ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

EPIDEMIOLOGICAL INVESTIGATIONS OF BRUCELLOSIS IN RUMINANTS AND HUMANS IN YABELLO DISTRICT OF BORENA PASTORAL AREA, OROMIA NATIONAL REGIONAL STATE, SOUTHERN ETHIOPIA

BY

YOHANNES GIRMA

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Epidemiology

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LIST OF ABBREVIATIONS

16srRNA	Sixteen sub unit Ribosomal Ribo Nucleic Acid
CD	Conjugate Diluents
CFT	Complement Fixation Test
DNA	Deoxy Ribonucleic Acid
FAO	Food and Agricultural Organization
GPDI	Gayo Pastoral Development Initiatives
I-ELISA	Indirect Enzyme Linked Immuno Sorbent Assay
LPS	Lipo poly Saccharide
MLVA	Multilocus Variable Number Tandem Reppeat
mRBPT	Modified Rose Bengal Plate Test
NAHDI	National Animal Health and Diagnostic Center
OD	Optical Density
OIE	Office International des Epizootics
OPADC	Oromia Pastoral Area Development Commission
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
SD	Sample Diluents
SPSS	Statistical Package for Social Sciences
TNF	Tumor Necrosis Factor
VNR	Variable Number Tandem Repeat
WHO	World Health Organization

ABSTRACT

A cross sectional epidemiological study design was employed to investigate a seroprevalence and risk factors associated with the occurrence of brucellosis in ruminants (cattle, sheep and goat), and humans from October 2011 to March 2012, in Yabello district and hospital of Borena pastoral area, respectively. Accordingly, a total of 1360 ruminant's sera (634 from cattle, 221from sheep and 505 from goats) and 138 humans were included in the study. Modified RBPT and I-ELISA was used as the screening and confirmatory test, respectively. The result of the present study indicates an overall animal *Brucella* antibody prevalence rate of, 10.3% (n=140) and 8.2% (n=112) by using RBPT and I- ELISA, respectively. Seroprevalence of 9.1% and 6.6% was recorded for cattle using mRBPT and I-ELISA, respectively. Similarly, a prevalence of 13.7% and 11.7% for goats and 5.9% and 4.9% for sheep was recorded by using mRBPT and I-ELISA respectively. An overall human *Brucella* antibody prevalence of 11.6% (n=16) and 3.6 %(n=5) was recorded using mRBPT and I-ELISA, respectively in Yabello Hospital. A multivariable logistic regression analysis of the risk factors identified age, parity status, and abortion history and species difference to be the major risk factors for overall animal brucellosis seropositivity ($P \leq 0.05$). However the seropositivity recorded for human in Yabello hospital was not statistically significant ($P \geq 0.05$). The result also indicates that goats are at higher risk of getting *Brucella* infection than sheep, with twice more likely to harbor *Brucella* infection than sheep. Increase in herd size was also found to be a major risk for small ruminant seropositivity. Thus the present study suggests that brucellosis is highly prevalent in the study districts among different species of animals and human patients admitted to Yabello hospital. The seropositivity in both animals and human could give an insight that brucellosis could pose a public health hazards. This warrants further extensive epidemiological and molecular investigation to identify the specific *Brucella* species in both animals and humans in the area.

Keywords: *Brucell*a, Cattle, Goat, Human, Sheep, Yabello

1. INTRODUCTION

Brucellosis is one of the most important bacterial zoonoses worldwide and in particular in developing countries the disease may have important economic, veterinary and public health consequences (Pappas *et al*., 2006; Franco *et al*., 2007). Brucellosis 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. Furthermore, since brucellosis is an important cause of abortion especially in first calf heifers, the disease can also cause important economic losses in developing countries (Smits *et al*., 2007*).*

Infection in animals is strongly correlated with abortions in the last trimester of pregnancy. The most commonly affected animals are cows (*B. abortus*), sheep and goats (*B. melitensis*), pigs (*B. suis*), and some other domestic animals including Camels. Some wild animals might also be affected by this zoonosis, but these are rarely implicated as sources of human disease. In animals, the primary sign of infection in females is abortion and in males epididymitis and orchitis and diagnosis can only be confirmed by laboratory tests that may even confirm latent infections (Corbel, 2006; Denov *et al*., 2010). Cross-transmission of brucellosis can occur between cattle, sheep, goats, camels and other species (Dawood, 2008).

Brucellosis in humans is known as "undulant fever" or "Mediterranean fever", "Malta fever" or "Bangs disease". Infections of humans are high and occur contagious through direct or indirect contact with infected animals or their products, causing a debilitating illness. It 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).

The incidence of human cases is significantly correlated to the level of *B. melitensis* infection in small ruminants. This is especially true among pastoral, rural and peri-urban communities where traditions and cultural practices contribute to disease transmission and where people live in close occupational contact with infected animals. Humans may be exposed to animal tissues, discharges or fetus membrane and fluids following abortion, or due to consuming raw contaminated milk, fresh cheese and other dairy products, and rarely raw meat or partly cooked offal (liver) from such animals. Aerosol and hand-to-mouth transmission may occur in abattoirs or laboratories. (Donev *et al*., 2010; Corbel, 2006).

This insidious and frustrating zoonosis, often neglected, under recorded and under reported, has wide spread impact on human and animal health and plays a significant role in the national economy of many developing countries.The reported annual incidence rate of human brucellosis in the endemic-disease areas in the world varies widely, from < 0.01 to > 200 cases per 100,000 populations. The low incidence rate reported in known brucellosis-endemic countries of Africa and Asia may reflect the absence or the low levels of surveillance and reporting systems (Pappas *et al*., 2006; Donev *et al*., 2010).

Estimates of the prevalence of animal and human brucellosis are not available for many countries of the world. Good quality data on the impacts of different diseases and their control on animal and human populations in sub-Saharan Africa including Ethiopia are usually lacking. However, a recent study has attempted to assemble expert advice on the potential impacts of animal diseases in the developing world including Ethiopia (Mc Dermott and Arimi, 2002).

Many developing countries with limited resources, including Ethiopia, are facing other priority diseases that are more spectacular and have not yet fully launched programs featuring any aspects of brucellosis intervention. The epidemiology of the disease in livestock and humans as well as cost-effective prevention measures are not well understood and available data are limited particularly in sub-Saharan countries (McDermott and Arimi, 2002). Hence, brucellosis remains widespread in domesticated and wildlife animal population and presents enormous economic and public health problems in African countries. Thus, the economic and public health impact of brucellosis remains of particular concern in developing countries (WHO, 2006). The disease poses a barrier to trade of animals and animal products, public health hazards and impediment to free animal movement. It could seriously impair socio-economic development for livestock owners, which represent a vulnerable sector in rural populations in general and pastoral societies in particular (Benkirane, 2006).

In Ethiopia, like many African countries, zoonotic diseases are among the major diseases of veterinary and public health importance accorded a very little attention. To that effect, little is known about its epidemiological status in animals and humans and factors contributing to its cross-species and zoonotic transmission (Megersa *et al*., 2011)

Antibodies against *Brucella* species were found repeatedly in sera of animals in Ethiopia, with large disparities between different regions, production systems, authors and time periods as generally seen in Sub-Saharan Africa (McDermott and Arimi, 2002). The evidences of *Brucella* infections have been serologically demonstrated in animals and humans in Ethiopia (Megersa *et al*., 2005; Berhe *et al.*, 2007; Ashenafi *et al.*, 2007). Most of those previous studies were based on seroprevalence of the disease that covered limited geographical localities and specific animal groups, which were easily accessible. Thus, the depth of information on animal and human Brucellosis is so limited to provide good epidemiological picture of the diseases and consequent socioeconomic impacts in

Ethiopia. In particular, such information is almost nil for pastoral areas, where the disease is of great economic and public health significance (Megersa *et al*., 2005).

A huge and diverse livestock species of Ethiopia are maintained under different agro-ecological zones, management, migration and animal health care system. The predominately extensive animal husbandry practices of the country provide ample opportunities for intermixing of different animal species, communal grazing areas and water points, and composite holding of livestock maintained by nearly 80% of the rural community (Samui *et al*., 2007).

Traditionally, the pastoralists and rural farmers keep mixed livestock species including cattle, camels, sheep, goats and equine species. In view of the widespread risk factors for establishment and transmission of pathogen infection owning to high stock density and multi-species composition, favorable climate and lack of controlling measures, it might not be difficult to estimate a high economic burden that the infection is posing to the Ethiopian livestock industry (Benkirane, 2006).

More importantly, a close human-animal contact and tradition of raw animal product consumption make zoonosis among the major public health hazards, with particular implication to pastoral area. This requires a thorough epidemiological investigations including due consideration to identifying the major risk factors that predominantly influence the disease occurrence, and thus contribute to designing appropriate and feasible national controlling strategies. This epidemiological investigation was therefore, designed with the aim to:

- Investigate epidemiological distributions and associated risk factors of Brucellosis in cattle, sheep and goats and human patients at Yabello hospital, in Yabello district.
- Determine the seroprevalence status of brucellosis in cattle, sheep and goats in Yabello district.
- Determine the seroprevalence status of human brucellosis at Yabello Hospital.

2. LITERATURE REVIEW

2.1. The aetiologic agent

Brucella species are facultative intracellular gram-negative cocco-bacilli, non-spore-forming and non-capsulated. Although *Brucella* species are described as non-motile, they carry all the genes except the chemotactic system, necessary to assemble a functional flagellum. Nine *Brucella* species are currently recognized, seven of them that affect terrestrial animals are: *B. abortus, B. melitensis, B. suis, B. ovis, B. canis,B. neotomae*, and *B. microti* (Sriranganathan *et al*., 2010) and two that affect marine mammals are: B. ceti and *B. pinnipedialis* (Foster *et al*., 2007).

The first three species are called classical *Brucella* and within these species, seven biovars are recognized for *B. abortus*, three for *B. melitensis* and five for *B. suis*. The *Brucella* have no classic virulence genes encoding capsules, plasmids, pili or exotoxins and compared to other bacterial pathogen relatively little is known about the factors contributing to the persistence in the host and multiplication within phagocytic cells. Also, many aspects of interaction between *Brucella* and its host remain unclear (Seleem *et al*., 2008 ; Sriranganathan *et al.*, 2010).

2.2. Morphology

Brucellas are coccobacilli or short rods 0.6 to 1.5 μm long by 0.5 to 0.7 μm in width. They are arranged singly and less frequently in pairs or small groups. The morphology of *Brucella* is fairly constant except in old cultures, where pleomorphic forms may be evident. *Brucella* spp. are non-motile. They do not form spores, flagella, or pili. True

capsules are not produced. *Brucella* are Gram-negative and usually do not show bipolar staining. They are not truly acid-fast but resist decolouration by weak acids, thus stain red by the Stamp's modification of Ziehl-Neelsen method, which is sometimes used for the microscopic diagnosis of brucellosis from smears of solid or liquid specimens (Europian Commission, 2001).

2.3. Genome 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 3.3 Mbp 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) (Bohlin *et al.,* 2010). Analysis of 16S rRNA sequences places *Brucella* spp. As members of the alpha-2 *Proteobacteria (*Shirley *et al*., 2005). The genus *Brucella* has six recognized species, all of which exhibit distinct host preferences (Shirely *et al*., 2005).The high degree of similarity among the brucellae (Gandara *et al*.,2001; Bricker,2002; Paulsen *et al*.,2002) lends support to the proposal that the classical species of *Brucella* are actually strains of *Brucella melitensis.* However, this view conflicts with the hypothesized evolutionary isolation of these classical species due to their intracellular existence and host preference (Shirley *et al*., 2005).

Pulsed-field gel electrophoresis (PFGE) maps of the classical *Brucella* spp. genomes are composed of two circular chromosomes of approximately 2.1 and 1.2 Mbp, with the exception of *B. suis* biovar 3, which has a single chromosome of 3.1 Mbp. PFGE studies revealed other differences, including a 640-kb inversion in the small chromosome of *B.*

abortus 544 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 (DelVecchio *et al*., 2002). 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 (Shirley *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).

2.4. **Epidemiology**

2.4.1. World distribution

The geographical distribution of brucellosis is constantly changing, with new foci emerging or re-emerging. The epidemiology of human brucellosis has drastically changed over the past few years because of various sanitary, socioeconomic, and political reasons, together with increased international travel. New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening (Pappas et al., 2006). The disease 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. 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 and official numbers constitute only a fraction of true incidence of the disease. Thus the true incidence of human brucellosis is unknown and the estimated burden of the disease varies widely, from <0.03 to >160 per 100,000 population (Pappas *et al*., 2006). Although brucellosis has been, or is close to being, eradicated from a number of developed countries, it continues to be a major public and animal health problem in many regions of the world , particularly where livestock are a major source of food and income. There are many reasons why brucellosis remains endemic. These include expansion of livestock herds and flocks, with associated uncontrolled movements; lack of veterinary support services and vaccines; and husbandry practices. Published studies on the relative occurrence of brucellosis are largely confined to serological surveys, and are much more commonly conducted for bovine brucellosis, occasionally for shoats and rarely for pigs and Camels (McDermot and Arimi, 2002).

Table 1 Prevalence status of brucellosis in different livestock species and human in some countries of the world.

Species	Authors	Country	Prevalence (%)	Date
Cattle	Al-Majali *et al*.	Jordan	10.1	2008
	Aulakh *et al*.	India	20.7	2008
Sheep	Celebi and Atabi	Turkey	36.7	2009
Goat	Gupta *et al.*	India	59	2006
	Bokaie *et al*.	Iran	3.4	2008
Human	Vancelik *et al.*	Turkey	5.4	2008

2.4.2. Distribution in Africa

Brucellosis exists throughout sub-Saharan Africa, but essentially nothing is known about its prevalence. 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).

Table 2 prevalence status of brucellosis in cattle, sheep, goat and human in some African countries

Species	Author	Country	Prevalence (%)	Date
Cattle	Kungu *et al*.,	Uganda	46.1	2010
	Samah *et al.*		5.4	2008
	Omer *et al*	Eritrea	5.6	2000a &b
	Weinhaupl,	Tanzania	14	2000
	Swai and Schoonman	Tanzania	5.3	2010
	Schelling *et al*	Chad	7	2003
Sheep	Unger *et al*.	Gambia	1.1	2003
	Bertu *et al*.,	Nigeria	14.5	2010
Goat	Abdel-El *et al*	Egypt	3.5	2010
	Bertu *et al*.,	Nigeria	16.1	2010
Human	Schelling *et al*	Chad	3.8	2003

2.4.3. Status of Brucellosis in Ethiopia

Brucellosis in highland areas of Ethiopia

The limited number of survey that was conducted in some extensive and intensive production systems of Ethiopia are also given in the table below. Virtually all of the surveys were limited to the study of bovine brucellosis and few on human brucellosis. Most of the studies conducted were also based on seroprevalence (Table 3.)

Table 3 Prevalence status of brucellosis in cattle and human in Ethiopian highlands

Species	Author	Place	Prevalence (%)	Date
Cattle	Gelaye *et al.*	Sidama zone	1.7	2010
	Nuradis *et al*.	Jimma zone	3.1	2009
	Berhe *et al*.	Tigray	3.19	2007
	Shewit *et al.*	W.Tigray	4.9	2008
	Teshale *et al.*	Central Oromia	2.9	2007
	Gebreyesus *et al.*	NW. Ethiopia	12	2001
Human	Kassahun	Sidama zone	5.8	2004
	Tadele	Jimma zone	3.4	2004
	Musie	Debrezeit	9	2005
	Hailemelekot	Bahir Dar	3.8	2005

Brucellosis in pastoral and agro pastoral areas of Ethiopia

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 Borena Oromos (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).

Even though, several serological surveys have showed bovine brucellosis is an endemic and widespread disease in urban, peri-urban, highland and lowland, extensive and intensive farming, small holder farms and ranches of the country (Kebede *et al.*, 2008; Hunduma and Regassa, 2009). Different studies revealed that in sub-Saharan Africa, the

highest incidences of brucellosis are found in pastoral production systems (McDermott and Arimi, 2002; Mangen *et al*., 2002). In seroprevalence study using RBPT, Hunduma and Regassa (2009) noted that bovine brucellosis is a widespread and well-established infection in both pastoral and agro-pastoral production systems. Especially cattle herders in pastoral areas are in close contact with their animal, consumption of raw milk and handling of aborted materials is common. Small ruminant brucellosis is mostly caused by *Brucella melitensis* (Omer *et al*., 2002). *Brucella ovis* is also an important cause of orchitis and epididymitis in sheep but it is not recognized as a cause of natural infection in goats (Teshale *et al*., 2006).

Most of the studies which conducted on brucellosis were entirely based on estimation of seroprevalence of the disease. There are very few reports on the Seroprevalence of brucellosis, which are conducted on different livestock species and human in pastoral and agro-pastoral areas of the Ethiopia. Additionally, pastoral households often keep a diverse composite of livestock species as part of a coping mechanism for uncertainties and risks. Such conditions certainly increase aggregation and interaction of different animals at villages, grazing fields and water points, thus, facilitate transmission of the disease. The dynamics and frequent migration of pastoral herds might increase the chance of coming into contact with other potentially infected herds and exposure to geographically limited or seasonally abundant diseases. Mobility also increases the opportunity of interactions with wild animals. This has already been confirmed by (Samui *et al*., 2007; Megerssa *et al*., 2011), in that herds coming into contact with wildlife had higher likelihood of acquiring infection than those without contact.

Table 4 Seroprevalence of brucellosis in animals and humans in different pastoral and agro pastoral areas of Ethiopia

Species	Author	Place	Prevalence (%)	Date
Cattle	Hunduma and Regassa	E. shoa	15.5	2009
	Megersa *et al*.		4.1	2011
		Borena	4.7	
		Jijiga	3	
		ShinilIe	6.6	
		S. Omo	3.4	
Sheep	Teshale *et al.*	Afar	14.6	2006
		Somali	3.2	2007
Goat	Teshale *et al*.		1.6	2006
		Somali	1.7	2006
	Ashenafi *et a*l.	Afar	5.8	2007
	Teshale *et al*.	Afar	16.5	2007
Human	Smits *et al.*	Metema	3	2009
		Borena	34.9	
		Hamer	29.4	
	Yimer *et al*.	Afar	16.5	2008

2.4.4. Mode of transmission

In cattle and other Bovidae, *Brucella* is usually transmitted from animal to animal by contact following an abortion. Pasture or animal barn may be contaminated and the organisms are probably most frequently acquired by ingestion and inhalation, conjunctival inoculation, skin contamination and udder inoculation from infected milking cups are other possibilities. The use of pooled colostrums 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 (Robinson *et al*., 2003).

In sheep and goats, *B. melitensis* is nearly always the infecting species. *B.ovis* can also infect sheep but is of little significance in relation to human disease. The mode of transmission of *B. melitensis* in sheep and goats is similar to that in cattle but sexual transmission probably plays a greater role. The transmission of disease is facilitated by commingling of flocks and herds belonging to different owners and by purchasing animals from unscreened sources. The sharing of male breeding stock also promotes transfer of infection between farms. Transhumance of summer grazing is a significant promoting factor in some areas, as, is the mingling of animals at markets or fairs. In cold climates, it can be the custom to house animals in close space and this also facilitates transmission of infection (Corbel, 2006).

Transmission of infection to humans occurs through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted foetuses or placentas. Food-borne infection occurs following ingestion of raw milk and other dairy products, but rarely from eating raw meat from infected animals. Occupational airborne infection in laboratories and abattoirs has also been documented. Accidental inoculation of live vaccines (such as *B. abortus* Strain 19 and *B. melitensis* Rev.1) can also occur, resulting in human infections. There are also case reports of venereal and congenital infection in humans (Robinson *et al.*, 2003).

2.4.5. Host diversity of brucellosis

Animals

In livestock species (cattle, sheep, goats, swine, and camel), the most frequent clinical sign following infection with *Brucella* is abortion. The principal strain that infects cattle is *B. abortus*, cattle can also become transiently infected by *B. suis* and more commonly by *B. melitensis* when they share pasture or facilities with infected pigs, goats and sheep. *B. melitensis* and *B. suis* can be transmitted by cow's milk and cause a serious public

health threat .The main symptoms in pregnant females is abortion (premature or full term birth of dead or weak calves) usually in the second half of gestation with retention of placenta and metritis (Acha *et al*., 2003; Sriranganathan *et al.*, 2010). The *Brucella* localize in the supra-mammary lymph nodes and mammary glands of 80% of the infected animals and thus continue to secrete the pathogen in milk throughout their lives. Most infected cows abort only once although the placenta will be heavily infected at subsequent apparently normal calvings (Sriranganathan *et al*., 2010).

The main etiologic agent of brucellosis in goats is *B.melitensis*. In certain countries like Brazil where there is no *B. melitensis*, goats can get infected with B. abortus. As in cattle, brucellosis in goats is characterized by late abortion, stillbirths, decreased fertility and low milk production (Lilenbaum *et al*., 2007). Sheep brucellosis can be divided into classical brucellosis and ram epididymitis. Ram epididymitis is caused by non-zoonotic agent *B. ovis*, while classical brucellosis is caused by *B. melitensis* and constitutes a major public health threat equal to goat brucellosis (Acha *et al*., 2003).

Zoonoses

Five out of the nine known *Brucella* species can infect humans and the most pathogenic and invasive species for human is *Brucella melitensis*, followed in descending order by *B. suis, B. abortus and B. canis* (Acha *et al*., 2003; Sriranganathan *et al*., 2010). The zoonotic nature of the marine *brucellae* (*B. ceti*) has been documented. *Brucella. melitensis, B. suis and B. abortus* are listed as potential bio-weapons by the Centers for Disease Control and Prevention in the USA. This is due to the highly infectious nature of all three species, as they can be readily aerosolized. Moreover, an outbreak of brucellosis would be difficult to detect because the initial symptoms are easily confused with those of influenza (Sriranganathan *et al*., 2010).

In places where brucellosis is endemic, humans can get infected via contact with infected animals or consumption of their products, mostly milk and milk products especially

cheese made from unpasteurized milk of sheep and goats and rennet from infected lambs and kids. Some specific occupational groups including farm workers, veterinarians, ranchers, and meat-packing employees are considered at higher risk (Tabak *et al*., 2008). *B. abortus* and *B. suis* infections usually affect occupational groups, while *B. melitensis* infections occur more frequently than the other *Brucella* species in the general population. Because person-to-person transmission rarely occurs, infected persons do not pose a threat to their surroundings (Sriranganathan *et al*., 2010)

2.5. Pathogenesis

The pathogenicity of *Brucella* is due to its ability to adapt to the environmental conditions encountered in its intracellular replicative niche including low levels of nutrients and oxygen, acidic pH and reactive oxygen intermediates (Kohler *et al*., 2002).

Smooth *Brucella* inhibit host cell apoptosis, favoring bacterial intracellular survival by escaping host immune surveillance, while rough *Brucella* mutants (*Brucella canis* and *Brucella ovis* are two exceptions) induce necrosis in macrophage (Pei *et al*., 2006).

However, the mechanisms and virulence factors that mediate macrophage cell death have not been identified. In contrast to other pathogenic bacteria, no classical virulence factors, such as exotoxins, cytolysins, capsules, fimbria, plasmids, lysogenic phages, drug resistant forms, antigenic variation, endotoxic lipopolysaccharide (LPS) have been described in *Brucella* (Moreno and Moriyon, 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 signalling, gene regulation, and transmembrane transportation are among the factors suspected to be involved in the virulence of *Brucella* (Lapaque *et al*., 2005).

Lipopolysaccharide is vital to the structural and functional integrity of the Gram-negative bacteria outer membrane (Cardoso *et al*., 2006). 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 defence system. The LPS of *Brucella* exhibits properties distinct from other LPSs. In contrast to classical entero bacterial LPS, those of *Brucella* are several hundred-times less active and less toxic than Escherichia coli LPSs (Lapaque *et al*., 2005).

Research suggests that the smooth, non-endotoxic lipopolysaccharides help block the development of innate and specific immunity during the early stage of infection, and protect the pathogen from the microbicidal activities of the immune system (Porte *et al*., 2003). *Brucella. melitensis* LPS does not stimulate production of tumor necrosis factor-a or nitric oxide (Tumurkhuu *et al.,* 2006). *Brucella* LPS plays a role in protecting against bactericidal cationic peptides (defensin NP-2, lactoferrin, cecropines, lysozyme, bactenecin-derived peptides, and the defensin-like antibiotic polymyxin B, and the crude lysosomal extracts from polymorphonuclear leukocytes) (Lapaque *et al*., 2005).

Smooth *Brucella suis* interacts with lipid-rafts through an unknown receptor on the surface of macrophages and enters cells via a pathway allowing it to avoid fusion with lysosomes (Lapaque *et al*., 2005). In contrast, the rough strain (lacking O-side chain) seems not to enter by lipid-rafts, and fuses rapidly with lysosomes. Although naturally rough strains of *Brucella* (*B. ovis and B. canis*) lack O-side chain in their LPS, they are pathogenic for rams and dogs, respectively, and induce long-lasting infections with high levels of splenic colonization in laboratory animals (Caro-Hernandezet *et al*., 2007).

The two-component BvrR/BvrS gene sensing system that 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; however, the transported effector substrate in *Brucella* has not yet been identified and it is very unlikely that the transported molecule is a classic virulence factor. 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 (Celli *et al*., 2005). 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 (Arenas *et al*., 2000; Boschiroli *et al*., 2002).

2.6. Diagnostic methods

Diagnosis and control of the disease in animals must be carried out on a herd basis. There may be a very long incubation period in some infected animals and individuals may remain serologically negative for a considerable period following infection. The identification of one or more infected animals is sufficient evidence that infection is present in the herd, and that other serologically negative animals may be incubating the disease and present a risk. Diagnostic tests fall into two categories: those that demonstrate the presence of the organisms and those that detect an immune response to its antigens (Corbel, 2006).

2.6.1. Bacteriological methods

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of a vaccination programme. 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).

Stained smears

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) or Kosters' methods. The presence of large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as *Coxiella burnetii* or *Chlamydia* may superficially resemble *Brucella* (Corbel, 2006).

Culture

Brucella may most readily be isolated in the period following an infected abortion or calving, but isolation can also be attempted post-mortem. *Brucellas are* excreted in large numbers at parturition and can be cultured from a range of material including vaginal mucus, placenta, fetal stomach contents and milk using suitable selective culture media. It is of the utmost importance that faecal and environmental contamination of the material is kept to a minimum to give the greatest chance of successfully isolating *Brucella*. If other material is unavailable or grossly contaminated, the contents of the fetal stomach will usually be otherwise sterile and are an excellent source of *Brucella*. In some circumstances it may be appropriate to attempt the isolation of *Brucella* post-mortem. Suitable material includes supramammary, internal iliac and retropharyngeal lymph nodes, udder tissue, testes and gravid uterus (Corbel, 2006).

2.6.2. Serological methods

Rose Bengal plate test

The RBT is one of a group of tests known as the buffered *Brucella* antigen tests which rely on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low pH. The RBT is a simple spot agglutination test where drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a

positive reaction. The test is an excellent screening test but may be oversensitive for diagnosis in individual animals, particularly vaccinated ones (Corbel, 2006).

Enzyme linked immune sorbent assays test

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).

Complement fixation test

The sensitivity and specificity of the CFT is good, but it is a complex method to perform requiring good laboratory facilities and trained staff. If these are available and the test is carried out regularly with good attention to quality assurance, then it can be very satisfactory. It is essential to titrate each serum sample because of the occurrence of the prozone phenomenon whereby low dilutions of some sera from infected animals do not fix complement. This is due to the presence of high levels of non-complement fixing antibody isotypes competing for binding to the antigen. At higher dilutions these are diluted out and complement is fixed. Such positive samples will be missed if they are only screened at a single dilution. In other cases, contaminating bacteria or other factors in serum samples fix or destroy complement causing a positive reaction in the test, even in the absence of antigen. Such “anti-complementary” reactions make the test void and a CFT result cannot be obtained (Corbel, 2006).

2.6.3. Molecular methods

Due to high genetic homogeneity among species of *Brucella*, strain identification is a difficult task. Classical bacteriology allows for identification of only a small number of subtypes below the species level. Furthermore, certain subtypes may dominate a geographic area. Thus, differential PCR-based assays are particularly useful for epidemiological trace back, or for species-specific eradication programs. In the following years many genus-specific PCR assays were developed, targeting regions and genes such as 16SrRNA, BCSP31, omp2a and omp2b and IS711. One of these types of assays was later included in a more complex assay for detection and differentiation of four different bacterial pathogens: *C. burnetii*, *B. melitensis*, *B. anthracis*, and *Y. pestis* (Jamba, 2008).

PCR assays differentiating between *Brucella* species and/or biovars tend to be more complex and consequently more difficult to perform because appropriate target sites are rare in *Brucella* due to the remarkable homogeneity of the genus. Discrimination of multiple species simultaneously utilises one of two approaches. The first approach includes complex reaction mixtures containing multiple primer pairs, each targeting a unique species-specific DNA sequence polymorphism. The second approach uses a single primer pair to amplify a DNA sequence containing internal species-specific polymorphism. Subsequently, the internal polymorphism is confirmed by some other method downstream. Based on these two approaches, multiplex PCR assays for identification and differentiation of *Brucella* species and/or biovars such as AMOS and BaSS were developed (Jamba, 2008).

PCR-RFLP assays, targeting omp2, omp25, omp31, dnaK genes were also successfully developed to differentiate the *Brucella* species. Several alternative molecular approaches have also been developed in recent years exploiting regions of hyper variability for strain identification. Restriction mapping pulsed gel electrophoresis, ribo typing, IS-RFLP typing were all successful in identifying the variations to some degree. More recently,

promising results in the typing of *Brucella* strains for epidemiological trace-back were obtained using variable number of tandem repeats analysis (VNTR), the methods being multilocus VNTR (MLVA) analysis and the hyper variable octameric oligonucleotide finger-prints (HOOF-Prints) as its variant (Jamba, 2008 ;Bricker *et al*., 2003).

2.7. Treatment

Due to intracellular localization of *Brucella* and its ability to adapt to the environmental conditions encountered in its replicative niche e.g. macrophage (Seleem *et al*., 2008; 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 (Pappas *et al*., 2005 and 2006a; Seleem *et al*., 2009). 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 (Ersoy *et al*., 2005;Seleem *et al*., 2009; Sriranganathan *et al*., 2010). Neither streptomycin nor doxycycline alone can prevent multiplication of intracellular *Brucella*.

Although the DS regimen is considered as the gold standard treatment, it is less practical because the streptomycin must be administered parenterally for 3 weeks. A combination of doxycycline treatment (6 weeks duration) with parenterally administered gentamicin (5 mg/kg) for 7 days is considered an acceptable alternate regimen (Glynn and Lynn, 2008).

2.8. Economic impact

Despite the existence of effective vaccines for cattle (S19 and RB 51) and goats (Rev 1), control efforts in economically poor endemic areas have failed as a result of the absence

of vaccine provision, inconsistent infrastructure and lack of funding. Notably, most cases of human brucellosis in non-endemic developed countries result from dairy products imported from endemic areas146 or from patients who import the disease. 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 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 programmes are shared between the public and private sectors and include international aid, they are likely to be profitable and cost effective (Roth *et al*., 2003; Smits *et al*., 2007).

The economic impact in terms of human disease has been even harder to gauge; not only must the cost of treatment and diagnosis be considered, but also the cost in terms of disability-adjusted life years. Regardless of the measures used, the economic burden of human brucellosis in endemic areas is high and justifies widespread and sustained control efforts (Roth *et al*., 2003; Smits *et al*., 2007).

2.9. Prevention and control

It is nearly always more economical and practical to prevent diseases than to attempt to control or eliminate them. For brucellosis, the measures of prevention include: Careful selection of replacement animals. These, whether purchased or produced from existing stock, should originate from *Brucella*-free herds or flocks. Pre-purchase tests are necessary unless the replacements are from populations in geographically circumscribed areas that are known to be free of the disease. Isolation of purchased replacements

animals for at least, 30 days. In addition a serological test prior to commingling is necessary. Prevention of contacts and commingling with the herds or flocks of unknown status or those with brucellosis. If possible, laboratory assistance should be utilized to diagnose causation of abortions, premature births, or other clinical signs. Suspect animals should be isolated until a diagnosis can be made. Herds and flocks should be included in surveillance measures such as periodic milk ring tests in cattle (at least four times per year), and testing of slaughtered animals with simple screening serological procedures such as the RBT. Proper disposal (burial or burning) of placentas and non-viable fetuses. Disinfection of contaminated areas should be performed thoroughly. Cooperation with public health authorities to investigate human cases. Animal brucellosis, especiall y when caused by *B. melitensis*, can often be identified through investigations of cases in humans (Corbel, 2006).

Human brucellosis is usually prevented by controlling the infection in animals. Pasteurization of dairy products is an important safety measure where this disease is endemic. Unpasteurized dairy products and raw or undercooked animal products (including bone marrow) should not be consumed. Good hygiene and protective clothing/equipment are very important in preventing occupational exposure. Precautions should be taken to avoid contamination of the skin, as well as inhalation or accidental ingestion of organisms when assisting at a birth, performing a necropsy, or butchering an animal for consumption. Particular care should be taken when handling an aborted fetus or its membranes and fluids. Risky agricultural practices such as crushing the umbilical cord of newborn livestock with the teeth or skinning aborted fetuses should be avoided (OIE, 2009).

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The Strain 19 *B. abortus* vaccine and *B. melitensis* Rev-1 vaccine must be handled with caution to avoid accidental injection or exposure. Adverse events have also been reported with the *B. abortus* RB51 vaccine, although it is safer than Strain 19. Persistent infections with vaccine strains have occasionally been reported in vaccinated animals. These strains

can be shed in the milk or aborted fetuses and can infect humans. Obstetricians should also take precautions when assisting at human births, particularly in regions where brucellosis is common. Recently, an obstetrician became infected by ingesting amniotic fluid and secretions from a congenitally infected infant. In the laboratory, *Brucella* spp. should be handled under biosafety level 3 conditions or higher. Human vaccines are not available (OIE, 2009).

The aim of an animal control programme is to reduce the impact of a disease on human health and the economic consequences. A major issue is that control measures should continue for a long period of time and be complemented with a monitoring system that may be hard to keep in place once the number of cases begins to decrease In many countries, methods for the control of brucellosis are backed by governmental regulation/legislation. In others, no authorities exist. Therefore, the procedures for management of infected herds and flocks may vary widely. Nevertheless, certain principles apply, namely, the reduction of exposure to *Brucella* species and the increase of the resistance to infection of animals in the populations. These procedures may be further classified under the general categories of test and isolation/slaughter, hygiene, control of animal movement, vaccination *(*Lundervold *et al.,* 2004*).*

Hygienic methods, to the control of brucellossis are also applied, to reduce exposure of susceptible animals to those that are infected. Owners should be informed about disease transmission and recommendations, such as separation of parturient animals, pasteurization of milk for consumption, avoidance of handling of parturient materials.

Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. Similarly, importations into clean areas must be restricted to animals that originate from brucellosis-free areas, that have a herd/flock history of freedom from the disease and that have given negative reactions to recently performed diagnostic tests. In practice, it is much more difficult to control the movement of livestock kept under pastoral and agropastoral conditions than that of beef or dairy cattle kept under intensive

conditions. Because the owners of herds and flocks may be accustomed to seasonal migrations which may cross national boundaries (Corbel, 2006).

There is a general agreement that the most successful method for prevention and control of brucellosis in animals is through vaccination. While the ideal vaccine does not exist, the attenuated strains of *B. melitensis* strain Rev.1 for sheep and goats and *B. abortus* strain 19 and the non-agglutinogenic *B. abortus* strain RB51 proven to be superior to all others.It is often recommended that vaccination with strains 19 and Rev.1 should be limited to sexually immature female animals. This is to minimize stimulation of post vaccinal antibodies which may confuse the interpretation of diagnostic tests and also to prevent possible abortions induced by the vaccines. Evaluation of the procedures used for the prevention and control of animal brucellosis should be performed. This should include surveillance of animals and humans and investigations of outbreaks. Procedures, including case definition and diagnostic tests, should be standardized and should be flexible enough to allow modification when new information becomes available (Corbel, 2006).

Eradication means the elimination of a pathogenic agent from a country or a zone. A highly organized effort is needed to reach eradication in either a territory and in a population. It is based on sanitary measures and on an organization of activities completely different from those implemented for a control programme. Crucial factors for the success of an eradication programme are the implementation of an effective surveillance system with adequate laboratory support, and the understanding and sharing of objectives for eradication by the decision-makers, farmers, and all other stakeholders (Corbel, 2006).

Disease control in pastoralists and migratory populations has proven especially challenging. Patients may not have access to medical services, and staff at local health-care centers may not be able to make or confirm the diagnosis (Arimi *et al.*, 2005).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted at Yabello, district of Borena zone, Oromia National Regional State from October 2011 to March 2012.Yabello district is geographically located at 4° 53´49´´ N, latitude and 38° 5´ 28´´ E, longitude, at a distance of 565 km South of Addis Ababa and. Generally, the Borana plateau represents a lowland area where the altitude gently slopes from Northern (1650 m.a.s.1.) to the Southern (1000 m.a.s1.).The climatic conditions of the area vary from arid to semi arid lowland. The area has a bimodal rain pattern with annual average rainfall ranging from 300 mm to 700 mm. The main rainy season (65% of precipitation) extends from March to May, and there is a smaller rainy season between mid September and mid November. The main dry season extends from December to February. The vegetation is dominated by savannah type containing mixture of perennial and woody plants with shifting in composition with response to intensity of grazing and browsing (Coppock, 1994).

Considerably, variable number and diversity of animal species are maintained under traditional management, migration and health care delivery system by households at pastoral villages in Borana. The pastoral villages in Borena are characterized by clustering of households with close proximity of houses (huts). Livestock production system is generally predominated by extensive pastoral or agro-pastoral system in which indigenous animals are allowed to forage freely during day time and kept in open enclosure during the night. The dynamic nature of this system is characterized by keeping diverse species of livestock with seasonal herd mobility, particularly during the dry periods. Stock composition varies between keeping cattle as major stock with small ruminants and camels (Megersa *et. al*, 2008).

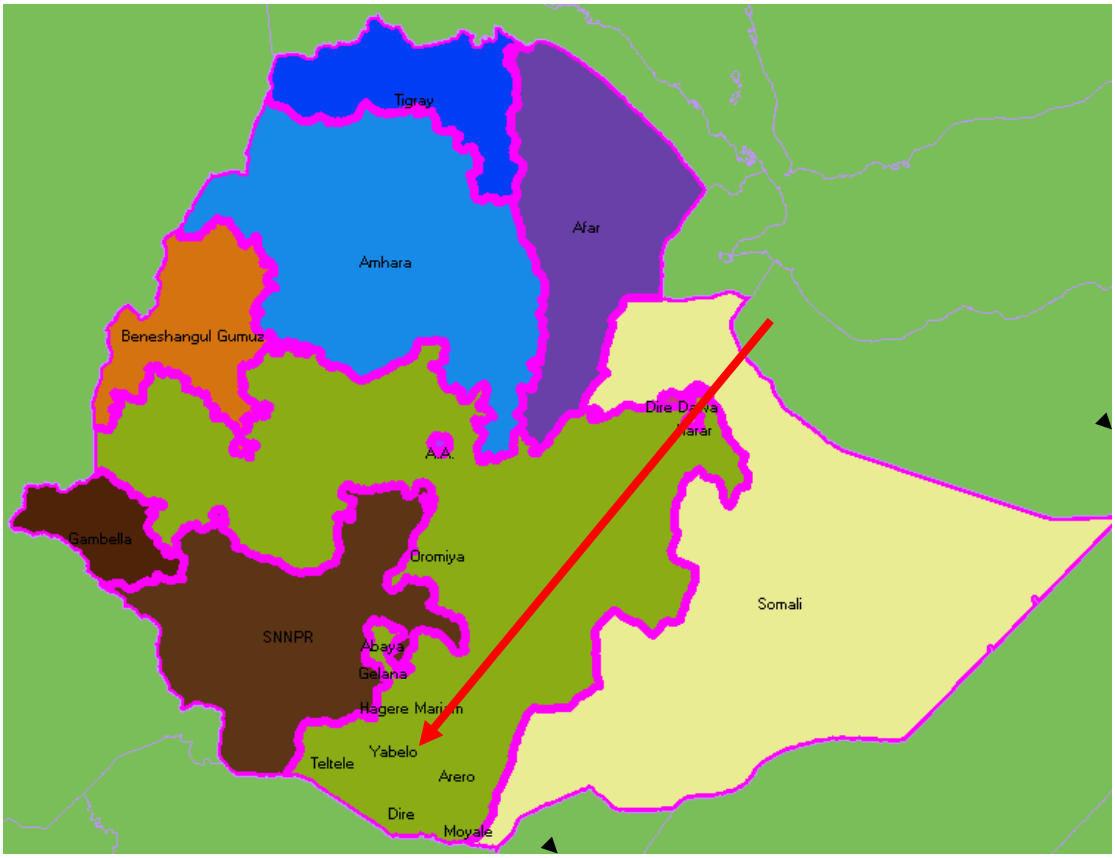

Figure1. Map of Ethiopia, indicating the study area (Yabello, district).

3.2. Study population

The study population was livestock species including cattle, sheep and goats above one year of age and human patients, who were admitted to Yabello hospital, with complaints of fever of unknown origin, back pain and join pain and those human patients, who were admitted to Yabello hospital, with various health problems but identified as a high risk groups. All the target livestock species were managed under the extensive pastoral production systems.

Table 5 Livestock population of Borena zone

No.	Districts	Cattle	Camel	Sheep	Goat	Equines
1.	Abaya	130,000	0	2000	14,000	0
2.	Arero	14,000	4,506	39,073	98,872	6,286
3.	Bule Hora	168,000	0	8,000	41,000	0
4.	D/Dawa	125,428	17,325	30,496	98,872	10,352
5.	Dhas	200,175	25,678	30,011	65,000	24,434
6.	Dillo	98,848	7,201	83,720	80,440	14,732
7.	Dire	89,398	4,506	29, 912	59,874	6,286
8.	Gelana	73,000	0	8000	21,000	0
9.	Melka Soda	220,767	6,572	30,112	78,256	27,312
10.	Miyo	134,650	10,743	13,737	42,657	6,000
11.	Moyale	48,311	5,864	5,468	18,388	2,924
12.	Teltele	197,956	910	59,416	127,640	741
13.	Yabello	232,949	22,972	39,073	98,872	6,986

Source: OPADC livestock population data

3.3. Study design

A cross-sectional epidemiological studies design and structured questionnaire survey were employed for this study from, October 2011 to March 2012, for sampling of both livestock species and human patients in Yabello district and Yabello hospital, respectively.

3.4. **Sampling and sample size determination**

The study area was selected conveniently based on the availability of huge population of livestock, veterinary laboratory (to easily transport and store sample) and hospital with skilled man power for human sample collection. Yabello district mostly entertains large population of livestock, as most livestock of border districts frequently move to it especially during shortage of feed and water, because of its relatively better pasture, mostly after others used up their own and it's relatively many alternative water wells.

A multistage sampling combined with the convenient sampling strategy was employed for sampling of individual animal species. Pastoral association is the smallest administrative unit in the study district. Pastoral associations for the study were selected by randomization after obtaining the total number of PA's in the district. Subsequently six PA's were considered for the present study. As sampling frame for pastoral herds were not available, all accessible herds and individual animals of the herds within the selected PA's were sampled.

Sampling of human patients was performed purposively. As a result, patients admitted to the hospital with signs of fever of unknown origin, join pain and back pain were sampled after reaching verbal consent.

As published, materials on the prevalence of the diseases were not available for both animals and human in the study area. Formula for simple random sampling was adopted for obtaining the desired sample size. Thus sample size (n) was determined based on the formula given by (Thrusfield, 2007). As a result expected prevalence of 50% with 5% desired absolute precision at 95% confidence level was assumed to calculate the desired samples size in ruminants (cattle, sheep and goats).

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

Thus, d = 0.05, 1.96 (CI = 95%), expected prevalence, p = 0.95, 1 - p = 0.05, the desired sample size for p = 0.05 will be n = 384. Thus with the aim of increasing the precision, increasing of sample size was practically possible (Thrusfield, 2007). Accordingly by doubling the sample, it becomes, n=384*2=768.Thus 768 animals was determined to be sampled for each small ruminants and cattle separately from the six PA's. Thus, a sample of n=634 for cattle and n= 736 for small ruminants were finally included in the study. This gaves a total of n =1360 ruminants (cattle, sheep and goat) sampled for this particular study.

Furthermore, human samples were obtained by sampling every individual with suggestive symptoms for brucellosis and those high risk groups with various health problems, admitted to the hospital during the study period. Accordingly, 138 human serum samples were obtained and tested for the presence of *Brucella* seropositivity.

3.5. Study methodology

3.5.1. Blood Sample Collection

Human blood sample was collected from those patients with the history of fever of unknown origin and from the risk groups admitted with different health problems to Yabello hospital. Before blood sample was taken a questionnaire prepared for this purpose was filled by interviewing each individuals at the case team office, during or after they fully complained for their main health problems to the physician. Then after reaching informed consent with the patient or his/her parents, he or she was then sent to the laboratory for blood sampling with the filled questionnaire. Subsequently, about 5ml of blood was collected from each selected individuals aseptically using plain vacutainer tube and left at room temperature overnight to allow clotting for serum separation. The

serum was then collected into cryovials with plastic pipettes and stored at Yabello hospital laboratory at –20°C until transported for serological tests to NAHDIC, Sebeta.

From each animal species, approximately 7-10 ml of blood was aseptically collected from the jugular vein using plain vacutainer tubes and clotted at room temperature for 12 hours. The collected sera was then collected in sterile test tubes and stored at Yabello regional veterinary laboratory at -20°C again until transported to NAHDIC, Sebeta.

Finally, both human and animal serum samples were transported safely in ice box packed with ice and the samples wrapped around with plastic materials to prevent damage of the samples and the labeling, which could arise from leakage, then transported to the National Animal Health And Diagnostic Laboratory (NAHDIC), Sebeta for further processing.

3.5.2. Serological survey

Modified rose Bengal plate test (mRBPT) was used as the screening test and indirect enzyme linked immune sorbent assay as the confirmatory test to estimate the prevalence of brucellosis (I-ELISA) for both study animals and humans.

Modified Rose Bengal plate test (mRBPT)

All serum of livestock and human were first subjected to the modified Rose Bengal plate agglutination test (mRBPT). Serum sample of, 30 µl was mixed with 30 µl of antigen (*B. abortus, strain99,* Lillidale, Diagnostics, Bad, buryview, Bothenwood wimborne Dorset BH21 4HU, United Kingdom) on a white tile or enamel plate to produce a zone approximately 2 cm in diameter, this was performed for human and cattle sample but for sheep and goats 75µl of serum was mixed 25µl of the mRBPT antigen, to increase the sensitivity of the test in small ruminant. The mixture was then agitated gently for 4

minutes at ambient temperature, and then observed for agglutination. Any visible and slight agglutination was considered to be positive. The RBT can be used in all animal species but positive results should be confirmed by a quantitative test. Subsequently I-ELISA assay was performed for further confirmation

Indirect enzyme linked immuno sorbent assay

All sera samples that was positive for RBPT was subjected for a further confirmation by I-ELISA. A commercially available indirect enzyme-linked immunosorbend assay (ELISA) (CHEKIT®-Brucellose, Dr. Bommeli AG, Liebefeld-Bern, Switzerland) was used because it has a better reproducibility of results compared to the RBPT, but is technically simpler to perform than the complement-fixation test (CFT). This assay uses microtiter plates with wells precoated with a lipopolysaccharide–phenol extract of the *B. abortus* 99 Weybridge strain and, as conjugate, a monoclonal anti-ruminant-IgG (also reacting with IgG of different animal species, including human). The protocol of the ELISA manufacturer was followed. The optical densities (OD) of all samples were tested in duplicate to obtain the mean OD. Results were expressed as the percentage of the ratio between the corrected sample OD and positive control OD (S/P-ratio).

3.5.3. Questionnaire survey

Parallel to collection of serum and other appropriate samples, human cases at Yabello hospital were interviewed using a standardized structured questionnaire, which covers demographic data (variables including age, educational level and major occupation), consumption habit (milk, and other animal products), knowledge of zoonosis ,zoonotic risk of milk ,sourcea of milk consumed, close occupational contact with animals and mode of contact (delivery or handling of abortus), . Similarly informations related to the animals like PA, sex, age, herd size history of abortion, parity status, reproductive status and species were also collected using a separate questionnaire format prepared for this purpose.

3.6. Data analysis

Data that was obtained from, serological and questionnaire surveys of both animals and humans were primarily stored in Microsoft excel spreadsheet (Microsoft Corp).The data was summarized first by using a descriptive statistics. Analysis of the explanatory and the outcome variables was performed by using univariable and multivariable logistic regression model, chi square and Fishers exact test was used as appropriate, while questionnaires were analysed using descriptive statistics using the SPSS 15 statistical package (SPSS Inc., Chicago, IL, USA, 2002).

Dependent and independent binary variable were coded as 0 (=no) and 1 (=yes).The major explanatory variables or risk factors considered for all animals species in this study, includes sex, age ,species, parity status ,reproductive status, herd size and history of abortion. For human demographic data, contact with animal consumption of raw milk and its product, fever of unknown origin, delivery assistance and knowledge of zoonosis, ,zoonotic risk of milk and others were considered. Each of the independent variables was categorized in to some levels and studied for association with seropositivity. Screening of variables for logistic regression was also performed using a univariable logistic regression between the dependent and independent variables. Independent variables that had model p values < 0.05 was selected in a preliminary step. For variables that presented strong co-linearity ($p < 0.05$), one of the two variables was excluded based on biological relevance following actions based on the recommendations of Dohoo *et al*. (1996). Prevalence in both livestock species, as well as human, was estimated with the chi-square test or with appropriate, Fisher's exact test. Odds ratio was used to assess the strength of association between exposure variables, associated with seropositivty of the disease in both animals and human.

Potential risk factors significant at $p < 0.05$ in the univariable logistic regression analyses was evaluated using stepwise regression to build a multivariable logistic regression model (Wald test stepwise p-Wald value to enter <0.05). The logistic model was then developed using the stepwise approach. Backward elimination followed by a forward selection was performed simultaneously variable removal was done, using a likelihood ratio test at each step with 0.05 (two-tailed; alpha = 0.05) as significance level for a variable entry and 0.10 was set to be a significance level for a variable removal, for those variables that had exceeding this significance level. Hosemer and Lemeshow goodness of fit test was also evaluated. $P < 0.05$ was taken as significant, for all variables in this particular study.

4. RESULT

4.1. Serological results of animal brucellosis

Out of the total 1360 ruminants sampled (634 cattle, 505 goat and 221 sheep), 10.3% (n=140) ruminants were tested positive on screening using mRBPT. Further, confirmation using an indirect ELISA identified 8.2% (n=112) ruminants seropositivity to *Brucella* antibodies. An overall small ruminants *Brucella* antibody of 11.3% (n= 91) and 9.6% (n=70) was recorded by using mRBPT and I-ELISA, respectively (Table 6).

The highest individual species level seroprevalence of 13.7 % (n=69), was recorded in goats followed by cattle 9.1 % (n=58) and sheep 5.9% (n=13) using mRBPT (Table 6). Using an indirect ELISA the highest species level seroprevalence of 11.7 % (n=58) was again recorded in goats, followed by 6.6% (n=42) in cattle and 4.9 % (n=11) in sheep (Table 6).

The distribution of *Brucella* seropositvity among the different pastoral associations is shown in Table 6. In goats, highest seroprevalence was recorded in Surupha 18.5% (n=25), followed by 11.5% (n=26), 11.5% (n=12), 9.9% (n=8), 7.0% (n=5) and 6.9% (n=6) in Adegelchat, Ela-weya, Didayabello, Haraweyou and Didahara, respectively.

Table 6 Distribution of seroprevalence of *Brucella* antibodies among pastoral associations and animal species in Yabello district, using mRBPT and Indirect ELISA

PA	Species	No. sampled	mRBPT		I- ELISA	
			No. Positive	Prevalence (%)	No. Positive	Prevalence (%)
Adegelchat	Cattle	80	12	15	7	8.8
	Goat	26	3	11.5	3	11.5
	Sheep	16	2	12.5	2	12.5
Dida hara	Cattle	129	16	12.4	12	9.3
	Goat	87	8	9.2	6	6.9
	Sheep	33	1	3	1	3
Dida	Cattle	91	3	3.3	3	3.3
yabello	Goat	81	9	11.1	8	9.9
	Sheep	54	3	5.6	2	3.7
Ela-weya	Cattle	123	7	5.7	3	2.4
	Goat	104	14	13.5	12	11.5
	Sheep	43	2	4.7	2	4.7
Haraweyou	Cattle	123	7	5.7	6	4.9
	Goat	71	6	8.5	5	7
	Sheep	53	1	1.9	1	1.9
Surupha	Cattle	88	13	14.8	11	12.5
	Goat	139	29	21.3	25	18.4
	Sheep	22	4	18.2	3	13.6
Sub total	Cattle	634	58	9.1	42	6.6
	Goat	505	69	13.7	59	11.7
	Sheep	221	13	5.9	11	4.9
Grand	**Ruminants**	**1360**	**140**	**10.3**	**112**	**8.2**
Total	**Small ruminants**	**726**	**91**	**11.3**	**70**	**9.6**

More or less similar prevalence was recorded among the different pastoral associations of Yabello district, while the same prevalence was reported in Adegelchat and Ela-weya PA's. Furthermore, a similar prevalence of 12.5% (n=11), 9.3% (n=12), 8.8 % (n=7), 4.9% (n=6), 3.3% (n=3) and 2.4% (n=3) was recorded in cattle in Surupha, Dida hara, Adegelchat, Haraweyou, Didayabello and Ela-weya respectively. Likewise, in sheep a seroprevalence of 3.6% (n=3), 12.5 (n=2), 4.7% (n=2), 3.7% (n=2), 3% (n=1) and 1.9 %(n=1) was recorded in Surupha, Adegelchat, Ela-weya, Dida yabello, Dida hara and Haraweyou (Table 6).

4.2. Risk factors for seropositivity to animal brucellosis

Univariate logistic regression analysis of the risk factors

Table 7 shows, prevalence and univariable logistic regression analysis of associations of various risk factors with *Brucella* seropositivity. The major exposure variables that were considered to predict the response of the outcome variables includes, age, abortion history, herd size, species ,parity, PA's, reproductive status and sex of the animals. The prevalence of brucellosis is significantly varied in goats, 11.5 %,with the goats being almost 2 times more likely to be infected than sheep(OR=1.86;P≤ 0.05).

The result also shows that female animals (9.6%) are almost 4 times more likely to be seropositive to *Brucella* infection when compared with male animals (OR=3.97; P≤ 0.05).

Table 7 Univariate logistic regression analysis of risk factors and prevalence of *Brucella* seropositivity in ruminants in Yabello district (=1360)

Risk factor	Level	No. sampled	No. Positive	Prevalence (%)	OR	95%CI	P-value
PA	Adegelchat	122	12	9.8	-	-	-
	Dida hara	249	19	7.6	0.76	0.36,1.62	0.472
	Dida yabello	226	13	5.8	0.56	0.25,1.27	0.164
	Ela- waya	270	17	6.3	0.62	0.28,1.33	0.219
	Haraweyou	247	12	-	0.47	0.20,1.08	0.074
	Surupha	246	39	15.9	1.73	0.87,3.43	0.119
Species	Sheep	221	11	5	1	-	-
	Goat	505	59	11.7	1.86	1.23,2.82	0.003
	Cattle	634	42	6.6	0.74	0.34,1.46	0.384
Sex	Male	268	7	2.6	1	-	-
	Female	1092	105	9.6	3.97	1.82,8.62	0.000
Age	1-2 years	345	5	1.4	1	-	-
	3-4 years	422	36	8.5	6.34	2.46,16.34	0.001
	>4 years	593	71	12	9.25	3.69,23.13	0.000
Parity	No parity	219	4	1.8	1	-	-
	1-2	348	37	10.6	6.39	2.25,18.20.	0.001
	>4	525	64	12.2	7.46	2.68,20.76	0.001
Reproductive	Heifer/weaner	190	4	2.1	1	-	-
status	Pregnant	106	8	7.5	3.8	1.12,12.92	0.033
	Lactating	674	78	11.6	6.09	2.2,16.85	0.001
	Dry	122	15	12.3	6.52	2.12,20.15	0.001
Herd size	Small	139	16	11.5	-	-	-
	Medium	494	40	8.1	0.68	0.37,1.25	0.213
	Large	727	56	7.7	0.64	1.16	0.139
Abortion	Absent	985	81	8.2	1	-	-
history	Present	107	24	22.4	3.23	1.94,5.36	0.000

Similarly, *Brucella* seroprevalence was also significantly varied with the age groups, with the odd of seropositivity being 6.34 and 9.25 times higher in animals in 3-4 years and greater than 4 year, respectively, than animals in 1-2 years (Table 7).

Seroprevalence, rate of brucellosis was also significantly varied with parity, with the likelihood of infection being 6.39 and 7.46 times higher in animals between 1-2 and greater than 2 parity, respectively, than in animals with no parity (Table 7).

Animals at different reproductive status, also revealed, a clear higher significant association with *Brucella* seropositvity, with a pregnant, lactating and dry animals being, 3.8, 6.09 and 6.52 times more likely to acquire *Brucella* infection, respectively, compared to heifers or weaner animals (Table 7).

Animal species having a reported history of one or more abortion was found to be significantly associated with *Brucella* seropositivity, with 3.23 times more likely to harbour *Brucella* antibody than non aborting animals (OR=3.23; $P \leq 0.05$).

Although, a high and relatively comparable prevalence rate was recorded in each pastoral associations of the study area, there were no statistically significant associations' found with the reported *Brucella* seropositivity ($P \geq 0.05$).

Likewise, statistically significant variation was not observed among the various levels of herd sizes, when compared to the animals from small herd size; however, similar seroprevalence was recorded among the three levels of herd sizes (Table 7).

Table 8 Univariate logistic regression analysis and Seroprevalence of brucellosis in cattle according to its various risk factors (n=634).

Risk factors	Level	No. Sampled	No. Positive	Prevalence (%)	OR	95% CI	P-value
PA	Dida yabello	91	3	3.3			
	Dida hara	129	12	9.3	3.01	0.82,10.98	0.09
	Adegelchat	80	7	8.8	2.81	0.70,11.27	0.14
	Ela- waya	123	3	2.4	0.73	0.15,3.72	0.71
	Haraweyou	123	6	4.9	1.50	0.37,6.18	0.57
	Surupha	88	11	12.5	4.19	1.13,15.57	0.03
Sex	Male	184	4	2.2			
	Female	450	38	8.4	4.15	1.46,11.80	0.01
Age	1- 2 years	252	3	1.2			
	3-4 years	165	11	6.7	5.93	1.63,21.59	0.01
	>4 years	217	28	12.9	12.30	3.68,41.05	0.00
parity	No parity	144	2	1.4			
	1-2	120	15	12.5	9.04	2.08,39,21	0.00
	>2	186	21	11.3	10.14	2.27,45.31	0.00
Reproductive	Heifer	137	3	2.2			
status	Pregnant	39	4	10.3	5.12	1.09,23.87	0.04
	Lactating	221	27	12.2	6.23	1.85,20.91	0.00
	Dry	122	15	12.3	3.65	0.79,16.88	0.09
Herd size	Small	89	6	6.7			
	Medium	307	20	6.5	0.96	0.4, 2.50	0.94
	Large	238	16	6.7	0.99	0.40, 2.60	0.99
Abortion	Absent	238	16	6.7			
history	present	15	7	46.7	11.40	3.88,33.52	0.00

Table 8 presents results of univariate analysis showing the association of predictor variable and *Brucella* seropositivity. Seroprevalence, recorded for cattle, in surupha PA revealed a statistically significant variation (12.5%), with the odd of seropositivity being 4.19 times higher than Dida-yabello (OR=4.19; p≤0.05). The rest of PA's showed no statistically significant associations regardless of the seropositivity recorded.

Various reproductive status of the animals have also revealed statistically significant association with seropositvity to brucellosis, with the dry, lactating and pregnant, cows being, 3.7 5.12, 6.2 times more likely to encounter *Brucella* infection compared to heifers respectively (Table 8).

The variation observed between sex of cattle was also significant, with female cattle being 4.15 times more likely to be infected with *Brucella* organisms than male cattle (OR=4.15; P≤0.05).

Similarly, significant difference in seropositvity was observed among various age categories, with the cattle between 3-4 years and greater than 4 years of age being 5.9 and 12.3 times more likely to be seropositive, respectively, in comparison with the cattle between 1-2 years (Table 8).

Previous history of abortion in female cattle was found to be significantly associated with seropositivity. The prevalence of *Brucella* seropositivity is high (46.7%) in cattle having a history of abortion, with 11.40 times more likely to be seropositive than animals having no history of abortion. The prevalence observed among different herd sizes in cattle, were not statistically significant (P≥0.05). The prevalence rate was almost similar among the various herd sizes in cattle (Table 8).

Univariate analysis of risk factors associated with *Brucella* seropositvity in small ruminants and its prevalence was presented in Table 9. Significantly higher difference in *Brucella* prevalence was recorded for surupha (17.7 %) with the odd of seroprevalence

being 4.2 time higher than, Haraweyou. The seroprevalence of brucellosis in Adegelchat, was, 2.7 times more likely to be higher than Haraweyou; however, this variation was not statistically significant (P≥ 0.05). Similarly, the variations in the seroprevalence rate that was recorded for the rest of the study PA's was not statistically significant (P≥0.05).

The prevalence of brucellosis is significantly high in goats, (11.5 %), with goats being 2.53 times more likely to be seropositive than sheep (OR=2.53; P≤ 0.05). In female small ruminants the seroprevalence of brucellosis was, (10.4 %). However, the variation in seroprevalence was not statistically significant, for the study sub population of small ruminants (OR=0.32, p≥0.05).

Likewise, *Brucella* seropositvity was significantly varied, with the age of the animals in small ruminants, the seroprevalence being (9.7%) and (11.4%), with almost 5 and 6 times more likely to be *Brucella* seropositive, respectively for animals between 3-4 years and greater than 4 years, than animals between 1-2 years age (Table 9).

Significant variation in seropositivity was also observed in animals having greater than two parity (12.7 %), with 5.3 times more likely to be seropositive than animals with no parity(OR=5.3;P≤0.05). However, the variation in seropositivity recorded for animals having, 1 or 2 parity was not statistically significant (CI; OR=3.9, P≥0.05).The seroprevalence recorded for the different reproductive status in small ruminants revealed a significant difference, for animals in a dry reproductive stage (15.9%), with the odd of seropositvity being almost 10 times higher than the weaned animals (OR=9.9, P≤0.05). However, the variation in seropositivity, that was recorded for pregnant and lactating small ruminants were not statistically significant (P≥0.05).

Table 9 univariate logistic regression analysis of risk factors and prevalence of brucellosis in small ruminants in Yabello district (n=726).

Risk factors	Level	No. Examined	No. Positive	Prevalence (%)	OR	95%CI	P-value
PA	Haraweyou	124	6	4.8			
	Dida hara	120	7	5.8	1.22	0.40,3.74	0.73
	Dida yabello	135	10	7.4	1.57	0.55,4.46	0.39
	Ela- waya	147	14	9.5	2.07	0.77,5.56	0.15
	Adegelchat	42	5	11.9	2.66	0.77,9.21	0.12
	Surupha	158	28	17.7	4.24	1.69,10.59	0.00
Species	Sheep	221	11	5			
	Goat	505	59	11.7	2.53	1.30,4.91	0.01
Sex	Male	84	3	3.6			
	Female	642	67	10.4	0.32	0.10,1.03	0.06
Age	1-2 years	93	2	2.2			
	3-4 years	257	25	9.7	4.9	1.14,21.12	0.03
	>4 years	376	43	11.4	5.88	1.40,24.71	0.02
parity	No parity	75	2	2.7			
	1-2 parity	228	22	9.6	3.9	0.90,16.99	0.07
	>2 parity	339	43	12.7	5.3	1.26,22.40	0.02
Reproductive	Weaner	53	1	1.9			
status	Pregnant	67	4	6	3.3	0.36,30.46	0.29
	Lactating	453	51	11.3	6.6	0.89,48.75	0.06
	Dry	69	11	15.9	9.9	1.23,79.02	0.03
Herd size	Small	50	10	20			
	Medium	187	20	10.7	1.34	0.76,2,37	0.31
	Large	489	40	8.2	2.81	1.31,6.03	0.01
Abortion	Absent	550	50	9.1			
history	Present	92	17	18.5	2.27	1.24,4.14	0.01

Animals from the large herd size category, tends to show siginificant difference to *Brucella* seropositivity (8.2 %), with 2.8 times more likely to be infected with *Brucella* organisms, than animals from the small herd size category (OR=8.2, P≤0.05), while the seropositivity recorded for the animals in the medium herd size was not significant (P≥0.05). Small ruminants, having a history of one or more abortion, depicted both significant variation and association, with those having, no reported history of abortion and seropositivity response to *Brucella* antibody (18.5 %), with 2.3 times more likely of being seropositive to *Brucella* antibody than those with absent history of abortion (OR=2.23; P≤ 0.05).

Multivariable logistic regression analysis of risk factors

Tabele 10 presents results of multivariable logistic regression analysis of potential risk factors that were associated with seropositivity to *Brucella* species antibody. Explanatory variables with a p-value ≤ 0.05, in the univariable logistic regression analysis were included in the final multivariable logistic regression model and also after performing, co-linearity tests, those predictor variables that were found to be co-linear were hindered from entering in to the final multivariable model. Eventually, reproductive status and sex of the animals were found to be significantly correlated with one or more other predictor variables. Reproductive status showed co-linearity with age, sex and parity, while sex of with parity, age and abortion history of the animals.

Accordingly, predictor variable that would most likely to predict the occurrence of *Brucella* infection like age, history of abortion, parity status and species of the animals were included in the final logistic regression model. Thus, final selection of the best potential risk factors or the exposure variable that would likely best explains the response of the predictor variable was based on a stepwise forward selection followed by the backward elimination procedure.

As a result separate model was built for over all ruminant effect and for cattle and small ruminant groups, using the same risk factors fulfilling the criteria to be included in the final logistic regression model for each groups animal groups.

According to the multivariable logistic regression model fitted for the overall effect of ruminant *Brucella* positivity, increasing age of the animals was identified to be the most important risk factor for the exposure to *Brucella* infection, animals with greater than 4 years (OR=7.5, CI; 2.9, 19.3) are more likely to be infected than animals in the 3- 4 years (OR=4.2, CI; 1.4, 11.3) followed by the animals in 1-2 years (Table 10).

Table 10 Multivariable logistic regression analysis identifying potential risk factors for seropositivty to brucellosis in ruminants (cattle, sheep and goat).

Variables	Level	SE	OR	95% CI for OR	P-value
Age	1 -2 years				
	3- 4 years	0.50	4.24	1.59,11.29	0.00
	> 4 years	0.48	7.51	2.91,19.34	0.00
Parity	No parity				
	1-2 parity	0.53	5.41	1.86,15.64	0.00
	>2 parity	0.54	6.77	2.41,19.06	0.01
Abortion	Absent				
history	Present	0.27	2.66	1.56,4.52	0.00
	Sheep				
Species	Goat	0.34	2.5	1.28,4.88	0.01
	Cattle	0.35	1.97		

Similarly increasing in parity status of the animals was more likely to be associated with the increasing risk of getting *Brucella* infection when evaluated collectively for livestock species.

Thus animal with multiple parturition were at higher risk of encountering *Brucella* infection (OR=6.8, CI; 2.4, 19.1) followed by animals having a single or two parity (OR=5.4, CI; 1.9, 15.6) than animals with zero or no parity. A history of abortion that was reported for animals, when seen collectively, for all animal species was found to be associated with *Brucella* seropositivity (OR=2.7, CI; 1.6, 4.5).

Furthermore, the result of multivariate logistic regression model has indicated that, goats were found to be at higher risk of exposing to *Brucella* infection (OR=2.5, CI; 1.3, 4.9) than cattle and sheep (Table 10).

Table 11 presents results of multivariable logistic regression analysis showing important risk factors for *Brucella* seropositivity in cattle. Thus separate multivariable logistic regression model was built for cattle sub study population similar to that built for over all ruminants. Accordingly, explanatory variables with p-value ≤ 0.05 in the univariable logistic regression model where included in the separate multivariate logistic regression model fitted for cattle. As a result, parity, age and abortion history of the animals were, included in the final logistic regression model. The rest of the variables were not included in the final model, either because of its not significant or had showed co-linearity with one or more other explanatory variables.

The result of the multivariate logistic regression analysis depicts that animals involved in age group greater than 4 years are more likely to be at higher risk for *Brucella* infection than animals in 3-4 years and 1-2 years (OR=7.9,CI;1.7,35,7). Similarly, the multivariate analysis revealed that *Brucella* seropositivity is an important risk factor for the

occurrence of abortion in cattle with a reported history of *Brucella* infection (OR=CI; 3.7, 52.1).

Animals with the parity status between 1-2 were found to be an important risk factor for *Brucella* seropositivity (OR=10.1, CI; 2.27, 45.3) and those with a multiple parturition were also found to be at the next higher risk of acquiring *Brucella* infection, compared to animals having no any parity status (OR=9.0, CI; 2. 1, 39.2).

Table 11 Multivariable logistic regression analyses identifying potential risk factors to *Brucella* seropositivity in cattle

Variables	Level	SE	OR	95% CI for (OR)	P-value
Age	1 -2 years				
	3- 4 years	0.83	4.60	0.91,23.22	
	> 4 years	0.77	7.85	1.72,35.73	0.01
Abortion	Absent				
history	Present	0.67	13.94	3.73,52.11	0.00
Parity	No parity				
	1-2	0.76	10.14	2.27,45.31	0.00
	>2	0.75	9.04	2.08,39.21	0.00

Table 12 presents the multivariate logistic regression analysis showing the association of the risk factors with *Brucella* seropositivity in small ruminats. The risk factors that would most likely to predict the outcome of the response variable were also selected by referring to the results of the univariate regression analysis of the small ruminants to include in to multivariate regression analysis.

Here again co-linearity test was performed. As a result, reproductive status and sex had shown co-linearity with one or more variables and were not offered to multivariate regression model. Reproductive status had shown co-linearity with the age and parity,

while sex with the age, parity and abortion history. The rest of the variables those were significant at P-≤0.05, in the result of the univariate analysis of the small ruminants were offered to the final multivariate logistic regression model. Thus stepwise forward selections followed by back ward elimination of the predictor variables were performed.

Table 12 Multivariate logistic regression analyses of important risk factors associated with the *Brucella* seropositivity in small ruminants

Risk factor	Level	SE	OR	95%CI	P-value
Species	Sheep				
	Goat	0.36	2.2	1.11,4.33	0.02
Herd size	Small				
	Medium	0.33	1.7		
	Large	0.44	3.04	1.31,7.04	0.03
Age	1 -2 years				
	3-4years	1.04	6.55		
	> 4 years	1.03	10.1	1.34,76,01	0.00
Abortion	Absent				
history	Present	0.33	2.1	1.10,3.88	0.03

The result of the logistic regression analysis of small ruminant animals revealed that goats were at higher risk of getting *Brucella* infection (OR=2.2, CI; 1.1, 4) than sheep.

Similarly, the result shows that small ruminants that were maintained in the large herd category were more likely to encounter *Brucella* infection than those maintained in the medium and small herd or flock (OR=3.0, CI; 1.3, 7.0).

Seropositivity to *Brucella* infection was found to be highly associated with animals greater than 4 years age (OR=10.1, CI; 1.3, 76.0) than in those in 3-4years and 1-2 years.

Furthermore, aborting animals was also found to be at higher risk to *Brucella* seropositivity, than those animals with no reported history of abortion (OR=2.1CI; 1.10, 3.9).

4.3. Serological results of human brucellosis

Table 13 presents seroprevalence results of brucellosis in Yabello hospital using RBPT and I-ELISA, respectively. An overall prevalence of 11.6% (n=16) and 3.6 %(n=5) was recorded at Yabello hospital from various patients admitted to the hospital, using mRBPT and I-ELISA, respectively. Seroprevalence of 7.2% (n=11) and 2.5% (n=2) was recorded in female human patients by using mRBPT and I-ELISA, respectively. Similarly, in male sex the seroprevalence of 8.5 % (n=5) and 5.1 % (n=3) was recorded again using mRBPT and I-ELISA, respectively. However the variation in the seroprevalence was not statistically significant ($p \leq 0.05$).

Table 13 Distribution of seroprevalence of human brucellosis in Yabello hospital (n=138)

		mRBPT		Indirect ELISA			
Sex	No. Examined	No. positive	Prevalence (%)	No. positive	Prevalence (%)	X^2	P-Value
Female	79	11	13.9	2	2.5	0.36	0.65
Male	59	5	8.5	3	5.1		
Total	**138**	**16**	**11.6**	**5**	**3.6**		

Table 14 Seroprevalence of brucellosis in human patients admitted to Yabello hospital and its assocciation with demographic risk factors

Risk factor	No. Examined	MRBPT		I- ELISA		OR	95% CI	X^2	P-Value
		No. positive	Prevalence (%)	No. positive	Prevalence (%)				
Age									
<=15	8	2	25	1	13	0	10,35		
16 -30	45	4	8.9	1	2.2	2	2, 64		
31 - 45	48	4	8.3	2	4.2	1	0.9,10	2.78	0.60
46 - 60	21	4	19	1	4.8	1	4,14		
>60	16	2	13	0	0				
Sex									
Female	79	11	14	2	2.5	2	1.0,10.	0.36	0.65
Male	59	5	8.5	3	5.1	2	1.0,12		
Education									
Illiterates	97	13	13	4	4.1	1	0.0,8		
Primary	35	2	5.7	1	2.9	1	3.0,8.0	0.35	0.84
Secondary	6	1	17	0	0				
Occupation									
Livestock keeper	107	13	12	5	4.7	1	1.0,9.0	1.5	0.68
Civil servant	5	0	0	0	0				
Trader	16	1	6.3	0	0				
Student	10	2	20	0	0				

Table 14 presents seroprevalence status of human brucellosis in Yabello hospital and its association with various demographic risk factors, using I-ELISA to estimate the seropositivity. Higher prevalence rate was recorded for age group ≤15 years (12.5%), followed by a prevalence rate of 4.8%, 4.2%, 2.2% and 0% in age groups 46-60,31-45,16-30 and > 60 years. However the variation in seropositvity among different age groups revealed no significant difference (P≥ 0.05).

Similarly, the seroprevalence rate of 4.1%, 2.9% and 0% that was recorded for peoples found illiterate, primary and secondary levels of education, respectively, was not also showed significant variation (P ≥ 0.05). Higher prevalence rate was recorded for illitrate people (4.1%). Human patients having occupational tendency of livestock keeping had shown a prevalence rate of (4.7%). However, this finding was also not statistically justified (P≥ 0.05). In contrast, no seropositivity was recorded for traders, students and civil servants (Table 14).

Table 15 presents seropositivity to brucellosis in human at Yabello hospital and its association with knowledge of zoonosis and disease symptoms suggestive of brucellosis. In the the present study the prevalence of brucellosis seropositivity was high in those people having no knowledge about zoonotic risk of milk was (12%) by using RBPT However, the seropositivity recorded using I-ELISA (3.2%). However the seroprevalence recorded was not statistically significant. Similary, seropositivity of 9.5% and 4.8% was recorded by using RBPT and I-ELISA, respectively, in those people having the knowledge, which infected milk could be a source of zoonotic disease to human. However, the variation in prevalence between the two groups was not significant (P≥ 0.05).

Table 15 Seroprevalence of brucellosis in human patients admitted to Yabello hospital and its assocciation with knowledges of zoonosis and disease symptoms experienced

		MRBPT		I- ELISA					
Risk factor	No. Examined	No. positive	Prevalence (%)	No. positive	Prevalence (%)	OR	95% CI	X^2	P-value
Zoonotic risk of milk									
No	117	14	12	4	3.2	1.1	0.1,7.	0.09	0.57
Yes	21	2	9.5	1	4.8	0.8	4,14		
Disease symptom									
None	19	3	16	2	10.5	0.3	3,24		
Fever & sweat	35	5	14	2	5.7	1.6	2.0,13		
Fever and Insomnia	15	1	7	0	0				
Headache, fever sweat and	43	7	16	0	0		2,7	4.78	0.44
Headache, fever, back pain and joint pain	10	0	0	1	2.3	1.6			
Zoonotic risk of in fected animal									
No	95	12	13	4	4.2	0.8	0.1,8	0.3	1
Yes	43	4	9	1	2.3	1.6	2,7		
Zoonotic risk of Abortus									
No	114	11	10	3	2.6	1.4	0.1,10	1.85	0.21
Yes	24	5	21	2	8.3	0.43	3,20		

Seropositivity of the disease was high in those sampled people but having no any symptoms suggestive of brucellosis (10.5%).But this variation was not associated with seropositivity (P≥ 0.05). Likewise, the prevalence rate recorded in those people having knowledge of disease transmitted during handling of infected animal and handling abortion materials was also not statistically significant (P≥ 0.05). Table 16 presents the association of *Brucella* seropositivity with some human practices and behaviours. Thus higher prevalence was recorded in those patients rearing cattle sheep and goat (9.1%) by using mRBPT. However, the seropositivity was not statistically significant in this individual (P≥ 0.05).

Similarly, the seroprevalence of (7.4%) which was recorded for those herders rearing cattle, sheep, goat and camel, was not statistically significant (p≥0.05). In general, all prevalences, which were recorded for people living in close contact with animals, those taking part in delivery assistance and those consuming different type of milk products were not significantly (p ≥0.05) associated with seroprevalence of brucellosis recorded for each people (Table 16).

The Seroprevalence of 4.7% was recorded for those patients who get their milk at home from their own animals, while at the same time; no prevalence was recorded for those patients who purchase milk from market 0%. However, the difference in seroprevalence that was recorded for those using milk, which was milked at home and those who consume milk by purchasing from market was found to be not statistically significant (P≥ 0.05).

Table 16 presents the association between *Brucella* seropositivity and human behaviours, practices and attitude related risk factors

Risk factor	No. Examined	MRBPT		I- ELISA		OR	95% CI	X^2	P-value
		No. positive	Prevalence (%)	No. positive	Prevalence (%)				
Species owned									
No animal	25	0	0	0	0				
Cattle	25	5	20	1	4	0.89	4,12		
Goat	1	0	0	0	0			4.44	0.62
Cattle and Camel	11	0	0	0	0				
Cattle, goat and Sheep	22	2	9.1	0	0				
Cattle,camel,goat and sheep	54	9	17	4	7.4	0.48	0.1,14		
Preferred milk									
Cattle	98	13	13	4	4.1	0.89	0.1,8		
Camel	5	0	0	0	0			1.69	0.89
cattle and camel	16	1	6	0	0				
cattle and goat	19	2	10.5	1	5.2	0.48	7,22		
Close contact									
No	43	4	9	1	2.3	1.62	2,7	0.3	1
Yes	95	12	13	4	4.2	0.84	0.1,8		
Delivery assistance									
No	43	4	9	1	2.3	1.62	2,7	0.3	1
Yes	95	12	13	4	4.2	0.84	0.1, 8		
Milk consumed									
Raw milk	42	7	17	2	4.8	0.74	2,11		
Raw milk and Tea with milk	53	5	9	2	3.8	0.95	2,9	2.1	0.35
Raw milk. Yoghourt	19	2	11	0	0				
Raw milk, Butter milk	6	1	17	1	16.7	0.17	13,47		
Milk source									
Home	108	13	12	5	4.7	0.77	1,9	1.5	0.26
Purchased	30	3	10	0	0				

5. DISCUSSION

The present study recorded an overall ruminant *Brucella* antibody seroprevalence of 10.3% and 8.2 % in Yabello district of Borena pastoral area, by using mRBPT and I-ELISA as the screening and confirmatory test, respectively. Indirect ELISA was used in the present study as the final confirmatory test to report the *Brucella* seroprevalence in animals and humans. In the present study the seroprevalence of brucellosis in cattle was 9.5% and 6.6 % using mRBPT and I- ELISA, respectively. Similarly, an over small ruminants *Brucella* seropositivity of 11.3% and 9.6% was recorded by using mRBPT and I-ELISA, respectively (Table 6).

The prevalence detected by I-ELISA in cattle in the present study was moderately in agreement with the findings of some authors in different parts of Ethiopia: 4.9% by Shewit *et al. (*2008), 4.9% by Mekonnen *et al*.(2010) and 4.6% by Hailemelekot *et al*.(2007). However, a relatively lower prevalence of 3.5 % by Megersa *et al*. (2011), 3.2% by Berhe *et al*.(2007),3.1% by Ibrahim *et al*. (2010), 1.7% by Gelaye *et al*.(2010), 3.1% by Nuradis (2009),) and 2.9 % by Teshale *et al.(*2007) were reported.

A comparable finding to the present study was reported in other African countries: 6.6% in Ghana, by Kubufaor *et al. (*2000), 7.3% in Tanzania by Sewai *et al*. (2010) and 6.6% in Chad by Schelling *et al*. (2003). On the other hand, a consistent prevalence with the present finding was reported in Ethiopia: 8.1% by Asfaw *et al.* (1998), 8.9% by Molla (1989) and 10.8% by Bekele *et al.* (2000).

Higher prevalence than the present study was reported as 46.8% in Uganda by Kungu *et al. (2010),* 41% in Togo by Domingo (2000) and 14.2% in South Africa by Manhica (2011). The difference in the prevalences recorded in the different study area may be

associated with the differences in agro ecology, management system, tests used to detect *Brucella* seroppositivity and sample sizes used in each study.

In the present study, an overall prevalence of small ruminants *Brucella* seropositivity of 11.3% and 9.6% was recorded by using mRBPT and I-ELISA, respectively (Table 6). The overall small ruminants *Brucella* prevalence detected by I-ELISA in the present study is higher than the finding of 1.6% by Teshale *et al*. (2006) in Somali pastoral area, and 4.8% by Ashenafi *et al*. (2007) in Afari pastoral area. Conversely, higher overall small ruminant prevalence of 16% was recorded in, Afar pastoral area by Teshale *et al*. (2006).

Prevalence of 13.7% in goats and 5.9% in sheep by using mRBPT as screening test and 11.7% in goats and 4.9% in sheep by using I ELSIA as the confirmatory test was recorded in the present study (Table 6). The prevalence obtained in this study using I-ELISA is higher than the findings of, 1.7% in goats and 1.6% in sheep by Teshale *et al*. (2006) in Somali pastoral area, 5.8% in goats and 3.2% in sheep by Ashenafi *et al.* (2007) in Afar region, 1.3% in goats and 1.5% in sheep by Tekleye and Kasali (1989) in central highlands of Ethiopaia, 1.7% in goats and 1.6% in sheep by Yibeltal (2005) in Somali region and 3.2% in goats and 1.6% in sheep by Mengistu (2007) in southern Ethiopia.

However, a comparable prevalence of 3.2% by Ashenafi *et al*. (2007) in sheep was recorded in Afar region. In contrast a considerably higher prevalence of 45.8% by Ojo *et al*. (2007) was reported in goats in Nigeria, than that reported by the present study.

The variations in results obtained between the present study and others may be explained by the fact that, I- ELISA could differentiate whether the infection is chronic (IgG) or acute (IgM). Further, the I-ELISA does not use, whole cell antigens, but it uses cytosolic S-LPS fragments, thus decreasing the cross-reaction with other gram negative bacteria

that is seen with the other serologic tests better (Corbel, 2006; Franco *et al*., 2007; Araj, 2010).

The prevalence of 4.2% reported by Tadele *et al*. (2011) in goats in south omo zone was lower than the present finding of 11.7% using I-ELISA. Similarly, the present finding in prevalence is higher than the, findings of various authors: 4.1% in goats and 1.6% in sheep by Benkirane (2006) in Morocco and 3.8%in goat and 1.4% in sheep by Omer *et al*. (2000) in Eritrea.

These differences could be mainly due to the variation in sensitivity and specificity imparted by the various tests, agro-ecological location and amount of sampled study population, management and production systems.

The present finding is comparable to the finding of 16.5% by Teshale *et al*. (2006), and 16.2% % by Yibeltal (2005) in goats in Afar regions, respectively. Higher finding than the present study was reported as 14.6% by Teshale *et al*. (2006) and 14.6% by Yibeltal (2005) in sheep in Afar pastoral area. Similarly, comparable prevalence of 16.7% by Benkirane (2006) was reported in goats in Sudan, 12% in goats and 2.2% in sheep by Benkirane (2006) in Algeria, 18.0% in goats and 4.0% in sheep by Benkirane (2006) in Tunisia. However, lower *Brucella* prevalence of 1.5% by Sefinew *et al.* (2010) was reported in sheep in Wollo.

The univariate logistic regression analysis of the risk factors that would likely associated with the seropositivity to ruminant brucellosis revealed that species, sex age, reproductive status, parity and history of abortion to be significantly associated with the seropositivity ($P \leq 0.05$) to *Brucella* antibodies (Table 7). Similarly the multivariable logistic regression analysis of the risk factors in ruminants also identified that age, parity, presence of abortion and species to be the major risk factors that are involved in the seropositivity to brucellosis (Table 10).

Likewise, the univariate logistic regression analysis of risk factors for cattle revealed that Surupha pastoral association was significantly associated with prevalence recorded ($P \leq 0.05$). The odd of the disease in this PA was 4.2 times higher than the Dida Yabello PA or the reference category (Table 8).

Thus, the result of multivariable logistic regression analysis indicated that, the odd of seropositivity in animals in 3-4 years and greater than 4 years were almost 9 and 5 times more than that of the animals in 1-2 years (Table 10).

Similarly, aborted animals are 13 times more likely to be at risk of acquiring *Brucella* infection, when compared to none aborted. Multiple parities and 1-2 parities were also found to be the risk for encountering *Brucella* organisms, with 10 and 9 times more likely to be seropositive to *Brucella* organisms, respectively, when compared to animals with no parity (Table 10)

On the other hand, the univariate logistic regression analysis of the effect of the risk factors on seropositivity to *Brucella* identified that, the prevalences recorded for sex, age, reproductive status, parity and abortion history of cattle were significantly ($P\leq 0.05$) associated with the seropositivity to *Brucella* organisms (Table 8). Likewise, the multivariable logistic regression also revealed that cattle in the different age groups, abortion history and parity status of the animal to be the most important risk factors for acquiring *Brucella* infection in cattle (Table 11).

Female animals were found to be 4.2 times more likely seropositive than male animals. Similar to the result of the present study, higher seroprevalence of bovine brucellosis in females than males was recorded by Asfaw *et al.* (1998) by Tolosa *et al.* (2008) Kebede *et al.* (2008) and Nicoletti (1984). The reason was explained by Kebede et al. (2008) that

males are kept for relatively shorter time duration in breeding herd than females and thus the chance of exposure is lower for males.

A statistically significant difference was also observed in seroprevalence of cattle brucellosis among different age groups as identified by the multivariate and univariate logistic regression analysis ($P \leq 0.05$). The odd of the disease being 12.3 and 5.9 times higher in animals in 3-4 years and greater than 4 years, respectively, than in animals in 1-2 years .

A significantly higher seroprevalence in older than younger animals can be attributed to the practice of leaving youngers around home premises, when adult cattle were taken to graze communally, lessening the risk of youngers acquiring the infection from common grazing and watering grounds (Kiputa *et al*., 2007), this is common phenomenon in Borena pastoral areas.

Henk *et al*. (2004) also explained that age of cattle determines the extent of disease challenge in a herd and primarily governs the course of bovine brucellosis following animal exposure. Radostits *et al*. (2007) pointed out that sexually matured and pregnant animals are more susceptible to infection with *Brucella* organisms than sexually immature animals of either sex.

Parity of animals had also shown statistically significant variations in seroprevalence, with the odd of the disease being 10.0 and 9.0 times higher in multiple parities and in animals with 1-2 parity, when compared with animals with zero parity. The present finding is also in consistent with the finding of Shewit *et al*. (2008), who reported the association of seropositivity with parity. However, the finding of the association of parity numbers with the seropositivity in the present finding is inconsistent with that of Yilkal *et al*. (1998*).*

The univariable logistic regression analysis, which was performed for various reproductive status indicated that, the pregnant and lactating animals were highly associated with the seropositivity to *Brucella* antibodies. The odd of the disease being 5.1 and 6.2 times more than the heifers (reference category), while no association with seropositivity to *Brucella* antibodies was recorded for dry animals. This could be explained by the fact that, susceptibility of cattle to *B. abortus* infection is influenced by the age, sex, and reproductive status of the individual animal (Radostits *et al*., 2007).

Presence of higher seropositive pregnant animals may also be due to the preferential localization of *Brucella* in the uterus in which allantoic fluid factors such as erythritol could stimulate the growth of *Brucella* and elevate in the placenta and fetal fluid from about the second trimester of pregnancy (Coetzer and Tustin, 2004;Radostits *et al*., 2007).

The presence of abortion history in animals was also significantly associated with the seropositivity to *Brucella* antibodies. That is aborted cows were found to be 11 times more likely to be seropositive when compared to non aborted cows. This finding is consistent with the findings of Tolosa (2004) and Hailemelekot (2005) who have reported significant association between abortion and seropositivity in intensive and semi intensive management systems.

The univariate logistic regression analysis also identified that species, sex, age, parity, reproductive status, herd size and abortion history to be significantly ($P \leq 0.05$) associated with seropositivity to *Brucella* organism (Table 9) in small ruminants. On the other hand, the multivariate logistic regression analysis of the effect of the risk factors on small ruminant seropositivity to *Brucella* antibodies identified age, abortion history, species and herd size to be significantly associated with the seropositivity to *Brucella* antibodies (Table 12).

The present study, revealed a relatively higher seroprevalence of brucellosis detected by I-ELISA in goats (11.7%) than sheep (4.9%). The difference in seropositivity between sheep and goats in the present study was statistically significant ($P \leq 0.05$).) with the odd of the disease, as identified by multivariablee logistic regression analysis, being 2.2 times higher in goats than in sheep.

This difference in seroprevalence between sheep and goats could be associated with the difference in proportion of sheep and goats in the herd or flock, which were included in the present study. Similarly, Because of the higher preference to goats than sheep, Borena pastoralists, keeps relatively lower number of goats than sheep with in a herd of goats. In addition, in Borena pastoral area, goats and sheep are reared mixed, where both species graze and corralled together at night in the same pen.

In addition, sheep are more resistant than goats and they do not shade the bacteria for long time. Excretion from the vagina in goats is more copious and prolonged than in sheep and lasts for atleast 2 -3 months and the goats are considered the principal host of *Brucella melitensis*, notably in Latin America, where, sheep are not significantly infected even when kept in close contact with goats (Alton,1985).

In the present study larger herd sizes was found to be significantly associated with *Brucella* seropositivity in small ruminants (Table 9). The multivariable logistic regression analysis identified the odd of seropositivity in large herd sizes to be 3.0 times higher than the seropositivity in small herds (Table 12). The present finding agrees with the previous studies where brucellosis was associated with large herd size (Abela, 1999; Omer *et al*., 2000; Kabagambe *et l*., 2001; Al-Majali, 2005). Larger herds were more likely to have at least one positive animal than smaller herds (Al-Majali, 2005). The number of susceptible animals is usually greater in large herds (Koopman and Longini, 1994). Another possible explanation could be that in pastoral areas larger herds were usually kept closely in a small confinement during night rest.

These are typically more difficult to control and allow for closer contact between animals and their environment which increases potential exposure to infectious excretions; which can in part be attributed to confinement systems being more frequently used with large herds. These systems in turn increase this exposure.

On the other hand, the number of direct and indirect contacts that a small-ruminant has with potential outside sources of infection may increase as the herd expands in size. (Stegeman *et al*., 1999; Gardner *et al*., 2002). It is generally accepted that an increase in herd size is usually accompanied by an increase in stocking density and increase in risk of exposure to infection especially following abortions (Nicoletti, 1984; Salman and Meyer, 1984). Herd size is an important determinant of the potential for transmission between susceptible and infected animals (Omer *et al*., 2000).

Similarly, the variations observed in prevalence among the various age groups were also found to be significantly associated with *Brucella* seropositivity in small ruminant (Table9). The, multivariable logistic regression analysis also revealed an increase in age as an important risk factor for acquiring *Brucella* infection. The risk of acquiring *Brucella* infectio*n* being 10.1 times in small ruminants greater than 4 years when compared to those between 1-2 years (Table 12). This could be explained by the fact that infection causes disease only in adult and sexually mature female and males. Young animals may be infected but do not show any clinical sign and generally show only a weak and transient serological response. However, susceptibility increases after sexual maturity and especially with pregnancy (Radostits *et al*., 2007).

In the present study *Brucella* seropositivity was also found to be strongly associated with presence of abortion history in small ruminant animals, with the aborted animals being 2.1 times more likely to be seropositive to *Brucella* infection, when compared to non aborted animals (Table 12). This finding was supported with Radostits *et al*. (2007) who

stated that late abortion and premature or full term birth of dead or weak kids or lambs predominated in pregnant animals with brucellosis.

In general, the distribution of *Brucella* antibodies among different animal species and different pastoral associations of the study area was observed to be more or less similar. This might be attributed to the increased, repeated and constant in contact among different individual animals, species and herds, owing to the common grazing lands and water points. The current global climate change is being contributing a lot for the establishement and increasingly changing of the epidemiology of different infectious livestock and human diseases including brucellosis.

Increasing change in climate is resulting in the shortening of the drought cycles where either, serious or mild drought is common every year in the area. This is now a common phenomenon in lowland pastoral areas. Borena is one these areas being repeatedly and constantly hit by recurrent drought, which results in shortage of animal feed, water and increasing suceptiblity to infectious diseases, and increasing risk of getting infections.

Thus, as the means of drought mitigation and coping strategies, during hard times and shortages, there is a trend in pastoral setting, that pastoralists with their livestock are forced to seasonally move from the different pastoral association ,districts and even by crossing national borders to share water, grazing lands and even accommodations, by travelling several kilometers. This results in massive concentration of animals around water points and on the same grazing land, with a relatively better pasture. This in turn, may contribute to the increased transmission of *Brucella* organisms among different animal species and herds, resulting in a similar distribution of the *Brucella* infections among the various animal species in the different pastoral associations and again congregation of a large number of mixed ruminants at water points facilitates disease spread. Helland (1982) also pointed that in dry areas, water resources are sparsely distributed.

Mobility also increases the opportunity of interactions with wild animals. This has already been confirmed by (Samui *et al*., 2007; Megerssa *et al*., 2011) in that herds coming into contact with wildlife had higher likelihood of acquiring infection than those without contact.

Moreover, it was explained that mobile herds have greater opportunity to come into contact with other potentially infected herds during their movement into the different areas (Omer *et al*., 2000). Furthermore, migration increases the chance of coming into contact with geographically limited or seasonally abundant diseases and also increases the opportunity for interactions of domestic and wild animals (Macpherson, 1995)

In general, it was described that the prevalence of brucellosis is relatively high in pastoral production systems, and increased as herd size increases (McDermott and Arimi, 2002). Similarly, in other study, it was stated that herds of bigger size were found to be more frequently infected than smaller herds (Hellmann *et al*.1984).

In the present study an overall human *Brucella* seropositivity of 11.6% and 3.6% were identified by using mRBPT and I- ELISA, respectively (Table 13). The present seroprevalence recorded by using I-ELISA is fairly in agreement with the findings of 5.8% by Kassahun (2004) in Sidama zone, Southern, Ethiopia, 3.4% by Tolosa (2004), 3.8% by Hailemelekot (2005). Omer et al. (2002) also reported seroprevalence of 4.6% in Eritrea. However, in contrast to the present finding higher prevalence of human brucellosis was 34.9% and 29.4% by Smiths *et al*. (2009) in Borena and Hamer, pastoral areas respectively, 16.5% by Yimer *et al* (2008) in Afar pastoral area, 12% by Gebreyessus (2001) and 9.0% by Mussie (2005) at Debre zeit.

The prevalence of 2.5% and 5.1% recorded by the present finding in Yabello hospital, in female and male humans, respectively, were not statistically associated with the *Brucella*

seropositivity (Table 13). The seropositivity recorded in humans among the different age groups of the individuals, educational level and occupation were found to be not statistically associated ($P \geq 0.05$) with the *Brucella* seropositivity (Table 14).

Similarly, the prevalences recorded on the individual's knowledge on zoonotic risk of brucellosis, those experiencing various disease symptoms suggestive for brucellosis, the zoonotic risk of coming in contact with the infected animals and aborted materials, were again not statistically significant($P \geq 0.05$) with the *Brucella* seropositivity (Table 15).

Furthermore, rearing different livestock species, the consumption of different milk produced by different animal species, coming in close contact with the animal species, delivery assistance, consumption of different type of milk, and the sources of milk consumed were all not associated ($P \geq 0.05$) with the *Brucella* seropositivity (Table 16).

6. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the present study revealed that brucellosis is prevalent in the study area in cattle, sheep, goats and humans. The multivariable logistic regression analysis identified that age, species, parity and presence of abortion to be the major risk factors for *Brucella* seropositivity in cattle and small ruminants. The result also showed that goats are at higher risk of getting brucellosis than sheep. Increase in herd size was also found to be an important risk for acquiring *Brucella* infection in small ruminant species. The prevalence of human brucellosis obtained from Yabello hospital might give an insight that brucellosis could pose a public health hazard, especially in those high risk groups, mainly the pastoralists in the study area. Because of their constant and increased interaction with their animals, pastoralists could be at a high risk of occupational infection. Based on the present findings the following recommendations are worth mentioning:

- Different livestock species need to be kept and maintained separately to reduce the risk of transmission of brucellosis among them.
- Public education and awareness rising among pastoralists, on the public health hazard of brucellosis need to be undertaken.
- Further extensive epidemiological studies needs to be undertaken to investigate the transmission dynamics of brucellosis in human and animals in the study area
- Molecular investigations of brucellosis, needs to be conducted to identify the specific species prevailing in the study area.

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8. ANNEXES

Annex 1. Questionnaire Formats 1 for individual sampled animals

District _____PA _____Village _____GPS_____ Date_____

No	Owner	Species	Sex	Age	Parity status	Reproductive status*	Herd size	Abortion history	Blood sampel	Milk milk	Discharge /abortus
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											

Annex 2. Questionnaire Format 3 for patients admitted to health institutions with suspected symptoms of brucellosis.

Name _____Age___ Sex___ Education_____ District _____ PA____ Date____

Village _____ Occupation of interviewee: a. Livestock keeper b. Veterinary c. Civil servant d. Trader e. Students

1. Animal species owned: a. Cattle b. Camel c. Sheep d. Goat

2. Which one of the following milk products did you often drink during last 6 months?

a. Raw milk b. Yoghourt c. Butter milk (Areera) d. Tea with milk e. Butter

3. Which animal milk do you often drink? a. cattle b. camel c. goat d. sheep

4. Have you experienced one of the following symptoms, which lasted for 15 days,during the last 6 months? a. Headache b. Prolonged intermittent fever with sweat c. Back pain d. Lack of sleep /Insomnia e. Joint pain f. Weakness

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

6. Do you have close-contact with animals? a. Yes b. No

10. Do you assist animals during delivery? a. Yes .b. No

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

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

13. Where is the source of milk you consume? a. Milked at home b. Market/purchased

Annex 3. Rose Bengal plate test techniques

Description of the test

An antigen prepared from *B.abortus* (strain 99) stained with rose Bengal dye and suspended in acid buffer (PH 3.65). Used to detect *Brucella* antibodies in serum - using a plate agglutination test. It detects antibodies against *B. abortus , B.melitensis,* and *B.suis* in serum samples.

Test Procedure

- Bring the RBPT antigen and test (sera) to room temperature before beginning the test.
- Place 30µl of each serum sample on the agglutination plate.

- Shake antigen bottle gently before use and place 30µl of RBPT antigen next to the serum sample on agglutination plate. Mix the antigen and the serum.

- Shake the plate for five minutes and read.

Result Interpretation

No agglutination indicates negative sample.

Agglutination indicates positive sample

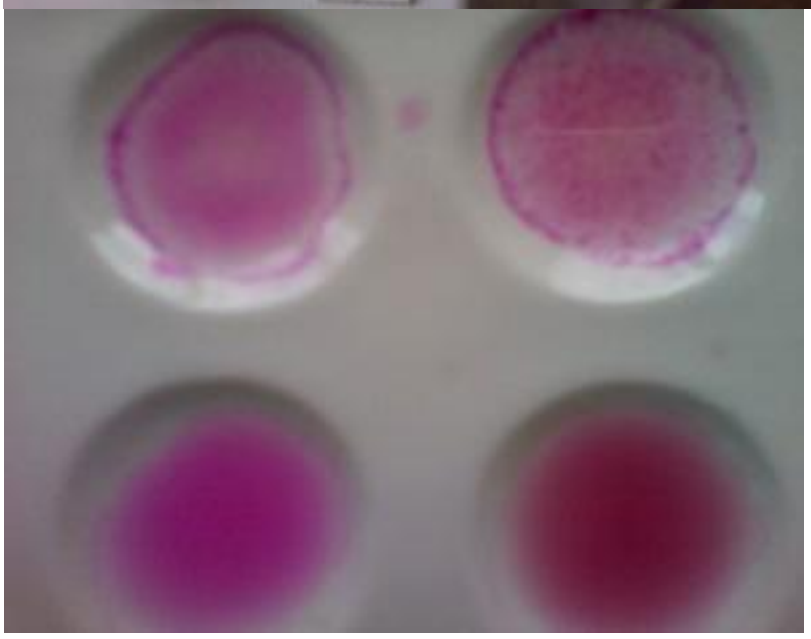
NB: Slight agglutination was also considered as a positive result

Validation of the Test VLA positive control (cat≠RAB1003 was used for each batch of samples and for newly opened antigen bodies

Antigen Presentation: 10ml of RBPT antigen per bottle

Storage: Store in the dark at $+2^{0c}$

Annex 4. Rose Bengal plate test showing agglutination (positivity)



Annex 5. Indirect enzyme linked immuno sorbent assay techniques

SERELISA® Brucela OCB Ab Mono Indirect: Kit for detection of Anti *Brucella* lipopolysaccharide antibodies in bovine sera (individual and pools,ovine and caprine sera(individual

Principle of the test

The "SEELISA® *Brucella* OCB Ab mono indirect kit uses an indirect immuno enzymatic

technique allowing the detection of brucellla lipopolysaccharides(LPS) antibodies in individual bovine in and caprine serum samplesor pools of 10 bovine sera(in accordance with the applied regulations .The reaction was composed of three steps

1. Each individual or pooled serum sample was placed in a well sensitized with *Brucella* LPS. Antibodies present in the sample bind to bacterial antigen coated to the well.
2. After a wash step, a peroxidase conjugate was added. It fixes to the immune globulin (antibodies) previously captured, forming a complex (AgLPS) –(Ab anti-LPS)-(peroxidase conjugate).
3. Excess conjugate was eliminated by a wash step. The enzyme linked to the complex was revealed by addition of a substrate.Which was transformed in to a coloured product.After stopping the reaction,the optical densities were measured. The presence or absence of antibodies was determined using threshold values obtained from the positive contro

II. Materials and reagents required

- Distilled or demineralized water
- Adjustable or set pipepettes to measure and deliver between 0 to 100µl.Measurement deviation must be≤10% for volumes ≤10µl and ≤5% for all other volumes
- Graduated cylinder (100ml and 1000ml).
- Manual, automatic or semi automatic washing devices for microtitration plates.
- Microplate reader, fitted with filters for bichromatic reading at 450 and 630 nm. It is also possible to use a monochromatic reader fitted with 450 nm filter.

IV. Samples

The reaction was performed on individual sera diluted at 1:100 or10 pooled samples diluted 1:20.

Sample storage:

Samples	Cold(+5^{0c})	Freeze(-20^{0C})	Lab.T^{OC} (-20^{0c})
Individual or pooled serum	Maximum 7 days	Yes	No

V. Procedure

Strictly comply with the procedure below .Use negative and positive control in duplicate for each test run, for each plate.

A. Preliminary steps

1. Carefully set up the distribution and identification of controls and sample.
2. Prepare the samples to be tested.

Individual samples protocol: dilution 1:10

Dilute the sample at 1:10 in samples diluents (SD) in a test tube or in to a dilution plate. Dilute again at 1:10 in a test tube or directly in to the wells: dispense 10µl of already diluted samples in 90µl of sample diluents (SD).

Pooled bovine samples protocol: dilution 1:20.

Add 10 µl of each individual serum to obtain 10µl of 10 pooled samples.

Dilute pooled samples at 1; 20 in a test tube or directly in to the wells: dispense 5µl of pooled samples in 95µl of sample diluents (SD).

B. Test procedure

Control and sample distribution

Control distribution

For the two following protocols, 100µl of diluted negative control was dispensed in wells A1 and A2, 100µl of positive control was dispensed in wells B1 and B2.

Individual samples protocol: dilution 1:100.

After shaking the vials dilute, the controls were diluted at 1:10 in a sample diuluent (SD). In a test tube or in to a dilution plate, then dilute again at, 1:10 in a test tube: dispense 10µl of already diluted controlin 90µ of sample diluents (SD).

2. Sample Distribution

Strictly comply with the procedure indicated in VI. A2., for the preparation of samples and the distribution directly in the plate.

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

Incubation of the plate

1 hour (±5^{0c}) at 37^{0c} (±3^{0c})

Washing

Wash buffer: dilute the concentrated washing solution (W) 1:10 in distilled or demineralized water.

Carefully remove the adhesive film and wash 4 times.

II. Addition of conjugates

1. preparation of conjugate:

<u>Individual samples protocol: dilution 1:200</u>

Dilute the concentrate (CJ) 1:200 in the conjugate diluents (CD).2ml were needed for one strip, meaning, 20µl of the conjugate in 1.98 ml of CD.

2. Distribution of the conjugate:

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

3. Incubation of conjugates

Incubate for 30 minutes (±5 min) at 37^{0c} (±3 min).

Carefully remove the adhesive film and wash 4 times.

III. Revelation

1. Addition of the substrate

Add 100µl of buffered peroxides substrate (PS) per well. Do not cover with adhesive films at this stage. Mix by gently shaking the plate manually or use a plate agitator to ensure correct homogenization.

2. Incubation of substrate:

   30 minutes ±5 minutes at laboratory temperature ($+20^0c \pm 5^{0c}$). Shielded from light.

3. Addition of stop solution

   Add 3µl of stop solution (S) per well.

   Mix by gentle shaking the plate manually or by using a plate agitator.

   Make sure that no bubbles occur in the wells.

4. Measure of the optical density: Measure the optical density (OD) bichromatically at 450and 630 nm or monochromatically at 450 nm (in the yellow band).

Reading bichromatically was strongly recommended. Should the monochromatic reader be used, ensure the cleanliness of the bottom of the wells prior to reading

VI. Test validation

The results of each test run were valid if:

ODP ≥0.5 and OD N<0.3*ODP

OD: Average of the ODs for the samples tested in duplicate

VII. Expression and interpretation of the result

The presence or the absence of antibodies against LPS of *Brucella* was determined by comparing the ODs, to the threshold values obtained from the positive control.

Two methods for calculating and interpreting the results were possible

METHOD 1": Index calculation

Positive threshold value in index=0

For individuals and pooled samples:

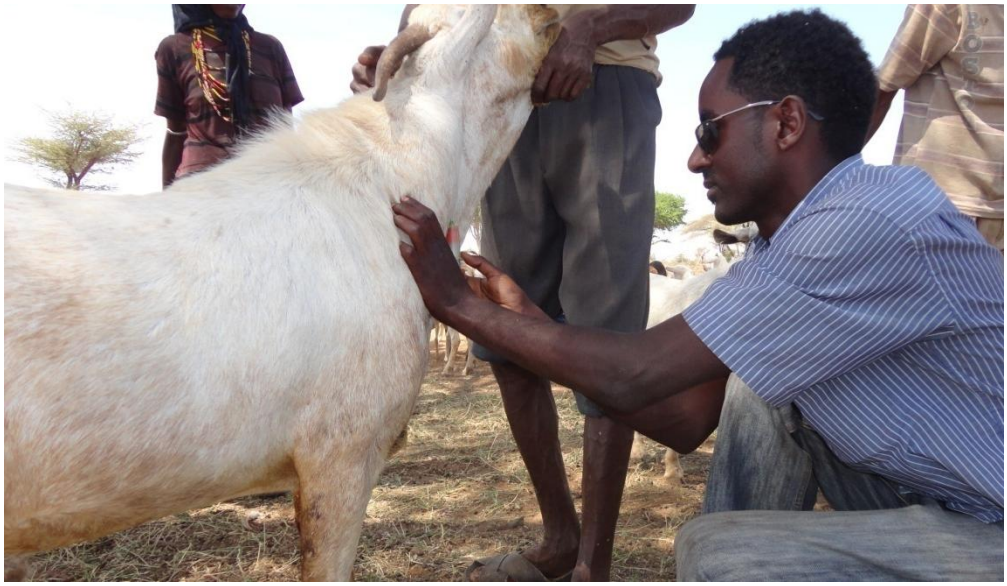
Sample index: =0.50*(sample OD-0.6*ODP)

Any samples or pool of samples presenting an index ≥0 was considered as positive

Any sample or pool of sample presenting and index ≤0 was considered as positive.

Method 2: analysis of the optical densities: For individual and pooled samples: Positive threshold value: $\alpha=0.6*(ODP)$,Compare each sample OD to this threshold value. Any individual or pooled sample presenting an OD $\geq \alpha$ was consiederd as positive. Any individual or pooled sample presenting an OD $< \alpha$ was considered as negative.

Annex 6. Animal and human sample collection at field and hospital



9. SIGNED DECLARATION SHEET

I under sign, declare that the thesis is my original work and has not been presented for a Degree in any University. All the resources and materials used are duly acknowledged.

Name _____

Signature _____

Date of Submission _____

This thesis has been submitted for examination with our approval as University advisors

Dr. Reta Duguma _____

Dr. Yasmin JIbril _____