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SEROPREVALENCE OF BRUCELLOSIS IN LIVESTOCK AND HUMAN IN ASAYITA AND MILLE DISTRICTS OF AFAR REGION, ETHIOPIA

MSc THESIS

BY

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SEROPREVALENCE OF BRUCELLOSIS IN LIVESTOCK AND HUMAN IN ASAYITA AND MILLE DISTRICTS OF AFAR REGION, ETHIOPIA

A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Masters of Veterinary Science in Veterinary Microbiology

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STATEMENT OF THE AUTHOR

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

AHRI	Armauer Hansen Research Institutes
ANRS	Afar National Regional State
^{0}C	Degree Centigrade
CC	conjugate control
CFT	Complement fixation Test
CSA	Central Statistics Authority
CIA	Central Intelligence Authority
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immune sorbentAssays
EPHC	Ethiopian population and housing census
FAO	Food and Agriculture organization
µL	Micro Litter
MM	Millimeter
PA	peasant association
PCR	Polymerase chain reaction
PFE	Pastoralist Forum Ethiopia
PBS	Phosphate buffer saline
RFLP	Restriction fragment length polymorphism
RT PC	Real-time PCR
SNNPS	South Nation National people State
WHO	World Health organization

ABSTRACT

Brucellosis is one of the neglected infectious zoonotic diseaseswith a serious public health importance worldwide. In pastoralist communities of Ethiopia, brucellosis is a major public health problem and has serious economic impact on livestock sectors. A cross-sectional study was conducted from November 2017 to April 2018 in one-health approach to estimate he sero prevalence and associated risk factors of brucellosis in livestock (cattle, camel, sheep and goats) and human in Mille and Asayita districts, Afar Region. A total of 562 livestock (goats, sheep, cattle and camel) and 172 human sera from pastoralists who own these livestock were tested using indirect/competitive ELISA. Based on the result, the overall seroprevalence of brucellosis in livestock (small ruminants, cattle and camel) was 9.07% [95% CI=6.6-11.45]. The highest seroprevalence was recorded in cattle 16.67% [95%CI:9.3-24] while the lowest seroprevalence was observed in camels 3.7% [95%CI: 0.4-7.9]. The differences in sero positivity between livestock species in study area were statistically significant (p=0.014). Other risk factors (districts and sex) did not show a statistically significant difference between the categories (p>0.05). On the other hand, the overall sero prevalence of human brucellosis in the pastoralist livestock owner was 48.8% [95%CI: 41.2- 56.3] using indirect ELISA test. Higher human brucellosis sero prevalence was recorded in Mille district (63.1%) than Asayita (35.2%).

Key words: *Afar, Brucellosis, ELISA, Pastoralists, Livestock*

1. INTRODUCTION

Brucellosis is an infectious disease of domestic and wild animals, with serious zoonotic implication in livestock. The disease is an important public health problem in many parts of the world. Cattle, goats, pigs, sheep, horses, and dogs play an important role in the transmission of brucellosis to man (Akbarmehr and Ghiyamirad, 2011).

Brucellosis is a public health problem in developing countries with adverse health implications both for animals and human beings as well as economic implications for individuals and communities. It is an occupational hazard with those particularly at risk such as laboratory workers, veterinarians, abattoir workers, farmers and animal keepers either living in close proximity with animals or handling aborted fetus and animal products that contaminated by *Brucella* agents(Radostits*et al*., 2000, FAO *et al*., 2006, Jim *et al*., 2012).

Brucellosis affects people of all age groups and of both sexes. The annual occurrence of human brucellosis in the world is more than 500,000 cases (Corbel, 2006; Pappas *et al*., 2006; Donev, *et al.*, 2010).

Millions of individuals are at risk worldwide, especially in countries where infection in animals has not been brought under control, procedures for heat treatment of milk such as pasteurization are not routinely applied, and standards of hygiene in animal husbandry are low. It has a considerable impact on animals and humans health, as well as wide socio-economic impacts especially incountries in which rural income relies largely on livestock breeding and dairy products (Gul& Khan, 2007).

Small ruminant brucellosis caused by *Brucella melitensis* occurs naturally in sheep and goats and is highly pathogenic for humans, causing one of the most serious zoonosis in the world. The disease is responsible for considerable economic losses to the small ruminant industry (Benkirane 2006, OIE 2009). Sheep and goats brucellosis is endemic in most countries of the Mediterranean basin, the Middle East and Central Asia (Al-Majali*et al*., 2005), Latin America, and parts of Africa (Benkirane *et al.,* 2006)

Teshale et al. (2006) investigated sero prevalence of brucellosis in 1442 camels in arid and semi-arid camel-rearing regions (Afar, Somali and Borena) of Ethiopia. The sero prevalence of small ruminant bucellosis reported elsewhere in Ethiopia including 12.35% reported in Afar region (Anteneh *et al*., 2014)

The overall sero prevalence of brucellosis in small ruminants that had history of recent abortion 12.4% and 7.52% with m RBPT and CFT, respectively. Was reported in Amihbara district, Afar Region (Muluken T, 2016)

Since then, brucellosis prevalence studies have been conducted in district of the region based on sero prevalence studies. But, until to date there is information gap to estimate sero prevalence of livestock and human brucellosis in afar region and not clearly known. There is no research done to get evidence by sero prevalence of livestock and human brucellosis

Therefore, thegeneralandspecificobjectives of this study were:

General objectives:

- Epidemiology and zoonatic important of brucellosis in Mille and Asayita districts of Afar region

. Specific objectives:

- To determine seroprevalence of livestock and human brucellosis in Asayita and Mille districts of Afar region
- To identify risk factors associated with seroprevalence of livestock and human brucellosis in the study area.

2. **LITERATURE REVIEW**

2.1. Etiology

The etiological agent of brucellosis is a bacterium of the genus *Brucella.* Currently ten species are recognized including the better known six classical species comprised *of Brucella (B)abortus, B. melitensis, B. suis, B. ovis, B. canis and B. neotomae.* More recently, new members to the genus include *B. Ceti and B. pinnipedialis, B. microti and B. Inopinata* (Godfroid *et al.,* 2011).

The Genus of *Brucella* are subdivided into six species categorized by antigenic variation and primary preferred host and these include *B. abortus, B. melitensis, B. suis, B. ovis, B. Canis and B. neotomae* (MorganandMacKinnon, 1979; Halling and Young, 1994).*)*. The ability of genus *Brucella* to replicate and persist in host cells is directly associated with its capacity to cause

persistent disease and to circumvent innate and adaptive immunity. There are different species of *Brucella* organism that cause disease in different animal species and humans. A single species can cause disease in different animal species and humans, which means it has a range of hosts (FAO, 2003).

Some *brucella* specie like *B. abortus, B. melitensis, B. Suis* an*d B. Can is* can affect a ranges of hosts in addition to their natural hosts resulting hazards on the health of animals including humans; due to this, infected countries are challenged and have been under difficulties to overcome or control brucellosis effectively. In addition to cattle, *B. Abortus* can affect other animals like sheep, goats, horses, camels, swine, dogs and humans. *B. Melitensis* also affects other animals like sheep, horses, swine, camels, dogs and humans. *B. Suis* also affects different animal species such as cattle, sheep, goats, dogs, camels, horses and humans.*B.ovis* affects only ovine while *B.canis* affects dogs and humans (FAO *et al.,* 2006).

2.2. Taxonomy and Classification of Brucella

The genus Brucella reside*s* within the family *Brucellaceae* order*Rhizobiales*, class *Alpha prote bacteria* and phylum *Proteo bacteria*. The *Proteo bacteria* are a major phylum of bacteria, which include a wide variety of pathogens, such as *Escherichia*, *Salmonella*, *Vibrio, Helicobactelr*. All *proteobacteria* are Gram-negative, with an outer membrane mainly composed of lipopolysaccharides (Bergey*et al.,* 1994)

3. EPIDEMIOLOGY

3.1 Global distribution

Brucellosis occurs worldwide, except in those countries where bovine brucellosis (*B. abortus*) has been eradicated. This is defined as the absence of any reported cases for at least five years. These countries include Australia, Canada, Cyprus, Denmark, Finland, The Netherlands, New Zealand, Norway, Sweden and the United Kingdom. The Mediterranean Countries of Europe, northern and eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America are still not brucellosis free (Pappas*etal*.,2006) 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).

3.2. Distribution in Africa

Brucellosis exists throughout sub-Saharan Africa Brucellosis occurs worldwide and 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). Also it is considered as a remerging problem in many countries such as Israel, Kuwait, Saudi Arabia, Brazil and Colombia, where there is an increasing incidence of *Brucella melitensis* or *Brucella suis* biovar1 infection in cattle (Cutler et al., 2005). According to WHO (1997) *B. melitensis is* considered to have the highest zoonotic potential, followed by *B. abortus, and B. suis* on those endemic regions. Most African countries are of poor socioeconomic status, with people living with and by their livestock, while health networks and surveillance and vaccination programmes are virtually non-existent. Moreover, there are far more morbid endemic infectious diseases, particularly malaria. Most febrile patients in these countries are initially empirically diagnosed as suffering from malaria, and only a small part of non-responders may be further tested for

brucellosis. Most of the data are derived from small sero epidemiological studies of patients with fever or high-risk populations (McDermott and Arimi, 2002).

According to data from OIE for 2004, Cameroon, Ethiopia, Kenya, Nigeria, Tanzania, and Uganda reported the existence of human cases of brucellosis, while in 2003 similar reports indicated that Ghana, Togo, and Chad are probably also endemic according to sero epidemiological studies (Schelling *et al*., 2003).

3.3. Occurrence of Brucellosis in the pastoral area of Ethiopia

In Ethiopia, the pastoralist and agro-pastoralist areas such as Borena, Afar and Somali are considered the traditional source of livestock, supplying 95% of livestock destined for the export market (Jemberu E. 2004).

The pastoral human population of Ethiopia makes up approximately 13.7% of the country's total population of 93.8 million (CIA, 2013 and PFE 2009). The pastoral population is heterogeneous in its ethnic composition and social structure. Livestock production, trading and 'take-a-chance' crop farming (subsistence rain-fed farming) are the pastoral livelihood systems (PFE, 2009).

The pastoral and agro-pastoral production system represent approximately 45-55% of the cattle, 75% of the small ruminants, 20% of the equines and 100% of the camels of the total national livestock population. The main mobile pastoralists in Ethiopia are the Somalis (Somali region) in the east, the Afars (Afar region) in the northeast, the BorenaOromos (Oromiya region) in the south and south-east and the Southern Omo people (SNNPS region) in the south and partly in the

Gambela and Benishangul regions and around the Dire Dawa Administration. Despite the large size of the regional livestock population, its economic contribution to the regional and national economy is not significant, mostly due to natural and human limitations (Amaha, 2006).

Goats and sheep are important domestic animals in tropical livestock production systems (Devendra and McLeroy, 1990), accounting for 21% of the global small ruminant population. According to statistics from the Central Statistical Agency (CSA, 2014), Ethiopia has over 27 million head of sheep and 28 million goats (CSA, 2014). 25% of the sheep and 73% of the national goat population inhabit the lowlands (mostly pastoral areas). Also according to Pastoralist Forum Ethiopia (PFE, 2004), most goat populations in Ethiopia are raised under pastoral communities. These small ruminants and their milk/meat products represent an important export commodity, which significantly contributes to the national economy.

Through limited sero-surveillances carried out so far indicated that, brucellosis may be one of the important diseases in goat raising community. A sero-surveillance study carried out in small ruminants in Afar and Somali Regions in 2005, clearly demonstrated that the disease exists in Ethiopia. The sero-surveillance findings were 14.6% in sheep, 16.2% in goats in Afar Region and 1.6% in sheep, 1.7% in goats in Somali Region (Yibeltal, 2005). In another work done in the central highlands of Ethiopia a sero-prevalence of 1.5% in sheep and 1.3% in goats is recorded (Tekeleye and kasali, 1990).

Animal brucellosis constitutes significant public health importance for a pastoral community where close intimacy with animals, raw milk consumption and low awareness on zoonoses

facilitate zoonotic transmission of the disease. Milk is a major staple food, and is an important source of protein and vitamins for households. Raw milk, which is the mode by which almost all the pastoral community consume it, is also a source of infection with milk-borne zoonoses such as brucellosis (Schelling, *et al*., 2003)

4. Diagnosis

For the diagnosis of brucellosis, the organism may be recovered from a variety of materials which usually depends on the presenting clinical signs (OIE, 2009). In animals, the placenta is the most infective and contains the greatest concentration of bacteria; this is followed by the lymph nodes and milk; and from blood in humans (Poiester *et al*., 2010). Furthermore, other materials rich in the organism include: Stomach contents, spleen and lungs from aborted foetuses, vaginal swabs, semen, and arthritis or hygroma fluids from adult animals. From animal carcasses, the preferred tissues for culture are the mammary gland, supra mammary, medial and internal iliac, retropharyngeal, parotid and pre scapular lymph nodes and spleen (OIE, 2009). All specimens must be packed separately, cooled and transported immediately to the laboratory in leak proof containers. The samples should be frozen until required for culture (OIE, 2009).

Definitive diagnosis of brucellosis is based on culture, serologic techniques or both. Presumptive evidence of brucellosis is provided by the demonstration by modified acid-fast staining of organisms of *Brucella* in abortion material or vaginal discharge, especially if supported by serological tests. Whenever possible, *Brucella* species should be isolated using plain or selective media by culture from uterine discharges, aborted fetuses, udder secretions or selected tissues, such as lymph nodes and male and female reproductive organs. Species and biovars should be identified by phage lysis, and by cultural, biochemical and serological criteria. Polymerase chain

reaction (PCR) can provide both a complementary and bio typing method based on specific genomic sequences (Alton *et al*., 1988; OIE, 2009).

4.1. Serological Diagnosis

Despite the development of numerous serological tests, no single test identifies all infected animals and a wide variation exists in estimates of their diagnostic accuracy (Adone and Pasquali, 2013; Abernethy et al., 2012). The current serological tests used for the diagnosis of *B. melitensis and B. ovis* in sheep and goats were initially developed for the diagnosis of *B. abortus* in cattle (OIE, 2012). Although not formally validated for use in sheep and goats, these tests, especially RBPT, CFT and more recently ELISA, have been widely used for the serological diagnosis of brucellosis in sheep and goats (Macmillan, 1990; Farina, 1985). They are also the official tests for international trade (European Commission, 2001; OIE Collective Manual, 2004).

Serological tests cannot differentiate between *Brucella* species and cannot therefore identify which species has induced host antibodies. Therefore, only isolation of the species or specific DNA detection by polymerase chain reaction (PCR), allows identification of the infecting strain (Godfroid*et al*., 2010; Plumb, 2013).

4.1.1. Rose Bengal plate test (RBPT)

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

agglutinins of bovine serum was inhibited whereas the activity of specific *Brucella* antibodies was not affected. Despite the scanty and sometimes conflicting information available (Alton, 1990), this test is internationally acknowledged as the test of choice for the screening of brucellosis in cattle as well as in small ruminants (Garin and Blasco, 2004; WHO, 2006).

However, the standardization conditions suitable for diagnosing cattle infection (European Commission, 2001; Garin and Blasco, 2004) are not adequate in sheep and goats and account for the low sensitivity of RBPT in small runinants. If the antigen is standardized differently, to give a higher analytical sensitivity, the diagnostic sensitivity to B. melitensis infection will be improved. 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 (Macmillan, 1990).

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

4.1.2. Enzyme linked immune sorbentAssays (ELISA)

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

4.1.3. Complement fixation test (CFT)

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

4.1.4. Milk ring test

The milk ring test is based on agglutination of antibodies secreted into the milk. This test allows screening of large number of cattle by using milk samples from tanks or pools from several cows. This test is useful for monitoring cattle herds or areas free of brucellosis so it is classified as surveillance or monitoring test (OIE, 2009a). Importantly, the number of false positive results is proportional to the number of cows secreting acidic milk due to colostrum's or mastitis (OIE, 2009).

4.2. Molecular typing

Despite the high degree of DNA homology within the genus *Brucella*, several molecular methods, including PCR, PCR restriction fragment length polymorphism (RFLP) and Southern blot, have been developed that allow, to a certain extent, differentiation between *Brucella* species and some of their biovars (OIE, 2009).

4.2.1. Multiplex PCR assay (Bruce-ladder)

Brucella bio typing and distinguishing vaccine strains by PCR can be accomplished satisfactorily but there has been limited validation of the PCR for primary diagnosis. The first species-specific multiplex PCR assay for the differentiation of *Brucell*a was described by Bricker &Halling. The assay, named AMOS-PCR, was based on the polymorphism arising from species-specific localisation of the insertion sequence IS711 in the *Brucella* chromosome, and comprised five oligonucleotide primers that can identify without differentiating *B. abortus, biovars*1, 2 and 4 but could not identify *biovars*3, 5, 6 and 9. Modifications to the assay have been introduced over

time to improve performance, and additional strain-specific primers were incorporated for identification of the *B. Abortus* vaccine strains, and other *biovars* and species (OIE, 2009).

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

4.2.2. Classical PCR

In Molecular technology like polymerase chain reaction (PCR) is a new approach and applied in many diagnostic works to overcome limitations and difficulties of bacterial culture and serological assays. In many works carried out PCR show high sensitivity, specificity and overcame the extraneous intervention of mimicry antibodies from sources other than actual infection. Antigen detection by use of primers, derived from the *OMP 31* gene sequence of the *B.melitensis* was developed successfully to diagnose from goat milk samples López et al., 2011).

4.2.3. Real- Time –PCR (RT PCR)

Real-time PCR is a valuable technique in quantification of nucleic acids in individual blood samples. It is highly reproducible, rapid, sensitive and specific (Wang Y, *et al*.,

2014). reported that the sensitivity of a SYBR Green I Light Cycler-based real-time PCR assay with serum samples was 93.3%, which is higher than 90% and 65% obtained by PCR-ELISA with whole blood samples and blood cultures, respectively (Queipo-Ortuno*et al.,* 2005).

The analytical sensitivity can be further increased by using real-time PCR assays, which can detect as few as five bacteria per reaction (Al Dahouk, *et al*., 2005; Navarro *et al*., 2006). Moreover, real-time PCR enables high-throughput screening of clinical samples and delivers results within a few hours. The *bcsp31* gene, coding for a 31-kDa immunogenic outer membrane protein conserved among all Brucella spp., is the most common molecular target in clinical applications (Baily, *et al*., 1992).Such a genus-specific PCR can help to avoid false-negative results in patients infected with unusual species and biovar.

4.2.4. DNA Sequencing

A primary molecular diagnosis must always be confirmed using a second gene target (Al Dahouk, *et al*., 2005). For confirmation and distinction from closely related microorganisms, 16S rRNA gene sequencing can be used (López-Goñi ,2008).Differential PCR assays, e.g. the conventional Bruce-ladder PCR (López-Goñi, 2011, López-Goñi , 2008, Mayer Scholl, 2011). Species specific real time PCRassays (Al Dahouk *et al*., 2007). It can also be used for confirmatory identification and differentiation of *Brucella specie.* Sub typing at the strain level can be useful for differentiating re-exposure from relapse (Al Dahouk,*et al*., 2005)

5. MATERIALS AND METHODS

5.1. Study area

Afar National Regional State is one of the 9 regions of Ethiopia and geographically located in the north-east of the country between 39°34' and 42°28' east longitude and 8°49' and 14°30' north latitude. The total area of the region is about 270,000 km^{2}. The regionshares common international boundaries in the North-East with Eritrea and in the east with Djibouti, as well as regional boundaries in the North-West with the Regional States of Tigray, in the South-West with Amhara, in the South with Oromia Regional State and in the South-East with Somali Region of Ethiopia (CSA, 2014).Afar National Regional State is characterized by an arid and semi-arid climate with low anderratic rainfall. The altitude of the region ranges from 120 m below sea level to 1500 m abovesea level. Temperatures vary from 20°C in higher elevations to 48C^{o}in lower elevations.Rainfall is bi-modal throughout the region with a mean annual rainfall below 500 mm in thesemi-arid western escarpments and decreasing to 150 mm in the arid zones to the east. The production system of the Afar region is dominated bypastoralism (90%) from which agro-pastoralism (10%). The region hasa total population of 1.5 million and administratively, divided into five zones, which arefurther subdivided into 32 districts and 358 pastoral associations (CSA, 2014).

The present study was conducted in two districts namely Mille and Asayita district which are located in Zone one of Afar National Regional State (ANRS) from November,2017 to April, 2018 (Figure 1).

Asayita also known as **Aussa**, is a district in north eastern Ethiopia. Aysaita district is one of 32 districts in Afar region and is about 655 Km far away from the capital city Addis Ababa and 65

kms north east of the capital city of Afar region, Samara, with the latitude and longitude of 11°34′N and 41°26′E, respectively. It has an elevation of 300 metres above sea level. Asayita is bordered on the south by Afambo district, on the west by Dubti district, on the north by the Awash River which separates it from Elidar district, and on the east by Djibouti. The climate of Asayita is characterize by high temperatures (reaching up to 43^{0}C) and low average rainfall of about 561mm on the western edge of the escarpment and 225mm on the lava plain and volcanic ash, where only camels and goats can be sustained in the fragile pastoral existence(Teshome *et al.*, 2003). The human population of Asayita is 65, 929 (CSA, 2004) and the district has 71383 cattle, 16943 sheep, 23086 goats, and 3277 camels (CSA, 2004). About 91.7% of population lives in rural area and pastorals and agro-pastoral system of livestock production is the dominant livelihood source.

Mille is one of the districts in the Afar Region of Ethiopia and Mille is bordered on the south by Gewane district, on the southwest by Telallak district, on the west by the Amhara Region, on the northwest by Chifra district, on the northeast by Dubti district, and on the southeast by the Somali Region.The human population of Mille is 84,407 (CSA, 2004) and the district has 135924 cattle, 248516 sheep, 304005 goats, and 65549 camels (CSA, 2004). More than 90% of populations in Mille district are pastoralist (Afar Afar Region Finance and Economy Bureau (ARFEB) (2007)

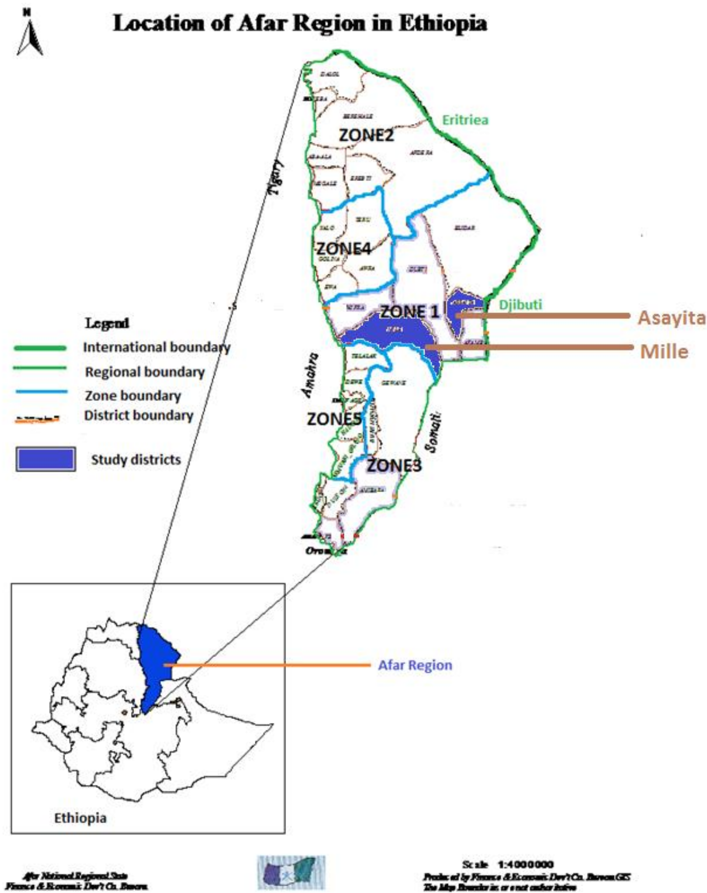

Figure 1. Map of study districts

5.2. Study subjects

The study population in this study were pastoralists who own livestock and indigenous Afar goat, sheep,cattleand camel owned by the pastoralists. For human participants, all age group above 7years old and both sex were included. For Ovine and caprine, small ruminants above 6 months of age and of both sex were included in the study. For cattle and camel, animals above the age of 3years and of both sexes were included in the study. Age, species, sex, and district of

the study population were recorded during the sampling. A total of 172 serum samples were collected from human of which 88 samples were from Asayita while 84 of them were from Mille. The study animals comprise a total of 562 heads oflivestockof which 317 were goat, 62 were sheep, 102 were cattle and 81 were camel from both Asayita and Mille districts.

5.3. Study design and sampling strategies

A cross-sectional study design was implemented from November 2017 to April 2018 to determine the sero-prevalence of brucellosis in sheep, goats, cattle and camel and their owners from the two selected study districts; Asayita and Mille. Two districts were selected purposively based on easier accessibility as well as ovine, caprine, cattle and camel populations. A multistage random sampling method were used in this study and pastoral villages were considered as primary unit, the herds as secondary units and individual animals as tertiary units. Similarly for human study, the pastoral village, the households and individual livestock owner were considered as primary, secondary and tertiary units. The numbers of animals included in the study were distributed proportionally over the pastoralist villages depending on the population of study subjects. Based on this, a total of 562 serum samples were collected from livestock among which 404 serum samples are from Mille, while 158 samples are from Asayita. On the other hand, 172 serum samples were also collected from humans of whom 88 samples were from Asayita while 84 of them were from Mille.

5.4. Sample size determination and sampling method

Multistage sampling technique will be used according to Dohoo *et al*. (2003) in the survey of livestock brucellosis (sheep goat, cattle and camel) brucellosis. The peasant association (PA) will

be considered as primary unit, the herds as secondary units and individual animals as tertiary units. Sheep and goat herd in 12 PAs from districts were sampled during the study based on the sheep, goats and cattle population of the district. In order to determine the desired sample size, there were no previous reports of prevalence in the districts. Therefore the average expected prevalence rate will be assumed to be 50% for the area within 95% confidence intervals (CI) at 5% desired accuracy as stated by Thrusfield (2007) formula:

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

Where: n= required sample size,

P_{exp}= expected prevalence

d^2= Desired absolute precision

Based on the above formula, the sample size to be selected is 384. But to increase precision serum sample were collected from a total sample of 562 animals.

Humansampling

Household members in those household where livestock had been tested were included in the study in order to assess association between animal and human brucellosis. Household members (at least 7 years old) were included if consent for enrolment is received. Household members ≥18 years of age provided their own consent, while both parent/guardian and the minor provided informed consent in order to participate when the participant is 7 to 18 years of age. A total of

172 human study subjects (88 from Asayita and 84 Mille district) were selected based on their willingness to participate and gave consent. All participants either own a minimum of seven heads of livestock or member of a pastoralists family who own livestock in the selected household.

5.5. Methodology

5.5. 1. Blood sample collection

Approximately 7 ml of blood from jugular vein of cattle and camel, about 5ml of blood from jugular vein of sheep and goat was collected aseptically using sterile plain vacutainer tubes based on the consent of the owner. In case humans, about 7ml of blood was collected from each individual if he/she was adult and only 4ml of blood sample from young aged individual with strict aseptic techniques from median cubital vein by using sterile syringe after properly disinfecting the site of vein puncturing after verbal agreement . The blood sample from human being was taken by Nurse located in each district clinic. Each sample was individually coded and identified by district, village, species, and age of the study animals. Collected blood samples were kept and transported by cold chain (ICE -box) to Semera regional laboratory where they were slanted and allowed to stay at room temperature overnight. After the period of 24 hrs the sera were separated stored at –20 ^{0}C. Then the sample was transport to Addis Ababa AHRI laboratory. After arrival at AHRI my first activity was self-introduction with immunology department which is situated in the AHRI and later the sample was submitted.

5.5.2.Enzyme linked immunosorbent Assay (ELISA)

After submission of samples to AHRI, two type of serological test were performed. Serum samples from human was tested using human IgG- based ELISA(SVANOVIR®), serum

samples from small ruminants and camel were tested using competitive ELISA ((SVANOVIR®), while serum samples from cattle were tested indirect ELISA test(SVANOVIR®) based on the manufacturer's instructions. The indirect ELISA detects the presence of antibody in a sample. The antigen for which the sample must be analysed is adhered to the wells of the microtiter plate. The primary antibody present in the sample bind specifically to the antigen after addition of sample. All the reagents were equilibrated to room temperature (18-25) before use. About 45µL of sample dilution buffer was added into each well that will be used for serum samples. About 5µL of positive, weak positive and negative serum controls were added into each of the appropriate well, respectively. Then about 5µL of sample dilution buffer was added into two appropriate wells (designated as conjugate control (CC). Then 5µL of test sample were added to each of the appropriate wells. Then after 50µL of mAb solution was added into wells used for controls and samples. The plate was sealed and the reagent mixed thoroughly for 5 minute by using plate shaker. Then the plate was incubated at room temperature (18-25 ^{0}C) and allowed to stay for 30 minutes. Then the plates were rinsed 4 times with PBS-Tween buffer. The solution was washed to remove unbound antibodies and then enzyme-conjugated secondary antibodies were added. The substrate for enzyme was added to quantify theprimary antibody through a colour change. The concentration of primary antibody present in the serum directly correlates with the intensity of the colour that was read by ELISA reader.

5.8. Ethical Clearance

The study protocol was approved by the Armauer Hansen Research Institute Ethical Clearance Committee as a part One HealthNationalBrucellosis Research project in the Pastoral regions of Ethiopia.Written informed consent was obtained from human study participants (livestock

owners) who involved in this study. Similarly, verbal consent was obtained from livestock owners for livestock blood sampling and information gathering regarding description of individual animal involved in the study and livestock related information.

5.8. Data analysis

We will use a unique field ID number as labelling system. During field data collection (interviews and biological samples), a unique numerical ID number was assigned to each participant. This number was written on each consent form, corresponding biological samples. These IDs was used during data management and laboratory analysis at AHRi and All survey and laboratory data will be double entered, cleaned and stored in Microsoft Access. Statistical analysis will be performed using STATA 10 Software.

6. RESULTS

6.1. Seroprevalence of brucellosis and association risk factors in livestock

In present study, the overall seroprevalence of brucellosis in livestock (small ruminants, cattle and camel) was 9.07% [95% CI=6.6-11.45]. Out of the 562 livestock serum samples were collected 51 (9.07%) were seropositive for brucellosis which was confirmed by detection of antibodies against brucellosis using I-ELISA and C-ELISA(Table1). The highest seroprevalence was recorded in cattle 16.67% [95%CI:9.3-24] while the lowest seroprevalence was observed in camels 3.7% [95%CI: 0.4-7.9]. The differences in sero positivity between livestock species in study area were statistically significant (p=0.014). Other risk factors (districts and sex) did not show a statistically significant difference between the categories (p>0.05) as presented in Table

Table 1: Association of risk factors with seroprevalence of brucellosis in livestock in the Asayita and Mille districts

Variable study	№ of examined animals	№ (%) of positive	χ2 value	P- value
District			0.29	0.587
Asayita	158	16(10.13)		
Mille	404	35(8.66)		
Species			10.59	**0.014***
Goat	317	27(8.52)		
Sheep	62	4(6.45)		
Cattle	102	17(16.67)		
Camel	81	3(3.7)		
Sex			0.093	0.760
Male	18	2(11.11)		
Female	544	49(9.01)		

*statistically significant

In multivariable logistic regression analysis of different risk factors with livestock brucellosis sero positivity revealed that cattle had 2.15 time the odds of being seropositive compared with goat (adjusted OR=2.15; 95% CI: 1.12-4.15) which was statistically significant (Table 2). In relation to other factorsconsidered, district and sex, there were no statistical significance differences of brucellosis sero positivity between groups

Table 2.Multivariable logistic regression analysis of risk factors of brucellosis with sero positivity in livestock of the study area

Variable study	№ of examined animals	№ (%) of positive	Crude OR(95%CI)	Adjusted OR(95%CI)
District				
Asayita	158	16(10.13)	1	1
Mille	404	35(8.66)	0.84(0.45-1.56)	0.93(0.49-1.76)
Species				
Goat	317	27(8.52)	1	1
Sheep	62	4(6.45)	0.74(0.24-2.19)	0.72(0.24-2.16)
Cattle	102	17(16.67)	2.14(1.12-4.13)	**2.15(1.12-4.15)***
Camel	81	3(3.7)	0.41(0.12-1.39)	0.42(0.12-1.44)
Sex				
Female	544	49(9.01)	1	1
Male	18	2(11.11)	1.26 (0.28-5.65)	1.47 (0.32-6.74)

*statistically significant

6.2. Seroprevalence of brucellosis and Association of risk factors in of human

In the present study, a totalof 172 human serum samples were collected from pastoralist who own the livestock and the overall seroprevalence of human brucellosis was 48.8% [95%CI: 41.2- 56.3] using indirect ELISA test. A higher human brucellosis seroprevalence was recorded inMille district (63.1%) than Asayita (35.2%) (Chi-square ($\chi2$) = 13.35, P=0.000). The difference in human brucellosis sero positivity between the districts was statistically highly significant ($\chi2$ =13.35 P = 0.000) as presented inTable 3. The seroprevalence was higher in age group of >40years(55%) and 13-40years old human (48.25%) than<13years (0%). Allthe six sera collected from human less than 13years old were negative. This difference in sero positivity of human brucellosis among the age groups was statistically significant (p=0.034) as presented in Table 3

Table 3. Association of risk factors with sero positivity of brucellosis in Human

Variables	№ of examined humans	№ (%) of positive	$\chi2$ value	P- value

Districts				
Asayita	88	31(35.23)	13.35	**0.000***
Mille	84	53 (63.1)		
Sex			0.4472	0.504
Male	100	51 (51)		
Female	72	31(45.83)		
Age			6.7433	**0.034***
<13yrs	6	0 (0)		
13-40yrs	114	55(48.25)		
>40yrs	52	29(55.77)		

*statistically significant

In multivariable logistic regression analysis risk factors (districts, sex and age) with human brucellosis sero positivity showed that pastoralists in Mille districts had 3.3 times the odds of being seropositive compared to those in Asayita district (Adjusted OR=3.3; 95% CI:1.74-6.26) which was statistically highly significant (Table 4). Sex of the study participants as a risk factor did not show statistical significant differences in sero positivity of human brucellosis among the groups considered. The age category did not fit to the multivariable logistic regression model and omitted during analysis.

Table 4: Multivariable logistic regression analysis of risk factors of brucellosis with sero positivity in Human

Variables	**№ of examined humans**	**№ (%) of positive**	Crude OR(95%CI)	Adjusted OR(95%CI)
Districts				

Asayita	88	31(35.23)	1	1
Mille	84	53 (63.1)	3.14 (1.68 -5.86)	**3.3 (1.74-6.26)***
Sex				
Female	72	31(45.83)	1	1
Male	100	51 (51)	1.23 (0.67 -2.25)	1.23 (0.63—2.40)
Age				
<13yrs	6	0 (0)	-	-
13-40yrs				
	114	55(48.25)	-	-
>40yrs	52	29(55.77)	-	-

*statistically significant

7. DISCUSSION

Brucellosis was considered by FAO, WHO and OIE as one of the most widespread zoonoses in the world (Schelling *et al.,* 2003). According to OIE, it was the second most important zoonotic disease in the world after rabies (OIE, 2009). The pastoralist whose livelihood depends on the livestock are at highly exposed to zoonotic brucellosis due to their habit of consumption of dairy

products and contact with infectious uterine discharges and *brucell*a contaminated tissues from livestock. As brucellosis affects different species of livestock including cattle, sheep, goats and camels, it is important to understand the epidemiological status of the disease in different species of livestock owned by pastoralists combined with assessing the status of the brucellosis in pastoralists owning the livestock in order to reveal the risk of zoonotic transmission of brucellosis to human in pastoralist setting.

Hence, the present cross sectional study revealed a high seroprevalence of human brucellosis 48.8% [95%CI: 41.2- 56.3] using indirect ELISA test in pastoralist and a 9.07% [95% CI=6.6-11.45] overall seroprevalence of brucellosis in their livestock (small ruminants, cattle and camel) in Asayita and Mille districts of Afar Region. The high prevalence of human brucellosis in the Afar pastoralists reported in this study was comparable with previous reports from Somali area (34.9%) Ghanem, *et al.*, 2009) .The similarity in high prevalence in human could be due to the similarities of the pastoral setting in livestock husbandry and cultural habits of consumption of raw milk and milk products and close contact with livestock among pastoralist of Ethiopia. On the other hand, the result of human brucellosis of the present study was much higher than previous reports from human in highland part of Ethiopia; 5.8% in Sidama zone (Kassaun *et al.,* 2007.); 9% in Bishoftu (Musie *et al.,* 2007) and 3.8% in Bahir Dar (Hailemelekot *et al.,* 2007). The difference might be due to the variation in the existence of potential risk for human infection of zoonotic brucellosis including the common habit of consumption of raw dairy products, the presence and ownership of large number and different species of livestock by pastoralist combined with their day today close contact with livestock in Afar pastoralists might increase the

rate of exposure to zoonotic diseases like brucellosis as compared to the highland dwellers might result the differences in human seroprevalence of brucellosis.

Association of risk factors for human brucellosis showed that pastoralist in Mille district had a high sero positivity (63.1%) than those from Asayita pastoralists (35.2%). The difference between the districts might be related to the variation in the rate of exposure which might related to the existence of large number of livestock population in Mille than Asayita, more over the camels found in Asayita are mainly male camel and few in number whereas in Mille districts the camels were mainly dairy camels kept for milk in which case it might favour the easy transmission of brucellosis through consumption of raw milk and milk products.

With regard to livestock brucellosis, the present study showed a moderately high overall prevalence 9.07% [95% CI=6.6-11.45] in livestock of Asayita and Mille districts of Afar region. Though this combined overall prevalence is difficult to compare with other studies as there are few studies which report a combined seroprevalence result with consideration all four species common to pastoral (cattle, goat, sheep and camel), the result was comparable to previous reports of 10.6% and 9.6 % seroprevalence of brucellosis in cattle and camels of Borena zone and Somali region, respectively (Megeresa *et al.,* 2011; Gumi *et al.*, 2013).However, the result obtained in the present study was lower than previous report from Amibara district of Afar region which reported 18.2% overall prevalence of brucellosis in cattle and camels of the district using c-ELISA (Yimer, 2016). The difference might be related to the difference in geographic location and livestock species considered as in the present study in addition to cattle and camel the study included sheep and goats in the overall seroprevalence analysis. In the present study, the seroprevalence of cattle was 16.6% which was the highest while that of goats and sheep were

8.52% and 6.45%, respectively.In multivariable logistic regression analysis of different risk factors with livestock brucellosis sero positivity revealed that cattle had 2.15 time the odds of being seropositive compared with goat which was statistically significant .The difference in the prevalence among the species might be related to the type of circulating in the area in which in our case *Brucella abortus* might be predominant in the study site which may establish itself in its classical host, the bovine better than the goats. Another possible explanation might be the difference in the duration of exposure as cattle live longer life than small ruminants this might result the high rate of exposure in cattle. The lowest seroprevalence in the present study was from camel (3.7%). This low seroprevalence in camel was in agreement with the previous report of 3.42% from Jijiga and Babile area of Eastern Ethiopia (Tilahun *et al.,* 2013). However, this camel seroprevalence result of the present study (3.7%) was lower than the previous reports from Afar region which reported 11.9% (Sisay and Mekonen, 2012) and 13.5% (Yimer, 2017). The difference might be related to the variation in the risk factors of infection of camel in geographic districts where the study carried out as the current study was carried out in camels of Mille and Asayiat while the other previous studies were done in Amibara district.

Generally, the current findings revealed that a high rate of zoonotic brucellosis (48.8%) in pastoralist community of Mille and Asayita districts of Afar region with moderately high seroprevalence (9.07%) and wide occurrence of brucellosis in their livestock cattle, camel, goat and sheep). The finding of this study indicating high prevalence of zoonotic brucellosis in human might be the result of the common cultural habit of consumption of raw milk (Radostits *et al.,* 2000) and unprotected contacted with infectious discharges and tissues from infected animals in pastoralist communities of the study area.

6. CONCLUSIONS AND RECOMMENDATIONS

In the current study, a high seroprevalence of human brucellosis (48.8%) was observed in the Mille and Asyaita district Afar Region and simultaneously a widespread occurrence of animal brucellosis in different species of livestock (cattle, camel, sheep and goat) owned by the pastoralists. A higher prevalence of brucellosis was detected in cattle (16.6%) as compared to small ruminants and camels owned by the pastoralists, suggesting the major role of cattle in livestockbrucellosisinthestudyareaIn Ethiopia, in spite of a number of research reports onseropr evalence of brucellosis in separated species of livestock and there only few reports were studies underway in such one-health approach. The present study tried to combine the investigation of brucellosis in livestock and their owners in a setting where risk factors such as consumption of raw milk and direct close contact exists for zoonotic transmission are widely observed. In conclusion, in pastoralist setting of Afar, the present study showed a high level of human brucellosis which might have not been detected by the health service of the region.

Therefore based on the above conclusions the following recommendations were forwarded:

- ❖ The high prevalence of brucellosis in human suggests the urgent need to design control strategies for control of zoonotic brucellosis through control of animal brucellosis.
- ❖ Seropositive human cases should be treated with standard medication for human brucellosis in the health facilities.
- ❖ Awareness creation for pastoralist on route of transmission and prevention of infection zoonotic brucellosis should be given by the relevant bodies.
- ❖ Further nationwide investigation and control strategies should be designed particularly in pastoralists settings of Ethiopia.

❖ Further in depth research should be carried out isolate and characterize the causative agent of brucellosis in livestock and human to design vaccine based control strategies.

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