majority of respondents, 265(93.3%) had no information about brucellosis while 19(6.7%) of respondents had information and knowledge about brucellosis in human and animal. The analysis result showed that most of the dairy animal owners, 173(60.9%) consumed raw milk and 111(39.1%) had not reported to consume raw milk. Respondents also reported that milk from aborted cows were not consumed by 128(45.1%), consumed without boiling by 80(28.2%) of respondents and 76(26.8%) of respondents were boiled before consuming. Majority of the respondents, 250(88%) mentioned that they had poor knowledge on zoonotic diseases and its transmission mechanism via dairy animals and animal products. Only 27(9.5%) of respondents had knowledge about zoonotic diseases transmitted through raw milk consumption.

In the current study 168(59.2%) of respondents handled aborted fetus without using personal protective equipment, while 80(28.2%) used plastic, 25(8.8%) used glove and the remaining 11(3.9%) had removed aborted fetus using other materials. It was also found that the majority of the farm respondents, 225 (79.2 %) were removing fetal and aborted materials on the open field while some others were burial after birth or abortion, and limited respondents feed to dogs especially those of small holder farmers and medium herd size farms owners (Table 13).

Table 13: Knowledge, Attitudes and Practices (KAP) of respondents regarding brucellosis

Variables	**No. Respondents**	**Frequency (%)**
Drinking raw milk		
No	111	39.1
Yes	173	60.9
Milk from aborted cow		
Boiling before drinking	76	26.8
Drink without boiling	80	28.2
Not drink	128	45.1
Milk from cow with RFM		
Boiling before drinking	118	41.5
Drink without boiling	166	58.5

...Continued

Variables	No. Respondents	Frequency (%)
Knowledge on Brucellosis		
No	265	93.3
Yes	19	6.7
Knowledge on zoonotic diseases transmitted by handling of infected animal and animal products		
No	250	88
Yes	34	12
Knowledge on zoonotic diseases due to raw milk consumption		
No	257	90
Yes	27	9.5
Contact with aborted fetus		
No	116	40.8
Yes	168	59.2
Way of contact with aborted fetus		
Bare hand	168	59.2
Wear glove	25	8.8
Wear plastic	80	28.2
With other material	11	3.9
Method of disposing aborted fetus		
Feeding for dog	12	4.2
Burial	32	11.3
Incineration borrow	15	5.3
Remove on open field	225	79.2
Hand washing after contact with aborted fetus		
No	259	91.2
Yes	25	8.8

4.1.4. Occupational risks among farm works and dairy cattle owners

In study area about 95 (37.4%) of small holder farmers had common housing with dairy animals. The result showed that only 8(42.1%), 1(9.1%) and 15(5.9%) of respondents from large scale, medium scale farms and small holder animal owners were aware of brucellosis respectively. Up to 11(57.9%), 10(90.9%) and 239(94.1%) of the respondents from large scale and medium scale farms, and small holder animal owners were reported that they had poor knowledge about brucellosis respectively. There was statistically significant variation between the indicated variables ($P < 0.05$) as shown in (Table 14).

Table 14: Occupational risks and awareness among farm works and owners about brucellosis

Variables	**Proportion of Respondents**			**X^2-value**	**P-value**
	Large scale farm (%)	**Medium scale farm (%)**	**Small holder farmer (%)**		
Human housing					
Common with dairy animals	0	0	95(37.4)	7.54	0.016*
Separate	2(100)	11(100)	159(62.6)		
Awareness about zoonotic brucellosis					
No	11(57.9)	10(90.9)	239(94.1)	29.9	0.000***
Yes	8(42.1)	1(9.1)	15(5.9)		
Awareness about zoonotic diseases transmission through drinking raw milk					
No	8(7.2)	10(9)	93(83.3)	13.3	0.001**
Yes	11(6.4)	1(0.6)	161(93.1)		
Contact with aborted fetus					
No	17(14.7)	10(8.6)	89(76.7)	33.5	0.000***
Yes	2(1.2)	1(0.6)	165(98.2)		
Contact with RFM					
No	19(9.6)	11(5.2)	180(87.5)	11.8	0.003**
Yes	0	0	74(100)		

5. DISCUSSION

Brucellosis is a serious zoonotic disease affecting human and all domestic animals. It is considered to be one of the great public health problems all over the world (WHO, 2006). In Ethiopia, bovine brucellosis has been extensively studied in intensive dairy cattle (Jergefa *et al*., 2009). However, little attention has been paid to this disease in small holder dairy farm and in animal owners and farm workers in central highland of Ethiopia. Control of brucellosis in humans depends on the availability of reliable and up to date information on its occurrence and distribution in animals.

The present study revealed that the overall seroprevalence of *Brucella* antibodies determined with RBPT and CFT in Holeta Town, Wolmera District and Adea Berga EIAR dairy farm, Oromia Regional State, Ethiopia were 1.2% and 0.6% in animals with history of recent abortion. Since CFT is recommended as confirmatory test for brucellosis with high specificity (Smith and Sherman, 1994; Radostits *et al*., 2007), the overall seroprevalence of bovine brucellosis in the study area was 0.6%.

The seroprevalence in this study was slightly higher than the finding of Yayeh, (2003) who reported an overall prevalence of 0.14% in North Gondar Zone, Bashitu *et al*., (2015) 0.2 % in Ambo and Debreberhan, (Edao *et al*., 2018) 0.06% in Addis Ababa area. While in other ways Alem and Solomon, (2002); Belihu, (2002) failed to find any seroreactive cattle in Eastern and Western Showa zones of central Ethiopia and in intensive dairy farms in Addis Ababa area, respectively. This variation in seroprevalence might be seen due to the difference in the study animal management system. Most of reactive animals in my study were from small holder farmers kept under extensive management system. The dependency of most of the farmers on outside sources for stock replacement could be one possible way of introduction of the disease into unaffected herds. This could also be due to differences between the study areas regarding conditions that could facilitate the rate of transmission of the disease (Radostits *et al*., 2000).

The finding of my study was in close agreement with the findings of Tesfaye, (2003) (0.69 %); Tolosa *et al*., (2008) (0.77 %); Gumi *et al*., (2013) (0.9%); Adugna *et al*., (2013) (1.0%); from Ethiopia and Kang'Ethe *et al*., (2007) (1.0%) from Kenya. On the other hand, there were reports with a relatively higher seroprevalence of bovine brucellosis in other parts of the country; Berhe *et al*., (2007) (3.19%); Kebede *et al*., (2008) (11.0%); Jergefa *et al*., (2009) (2.9%); Hailesillasie *et al*., (2010) (4.9%); Ibrahim *et al*., (2010) (3.1%); Degefu *et al*., (2011) (1.38%); Haileselassie *et al*., (2011) (6.1%); Megersa *et al*., (2011b) (3.5%); Asmare *et al*., (2013) (1.9%); Tibesso *et al*., (2014) (4.3%); Geresu *et al*., (2016) (1.4%). Similarly, relatively higher seroprevalence were reported in other African countries by other authors: 8.5% Omer *et al*., (2002) from Eritrea, 24.5% Angara *et al*., (2004) from Sudan; 24.0%, Matope *et al*., (2011) from Zimbabwe; 5.5% Mai *et al*., (2012) from Nigeria were some of the reports.

The difference observed in seroprevalence could be due to variation in production systems and animal management. Most of the previous higher prevalence studies which reported by different authors were in extensively managed herds, where cattle from several owners mingle at grazing or watering points. According to the result in my study all of the confirmed case was from extensively managed small holder dairy animal owners. Hence, the reasons for the low prevalence of bovine brucellosis in this study areas could possibly be explained by better hygienic practices, separation of cows during parturition, cleaning and disinfection activities, culling of infected animals and depending on own herds for replacing stock in the two large scale and eleven medium scale farms, and the prevailing management differences between the intensive, semi-intensive and extensive production systems. This is also reflected the relatively good hygienic status of the farms and practices in disposing aborted materials to ward off contact with animals.

In addition to estimation of seroprevalence, this study was also carried out to assess the risk factors associated with disease occurrence. The previous history of abortion stage had statistically significant association with seropositivity of bovine brucellosis. This was in agreement with previous reports of Geresu *et al*., (2016). This could be explained by the presence of higher seropositivity in cows in the last trimester which may be due to the preferential localization of *Brucella* in the uterus, in which allantoic fluid factor, erythritol stimulate the growth of *Brucella*

and elevate in the placenta and fetal fluid from about the 5th month of gestation (Swanepoel *et al*., 2004; Radostits *et al*., 2007).

In addition to this there was a highly significant association between cows with history of retained fetal membrane and seropositivity of brucellosis in present study. Retention of the placenta and inflammation of the wall of the uterus (metritis) are common sequelae to abortion due to brucellosis, Aparicio, (2013) reported that *Brucella* infected cows were expected to abort 3 to 4 times more than unexposed cows. This could also be explained by the fact that retained fetal membrane is typical outcomes of brucellosis. Other studies have also shown a significant association between seropositivity and retained fetal membrane (Berhe *et al*., 2007; Tolosa *et al*., 2008; Ibrahim *et al*., 2010; Megersa *et al*., 2011a; Adugna *et al*., 2013; Alemu *et al*., 2014).

There were statistically significant differences in seroprevalence of brucellosis seropositivity and breeding methods. In the present study area most farms used Artificial insemination (38.9%) than bull (22.7%) for breeding purpose. There was higher seroprevalence rate in bull service, whereas no seropositive record in AI mating method. The sources of replacement stock ($p=0.021$) were shown to significantly affect the prevalence of bovine brucellosis in study areas. Those animals purchased from other area were relatively suffer of brucellosis than cattle grown and replaced the stock.

There was no statistically significant difference ($P>0.005$) between seroprevalence of brucellosis and dairy cow origins. The finding was in line with report by Asgedom *et al*., (2016) that, there was no statistically significant variation in seroprevalence of brucellosis among different origin. Even though, there was no statistically significant association, high prevalence of brucellosis was observed in dairy cows of Holeta Town Burka Harbu kebele (0.6%) than all other kebeles and farms. The reasons for the variations in brucellosis seroprevalence among the study areas might be related to the difference in management practice performed in the three study sites. In addition, different studies revealed that the seroprevalence of brucellosis is affected by different agro climate conditions, which determine survival of *Brucella* organisms (Radostits *et al*., (2007).

According to the result of my study *Brucella* infection did not show significant variation between breeds. The present finding agrees with the previous reports of Kebede *et al*., (2008); Amenu *et al*., (2010); Asmare *et al.*, (2013); Geresu *et al*., (2016) who reported seropositivity of *Brucella* infection was independent of the breeds. The variation in number of animals sampled per breed group might be accountable for the absence of significant variation of *Brucella* infection between the breeds. On the other hand in my study site cross breed were increasing and the farmers give high care for cross breed cow than local breeds.

According to the present study, there was no statistically significant different among age groups to *Brucella* seropositivity. All positive cows (0.6%) were found among adult age group whereas no *Brucella* seropositivity was observed in young and old age group of dairy cattle in the study sites. Similar findings were also reported by Jergefa *et al*., (2009); Amenu *et al*., (2010); Geresu *et al*., (2016) where age was not significantly associated with *Brucella* seropositivity.

The higher seroprevalence of brucellosis among adult cows may be related to the higher number in adult cow included in the study. In addition, may related to their advanced age as the organism may remain latent or chronic for an unspecified period before manifesting as clinical disease. The other justification also possible as age is one of the intrinsic factors which influence the susceptibility to *Brucella* infection. Brucellosis appears to be more associated with sexual maturity (Radostits *et al*., 2000). It is essentially a disease of sexually mature animals and susceptibility increases with sexual maturity and pregnancy due to the influence of sex hormones and placenta erythritol on the pathogenesis of brucellosis *(Radostits et al*., 2007). On the other hand, younger animals tend to be more resistant to infection and frequently clear infections, although latent infections could occur (Walker, 1999).

The parity status was not found to be a significant determinant of seroprevalence. In present study, 1 of the seropositive cow was in their pluripareous parity status whereas the other 1 from primipareous. Furthermore, of parity status 83.8 % (n=295) of cows were pluripareous whereas about 12.2% (n= 57) were primipareous. Hence, the present finding is in line with reports of Tolosa *et al*., (2008); Adugna *et al*., (2013); Asmare *et al.*, (2013); Geresu *et al*., (2016) where parity status were not significantly associated with *Brucella* seropositivity. The reason of the

similarity of *Brucella* infection among the parity could be due to the difference in the number of animals included for the study.

The finding of this study revealed that relatively higher seroprevalence of brucellosis was observed in extensive (0.3%) and semi-intensive (0.3%) management system than intensive management systems though the finding was not statistically significant. A relatively higher seroprevalence observed in this study, in the extensive and semi-intensive management system could be partly explained by the fact that contact between animals increases in communal grazing practices which was the predominant feeding system in the extensive and semi-intensive type of management. In such circumstances, cattle of unknown disease status might mix and often grazed together and resulted in spreading and transmission of disease among herds. About 90.9 % of the farms in the study area shared the communal grazing system. It has also been indicated that free grazing which allows unrestricted contact between animals may have contribution to the spread of brucellosis in extensive management system (Silva *et al*., 2000). In addition, the observation made by Kagumba and Nandokha, (1978) indicates that the prevalence of brucellosis was higher in communally grazed large herds of cattle.

The lower prevalence recorded in the intensive management system in the study area could be due to the better hygienic practices in the intensive management system which was expressed by the relatively better proportion of farm owners having separate parturition pens, separating cows during parturition, performing cleaning and disinfection duties, depending on own herd for replacing stock and having better knowledge about the disease. Brucellosis has been labeled to be a disease of poor hygienic condition that would exposure animals to aborted fetus, placentas, vaginal discharges or newborn calves from infected cows. Likewise, the use of maternity pens at calving is proved to be associated with a decrease in prevalence of infection, presumably due to decreasing the exposure of infected and susceptible animals (Radostits *et al*., 2000).

Farm hygiene was observed based on manure disposal, drainage, and physical appearance of the animal and ventilation status of the farms. Accordingly, most of the farms' hygiene was fair, and some were good while the other some were poor. Even though this risk factor had no any statistical significant association with occurrence of brucellosis the high seroprevalence was

recorded in poor hygienic barns (0.6%). Though, keeping good hygiene at dairy farm (Mugizi, 2009) are considered as a protective factor for brucellosis, unhygienic practices were identified as factors that will facilitate the spread of *Brucella* infections (Adesokan *et al*., 2013).

Serum samples from 149 recently aborted dairy cow owners and 17 government farm employees were examined for the presence of brucellosis. Of the total examined human, seroprevalence of brucellosis using RBPT and CFT was found to be 4.2% and 1.2%, respectively. Since CFT is recommended as confirmatory test for brucellosis with high specificity (Smith and Sherman, 1994; Radostits *et al*., 2007), the overall seroprevalence of human brucellosis in the study area was 1.2%.

It is not surprising to get people infected with brucellosis in an area where there are infected animals since the seroprevalence of brucellosis in human is largely influenced by the seroprevalence of disease among domestic animals around (Omer *et al.*, 2002). The findings of this study are relevant for the country towards the development of a national brucellosis control program by the medical and veterinary sectors. Our study provides evidence that brucellosis is one of the public health problems among the rural and urban population of Ethiopia.

In Ethiopia, very few studies have been conducted to determine seroprevalence of human brucellosis. The similar studies by Haileselassie *et al*., (2011) (1.2%) in Western Tigray; Tibesso *et al*., (2014) (1.2%); Amanuel *et al*., (2017) 1.3% in Debrezeit and Mojo were reported. Compared to the present study, however, the relatively higher seroprevalence of brucellosis observed by Yirgu (1991) (12.5%); Tolosa, (2004) (3.4%); Kassahun *et al*., (2006) (4.8%); Hailemelekot *et al*., (2007) (3.8%); Eshetu *et al*., (2008) (16.5%); Haileselassie *et al*., (2011) (2.2%); Gebawo *et al*., 2014) (2.2%) might be attributed to the large sample size involved and/or the different confirmatory tests used by the two studies, CFT versus 2–mercaptoethanol test (MET). The lower prevalence in our study could also be due the fact that the number of livestock, level of contact with animals and frequency of consumption of dairy products is low as compared to the mentioned study.

Our findings were lower compared to studies in Uganda that recorded a seroprevalence of 5.8% in Mbarara; 9% in Kampala as reported by Mugabi, (2012); a seroprevalence of 11% carried out in the Southwest Uganda by Miller *et al*., (2016)**.** However, in these studies, the study populations were high risk livestock keeping populations with a high dairy industry and with a high consumption of milk as well as locally made milk products. Rather, very high *Brucella* infection was reported in abattoir workers in other countries, Agasthya *et al*., (2007); Mukhtar and Kokab, (2008) reported prevalence of 19.69% and 21.7% among slaughter house workers in India and Pakistan, respectively. This might be correlated exposure of abattoir worker for brucellosis infected animal and animal discharges. Brucellosis is an occupational disease, occurring most often in veterinarians, farmers, stock inspectors, abattoir workers, laboratory personnel, butchers (Bishop *et al*., 1994).

In present study, strong statistically significant association was seen between human brucellosis seroprevalence and human housing, contact with aborted fetus, drinking row milk, drinking raw milk from aborted animals, drinking raw milk from cow with RFM and history of testicular swelling.

Statistically significant association seen between human *Brucella* seroprevalence and human housing and contact with aborted fetus was agreed with reports of Yirgu, (1991); Eshetu *et al*., (2008) from Ethiopia and Staak, (1990) from other country. This may be due more cases of human brucellosis occurred in rural areas where most of the people are farmers or in close contact with animals. The other possible explanation could be given from this finding is that both farmers, animal health personnel and farm government employees could be infected while helping infected cows during parturition either through abrasions or the conjunctiva, acquire infections by handling tissues containing *Brucella* organisms, and also can contracts brucellosis either by handling infected animals or by living with infected animal in similar house.

In the present study, strong association was observed between seropostivity and handling of parturient materials, drinking row milk, drinking raw milk from aborted animals and drinking raw milk from cow with RFM which is in agreement with other studies by Kassahun , 2004); Tadelle, (2004). The possible reason for this may be in our study area most of participants were drink raw

milk including raw milk from aborted and retained fetal membrane cow as it was assessed with interview question. The primary method of transmission of *Brucella* is through raw milk and contact with aborted materials.

The clinical manifestations pertaining to human brucellosis found in this study was similar to previous findings reported by Eshetu *et al*., (2008); Kassahun, (2004); Mussie, (2005). Apart from general clinical manifestations, participants with a history of testicular swelling had a statistically significant association with *Brucella* seroprevalence. This might be due the fact that the primary manifestation of brucellosis in man is chronic head ache at early stage and swelling of testicle due to Orchitis.

There was no statistically significant association between sexes even though high prevalence was seen in male. This finding was in agreement with Corbel, (1997); Mussie, (2005). All positive sera were from male participants. This may reflect cultural and social behavior patterns whereby the males are actively involved in caring for domestic animals in central high land areas. In this study, higher proportion of seropostivity was observed in age category of 31-45 years age, although the different age categories did not differ significantly. This finding was agreed with the finding of Haddad and Smith, (1986); Mussie, (2005); Kassahun *et al*., (2006). This difference might have been associated with the fact that in the study area age category that were between 31-45 years of age were responsible to handle aborted animals, milking of cows, and continuous contact with animals.

A total of 267 cattle owners and 17 government farm workers were interviewed to assess their awareness levels regarding animal management, brucellosis and occupational risks using structured interview question. Knowledge of diseases is a crucial step in the development of prevention and control measures (Prilutski, 2010). Despite huge efforts of the government and non-government institutions to improve animal production in the areas, general knowledge of brucellosis among the farmers was still poor. The educational status attained by majority of the respondents was low which falls between read and write and lower grades. In addition to this, barn hygiene, proper disposal of aborted materials and the use of a separate parturition pen were not well cooperated especially in small holder farmers. These could have led high risks of

transmitting the disease within and between the herds and human. This is in agreement with previous studies of Regassa *et al*., (2009); Megersa *et al*., (2011b); Adugna *et al*., (2013). Likewise, mixing of different animal species has its own economic importance by increasing the chances of transmission of brucellosis to the cattle.

Concerning knowledge of brucellosis and other zoonotic diseases, interview question based data were collected. The overall awareness level of brucellosis and other zoonotic diseases among small holder farmers, medium scale farm owners and government farm employees were found to be relatively low. In addition, most of them were not wear protective gloves or other material while handling aborted animals and aborted material. Similar finding was also reported by Tuli *et al*., (2017). This might be attributed to their educational status, since most of the farmers were could not read and write or primary education background. Additionally, it could also be due to a lack of awareness creation program about zoonotic diseases. Generally, a lack of sufficient knowledge of brucellosis and other zoonotic diseases, unprotected working conditions, regular exposure from aerosol and contact through cuts and abrasion to infected materials such as aborted materials, carcasses, viscera, organs, blood and urine are considered as fertile grounds for exposure and transmission of the diseases to humans. In this regard, very little has been done by way of awareness creation of brucellosis.

The result of knowledge assessment showed that brucellosis was not well known by the general community in the present study area, since around 93.3% of the study respondents had not information about brucellosis. This is similar to findings of previous studies done in Kenya, 80% of respondents were no knew of the existence of brucellosis whereas in Tajikistan 85% of the respondents had never heard of brucellosis Lindahl *et al*., (2015).

Most of the respondents from small holder dairy farms relatively did not understand the methods of zoonotic disease transmission including brucellosis. Farmer's lack of awareness about brucellosis, improper handling of aborted materials and the habit of consuming raw milk, among other factors, might contribute to further spread of brucellosis in their livestock and expose the community to a public health hazard (Megersa *et al.*, 2011b). This low awareness is a limiting factor if control strategies are to be implemented. Lack of knowledge on the causative agent,

mode of transmission and preventative measures against brucellosis can be detrimental. It is therefore important to establish an educational campaign in the study areas to enlighten the communities on the disease, risk factors as well as control strategies particularly in both livestock and humans.

Based on the questionnaire survey, most of the respondents were handling abortion and placental retention without using personal protectives (59.2%); even though using personal protectives are the most known measures against zoonotic brucellosis (Radostits *et al*., 2007). These factors combined with the poor hand cleaning practice after contact with aborted materials (91.2%) by the owners could pose a great risk of the spread of the disease to unaffected animals (Tolosa, 2004). In addition, most of the respondents in this study did not bury afterbirth (aborted fetus, still birth and retained fetal membrane) rather they left them on open field (79.2%).

Studies also assessed the occupational risks that, the majority of the participants in both types of farming systems have the habit of drinking raw milk, contact with aborted fetus and retained fetal membrane. This might be attributed to the culture and tradition of consuming raw milk and milk product and also the lack of knowledge on zoonotic disease via milk and aborted materials. The seroprevalence study research conducted in high risk group such as farmers (livestock owners), veterinary professionals, meat inspectors and artificial insemination technicians in Addis Ababa (Kassahun *et al*., 2006); Mussie *et al*., (2007) in Amhara Regional State found a seroprevalence of 5.30%, and 4.8% by screening sera from 238, and 336 individuals respectively.

Limitations of this research

Diagnosis of bovine brucellosis depends on isolation and identification of the causative agent or demonstration of specific antibody using serological tests. I cultured two vaginal swab samples taken from two recently aborted cows in *Brucella* laboratory of Addis Ababa University Institute of Pathobiology. Even though one of the samples showed the growth of the pathogen, I could not proceed to further processing for biochemical test and molecular detection of the colony due to CVID 19 (Annex 3). So, the suspected colony was preserved and the remaining biochemical and molecular tests are supposed to continue after the issue of this outbreak is resolved.

6. CONCLUSION AND RECOMMENDATIONS

The present study revealed that the overall seroprevalence of bovine brucellosis with recent abortion history and human brucellosis from animal owners and farm workers were 0.6 and 1.2% in Holeta towns, Wolmera District and Adea Berga EIAR dairy farm Oromia Regional State, Ethiopia. The finding of positive serological reactors did not only suggest the presence of the disease in the cattle population in the areas, but also indicated the presence of foci of infection that could serve as sources of infection for the spread of the disease into unaffected animals and humans. In this finding stage of abortion, retained fetal membrane, source of stock replacement, and breeding methods were statistically significant risk factors associated with dairy animal brucellosis seropositivity. On the other hand human housing, contact with aborted fetus and RFM, drinking row milk, drinking raw milk from aborted animals and drinking raw milk from cows with RFM were statistically important risk factors associated with human brucellosis seropositivity. This study also provided important information on knowledge, attitude and practice of livestock owners and occupational workers about brucellosis that result in significant zoonotic importance of using raw milk for human consumption. This emphasizes impact of brucellosis in animals, public health and the need to control and prevent brucellosis in the study areas.

Based on the above conclusions, the following recommendations are forwarded to curb further spread of the disease in both cattle and human populations:

- ✓ Isolation of aborted animals and proper disposal of aborted fetuses and fetal membranes, preferably, by incineration.
- ✓ Replacement stock should be purchased from herd known to be free of brucellosis.
- ✓ Strict movement control of animal from one area to another in order to prevent the spread and transmission of the disease from infected cattle to the non-infected ones.
- ✓ The implementation of test and slaughter policy with compensation payment to the farmers as the prevalence of the disease is low in the study area.
- ✓ Adoption of replacement stock vaccination with the aim of eradicating the diseases and prevention of its impact on the public and economic sector.

- ✓ Awareness creation among farmers, butchery men, abattoir workers and animal health workers about the nature and effect of the disease through formal and informal educational channels is required.

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8. ANNEXES

Annex 1: Rose Bengal Plate Test Procedures

Reagents:

- ✓ RBT *Brucella* antigen
- ✓ Positive control sera (from previously positive serum)
- ✓ Negative control sera (from previously negative serum)
- ✓ Test sera

Materials:

- ✓ Plate
- ✓ Micro pipette of 30 µl
- ✓ Micro pipette tips
- ✓ Applicator
- ✓ Magnifying glass
- ✓ Tube of serum collection
- ✓ Vacutainer tubes fitted with handle and needles
- ✓ Rack

Procedure

1. Sera (control and test sera) and antigen for use were left at room temperature for half an hour before testing; since active materials straight from the refrigerator react poorly. 30µl serum was mixed with 30 µl volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter.
2. The antigen and serum were mixed thoroughly using an applicator stick (a stick being used only once).
3. Rock plate by hand for about 4 minutes.
4. Examine for agglutination in a good light.
5. Use magnifying glass when micro agglutination suspected.

Interpretation:

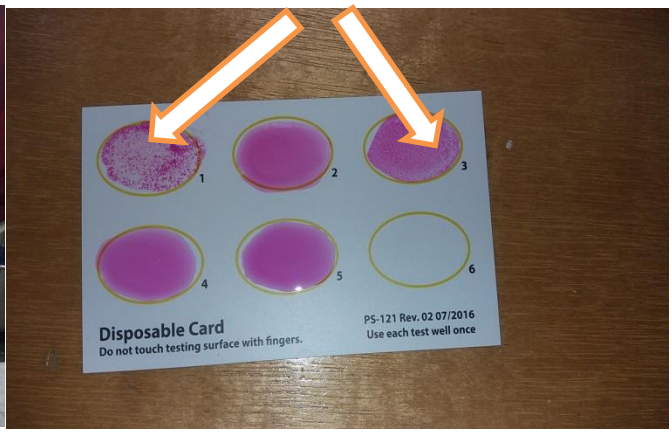
0 = no agglutination

+ = barely perceptible

++ = fine agglutination, some clearing

+++ = coarse clumping, definite clearing

RBPT Positive



Annex 2: Complement Fixation Test Procedures

In the CFT, all reagents were evaluated by titration. The preparation of sheep red blood cells (SRBC), the methods of CFT test, and preparation of reagents were according to the protocol of BgVV Service Laboratory (2000).

Reagents:

- ✓ Veronal buffered diluent (prepared by mixing 1vial of the constituents into 1 liter of distilled water (PH 7.25).
- ✓ Working strength antigen
- ✓ Complement
- ✓ Ambocepter
- ✓ Alsever's solution
- ✓ Positive control sera
- ✓ Negative control sera (from previously tested

Materials:

- ✓ Micro titer plate (U-shaped)
- ✓ Shaker housed in an incubator (37 ºC)
- ✓ 0-100 µl adjustable single channel pipette
- ✓ 25 µl multi-channel pipette
- ✓ 1000 µl adjustable single channel pipette

- ✓ Pipette tips
- ✓ Plastic troughs
- ✓ Traditional glass pipette
- ✓ Centrifuge tubes
- ✓ Measuring cylinders
- ✓ Glass and plastic beakers
- ✓ Water bath (58 ºC)
- ✓ Centrifuge
- ✓ Refrigerator
- ✓ Rack

i. Preparation of SRBC for hemolytic system

10 ml of SRBC in Alsever's solution were centrifuged at 2500 rpm for 5 minutes. The supernatant was discarded and replaced by veronal buffer diluents (VBD). The SRBC were re-suspended in the diluent and centrifuged again. This procedure was repeated 4 times. Before discarding the supernatant after the last washing, the packed cells volume was measured. The volume of the packed cells was read by placing an identical tube next to the blood containing tube and filled up to the level of the blood by a measured amount of water. Finally, a 2% suspension of SRBC was prepared.

ii. Amboceptor titration

1. Two rows of 5 test tubes each were arranged on a rack
2. In two other test tubes, 1: 500 and 1: 750 prediluted were mad
3. 1ml of 1: 500 prediluted amboceptor was transferred to the first test tube of row 1 and 1 ml of 1: 750 prediluted amboceptor was transferred to the first test tube of row 2
4. 0.5ml VBD was added to each of the rest of tubes of both rows
5. Amboceptor was then diluted serially from tube 1 to tube 5 in 0.5 ml amount in both rows. Thus the dilution ran from 1: 500 to 1: 8000 and 1: 750 to 1: 12000 in row 1 and row 2, respectively
6. To each tube of the two rows, 1ml of VBD was added
7. Following, 0.5 ml of 2 % SRBC was added to each test tubes of the two rows and were shacked well
8. The tube were left on the table for 10 minutes
9. 1 ml of complement at working dilution was added and incubated at 37 0C for 30 minutes
10. The last tube showing complete hemolysis, minimum hemolytic dose (MHD) was read. The working dilution of amboceptor is 4 times MHD (BgVV Service Laboratory, 2000)

iii. Evaluation of complement

1. Freeze dried complement was reconstituted according to its instructions
2. A 1: 100 complement dilution was prepared
3. Complement was added into 9 wells increasing by 5µl every time, starting with 10µl
4. Diluent was added into the 9 wells in decreasing amounts by 5 µl , starting with 40 µl
5. 25µl of a diluent was added into the wells with Cornwall syringe
6. The plate was placed in water bath at 37^0C for 1 hour
7. 25µl 2 % SRBC was added to all wells
8. 25µl amboceptor at working dilution 1:1000 was
9. added to all wells
10. Components were mixed by shaking and incubated again in water bath at 37 oC for 30 minutes

The test was read by recording minimum hemolytic dose of complement (MHD) which was represented by the first well showing complete hemolysis. The next well contains the full hemolytic dose (FHD). The working dilution of complement was then computed: complement dilution= 2FHD/ initial dilution of complement.

iv. Antigen titration

Micro titer plate I:

1. 25µl of VBD was first placed to every walls of a micro titer plate
2. 25µl of a pre diluted antigen was added to all wells of row A
3. By serial doubling (two fold) dilution 25µl of antigen was transferred from row A to B and from row B to C until row G by multi-channel pipette; 25µl mixture was discarded from row G

Micro titer plate II

1. 50µl VBD was added in all wells
2. 50µl of prediluted inactivated positive control serum was added to all wells of column 1
3. 50µl was serially transferred by two fold dilution, from column 1to column 2, and again

from column 2 to column 3, until column 11 from where 25 µl was discarded (column 12 had only VBD)

Mix plate I and II

1. 25µl was transferred from plate II to plate I
2. 25µl of complement in at working dilution (1:40) was added to all wells of plat I
3. Plate I was incubated 37^0C for 30 minutes (sealed)(warm fixation)
4. The following, 25µl of equal volumes of 2% SRBC and amboceptor (working dilution) pre-mixed were added to all wells
5. The plates were covered with sealing tape and shacked placed in an incubator (37^oC) for 30 minutes (warm fixation)

The interpretation was performed as follow:

The last wells with 50% sedimentation was read and recorded. This was regarded as the right corner value. In this case, the corner value was 1: 25 dilution and was used throughout the test.

The 50% sedimentation was taken as one unit and the working dilution of the antigen was two units.

Test procedure:

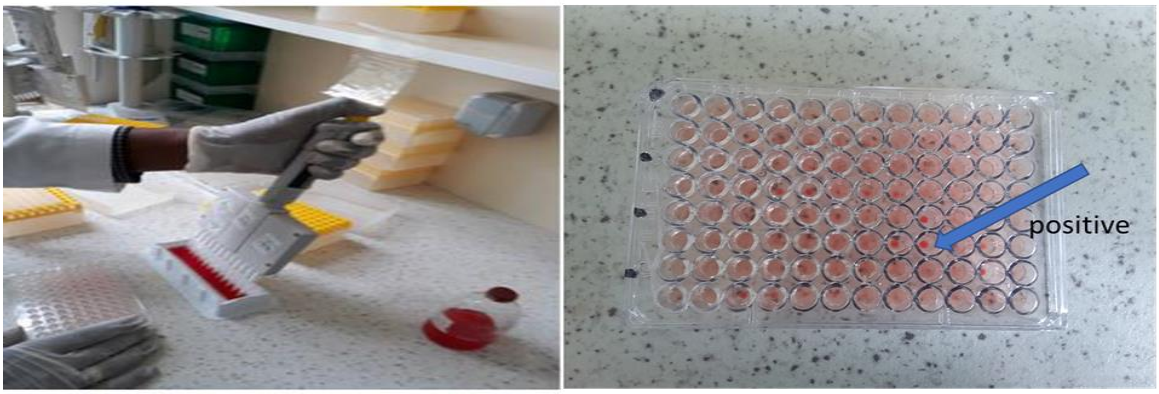
1. The sera were pre diluted at 1:2.5 and incubated at 58^oC in a water bath for 30 minutes in order to inactivate the native complement
2. 25µl of diluted test sera was placed in wells of first and second rows of U-bottom plate, and 25µl of veronal buffer was added to all wells except those of the first row
3. Serial doubling dilution were then made by transferring 25µl volumes of serum from 2nd row on wards continuing for at least four dilution
4. 25µl of antigen diluted to working dilution excluding those of anticomplemetary controls, which received 25µl VBD was added to all wells
5. 25µl of complement (1: 40 working dilution) in working dilution was added to all wells except control wells
6. Control wells containing: serum control has serum + complement + diluent + and antigen control has antigen + complement + diluent. Complement control has complement + diluent and hemolytic system has diluent set up to contain 75µl total volume in each case

before hemolytic system was added

7. The plates were incubated for 30 minutes at 37 ^{0}C with agitations (warm fixation)
8. 25µl of 2 % SRBC and amboceptor (hemolytic system) mixture was added into all the wells
9. Plates were sealed with sealing tape and placed on a shaker and incubator (37 ^{0}C) for 30 minutes
10. Before reading the result the plates were left in the refrigerator at +4^{o}C for one hour in order to allow non lysed cells to settle
11. Plates were taken out from refrigerator and results were read after being left on the table for 10 minutes at room temperature
12. Positive reactions were indicated by the absence of hemolysis, sedimentation of SRBC, and negative reactions by the hemolysis of SRBC

The interpretation was performed as follow:

Sera with at least 50% fixation of the complement at a dilution of 1:10 were taken as positive. A hemolytic reaction of 50% or less at a dilution of 1:5 was considered as the minimum sero-positive threshold (Dohoo *et al*., 1985).

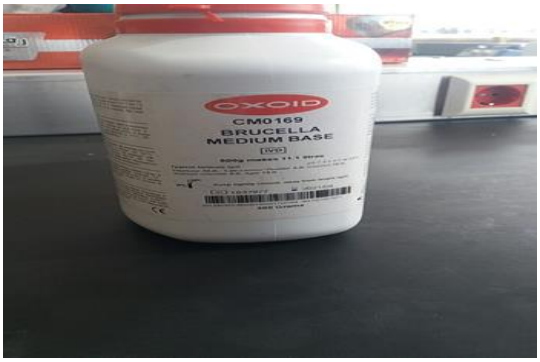


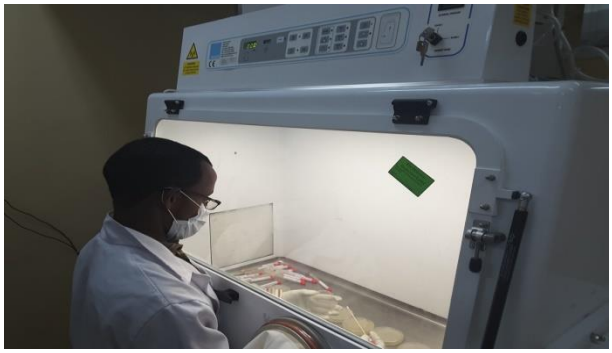
Annex 3: Preparation of *Brucella* medium agar

Procedure

Suspend 22.5 grams Brucella Agar Base in 500 ml distilled water and boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add sterile 5% v/v inactivated Horse Serum (inactivated by heating at 56°C for 30 minutes) and rehydrated contents of one vial of Brucella Selective Supplement (FD161) was added and the media is then poured in to sterile Glass petridishes.

Unconfirmed Brucella colony





Annex 4: Interview Question

Seroprevalence and isolation of *brucella* species from cattle and its public health significance in holeta and its surrounding, Oromia region, Ethiopia

Date_____/_____/_____

Code _____

Enumerator Name _____

I, General information

1. Name of respondent_____

1.1. Age: _____1.2. Sex: _____1.3 Address----------

1.4. Educational background a) Primary school, c) College/University,

b) Secondary school, d) nonacademic/informal, e) Illiterate

II. Response of Respondent on Livestock Management

1. Type of farming; A) Intensive, B) Semi-intensive, C) Extensive

A) Dairy only, B) beef, C)crop-livestock mixed, D)other

2. Herd size: a) <10 b) 10-20 c)>20

3. Breed: A) Local, B) Cross breeds

4. Source of replacement A) From local market B) From governmental farms, C, rise own animal

5. What is parity status?

Identity of the cows	Parity status	Calving interval

6. What type of insemination is most commonly used in the farm? a) AI b) Natural C) both

III. Husbandry Practice

1. Type of housing a) separate, b) common

2. Human housing

A) Housed with family and livestock

B) Housed in family without livestock

3. General Hygiene of the House: A) Very good, B) Good C), Satisfactory D) Poor

4. Do you separate cows during parturition? Yes/ No

5. What are the commonly encountered disease affecting cattle in order of importance?

A._____ C._____

B_____ D_____

6. Do you know disease that causes abortion in cattle? Yes/No

7. What are the names of the disease locally?

A)._____

B)_____

C)_____

8. Was any event of abortion, still birth and retained fetal membrane in your farm? yes/no

Problems	Age	Time since it occur
Abortion		
Still birth		
Retained fetal membrane		

11. Do you know disease Brucellosis A) yes, B) no

10. Do you have direct contact with aborted/still birth/RFM?

11. If yes for Q10, how do you make a contact? A) Wear glove, B) Wear plastic, C) Bare hand

12. If yes for Q10, do you wash your hand? A) Yes, B) No

13. Where do you dispose the aborted/still birth/RFM birth or retain placenta?

A) Burying B) Open dump C) Fed to Dogs D) Through over the field, E) Other_____

14. How do you dispose the aborted fetus? A) Using protective B) Bare hand

15. What are the reasons of culling in the farm? A) Disease, B) Old age, C) Infertility,

D) Poor production, E) Others

16. Do you know any disease transmitted from animal to human through handling of infected animals and its products? a. Yes b. No _____

17. Do you know any zoonotic diseases that transmit through milk consumption a. Yes b. No

_____

18. Do you know any diseases that transmit during handling of delivery or abortion? a. Yes b. No

_____

19. Have the farm/ herd been tested for brucellosis? a. Yes b. No. When_____

20. What do you do with the known *Brucella* infected Animals? A). Segregation, A) Left with herd, B) Slaughter, D. Both

21. What do you do to the calving pen after parturition? A) Flushing with water, B) Disinfecting with detergents, C) Both, D,non

22. What do you do for the milk from aborted/still birth caw? A) Drink without boiling, B) Boiling, C) Did not drink

Annex 5: Format used for individual human sampling

No/	Farm/owner Name	Age	Sex	Abortion	Chronic headache	Orchitis	Knee pain	Sample type	Test type	Result

Annex 6: Format used for individual Animal sampling

No/	Farm/owner Name	Age	Parity	Abortion	Still birth	RFM	MGT system	Sample type	Test type	Result

Annex 7: Ethical clearance certificate for animal

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/10/01/12/2020

Name of Applicant: Temesgen Kassa (DVM, MVSc fellow)

Address: College of Veterinary Medicine and Agriculture (Addis Ababa University)

Title of the project: *Seroprevalence and isolation of Brucella species from cattle and its public health significance in Wolmera and Adda Berga districts, Oromia Region, Ethiopia*

Date of application:	**15/10/2019**
Nature of the project:	**mildly invasive**
Target animal species:	**cattle**
Number of animals involved:	**384**
Study area:	**Wolmera and Adda Berga districts, Ethiopia**

Minutes No. and date of review: **VM/ERC/01/12/020, 03/01/2020**

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is ethically sound to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when this is deemed necessary
3. A separate clearance is required for any work (except questionnaire) on human subjects

Dr Getachew Terefe
Chairman

Signature

The Federal Democratic Republic of Ethiopia

መልስን በሚጽፉልን ጊዜ ከላይ የሚገኘውን የደብዳቤ ቁጥር ይጥቀሱልን
Please quote Our Ref. No. when replying

ፋክስ	ስልክ	ፖ.ሣ.ቁ	ቢሾፍቱ/ደብረዘይት፣ ኢትዮጵያ
Fax 251-11-4339933	Tel +251 114338450	P.o.x. Box}34	Bishoftu/Debre Zeit, Ethiopia

Annex 8: Ethical clearance certificate for human

Biiroo Eegumsa Fayyaa Oromiyaa
Oromia Health Bureau
Saarbet (Calcalii) - Finfinnee

የኦሮሚያ ጤና ጥበቃ ቢሮ
ሳርቤት(ጨሰጨለ) - ፊንፊኔ

Lakk/Ref.NO BEFO/HBTQM/1468/2014
Guyyaa/Date 24/5/2014

W/E/F/God/Shavaa Licaa tiif
W/E/F/Bul/Mag/Holataa tiif
W/E/F/God/ Ad/Naan/ Finfinnee tiif

Bakka jirtanitti

Dhimmi: Xalayya Deggersa Ilaala.

Akkuma beekamu Biiroon Keenya Ogeyyii, dhabbile akkasumas ramoota qorannoo geggessuuf propozaala dhiyeffatan propoozaala isaanii madaaluun akkasumas iddo biratti ilaalchisani fudhatama argatan (approved) dhiyeffatan, propoozaala isaanii ilaaluudhaan waraqa deggersa ni kenna. Haaluma kanaan mata dure''**SEROPREVALENCE AND ISOLATION OF BRUCELLA SPECIES FROM CATTLE AND ITS PUBLIC HEALTH SIGNIFICANCE IN WOLMERA AND ADDA BERGA DISTRICT, OROMIA REGION, ETHIOPIA**''Jedhamu irratti ''**Dr.Temesgen Kassa**'' aanaalee Walmaraa fi Ada'aa Bargaa keessatti qorannoo geggessuuf propoozaala isaanii koree "Health Research Ethical Review Commite' Biiroo keenyatti dhiyeffatani jiru. Haaluma kanaan koreen "Health Research Ethical Review Committee" Biiroo keenyaa piropoozaal kana ilaaluun mirkaneessee qorannoon Kun akka hojii irra oolu murteessee jira.

Kanaafuu, hojji qorannoo kana irratti deggersa barbaachisa ta'e gootaniifii hordoftan jechaa, ''**Dr.Temesgen Kassa**''qorannoon Kun qaceffamee eerga xumuramee booda firii isaa koppii tokko BEFO tiif akka galii godhan galagalcha xalayaa kanaan isaan beeksifna.

Haaluma kanaan anis''**Dr.Temesgen Kassa**'' wayitti qorannoon Kun qaceffame xumuramu firii isaa koppii tokko BEFO tiif gaii gochuuf mallattoo kootiin nimirkanessa.

Nagaa wajjin!

Maqaa ''**Dr.Temesgen Kassa** ''
Mallattoo_____
Bilbila 0911199867
G/G
'' Dr.Temesgen Kassa'' tiif
Finfinnee

Oromia Regional Health Bureau Emergency Management & Health Research directorate

Gammachuu Shuumii
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