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BRUCELLOSIS IN BORENA CATTLE: - SEROPREVALENCE AND AWARENESS OF THE PASTORAL COMMUNITY IN YABELLO ETHIOPIA

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Roba Jilo

Addis Ababa University, College of Veterinary Medicine and Agriculture,
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Microbiology, Immunology and Public health

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

By

Roba Jilo

June, 2017
Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Veterinary Medicine and Agriculture

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by **Roba Jilo** entitled: **Brucellosis in Borena Cattle: - Seroprevalence and Awareness of the Pastoral Community in Yabello Ethiopia.** And recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Veterinary Public Health.

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SIGNED DECLARATION SHEET

First I declare that this thesis is my original work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for a post graduate (MSc) degree at Addis Ababa University College of veterinary medicine and agriculture and is deposited at the university/college library to be made available to the borrowers under the rule of the library. I solely declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. Brief quotations from this thesis are allowed without special permission provided that proper acknowledgement of sources is made. Request for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the dean of the college when his or her judgment. The proposed use of the material is in the interest of scholarship. In all other interests however permission must be obtained from the author

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LIST OF ABBREVIATIONS

CFT	ComplementFixationTest
^0C	Degree Centigrade
DNA	DeoxyRibonucelicAcid
ELISA	Enzyme LinkedImmunosorbentAssay
H_2S	Hydrogensulfide
IgA	Immunoglobulin A
IgG1	Immunoglobulin G_1
IgG2	Immunoglobulin G_2
IgM	Immunoglobulin M
Ml	Mili liter
MRT	Milk Ring Test
MZN	Modified ZeihlNeelsen Staining
OIE	Office International des Epizootics
PAHO	Pan American Health Organization
RBPT	Rose Bengal Plate Test
SAT	Serum Agglutination Test
SDHT	Skin- Delayed-type Hypersensitivity Test
WHO	World Health Organization

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ABSTRACT

There is insufficient information on brucellosis on Borena cattle at Dida Xuyura ranch and its surroundings, despite its impact to the development of the cattle industry in Ethiopia. The present study was conducted from November 2015 to May 2016 in Yabello district, Borana zone, Ethiopia. The study was cross-sectional and the objectives of the study were: estimation of prevalence of bovine brucellosis, assessment of risk factors and assessment of knowledge of pastoralist about the disease and its risk factors in Borana cattle at Dida Tuyura ranch and its surrounding. The study animals were selected by multi-stage sampling. Blood was collected from selected animals and serum was extracted. The Sera samples were screened using the rose Bengal plate (RBPT) test and those which tested positive were further tested using Complement fixation test (CFT) for confirmation. Sixteen (16) cattle out of 661 (2.4%; 95 5 CI: 1.39, 3.9) tested using RBPT were found to be positive. The sero-prevalence was 2.94% (95% CI: 1.42, 3.53) in animals sampled from Dida Tuyura Ranch where as it was 1.86% (95% CI: 0.68, 4.01) in cattle sampled from pastoralist' herd surrounding the ranch. However, only 5 animals were found positive with CFT in animal sampled from Dida Tuyura Ranch yielding a prevalence of 1.47% (95% CI: 0.48, 3.41). from Six animals which gave positive reaction to RBPT from pastoralists' herd in the vicinity of the ranch only two gave positive reaction to CFT yielding a prevalence of 0.62% (95% CI: 0.162, 4.73). Taken together the seroprevalence of bovine brucellosis as revealed by CFT 1.1% (95% CI: 0.43, 2.17). Univariable logistic regression analysis showed that previous history of abortion and retained fetal membranes were significantly associated with sero-positivity to brucellosis ($P<0.05$) whereas sex, age, parity, body condition and PAs were not associated with infection with *Brucella (P>0.05)*. In the multivariable analysis, only abortion (OR=13.46, $p<0.05$)) remained to be independently associated with brucellosis seropositivity whereas other not. The results of questionnaire survey revealed that the majority of the pastoralists or cattle attendants do not have sufficient knowledge about brucellosis and are at risk of acquiring the infection. Therefore, educating the pastoralists about the disease through extension service on the handling of aborted fetuses and assistance of delivery is important. In addition breeding animals must be tested before distributed to pastoralists.

Keywords: Bovine Brucellosis, knowledge of Pastoralists, Sero-prevalence, Borana, Ethiopia

1. INTRODUCTION

Cattle are an important component of the livestock sector and are mainly kept in different agro ecological zones of Ethiopia. They provide various benefits particularly to smallholder farmers and the country as a whole. The current report of the Central Statistical Agency (CSA) Ethiopia hosts over 50 million heads of cattle. They are important collaterals and insurance in case of crop failures. Besides, they are important source of cash and high quality proteins to the rural people (Berhanu *et al*., 2006; Tsedeke, 2007).

The level of product obtained from cattle at present is suboptimal in all regions and production systems of the country. In the first place, the national cattle productivity is one of the lowest in Africa. Secondly, the contribution of cattle to the national economy does not commensurate with its size. All together, level of foreign currency obtained from international marketing of cattle and cattle products is much lower than would be expected, given the size of the cattle population (Berhanu *et al*., 2007). This sub-optimal productivity of Ethiopian cattle is due to several technical and non-technical factors. Infectious diseases are among the technical factors impairing cattle production. Brucellosis is one of these infectious diseases of live stock and human in Africa and other parts of the developing world. Its importance is emanated from its wide spread distribution and impact on multiple animal species, such as cattle, sheep, goat, pig and human beings (Asmare *et al*., 2013; Mc Dermot and Arimi, 2002).

In cattle Brucellosis is primarily a reproductive disease characterized by abortion late in pregnancy, frequently followed by fetal membrane retention and endometritis which may be the cause of infertility in subsequent pregnancies (Radostits *et al*., 2007). The serological differences are related to the amounts of A and M antigens that a *Brucella* strain possesses. There are about nine biotypes being recognized and a number of strain variants. About 85-89 % of the infection are from biotype1 (Ocholi *et al.*, 2004). *Brucella abortus* affects many animal species on every continent and has zoonotic and economic importance, a/;s well as a public health hazard (OIE, 2008).

Bovine brucellosis is widespread throughout the world except for a number of countries (Japan, Canada, USA) where eradication has been successful (WHO, 1986). It is an economically important disease of livestock causing reproductive wastage through infertility, delayed heat, loss of calves, reduced meat and milk production, culling and economic losses from international trade bans (McDermott and Armini, 2002).Many countries have made considerable effort with their eradication programs and some have eradicated the disease (Radostits *et al*., 2007). Most European countries are free of Bovine Brucellosis (PAHO WHO, 2001).

Brucellosis is of major public health importance in most developing countries, which have no national brucellosis control and eradication program (Radostits *et al*., 2007). In addition the policy of many developing countries, importing exotic, high production animals, without having the required veterinary infrastructure and appropriate level of development of socio-economic situations of the animal holders aggravates the situation (Seifert, 1996). In most developing countries, resource is short falling to control brucellosis. Although, information on the prevalence of brucellosis is inadequate, there are indications of a very high incidence in many areas, particularly in the tropical countries where the loss in milk and animal protein that accompanies this disease is least affordable. The prevalence of infection varies considerably between herds, areas, management and countries (WHO, 1986).

In Ethiopia, information on economic and zoonotic importance of brucellosis is not well established quantitatively as well as qualitatively as compared to the degree of the risks of the disease expected due to high animal population of the country and the greater tendency of private as well as government farms to expand high producing exotic dairy farms to satisfy the ever increasing milk demand of the urban population.

However, the existence of bovine brucellosis in state dairy and privately owned dairy farms, different ranches and research institutions is reported. The first report was given in 1970 by the veterinary section of the US Navy Medical Research Unit which shows that

the overall prevalence of bovine brucellosis was 11.7% out of 1328 bovines tested for brucellosis in different regions of the country (MoA, 1970). Though the team had reported that it had conducted the test in all domestic animals, they have reported bovines as the only species to give positive reaction for the test. According to their study, the result of the test in different regions of Ethiopia was 2 % (1:43) for Eritrea, 8 % (24:293) for Harar, 5% (2:40) for Illuababor, 7 % (10:141) Kaffa, 8 % (28:349) for Shoa, 21 % (90:418) for Sidamo and 2 % (1:40) for Wallo (MoA, 1970). According to a recent Studies, prevalence rate as low as 0.2% is reported in Jimma (Taddele, 2004) and 1.66 was reported in Sidama Zone (Kassehun, 2004).

The evidences of *Brucella* infections in Ethiopian cattle have been serologically demonstrated by different authors (Berhe *et al*., 2007; Jergefa *et al*., 2009; Mekonen *et al*., 2010). A relatively high seroprevalence of brucellosis (above 10%) has been reported from smallholder dairy farms in central Ethiopia (Kebede *et al*., 2008). While most of the studies suggested a low seroprevalence (below 5%) in cattle under crop-livestock mixed farming (Ibrahim *et al*., 2010; Hailemelekot *et al*., 2007; Asmare *et al*., 2007). There is a scarcity of published literature on the status of cattle brucellosis in pastoral areas of the country where large population of cattle are reared. So far, a study carried out in east Showa zone of Ethiopia showed a relatively higher seroprevalence in pastoral than agropastoral system (Dinka and Chala, 2009).

The limited studies (the surveys) so far conducted on brucellosis are not sufficient to show the exact national picture and significance except highlighting the existence of the disease in very limited areas of the country which were selected not based on strategic national disease survey approach but on personal preference and motives of the investigators or researchers. Moreover, most of the studies so far conducted were based on serological diagnostic technique; most of which were not according to OIE recommendation for international trade for their sensitivity and specificity. The overall infection risk is also influenced by the pattern of *Brucella* spp. present; as *B.melitensis* often represents a more serious public health hazard than *B. abortus* (WHO, 2006). To date, the occurrence of brucellosis has not been investigated in different livestock species

sharing common ecozone and management under a pastoral setting in Ethiopia. The present study therefore aimed at investigating the seroprevalence situations of brucellosis in the major livestock species kept together in the Borana pastoral system of Ethiopia. Hence, taking into account the above mentioned scenarios, this research (study) on the seroprevalence of bovine brucellosis and its zoonotic importance was under taken by using two currently OIE recommended serological methods, Rose Bengal plate Test (RBPT) and Complement Fixation Test (CFT), and questionnaire survey on potential risk factors for the disease in animals and zoonotic significance in humans, in the study area with the following objectives.

Objectives:

- ✓ To estimate overall sero-prevalence of bovine brucellosis in the Dida Xuyura Ranch and adjacent pastoral herd.
- ✓ To assess the potential risk factors of bovine brucellosis in the study areas
- ✓ To assess the knowledge of pastoralist about the disease and its risk factors in the study area

2. LITERATURE REVIEW

2.1. Historical Overview of Brucellosis

Brucellosis is an ancient disease that can possibly be traced back to the 5th plague of Egypt around 1600 BC. Recent examination of the ancient Egyptian bones, dating to around 750 BC, showed evidence of sacroiliitis and other osteoarticular lesions, common complications of brucellosis (Pappas and Papadimitriou, 2007).

Eighteen centuries later, Sir David Bruce isolated *Micrococcus melitensis* (now *B.melitensis*) from the spleen of a British soldier who died from a febrile illness (Malta fever) common among military personnel stationed on Malta, an island not far away from Herculaneum (Godfroid *et al.,* 2005). The zoonotic nature of the brucellosis was accidentally demonstrated in 1905 by isolating *B. melitensis* from goat's milk used for the production of soft cheese in Malta (Rust, 2006). In 1953, *B.ovis* was identified as a cause of epididymitis in rams (Nicolleti, 2002). In the last 15 years 3 new non-classical species of *Brucella* has been identified (Scholz *et al*., 2008).

2.2. *Brucella* species and Their Host Preferences

Brucella have definite host preferences (Table 1). Secondary hosts play a minor role in the maintenance and spread of a particular *Brucella* species. *Brucella abortus* mainly infects cattle and is the main cause of contagious abortion. However, sheep, goats, dogs, camels, buffaloes as well as feral animals may also contract *B. abortus* infections (Radostits *et al*, 2007). Although sheep do not easily become infected with *B. abortus*, they may become carriers and excrete brucellae for up to 40 months once they have acquired the infection. This organism is a Gram negative, facultative intracellular pathogen, and contains three biovars (Acha and Szyfres, 2001). All these biovars can cause disease in small ruminants, but their geographic distribution varies. Isolation of *B. abortus* from swine,horses (Radostits *et al.*, 20007) and camels in areas with enzootic brucellosis clearly indicates that these species may acquire infection with *B. abortus.*

However, their significance as a host for *B. abortus* is doubtful, in areas where these animal species usually do not intermingle with cattle. Dogs with naturally acquired *B. abortus* infections play an important role in the epidemiology of cattle brucellosis. The relationship between infected dogs and outbreaks of brucellosis in cattle has not only been reported but has also been demonstrated (Radostits *et al.*, 2007).

Table 1: Worldwide geographical distribution of *Brucella* species and its biotypes.

Species	**Host (s)**	**Disease**	**Geographical distribution**
B. abortus	CATTLE*	Abortion & Orchitis	Biotypes
	Sheep, goats and pigs	Sporadic abortion	1: Worldwide (common)
			2: Worldwide (not common)
	Horses	Associated with bursitis	3: India, Egypt & East Africa
	Humans	Undulant fever	4: Britain & Germany
			Other biotypes are infrequently isolated
B. melitensis	GOATS*	Abortion	Many sheep and goat raising regions except New Zealand, Australia and North America
	Sheep's		
	Cattles	Occasional abortion and excretion in milk	
	Humans	Malta fever	
B. suis	PIGS*	Abortion, orchitis, arthritis, spondylitis and herd infertility	Biotypes
			1: Worldwide
	Humans	Undulant fever	2: Western and Central Europe
			3: USA, Argentina and Singapore
			4: Arctic Circle (Canada, Alaska and Siberia) in reindeer and caribou
B. ovis	SHEEPS*	Epididymitis in rams and sporadic abortion in ewes	New Zealand, Australia and some other sheep-raising countries: USA, Romania, Czechoslovakia, South Africa & South America

B. canis	DOGS*	Abortion, Epididymitis, disco-spondylitis and permanent infertility in male	North America and parts of Europe Becoming worldwide but not common
	Humans	Undulant fever	
B. neotomae	Desert room rat (neotoma lepida)	Non-pathogenic for the wood rat and has not been recovered from any other animal species	USA (Utah)

*Natural host given in capital letters

Source: Quinn *et al.,* 2002

2.2.1. Characteristics of Brucella Organisms

Brucellaea are facultative intracellular bacteria comprising of various species belonging to order Rhizobials and family alpha *proteobactereacea*. *Brucellaea* are a small (0.6x0.6 to 1.5 µ m) gram negative, non-motile, non-spore forming, rod shaped (cocco bacillary) bacteria. They are partially acid fast positive because they are not decolorized by 0.5% of acetic acids in Modified Ziehl-Neelsen (MZN) staining techniques. In MZN stained smears, the organisms appear as cluster of red staining coccobacilli because they retain the carbol fuchsine (Quinn *et al.,* 2002: OIE, 2012).

The genus *Brucella* comprises of ten species based on their difference in host specificity. Of these, six of them are called classical *Brucella* species affecting terrestrial animals. These include of *Brucella melitensis* (goats, sheep, biovars 1-3), *B. abortus* (cattle, biovars 1-6 and 9), *B. suis* (pigs, reinders and hares, biovars 1-5), *B. ovis* (sheep), *B. canis* (dogs) and *B. neotomae* (desert wood rats) (Godfroid *et al.,* 2011).

Recently, four additional new *Brucella* species has been identified. These are *B. ceti* and *B. pinnipedialis*, which were recently identified in marine mammals with Cetaceans (dolphin, porpoise, and whale species) and Pinnipeds (various seal species) as preferred hosts respectively. *B. microti* was isolated from the common vole and *B. inopinata* was

isolated from a breast implant wound of a female patient (Foster *et al.*, 2007; Scholz *et al.*, 2008; Scholz *et al.*, 2010).

2.2.2. Growth and biochemical properties

Brucellaea are aerobic, but some strains require an atmosphere containing 5-10% carbon dioxide (CO2) for growth, especially on primary isolation. The optimum pH for growth varies from 6.6 to 7.4, and culture media should be adequately buffered near pH 6.8 for optimum growth. The optimum growth temperature is 36-38°C, but most strains can grow between 20°C and 40°C. Brucellaea require biotin, thiamin and nicotinamide for growth. The growth is improved by serum or blood, but haemin (V-factor) and nicotinamide-adeninedinucleotide (X-factor) are not required. The growth of most *Brucella* strains is inhibited on media containing bile salts, tellurite or selenite. Growth is usually poor in liquid media unless culture is vigorously agitated. Growth in static liquid media favors dissociation of smooth-phase cultures to non-smooth forms. Continuous and vigorous aeration will prevent this, provided a neutral pH is maintained. In semi-solid media, CO_2-independent *Brucella* strains produce a uniform turbidity from the surface down to a depth of a few millimeters, while cultures of CO_2-requiring strains produce a disk of growth a few millimeters below the surface of the medium (European commission, 2001;Quinn *et al.*, 2002; OIE, 2012).

On suitable solid media *Brucellaea* colonies are visible after 2 days of incubation. After 4 days of incubation, *Brucellaea* colonies are round, 1-2 mm in diameter, with smooth (S) margins, translucent and a pale honey color when plates are viewed in the daylight through a transparent medium. When viewed from above, colonies appear convex and pearly white. Later, colonies become larger and slightly darker (European commission, 2001; OIE, 2012)

Smooth Brucellaea cultures, such as *B. melitensis* cultures, have a tendency to undergo variation during growth, especially with subcultures, and dissociate to rough (R) forms, and sometimes mucoid (M) forms. Colonies are then much less transparent with a more granular, dull surface (R) or a sticky glutinous texture (M), and range in color from matt white to brown in reflected or transmitted light. Intermediate (I) forms between S, R and

M forms may occur in cultures undergoing dissociation to the non-smooth state. Changes in the colonial morphology are generally associated with changes in virulence, serological properties and phage sensitivity (European commission, 2001; OIE, 2012).

The metabolism of *Brucellaea* is oxidative and *Brucellaea* cultures show no ability to acidify carbohydrate media in conventional tests. The *Brucella* species are catalase positive and usually oxidase positive, and they reduce nitrate to nitrite (except *B. ovis* and some *B. canis* strains). The production of H_2S from sulphur containing amino-acids also varies. *B. melitensis* does not produce H_2S. Urease activity varies from fast to very slow. Indole is not produced from tryptophane and acetylmethycarbinol is not produced from glucose (European commission, 2001; OIE, 2012).

2.2.3. Antigenic characteristics

The outer cell membrane of the virulent and zoonotic species such as *B. abortus, B. meletensis* and *B. suis* is made of smooth lipopolysaccharide (S-LPS) motifs. The S-LPS contains an O15 polysaccharide (OPS) that is chemically defined as a homo polymer of 4,6-dideoxy-4-formamide-alpha-D-mannose, linked via 1,2-glycosidic linkages (Nielson, 2002) whereas the non-smooth species such as *B. ovis* and *B. canis* lack the OPS on their LPS and have a rough lipopolysaccharide (R-LPS) instead. The lack of the OPS rendered these rough species more immunogenic following infection of the hosts. It is believed that the S-LPS are able to evade innate immunity and to be a less potent inducer of inflammatory cytokines. These activities protect the bacterium against the initial immune response and enhance its ability to survive in the host. Once inside a phagocytic cell, the S-LPS species are able to prevent the infected cell from antigen presentation to T helper cells via the major histocompatibility complex II (MHC II). The S-LPS enables the bacteria to hinder apoptosis by the infected cell as well. These evasion techniques add to the S-LPS *Brucella* spp. virulence in comparison to the R-LPS strains. The latter are unable to inhibit the host immune response and are greatly impacted by the innate immune system, and are thus prevented from having a more severe effect on the host (Franco *et al.,* 2007).

2.2.4. Molecular characteristics

Over 40 *Brucella* phages have been reported to be lytic for *Brucella* members. All phages are specific for the genus *Brucella*, and are not known to be active against any other bacteria that have been tested. Thus, lysis by *Brucella* phages is a useful test to confirm the identity of *Brucella* spp. and for speciation within the genus. The *Brucella* phages currently used for *Brucella* typing are: Tbilisi (Tb), Weybridge (Wb), Izatnagar1 (Iz1) and R/C. The three former phages are used for differentiation of smooth *Brucella* species. R/C is lytic for *B. ovis* and *B. canis* (European commission, 2001; OIE, 2012).

The genome of most *Brucella* species consists of two circular chromosomes or replicons of 1.1 and 2.2 Mb (Lindquist *et al*., 2007). The genome of *B. melitensis* strain 16 M contain 3,294,935 (base pairs) bp distributed over two circular chromosomes of 2,117,144 bp and 1,177,787 bp encoding 3,197 open reading frames (ORFs). The complete sequencing of the *B. melitensis* genome was achieved in 2002 (Del Vecchio *et al.,* 2002).Natural plasmids have not been detected (Corbel, 1997). Sequencing and annotation of the genomes of *B. suis*, *B. melitensis*, and *B. abortus* has been completed; the majority of the open reading frames share greater than 99 percent sequence identity between species(Del Vecchio *et al.*, 2002; Paulsen *et al.*, 2002; Halling *et al.*, 2005).

2.3. Brucellosis in Animals: Clinical Characteristics

Brucellosis is a sub-acute or chronic disease which may affect many species of animals. In cattle, sheep, goats, other ruminants and pigs the initial phase following infection is often not apparent. In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy, and epididymitis and orchitis in the male (Shareef *et al.*, 2006; Foster and Ladds, 2007). Clinical signs are not pathognomonic and diagnosis is dependent upon demonstration of the presence of *Brucella* spp. either by isolation of the bacteria or detection of their antigens or genetic material, or by demonstration of specific antibody or cell-mediated immune responses (Silva *et al*., 2000).

Characteristic but not specific signs of brucellosis in most animal hosts are abortion or premature births and retained placenta. In some areas, abortion is relatively uncommon. In some parts of Africa, hygromas and abscesses are the major clinical signs in nomadic or semi-nomadic cattle herds infected with *B. arbortus* biovar 3. There is lowered milk production due to premature births. Interference with fertility is usually temporary and most infected animals will abort only once and some are unaffected. The udder is often permanently infected, especially in the case of cows and goats. Shedding of organisms in milk is frequent. Localized infections in sheep result in orchitis or epididymitis in the case of *B. melitensis* and *B. ovis*. In goats, cattle, swine and dogs similar complications may follow infection with *B. melitensis, B. abortus, B. suis* and *B. canis* respectively. Arthritis may also be a rare sign in *B. melitensis*-infected sheep and goats. In horses, local abscess formation in bursae may be the only clinical sign and infection in this species is often asymptomatic. Camels infected with *B. melitensis* shed the organisms in milk and in some countries this is a serious public health problem. Clinical signs of brucellosis in camels appear to be very rare (Radostits *et al*., 2007).

The severity of the disease depends upon many factors such as previous vaccination, age, sex and management such as herd or flock size and density. Abortions are more prevalent in unvaccinated animals and numbers of organisms shed are much greater. The bacteria are found in tissues and fluids associated with pregnancy, the udder and the lymph nodes which drain the relevant areas. Most infections result from ingestion of bacteria either from diseased animals or contaminated feedstuffs (Szyfres, 2001; Radostits *et al*., 2007). However, infection may also be acquired by respiratory exposure and by contamination of abraded skin and mucosal surfaces. Natural breeding transmits infection in swine and dogs and, to a lesser extent, sheep and goats. Persistent bacteraemias are also more common in the first two species**.** Bacteraemia occurs during the course of infection in other species but is usually intermittent and of short duration (Degefa *et al*., 2011).

2.4. Pathogenesis

An important aspect of *Brucella* infection is its ability to persist and replicate within phagocytic cells of the reticuloendothelial system as well as in non-phagocytic cells such

as trophoblasts. This ability involves a temporary fusion of the *Brucella*-containing vacuole with the lysosome, and subsequent exclusion of the lysosomal proteins (Starr *et al.,* 2008). Soon after internalization by the phagocytes, the *Brucella*-containing vacuole interacts with early and late endosomes. The majority of phagocytosed *Brucella* is destroyed by bactericidal action of free radicals of oxygen, nitric oxide and enzymes inside phagolysosomes. However, a certain number of bacteria resists these bactericidal mechanisms, and after transient fusion with the lysosome can actively exclude lysosomal proteins and redirect intracellular trafficking of *Brucella*-containing vacuole to the endoplasmic reticulum, where the organism is capable of replicating (Franco *et al.,* 2007). Replication leads to release of the bacteria from the cells, thus resulting in a bacteremic phase (Ragan, 2002).

The method of escape from the phagocytic cells is yet undiscovered but the overall result is cell lysis. The bacteremia allows for colonization of the bacteria in multiple tissues, but in livestock the bacteria most frequently colonize in the lymphoid tissues, mammary gland and reproductive tract (Ragan, 2002). The *Brucella* virulence factor is thought to play a significant role in these intracellular survival events. The *Brucella* virulence factor is a secretory pump that selectively pumps proteins and macromolecules across membranes and is critical in pathogenesis and virulence of *Brucella* infections. It helps ensure the survival of the bacteria in the phagolysosome and establishing the infection (Franco *et al.,* 2007). Lipopolysaccharide is another virulence factor of *Brucella* that contributes to initial survival of bacteria in macrophages (Lapaque *et al.,* 2005)

The localization of *Brucella* spp. in the reproductive tract leads to colonization of the chorionic trophoblast of the placenta in pregnant livestock. This affinity for the chorionic trophoblastic cells is due to the presence of steroid hormones and erythritol which is an important substance present in allantoic fluids that stimulates the replication of *Brucella* spp (Xavier *et al.,* 2009). The stimulation of *Brucella* spp. seen in the presence of erythritol is due to the preferential use of erythritol by *Brucella* spp. as an energy and carbon source, even in the presence of glucose and other metabolites. The reason for the preferential use of erythritol is due to its ease of uptake by the bacteria, as compared to glucose. This makes erythritol more readily available to the bacteria for energy

consumption (Quinn *et al.,* 2002). Growth of *Brucella* inside the trophoblast also become prominent when the concentration of steroid hormones of $PGF_2\alpha$, estrogen and cortisol and erythritol is higher and acts together during late gestation of ruminants which results in late term abortion or birth of weak offspring in case of female animals and epididymitis and orchitis in male animals. The resulting placentitis caused by replicating bacteria results in ulceration of the chorioallantoic membrane while sparing the endometrium of the uterus. The resulting pathology leads to late gestation abortions in naïvely infected livestock (Radostits *et al.,* 2007).

2.5. Diagnosis of Bovine Brucellosis

2.5.1. Bacteriological detection 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. 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* (Quinn *et al.,* 2002; Poiester *et al.,* 2010).

Isolation may be performed by culturing body tissues or secretions like blood, milk and virginal discharge (Poiester *et al.,* 2010). *Brucella* species can also be cultured from pus, cerebrospinal fluid, and pleural, joint and ascitic fluids. Growth of the bacteria in culture media is an unequivocal proof of infection (Poiester *et al.,* 2010). The identification of *Brucella* species in culture depends on a great deal of phenotypic traits such as: CO_2 requirement, phage typing and biochemical tests, which, among other problems, involve time, bio-safety, trained personnel and somewhat ambiguous results (Bricker, 2002). Broth or agar can be prepared from powder media for culture of *Brucella* organisms. Due to the low *Brucella* load in

the blood and other body fluids, broth or a biphasic medium are preferable for their culture. However, for other specimens, solid media with 2.5% agar facilitates the recognition of colonies and discourage bacterial dissociation (Poiester *et al.,* 2010). Optimum pH for growth of *Brucella* varies from 6.6 to 7.4, and culture media should be adequately buffered near pH 6.8 for optimum growth. The optimum growth temperature is 36-38°C. However, most strains grow between 20 and 40°C (European Commission, 2001).

The most widely used selective medium is the Farrell's medium (Farrell, 1974), which is prepared by the addition of six antibiotics to a basal medium. On suitable solid media, *Brucella* colonies can be visible after 2–3-days of incubation. After 4 days' of incubation, *Brucella* colonies are round, 1–2 mm in diameter, with smooth margins. They are translucent and a pale honey colour when plates are viewed in the daylight through a transparent medium. When viewed from above, colonies appear convex and pearly white. Later, colonies become larger and slightly darker. Smooth (S) *Brucella* cultures have a tendency to undergo variation during growth, especially with subcultures, and to dissociate to rough (R) forms. Colonies are then much less transparent, have a more granular, dull surface, and range in colour from matt white to brown in reflected or transmitted light (OIE, 2012).

2.5.2. Serological diagnosis

The detection of specific antibody in serum or milk remains the most practical means of diagnosis of brucellosis. The most efficient and cost-effective method is usually screening all samples using a cheap and rapid test which is sensitive enough to detect a high proportion of infected animals. Samples positive to screening are then tested using more sophisticated, specific confirmatory tests for the final diagnosis to be made. It is absolutely essential that only internationally recognized tests using antigens standardized against the 2nd International anti-*B. abortus* Serum are used. Appropriate quality control sera should be included with each batch of tests, and tests should be repeated if the quality control criteria are not met. Serological results must be interpreted against the

background of disease incidence, use of vaccination and the occurrence of false positive reactions due to infection with other organisms (Godfroid *et al.,* 2002; See *et al.*, 2012).

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 and other tests such as the buffered plate agglutination tests and the card test play a major role in the serological diagnosis of brucellosis worldwide. 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. The procedure can be automated but this requires custom-made equipment (Diaz *et al.,* 2011).

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 (WHO, 2006).

Complement fixation test (CFT) is another commonly used serological methods. 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 (OIE, 2012).

In addition to these commonly used sero-diagnostic methods, supplementary tests such as Milk ring test, Fluorescence polarization assay, Intradermal test, Serum agglutination test (SAT) and milk ELISA have been used seldom for diagnosis of bovine brucellosis (WHO, 2006).

2.5.3. Molecular biology techniques

The polymerase chain reaction (PCR) is a recent and promising technique that allows for rapid and accurate diagnosis of bovine brucellosis without the limitations of the conventional methodology (Baddour, 2012). Several genus-specific PCR systems using primer pairs that target 16S RNA sequences and genes of different outer membrane proteins have been developed (Queipo-Ortuρo *et al.,* 2005). The first species-specific multiplex PCR was called Abortus-Melitensis-Ovis-Suis (AMOS-PCR) assay, which is used to identify and differentiate *B. abortus* biovars 1, 2 and 4, *B. melitensis*, *B. ovis* and *B. suis* biovar 1. The PCR is based on the polymorphism arising from species specific localization of the insertion sequence IS711 in the *Brucella* chromosome. A Bruce-ladder multiplex PCR assay was also developed for identification and differentiation of *Brucella* species and vaccine strains in a single step (Weiner *et al.*, 2011).

2.6. Epidemiology of Brucellosis in Animals

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 but 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. 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 (Alton *et al.,* 1975). 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. Swine brucellosis is transmitted by direct contact with recently aborted sows, by ingestion of contaminated food or exposure to a contaminated environment. However, sexual transmission is particularly important. Brucellosis may be introduced on to farms through the communal use of boars or by purchase of infected animals (Chukwu, 1985; Chukwu, 1987).

For all species, embryo transfer is safe provided that recommended procedures are followed. *B. canis* can be a major problem in dog breeding kennels. Transmission is by contact with recently aborted animals or with food or environment contaminated by abortions or excreta. Sexual transmission is also an important means of spread and males can excrete the organisms in large numbers in their semen (WHO, 2006).

Urinary excretion also occurs and is a potential hazard to humans. However, in some countries where *B. canis* is present in the dog population, overt human disease caused by this organism seems to occur infrequently (WHO, 2006). It should be remembered that dogs can acquire infection with *B. abortus, B. melitensis* or *B. suis* from aborted ruminants or swine, usually by ingesting fetal or placental material. They can then excrete these bacteria and may present a serious hazard to humans and domestic livestock (Baumgarten, 2002). *B. suis* biovar 4 causes brucellosis in caribou and reindeer. The

epidemiology is similar to that of bovine brucellosis. Transmission to people can occur through the usual routes. However, ingestion of raw or undercooked reindeer bone marrow has also been implicated as a source of human infection (Boschiroli *et al.,* 2001).

In cattle, sheep, goats and swine, susceptibility to brucellosis is greatest in sexually mature animals. Young animals are often resistant, although it should be noted that latent infections can occur and such animals may present a hazard when mature. Breed may also affect susceptibility, particularly in sheep (Boschiroli *et al.,* 2001). The milking breeds seem to be the most susceptible to *B. melitensis.* Breed differences in susceptibility have not been clearly documented in cattle although genetically determined differences in susceptibility of individual animals have been demonstrated. Polymorphism of the natural resistance associated monocyte protein (NRAMP) gene has been shown to influence substantially susceptibility to brucellosis in cattle and pigs (Chukwu, 1985). However, management practices are far more important in determining the risk of infection. Latent or inapparent infections can occur in all farm animal species. These usually result from infection in utero or in the early post-natal period. Such animals can retain the infection for life and may remain serologically negative until after the first abortion or parturition. Latent infection has been estimated to occur in the progeny of about 5% of infected cows. The extent of the problem in other species is not known, but latency has been documented in sheep (Chukwu, 1985; WHO, 2006).

Acquired immunity has a substantial effect on susceptibility. Vaccination of cattle with *B. abortus* strain 19 or RB 51, or sheep and goats with *B. melitensis* Rev 1 can reduce susceptibility a thousand fold or more to the homologous species. *B. abortus* strain 19 does not protect cattle against *B. melitensis*. However, there is little information on the use of Rev1 vaccine in cattle. The efficacy of this vaccine against the *B. melitensis* strains prevalent in some areas has also been questioned (Boschiroli *et al.,* 2001). Vaccines must be obtained from a reliable, internationally approved source. It is possible that strains of *B. melitensis* exist which can circumvent the immunity induced by this vaccine. However, it is at least as probable that variations in vaccine quality have affected protection rates. For the present, Rev 1 vaccine is the most effective vaccine available against *B. melitensis* and in many countries has given very good results. Its use is

recommended when uncontrolled *B. melitensis* infection exists in ruminant populations (WHO, 2006).

2.6.1. Epidemiology of bovine brucellosis in Ethiopia

In Ethiopia, brucellosis in animals and humans has been reported from different localities of the country, particularly associated with cattle in both intensive and extensive management systems (Domenech, 1977; Molla, 1989). These prevalence studies in animals and human were largely confined to serological surveys and commonly targeted bovine brucellosis, occasionally sheep and goats and rarely camels. So far, attempts to identify *Brucella* species in the country were unsuccessful; the distribution and proportion of their natural hosts was also not studied exhaustively. This is largely attributed to the degree of laboratory development and lack of consumables for laboratory tests.

The most significant economic losses are usually incurred following bovine brucellosis. In Ethiopia, information on losses specifically through brucellosis in the different types of production systems is sparse, with the exception of Tariku (1994) who reported an annual loss from brucellosis estimated to be 88,941.96 Ethiopian Birr ($5231 equivalent) among 193 cattle, largely due to reduced milk production and abortions (Chaffa State Farm, Wollo, from 1987 to 1993). Both husbandry systems as well as environmental conditions greatly influence the spread of *Brucella* infection (WHO, 2004). Ethiopia has several institutionally owned commercial dairy farms, mostly situated in and around Addis Ababa and in some regional towns. These farms have been the focus of most *Brucella* surveys, potentially producing a bias in reported findings.

Prevalence in intensive management system

Higher individual bovine brucellosis seroprevalence has been recorded in intensively managed cattle herds as compared to those in the extensive management system. In Borena Zone of Oromia Region, the highest seroprevalence (50%) was documented using ELISA in Didituyura Ranch (Alem and Solomon, 2002). A seroprevalence of 39% was

also recorded at the Institute of Agricultural Research in Western Ethiopia (Rashid, 1993), 22% in Dairy Farm in Northeastern Ethiopia (Tariku, 1994), 11 to 15% in dairy farms and ranches in Southeastern Ethiopia (Megersa *et al.,* 2010), 8.2% in Arsi area (Molla, 1989), 8.1% in dairy farms in and around Addis Ababa (Asfaw *et al.,* 1998), and 7.7% in Tigray region (Haileselassie *et al.,* 2010). On a cautionary note, different serodiagnostic methods might cause some variability. Similarly, some question the value of testing individual animals, instead preferring to class infection at the herd level rather than the individual animal.

Relatively low individual animal seroprevalence were recorded in some intensive farms in different parts of the country. Asmare and colleague (2013) documented 2.46% in Sidama Zone of Southern Ethiopia; Mussie *et al.* (2007) reported a prevalence of 4.63% in Northwestern part of Amhara Regional State. According to these authors, the reasons for the low prevalence of bovine brucellosis in these study areas were explained by better hygienic practices, use of maternity pen and/or separation of cows during parturition, cleaning and disinfection activities, culling of infected animals, depending on own herds for replacing stock and farm owners knowledge of brucellosis in these intensive farms. In contrast to the above reports, Alem and Solomon (2002) failed to find any seroreactive cattle after screening 564 animals in Eastern and Western Showa zones of central Ethiopia using rosebengal plate test (RBPT), serum agglutination test (SAT) and complement fixation test **(**CFT).

Prevalence in extensive management system

In Ethiopia, 95% of cattle are farmed under extensive systems. According to the available data, *Brucella* seroprevalence within extensive cattle rearing systems is lower than that of intensive systems. Tolosa *et al.* (2008) reported overall individual animal prevalence and herd prevalence of 0.77 and 2.9%, respectively in Jimma Zone. Recent reports from North West, Tigray region (Haileselassie *et al.,* 2010) and Southern Sidama Zone (Asmare *et al.,* 2013), recorded an overall prevalence of 1.2 and 1.66% following screening of 848 and 1627 cattle from extensive system, respectively. A cross-sectional epidemiological study carried out in Tigray Region of Ethiopia revealed that of 816

indigenous cattle sera examined, only 27 (3.3%) were sero-positive using RBPT, of which 26 (3.19%) were also positive by CFT. Overall herd-level prevalence was reported to be 42.31% and the within-herd prevalence varied from 0 to 15.15% based on CFT (Berhe *et al.,* 2007). In another study, Ibrahim *et al.* (2010) reported overall individual and herd level seroprevalences of 3.1 and 15.0%, respectively. Using CFT, Kebede *et al.* (2008) reported individual and herd animal prevalence of 11.0 and 45.9%, respectively. Dinka and Chala (2009) investigated bovine brucellosis using RBPT in four districts of East Showa Zone. In their study, *Brucella* antibody was detected in 8.7, 18.6, 5.1 and 10% of the samples in Fentale, Arsi Negele, Lume and Adami Tulu study districts, respectively. The overall herd prevalence was reported to be 11.2%. Jergefa *et al.* (2009) also conducted seroprevalence study using RBPT and CFT in three agroecological areas of central Oromiya namely: Walmara, Adami Tulu-Jido Kombolcha and Lume Districts. Their result revealed overall prevalence of 2.9 and 13.6% in individual animal and herd level, respectively.

2.7. Treatment, prevention and control

As the source of human brucellosis is direct or indirect exposure to infected animals or their products, prevention must focus on various strategies that will mitigate infection risks. To our knowledge, there has been no national program proposed for prevention and control of brucellosis in Ethiopia. Similarly at regional levels, no strategy is in place to control brucellosis. This is largely a result of lack of facilities and budget to run such a program. Moreover, many responsible bodies may not recognize the significance of brucellosis given the contradictory and sometimes low prevalence data. However, at this time, it is crucial to define geographical extent of the problem and then allocate resources and funds to initiate prevention and control strategies in this country. These strategies have been proposed as follows.

2.7.1 Classification of endemic areas based on prevalence

This will enable instigation of appropriate control method in endemic areas. Identification of low and high prevalence areas will greatly facilitate the implementation of appropriate control programs, and should ideally be combined with other strategies like accurate

livestock census data and a livestock identification system (either simple ear notches or more sophisticated ear labeling system) (Boschiroli *et al.,* 2001). Vaccine storage and quality control systems are also a priority coupled with surveillance systems and post-vaccination surveillance to identify the remaining foci of infection (the efficacy of post-vaccination surveillance is reliant upon existing records combined with reliable livestock identification). In areas where the disease is less prevalent (for example seroprevalence of less than 1%), test and cull policy with compensation may be recommend. For areas with high and moderate prevalence (>5%) under well-organized farming systems, we may recommend test and segregation policy by which animals with brucellosis will be isolated and products consumed after pasteurization, with animals being disposed of properly at the end of their productive live (WHO, 2006).

2.7.2. Characterization of Brucella species

One of the most successful methods for prevention and control of livestock brucellosis is through vaccination. In different parts of the world both live vaccines, such as *B. abortus* S-19, *B. melitensis* Rev-1, *B. suis* S-2, rough *B. melitensis* strain M111 and *B. abortus* strain RB51 and killed vaccines, such as *B. abortus* 45/20 and *B. melitensis* H.38 are available. Each vaccine has been reported to have its own advantages and disadvantages (Ward *et al.,* 2012), with protection following localized persistence of live vaccines preferred by most and showing efficacy in small ruminants (Smits, 2012; Ward *et al.,* 2012) and cattle (Cheville *et al.,* 1996). Use of the RB51 attenuated live vaccine has recently gained popularity for control of brucellosis in cattle (Cheville *et al.,* 1996), but on a cautionary note, the failure of this strain to induce serological reactivity, coupled with its inherent resistance to rifampicin, might complicate detection and management of zoonotic infection spilling into humans with occupational risk factors for acquiring brucellosis. Currently, despite huge research efforts, no vaccine has been approved for the prevention of human brucellosis. Treatment regimes for human brucellosis require combination of antibiotics. These have recently been compared using meta-analysis (Skalsky *et al.,* 2008). Currently, vaccination against animal brucellosis has yet to be explored in Ethiopia. As a prerequisite, *Brucella* species identification should be undertaken to inform selection of the most appropriate vaccine, for example, *B.*

melitensis has recently been found infecting cattle in Kenya (Muendo *et al.*, 2012) and to enable differentiation of vaccine and wild-type strains. Vaccination is generally recommended for seroprevalence rates between 2 and 10%. Whether a strategy of test and segregation alone for high seroprevalence rates is sufficient may depend on the farming conditions. This might be appropriate for farms in conjunction with appropriate hygienic measures, but supplementation with vaccination may be required to control the disease in extensive livestock conditions.

2.7.3. Application of farm Biosafety measures

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

2.7.4. Application of veterinary extension

The development of a national veterinary extension services in the country, is essential to promote awareness about brucellosis, its impact on livestock production and zoonotic risks, would provide a valuable prevention measure. This would help to unify both community/dairy cattle producers to control and eliminate brucellosis. Currently, many dairy cattle producers hide or dispose of animals with a history of abortion, potentially facilitating disease transmission between farms and regions. This seriously undermines efforts of controlling and preventing the disease.

2.8. Disease of Brucellosis in Humans

2.8.1. Clinical Manifestations

Brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia and prostration, and which, in the absence of specific treatment, may persist for weeks or months. Typically, few objective signs are apparent but enlargement of the liver, spleen and/or lymph nodes may occur, as many signs referable to almost any other organ system. The acute phase may progress to a chronic one with relapse, development of persistent localized infection or a non-specific syndrome resembling the "chronic fatigue syndrome". The disease is always caused by infection with a *Brucella* strain and diagnosis must be supported by laboratory tests which indicate the presence of the organism or a specific immune response to its antigens. Brucellosis in human beings now a day is associated with various clinical problems involving various body systems and organs. These various forms of brucellosis in humans are summarized below (WHO, 2004; WHO, 2006).

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

Gastrointestinal complications, especially due to *B. melitensis,* are often foodborne, and unpasteurized milk or dairy products, such as cheese, are common vehicles of transmission. Foodborne brucellosis resembles typhoid fever, in that systemic symptoms predominate over gastrointestinal complaints. Nevertheless, some patients with the disease experience nausea, vomiting, and abdominal discomfort. Rare cases of ileitis, colitis and spontaneous bacterial peritonitis have been reported (Yong and Corbel, 1989).

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

Aerosol inhalation is a recognized route of transmission of brucellosis, especially common in abattoirs where infected animals are slaughtered. A variety of pulmonary complications have been reported, including hilar and paratracheal lymphadenopathy, interstitial pneumonitis, bronchopneumonia, lung nodules, pleural effusions, and empyema. *Brucella* organisms are rarely isolated from expectorated sputum (WHO, 2006).

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

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

Cardiovascular complications with endocarditis are the most common cardiovascular manifestation, and it is said to be the most common cause of death from brucellosis. Endocarditis is reported in about 2% of cases, and can involve both native and prosthetic heart valves. The aortic valve is involved more often than the mitral valve. Direct invasion of the central nervous system occurs in about 5% of cases of *B. melitensis* infection, and meningitis or meningoencephalitis are the most common manifestations. *Brucella* meningitis can be acute or chronic. It often occurs late in the course of disease, but it can be the presenting manifestation (WHO, 2006).

Cutaneous complications with a variety of skin lesions have been reported in patients with brucellosis, including rashes, nodules, papules, erythema nodosum, petechiae, and purpura. Cutaneous ulcers, abscesses, and suppurative lymphangitis appear to be more common with *B. suis*. Although uncommon, a variety of ocular lesions have been reported in patients with brucellosis. Uveitis is the most frequent manifestation, and can present as chronic iridocyclitis, nummular keratitis, multifocal choroiditis or optic neuritis (WHO, 2006).

2.8.2. Epidemiology of brucellosis in humans

Brucellosis is a zoonotic disease; hence the ultimate sources of infection are infected animals. The key species are the major food-producing animals: cattle, sheep, goats, pigs. Others, including bison, buffalo, camels, dogs, horses, reindeer and yaks are less important, but they can be very significant local sources of infection in some regions. Recently, the infection has also been identified in marine mammals, including dolphins, porpoises and seals, and these may present an emerging hazard to persons occupationally exposed to infected tissues from them. The risk of disease and its severity is to a

significant extent determined by the type of *Brucella* to which an individual is exposed (WHO, 2004). This will be influenced by the species of host animal acting as source of infection. *B. melitensis* is the type most frequently reported as a cause of human disease and the most frequently isolated from cases. It is the most virulent type and associated with severe acute disease. It is recorded as endemic in several countries and accounts for a disproportionate amount of human brucellosis. The organism is normally associated with infection in sheep and goats, but other species, including dogs, cattle and camels can be infected (Yong and Corbel, 1989).

In some countries, particularly in the Middle East, *B. melitensis* infection of cattle has emerged as an important problem. Contrary to some traditional views, *B. melitensis* remains fully virulent for man after infecting cattle. The bovine infection presents a particularly serious problem because of the large volume of infected milk that can be produced by an individual animal and because of the extensive environmental contamination that even single abortions or infected births can produce. *B. abortus* is the most widespread cause of infection, but associated with much less human disease (WHO, 2006). Infection in man is often sub-clinical and, where disease does occur, it is usually less severe than that caused by *B. melitensis* or *B. suis*. Cattle are by far the most common source of *B. abortus* but bison, buffalo, camels, dogs and yaks are important in some areas. *B. suis* has a much more restricted occurrence than *B. melitensis* and *B. abortus*. It is locally important as a source of human infection which can be as severe as that produced by *B. melitensis*. The sources and virulence of the organism vary with its biovar (subtype defined by laboratory tests). Biovars 1, 2, and 3 are associated with pigs and also, in the case of biovar 2, with hares. This variant has a low pathogenicity for humans but biovars 1 and 3 are highly virulent and can cause severe disease. Biovar 4 is associated with infection of caribou and reindeer in Alaska, Canada and Northern Russia. It is infrequently reported as a cause of human disease. Naturally acquired human cases of biovar 5 infection have not been reported. *B. canis* is a widespread infection of dogs in many countries. It is infrequently associated with human disease. Reported cases have usually been mild. *Brucella* infection occurs in many species of wild animals but these are rarely implicated as sources of human disease (Young, 1990).

2.8.3. Transmission of brucellosis to humans

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

Person-to-person transmission

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

Infection from a contaminated environment

This is difficult to document but probably occurs more frequently than is recognized. Infected animals passing through populated areas or kept in close proximity to housing may produce heavy contamination of streets, yards and market places, especially if abortions occur. Inhalation brucellosis may then result from exposure to contaminated dust, dried dung etc., (WHO, 2004). Contact infection may also result from contamination of skin or conjunctivae from soiled surfaces. Water sources, such as wells, may also be contaminated by recently aborted animals or by run-off of rain water from contaminated areas. *Brucella* spp. can survive for long periods in dust, dung, water, slurry, aborted fetuses, soil, meat and dairy products. The precise duration of survival is dependent on many variables such as the nature of the substrate, number of organisms, temperature, pH, sunlight, the presence of other microbial contaminants (Young and Corbel, 1989; WHO, 2006).

Occupational exposure

Certain occupations are associated with a high risk of infection with brucellosis. These include people who work with farm animals, especially cattle, sheep, goats and pigs: farmers, farm labourers, animal attendants, stockmen, shepherds, sheep shearers, goatherds, pig keepers, veterinarians and inseminators are at risk through direct contact with infected animals or through exposure to a heavily contaminated environment. Infection may occur by inhalation, conjunctival contamination, accidental ingestion, skin contamination especially via cuts or abrasions, and accidental self-inoculation with live vaccines (Young and Corbel, 1989). The families of farmers and animal breeders may also be at risk as domestic exposure may be inseparable from occupational exposure when animals are kept in close proximity to living accommodation. Persons involved in the processing of animal products may be at high risk of exposure to brucellosis. These include slaughter men, butchers, meat packers, collectors of fetal calf serum, processors of hides, skins and wool, renderers and dairy workers (WHO, 2004). Direct and environmental contamination may present hazards through inhalation, ingestion, mucous contamination and skin contact or penetration. The preparation and use of live vaccines is also hazardous as strains such as *B. abortus* S19 and *B. melitensis* Rev 1 are not completely avirulent for humans. The rough vaccine strain *B. abortus* RB 51 appears to be of low pathogenicity but still presents a potential hazard through accidental injection and is rifampicin-resistant. The use of virulent strains to prepare diagnostic antigens should also be avoided where possible (WHO, 2006).

Foodborne transmission

This is usually the main source of brucellosis for urban populations. Ingestion of fresh milk or dairy products prepared from unheated milk is the main source of infection for most populations. Cow, sheep, goat or camel milk contaminated with *B. melitensis* is particularly hazardous as it is drunk in fairly large volume and may contain large numbers of organisms. Butter, cream or ice-cream prepared from such milk also presents a high risk. Soft cheeses prepared from sheep or goats milk by addition of rennet are a particularly common source of infection in Mediterranean and Middle Eastern countries (Young, 1990). The cheese-making process may actually concentrate the *Brucella* organisms, which can survive for up to several months in this type of product. Such

cheeses should be stored in cool conditions for at least six months before consumption. Hard cheeses prepared by lactic and propionic fermentation presents a much smaller risk. Similarly, yoghurt and sour milk are less hazardous. *Brucella* dies off fairly rapidly when the acidity drops below pH 4, and very rapidly below pH 3.5. In many countries, the consumption of "health foods" has become fashionable. These often include unpasteurized milk or milk products and may pose a particular risk. There is often considerable resistance to accepting that such "healthy" products can be dangerous. Raw vegetables may be contaminated by infected animals and present a hazard. In endemic areas, tourists consuming "ethnic" food products may be particularly at risk (Young and Corbel, 1989).

Travel-acquired Brucellosis

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

Bio-terrorism

B. melitensis and *B.suis* have been developed experimentally as biological weapons by state sponsored programmes. Their relative stability in aerosol form combined with low infectious dose make them suitable agents for this purpose. *Brucella* could be used to attack human and/or animal populations. The impact is likely to be greatest in those areas in which the disease is not endemic. The organism can be obtained from natural sources in many parts of the world. Health and veterinary authorities should be aware of this potential source of infection (WHO, 2006).

2.8.4. Human Brucellosis in Ethiopia

Brucellosis primarily affects livestock, but can be transmitted to humans by ingestion, close contact, inhalation or accidental inoculation. The prevalence of human brucellosis

differs between areas and has been reported to vary with standards of personal and environmental hygiene, animal husbandry practices, and species of the causative agent and local methods of food processing (WHO, 2006). The brucellosis 2003 International Research Conference estimated that 500,000 human infections occur per year worldwide, with incidences ranging from less than one case per 100,000 population in UK, USA and Australia, through 20 to 30 cases per 100,000 in southern European countries such as Greece and Spain, to more than 70 cases per 100,000 in Middle Eastern States such as Kuwait and Saudi Arabia (Pappas *et al.,* 2006). As compared to study of animal brucellosis, study of human brucellosis in Ethiopia is sparse with even less information on risk factors for human infection. For instance, out of 56 cases with fever of unknown origin, two (3.6%) were reported to be positive for *B. abortus* antibodies by RBPT and CFT (Tolosa *et al.,* 2007). A study conducted in traditional pastoral communities by Ragassa *et al.* (2009) using *B. abortus* antigen revealed that 34.1% patients with febrile illness from Borena, 29.4% patients from Hammer and 3% patients from Metema areas were tested positive using *Brucella* IgM/IgG Lateral Flow Assay. But they failed to include a parallel study of animal brucellosis. Studies conducted in high risk group such as farmers, veterinary professionals, meat inspectors and artificial insemination technicians in Amhara Regional State (Mussie *et al.,* 2007), Sidama Zone of Southern People Nations and Nationalities Sate (Kasahun *et al.,* 2007) and Addis Ababa (Kassahun *et al.,* 2006) found a seroprevalence of 5.30%, 3.78% and 4.8% by screening sera from 238, 38 and 336 individuals respectively. The discrepancy between Regassa *et al.* (2009) and others might be due to difference in milk consumption habits and sensitivity of test methods used. Furthermore Abebe *et al.* (2009) assessed the prevalence of major causative agents of acute febrile illness in 653 outpatients of four health centers in Northern Ethiopia. Among these febrile patients, *B. abortus* was detected in 6.3%, 3% and none of the patients in Finoteselam, Quarit, and both Dembecha and Jiga, respectively. It must be remembered that as these investigations were of acute cases and as such, they may not have had sufficient time to allow seroconversion. Similarly, seroreactivity may not correlate with the acute febrile episode for which the individuals were admitted.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Yabello district, Borana zone, Ethiopia (Figure 1). The Yabello district comprises about 23 pastoral associations (PAs), in which 48% (11 PAs) and 52% (12 PAs) of the peoples dwelling in and around the district practice pastoral and agro-pastoral activities, respectively. Yabello area is featured by semi-arid to arid climate and scarcity of water is standing problem. As a result livestock production plays important role in the livelihood of the community. Live stock is kept under extensive production system. Sometimes agriculture is practiced when there is sufficient rain during major rainy seasons. Major rainy season is from mid March to May, which is 'GANNA'. The minor rain season is from mid September to October, which they call 'HAGGAYA.' There are veterinary services provided by veterinary doctors, animal health assistants, community animal health workers. The estimated total human population of Borana zone is 480,000 with annual population growth of 2.5–3% (Homan *et al*., 2003). The Borana zone supports a total of 1,771,589 cattle, 1,991,196 goats, 699,887 camels and 52,578 donkeys (CSA, 2008). Cattle are the livestock species highly valued by the Borana pastoralist. To this end, the government has established Borana cattle breeding and improvement center at Dida Xuyura. Dida Xuyura ranch is the only Borana cattle breeding and improvement premise found in the southern rangelands. The ranch is situated at about 550 km south of Addis Ababa and 20 km north of Yabello town.

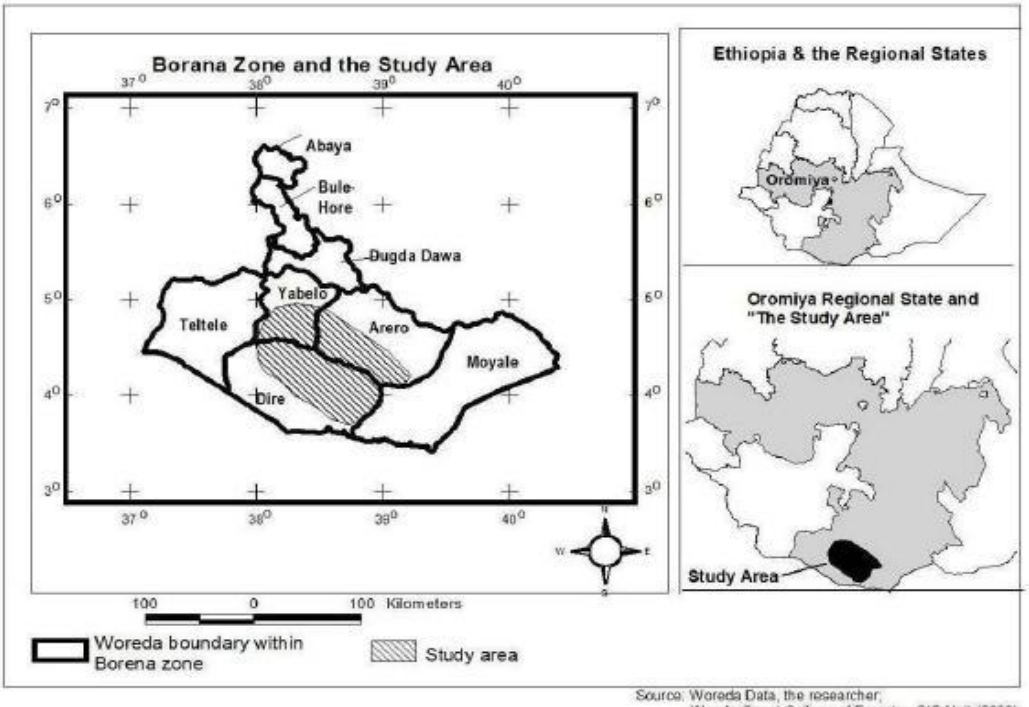

Figure 1. Map of the study area in Borana, southern Ethiopia.

3.2. Study Design

The study was conducted from November 2015 to May 2016 in Yabello district, Borana zone, Ethiopia. A cross-sectional sero-epidemiological study was carried out to determine the current prevalence of bovine brucellosis and risk factors associated with its occurrence in Dida Tuyura ranch and pastoral cattle herds in Yabello District. The study subjects were selected by multistage sampling. The primary units were villages in the agro-pastoral or crop-livestock mixed production system and pastoral communities in pastoral production system. The secondary units were cattle herds in selected villages. Individual samples were selected from the previously identified herds by simple random sampling method.

3.3. Study Population and sample size

The study population used in this study is the Borana cattle raised at the Dida Tuyura cattle breeding and improvement ranch and Dida Yabello peasant association. The sample size for this study was determined as described by Thrusfield (2005) as follows.

$$n = \frac{1.96^2 \, Pexp(1-Pexp)}{d^2}$$

Where

n=required sample size

Pexp=expected prevalence

d= desired absolute precision

A previous study on *Brucella* at yabello distric revealed an average prevalence of 11.2% (Hunduma and Regassa, 2009). Therefore using 11.2% expected prevalence and 5% absolute precision at 95% confidence level, the number of animals needed to estimate the prevalence of *Brucella* in Dida Tuyura cattle breeding and improvement ranch and Dida Yabello peasant association was calculated 153. In actuality we have sampled more than calculated depending on the resource we had. Hence, 661 cattle were included (339 from Dida Xuyura ranch and 322 from surrounding pastoral herds, Dida Yabello). Thus, the primary sampling unit was an animal in the selected herds.

3.4. Sample and Data Collection

In Dida Tuyara Ranch there are 35 herds each consisting of 40-50 cattle. From each herd 10 animals ≥ 3 years of age were randomly selected. Blood samples were collected from 350 animals. From each cattle about 10 ml of blood was collected from the jugular vein following standard procedures using plain vacutainer tubes. Besides blood samples, history of abortion, number services per conception, retained placenta, lactation stages were collected from the records for animals raised on the ranch. 11 samples which were not having clear information were discarded.

Whereas from Dida Yabello, 33 model cattle owner pastoralists were purposively selected and 322 blood samples were collected from their herds. Sample to be taken from

each pastoralist herd was decided based on herd size. Animals ≥ 3 years of age were randomly selected and sampled. Besides blood samples, information about history of abortion, number services per conception, retained placenta, and lactation stages were collected for sampled animals from owners. Therefore 661 individual animals were included into the study to investigate bovine brucellosis in the study area. Accordingly, the collected blood samples were kept at room temperature overnight for clot retraction and serum was harvested separately into sterile tubes. The sera samples were stored at -20°C until analyzed in the laboratory.

3.5. Laboratory Analysis

Rose Bengal plate test

All serum samples were screened using the RBPT, according to the procedures described by Alton and colleague (1975) and the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* of the World Organisation for Animal Health (OIE) (2004). The rose bengal antigen constituted a suspension of *B. abortus* (obtained from the Institut Pourquier, 326 rue de la Galéra, Parc Euromédecine, 34090 Montpellier, France). Thirty µl of serum was mixed with an equal volume of antigen suspension on a glass plate and agitated. After four minutes of rocking, any visible agglutination was considered a positive result.

Complement fixation test

All sera which tested positive to the RBPT were further tested using CFT for confirmation. The CFT was performed at the National Veterinary Institute, Debre Zeit, Ethiopia. A standard *B. abortus* antigen for CFT (Veterinary Laboratories Agency, United Kingdom) was employed to detect the presence of antibodies against *Brucella* in the sera. The control sera and complement were both obtained from the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Germany. Sera with a strong reaction – more than 75% fixation of the complement (3+) at a dilution of 1:5 and

with at least 50% fixation of the complement (2+) at dilutions of 1:10 and 1:20 – were classified as positive (+), according to the guidelines of the OIE *Manual* (2004).

Sensitivity and specificity of the tests

For RBPT sensitivity from 91% to 100% in affected areas (Faye *et al*., 2005), and from 96.7% to 100% on *Brucella*-free farms (20); specificity from 95% to 99% in affected areas (Faye *et al*., 2005), and from 79% to 91.9% on free farms (Mainar-Jaime *et al*., 2005). For the CFT sensitivity from 96.7% to 100% and specificity from 88.8% to 97.7% (Mainar-Jaime *et al*., 2005).

3.6. Data Analysis

Putative biological and environmental factors believed to be associated with *Brucella* infection were recorded and entered into Microsoft excel spread sheet. Additionally, sampled animal information on Pa, sex, age, body condition, parity, history of abortion and retained foetal membrane was collected. All the necessary statistical analysis was performed using STATA version11.0 for windows (Stata Corp, College Station, TX) or R. Association of *Brucella* seropositivity with aforementioned exposure variables was assessed using logistic regressions.

4. RESULTS

4.1. Results of Seroprevalence of Bovine Brucellosis and Associated Risk Factors

4.1.2. Results of seroprevalence of bovine brucellosis at animal level

In this study 16 cattle out of 661 (2.4%; 95 5 CI: 1.39, 3.9) tested using Rose Bengal Plate Test were found to be positive. The sero-prevalence was 2.94% (95% CI: 1.42, 3.53) in 339 animals sampled from Dida Tuyura Ranch where as it was 1.86% (95% CI: 0.68, 4.01) in 332 cattle sampled from pastoralist' herd surrounding the ranch. However, only 5 animals were found positive with Complement Fixation Test in animal sampled from Dida Tuyura Ranch yielding a prevalence of 1.47% (95% CI: 0.48, 3.41). From Six animals which gave positive reaction to Rose Bengal Test from pastoralists' herd in the vicinity of the ranch 2 also gave positive reaction to Complement Fixation Test yielding a prevalence of 0.62% (95% CI: 0.162, 4.73). Taken together the seroprevalence of bovine brucellosis as revealed by Complement Fixation Test was 1.1% (95% CI: 0.43, 2.17).

4.1.2. Results potential risk factors associated Brucella seropositivity at animal level

Table 2 present results of animal level *Brucella* seropositivity and their association with exposure variables using logistic regression. Accordingly, seroprevalence of bovine brucellosis did not show significant variations among Pa, parity, body conditions and sex ($P > 0.05$) using univariate logistic regression analysis. However, abortion history and RFM were the two potential risk factors significantly associated with Brucella seropositivity ($P<0.05$) by using univariable analysis of logistic regression. Animals that have abortion history were 10 times at risk of being infected with *Brucella* than animals did not have abortion history whereas animals that had suffered from retained fetal membranes were 9.7 times at risk of being positive to *Brucella* infection than animals without such history (Table 2).

Table 2. Univariable analysis of potential risk factors associated *Brucella* seropositivity

Variable category	No of animal studied	Seroprevalnce (%)	P value	OR(95%CI)
Pa				
Dida Tuyura	339	1.47	0.299	2.4(0.46,12.4)
Dida Yabello	322	0.62	-	
Sex				
Male	57	1.73	0.584	1.7(0.21,14.4)
Female	604	0.99	-	
Body conditions				
Poor	195	2.3	-	1.0
Medium	317	0.3	0.056	0.12(0.014,1.05)
Good	149	0.7	0.229	0.26(0.01,0.06)
History of abortion				
Yes	80	5	0.003**	10.2(2.22, 46.2)
No	581	0.51	-	1.0
RFM				
Yes	50	6	0.004**	9.7(2.1,45.5)
No	611	0.65		1.0
Parity				
No parity	57	1.75	-	1.0
1st parity	280	0.71	0.461	0.40(0.037,4.51)
2nd parity	216	1.38	0.838	0.75(0.08, 7.72)
Above three	108	0.92	0.649	0.52(0.03, 8.52)

***: statistically significant**

Table 3 presents results of other potential risk factors analyzed using Fisher' exact test that came up with zero out come in their category. Accordingly, in the present study age groups were not significantly associated with *Brucella s*eropositivity based on Fisher's exact test (P>0.05).

Table 3.Fisher's exact test results for association of potential risk factors with *Brucella* seropositivity

Variable category	No of animal studied	Seroprevalnce (%)	P value*
Age			
Young-adult	142	0	0.525
Adult	336	1.5	
Old	183	1.1	

4.1.3. Multivariable analysis of animal level risk factors with Brucella sero-positivity

A risk linked abortion history was observed in final model of animal level analysis. Thus, in the multivariable analysis, only abortion remained to be independently associated with brucellosis seropositivity whereas other not (Table 4).

Table 4. Multivariable model for risk factors of bovine *Brucella* seropositivity at animal level

Variable category	Coefficient	SE	CI (95%)	P value	OR
Sex					
Male	1.67	1.24	-0.76, 4.11	0.178	5.3
Body conditions					
Medium	-2.07	1.10	-4.25, 0.09	0.061	0.12
Good	-1.41	1.11	-3.59, 0.76	0.204	0.24
Abortion history					
Yes	2.60	0.88	0.87, 4.32	0.003*	13.46
Constant	-4.68	0.74	-6.14,-3.22	0.000	1.0

Constant: female, poor and no variable category (references for each variable)

*: statistically significant

4.2. Results of Questionnaire Survey

A total of 33 livestock owners in the Borana pastoral areas surrounding Dida Tuyura Ranch, who are among the ones to whom the animals breed on the ranch are distributed were interviewed, based on their willingness to participate in the survey. The owners revealed that extensive management system was exercised in both Dida Tuyura and Liban Kara villages (Ollas) of Yaballo district. Cattle are either kept alone or together with other species of animals mainly for milk production (30/33=90.91%; 95% CI: 75.67, 98.08) and income generation (3/33=9.09%; 95% CI: 13.29, 45.52) through marketing. The highest proportion (27/33=87.88%; 95% CI: 71.79, 96.59) of the cattle herds were reared along with camels, sheep and goats, while (6/33=18.18%; 95% CI: 6.98, 35.46) of cattle herds were kept only with small ruminants, while only very few herds were kept along with either only equine or camels. Seven of the 33 interviewed pastoralists (21.21%; 95% CI: 8.98, 38.90) do not separate animals during parturition while the remaining provide separate parturition area for pregnant female cattle. The larger proportion of the respondents (19/33 =57.57%; 95% CI: 39.22, 74.52 and 20/33 = 60.60%; 95% CI: 42.14, 77.09) respectively do not have calving room and practice poor hygienic practice during assisting of parturitions. Ten of the interviewee (30.30%; 95% CI: 15.59, 48.71) experienced abortion in cattle on their farms and 29/33 (87.88%; 95% CI: 71.79, 96.59) of the dispose the aborted fetus in the environment. The questionnaire survey showed that over all of the interviewed owner's stated that they drank fresh raw milk frequently. Most of the milk originated from their own cattle, the rest was purchased. Almost about half of the pastoralists have their cattle tested for brucellosis previously. They also indicated that they knew that brucellosis affects other animal species.

5. DISCUSSION

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

In the present study, all the 661 cattle were clinically normal at the time of sampling and according to the ranch attendants and owners, none had previously shown clinical signs of brucellosis. The seroprevalence of bovine brucellosis reported in Dida Tuyura Ranch and pastoral herds is 1.1%. This finding in apparently healthy animals indicates that many infected cattle might be silent carriers for brucellosis and their products may pose a serious health problem for the community. In consent to this study previous authors showed lower prevalence of brucellosis in cattle (Jergefa *et al*., 2009) and in camels (Bekele, 2004; Gumi *et al*., 2013).

The seroprevalence result of the present study is lower than many of the earlier reports in Ethiopia. For instances, higher prevalence than the current report was observed by various authors (4.2% in Borena, Oromia region by Teshome *et al*., 2003, 1.7% in Tigray by Bekele, 2004, 7.6 % in Afar by Zwold *et al*., 2012 and 2.43 % in Jijiga by Bekele *et al*., 2011). The findings in the present study were also lower than reported in other African countries. For instance a prevalence of 2.0 to 15.4% was reported in Kenya (Wanjiohi *et al*., 2012), 3.1% in Eritrea (Omer *et al*., 2000) and 3.1% in Somalia (Ghanem *et al*., 2009). Differences in seroprevalence observed in this study, as opposed to those recorded by previous researchers, might also be due to differences in herd size, sample size, tests used, agro ecological and management conditions, and the presence or absence of infectious foci, such as *Brucella*-infected herds, which could spread the disease among contact herds. In general the occurrence of brucellosis in cattle bear on ranch is significant. It may act as a source of infections for cattle

owned by pastoralists if the heifers distributed are not tested regularly. This may bear huge impact the economy the area in general.

There was no statistically significant difference (P>0.005) in seroprevalence of *Brucella* between Dida Xuyara ranch and Dida yabello pastoralist cattle. This finding is in line with report by Asgedom *et al*., (2016) that, there is no significant variation in seroprevalence of *Brucella* among PAs. Even though, it was not statistically significant, high prevalence of *Brucella* was observed in the cattle of Dida xuyara ranch (1.47) than dida yabello pastoralist (0.62%). This may be related to the report that, increase with reproductive diseases increases with the change from pure extensive to intensive management (Teklu and Gangwar, 2011).

Though in the present study the seroprevalence of bovine brucellosis *is not statistically significant* between the sexes, the result showed that infection was higher in male (1.73%) than female (0.99%).The similarity of the result could be due to similarity in management even though females is more susceptible to the infection than males. Therefore, contrary to this finding many previous studies showed that female are at higher risk of contracting brucellosis than male for example: Asgedom *et al*. (2016); Yilkal, (1998) Bekele *et al*., 2000) . Moreover, there was report that serologic response of male animal is limited and the test of infected male animals were usually observed to be non-reactor or shown to be low antibody titers. And also there were some reports that male cattle are more resistance than female (Asgedom *et al*., 2016). Still it could due to accidental appearance of positive animals in males sampled in small numbers

5% of animals with history of previous abortion and 6% of animals with history of retained foetal membrane had *Brucella* antibody in their serum according to recent study. Statistical analysis also revealed associated between *Brucella* seropositivity and history of previous abortion (P<0.003).With history of retained foetal membrane too (P<0.004). This finding is consistence with Tesfaye (1996) (6.1%) from Mekele dairy farm and Yayeh (2006) from north Gondar. But lower than the report of Asfaw,

(2014) (17.39) from in and around Asella and Bishoftu towns. This may be due to fact that the seroprevalence of brucellosis is lower in low-land agro-climate, which is unsuitable for survival of *Brucella* organisms than highlands (Radostits *et al*., 2000). Generally abortion or still birth and retained placenta are typical outcomes of brucellosis. In addition, in highly susceptible non vaccinated pregnant cattle, abortion after the 5th month of pregnancy is cardinal feature of the disease (Asfaw, 2014).

Asgedom *et al*., (2016); Megersa *et al*., 2011; Tsegay *et al*., 2015 and others reported that there was statistically significant difference among different age groups to *Brucella* seropositivity. There reason was mentioned as; brucellosis appears to be more associated with sexual maturity and higher prevalence reportedly reported in sexually mature animals. Seroprevalence may increase with age as a result of prolonged duration of antibody responses in infected animals and prolonged exposure to pathogen, particularly in traditional husbandry practice where female animals are maintained in herds for long period of time (Megersa *et al.,* 2011). But according to the present study, there was no statistically significant different among age groups to *Brucella* seropositivity. This may be due to fact that only sexually matured animals above the age of 3 years were sampled and majority was between 3-6 years of age.

Significant difference in sero positivity was not observed among 4 parity groups (P>0.05). This finding is similar with Berhe *et al*., (2007) but opposite to the findings of Asfaw, (1998) ; Teklu and Gangwar (2011) and Asfaw, (2014) who had reported significant difference in seropositivity among parity groups. Finally body condition had no significant association to *Brucella* seropositivity, findings by Joseph *et al*., (2016) and Tsegay *et al*., (2015) supports this.

The questionnaire survey has provided information regarding the knowledge and practices of cattle owners about brucellosis in Yabello district outheast Ethiopia. Knowledge of diseases is a crucial step in the development of prevention and control measures (WHO, 2004). Despite huge efforts of the government and non-government institutions to promote and improve animal production in the areas, this study

highlighted that general knowledge of brucellosis among the pastoralists was poor. Cattle rearing pastoralists in Borena zone of Oromia regional state practiced a high degree of ruminant diversification, i.e., in addition to cattle, they kept camels, sheep and goats. Mixing of animals although having its own economic importance increases the chances of transmission of brucellosis among the different species (Acha & Szyfres, 2001). In most of the areas in the study zones, animals had direct access to water sources like pond/dam water and contaminated it through discharges. This is shown by the fact that most of the pastoralists dispose the aborted fetuses in the environment freely. However, the exposure rate may not be very high due to the fact that cattle herds are mobile; this does not restrict them to a specific category of the water resources. Most of the pastoralists in the area indicated that they consume raw milk frequently. Moreover they indicated that their animals were tested previously for brucellosis but no action was taken. This adds to the problem of the pastoralists as they consider it not to be serious. On top of this most of them didn't have any knowledge about the transmission of brucellosis through consumption of raw milk. The pastoralists of the study area consume raw milk and often assist delivery by themselves despite practicing unhygienic producers. These practices expose pastoralists to brucellosis and clearly show the public health importance the disease in the pastoral areas.

6. CONCLUSION AND RECOMMENDATIONS

In the present study, respective 1.47% and 0.62% *Brucella* prevalence in apparently healthy cattle in Dida Tuyura Ranch and pastoralists' herd in the vicinity of the ranch was obtained. The study also revealed 1.1% over all prevalence to *Brucella* seropositivity in the area. This low seroprevalence of brucellosis in apparently healthy cattle observed in this study showed that these animals are reproducing normally and serve as permanent carriers of brucellosis. Previous history of abortion and retained fetal membranes were significantly associated with sero-positivity to brucellosis. Animals that have abortion history were 10 times at risk of being infected with *Brucella* than animals did not have abortion history whereas animals that had suffered from retained fetal membranes were 9.7 times at risk of being positive to *Brucella* infection than animals without such history. Finally the study clearly showed that the pastoralists have less knowledge of the disease and are at risk of acquiring the infection. The herding practices also showed that cattle could be good sources of *B. abortus* for other animal species. Since this low seroprevalence of bovine brucellosis is not the result of informed policy, there is no guarantee that it will continue unchanged. It is, therefore, an important period of consolidation for pastoralists and local authorities to keep the disease burden low. Therefore, the following are recommended:

- There should be education of the pastoralists about transmission, economic and public health importance of Brucellosis in the study area
- There should be extension service on the handling of aborted fetuses and assistance of delivery
- Animals must be tested and confirmed to be negative before distributed to pastoralists
- The public health authorities should teach the pastoralists to boil milk before consumption
- Further research on the isolation and characterization of circulating *Brucella* species in other livestock (small ruminants, camel, equine and dog) of Ethiopia should be initiated.

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8. ANNEXES

Annex 1: Rose Bengal Plate Test procedure

All serum samples collected will be screened using RBPT. The antigen consisted of a suspension of *Brucella abortus* (obtained from Institute purquier 326, Rue de la Galera 34097 MONTEPELLIER CEDEX 5 France). The procedure recommended by Alton *et al*. (1975) and OIE (2005) is used. Briefly the procedure is as follows:

The test sera and the antigen will be left at a room temperature for half an hour every time before the test is proceeded.

- 30 micro litter of each test serum will be taken and placed on a clean glass slide,
- 30 micro liter of RBPT antigen will be added to the side of each test serum using epindorf tube.
- Then the antigen and the test serum were mixed thoroughly by an applicator,
- The glass slide was shaked by hand for 4 minutes and
- Finally the result of each test was read by looking the presence or absence of agglutination and the degree of agglutination was also appreciated in a very good light source and when necessary magnifying glasses were used.

Interpretation: After four minutes rocking (shaking) any visible agglutination was considered positive.

Annex 2: Complement Fixation Test

Procedure

1. Test sera and appropriate working standards are diluted with an equal volume of veronal buffered saline in small tubes and incubated at 58°C for 50 minutes in order to inactivate the native complement.
2. Using standard 96-well U-bottom microtitre plates, 25 µl volumes of diluted test serum are placed in the wells of the first and second rows, and 25 µl volumes of veronal buffered saline are added to all wells except those of the first row.
3. Serial doubling dilutions are then made by transferring 25 µl volumes of serum from the second row onwards continuing for at least four dilutions.
4. Repeat steps ii and iii above for each serum to act as anticomplementary serum controls (see below).
5. Volumes (25 µl) of complement at 1.25 MHD, are added to each well and 25 µl of antigen, diluted to working strength, are added to all wells excluding those of the anticomplementary controls. These latter wells receive 25 µl of veronal buffered saline instead.
6. Control wells containing: diluent only, negative serum + complement + diluent, antigen + complement + diluent, and complement + diluent, are set up to contain 75 µl total volume in each case.
7. The plates are incubated at 37°C for 30 minutes with agitation at least for the initial 10 minutes, or at 4°C for 14- 18 hours. 99
8. Volumes (25 µl) of sensitised SRBC suspension are added to each well, and the plates are reincubated at 37°C for 30 minutes with agitation at least for the first 10 minutes.
9. The results are read after the plates have been left to stand at 4°C for up to 1 hour to allow unlysed cells to settle.

Interpretation

Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive and lack of fixation/complete hemolysis was considered as negative.

Annex 3: Questionnaire on assessment of Community awareness on Brucellosis in Borena Zone, Southern Ethiopia

Date _____

Name _____Sex_____ Age_____ District_____

Kebele_____ phone No._____

Make only right sign (tick) on selected options by individual for the following questions

1. If you are pastoralist which spp of animal you reared? =Cattle =sheep and goat =camel
2. If you have cattle what is your herd size? (Indicate in number) _____
3. Do you know bovine Brucellosis ? =yes =No
4. Which animals affected by bovine Brucellosis? =Cattle =all warm blooded animals =human =don't know
5. Do you have awareness on zoonotic importance of Bovine Brucellosis? =Yes =No
6. Which one of the following you expect as means of Brucellosis transmission from animal to animal? =Contact with infected domestic and wild animals =by inhalation of aerosol /coughing =contaminated feed = Coitus
7. Which one of the following you expect as means of Brucellosis transmission from animal to human? =Eating raw meat =drinking raw milk =by inhalation of aerosol during coughing =sharing the same house with infected animal/human
8. Life style of pastoralist in area in relation to their animals : =share the same house =not sharing
9. Milk drinking habit: =boiled milk =both boiled and raw milk
10. Meat eating habit: =cooked only =both cooked and raw meat
11. If Brucellosis suspected animal is died what do you do? =Burn all carcass =burring all carcass =cooking and eat the meat

12. Which control means of Brucellosis do know? =Test and culling

=pasteurisation of milk =improving sanitary and hygienic standards