ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

EPIDEMIOLOGY OF BOVINE BRUCELLOSIS IN SELECTED THREE AGROECOLOGIES IN CENTRAL OROMIA, ETHIOPIA

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ABBREVIATIONS

BgVV	Bundesinstitute for Gesundheitilichen Verbaucherschutz und Veterinarmedizine
CACC	Central Agricultural Census Commission
CFT	Complement FixationTest
DNA	Deoxy Ribo NucelicAcid
ELISA	Enzyme Linked Immunosorbent Assay
H_2S	Hydrogen sulfide
IgA	Immunoglobulin A
IgG1	Immunoglobulin G_1
IgG2	Immunoglobulin G_2
IgM	Immunoglobulin M
MRT	Milk Ring Test
MZN	Modified Zeihl Neelsen Staining
^{o}C	Degree Centigrade
OADB	Oromia Agricultural Development Bureau
OARDB	Oromia Agricultural and Rural Development Bureau
OIE	Office International des Epizootics
OR	Odds Ratio
PAHO	Pan American Health Organization
RBPT	Rose Bengal Plate Test
SAT	Serum Agglutination Test
SDHT	Skin- Delayed-type Hypersensitivity Test
VBD	Veronal Buffer Diluents
WHO	World Health Organization

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ABSTRACT

A cross-sectional sero-epidemiological study of bovine brucellosis was carried out in three difeferenmt agroecologies of central Oromia, Ethiopia. The study was conducted from September 2005 to March 2006 to determine the overall current epidemiological picture of the disease and the effect of potential risk factors in the study areas. A total of 176 households (farms) and the 1238 animals were selected randomly by one stage cluster sampling method from all the three agro-ecological areas. From the lowland areas, 59 households and 423 animals were selected, while 58 households and 385 animals were selected from the mid-highland areas and 59 households and 430 animals from the highland areas. Serum samples (1238) were collected once from all animals above six month of age and were tested using RBPT (as a screening test) and CFT (as a confirmatory test) to detect brucella seropositivity. Questionnaires were also administered to the 176 households to gather information on farm and cow attributes. The results showed that the overall seroprevalence of bovine brucellosis at cow level was 2.99%. The seroprevalence varied from 1.04% in the mid-highland, 3.48% in the highland and 4.26% in the lowland. The overall herd level seroprevalence was 13.64%. At herd level, the seroprevalence was 18.64% in the highland, 17% in the lowland and 5.17%, in mid-altitude agroecology. The result of univarate logistic regression showed that breed and management had statistically significant effect on cow level bovine brucellosis seroprevalence ($p<0.05$) in the overall studied areas. In the lowland, breed ($p<0.05$), management system ($p<0.01$), mating method ($p<0.01$) and source of replacement stock ($p<0.01$) had significant effect on the cow level seroprevalence of bovine brucellosis. In the multivariate logistic regression test, only management factor had significant effect in the lowland areas ($p<0.001$). The herd level seroprevalence was not affected by all the potential risk factors considered on the overall and agroecology specific seroprevalence ($p>0.05$). The results of questionnaire survey showed that farmers had low awareness on brucellosis and were not practicing the necessary precautions to prevent and control the disease. In conclusion, individual animal seroprevalence was low while moderate seroprevalence was observed at herd level. Breed, management system, herd size, mating method and sources of replacement stock were found to be important risk factors to

bovine brucellosis in the study areas.

Keywords: bovine, brucellosis, seroprevalence, agroecology, Oromia, Ethiopia

1. INTRODUCTION

Brucellosis is an important livestock and human disease in Africa and other part of the developing world (Perry *et al.*, 2002). Its importance is emanated from its widespread distribution and impacts on multiple animal species, such as cattle, sheep, goats, pigs and human beings (Dermot Mc and Arimi, 2002). Brucellosis is primarily a reproductive disease characterized by abortion, retained fetal membrane and impaired fertility in the principal animal host. The disease caused by *Brucella abortus* which is known as brucellosis, Bang's disease, contagious abortion, or infectious abortion. *Brucella abortus* was first isolated from cattle in 1897 by the Danish veterinarian Bernard Bang (Radostits *et al.*, 2000). It is to a certain extent distinguishable from other Brucella species by biochemical reactions and by serological means. The serological differences are related to the amounts of A and M antigens that a Brucella strain possesses. There have been about nine biotypes being recognized and a number of strain variants. About 85-89 % of the infections are from biotype1 (Ocholi.*et al.*, 2004). *Brucella abortus* affects many animal species on every continent and has zoonotic and economic importance, as well as a public health hazard (OIE, 2002).

Bovine brucellosis is widespread throughout the world except for a number of countries where eradication has been successful (WHO, 1986). Many countries have made considerable effort with their eradication programs and some have eradicated the disease (Radostits *et al.*, 2000). The disease has been eradicated from Finland, Norway, Sweden, Denmark, The Netherlands, Belgium, Switzerland, Germany, Australia, Hungary, Romania and Bulgaria as well as other countries (PAHO/WHO, 2001). 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 (WHO, 1986). The prevalence of infection varies considerably between herds, areas, management and countries.

Brucellosis has been known as a major economic importance in most developing countries, which have no national brucellosis control and eradication program (Radostits *et al.*, 2000). Moreover, 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 compounded by limited resource aggravates the situation (Seifert, 1996).

In Ethiopia, a number of research works (Gebremariam, 1985; Kabede, 1999; Kassahun, 2004) have been conducted to study the prevalence of bovine brucellosis, however, most of the research were not wide in their coverage. In addition, the works lack information on economic and zoonotic importance of brucellosis despite the fact that the importance of the disease is increasing due to the intensification of animal population in the country to respond to the ever increasing demand for milk by the urban population. The first report was given in 1970 by the veterinary section of the US Navy Medical Research Unit with an overall prevalence of 11.7% in a total of 1328 bovines tested for brucellosis in different regions of Ethiopia (MoA, 1970). The subsequent reports ranged from 0.2% in Jimma (Taddele, 2004) to 38% in the central highlands of Ethiopia (Rashid, 1993). In spite of the great potential for dairy development in the central highlands of Oromia and the tremendous efforts made by both governmental and non-governmental development institutions to promote the dairy industry, limited studies have been conducted on the epidemiology of bovine brucellosis in the different agro-ecological conditions.

The objectives of this study are thus:

- To determine overall sero-prevalence of bovine brucellosis;
- To determine the effects of potential risk factors on seroprevalence of bovine brucellosis in three different agroecologies of Central Oromia, Ethiopia.

2. LIERATURE REVIEW

2.1. Etiology

The organism responsible for brucellosis is a small Gram-negative coccobacillus, non-motile, non-encapsulated and non-spore forming, facultative intracellular organism (Quinn *et al.*, 2002 and Ocholi *et al.*, 2004). In infected tissue, it normally occurs singly or in small groups. The colonies are circular, and 2-4 mm in diameter. Smooth colonies appear translucent, honey-colored and bluish-green when stained with crystal violet and rough colonies appear granular, reddish-yellow and deep violet-red when stained with crystal violet whereas mucoid colonies are stringy and appear transparent, grayish and light bluish-red when stained with crystal violet (Quinn *et a*l., 2002).

Several commercially produced media suitable for the isolation of Brucella, among which is 5-10% blood agar, are available. To check up bacterial and fungal contaminants brucella selective medias are often used. The selective medias is nutritive media: blood agar based with 5% seronegative equine or bovine serum and antibiotic supplement (Quinn *et al.*, 2002). On primary isolation, it usually requires the addition of 5-10% carbon dioxide and takes 3-5 days incubation at 37°C for visible colonies to appear. *Brucella abortus* produces H_2S. It usually grows in the presence of basic fuchsine, but not always in the presence of thionin at standard concentrations, and is catalase and oxidase positive. It is lyzed by brucella-phages: Weybridge (Wb), Firenze (Fz), Tbilisi (Tb) and Berkeley (Bk) (Ocholi *et al.*, 2004).

Each Brucella is know to have a specific oxidative metabolic pattern that can be used to determine the ability of an isolate to utilize oxygen on various amino acid and carbohydrate substrates. Currently, there are six recognized species within the genus of Brucella: *B. aborts, B. melitensis, B. suis B. ovis, B. canis, B. neotome* and nine recognized biovars of *B. abortus*. The boivars are 1-6 and 7a while 7&8 are currently deleted by International Committee for Taxonmy of Brucellae (Quinn *et al.*, 2002; Ocholi

et al., 2004).

2.2. Hosts

Brucellae have been reported to have definite host preferences and secondary hosts play a minor role in the maintenance and spread of a particular Brucella species. *Brucella abortus* has been indicated to mainly infect 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*, 2000). 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 (Anderson, 1967). Naturally acquired *B. abortus* infections, is low in goats and this animal species is irrelevant as a host for *B. abortus* (Mathur, 1967). Isolation of *B. abortus* from swine, horses (Radostits *et al*, 2000) and camels (Al-Khalaf and El-Khaladi, 1989) 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 (Forbes, 1990; Radostits *et al.*, 2000). Feral animal such as buffalo, swine, deer, fox, hare and rodents are susceptible to Brucella, however, their role as a host for Brucella particularly in intensive cattle farming is not known (Thorpe and Sidewell, 1965). The role of small feral animals is not clear although studies of experimentally induced and naturally occurring Brucella infection in flies, arthropods and other parasites suggest that they may be susceptible to infection with Brucella (Thorpe and Sidewell, 1965).

Age, sex, stage of pregnancy and natural resistance to Brucella may influence the progression of infection. Heifers born to infected dams usually test seronegative for Brucella for a long period (Bercovich and Taaijke, 1990). Pregnant females are more likely to become infected than non-pregnant cattle and males due to the presence of a substance called erythritol in gravid uterus which sustains the growth of the organism

(Hirsh and Zee, 1999).

2.3. Transmission

Transmission of *B. abortus* mostly occurs via the oral route because cattle tend to lick aborted fetuses and the genital discharge of an aborting cow. Brucella organisms can also be transmitted through the transmammary route, while calves born to healthy dams are infected when fed on colostrum or milk from infected dams (*Radostits et al.*, 2000).

Bulls that remain fertile and functionally active after infection with Brucella shed the organisms with the semen during the acute phase of the disease. During the later stage, shedding may cease or become intermittent. In contrast to artificial insemination, bulls used in natural service may fail to spread the infection, since semen is not deposited in the uterus (Hirsh and Zee, 1999; Radostits, *et al* 2000).

Wildlife, birds and waterways contaminated with urine, uterine discharge, or slurry from aborting cattle are vectors for indirect exposure to Brucella organisms. Dogs carrying pieces of placenta or aborted fetuses from one place to another can disseminate the infection (Forbes, 1990). Contamination of a cowshed or pasture takes place when infected cattle abort or have full-term parturition. Although it is generally accepted that *B. abortus* is not excreted for considerable time before abortion occurs, excretion in the vaginal discharge of infected cattle may occur as early as 39 days after exposure. A massive excretion of Brucella starts after abortion and may continue for 15 days.

After the fetal membranes are expelled the uterine discharge diminishes and the number of Brucella organisms excreted decreases rapidly (Nicoletti, 1980; Radiostatis, 2000). The infectious material usually clears after 2-3 months from the genital tract, although some infected cattle remain carriers of Brucella and excrete it intermittently for many years (Herr *et al.*, 1990).

2.4. Survival of Brucella in the environment

The survival of Brucella in the environment plays a role in the epidemiology of the disease. Brucella organisms survive under various experimental and environmental conditions. Temperature, humidity, and PH influence the organism's ability to survive in the environment. Brucellae are sensitive to direct sunlight, disinfectants and pasteurization. In dry conditions, they survive only if embedded in protein (Davies and Casey, 1973). Brucella can survive in tap water for several months at 4 - 8 ^{o}C, 2.5 years at 0 ^{0}c, and several years in frozen tissues or medium. Brucella can also survive up to 60 days in damp soil and up to 144 days at 20 ^{0}c and 40% relative humidity (Radostits *et al.*, 2000). Brucellae can survive 30 days in urine, 75 days in aborted fetuses and more than 200 days in uterine exudate. Brucella will be destroyed at a temperature range of 56 ^{o}C-61 ^{0} C within 4.5 hours. However, there are conflicting reports as to its survival in liquid manure. *B. abortus* can survive at least for 8 months at 12^{0}c whereas another study indicates that Brucella could not be recovered from slurry after 3 months (Radostits *et al.*, 2000). The survival of Brucella is also subject to seasonal influences. It has been found that Brucella can survive in feces, slurry, or liquid manure for 85-103 days in winter, 120-210 days in spring, 30-180 days in summer, and 50-120 days in autumn (Kerimov, 1983). Although *B. abortus* is relatively resistant and may survive for a considerable time, the environment is not considered to be an important source of infection (Bishop *et al.*, 1994).

2.5. Distribution of the disease

2.5.1. Worldwide distribution

Bovine brucellosis has a worldwide distribution, but the disease has been eradicated from Finland, Norway, Sweden, Denmark, The Netherlands, Belgium, Switzerland, Germany, Australia, Hungary, Romania and Bulgaria, Japan, Canada and USA (WHO, 1986; Nicoletti, 1989; Radostits *et al.*, 2000 and PAHO/WHO, 2001). However, the disease is still found widely distributed with major economic importance in most developing

countries (Radostits *et al.*, 2000). Table 1 shows worldwide geographical distribution of Brucella species and bio-types.

Table 1. Worldwide geographical distribution of Brucella species and biotypes

Species	Host(s)	Diseases	Geographical distribution
B. abortus	CATTLE*	Abortion and orchitis	Biotypes:
	Sheep, goats and pigs	Sporadic abortion	1: Worldwide (common) 2: Worldwide (not common) 3: India, Egypt, East Africa
	Horses	Associated with bursitis (poll evil and fistulous withers)	5: Britain and Germany Other biotypes are infrequently isolated
	Humans	Undulant fever	
B. melitensis	GOATS, sheep	Abortion	Many sheep- and goat-raising regions except New Zealand, Australia and North America
	Cattle	Occasional abortion and excretion in milk	
	Humans	Malta fever	
B. suis	PIGS	Abortion, orchitis, arthritis, spondylitis and herd infertility	Biotypes: 1: Worldwide 2: Western and Central Europe
	Humans	Undulant fever	3: USA, Argentina and Singapore 4: Arctic Circle (Canada, Alaska and Siberia) in reindeer and caribou
B. ovis	SHEEP	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 males	North America and parts of Europe Becoming worldwide but not common
	Humans	Undulant fever	
B. neotomae	Desert wood 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)

2.5.2. Distribution in Africa

As it has been shown in Table 3, the disease is prevalent in many countries of Africa (Table 2) (Kagumba and Nandokha, 1978; Nicolletti, 1984; Chukwo, 1987; Seifert, 1996). High prevalence rates have been reported from Egypt (100%) and Sudan (35%). Moderately high prevalence rates have been reported from Nigeria (30%), Tanzania (28%) Zambia (27.9%) and Ghana (23.5%). On the other hand, low prevalence rates have been reported from Uganda (5%), Tanzania (5.9%), Kenya (9.9%) (Kagumba and Nandokha, 1978), Djibouti (4%) (Chantel *et al.*, 1994) and Eritrea (5.6%) (Omer *et al.*, 2000).

Table 2. Prevalence of Bovine brucellosis in Africa

Country	Host	N	Prevalence (%)	Tests used
Botswana	Cattle	--	18.0	RBPT
Chad	Cattle	246	13.0	SAT
Egypt	Cattle	50	100.0	SAT
Ghana	Cattle	11522	23.5	SAT
Guinea	Cattle	1861	6.9	SAT and RBPT
Kenya	Cattle	10361	9.9	RBPT
Nigeria	Cattle	983	30.0	SAT
Senegal	Cattle	768	35.0	MRT
Somalia	Cattle	5056	9.5	SAT
Sudan	Cattle	1522	57.4	MRT
Tanzania	Cattle	126	28.5	MRT
Zambia	Cattle	705	27.9	SAT

N= number of animals tested

Source: Adapted from Tadele (2004)

2.5.3. Prevalence of brucellosis in Ethiopia

In Ethiopia, available information on prevalence, incidence and economic importance of brucelllosis is not well representing the different situations in the country, except a single report by the veterinary branch of the American Medical Research Unit in 1970. Table 3 shows the summary of the prevalence studies in Ethiopia. High prevalence rate of 38.7% has been reported from central highlands of country (Rashid, 1993).

Table 3. Summary of prevalence of bovine brucellosis in Ethiopia

Breed	Location	N	Prevalence (%)	Tests used	Sources
Mixed	CHE	3577	2.1	SAT, CFT	Shiferaw, 1987
Mixed	Bahardar	678	9.8	CFT	Hadigo, 1987
Mixed	Arsi	2178	8.26	RBPT	Molla, 1989
Mixed	Arsi	NA	7.62	SAT	Molla, 1989
Zebu	CHE	NA	3	RBPT, SAT	Wondimu, 1989
Cross	SE	734	15.8	RBPT	Zewdu, 1989
Cross	SE	182	11.6	SAT	Zewdu, 1989
Cross	CHE	NA	38.7	RBPT, SAT	Rashid, 1993
Cross	CSF	182	22	RBPT, SAT	Sintaro, 1994
Cross	AA.sur	950	8.1	RBPT, CFT	Asfaw *et al.*, 1998
Zebu	EANRS	3644	1,8	RBPT	Kebede,1999
Crossbred	JIMA ZONE	508	0.2	RBPT, CFT	Taddele, 2004
Local	JIMA ZONE	1305	0.77	RBPT, CFT	Taddele, 2004
Intensive	SIDAMA,Z	811	2.4	RBPT, CFT	Kassahun, 2004
Extensive	SIDAMA,Z	1627	1.66	RBPT, CFT	Kassahun, 2004

CHE=Central highlands of Ethiopia,N=Number of observations, SE=Southern Ethiopia, CSF=Chafa State Farm, AA.sur=Addis Ababa surrounding, EANRS=Eastern Amhara National Regional State

2.6. Pathogenesis

Brucella is a facultative intracellular bacterium that can survive within host cells causing a chronic infectious disease that may persist throughout the life of an animal. It seems that the initiation of Brucella infection depends on exposure dose, virulence of the organism and natural resistance of the animal to Brucella (Hirsh and Zee, 1999; Radostits *et al.*, 2000). Resistance to infection is based on the host's ability to prevent the establishment of a mucosal infection by the destruction of the invading organism. Invading Brucella usually localize in the lymph nodes, draining the invasion site, resulting in hyperplasia of lymphoid and reticulo-endothelial tissue and the infiltration of inflammatory cells. Survival of the first-line of defense by the bacteria results in local infection and the escape of Brucella from the lymph nodes into the blood (Hirsh and Zee, 1999). During the bacteraemic phase which may last 2-8 weeks; bones, joints, eyes and brain can be infected, but the bacteria are most frequently isolated from supra-mammary lymph nodes, mammary lymph nodes, milk, iliac lymph nodes, spleen and uterus. In bulls, the predilection sites for infection are the reproductive organs and the associated lymph nodes. During the acute phase of infection, the semen contains large number of Brucella but as the infection becomes more chronic the number of Brucella excreted decreases and excretion may cease altogether. However, it also may continue to be excreted for years or just become intermittent. Usually, orchitis, epididymitis and infection of the accessory sex glands may also occur (Hirsh and Zee, 1999; Radostits *et al.*, 2000). The tropism of Brucella to the male or female reproductive tract was thought to be by erythritol, which stimulates the growth of the organism, but Brucella has also been found in the reproductive tract of animals with no detectable levels of erythritol. In the acute stage of infection, abortion occurs at about five months of pregnancy and cattle usually abort only once. Abortion and the subsequent retained fetal membrane, late abortions or birth of infected full-time calves have been reported to be common in herds with endemic brucellosis. Excretion of Brucella after parturition may persist for months or years and may re-occur after any consecutive normal parturition. Infected cattle excrete Brucella in the colostrum or milk although it cannot always be detected (Hirsh and Zee, 1999; Quinn *et al.*, 2002).

Abortion and expulsion of the fetus has been thought to be the results of placentitis caused by brucella. Proliferation of brucella in the uterus induces necrosis and destruction of the fetal and maternal placental membranes resulting in death and then expulsion of the fetus. The pathologic changes in the caruncules and cotyledons prevent normal separation and expulsion of the placenta. Although placentitis impairs the normal function of the placenta, Brucella endotoxins may also play a role in inducing abortion (Anderson *et al.*, 1986). *Brucella abortus* may induce production of high concentration of cortisol that decreases progesterone production and increases estrogen production. Decreases in progesterone level and increases in estrogen levels induce a premature parturition (Enright *et al.*, 1984).

2.7. Economic importance

Brucellosis is recognized as an important disease worldwide resulting in heavy economic losses to livestock industry. The economic loss from brucellosis in developed counties arises from the slaughter of cattle herds that are infected with Brucella. The economic loss from brucellosis in developing countries arises from the actual abortion of calves and resulting decreased milk yield, birth of weak calves that die soon after birth, retention of the placental membrane, impaired fertility and sometimes arthritis or bursitis. Due to its effects on multiple animal species and humans, the impact of brucellosis is considered great in Africa (Perry *et al.*, 2002).

It is difficult to estimate the financial loss caused by brucellosis, as it depends on the type of cattle farming, herd size, and whether it is an intensive or extensive cattle farm (Radostits *et al.*, 2000; Roth, *et al*, 2003). Studies in Southern Sudan have shown that calves produced from Rose Bengal test positive and negative cows in a transhuman system in had, approximately 10% less calves than negative. 22% abortion rate has been documented in positive versus 11% in negative cows (McDermott *et al.* (1987).

2.8. Zoonotic importance

Brucellosis is recognized as an important zoonotic disease worldwide posing serious health hazards. Brucellosis in human is known as Malta fever or Undulant Fever (Ansorg *et al.,* 1983). Humans usually acquire brucellosis by consumption of raw milk or milk products. Brucellosis is also recognized as an occupational hazard for farmers, veterinarians and workers in the meat processing industry. Farmers and workers in the meat industry may contract brucellosis percutaneous, conjunctival or by nasal mucous membrane infection. Veterinarians may become infected with *B. aboruts* when handling aborted fetuses or apparently healthy calves born to infected cows and by performing gynecological and obstetrical or rectal examinations of infected cattle. Because cattle and small ruminants are the major source of human infection, program to eradicate human brucellosis have been largely aimed at these animal species (Radostits *et al.*, 2000; Roth *et al.*, 2003). In rural areas, human population is highly correlated with livestock populations in the different livestock production systems. In both pastoral and mixed livestock production systems, people live very closely with livestock populations having a high incidence of brucellosis and are thus at high risk of infection (Yinnon *et al.*, 1993).

Brucellosis in humans is a multisystemic, acute to chronic, disease characterized by fever, headache, joint pains, musculo-skeletal pains, sweating, malaise and body wasting. Because of these rather non-specific signs, this has caused tremendous problems with the clinical diagnosis of brucellosis in sub-Saharan Africa, where it is constantly mis-diagnosed as malaria, which is very prevalent (Maichomo *et al.*, 1998; Oomen,1976). Other diseases from which it is difficult to distinguish clinically are typhoid, rheumatic fever and other conditions causing pyrexia, frequently diagnosed as pyrexia of unknown origin (PUO) (Baba *et al.,* 2001; Hendricks *et a*l., 1995; Maichomo *et al* 2000; Muriuki *et al.*,1997; Mutanda, 1998) .

2.9. Diagnosis and diagnostic techniques

2.9.1. Clinical findings

Clinical findings are dependent upon the immune status of the herd. Abortion storm after 5 months of pregnancy is a typical feature in highly susceptible non-vaccinated animals. In subsequent pregnancies the fetus is usually carried to the full term although second or third abortion in some animals may occur. Retention of placental membrane and metritis are common sequel to abortion. Death could occur due to acute metritis and septicaemia caused by superimposed secondary mixed infection or sterilities in chronic cases. History of an out break in a herd has been usually indicated as an introduction of an infected cow, less commonly also the source might be traced to infected bulls or horses with fistulous withers. In chronic cases, abortion is limited to firstheifer-calves and newly introduced healthy cows. Orchitis and epididymitis has been demonstrated to occur occasionally in male animlas. In one or both scrotal sacks acute painful swelling to twice normal size with gross enlargement may occur. The testes undergo liquefaction necrosis and eventually destroyed. Synovitis and hygromatous swelling of the knees in chronically infected cow or bull is not uncommon. In horses, *B. abortus* causes chronic bursal enlargement of the neck and wither (fistulous wither) or the navicular burcae with intermittent lameness (Hirsh and Zee, 1999; Radostits *et al.*, 2000)

2.9.2. Direct bacteriological examination

Smears are made from fetal stomach contents, fetal lesions, cotyledons and uterine discharges and specimens are stained by the Modified Ziehl Neelsen (MZN) stain to look for small, red-colored coccobacilli in clumps since they are intracellular growing organisms (Quinn *et al.*, 2002)

Bacteriological examination of lochia of aborting cattle is the method of choice for diagnosing early infections (Erasmus, 1986). However, the author has shown that the procedure is laborious, time consuming, costly and cannot routinely be used as a

diagnostic procedure.

2.9.3. Isolation and identification

Colonial morphology

When culturing Brucella liquid specimens could be inoculated straight on to the media plates. Scrapings from cotyledons are used, tissue samples are homogenized and aliquots used for culture. Centrifuged milk or colostrums and loop full of cream and from a positive Brucella milk ring test often yields *B. abortus* (Quinn *et al.*, 2002). The colonial morphology of the Brucella organisms after 3-5 days of incubation on selective serum agar are pinpoint, smooth, glistening, bluish and translucent. As they are aging, the colonies become opaque and about 2-3mm in diameter. Strains of Brucella *abortus*, *Brucella suis* and *Brucella neotome* are usually in the smooth form on first isolation. On sub-culturing, they may become rough but *Brucella ovis* and *Brucella canis* are always in the rough form; which are dull, yellowish opaque and when touched with inoculation loop are found to be friable. Brucella are also non hemolytic on blood agar (Quinn *et al.*, 2002, Hirsh and Zee, 1999). Moreover, the probability of successful recovery of *B. abortus* is strongly reduced when the material is heavily contaminated and negative culture results do not exclude infection (Erasmus, 1986).

Biochemical tests

Together with colonial morphology and staining properties, biochemical tests are used to identify and isolate Brucella organisms from other species of bacteria. They are non-motile, catalase positive, oxidase positive (except *B. ovis* and *B. neotome*), give rapid urase activitiy (except *B. ovis* and B. *melitensis*), reduce nitrate and are indole negative (Quinn *et al.*, 2002; Ocholi. 2004).

Brucella biotyping.

Biotyping definitely identifies the species and the biotypes. This type of diagnosis is usually carried out in reference laboratories and is based on CO_2 requirement, H_2S production, growth in the presence of dyes (thionine and basic fuchsine), agglutination in

mono-specific sera (AMR), lyses by phage Tibilisi and oxidative metabolic rates. The oxidative metabolic rates on selected substrates are conducted in reference laboratories, if the above mentioned tests are equivocal (Quinn *et al.*, 2002; Ocholi *et al.*, 2004). Differential characteristics of the species and biotypes in the genus Brucella is shown in Table 4.

Table 4. Differential characteristics of the species and biotypes of Brucella

	Biotype	CO_2 required	H_2S production	Urease activity (hours)	Growth in presence of dyes: Thionin (20 µg/ml)	Growth in presence of dyes: Basic fuchsin (20 µg/ml)	Agglutination in monospecific sera: A	Agglutination in monospecific sera: M	Agglutination in monospecific sera: R	Lysis by phage Tbilisi: RTD	Lysis by phage Tbilisi: 10^4 x RTD	Main host(s)
B. abortus	1	(+)	+	1–2 +	–	+	+	–	–	+	–	Cattle
	2	(+)	+	1–2 +	–	–	+	–	–	+	+	Cattle
	3	(+)	+	1–2 +	–	–	+	–	–	+	–	Cattle
	4	(+)	+	1–2 +	–	(+)	–	–	–	–	–	Cattle
	5	–	–	1–2 +	–*	–	–	–	–	+	+	Cattle
	6	–	(+)	1–2 +	–*	–	+	–	–	+	+	Cattle
	7[a]	(+)	–	1–2 –	–*	–	–	+	–	–	+	Cattle
Strain	19	–	+	1–2 +	–	–	+	–	–	–	+	Vaccine
B. melitensis	1	–	–	v	–*	–	–	+	–	–	–	Goats, sheep
	2	–	–	v	+*	–	–	–	–	–	–	Goats, sheep
	3	–	–	v	–*	+	–	+	–	–	–	Goats, sheep
B. suis	1	–	+	0–0.5 +	–	(–)	+	–	–	–	+	Pigs
	2	–	–	0–0.5 +	–*	–	–	–	–	–	–	Pigs, hares
	3	–	–	0–0.5 +	–	+	+	–	–	–	+	Pigs
	4	–	–	0–0.5 +	–	(–)	–	–	–	–	+	Reindeer, caribou
	5	–	–	0–0.5 +	–	–	–	–	–	–	–	Rodents
B. ovis		–	–	–	+*	(–)	–	–	+	–	–	Sheep
B. canis		–	–	0–0.5 +	+		–	–	–	–	–	Dogs
B. neotomae		–	+	0–0.5 +	–	–	–	–	–	–	–	Desert wood rat

+ = positive, (+) = most strains positive, (–) = most strains negative, – = negative, v = variable reactions, * = inhibited by 40 µg/ml thionin, a = formerly *B. abortus* biotype 9, biotypes 7 and 8 were deleted by International Committee on Bacterial Taxonomy, Subcommittee on Taxonomy of *Brucella* (1986), *Int. J. Syst. Bacteriol.*, 38: 450–452.
A = *B. abortus* antigen, M = *B. melitensis* antigen, R = rough, RTD = routine test dilution.

Source: Quinn *et al.*, 2002

Animal inoculation

Guinea pig inoculation is the most sensitive test for isolating pathogenic Brucellae. It is inoculated intramuscularly with 0.5-1.0 ml of suspected tissue homogenate. Usually two animals are used per sample and are euthanised at 3and 6 weeks after inoculation. Then, serum is taken for serology and spleen and any other abnormal tissue is collected for bacteriological examination (Quinn *et al.*, 2002).

2.9.4. Serological tests

Body fluids such as serum, uterine discharge, vaginal mucus, milk, or semen plasma from suspected cattle may contain different quantities of antibodies of the M, G_1, G_2, and A types directed against Brucella (Beh, 1974). Because infected cattle may or may not produce all antibody types in detectable quantities, several tests are used to detect brucellosis. The commonly used tests are the Milk Ring Test (MRT), Serum Agglutination Test (SAT), Complement Fixation Test (CFT), Rose Bengal Plate Test (RBPT), Anti-globulin (Coombs) Test, 2-MercaptoethanolTest, Rivanol Test, and the Enzyme Linked ImmunosorbentAssay (ELISA). The use of several tests to reliably detect brucellosis suggests shortcomings in each of the tests.

The Milk Ring Test

The Milk Ring Test (MRT) is cheap, easy, simple and quick to perform. It detects lacteal antibrucella IgM and IgA bound to milk fat globules. However, it tests false positive when milk that contains colostrum, milk at the end of the lactation period, milk from cows suffering from a hormonal disorder or milk from cows with mastitis are tested (Bercovich and Moerman, 1979). Milk that contains low concentrations of lacteal IgM and IgA or which is lacking the fat-clustering factors tests false negative (Patterson and Deyoe, 1978). Because lacteal antibodies rapidly decline after abortion or parturition, the reliability of the MRT, using 1 ml milk, to detect Brucella antibodies in individual cattle or in tank milk is strongly reduced. Although the MRT performed with 8 ml milk improved the detection of brucellosis in tank milk, it may test false positive when traces

of colostrum are present in tank milk (Bercovich and Moerman, 1979).

The Serum Agglutination Test (SAT)

The Serum Agglutination Test (SAT) is the principal serological test used to detect brucellosis. It measures agglutinating antibodies of the IgM, IgG_1, IgG_2, and IgA types (Levieux, 1974). The SAT is relatively simple and easy to perform, but it requires basic laboratory equipment. It has been reported to be used to detect acute infections, as antibodies of the IgM type usually appear first after infection and are more reactive in the SAT than antibodies of the IgG_1 and IgG_2 types (Beh, 1974; Levieux, 1974). However, because the SAT may yield both false negative or false positive results, it effectively detects brucellosis only on a herd basis (Corbel *et al.* 1984).

The Complement Fixation Test (CFT)

The Complement Fixation Test detects specific antibodies of the IgM and IgG_1 type that fixes complement. The CFT is highly specific, but it is laborious and requires highly trained personnel as well as suitable laboratory facilities. This makes the CFT less suitable for use in developing countries. Although its specificity is very important for the control and eradication of brucellosis, it may test false negative when antibodies of the IgG_2 type hinder complement fixation (MacMillan, 1990). The CFT measures more antibodies of the IgG_1 type than antibodies of the IgM type, as the later are partially destroyed during inactivation. Since antibodies of the IgG_1 type usually appear after antibodies of the IgM type, control and surveillance for brucellosis is best done with SAT and CFT.

The Rose Bengal Plate Test

The Rose Bengal Plate Test is a spot agglutination technique. Because the test does not need special laboratory facilities and it is simple and easy to perform, it is used to screen sera for Brucella antibodies. The test detects specific antibodies of the IgM and IgG types and is more effective in detecting antibodies of the IgG_1 type than IgM and IgG_2 types

(Levieux, 1974). Although the low pH (3.6) of the antigen enhances the specificity of the test, the temperature of the antigen and the ambient temperature at which the reaction takes place may influence the sensitivity and specificity of the RBPT (MacMillan, 1990).

The Anti-globulin (Coomb's) Test

The Anti-globulin (Coombs) Test detects antibodies of the IgG_2type and is used to confirm SAT results. The Coombs Test, although laborious, is particularly important when the SAT is positive and CFT results are negative or inconclusive (Kiss, 1971). However, Coombs Test results are indicative for infection only when its titers are at least two times the titers of the SAT. This is the tests main limitation, as not all infected cattle show this ratio. The 2-Mercaptoethanol and the Rivanol tests detect specific IgG (Rossi and Cantini, 1969) and are usually used to differentiate between infected and vaccinated cattle.

Enzyme Linked Immunosorbent Assay (ELISA)

The ELISA has proven to be specific and as sensitive as the MRT and SAT in detecting Brucella antibodies in milk and serum. ELISA results are usually also in agreement with CFT results. The test can be used for screening and confirmation of brucellosis in both milk and serum. However, depending on the presence of traces of colostrum in the milk or the presence of low concentrations of lacteal immunoglobulin, the ELISA may give false positive or false negative (Bercovich and Taaijke, 1990). It seems that the ELISA is less sensitive than the CFT, as some infected cattle that test positive with the CFT may test negative with the ELISA. Sutherland *et al.* (1986) has reported that the main advantage of the ELISA when compared with the CFT lies in its relative simple test procedure. The assay is very costly when only a few samples are tested; therefore, it is unsuitable for testing individual animals but it is the ideal test for screening suspected herds.

2.9.5. The Skin-Delayed-type-Hypersensitivity (SDTH) Test

Since the reliability of serological tests to detect brucellosis depends on antibodies that may or may not be present at the time of examination, inevitably some infected animals may elude detection. Because the skin-delayed-type-hypersensitivity (SDTH) test is independent of circulating antibodies and it should be added to the serological tests to improve detection of brucellosis. The SDTH test confirms serologic test results, confirms brucellosis in cattle with ambivalent serologic test results and detects latent carriers of Brucella. Furthermore, the SDTH test does not sensitize cattle for several consecutive SDTH tests (Bercovich, 1999). Therefore, the SDTH test should be the test of choice in developing countries, as cattle in those countries are usually not tagged so that serological test results could be related to the individual animal. Where the animals are tagged a combined use of the SAT and SDTH tests increase the reliability of brucellosis diagnosis (Bercovich, 1999).

2.9.6. Molecular diagnosis

This is based on DNA sequence analysis called Brucella "Hoof Printing". It is based on the difference of the genomic profile of Brucella. This difference is found on different loci in both lower and upper strand of the chromosome of the brucella DNA. It is an eight base pair hotspots (octameric) variable number tandem repeating loci of the DNA chromosomes. It is highly discriminative, rapid and sensitive molecular diagnostic tool recently discovered. It is being advocated to be used by epidemiologists as a herd diagnostic tool and trace back of the origin of brucellosis outbreak. The procedure is based on PCR technology. According to a study made in USA, DNA fingerprints of Brucella strains from Florida herd showed difference genetically. This study showed that DNA finger printing is highly discriminating method of characterization of Brucella strains far surpassing the capabilities of bio-typing. Hence, this technique is being advocated and available tool for epidemiologists trying to study the origins and spread of brucellosis outbreaks (Briker *et al.*, 2003; Briker and Ewalt, 2004).

2.10. Prevention, control and eradication

2.10.1. Treatment

Previous studies have shown that treatment is mostly not successful because of the intracellular sequestration of the organisms in the lymph nodes, the mammary glands and reproductive organs. But treatments have been indicated generally according to the situations and the circumstances. The treatments often given are bovine plasma, sulphadiazine, streptomycin, chlortetracycline and chloramphenicol (Radostits *et al.*, 2000, Hirsh and Zee, 1999).

2.10.2. Control

Brucella abortus strain 19, a live vaccine (liquid or lyophilized) and RB51, have been used for vaccination to control brucellosis. Its main value is that it is used in infected herds to protect the uninfected animals in the contaminated environment, enabling infected animals to be disposed off gradually. This makes vaccination with strain 19 advantageous over the test and slaughter methods (Radostits *et al.*, 2000; Hirsh and Zee, 1999). Four to 8 months has been reported to be optimum age for vaccination. About 65-75% of animals vaccinated with strain 19 are resistant to any kind of exposure while the remaining proportion (25-35%) may be infected after vaccination but don't abort usually. This has been shown to be a very good efficiency when it is compared to k45/20 vaccine, which can protect only 30% of the vaccinated animals. Strain 19 has been used to decrease the incidence of infection in large dairy herds, where it is impossible to institute management procedures for the ideal control of brucellosis, such as test and slaughter due to high incidence and due to high density of animals in small area (Radostits *et al.*, 2000).

2.10.3. Eradication

An area based eradication has been recommended to be instituted when the level of infection is below 4% of the cattle population. Farm based eradication programe can be engaged when the incidence in the farm is very low and the incidence on the surrounding

farm is below 5%. Management of the infected animals, disposal of their contaminants and disinfection of contaminated area is very important in the eradication processes of brucellosis. Testing all cattle, horses and pig brought into the farm and isolation for 30 days and retesting are important in the prevention and control of brucellosis. Keeping cows in advanced pregnancy isolated from the herd until after parturition since there is latency in showing positive serum until after parturition in some infected cows posing a problem (Radostits *et al.*, 2000).

3. MATERIALS AND METHODS

3.1. The study area

The study was conducted in West and East Shoa zones, Central Oromia, Ethiopia. Walmara district was the study area in West Shoa Zone, while Lume and Adami Tulu-Jido-Kombolcha districts in the East Shoa Zone. The description of the specific study areas is presented hereunder.

3.1.1. Walmara District

Walmara district is situated within the West Shoa Zone of Oromia forming part of the central highlands of Ethiopia, at 38^0 3' E and 9^0 3'N rising to the height of 2400 above sea level. The administrative town of Walmara district is Holeta, which is located 44 kms west of Addis Ababa on the main road to Ambo and Naqampte. The district is characterized by mild tropical weather with the minimum and maximum temperature ranging from 2^0C to 9^0C and 20^0C to 27^0C, respectively. The area experiences bimodal rainfall with the short rainy season covering from March and April followed by the long rainy season from June to September. The long dry period lasts from November to February and the short dry spell is during May. The average annual rainfall of Holeta during the last 10 years was 1000-2200mm. The vegetation of the area is made of annual legumes and perennial grass species. Major crops include wheat, barley, lentil, tef and maize. According to a study conducted in 1992/93 by ILRI, the production systems of the area were classified as mixed crop livestock, market oriented specialized dairy production and urban dairy production (Yoseph *et al.*, 1999).

3.1.2. Lume District

Lume district is found in Central Oromia, Ethiopia, at 39'01-39'17^{0}E and 8'12-8'5^{0}N. The District is composed of 15% highland, 50% mid-highland (transitional scrap slope) and 35% semi-arid low land. The present study concentrated totally on the mid highland

(transitional scrap slope) area of the district. The administrative town of Lume district is Modjo, which is located at about of 70 kms South of Addis Ababa. Currently, there are about 35 peasant Associations in the district. The district is well known for its high production of cereal crops especially tef, wheat, beans, peas and lentils whose cultivation is totally dependant on the traction power of oxen. There is one dairy farmers milk marketing cooperative established by smallholder dairy farmers keeping crossbred dairy cattle (OADB, 1996).

3.1.3. Adami Tulu-Jido-Kombolcha District

The District is found in the mid rift valley at 7^0 9' N and 38^0 7' E. The administrative town of the district is Zeway, which is located at 167 kms south of Addis Ababa just on the main read to Awassa. The altitude of the town is about 1650 meters above sea level and has a bimodal unevenly distributed mean annual rainfall of 760.9mm. The rainfall extends from February to September with dry period from May-June. The total area of the District is estimated to be about 75,223 ha; of which 36,661 ha is under crop production and 17,113 ha is used for grazing; the rest for different purposes. The total human population of Adami Tulu-Jido-Kombolch district is about 111,926. About 72% of the population lives in the rural areas. There are about 16,479 households with an average family size of 9.58. There are about 254,694 cattles, 11,384 sheep, 133,902 goats, 12,505 equines and 49,966 poultry in the District (OADB, 1996).

Livestock production is dominant farming system in the area as crop production is a latest introduction. Crop production was not a known practice for the last 50-60 years. The major crops being cultivated in the area now a day are maize, tef and haricot bean while the minor crops include wheat, barley and sorghum. Adami Tulu Agricultural Research Center and Abernosa Cattle Breeding Ranch are two important government organizations dealing with livestock research and improvement in the District. The institutions are established to improve livestock productivity through crossbreeding of local cattle with Holstein-Frisian and other exotic cattle breeds (OADB, 1996).

3.2. Study population

The cattle population in the study areas which are 6 months or older in age were the study population to study the seroprevalence of bovine brucellosis. In addition, all households keeping cattle for different purposes were also the study population for the questionnaire survey. The cattle population in different districts of West and East Shoa Zones of Oromia are presented in Table 5.

Table 5. Cattle population in the study areas

Zone	Cattle population in the Zone	Districts	Cattle population in the districts
West Shoa Zone	2,431,376	Walmara	88,448
East Shoa Zone	1,416,551	Lume	65,992
		Adami Tulu-Jido-Kombolcha	131,706

3.3. Study design

Cross-sectional seroepidemiological study was carried out on bovine brucellosis in the selected districts of Central Oromia, Ethiopia, during the period covering from October 2005 to April 2006 using RBPT, CFT and questionnaire survey methods. Blood samples were collected once from all cattle above six months of age included in the study. Screening of serum for bovine brucellosis was conducted by using Rose Bengal Plate Test (RBPT) and confirmation was made by Complement Fixation Test (CFT). Information regarding individual animal and herd attributes were collected by questionnaire survey.

3.3.1. Sample size determination

One stage cluster sampling method was used to calculate the total number of herds to be included in the study. Herd was considered as a cluster and the average herd size was

considered to be six as estimated by OADB (1996). The numbers of clusters to be sampled from each district is determined by the formula previously described by Thrusfield (1995):

$$g = 1.96^2 (nv_c + Pexp(1-Pexp)/nd^2$$

Where, g= no clusters to be sampled,
Pexp =expected prevalence, taken as 12.3% for this study
d = desired absolute precision, taken as 0.05% for this study
Vc =between cluster variance, taken as 0.0008 for this study

Information about the between cluster variation (Vc) in the study area was not available. The between cluster variance was determined, therefore, from the prevalence (12.3%) (OADB, 1996) according to Thrusfield (1995).

Accordingly the number of clusters (herds) to be sampled from each study area was 29 and total number of clusters to be sampled from the three study areas was determined to be 87. When this value is multiplied by 6, which is the average herd size, the total number of animals to be included in the study was 552. To increase the precision, a total of 176 herds were sampled, from which 59 were from the lowland, 58 from the mid-highland and 59 from the highland. Thus, the total number of animals sampled was 1238, from which 430 were from Walmara District (highland), 423 from Adami Tulu-Jido-Kombolcha District (lowland) and 385 from Lume District (mid-highland).

3.4. Data collection

3. 4.1. Blood sample collection

About 10 ml of blood sample was collected from the jugular vein of each of the selected animals using plain vacutainer tubes. The collected blood samples were kept at a room temperature overnight for clot retraction and serum separation. The separated serum was

collected by plastic pipette. Then, the separated sera were stored at -20^{0}c until tested by RBPT and CFT.

3.4.2. Serological tests

Rose Bengal Plate Test

Test procedure

All serum samples collected were screened using RBPT according to the procedures described by Alton *et al.* (1975) and OIE (2005). The antigen consisted of a suspension of *Brucella abortus* (obtained from Institute purquier 326, Rue de la Galera 34097 MONTEPELLIER CEDEX 5 France). Materials used and the details of the procedure are presented in Annex 2.

Interpretation

After four minutes rocking (shaking) any visible agglutination was considered as positive (OIE , 2005).

Complement Fixation Test

The CFT is a widely used and accepted confirmatory test, although it is complex to perform, requiring good laboratory facilities and adequately trained staff. Therefore, sera that were positive to RBPT were further retested by CFT for confirmation. Complement Fixation Test had been under taken at the National Veterinary Institute of Ethiopia. Standard *Brucella abortus* antigen for CFT, S 99 (CVL, New Haw Weybridge, and Surry Kt15 3NB, UK) was used. Antigen control sera and complement were obtained from the BgVV, Berlin, Germany. Materials used and the details of the procedure are presented in Annex 3.

The test proper, multiple sera technique

1. 25 µl of VBD was added to all cups.
2. 25 µl of control test sera was added simultaneously to col.1, 4, 5, 8, 9 and 12. The sera were pre-diluted to 1:2.5 and was inactivated at 58^0c in a water bath for 30 min.
3. 25 µl sera were serially transferred from col.1 to3, 5, 7, 9 and 11. 25 µl VBD was added to col.4, 8 and12.
4. 25 µl of antigen in working dilution of 1:100 was added to col. 1-3, 5-7 and 9-11.
5. 50 µl of complement in working dilution was added to all cups.
6. The plate was placed at 4^0c overnight covered with a 2^{nd} empty plate (cold fixation).
7. The following day 50µl of equal volume pre-mixed 2% sheep red blood cells and amboceptor at working dilution of 1:2000 were added.
8. The plate was sealed, shaken well and kept in water bath at 37^0c for 30min.

Interpretation

Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 and at least with 50% fixation of complement (2+) at a dilution of 1:10 and at dilution of 1:20 were classified as positive (+) (OIE , 2005).

3.4.3. Questionnaire survey

A structured questionnaire format was prepared and the following information was collected by interviewing farmers. The collected information include descriptors of study area, cow attributes (breed and age), management system, herd size and composition, housing system, farm hygienic practices, level of awareness on brucellosis, sources of replacement stock and others (Annex 1).

3.5. Data Analysis

Data obtained from cross-sectional seroprevalence study and questionnaire survey were entered into the Microsoft Excel program (2000). Descriptive statistics were conducted by SPSS for Windows (release 11.5, 2002). The analysis of the effects of different potential risk factors on individual animal and herd seroprevalence of bovine brucellosis was performed by Intercooled Stata 7 for windows (2002).

4. RESULTS

4.1. Seroprevalence of bovine brucellosis

4.1.1. Overall individual animal seroprevalence in the study areas

The overall individual animal's seroprevalence in the three agro-ecological areas of the study is 2.99% of 1237 bovines tested by RBPT and CFT (Table 6). The highest seroprevalence (4.26%) is recorded in the lowland areas followed by the highland (3.48%) and mid-highland areas (1.04%) in descending order.

Table 6. Overall individual animal seroprevalence of bovine brucellosis in the three agro-ecological zones

Agroecology	N	No. of seropositive animals (prevalence)	
		RBPT	CFT
Lowland	423	24 (5.67%)	18 (4.26%)
Mid-highland	385	10 (2.60%)	4 (1.04%)
Highland	430	27 (6.26%)	15 (3.48%)
Total	1238	61 (4.92%)	37 (2.99%)

N=number of animals tested

4.1.2. Overall herd level seroprevalence in the study areas

The overall seroprevalence at herd level in the three agro ecological zones is presented in Table 8. A total of 24 herds (13.64%) had at least one animal reacting positively for both RBPT and CFT. The highest herd level seroprevalence (18.64%) was found in the highland followed by the lowland (17%). The least value was recorded for the mid-highland areas (5.17%).

Table 7. Overall herd seroprevalence of bovine brucellosis in the three agro-ecological zones

Agroecology	N	No. of seropositive herds (prevalence)	
		RBPT	CFT
Lowland	59	14 (23.7 %	10 (17%)
Mid-highland	58	6 (10.3 %)	3 (5.17%)
Highland	59	8 (13.6%)	11 (18.64%)
Total	176	28 (16%)	24 (13.64%)

NF=number of farms tested

4.2. Factors affecting the overall seroprevalence of bovine brucellosis in the study areas

4.2.1. Factors affecting the overall individual animal seroprevalence

The effects of different potential risk factors on the seroprevalence of bovine brucellosis in the three agroecological zones were analyzed using univariate logistic regression. The results revealed that breed ($p<0.05$) and management system ($p<0.05$) had significant effects on the overall individual animal seroprevalence (Table 8). The prevalence was higher (4.91%) in crossbred animals than indigenous (2.24%). It was also higher in those under intensive management (4.88%) than those under semi-intensive (2.86%) and extensive managements (2.44%). All other factors including age, herd size, agroecology, mating method and sources of replacement stock had no significant effect ($p>0.05$). When the two factors having significant effect in univariate analysis were fitted in a multivariate model of logistic regression, both factors had no significant effect ($p>0.05$).

4.2.2. Factors affecting the overall herd seroprevalence

Farm attributes including herd size, management system, mating method, sources of replacement stock were considered as potential risk factors and analyzed using univariate logistic regression. However, all the factors had no significant effect on the overall herd seroprevalence of bovine brucellosis ($p>0.05$).

Table 8. Factors affecting the overall individual animal seroprevalence in the study areas (univariate logistic regression analysis)

Risk factors	Group	N	Number positive (prevalence)	95% CI	P-value	OR
Breed	Indigenous	892	20 (2.24%)	1.16-4.26	0.017	2.22
	Crossbred	346	17 (4.91%)			
Age	0.5-3 years	412	17 (4.12%)	0.36-1.11	0.115	-
	3-10 years	729	18 (2.47%)			
	> 10 years	97	2 (2.06%)			
Herd size	1-6	296	16 (5.41%)	0.72-2.39	0.373	-
	7-16	537	9 (1.68%)			
	>16	405	12 (2.96%)			
Management system	Intensive	266	13 (4.88%)	1.00-2.03	0.046	1.43
	Semi-intensive	70	2 (2.86%)			
	Extensive	902	22 (2.43%)			
Agroecology	Lowland	423	18 (4.26%)	0.59-1.30	0.519	-
	Mid-highland	385	4(1.04%)			
	Highland	430	15(3.49%)			
Mating method	Natural	894	20(2.2%)	0.87-1.86	0.213	
	Artificial	116	11(9.5%)			
	Both	228	6(2.6%)			
Source of replacement stock	Regional market	891	20(2.24%)	0.91-1.70	0.176	-
	Village breeders	236	13(5.51%)			
	Government farms	6	1(16.67%)			
	Urban dairy farms	105	3(2.86%)			

4.3. Factors affecting seroprevalence of bovine brucellosis in the three agroecological zones

4.3.1. Factors affecting individual animal seroprevalence in the lowland areas

The results of univariate logistic regression showed that breed ($p<0.05$), herd size ($p<0.01$), management system ($p<0.01$), mating system ($p<0.01$) and sources of replacement stock ($p<0.01$) had significant effect on the seroprevalence in the lowland area (Table 9). The prevalence was higher in crossbred (10.3%) than indigenous animals (2.7%) (Anne 4). Regarding the effects of farm attributes, the prevalence was higher in animals kept by farms with small herd size (10.2%) than medium (2.8%) and large

(2.2%) herd sizes, in those managed intensively (10.3%) than extensively (2.7%), in animals kept in farms using artificial insemination (10.3%) than those using natural mating (2.7%) and in those kept by farms replacing animals from village breeders (10.3%) than the others (2.7%).

Table 9. Factors affecting individual animal seroprevalence in the lowland areas (univariate logistic regression analysis)

Risk factors	Group	N	Number of positives (prevalence)	95% CI	P-value	OR
Breed	Indigenous	336	9(2.7%)	1.61-10.91	0.015	4.19
	Crossbred	87	9(10.3%)			
Age	0.5-3 years	143	7(4.9%)	0.35-1.66	0.498	-
	3-10 years	237	10(4.2%)			
	> 10 years	43	1(2.3%)			
Herd size	1-6	98	10(10.2)	0.22-0.77	0.006	0.41
	7-16	143	4(2.8%)			
	>16	182	4(2.2%)			
Management system	Intensive	87	9(10.3)	1.27-3.30	0.003	2.05
	Extensive	336	9(2.7%)			
Mating method	Natural	335	9(2.7%)	1.59-10.74	0.004	
	Artificial	88	9(10.3%)			
Source of replacement stock	Regional market	335	9(2.7%)	1.59-10.74	0.004	-
	Village breeders	88	9(10.3%)			

When all the risk factors with significant effect in univariate analysis were fitted in a multivariate model of logistic regression, management system was found as the only factor determining the individual animal seroprevalence in the lowland areas ($p<0.001$).

4.3.2. Factors affecting individual animal seroprevalence in the mid-highland and highland areas

The results of univariate logistic regression to analyze the effects of potential risk factors on the seroprevalence in the mid-highland areas (Table 10) and highland areas (Table 11) revealed none of the considered factors had significant effect ($p>0.05$) on individual

animal seroprevalence. All the factors with the exception of age had marginal p-values in the mid-highland areas.

Table 10. Factors affecting individual animal seroprevalence in the mid-highland areas (univariate logistic regression analysis)

Risk factors	Group	N	Number positive (prevalence)	95% CI	P-value	OR
Breed	Indigenous	280	1(0.4%)	0.97-9.86	0.056	-
	Crossbred	105	3(2.9%)			
Age	0.5-3 years	104	2(1.9%)	0.06-2.28	0.279	-
	3-10 years	253	2(0.8%)			
	> 10 years	28	0			
Herd size	1-6	112	3(2.7%)	0.02-1.35	0.092	-
	7-16	198	1(0.5%)			
	>16	75	0			
Management system	Intensive	105	3(2.9%)	0.92-8.93	0.070	-
	Extensive	280	1(0.4%)			
Mating method	Natural	283	1(0.4)	0.94-9.12	0.065	-
	Artificial	102	3(2.9%)			
Source of replacement stock	Regional market	280	1(0.4%)	0.95-4.30	0.070	-
	Urban dairy farm	105	3(2.9%)			

4.3.3. Factors affecting herd seroprevalence in the three study areas

Herd attributes including herd size, management system, mating methods and sources of replacement stock were identified as potential risk factors for herd level seroprevalence. The results of univariate logistic regression showed that all the factors had no significant effect on herd seroprevalence in all the studied areas ($p>0.05$).

Table 11. Factors affecting individual animal seroprevalence in the highland areas (Univariate Logistic Regression Analysis)

Risk factors	Group	N	Number positive (prevalence)	95% CI	P-value	OR
Breed	Indigenous	276	10(3.6%)	0.48-2.23	0.937	-
	Crossbred	154	5(3.2%)			
Age	0.5-3 years	165	8(4.8%)	0.25-1.59	0.334	-
	3-10 years	239	6(2.5%)			
	> 10 years	26	1(3.8%)			
Herd size	1-6	86	3(3.5%)	0.70-3.13	0.306	-
	7-16	196	4(2%)			
	>16	148	8(5.4%)			
Management system	Intensive	286	12(4.2%)	0.0.25-1.41	0.232	-
	Semi-intensive	70	2(2.9%)			
	Extensive	74	1(1.4%)			
Mating method	Natural	276	10(3.6%)	0.46-1.56	0.608	-
	Artificial	28	2(7.1%)			
	Both	126	3(2.4%)			
Source of replacement stock	Regional market	276	10(3.6%)	0.42-2.99	0.830	-
	Village breeders	148	4(2.7%)			
	Government farm	6	1(16.7%)			

4.4. Description of farm characteristics

The herd size pattern in the lowland area revealed that most of the intensive farms (85.7%) had a herd size of 6 or less while most of the extensive farms (71%) had a herd size greater than or equal to 7. In the case of mid-highland areas, nearly all the intensive farms had a herd size of 6 or less, while the majority of extensive farms (86.2) had a herd size of 7 or more. In the highland agroecology, the majority of the farms in the intensive system (54.6%) had a herd size of 7 or above, in the semi-intensive system higher proportion of the farms (64.3%) had a herd size of 6 or less and in the extensive system

most of the farms (72.1%) had a herd size of 7 or more.

In all the study areas most of the farmers (84.75-89.83%) were not aware of bovine brucellosis. More than half of the farmers in the highland area properly disposed aborted materials, while farmers in the other areas practiced at lesser extent (32.2-43.1%). Nearly all the farmers in all the agroecological zones (81.34-91.53%) had no a separate parturition pen. The practice of regular cleaning of the barn was performed by 50.85-69.49% of the farms in all the studied areas. The occurrences of abortion, stillbirth and retained fetal membranes were also less than 10.34%, 6.88% and 6.88%, respectively (Table 12).

Table 12. Farm characteristics in the three agroecological zones

Variables	Proportion of respondents (n)			
	Category	Lowland	Mid-highland	Highland
Awareness on brucellosis	Yes	15.25 (9)	10.34 (6)	10.34 (6)
	No	84.75 (50)	89.66 (52)	89.83 (53)
Proper disposal of aborted materials	Yes	32.20 (19)	43.10 (25)	50.85 (30)
	No	77.8 (40)	56.9 (33)	49.15 (29)
Presence of parturition pen	Yes	8.47 (5)	17.24 (10)	18.64 (11)
	No	91.53 (54)	82.76 (48)	81.34 (48)
Regular cleaning of barn	Yes	50.85 (30)	60.34 (35)	69.49 (41)
	No	49.15 (29)	39.66 (23)	30.51 (18)
Occurrence of abortion	Yes	6.88 (4)	10.34 (6)	8.47 (5)
	No	93.22 (55)	89.66 (52)	91.53 (54)
Occurrence of stillbirth	Yes	6.88 (4)	3.44 (2)	3.39 (2)
	No	93.22 (55)	96.66 (56)	96.61 (57)
Occurrence of retained fetal membrane	Yes	6.88 (4)	3.44 (2)	8.47 (5)
	No	93.22 (55)	96.66 (56)	91.53 (54)

n=number of respondents

5. Discussion

5.1. Overall seroprevalence

The seroprevalence rates at individual animal level found in the present study for the overall studied areas (2.99%), highland (3.48%), mid-highland (1.04%) and lowland (4.26) are in disagreement with most of the reports from the central and other parts of Ethiopia. The Veterinary Section of the US Medical Team reported an over all prevalence rate of 11.7% from samples collected from different parts of the country (MoA, 1970). According to this study, the results of the test in different regions of Ethiopia were 8 % for Harar, 5% for Illuababor, 7 % for Kaffa, 8 % for Shoa, 21 % for Sidamo and 2 % for Wallo. Several researchers also have reported high prevalence of bovine brucellosis in different part of Ethiopia. Prevalence rates as high as 38.7% and 22% were reported by Rasid (1993) and Sintaro (1994) in central highlands of Ethiopian and Chaffa State Dairy Farm, respectively. Similarly, higher prevalence rates were reported by Gebremariam (1985) in crossbred cattle around Addis Ababa (18.4%) using RBPT and CFT, Zewdu (1989) in Sidamo (15.8%) using SAT, (Asfaw *et al.*, 1998) in urban and peri-urban dairy farms in and around Addis Ababa (8.11%) using RBPT and CFT and Molla (1989) in indigenous and crossbred cattle in Arsi (7.62%) using SAT. Lower prevalence rates were also reported by Tadelle (2004) in Jimma (0.2-0.77%), Kebede (1999) in eastern part of Amhara Regional State (1.0%), Kasshun (2004) in Sidama Zone and Gebretsadik (2005) in intensive farms in Tigray (0.2%). Prevalence rates very close to the finding in this study were reported by Wondimu (1989) (3%) and Shiferaw (1987) (2.1%) in the central highlands of Ethiopia, Mussie (2005) in Amhara Region (4.63%) and Gebretsadik (2005) in extensive farms in Tigray (3.19%).

There are also a number of reports of the prevalence of the disease from different African countries. Slightly higher prevalence of bovine brucellosis; 5%, 5.6%, 5.9%, 6.5% and 9.9% were reported in Uganda, Eritrea, Tanzania, Sudan and Kenya, respectively (Kagumba and Nandokha, 1978; Hellman *et al.*, 1984; Omer *et al.*, 2000). The

prevalence rate reported in Djibouti (4%) is very close to the finding of this research (Chantel *et al.*, 1994). With regard to the herd level seroprevalence, the 13.64% we found in the overall studied areas is in agreement with the previous findings of 13.7-14.96% by Musie (2005), Tadele (2004) and Kassahun (2005).

Variations in seroprevalence reports among the various researchers in the different parts of the country as compared to the present finding is probably due be to the difference in management, herd size, the presence or absence of the infection foci such as brucellosis infected dairy farms and ranches in the surrounding. The fact that source of stock replacement is found to be associated with brucellosis infection (OR=1.05) substantiates the above statement (Radostits *et al.*, 2000).

5.2. Risk factors of bovine brucellosis in the study area

Among the potential risk factors considered, breed of cows had significant effect on the seroprevalence of bovine brucellosis ($p<0.05$) in the overall study area and the lowland agroecology. In both cases, the seroprevalence was higher in crossbred animals than in indigenous ones. Similar finding was reported by Bukanzi *et al.* (1993) from Uganda. On the other hand, there are also reports indicating absence of association between seroprevalence and breed (Madson, 1989; Radostits *et al.*, 2000). The significant difference in the two breed groups in this study could be the reflection of the compounded effect of management systems and mating systems. In the studied area , farmers owning crossbred animals tend to follow the intensive type of management and prefer to use AI as a mating method. As indicated below management system and mating system had significant effect on seroprevalence. Another important risk factor with significant effect in the overall study areas ($p<0.05$) and the lowland agroecology ($p<0.01$) was management system. The seroprevalence in this study was higher in the intensive management system than the extensive management system. This finding is in agreement with the reports of Takleye *et al.*, (1989) in which low prevalence was found in extensive management system (0.054%) than and in intensively managed systems (10.3%) in central Ethiopia. Similarly Jiwa *et al.* (1996) reported higher prevalence of

brucellosis in intensive dairy farms (6.3%) and ranches (15.8%) than in animals kept under extensive grazing conditions (4.3%) in Tanzania. The finding in this result is not in line with that of Gebretsadik (2005) for Tigray Region who reported a higher prevalence rate in the extensive system. The high seroprevalence in the intensive system in this study and other previous works can be explained by the fact that the incidence of brucellosis increases with change from purely extensive system to a more intensive system of cattle management which increases the chance of cattle contact within a herd (Thimm and Wundt, 1976). The difference of the finding of this study from that of Gebretsadik (2005) could be due to the mobility of herders (transhumance) in the extensive system of his study area which might increase the chance of exposure to sources infection through contact with other herds. There was also a significant decrement in seroprevalence with the increment of herd size in the lowland agroecology in this study ($p<0.01$). This finding is contradictory to that reported by Yilkal (1998) and Tadele (2005). This could be related to the typical herd size pattern in the lowland agroecology where extensive farms had larger herd size and intensive farms had lower herd size. The type of mating method used by farmers ($p<0.01$) and sources of replacement stock ($p<0.01$) in the lowland agroecology in this study also had significant effect on seroprevalence. The seroprevalence was higher in animals bred using AI and animals kept by farms replacing stock from village breeders. However, reports substantiating this finding are lacking and hence need further investigation.

5.3. Questionnaire survey

The findings in this study regarding lack of awareness about brucellosis and the importance of precaution measures against the disease such as dependence on outside replacement stock, improper disposal of aborted materials and absence of separate parturition pen are in agreement with the reports of Yilkal (1998), Tadele (2004) and Gebretsadik (2005). This indicates that less attention has been given to brucellosis and hence there is lack of extension work to increase the awareness of the importance and associated risks of the disease.

7. CONCLUSSIONS AND RECOMMENDATIONS

In the present study, the overall seroprevalence of bovine brucellosis at individual animal level for the three selected agro ecological areas of central Oromia was low while the herd level seroprevalence ws moderate. The lowland areas had relatively higher individual animal seroprevalence than the other agroecologies. Management and breed of animals were the most important risk factors associated with bovine brucellosis in the overall studied areas while breed, management system, type of mating used and source of replacement stock were the most important risk factors associated with seropsitiveity in the lowland area.

Based on the above conclusions the following are recommended:

- Awareness creation and continuous extension education for the farmers and the community for prevention and control of the disease from being transmitted to healthy animals and healthy heard;

- All animals with sign and symptom of brucellosis should be tested and the positive ones should be removed swiftly from the healthy animals to hamper the transmission of the disease;

- Breeding animas and semen from AI should be tested and must be confirmed to be free of bovine brucellosis before being transported to non infected areas and farm;

- Proper management, such as regular cleaning of the housings and animal premises, proper disposal of aborted materials and after births, isolation of pregnant animals several weeks before and after calving should be practiced by the cattle keeping farmers;

- Test and slaughter policy of sero-reactor animals should be applied in the area since the prevalence is very low;

- Further study on the determination of the specific biotypes of the causative agent should be carried out, which is essential to devise some control and prevention methods against

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6. ANNEXES

Annex 1. Questionnaire on Epidemiological study of bovine brucellosis in cattle

Date..... Code.....

1. Name of the District.....
2. Name of the farmer's association.....
3. Name of the village.....
4. Name of the owner (household head).....
5. Type of farming.....
6. Dairy only/ crop livestock mixed farming/other.....
7. Is the farming land is fenced? Yes/ No
8. Animal identification:
    8.1. Age of the animal.....
    8.2. Sex.....
    8.3. 8.3. Breed: a) Indigenous (local) b) Cross Breed Dairy
9. Herd size: ----------
10. Herd structure

10.1. Number of adult male .....

10.1.1. Number of adult male (bulls) used for service.....

10.1.2. Number of adult male (castrates):----------

10.1.3. Number of young male (bullocks):----------

10.2. Number of adult female (cows).....

10.2.1. Number of productive female (cows).....

10.2.2. Number of unproductive females.....

10.2.3. Number of served heifers.....

10.2.4. Number of replacement heifers (weaning to service).....

10.2.5. Number of calves (birth to winning).....

11. Why do you keep animals (cattle)? For dairy/beef/for drought/for all

12. How did you start cattle keeping?

a) Given from the family

b) Bought from local market

c) Other

13. How did you get skills of cattle management?

a) From learning institutions. b) From extension agents.

b) c) Traditionally from parents d) Others.

14. What are the objectives of cattle keeping in your farm?

a) To generate cash b) to supplement family food

c) as a sole means of existence d)for drought e)Others

15. Which breed of cattle do you own?

Breed of cattle	Number	Sex	Adult	heifer	bull	calf
Local						
25%cross						
50% cross						
75%cross						
More than 75% cross						
100% exotic						

16. What is the Parity status and average calving interval of the dairy cows in your farm?

Identity of the cow	Parity status	Calving interval

17. What type of insemination is used in your farm? a) AI b) natural c) both

18. Management and housing

18.1. Type of housing:

18.1.1. Barn

a) Separate Barn for cattle. b) Barn shared with other live stocks

18.1.2 Open field

a) Separate open field b) open field with other livestock

18.1.3. Human housing

a) Housed in the house of the family mixed with other lives tocks b) housed in the house of the family without being mixed

19. General hygiene of the house: good, satisfactory, poor

20. Do you separate cows during parturition? Yes? No

21. Do you put them in separate calving room maternity pen? Yes/No

22. Do you clean the maternity pen after the cows and the calve left? Yes? No

23. Is maternity pen made clean, dry and well bedded Yes/ No?

24. How is the feeding style of your cattle? a) Grazing separately b) grazing mixed with other livestock

23. Do you mix your animals (cattle) with other herd? Yes/No

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

1.....2.....3.....

4.....5.....6.....

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

27. What are the names of the disease locally?

28. Was any event of abortion in your farm? Yes/No

28.1 If yes which animals and at what time (months) does it occurred?

Cow identification	Time of abortion

29. How many abortions (how many animals) have aborted in your farm during the last three years?

.....

30. What do you do with them after abortion?

3.1. How do you dispose the after birth?

.....

.....

.....

30.2. How do you dispose the aborted fetus?

.....

.....

.....

31. What are the reasons of culling in your farm? a) Disease b) old age

c) infertility d) poor production e) other

32. From where do you get your replacement stock?

.....

.....

.....

.....

33. Have you ever tested your farm for brucellosis up to now? Yes/ No

34. Have you ever vaccinated your animals for brucellosis up to now? Yes/ No

Annex 2. Materials used and procedures of RBPT

Materials used:

RBPT brucella antigen
Known positive control serum
Known negative control serum,
Glass slide
Micropipette
Micropipette tip
Mixing applicator

Procedure:

- The test sera and the antigen were left at a room temperature for half an hour every time before the test is started
- 30 µl of test serum was taken and placed on a clean glass slide
- 30 µl of RBPT antigen was added to the side of each test serum
- Then the antigen and the test serum were mixed thoroughly by an applicator,
- The glass slide was shacked 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 glass was used.

Annex 3. Materials used and procedures of CFT

Materials used

1. Micro well plates (U-shaped), Multi channel and single channel micropipettes, pipette tips
2. Flasks and measuring cylinders
3. Beam balance (Digital balance)
4. Incubetor, water bath, deep freezer, Centrifuge
5. Vernal buffer, Alsever solution,
6. Complement, Hemolysin (Amboceptor), Control Serra, Sheep RBC
7. CFT Antigen

Preparation of sheep red blood cells for the hemolytic system

1. Ten µl of sheep red blood cells in Alsever's were centrifuged at 2500rpm for 5 minutes.
2. The supernatant was discarded and replaced by Veronal buffer diluents (VBD).
3. The sheep red blood cells were resuspended in diluents completely. This procedure is to be repeated 4 times.
4. Before discarding the supernatant after the last washing, the volume of the packed cell will be measured.
5. The volume of the packed cell was measured by placing an identical tube next to the blood containing tube filled up to the level of blood by a measured amount of water.
6. By addition of calculated amount of water, a 2% sheep red blood cell suspension was prepared.

Amboceptor titration

1. Pre-dilution of amboceptor was made in jumping dilution.
2. Prepared 1:500 dilution was made up to 1:8000.
3. From these dilutions 500µl was transferred to a set of tubes, always starting with the dilution 1:12000 dilutions.
4. 1µl of diluents was added to each of the test tubes.
5. 0.5µl 0f 2% sheep red blood cells was added, and shacked well.
6. The tubes were kept on the bench for ten minutes.
7. 1µl of complement at a dilution of 1:40 is was added.
8. The last tube showing complete hemolysis, minimum hemolytic dose (MHD) was read and recorded.

Evaluation of complement

1. Set up of 3 rows of 9 tubes each was prepared.
2. A 1:40 complement was prepared.
3. Complement was added into the 9 tubes increasing by 0.05ml every time, starting with 0.1ml.
4. Diluent was added into the 9 tubes in decreasing amount by 0.05ml, starting with 0.4ml.
5. 1.5µl of diluents was added into the tubes with the corn well syringe.
6. The set tubes were placed in a 37^0c water bath for 1hr.
7. 0.5µl of 2% sheep red blood cell was added in all tubes
8. 0.5µl of amboceptor at working dilution 1:1000 was added in all tubes.
9. The tubes were properly mixed and put again in the water bath of 37^0c for another 30 minutes.
10. The test was read by recording the minimum hemolytic dose of complement (MHD), which was represented by the first tube showing complement hemolysis. The next tube contains the full hemolytic dose (FHD).

The complement dilution=2FHD/dilution of complement.

Titration of antigen

- Microtiter plate I

1. 25 µl of VBD was added in all cups (wells).
2. 25 µl pre-diluted antigen wase added to all cups of row A.
3. By serial doubling (two fold) dilution 25µl of antigen was transferred from row A to B, and again from row B to C, etc; until row G by multichannel pipette. 25µl mixture was discarded from row G (row H will only contain the diluents)

- Microtiter plate II

1. 50 µl of VBD was added to all cups.
2. 50 µl of pre-diluted (1:2.5) in activated positive control serum was to all wells of col.1
3. 50 µl was serially transferred by two-fold dilution from col.1 to col.2, and again from col. 2 col. 3 etc. until col.11. 50µl is to be discarded from col. 11.

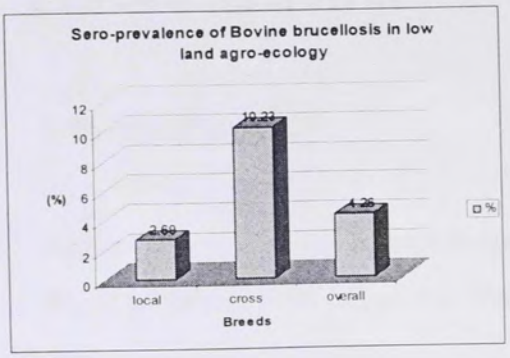
- Mix plate I and II

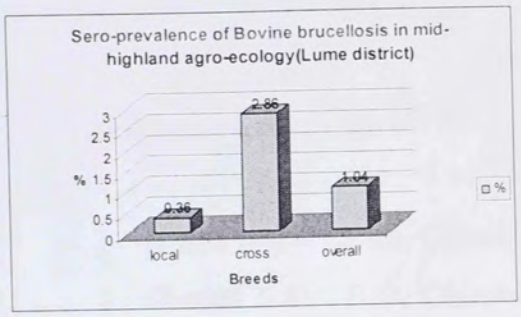
1. 25 µl was transferred from plate II to Plate I.
2. 50 µl of complement in 1:40 dilution was added to all cups of plate I
3. Plate I is to be kept in a refrigerator, covered with second empty plate (cold fixation)
4. The following day, 50µl of 2% sheep red blood cells, amboceptor's premixture, equal volume (i.e.25µl) of sheep red blood cells, 25µl of a 1:100 working dilution of amboceptor was added to all cups.
5. The plate was covered with sealing tape, shaken well and was kept in

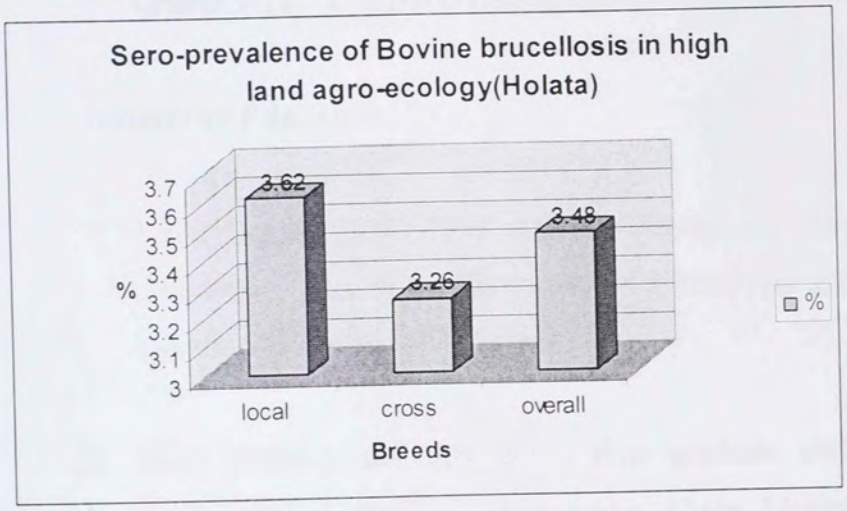
water bath at 37^0c for 30 min.

6. The last cup with 50+ sedimentation was read and recorded. The highest dilution of antigen with 50+ sedimentation is the limiting antigen concentration or the right corner value.

Annex 4. Seroprevalence of bovine brucellosis by breed in the three agroeclogies







9. CARRICULUM VITAE

I. Personal data

Name	Tujuba Jergefa Oncho
Place of Birth	Ghimbi, Wollaga, Ethiopia
Sex	Male
Martial Status	Married
Nationality	Ethiopian

II. Academic background

1 Grade 1-6: Biftu Ghimbi Primary and Junior Secondary School
2 Grades 7- 8: Biftu Ghimbi Primary and Junior Secondary School
3 Grades 9-12: Ghimbi comprehensive Senior Secondary School

University Education

1 From October 1987-1993 August: Attended higher education at Adds Ababa University, Faculty of Veterinary Medicine and graduated With the Degree of Doctor of Veterinary Medicine.

2 From October.2005-July 2006: Post graduate studies on Tropical Veterinary Epidemiology (M.Sc. TVE), Addis Ababa University, Faculty of Veterinary Medicine

III. Research experience and papers Produced:

1 Seminar on hydatidosis in livestock and zoonotic importance in human population (1991)

2 Problems related to sheep keeping and their economic importance in and around Awasa, DVM Thesis, Addis Ababa University Faculty of Veterinary Medicine, Debre-zeit, Ethiopia, 1992

3 Epidemiological study of Bovine brucellosis in Central Oromia, Ethiopia in partial fulfillment of the degree of Master of Science in Tropical Veterinary Epidemiology

IV. Work Experience:

1 General veterinary practice in Sidama Region as extern student for 10 months (1992)
2 District Veterinarian in Darimu District Ilu-abba Bora Zone, Western Oromia, Ethiopia (May1996 -2000 May)
2 District Veterinarian in Walmara District West Shoa Zone, Central Oromia, Ethiopia (June2000 -2002)
3 Field Service Veterinarian & privatization Officer in Oromia Regional State Agricultural and Rural Development Veterinary Service Department, Fifinnee (Addis Ababa), Ethiopia (June 2000 -2004 Oct.)
4 Assistant Researcher at Addis Ababa University, Faculty of Veterinary Medicine (June 2005-July 2006
5 MSc research work on Bovine brucellosis in central Oromia, at Addis Ababa University Faculty of Veterinary Medicine (October 2005-July2006)

V. Further Training:

1 Tsetse and typanosomiasis control training for 10 days

VI. Language Proficiency:

No	Language	Reading	Writing	Speaking	Listening
1	Afaan Oromo	Excellent	Excellent	Excellent	Excellent
2	Amharic	V. good	V. good	V. good	V. good
3	English	V. good	V. good	V. good	V. good

VII. References:

Dr. Merga Bekana, Associate Professor, Dean, Faculty of Veterinary Medicine, Addis Ababa University, Debre-Zeit, Ethiopia,

Dr. Kelay Belihu, Assistant Professor, Associate Dean for Research and Graduate Programs, Faculty of Veterinary Medicine, Addis Ababa University, Debre-Zeit, Ethiopia,

Dr. Dagninet Yimenu, National Coordinator for Pan African Control of Epizootics (PACE), Ethiopian Federal Democratic Republic, Ministry of Agriculture and Rural Development, Animal Health Department, Addis Ababa, Ethiopia,

10. SIGNED DECLARATION

I, the undersigned, declare that the thesis is my original work and has not been presented for a degree in any university.

Name: Tujuba Jergefa Oncho

Signature_____

Date of submission _____June 2006

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

Dr. Kelay Belihu _____

Dr. Merga Bekana _____

Epidemiolgy Of Bovine Brucello sis In Selecte Three Agroecologies In Central Oromia Ethiopia.

Tujuba Jergefa