ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

SEROPREVALENCE STUDY OF BOVINE BRUCELLOSIS AND ITS PUBLIC HEALTH SIGNIFICANCE IN SELECTED SITES OF JIMMA ZONE, WESTERN ETHIOPIA

BY
TADELE TOLOSA FULASA

JUNE, 2004
DEBRE ZEIT, ETHIOPIA
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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of science in Tropical Veterinary Medicine

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Board of Examiners **Signature**

1. Prof. Ph. Dorchies

_____

2. Prof. Feseha Gebreab

_____

3. Dr. Wondweson Abebe Gebreyes _____

4. Dr. Giles Innocent

_____

5. Dr. Andy Catley

_____

6. Dr. David Barrett

_____

Academic Advisors

Dr. Fekadu Regassa

_____

Dr. Kelay Belhu

_____

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ACRONYMS

AASUR	Addis Ababa and Surroundings
AI	Artificial insemination
ANRS	Amhara National Regional State
BgVV	Bundesinstitut für Gesundheitlichen Verbraucherschutz und Veterinärmedizin
CHE	Central Highland of Ethiopia
CFT	Complement Fixation Test
CSA	Central Statistics Authority
CSF	Chaffa State Farm
FAO	Food and Agricultural Organization
FHD	Full hemolytic dose
EANRS	Eastern Amhara National Regional State
EEC	European Economic Community
ESAP	Ethiopian Society of Animal Production
HIT	Haemagglutination Inhibition Test
ICFTU	International Complement-Fixation Test Units
IDF	International Dairy Federation
Ig	Immunoglobulin
ISABS	International Standard for Anti-*Brucella abortus* serum
MHD	Minimum Hemolytic Doses
MRT	Milk Ring Test
NA	Not Available
NVI	National Veterinary Institute
OIE	Office International Des Epizootics
OIEISS	Office International Des Epizootics International standard Sera
PAHO	Pan American Health Organization
RBT	Rose-Bengal Test
SAT	Serum Agglutination Test
SEE	South Eastern Ethiopia
SRBC	Sheep Red Blood Cells
VBD	Veronal buffer diluent
WHO	World Health Organization

DEDICATION

This paper is dedicated:

To my mother, Alemi Tolosa and my father Tolosa Fulasa, who never went to school themselves for raising me up and sending me to school and supporting me financially and morally throughout my academic career.

To my beloved wife, Lemlem Dhunfa, and my $F_{1,}$ Robsen Tadele.

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ABSTRACT

The prevalence of bovine brucellosis was measured in cross sectional study in Jimma zone, Western Ethiopia using RBT and CFT from October 2003 to April 2004. The study animals consisted of 1813 cattle among which were 1305 local breed found in extensive system in five districts and 508 cross breed in 46 farms found in Jimma town. The overall individual animal prevalence of 0.77 % and 0.2 % were recorded in five districts and Jimma town, respectively. When the two management systems were considered together an overall individual animal and herd prevalence of 0.61 % and 2.9 % were found, respectively. Higher prevalences were observed in larger herd size ($P< 0.001$); older age group were affected more than younger animals ($P< 0.05$) in the extensive management system. No reactors were observed in male. Seropositivities of 8 % were observed in animals with previous history of abortion. In the study of the disease in human beings, classified in four high-risk occupational groups, using RBT and CFT, the highest prevalence (14.3%) was observed among animal health workers and followed by farmers (3.2 %) and there was no reactors found among butchers and abattoir workers. An overall prevalence (2.4 %) was found in the area. There was a significantly higher risk of acquiring the infection when handling parturient animals ($P< 0.05$). The study demonstrates that the prevalence of brucellosis in the area is low; however, there is probable risk of spread of the disease in the unaffected cattle population since there are no precaution measures taken in the areas that should have been practiced by farmers. Since the prevalence is below 2 %, test and slaughter with compensation payment to farmers is recommendable, while in case of human brucellosis, since its presence is confirmed and the risk factors are identified, the medical personnel should give attention as to differential diagnosis of the disease which have been overlooked so far.

Key words: Ethiopia, Bovine brucellosis, cattle, Risk factors, Cross-sectional study, Sero prevalence, Reproductive status, Extensive management, Intensive management, Human brucellosis, occupational groups.

1. INTRODUCTION

Bovine brucellosis is a contagious disease usually caused by *Brucella abortus*, less frequently by *Br. melitensis*, and rarely by *Br. suis*, all of which are Gram-negative, facultative, intracellular coccobacilli bacteria (OIE, 2000). *Brucella abortus* is mainly infective for cattle, but occasionally other species of animals such as sheep, swine, dogs and horses may be infected. In horses, *Br. abortus* together with *Actinomyces bovis* is commonly present in poll evil and fistulous withers (Roberts, 1971; Radostits *et al.,* 2000).

The etiological agent *Brucella melitensis* discovered by Bruce in 1886 presented the first clear-cut picture of the disease. In 1897, Bernhard Bang, a Danish veterinarian and physician, identified *Br. abortus* as the cause of abortions in cattle in Denmark. At the beginning of the century, Zammit identified goats as the reservoir of brucellosis in Malta. The relationship between the agents of Malta fever and Bang's disease was recognized by Alice Evans, who renamed the genus *Brucella* to honor Bruce (Isselbacher *et al.*, 1980).

Sources of infection for the transmission of the bovine brucellosis are aborted fetuses, the fetal membranes after birth, and vaginal discharges and milk from infected animals (Radostits *et al*, 2000; PAHO/ WHO, 2001). The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water, and after birth, fetuses, and newborn calves, all of which may contain a large number of the organisms and constitute a very important source of infection (PAHO/WHO, 2001).

The disease in cattle is characterized by abortion in late pregnancy and subsequent high rate of infertility in females and varying degree of sterility in the male, leading to a significant economic loss (Bakunzi *et al*., 1993). The same organism also causes undulant fever in man from drinking raw or un-pasteurized infected milk or milk products or from exposure of farmers, packing house workers, veterinarians and others to infected discharges or tissues (Roberts, 1971; Weidmann, 1991).

Following penetration to the intact mucous membranes via the lymph system, the *Brucella* can spread out through the body and then early bacteremia is followed by localization of infection in the genital organs and cells of the monocytes–macrophage series.

The controlling of brucellosis under most conditions is best accomplished by a wide spread use of vaccines. Its control and eradication are difficult because of many technical and other aspects of the disease (Nicoletti, 1984). Modifications of environment, livestock management, social customs and resources will be necessary before many countries can consider eradication program.

Bovine brucellosis is of major economic importance in most countries of the world (Nicoletti, 1984; OIE, 1997). It affects approximately 5% of the livestock population worldwide and continues to increase in distribution (OIE, 1997). Most European countries are free of bovine brucellosis (PAHO/WHO, 2001), however, the disease still exists worldwide especially in the Mediterranean basin, the Arabian Peninsula, the Indian subcontinent, in parts of Mexico, Central and South America (WHO, 1986). The disease is thus a major problem confronting food production in tropical and subtropical regions of the world. Its occurrence is increasing in developing countries in an even aggravating epidemiological situation. This depends on the policy of many developing countries of importing exotic high production breeds without having the required veterinary infrastructure and the appropriate level of development of the socio-economic situation of the animal holder (Seifert, 1996).

The significance of brucellosis as a zoonosis has even increased in recent times. Some of the reasons for this are the expansion of international commerce in animals and animal products, increasing urbanization with growing numbers of animals in close proximity to people, increasing tourism (consumption local animal products), new ways of land use (irrigation) and new methods of cattle production involving higher animal concentration, which is a main factor in the spread of the disease (Weidmann, 1991; Seifert, 1996). The disease has, therefore, a significant negative impact on human productivity (Nicoletti, 1984).

In Africa, brucellosis is considered to be one of the most serious disease problems facing the veterinary profession. The prevalence is often high because of close human-animal contacts and food consumption customs (Nicoletti, 1984) The high prevalence is probably due to the fact that many countries have not yet started control or eradication schemes. However, only a few African countries have ever carried out an extensive survey of the prevalence of brucellosis in animals or man. According to Staak (1990), brucellosis is perhaps the most widespread and economically important disease in tropical and sub-tropical regions. The direct loss of meat (as a result of

abortion, infertility, and weight loss) in infected herds of cattle was estimated to be 15% while that of milk (reduced milk production) was 20%.

Ethiopia, one of the developing countries in sub-Saharan Africa, stands first in livestock population in Africa and tenth in the world. The livestock population of Ethiopia is estimated at 30 million head of cattle, 21.7 million sheep, 16.7 million goats, 7.02 million equines and 1 million camels (C.S.A, 1998). However, livestock output is still relatively poor, despite the implementation of numerous livestock development projects, due to a combination of constraints including under- nutrition, little or no improvement in management practices and high attrition from disease. Extension in livestock management and disease prevention measures, including for zoonoses, is minimal and coverage of the total area by veterinary services is limited.

Brucellosis is known to be an endemic and growing problem in domestic livestock (local and cross breed) herds in Ethiopia. The presence of bovine brucellosis in Ethiopia is well established. Meyer (1980) reported positive reactors of 39% out of 1010 cattle. Gebre-mariam (1985) also reported 18.4% positive reactors around Addis Ababa. Other survey from Arsi by Molla (1989) showed 7.62% positive reactors. 15.8% positive reactors were reported from selected sites of Sidamo (Zewdu, 1989). Bekele *et al*.,(1989) found 4.2% positive reactors from zebu cattle. The presence of the disease in different ranches has been reported (Hadigo, 1987; Molla, 1989; Bekele *et al.*, 2000). Higher prevalence was reported in large farm than smaller farms (Asfaw *et al*., 1998).

Although much work has been done and reports are available, there is no information on the status of bovine brucellosis in Western parts of the country. Only fragments of information are available from Oromia Agricultural Development Bureau that the disease is recorded in all zones in the Region with apparently low incidence. Therefore, the objectives of this study were:

- To determine the prevalence of brucellosis in the cattle population in the study area
- To identify risk factors and quantify their degree of association with brucellosis in cattle
- To assess the public health implication of the disease in the study area.

2. LITERATURE REVIEW

2.1. Epidemiology

2. 1. 1. Etiology

Brucella abortus is the causative organism for bovine brucellosis and at least nine biotypes have been recognized including a number of strain variants (Radostits *et al.,* 2000). *Brucella abortus* is mainly infective for cattle, but occasionally other species of animals such as sheep, swine, dogs and horses may be infected. Cattle can be also become infected by *Br. suis* and *Br. melitensis* when they share pasture or facilities with infected pigs, goats, or sheep. The infections in cattle caused by heterologous species of *Brucella* are usually more transient than that caused by *Br. abortus (PAHO/WHO, 2001).*

2.1.1.1. Resistance and survival properties

Under appropriate conditions, *Brucella* organisms can survive in the environment for a very long period. Their ability to withstand inactivation under natural conditions is relatively high compared with most other groups of non-sporing pathogenic bacteria. (WHO, 1986). *Brucella abortus* is sensitive to pasteurization temperatures 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 (Bishop *et al.*, 1994; Walker, 1999).

Survival is prolonged at low temperatures and organisms will remain viable for many years in frozen tissues. *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 minutes exposure at 37^0C. Formaldehyde solution is the most effective of the commonly available disinfectants, provided that the ambient temperature is above 15^0C (WHO, 1986).

2.1.2. Occurrence and prevalence of infection

Bovine brucellosis is widespread throughout the world except for the growing number of countries where eradication has been achieved (WHO, 1986). Many countries have made considerable progress with their eradication programs and some have eradicated the disease (Radostits *et al.*, 2000). The disease has been eradicated in Finland, Norway, Sweden, Denmark, The Netherlands, Belgium, Switzerland, Germany, Australia, Hungary, Rumania and Bulgaria as well as other countries. Most European countries are free of bovine brucellosis (PAHO/WHO, 2001).

It is of major economic importance in most developing countries, which have not had a national brucellosis eradication program (Radostits, *et* a*l*., 2000). In addition, the policy of many developing countries of importing exotic high production breeds without having the required veterinary infrastructure and the appropriate level of development of the socio-economic situation of the animal holder aggravates the situation (Seifert, 1996). In most developing countries, resources have not been sufficient to control brucellosis. Although information on prevalence is inadequate, there are indications of a very high incidence in many areas, particularly in tropics; in countries, that can least afford the loss in milk production and animal protein that accompanies this disease (WHO, 1986). The prevalence of infection varies considerably between herds, areas and countries.

The disease is prevalent in many countries of Africa (Kagumba and Nandokha, 1978; Nicoletti, 1984; Chukwu, 1985; Seifert, 1996). The reason for high prevalence is probably due to the fact that many countries have not yet started control or eradication schemes (Chukwu, 1985). Surveys on the prevalence of bovine brucellosis have been carried out in a few African countries (Table 1). A prevalence of 5 %, 5.9 %, and 9.9 % was reported in Uganda, Tanzania, and Kenya (Kagumba and Nandokha, 1978) respectively. Four hundred ninety nine sera sampled from cattle in Djibouti Republic revealed prevalence of 4 % (Chantal *e*t *al*., 1994). In Southern Sudan, 6.5 % prevalence was found in Dinka cattle of which 9.4 % of female Dinka cattle have been slaughtered because of infertility caused by brucellosis (Hellmann *et al*., 1984). In Eritrea 5.6 % seropositive animals to *Brucella* species have been detected in cattle (Omer e*t al*., 2000). The prevalence of bovine brucellosis in Africa from different sources is summarized (Table 1).

Table 1: Prevalence of bovine brucellosis in some African countries

Country	Host	prevalence %	No. tested	tests used	reference
Kenya	Cattle	9.9	10361	RBT	(Kagumba et al., 1978)
Uganda	Cattle	5	1739	RBT	(Kagumba et al., 1978)
Botswana	Cattle	18	NA	RBT	(Chukwu , 1985)
C. Africa	Cattle	40	8800	RBT, SAT, CFT	(Chukwu , 1985)
Chad	Cattle	13	240	SAT	(Chukwu , 1985)
Egypt	Cattle	0	510	SAT	(Chukwu , 1985)
	Cattle	100	50	SAT, MRT	(Chukwu , 1985)
Ghana	Cattle	23.5	1521	SAT	(Chukwu , 1985)
Guinea	Cattle	6.9	1861	RBT, SAT, CFT	(Chukwu , 1985)
Ivorycoast	Cattle	10.8	12343	RBT, SAT, CFT	(Chukwu , 1985)
	Cattle	45	1180	RBT	(Chukwu , 1985)
Kenya	Cattle	7.73	220	RBT	(Chukwu , 1985)
	Cattle	9.2	2372	SAT	(Chukwu , 1985)
	Cattle	30	983	SAT	(Chukwu , 1985)
	Cattle	11.8	1735	SAT	(Chukwu , 1985)
	Cattle	8.9	1070	SAT	(Chukwu , 1985)
	Cattle	3.74	2350	SAT	(Chukwu , 1985)
Nigeria	Cattle	8.06	1650	SAT	(Chukwu , 1985)
	Cattle	8.77	741	SAT	(Chukwu , 1985)
	Cattle	0-60	1989	CFT, SAT, HIT	(Chukwu , 1985)
	Cattle	79.70	133	SAT	(Chukwu , 1985)
	Cattle	38	133	SAT	(Chukwu , 1985)
Zimbabw	Cattle	18.4	892	SAT	(Chukwu , 1985)
Togo	Cattle	41	1056	RBT, SAT, CFT	(Chukwu , 1985)
Rwanda	Cattle	34.9	654	CFT	(Akakpo et al., 1987)
Uganda	Cattle	3	756	RBT, CFT	(Oloffs et al., 1998)
Ghana	Cattle	6.6	183	RBT	(Kubuafor, 2000)

--------- **Continued**

Country	**Host**	**prevalence %**	**No. tested**	**tests used**	**reference**
	Cattle	35	768	MRT	(Chukwu , 1985)
Senegal	Cattle	13	1051	SAT	(Chukwu , 1985)
	Cattle	9.4	NA	SAT, MRT	(Chukwu , 1985)
Sierra	Cattle	10.4	226	SAT	(Chukwu , 1985)
Leon	Cattle	2.1	96	SAT	(Chukwu , 1985)
	Cattle	11.9	2184	SAT	(Hussein *et al*., 1978)
	Cattle	2.7	902	SAT	(Hussein *et al*., 1978)
Somalia	Cattle	9.5	5056	SAT	(Wernery *et al.*, 1979)
	Cattle	12	576	MRT	(Wernery *et al*., 1979)
	Cattle	15	660	SAT	(Chukwu , 1985)
	Cattle	13.9-18	5689	SAT	(Chukwu , 1985)
	Cattle	38.6	76	MRT	(Chukwu , 1985)
	Cattle	57.4	1522	MRT	(Chukwu , 1985)
Sudan	Cattle	14.2-66.	2064	MRT	(Hellmann *et al*., 1984)
	Cattle	6.5	5982	SAT, CFT	(Hellmann *et al.*,1984)
	Cattle	22.5	1228	SAT, CFT	(Hellmann *et al*., 1984)
	Cattle	9.14	776	MRT	(Kagumba *et al.*, 1978)
	Cattle	14.2	368	SAT	(Jiwa *et al*., 1996)
Tanzania	Cattle	10.8	13087	SAT	(Jiwa *et al*., 1996)
	Cattle	5.9	23017	RBT	(Kagumba *et al*., 1978)
	Cattle	14.1	341	SAT	(Weihaupl *et al.*, 2000)
Djibouti	Cattle	4	499	CFT	(Chantal *et al*., 1994)
	Cattle	11.3	1879	RBT, SAT, CFT	(Chukwu , 1985)
Zambia	Cattle	27.9	705	SAT	(Chukwu , 1985)
	Cattle	11.1	432	SAT	(Chukwu , 1985)
	Cattle	9.5	788	SAT	(Chukwu , 1985)
Eritrea	Cattle	5.6	2427	RBT, CFT	(Omer *et al*., 2000a)
	Cattle	8.5	1294	RBT, CFT	(Omer *et al*., 2000b)

2.1.3 Risk factors for infection

The risk factors that influence the initiation, spread, maintenance and/ or control of bovine brucellosis are related to the animal population, management and to biology of the disease (Radostits *et al.,* 2000).

2.1.3.1 Agent risk factors

Brucella abortus is a facultative intracellular pathogen, which is capable of multiplication and survival within host phagocytes (WHO, 1997). The organisms are phagocytosed by polymorphonuclear leukocytes in which some survive and multiply. These are then transported to lymphoid tissues and fetal placenta. The inability of the leukocytes to effectively kill virulent *Br. abortus* at the primary site of infection is a key factor in the dissemination to regional lymph nodes and other sites such as the reticuloendothelial system and organs such as the uterus and udder. The organism is also able to survive within macrophages because it has the ability to survive phagolysosome. *Brucellae* are able to survive within host leukocytes and may utilize both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanism during the period of haematogenous spread (Radostits *et al.,* 2000).

2.1.3.2 Host risk factors

Susceptibility of cattle to *Br. abortus* infection is influenced by age, sex, breed, and reproductive status of the individual animal. Infection occurs in cattle of all ages but persists most commonly in sexually mature animals (Radostits *et al*., 2000). Younger animals tend to be more resistant to infection and frequently clear infections, although latent infections do occur (Walker, 1999). Only 2.6% of animals infected at birth remain infected as adults. Sexually mature and pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex (Radostits *et al.,* 2000). Susceptibility increases with pregnancy and as stage of gestation increases (Bishop *et al.,* 1994). Natural exposure to field strains occurs primarily at the time of parturition of infected cows. The greater the number of infected cows that abort or calve, the greater the exposure risk to the other cattle in the herd (Radostits *et al.,* 2000). All breed of cattle appear to be comparable in susceptibility to brucellosis and apparently no specific breed resistance to brucellosis is known (Radostits *et al.,* 2000). In contrary to this, there is a report that

genetic resistance demonstration to brucellosis and selection is possible for this trait in livestock in the near future (WHO, 1997).

2.1.3.3 Management risk factors

The spread of the disease from one herd to another and from one area to another is usually due to the movement of an infected animal from an infected herd into a non-exposed herd (Radostits *et al.,* 2000). Whether a herd raises its own replacement animals or purchases replacement animals affects the potential for introduction into the herd (Walker, 1999). The unregulated movement of cattle from infected herds or areas to brucellosis-free herds or areas is the major cause of breakdowns in brucellosis eradication programs. Once the herds are infected, the time required to become free of brucellosis is increased by large herd size, by active abortion, and by loose housing (Radostits *et al.*, 2000).

Large numbers of organisms are shed from the reproductive tract when infected cows abort. In cows which lactate following abortion, milk, including colostrum, is an important source of infection, and bacteria are excreted intermittently in milk throughout the lactation period. The fluid in hygromas caused by *Br. abortus* infection may contain large numbers of organisms, but because of being restricted to the lesion they do not seem to be important in the spread of the disease (Bishop *et al*., 1994).

A contaminated environment or equipment used for milking or artificial insemination is further sources of infection. Permanent calving camps and lush pastures, particularly if they are wet and muddy, may play a very important role in the spread of the disease (Bishop *et al*., 1994).

2.1.4. Transmission

2.1.4.1. Sources of infection

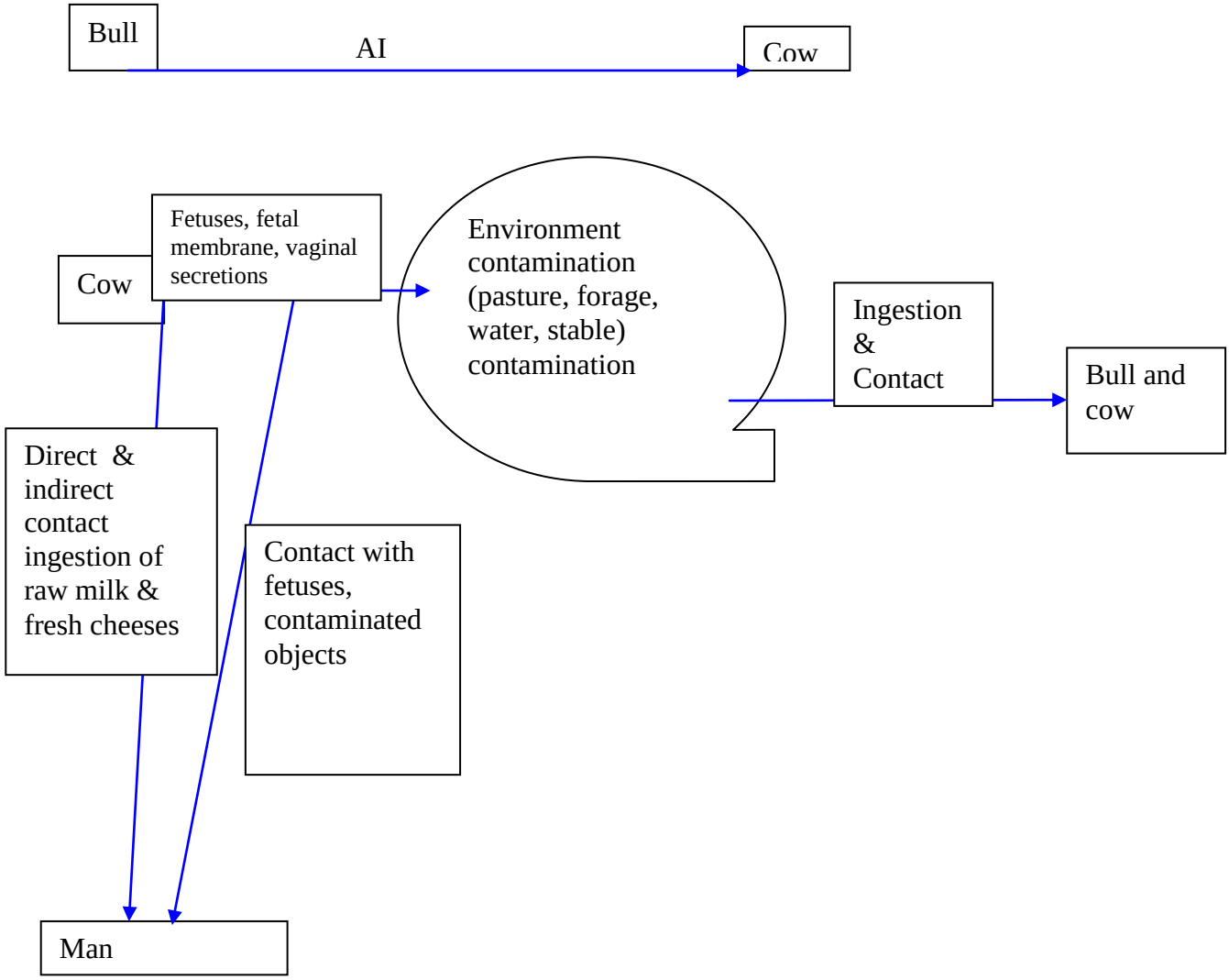
The risk associated with exposure of susceptible animals to the disease following parturition or abortion of infected cattle depends on three factors: the number of organisms excreted, the survival of these organisms under the existing environmental condition, and the probability of susceptible animals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

achieves its greatest concentration in the contents of the pregnant uterus, the fetus and the fetal membranes after birth (Radostits *et al*., 2000). In addition, vaginal discharge and to a lesser extent, farm areas contaminated by fecal matter of calves fed on contaminated milk could be considered as main source of infection (PAHO/WHO, 2001). Infected animals also shed organisms in the milk. Therefore, raw milk or raw milk products of bovine origin are ready sources for infections in humans (Walker, 1999). There can be also accidental self inoculation with live *Brucella* vaccine strains that result in the disease.

2.1.4.2. Mode of transmission and route of infection

The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water (Fig. 1). Moreover, cows customarily lick after birth, fetuses, and newborn calves, all of which may contain a large number of the organisms and constitutes a very important source of infection. Bulls do not usually transmit infection from infected cows to non-infected mechanically. The use of infected bulls for AI constitutes an important risk, since the infection can be spread to many herds (PAHO/WHO, 2001). Humans are infected from drinking raw or un-pasteurized infected milk, from exposure to infected discharges or tissues (Roberts, 1971).

Fig 1: Bovine Brucellosis (*Br. abortus*) mode of transmission



Source: PAHO/WHO, 2001

2.2. Pathogenesis

Following exposure, *Brucella* penetrates intact mucosal surfaces (Walker, 1999) and survives and multiplies in cells of the reticuloendothelial system, such as the bone marrow, lymph nodes, liver, spleen, and also kidney (Isselbacher *et al*., 1980). Multiplication of the organisms here may last for several months, resolve itself, or be recurrent for at least two years in 5-10% of animals. Recurrence occurs particularly at the time of parturition. During the bacteraemia, organisms are

carried intracellularly in neutrophils and macrophages or free in the plasma and localize in various organs, especially the gravid uterus, udder, and suprammamary lymph nodes.

Localization may also occur in other lymph nodes and the spleen, testes, and male accessory sex glands. Occasionally bacterial localization occurs in synovial structures causing a purulent tendovaginitis, arthritis, or bursitis (Bishop *et al.,* 1994).

The preferential localization to the reproductive tract of the pregnant animals is due to the presence of unknown factors in the gravid uterus. These are collectively referred to as allantoic fluid factors that would stimulate the growth of *Brucella*. Erythritol, a four-carbon alcohol, is considered to be one of these factors (Walker, 1999) which are elevated in the placenta and fetal fluid from about the fifth month of gestation (Bishop *et al.,* 1994). The preferential replication of *Br. abortus* in the extraplacentomal site within trophoblasts of the chorioallantoic membrane results in rupture of the cells and ulceration of the fetal membrane. The damage to placental tissue together with fetal infection and fetal stress will induce maternal hormonal changes. As a result, abortion occurs principally in the last three months of pregnancy, the incubation period being inversely proportional to the stage of development of the fetus at the time of infection (Radostits *et al.,* 2000).

2.3. Immunity

2.3.1. Humoral immune response

Naturally infected animals and those vaccinated as adults with strain 19 remain positive to the serum and other agglutination tests for long periods. The serum of infected cattle contains high levels of IgG_1, IgG_2, IgM, and IgA isotypes of antibody (Radostits *et al.*, 2000). Similar isotypes at different relative concentrations occur in milk, although most of the IgA is present in secretory form. The first isotype produced after an initial heavy infection or strain 19 immunization is IgM and is soon followed by IgG antibody. IgG_1 immunoglobulin is the most abundant in serum and exceeds the concentration of IgG_2. The magnitude and duration of the antibody response following immunization is directly related to the age at immunization and the number of

organisms administered. Following immunization with a standard dose of strain 19 during calf hood, IgG antibody concentrations usually decline to diagnostically insignificant levels over 3-6 months. Residual antibody if present, is usually predominantly of the IgM class. Following exposure to virulent *Br. abortus*, antibody may appear in 4-10 weeks or longer, depending on the size and route of entry of the inoculums and the stage of pregnancy of the animal. Antibodies of IgG, IgM, IgG_1 and IgG_2 isotypes can all react in the tube agglutination test, but those of the IgM class are by far the most efficient (WHO, 1986; WHO, 1997).

2.3.2. Cellular immune response

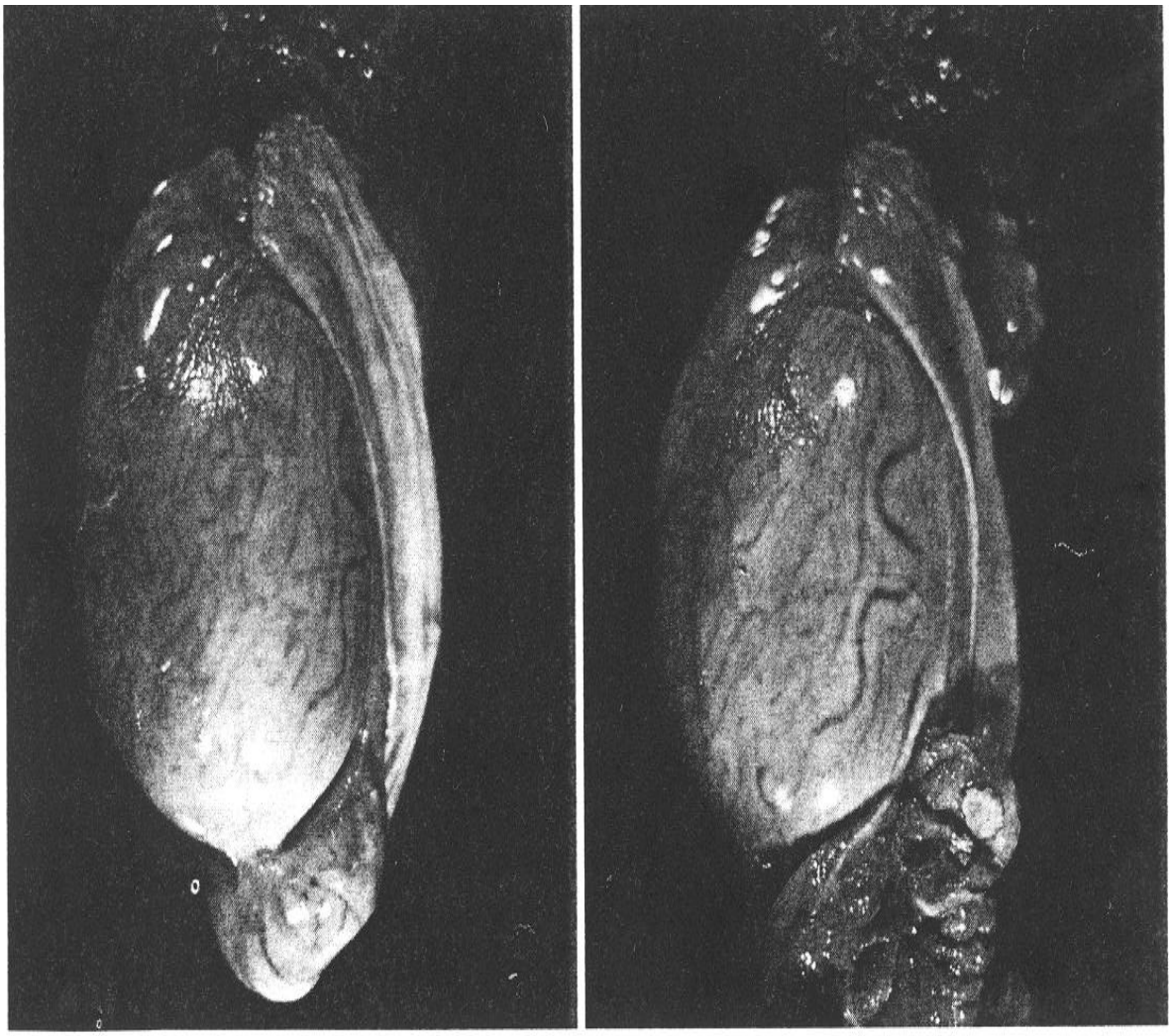
Brucella species are facultative intracellular pathogens. They are readily phagocytised by macrophages and polymorphonuclear leukocytes and, in the case of virulent strains, are capable of surviving within these cells, and phagocytosis is promoted by antibody. However, since virulent *Brucella* can survive within normal macrophages for long periods, recovery from infection is likely to be dependent upon the acquisition of increased bactericidal activity by phagocytic cells. Macrophage activation occurs when T-lymphocytes of the appropriate subset are stimulated to release lymphokines (interleukins) (WHO, 1986; WHO, 1997).

The release of these activating factors is dependent upon recognition of the appropriate antigen by the T- lymphocyte and is subject to regulation through the major histocompatibility complex. Live organisms capable of establishing persistent intracellular infection and certain types of antigen, with or without adjuvant, are the most effective inducers of cell-mediated immunity. The role of cytotoxic cells, including cytotoxic T-lymphocytes, natural killer (NK), and killer (K) cells, in the cell-mediated immune response to *Brucella* has not been elucidated. Further studies are also needed to determine the basic processes underlying the development of protective immunity to *Brucella* in the natural host species (WHO, 1986; WHO, 1997).

2.4. Clinical features

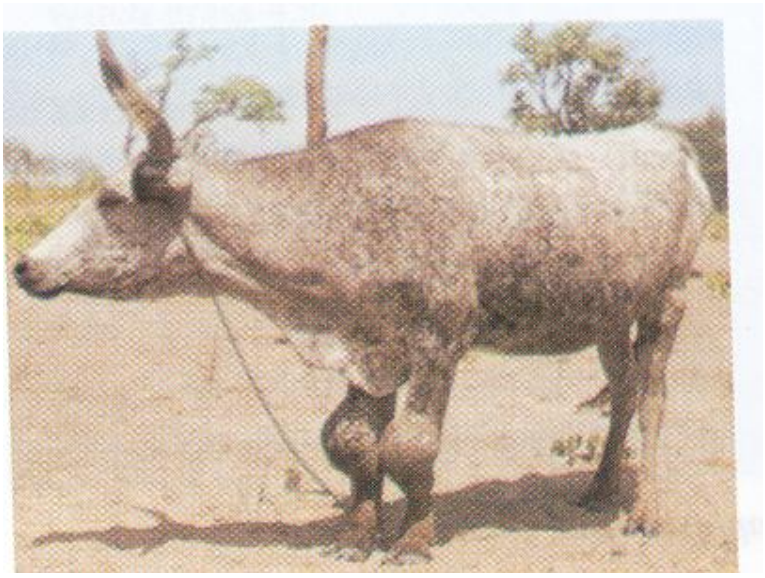
The incubation period varies between 14 and 120 days (Seifert, 1996). Primary clinical manifestations of brucellosis are related to the reproductive tract. In highly susceptible non-vaccinated pregnant cattle, abortion after the 5th month of pregnancy is cardinal feature of the disease (Radostits *et al.*, 2000). Retention of placenta and metritis are common sequels to abortion (Walker, 1999). Females usually abort only once, presumably due to acquired immunity. In general, abortion with retention of the placenta and the resultant metritis may cause prolonged calving interval and permanent infertility. In male animal, the epididymis (Fig. 2) is usually affected, but also the accessory sexual glands, with painful, necrotic tissue degeneration and a decrease in semen quality (Weidmann, 1991). Chronic infection with *Br. abortus* in cattle can result in hygromas (Fig. 3) (Walker, 1999).

Fig.2: Swelling and irregular conformation of the tail of the epididymis of an infected animal with *Brucella*. Cross-section of the tail of epididymis.



Source: Walker, 1999.

Fig. 3: Brucellosis: Characteristic hygromas in a cow.



Source: Seifert, 1996.

2.5. Diagnosis

There are basically two main groups of diagnostic methods for detecting brucellosis: Identification of the agent and serological tests.

2.5.1. Identification of the agent

2.5.1.1. Microscopic examination

This is a useful procedure for examination of abortion materials. Smears of placental cotyledon, fetal stomach contents or uterine exudates should be heat fixed and stained by a Stamp's modification of the Zeihl-Neelsen stain. It is a small, Gram-negative coccobacilli or short rod measuring 0.6 to 1.5μm by 0.5 to 0.7μm (Bishop *et al.*, 1994; Radostits *et al.*, 2000). No capsules, flagella, or spores are produced; however, an external envelope has been demonstrated by electron microscopy around *Br. abortus*, *Br. melitensis*, and *Br. suis* (Walker, 1999). *Brucellae* are also non-motile (Freeman, 1979; IDF, 1994).

2.5.1.2. Isolation of *Brucella*

- Culturing of the organism: from milk sample, tissue sample, and genital discharges, fluid from hygromas, fetal stomach contents and semen, etc; is possible and can be cultured directly or after centrifugation where appropriate, and the use of selective medium is recommended.
- Inoculation: Into Guinea pig and mouse is the technique that has value for the isolation of *Brucella* when specimens are derived from potentially contaminated sources such as milk, cheese, semen, or genital discharges. Inoculation should be made subcutaneously into Guinea pig or intravenously (0.1ml), or subcutaneously if the material is heavily contaminated, into mice. A guinea pig is killed 3 weeks post infection and the second 6 weeks after inoculation. A blood sample for serological examination is taken at the time of killing; macroscopic lesions are recorded and the spleen is cultured. The mice are killed 7 days after inoculation and the spleen and liver removed for culture on nutrient medium.

Most strains are fastidious and slow growing, and require Co_2 (5-10%) supplementation for primary isolation at an optimal growth temperature of 20-40^{0}C. Complex medium containing serum is required on sheep blood agar, the colonies not be as distinctive as when grown on serum dextrose agar (Bishop *et al.*, 1994). The optimum PH is 6.6 to 7.4 (Walker, 1999). Colonies have smooth or non-smooth morphology. Non-smooth colonies have intermediate, rough or mucoid forms. Smooth forms are often markedly pathogenic, whereas the rough variants are usually less

so (Bishop *et al*., 1994). The mucoid colonies are similar to the rough colonies except for having a glutinous texture (Walker, 1999).

The metabolism of *Br. abortus* is oxidative and *Brucella* culture shows no ability to acidify carbohydrate in conventional tests. They are catalase positive and usually oxidase positive and reduce nitrate to nitrites. The production of H_2S from sulfur containing amino acids also varies, showing some correlation with nomen-species and biovars urease activity varies from fast to very slow (IDF, 1994).

2.5.2. Serological tests

When bacteriological diagnosis is not practicable diagnosis has to be based on serological methods, e.g. in surveys or eradication programs.

2.5.2.1. Herd surveillance test/ Milk ring test (MRT)

It is the most practical and economical method for locating infected dairy herds (Weidmann, 1991) and for surveillance of brucellosis free herds. If performed on pooled milk 3 or 4 times a year on each herd, it will detect the majority of infected herds. Herds with a positive milk-ring test can then be examined by individual serum or milk tests to identify the infected individuals. Milk from individual animals can be serially diluted in *Brucella*-free milk to determine the end-titre of the milk-ring reaction. Titres above 1:10 are suggestive of infection.

2.5.2.2. Tests for individual animal diagnosis

Tube agglutination test (TAT): Most widely used procedure for the purposes of international trade. It measures the total quantity of agglutinating antibodies and has the disadvantage of reacting to post immunization agglutinins and sometimes to those caused by heterospecific antigens.

Plate agglutination test (PAT): Simple and more rapid than the TAT but affected by the environmental conditions (Weidmann, 1991). Sensitivity and specificity are similar to those of tube tests. The use of the PAT should be discouraged, except when serum quality is inferior and its use is essential.

The Rose Bengal Plate Test (RBT): Applied for a screening test for individual diagnosis in herds of cattle. It has also been used in surveys and surveillance (Weidmann, 1991). It is generally considered to have poor specificity, especially in cattle immunized with strain 19. For this reason sera positive in the Rose Bengal Test are usually retested by a definitive test, such as the complement-fixation test.

The Complement Fixation test (CFT): It is the most reliable diagnostic test now in routine use for individual animals. It is relatively insensitive to antibody resulting from strain 19 immunizations. The workload resulting from the technical complexity of the CFT can be greatly reduced by using it only as a definitive test on samples that have been found positive in a preliminary screening test. Either warm or cold fixation may be used for the reaction between test serum, antigen, and complement. In warm fixation the mixture is held at 37 ^{0}C for half an hour. In cold fixation, the mixture is held at approximately 4 ^{0}C for 14-18 hours. A number of factors affect the choice of method.

1. Anticomplementary activity in serum samples of poor quality is more evident with cold fixation

2. Fixation at 37 ^{0}C increases the frequency and intensity of prozones and several dilutions must be tested for each sample.

3. Fixation in the cold produces higher titres in positive sera.

4. With cold overnight fixation the working week is reduced by one day.

When the complement-fixation test has been as the principal definitive diagnosis test in eradication campaigns, warm fixation has generally been used. Bovine serum is usually inactivated at 58 ^{0}C for 30 minutes. Higher temperatures reduce anticomplementary activity but also reduce the complement-fixing activity of IgM.

The classical complement-fixation procedure in tubes with a total volume of 1 or 2 ml is practical only for testing a few individual samples, e.g. in the diagnosis of human brucellosis. In veterinary practice, the numbers of samples involved in eradication programs necessitate some degree of automation, which may be based on either continuous flow or micro-methods. Micro-methods are generally preferred. They represent the classical technique reduced to small volume and allow various degree of automation to be applied, such as mechanical dilution of serum and addition of reagents. The continuous flow method, on the other hand is entirely automatic standardization of the complement-fixation test. A unitage system has been recommended based on the second International Standard for Anti-*Brucella abortus* serum (ISABS). The second ISABS is taken to contain 1000 International Complement-Fixation Test Units (ICFTU) and if this serum is tested in a given method and gives a titre of say 500, then the factor for an unknown serum tested by that method can be found from the formula:

1000/500*titre of test serum = number of ICFTU of antibody in the test serum.

The European Economic Community (EEC) has adopted this unit as the EEC unit. The ISABS contains only IgG; national standard sera should also depend on this isotype for their specific complement-fixing activity.

Difficulties in standardization arise because different techniques selectively favor complement fixation by different immunoglobulin isotypes. It is recommended that any country using the complement-fixation test on a national scale should obtain agreement between the different laboratories performing the test by a standard method. This allows the same level of sensitivity to be obtained.

2.5.3. Supplementary Test (WHO, 1986)

2.5.3.1. Ethacridine/ Rivanol/ Agglutination Test

The principle of the test is to eliminate the reactions caused by IgM antibodies that persist following stain 19 immunizations. In the USA, the Ethacridine test is often performed on sera that are positive in screening procedures, such as the buffered antigen tests. Equal quantities of serum and Ethacridine solution are mixed in a tube. A precipitate is formed after which charcoal

is added and the tube is centrifuged. The supernatant is then tested with Ethacridine plate test antigen. Different dilutions are tested on a glass plate. The Ethacridine agglutination test has been extensively evaluated and can often replace more difficult procedures, such as the Complement-Fixation Test.

2.5.3.2. Enzyme Immunoassay

It has been extensively investigated as a definitive test for the detection of antibody to *Brucella* in bovine sera. The test shows great promise of increased sensitivity and specificity. Both whole-cell and purified lipopolysaccharide antigens have been used and a variety of antiglobulin conjugates and substrates. A great deal of work is still required on standardization of reagents Enzyme immunoassay is a valuable research tool that can employ purified *Brucella* antigens and specific and sensitive anti-immunoglobulin reagents, thus permitting the measurement of immunoglobulin subclass of *Brucella* antibody to define antigens. These methods promise to provide a basis for the development of a new generation of simple tests that can be conducted with inexpensive equipment and reagents.

2.5.3.3. The Anamnestic Test

This test has been developed to detect latent infection in cattle in eradication programs. It is based on the principle that it is possible to distinguish serologically between a primary and a secondary responsible to the injection of *Brucella* antigen. In practice, the cattle are tested by the routine serological method, usually the complement fixation test, and reactors are removed. The negative cattle are immunized, usually with *Br*. *abortus* 45/20 vaccine. Approximately 6 weeks later, they are again tested and any further reactors removed. The anamnestic test cannot be used on cattle previously immunized with strain 19. This test has been used with success to brucellosis-free groups of weaned heifers in range cattle that cannot be mustered regularly for serological testing.

Various other serological tests have been used experimentally without so far being accepted for routine use. These tests include the indirect hemolysis test, a sensitive and specific procedure that gives little reaction with antibody resulting from strain 19 immunization. The hemolysis-in-gel test is a similar procedure in gel, but is considerably more sensitive to vaccinal antibody than the

indirect hemolysis test. The radial immunodiffusion test is a simple gel-diffusion test utilizing a polysaccharide (poly B) hapten. It is especially insensitive to vacinnal antibody, and detects a high proportion of cattle excreting *Br*. *abortus* in the milk.

2.6. Significance of the disease

2.6.1. Economic importance

On an average, outbreak of bovine brucellosis resulted in a loss of milk production of the herd as much as a 20% and this can reach 40-50% in early abortion (Bishop *et al.*, 1994). In addition to the loss of milk production, there is the loss of calves and interference with the breeding programs. This is of greater importance in beef herds where calves represent the sole source of income (Radostits *et al*., 2000). The common sequel of infertility increases the period between lactations, and in an infected herd the average inter calving period may be prolonged by several months.

Losses in animal production due to the disease can be of major importance, primarily because of the decreased milk production by aborting cows (Radostits *et al.*, 2000) and this is often associated with retained placenta, metritis and a subsequent period of infertility (OIE, 1997).

In general, economic losses due to brucellosis are usually caused by (Chukwu, 1987):

- ♠ Losses due to abortion,
- ♠ Diminished milk production,
- ♠ Cull and condemnation of animals due to breeding failure,
- ♠ Endangering animals export trade of a nation,
- ♠ Human brucellosis causing loss of some hours and medical costs,
- ♠ Government costs on research and eradication schemes

2.6.2. Public health importance

Brucellosis is a disease of animals in which man is infected as terminal host. The incidence of brucellosis in man is clearly correlated to the degree of incidence in the domestic animals around him (Weidmann, 1991). In developing countries, brucellosis is a relatively common disease among animals and man, and in these countries, it constitutes a large and uncontrolled public health problem (WHO, 1986). According to world health organization, about half a million cases of human brucellosis occur each year (FAO/WHO, 1986).

Man becomes infected when there is indirect contact with cows at abortion, parturition, or in the post parturition period from splashing of infected droplets into the eyes (Sewell and Brocklesby, 1990) or drinking unpasteurized milk or milk products (Roberts, 1971). Brucellosis is an occupational disease, occurring most often in veterinarians, farmers, stock inspectors, abattoir workers, laboratory personnel, butchers (Sewell and Brocklesby, 1990; Bishop *et al.,* 1994). The disease in humans is characterized by a multitude of somatic complaints, including fever, sweat, anorexia, malaise, weight loss, depression, headache and joint pains (WHO, 1997) and is easily confused with malaria and influenza (Sewell and Brocklesby, 1990). Reports on human brucellosis in some African countries are summarized in Table 2.

Table 2: Prevalence of human brucellosis in some African countries

Country	Number tested	Prevalence %	Tests	Reference
Nigeria	13999	7.6-29.8	SAT	(Chukwu, 1985)
	738	5.55	SAT	(Chukwu, 1985)
Tanzania	540	22.6	SAT	(Chukwu, 1985)
	80	20	SAT	(Chukwu, 1985)
Uganda	3164	6.4	SAT	(Chukwu, 1985)
Somalia	353	0.6	SAT	(Hussien *et al*., 1987)
Djibouti	108	6.5	CFT	(Chantal *et al.*, 1996)
Eritrea	130	7.1	CFT	(Omer *et al*., 2002)
	21	4.6	CFT	(Omer *et al*., 2002)
	105	3	CFT	(Omer *et al.*, 2002)

2.7. Control and eradication

2.7.1. Chemoprophylaxis

An effective treatment for animals with brucellosis is not known to date (Weidmann, 1991). The treatment of brucellosis in the cow has generally been unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs and

the bacteria are facultative intracellular which survive and multiply within the cells (Radostits *et al.,* 2000). Generally, treatment of infected livestock is not attempted because of the high treatment failure rate, cost, and potential problems related to maintaining infected animals in the face of ongoing eradication programs (Walker, 1999). Man can be treated with antibiotics (doxicyclin with rifampicin), however, relapses are not impossible (Weidmann, 1991).

2.7.2. Immunoprophylaxis

The strategies for preventing brucellosis have to be adapted to the animal production system (Seifert, 1996).The successful prevention of this disease, which is so difficult in cattle production in the tropics, requires that, as far as possible, all available steps be taken to combat it (Weidmann, 1991). Failures of disease control are mostly due to the application of a scheme for which neither the veterinary infrastructure, nor the required reliable serological laboratories exist and the animal holder does not have the socio- economic prerequisites. Principally two alternatives exist (Seifert, 1996):

2. 7. 2. 1. Test and slaughter, involves recognition of all animals which have responded immunologically to a *Brucella* infection and subsequent culling of the reactors According to Weidmann (1991) these method could be achieved when the rate of infection is reduced to an acceptable level (about 1-2%). Part of the scheme has to be a careful control of all animals which will be newly added to the herd as well as a production system which prevents contact with infected neighbouring farms and/or contaminated feed or pasture.

2. 7. 2. 2. Vaccination of exposed herds with inactivated or live vaccines.

- Calf hood vaccination- only performed on heifer calves between ages of 4-10 months. Vaccinated calves must be identified by a tattoo and ear tag.
- Adult vaccination the whole herd is vaccinated whenever there are certain problem herds. Herds have to be maintained in quarantines until all vaccinated animals have been removed from the herd.

The following are some of the vaccination available against brucellosis:

❖ Live *Br. abortus* strain 19 vaccines

A single dose at 3 to 7 months of age is required with *Br. abortus* strain 19. Adult animals vaccinated with strain 19 develop a better immunity than calves. However, due to the danger of abortion in pregnant animals, vaccination has thus so far been performed, above all, in calves, resulting in an average protection from infection of about 70 %(Weidmann, 1991). Bulls should not be vaccinated because orchitis can develop (Walker, 1999).

❖ Killed *Br. abortus* 45/20 vaccines

Two doses administered 6 weeks apart in animals over 6 months of age are required with *Br. abortus* 45/20. Adult cow vaccination is sometimes performed as a regulatory effort to control infection in a herd (Walker, 1999).

❖ *Brucella abortus* strain RB51 vaccines

This is a recently developed vaccine and has replaced *Br. abortus* strain 19 in a number of countries as the approved calf hood vaccine because it does not interfere with serological evaluation (Walker, 1999). *Brucella abortus* strain RB51 is a live stable rough mutant of *Br. abortus* strain 2308, which lacks much of the lipopolysaccharide O-side chain and has been investigated as an alternative to strain 19 vaccines (Radostits *et al.*, 2000). Adult vaccinations with *Br. abortus* strain RB51 only rarely causes abortion.

2.7.3. Hygienic Prophylaxis

Experience shows that vaccination alone cannot bring about the eradication of the disease (Weidmann, 1991). From the epidemiology of the disease, important steps were derived at an early stage as hygienic prophylactic measures. These include:

- The isolation of calving animals' in separate calving pens which are subsequently disinfected with 2.5 % formalin (Weidmann, 1991; Bishop *et al.*, 1994).
- Wet and well- grassed calving camps should be avoided, and vehicles used for transporting infected animals should be disinfected after use (Bishop *et* a*l*., 1994).

- Aborted fetuses, placentas, and uterine discharges mus*t* be disposed of, preferably by incineration (Weidmann, 1991; Bishop *et al.,* 1994; Radostits *et al*., 2000).

- All cattle, horses, and pigs brought to the farm should be tested, isolated for 30 days, and retested (Radostits e*t al*., 2000).

- Cows, which are in advanced pregnancy, should be kept in isolation until after parturition, since occasional infected cows may not show a positive serum reaction until after calving or abortion (Bishop *et* a*l*., 1994; Radostits *et al*., 2000).

- Replacement stock should be purchased from herd free of brucellosis (Bishop *et al*., 1994).

- Chlorhexidine gluconate is an effective antiseptic against *Br*. *abortus* and is recommended for washing the arms and hands of animals attendants and veterinarians who are exposed to contaminated tissues and materials (weidmann, 1991; Bishop *et* a*l* 1994; Radostits *et a*l., 2000).

2.8. Status of bovine brucellosis in Ethiopia

According to a report from WHO (1986) the incidence of animal and human brucellosis in the last two decades has increased as rapidly as urbanization and improved transportation has concentrated herds that were traditionally small and dispersed. In many developing countries the problem is compounded by an absence of national surveillance programs, diagnostic facilities or reliable data.

In Ethiopia, in the past, information concerning the prevalence of brucellosis was lacking. In recent years, however, increasing demand to milk and milk products necessitated great attention to be given to dairy farms by both public and private sectors (Shiferaw, 1987). In Ethiopia, a number of works have been done on sero-prevalence of brucellosis in different parts of the country. However, the economic impact of the disease on animal productivity and production is not yet assessed. So far, only one study has been made at Caffa State farm, Wollo, from 1987 to

1993. The same paper indicated that there was an annual loss estimated to be 88,941.96 Eth. Birr due to reduced milk production and abortions in the farm on 193 study animals (Sintaro, 1994).

Serological investigation on the prevalence of brucellosis in Ethiopia has been carried out in different part of the country for the last 24 years. Pioneer survey was done by Meyer (1980) and reported a positive reaction of 39% out of 1010 cattle owned by the then Institute of Agricultural Research (IAR). The survey conducted by Gebre-mariam (1985) on prevalence of bovine brucellosis in four different farms around Addis Ababa showed that 18. 4% were positive reactors out of 178 tested animals.

The other survey conducted on sero-epidemiological prevalence of bovine brucellosis in Arsi region on 2178 animals showed that 7.62% were positive reactors (Molla, 1989). Zewdu (1989) in his study on bovine brucellosis in selected sites in Sidamo region indicated that out of 734 tested animals 15.8% were found to be positive reactors. Generally speaking, the disease seems to be more prevalent in large improved herd than smaller indigenous herd (Wondimu, 1989; Asfaw *et al*., 1998).

The prevalence is also high in ranch animals (Hadigo, 1987; Molla, 1989; Bekele *et al*., 2000). The fact that infected ranches are breeding centers definitely favors the spread of brucellosis (Bekele *et al*., 2000). In addition, the distribution of F_1 heifers from the ranches would most likely exacerbate the situation in other production systems. These production units will therefore continue to spread brucellosis to other areas until effective measures are instituted which might change the situation (Molla, 1989; Bekele *et al*., 2000). However, there is no information available so far on the measures taken to combat brucellosis in Ethiopia. In Ethiopia, reliable data on human Brucellosis is lacking. Summary of values of the prevalence of Bovine Brucellosis in Ethiopia is presented in Table 3.

Table 3: Prevalence of Bovine Brucellosis in Ethiopia

Breed	Location	No. of animal tested	Prevalence %	Tests	Reference
Cross	CHE	1010	39	RBT	Meyer, 1980
Crosses	CHE	178	18.4	RBT, CFT	Gebre-mariam, 1985
Mixed	CHT	3577	2.1	SAT, CFT	Shiferaw, 1987
Mixed	Bahirdar	678	9.8	CFT	Hadigo, 1987
Zebu	CHE	1609	4.2	RBT	Bekele *et al*., 1989
Mixed	Arsi	2178	8.26	RBT	Molla, 1989
Mixed	Arsi	NA	7.62	SAT	Molla, 1989
Cross	CHE	NA	15	RBT, SAT	Wondimu, 1989
Zebu	CHE	NA	3	RBT, SAT	Wondimu, 1989
Cross	SE	734	15.8	RBT	Zewdu, 1989
Cross	SE	182	11.6	SAT	Zewdu, 1989
Cross	CHE	NA	38.7	RBT, SAT	Rashid, 1993
Cross	CSF	182	22	RBT, SAT	Sintaro, 1994
Cross	AASUR	950	8.1	RBT, CFT	Asfaw *et al*, 1998
Zebu	EANRS	3644	1.8	RBT	Kebede, 1999
Mixed	SE	4243	4.9	RBT, CFT	Bekele *et al*., 2000
Zebu	ANRS	NA	8.23	RBT	Mekonnen, 2001

NA= not avialable

3. MATERIALS AND METHODS

3.1. Study Materials

3.1.1. Study area

The study area, Jimma zone, is located about 335 km southwest of Addis Ababa at 7^0 13′ and 8^0 56′N latitude and 35^0 52′and 37^0 37′E longitudes. Altitude ranges from 880 meter above sea level in the Omo valley to 3360 meter above sea level at May Gudo in Nadda-Dedo mountain chains. The zone has 13 administrative districts. The annual rainfall of the zone is 1637 mm in average and the temperature ranges from mean minimum annual temperature of 11.43 ^{0}C to mean maximum annual temperature of 26.2 ^{0}C with a general feature of short and long rainy season shifting with dry season that indicate the area to have bimodal pattern of rainfall.

Agro-ecologically, the zone is divided into three: high altitude (*dega*) 16%, mid altitude (*weinadega*) 62%, and low altitude (*kola*) 22%. There are three types of soils in the zone: Orthic Aerosols (49%), Dystric Nithosols (38%) and Vertisols (13%). The zone has remarkable potential of surface water and expected to possess dependable source of ground water. It is bounded with three major rivers: Ghibe, Gojeb, and Deddessa, which have a numerous dendrites like tributaries that drain to the rivers.

Coffee is the major cash crop but there are also other crops of minor importance including mainly maize, teff, wheat, barely, and sorghum. Seven districts are engaged in coffee – crop – livestock type of agricultural production system: Mana, Gomma, Limmu-Seka, Limmu-Kossa, Gera, Seka-Chokorsa, and Kersa. The rest six districts are known for their mixed crop – livestock agricultural production system (Omo –Nadda, Dedo, Sokorru, Tiro – Afeta, Sigmo, and Setema).

Five districts and excluding Jimma town were selected by clustering sampling. These were Mana, Kersa, Seka-Chokorsa, Limu-kosa and Dedo (Fig 4). All Five districts were located at various km distances from Jimma town.

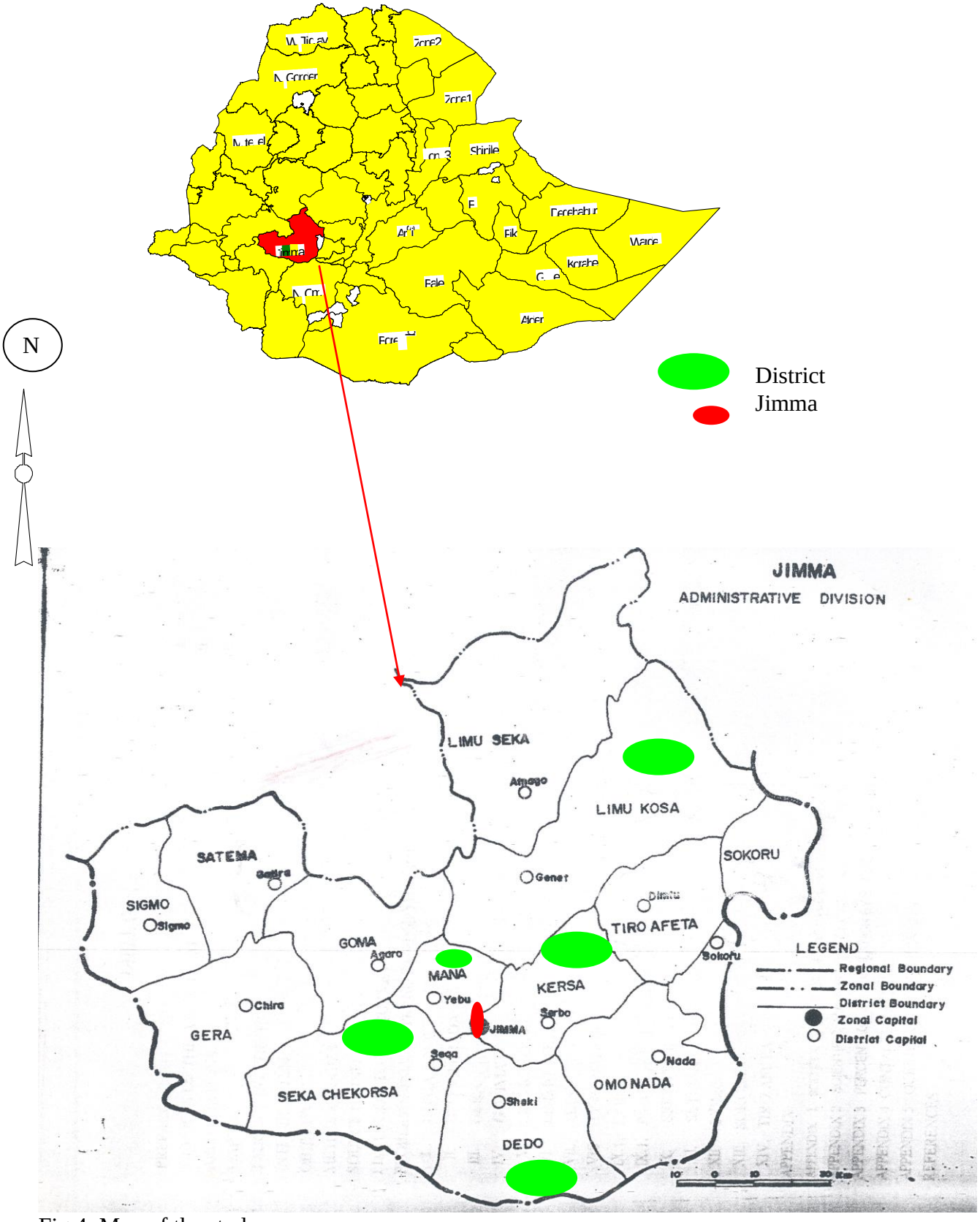


Fig.4: Map of the study area

3. 1. 2. Study population

The cattle population in Jimma Zone is the study population. The zone has 983,101 cattle and the distribution of cattle population in study areas is indicated in Table 4. Mixed crop- livestock, extensive system is the main production system practiced in the area. Communal grazing and watering, poor shelter, under feeding, etc., are livestock management problems, which could have their own share effect as determinant for various animal diseases. All types of livestock species are being reared in the zone. Of all species, cattle are the predominant in the area. Cattle are used as assets and are the only source of traction power besides milk and meat. There are about 1200 cross bred cattle distributed in Jimma town and its surroundings (Office of Planning and Economic development of Jimma zone, 2002).

Table 4: Cattle population of the selected districts.

District	**Cattle population**
Kersa	179441
Seka-Chokorsa	115069
Dedo	91226
Limu kosa	120000
Mana	31315
Jimma town	1200
Total	**538251**

3. 2. Study Methodology

3. 2. 1. Study design

A cross-sectional epidemiological study was conducted between October 2003 and April 2004 to estimate the overall prevalence, herd prevalence, and within herd prevalence of brucellosis. The prevalence was determined in respect to risk factors: age, sex, parity, herd size, district, and management system.

3. 2. 1. 1. Sampling Procedure

Multi stage sampling procedure was followed to select study animals. The numbers of animals to be sampled from each district (excluding Jimma town) were determined by the proportion of the cattle population existing in each district. From each district, three peasant associations (PA's) were taken randomly. The number of animals to be sampled from each PA was also determined by the proportion of the livestock population within the PA. From each PA two villages were taken which made a total of 30 villages included in the sample. A total of 270 herds with an average herd size of 5 cattle (ranging from 2 to 10), were sampled randomly. In addition, 46 farms owning mainly crossbred dairy cattle were selected randomly and included in the sample from Jimma town. All animals above six months of age kept for breeding purpose were sampled from the selected herds and farms. The total sample size was determined using the formula for simple random sampling technique (Thrusfield, 1995) and with an estimated *Brucella* infection rate of roughly 4%, a precision level of 1% and a 95 % confidence interval.

A total of 1813 cattle from the study site were sampled to determine the prevalence of the disease under investigation (Table 5). All animals under individual ownership, or management were handled as one herd. The herd was categorized into four classes: cows, calves, bulls, and heifers. Each class was defined as follows.

Cow: - female cattle after first calving.

Calf: - cattle of both sexes above 6 months but under one year of age.

Heifer: - female above one year of age but has not calved.

Bulls: - un castrated male cattle above one year of age.

Blood sample was collected from four different groups assumed to be at risk and a total of 126 individuals were included in the sample by purposive sampling. These include abattoir workers, butchers, farmers and animal health workers. 56 farmers patients came to Jimma hospital and diagnosed with fever of unknown origin, 38 participants abattoir workers from Jimma abattoir, 25 butchery men from Jimma town and 7 animal health workers from different districts in the zone were considered in this study.

Table 5: The number of animals sampled from each districts

District	Number of animal sampled
Kersa	449
Seka-Chokorsa	309
Dedo	172
Limu kosa	320
Mana	55
Jimma town	508
Total	**1813**

3. 2. 1. 2. Measurements

3. 2. 1. 2. 1. Questionnaires

A questionnaire was designed to collect information on factors that are believed to influence the spread and prevalence of *Brucella* infection. These include herd size and composition, management system, age of the animal, purchase source and replacement dairy cattle, handling of animal product and handling of calving/abortion. With regard to the public health significance of the disease, the presence of symptoms suggestive of brucellosis in humans (fever, sweat, anorexia, malaise, weight loss, depression, headache and joint pains), the habit of consumption of un-pasteurized milk, contact with aborted animals or abortion materials and handling of parturient animals were considered. The questionnaire was administered in person to individuals (annex 1).

3. 2. 1. 2. 2. Collecting and handling of blood

Approximately 10 ml of blood sample was collected from the jugular vein of each animal using plain vacutainer tube and needle. Each sample from each animal was labeled by using codes describing the specific animal and herd/ farm. The tubes were set tilted on a table overnight at a room temperature to allow clotting. Next morning, the clotted blood in the tubes was centrifuged to obtain clear serum. The obtained serum was stored at -20 ^{0}C until they were tested by both Rose Bengal PlateTest and Complement Fixation Test. About 5 ml of blood was collected from

each person in evacuated plain vacutainer tubes and the blood samples were left over night to clot and the sera taken and stored at -20 ^{0}C until tested. The questionnaire survey and blood sampling in humans was carried out by professional teams from Jimma Specialized Hospital and Jimma Health Center.

3. 2. 1. 2. 3. Serological Tests

❖ Rose Bengal PlateTest

The RBT antigen was obtained from INSTITUT POURQUIER 325, rue de la galéra 34097 MONTPELLIER CEDEX 5, France. The method prescribed by BgVV Service Laboratory (2000) was followed to undertake RBT. The test was undertaken at Faculty of Veterinary Medicine, Department of Microbiology, Debre zeit.

Test Procedures:

Sera (control and test sera) and antigen for use were left at room temperature for half an hour before testing, since active materials straight from the refrigerator react poorly

1) 30 µl serum was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter.
2) The antigen and serum were mixed thoroughly using an applicator stick (a stick being used only once)
3) The plate was rocked by hand for about 4 minutes
4) The tests were read by examining for agglutination in a good light
5) Magnifying glass was used to detect micro agglutination when suspected

The interpretation was performed as follows:

0 = no agglutination
+ = barely perceptible
++ = fine agglutination, some clearing
+++ = coarse clumping, definite clearing

Those samples identified with no agglutination were recorded as negative those with +, ++, +++, ++++ were recorded as positive.

❖ Complement Fixation Test (CFT)

In the CFT, all reagents were evaluated by titration. The preparation of sheep red blood cells (SRBC), the methods of CFT test, and preparation of reagents were according to the protocol of BgVV Service Laboratory (2000). The CFT test was conducted at the National Veterinary Institute, Department of Immunology, Debre Zeit.

i. Preparation of SRBC for hemolytic system

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

ii. Amboceptor titration

1. Two rows of 5 test tubes each were arranged on a rack
2. In two other test tube, 1: 500 and 1: 750 prediluted were made
3. 1ml of 1: 500 prediluted amboceptor was transferred to the first test tube of row 1 and 1 ml of 1: 750 prediluted amboceptor was transferred to the first test tube of row 2
4. 0.5 ml VBD was added to each of the rest of tubes of both row
5. Amboceptor was then diluted serially from tube 1 to tube 5 in 0.5 ml amount in both rows. Thus the dilution ran from 1: 500 to 1: 8000 and 1: 750 to 1: 12000 in row 1 and row 2, respectively
6. To each tube of the two rows, 1 ml of VBD was added
7. Following, 0.5 ml of 2 % SRBC was added to each test tubes of the two rows and were shacked well
8. The tube were left on the table for 10 minutes
9. 1 ml of complement at working dilution was added and incubated at 37 ^{0}C for 30 minutes

10. The last tube showing complete hemolysis, minimum hemolytic dose (MHD) was read. The working dilution of amboceptor is 4 times MHD (BgVV Service Laboratory, 2000)

iii. Evaluation of complement

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

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

iv. Antigen titraion

Micro titer plate I:

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

Micro titer plate II

1. 50µl VBD was added in all wells

2. 50µl of prediluted inactivated positive control serum was added to all wells of column 1
3. 50 µl was serially transferred by two fold dilution, from column 1to column 2, and again from column 2 to column 3, until column 11 from where 25 µl was discarded (column 12 had only VBD)

Mix plate I and II

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

The interpretation was performed as follow:

The last wells with 50 % sedimentation was read and recorded. This was regarded as the right corner value. In this case, the corner value was 1: 25 dilution and was used through out the test. The 50% sedimentation was taken as one unit and the working dilution of the antigen was two units.

Test procedure:

1. The sera were pre diluted at 1:2.5 and incubated at 58 ^{0}C in a water bath for 30 minutes in order to inactivate the native complement
2. 25 µl of diluted test sera was placed in wells of first and second rows of U-bottom plate, and 25 µl of veronal buffer was added to all wells except those of the first row
3. Serial doubling dilution were than made by transferring 25 µl volumes of serum from 2nd row on wards continuing for at least four dilution
4. 25 µl of antigen diluted to working dilution excluding those of anticomplemetary controls, which received 25 µl VBD was added to all wells
5. 25 µl of complement (1: 40 working dilution) in working dilution was added to all wells except control wells

6. Control wells containing: serum control has serum + complement + diluent + and antigen control has antigen + complement + diluent. Complement control has complement + diluent and hemolytic system has diluent set up to contain 75µl total volume in each case before hemolytic system was added
7. The plates were incubated for 30 minutes at 37 ^{0}C with agitations (warm fixation)
8. 25 µl of 2 % SRBC and amboceptor (hemolytic system)mixture was added into all the wells
9. Plates were sealed with sealing tape and placed on a shaker) and incubator (37 ^{0}C) for 30 minutes
10. Before reading the result the plates were left in the refrigerator at +4^{o}C for one hour in order to allow non lysed cells to settle
11. Plates were taken out from refrigerator and results were read after being left on the table for 10 minutes at room temperature
12. Positive reactions were indicated by the absence of hemolysis, sedimentation of SRBC, and negative reactions by the hemolysis of SRBC

The interpretation was performed as follow:

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

3. 2 .1. 2. 4. Data analyses

The total prevalence was calculated based on the RBT+/CFT positive results; by dividing the number of RBT+/CFT positive animals by the total number of animals tested. The within herd prevalence was calculated by dividing the number of RBT+/CFT reactors within a herd by the number of serum samples tested in that herd (Thrusfield, 1995).

The Fisher's exact test was applied to test the existence of associations between seropositivity and risk factors such as age, sex, parity, herd size, district and management system in cattle and was used to determine the association between infection rate and contact with animals in humans.

In addition, logistic regression analysis was used to calculate odds ratio (OR) to measure the degree of association between risk factors and the disease in cattle and humans using computer program STATA 7 (2001), stata cooperation 4905, Lake way Drive college station, Texas 77845, USA.

4. RESULTS

4. 1. Questionnaire surveys

Questionnaires were administered to 23 intensive and 40 extensive farm owners. The questionnaires addressed a number of aspects of management and husbandry practices, housing conditions, educational status of the farmers and breeding status of the study animals. History of abortion was taken during blood collection for all cows. About 14% and 19.6% of the cows in the intensive and extensive farms, respectively, were reported to have had previous abortions (Table 6).

Table 6: Summary of proportional distribution of dairy cattle by reproductive status and abortion Prevalence

Breeding status	Intensive farm	Extensive farm	Total
Proportion of lactating cows	191/289 (66.1%)	316/671 (47.1%)	507/960(52.8%)
Proportion of pregnant cows	145/289 (50.2%)	192/671 (28.6%)	337/960(35.1%)
History of abortion	31/222 (14%)	77/393 (19.6%)	108/615 (17.6%)

n= number of farms

4. 1. 1. Farm characteristics

Fifty-four percent of the farmers started farming by purchasing animals and 36.5% by inheriting from parents or gifts from relatives. In addition, 82.5% had acquired skills of keeping animals from their parents and 17.5% had initial training from Ministry of Agriculture and development agents.

Of the 63 farmers studied, 63.5% of them in the area had only primary education while 36.5% had formal education above 8^{th} grade. About 60% of the intensive farmers had completed 12^{th} grade while none from the extensive farmers had reached this level (Table 7).

Table 7: Summary of proportional distribution of educational status of farmers, farm management and husbandry practices in the study areas

Management activities	Intensive farms (n=23)	Extensive farms (n=40)	Total
Educational status of farmers			
< 8th grade	4 (17.4%)	36 (90%)	40 (63.5%)
> 8th grade	19 (82.6%)	4 (10%)	23 (36.5%)
Total	23 (100%)	40 (100%)	63 (100%)
Mating practice			
Use of natural mating	5/23 (21.7%)	40/40 (100%)	45/63 (71.4%)
Use of AI	5/23 (21.7%)	-	5/63 (8%)
Use of AI +natural mating	13/23 (56.5%)	-	13/63 (20.6%)
Awareness on brucellosis			
Yes	8/23 (34.8%)	2/40 (5%)	10/63 (15.9%)
No	15/23 (65.2%)	38/40 (95%)	53/63 (84.1%)
Presence of parturition pens	5/23 (21.7%)	2/40 (5%)	7/63 (11.1%)
Separation of cows during parturition	6/23 (26.1%)	5/40 (12.5%)	11/63 (17.5%)
Cleaning and disinfection of premises	15/23 (65.2%)	5/40 (12.5%)	20/639 (31.7%)
Proper disposal of after birth			
Yes	19/23 (82.6%)	5/40 (12.5%)	24/63 (38.1%)
No	4/23 (17.4%)	35/40 (87.5%)	39/63 (61.9%)
Stock replacement strategy			
Out side source	11/23 (47.8%	20/40 (50%)	31/63 (49.2%)
Own farm	8/23 (34.8%)	5/40 (12.5%)	13/63 (20.6%)
Out side source and own farm	4/23 (17.4%)	15/40 (37.5%)	19/63 (30.2%)
Culling animals for various reasons	23/23 (100%)	25/40 (62.5%)	48/63 (76.2%)

n= number of farms

It was also found out that all the farmers in the extensive management system were dependent on natural mating using bulls while nearly half of the farmers in the intensive management system were using both AI and natural mating. Equal proportions of the remaining farmers in the intensive management system were using either AI or natural mating. Slightly more than half of the farmers

in the intensive management system were not aware of brucellosis while nearly no farmers in the extensive management system were aware. The practices of provision of separate parturition pens, separation of cows during parturition and cleaning and disinfection of contaminated areas was done in a relatively better way by farmers in the intensive management system. In addition, most of the farmers in the intensive management system were disposing afterbirth and abortion materials properly while few farmers in the extensive management system were doing so. Nearly half of the farmers in the extensive and intensive management system were dependent on external sources for replacement stock and culling was practiced by all farmers in the intensive management system and more than half of the farmers in the extensive management system.

4. 2. Seroprevalence of brucellosis

Of 1813 sera tested using RBT, 17 (0.94%) animals reacted positively to brucellosis. These reactors were further retested using CFT and 11 (0.61%) animals were confirmed to be seropositive for brucellosis (Table 8). The highest prevalence rate was found in Limu-Kosa district (2.5 %). All male tested were negative for *Brucella* antibody.

Table 8: Results of RBT and CFT for brucellosis by study districts

		RBT	CFT*
District*	N	Number (%) positive	Number (%) positive
Kersa	449	3 (0.67%)	0 (%)
Seka chokorsa	309	2 (0.65%)	0 (%)
Limu Kosa	320	9 (2.8%)	8 (2.5%)
Dedo	172	2 (1.16%)	2 (1.16%)
Mana	55	0 (0%)	0 (0%)
Jimma town	508	1 (0.2%)	1 (0.2%)
Total	1813	17 (0.94%)	11 (0.61%)

N= number of animals tested, *= Fisher's exact test, P<0.001

4. 3. Seroprevalence at herd level in the extensive management system

The overall herd prevalence, based on RBT+/CFT, was 2.96%. The prevalence were established only in two districts: viz, Limu kosa (12.5%) and Dedo (2.8%) (Table 9). The herd seroprevalence in farms that kept more than five animals were found to be 14.5% (8/55) while it was nil in farms keeping less than five animals. This difference was statistically significant ($p<0.001$). The within herd prevalence recorded was ranging from 0% to 33.3%. One or two reactors were recorded in the seroposirtive herds.

Table 9: Seroprevalence of brucellosis at herd level in districts practicing only extensive management system

District*	NF	N	CFT	CFT
			Number (%) of positive animals	Number (%) of positive farms
Kersa	92	449	0 (0%)	0 (0%)
Seka chokorsa	72	309	0 (0%)	0 (0%)
Limu kosa	56	320	8 (2.5%)	7 (12.5%)
Dedo	36	172	2 (1.16%)	1(2.8%)
Mana	14	55	0 (0%)	0 (0%)
Total	270	1305	10 (0.77)	8 (2.96%)

N= number of animals tested, NF= number of farms tested, *=Fisher's exact test, $P<0.05$

4. 4. Seroprevalence at individual animal level in extensive management system

The individual animal seropositivity was established by using the RBT positive sera with CFT positive serial serological test results. The overall individual seropositivity in the study area was established at 0.77%. Prevalences were compared between study sites; the prevalence for Dedo, and Limu-kosa were 1.16%, and 2.5% respectively. No reactor animal was detected from Kersa, Mana, and Seka-Chokorsa districts. The difference between districts was statistically significant ($P<0.05$).

A logistic regression analysis revealed that positive reactors were significantly higher ($p<0.05$) in older age category than younger ages (Table 10). The seroprevalence was 1.1 % in animals between 3-6 years and 1.6% in animals above 6 years. The seroprevalence of brucellosis by age category showed that there is strong linear trend (Fig 5).

Table 10: Seroprevalence of brucellosis according to risk factors (sex, age, herd size) in extensive management system

		CFT			
Risk factors	N	Number (%) of positive animals	CI (95%)	p-value	OR
Sex					
Male	273	-	0-1.3	0.1	-
Female	1032	0.97% (10)	0.5-1.8		
Age					
0.5-<3	489	-	0-0.8	0.017**	3 (1.2-7.7)
>3-6	566	1.1% (6)	0.4-2.3		
>6	249	1.6% (4)	0.4-4.1		
Herd size					
1-5	948	0 (0%)	0-0.4	0.000***	6.7 (2.1-9.4)
>5	357	10 (2.8%)	1.4-5		
Total	1305	10 (0.77 %)			

N= number of animals tested, CI= confidence interval, OR= odds ratio

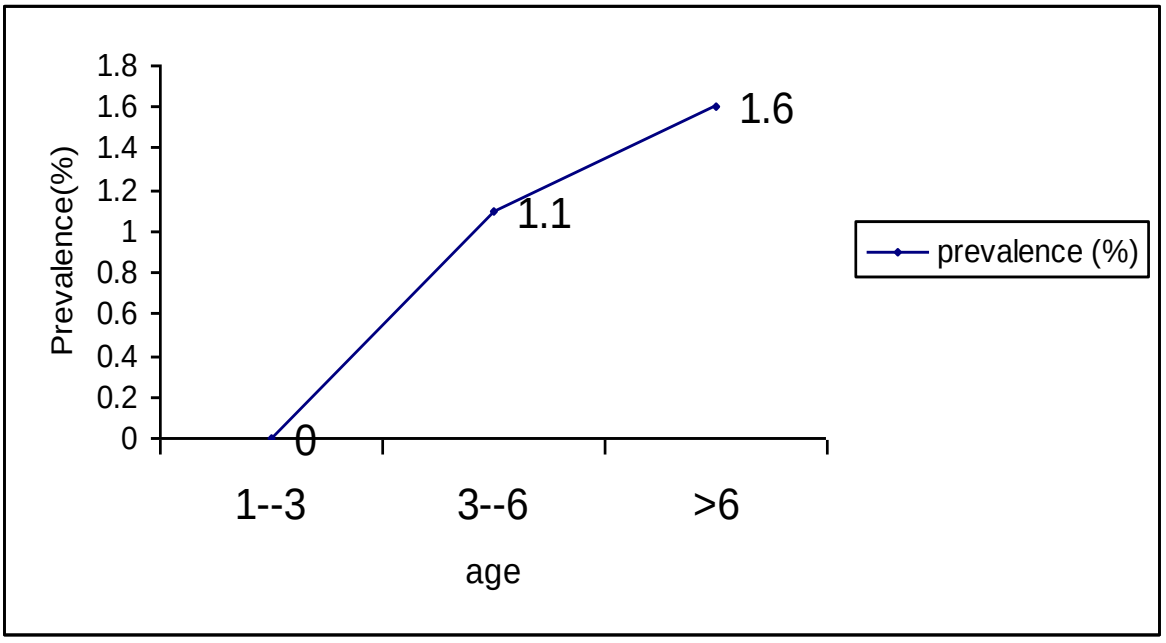
In addition, herd size had significant effect on the prevalence of brucellosis in individual animals ($p< 0.001$). All infected animals were in herds with more than five animals in the extensive management system and no infection was established in herds keeping less than five animals (Table 10).

Table 11. Multivariate logistic regression estimates for risk factors in extensive management system

Risk factors	CI	Odds ratio	P-value
Sex	0.08-6.86	0.75	0.800
Age	1.23-7.40	3	0.016
Herd size	3.98-242.1	31	0.001

The results of multivariate logistic regression indicated that age ($p < 0.05$) and herd size ($p < 0.01$) affected significantly of bovine brucellosis in the extensive management system. This result was not different from that of the univariate analyses (Table 11).

Fig. 5: Association between age category and *Brucella* seropositivity in individual animals in extensive management system



4. 5. Seroprevalence at herd and individual animal level in intensive management system

The overall herd prevalence, based on RBT+/CFT in the intensive system was 2.2 % (1/46). The within herd prevalence established was 4.5 %. The overall prevalence in individual animals was 0.2 %. Positive reactors were recorded in farms keeping more than ten animals (0.3 %) (Table 12). A logistic regression was run to determine the association between risk factors including sex, age and herd size but none of the factors considered were significantly associated ($p > 0.05$) with the seroprevalence of brucellosis in the intensive management system (Table 13). However, it was difficult to draw firm conclusion from these results since small numbers of animals have been screened from the intensive farms. This is because the numbers of intensive farms in Jimma town were small.

Table 12: Seroprevalence of brucellosis in intensive management system in Jimma town

Farm category	N	Number (%) positive
Small scale	62	0 (0%)
Medium	76	0 (0%)
Large	370	1(0.3%)
Total	508	1(0.2%)

N= number of animals tested

Table 13: Seroprevalence of brucellosis according to risk factors (sex, age, herd size) in intensive management system in individual animals

Risk factors	N	CFT		
		Number (%) of positive animals	CI (95%)	p-value
Sex				
Male	75	0	0-4.8	0.7
Female	433	2.35% (1)	0.006-1.3	
Age				
0.5-<3	231	0	0-1.6	0.6
>3-6	180	5.6% (1)	0-3.7	
>6	97	0	0	
Herd size				
1-5	62	0	0-5.8	0.8
5-10	76	0	0-4.7	
> 10	370	0.3% (1)	0.007-1.5	

N= number of animals tested, CI= confidence interval

4. 6. Reproductive status and brucellosis infection rates in extensive management system

Odds ratio (OR) was calculated to measure the likely association that could exist between reproductive status and brucellosis. Significant association was found between brucellosis and occurrence of previous abortion ($p< 0.001$). However, factors including pregnancy status, lactation status and parity were not significantly associated with the prevalence of brucellosis. Although no significant association ($P>0.05$) was observed between Brucella seropositivity and parity, majority of the positive animals were in the cows with 2nd parity compared to those in their 1st parity (Table 14).

Table 14: Association between reproductive status and brucellosis prevalence

Risk factors	N	CFT			
		Number (%) of positive animals	CI (95%)	p-value	OR
Pregnancy status					
Pregnant	192	1.6% (3)	0.4-1.9	0.8	1.2(0.4-3.4)
Non-pregnant	763	0.92%(7)	0.3-4.5		
Lactation status					
Lactating	316	1.3% (4)	0.4-3.2	0.6	1.4(0.4-4.8)
Non-lactating	639	0.94% (6)	0.3-2		
Parity					
0	318	0	0-1.2	0.06	2.9(1-8.9)
1	138	1.4% (2)	0.2-5		
2	496	1.6% (8)	07-3		
3	2	0	0-8.4		
Previous abortion					
Yes	77	8% (6)	2.95-16.4	0.000***	18.7 (5.2-67.8)
No	878	0.5% (4)	0.1-1.2		

N= number of animals tested, CI= confidence interval, OR= odds ratio

4. 7. Occurrence of brucellosis in human

A total of 126 individuals (100 males and 26 females) were interviewed and tested. These were 30.2% abattoir workers, 19.8% butchers, 44.4% farmers, and 5.6% animal health personnel. Of the 126 serum samples screened by RBT 4 (3.2%) were positive for brucellosis. The RBT+ sera were further retested by CFT and three (2.4%) were found seropositive for *Brucella* antibody (Table 15). All the positive reactors were males. High seropositivity (14.3%) was observed in animal health workers. Seropositity of 3.4 % was also recorded in farmers. However, there was no positive reactor from butchers and abattoir workers included in this study.

Table 15: Seroprevalence of brucellosis in different groups of people in the study areas assumed to be at high risk for *Brucella* exposure

Groups	Number of Samples examined	Number (%) of Positive individuals
Abattoir workers	38	0 (0%)
Butchers	25	0 (0%)
Farmers	56	2 (3.4%)
Animal health workers	7	1(14.3%)
Total	126	3 (2.4%)

A further analysis to determine the association between selected risk factors and prevalence of brucellosis indicated that there was no significant association between seroprevalence and risk factors including drinking raw milk, consumption of milk products and animal contacts. Strong statistical association ($P<0.05$) was recorded between *Brucella* seropositivity and handling of parturient animals (Table 16).

Table 16: Association of *Brucella* seropositivity with selected risk factors

Risk factors	p-value
Drinking raw milk	0.2
Consumption milk product	0.5
Animal contact	0.7
Handling parturient animals	0.01*

* Fisher's exact test

5. DISCUSSION

Most of the respondents (71 %) in this study were using bulls for service. All these respondents were from the extensive management system and had no other options, while 21 % of the farmers in the intensive management system had the opportunity to use artificial insemination. In addition, most of the respondents (61.9%) did not bury afterbirth and aborted fetus and aborted materials rather left them on the ground or given to dogs. Most of the farmers were not also separating cows during parturition. These factors combined with the unawareness of most of the farmers on brucellosis and the poor cleaning and disinfection practice by farmers could pose a great risk of spread of the disease to unaffected animals. The dependency of most of the farms on outside sources for stock replacement could be one possible way of introduction of the disease into unaffected herds. According to the observation made, out of 77 female animals in the extensive management system with abortion history 6(8 %) of them were detected positive for brucellosis; on the other hand none of the animal from 31 cross breed cows with history of abortion were detected positive for brucellosis. It has been reported that numerous infectious and non- infectious agents can cause fetal loss or abortion in cattle (Radostits *et al*., 2000). Besides, abortion rate in infected herds is dependent on many factors and varies according to the susceptibility of the pregnant females, management practices, the severity of the challenge, the period for which the herd has been infected and various environmental factors (Bishop *et al*., 1994).

Diagnosis of bovine brucellosis depends on isolation and identification of the causative agent or demonstration of specific antibody using serological tests. However, pathogen identification has proven too complicated, time-consuming and expensive for mass studies (Weidman, 1991). The RBT was used in this study because it is fast, sensitive, easy, and allows processing many samples per day (PAHO/WHO, 2001). Those sera testing positive with RBT were further retested by CFT for confirmation (Dohoo *et al*., 1986) since such serial testing procedure maximizes the specificity of the tests. The specificity of the CFT has been shown to approximate 100 % in unvaccinated population (Dohoo *et. al*., 1986). An animal was regarded as sero positive if it was positive by both the RBT and CFT.

The overall sero prevalence of brucellosis in individual animals was 0.61 % (n=1813) in the zone in both management system. Different findings on serological prevalence of brucellosis have been reported for the last 24 years from different corners of Ethiopia. Most of the works reported so far were from southeastern and central highland and with few reports from northern parts of the country. Much higher seroprevalence were reported by Meyer (1980) for cattle owned by the then Institute of Agricultural Research (IAR) (39 %), Gebre-mariam (1985) from four crossbred dairy farms around Addis Ababa (18.4 %) by using RBT and CFT, Zewdu (1989) in Sidamo region (15.8 %), Molla (1989) in different breeds of cattle (indigenous and crossbred) from Arsi (7.62 %) by using SAT, Bekele *et al*. (1989) from indigenous zebu cattle in central Ethiopia (4.2%) , Sintaro (1994) from Chaffa State Dairy Farm (Wollo) (22%), and also in ranch animals (Hadigo, 1987; Molla, 1989; Bekele *et. al*.; 2000). Closer values of prevalence were reported by Kebede (1999) who found an overall prevalence of 1.8 % from Eastern Amhara Region (ranging from 0.2 % in the highlands to 3 % in the lowlands). The prevalence in this study was in agreement with the findings of Tesfaye (2003) who reported an overall prevalence of 0.69 % in cattle in extensive and intensive managements in Tigray region and slightly higher than the finding of Yayeh (2003) who reported an overall prevalence of 0.14 % in cattle in extensive and intensive managements in selected areas of North Gondar Zone, Ethiopia.

The difference in prevalence observed between the reports from different parts of Ethiopia and the present study may be due to differences in management and husbandry condition in the area and the intensity of dairy production, which is not to a higher degree in the present study area. There can also be differences between the study areas regarding conditions that could facilitate the rate of transmission of the disease (Radostits e*t al*., 2000).

Higher prevalence were recorded specially for two districts in Limu-Kosa (2.5 %), and Dedo (1.16 %). This could be partly related to the existence of a ranch in the area (Limu-Kosa) which had been closed some time before due to health problems encountered in the ranch herd suspected to be brucellosis.

In the present study, males were non-reacting for both tests. This observation was in agreement with the work of Tesfaye (2003) and Yayeh (2003) who reported only female reactors. Sex has been one of the risk factors affecting susceptibility of cattle to *Br. abortus* infection (Radostits *et al*., 2000). It is well known that female cattle are more susceptible to *Brucella* infection than

males. The probable reason could be the preferential growth of *Brucella* organism in gravid uterus especially if it is pregnant than in testes (Roberts, 1971).

A relatively higher seroprevalence observed, in this study, in the extensive management system could be partly explained by the fact that contact between animals increases in communal grazing practices which was the predominant feeding system in the extensive type of management. In such circumstances, cattle of unknown disease status might mix and often grazed together and resulted in spreading and transmission of disease among herds. About 85 % of the farms in the study area shared the communal grazing system. It has also been indicated that free grazing which allows unrestricted contact between animals may have contribution to the spread of brucellosis in extensive management system (Silva *et al.*, 2000). In addition, the observation made by Kagumba and Nandokha (1978) indicates that the prevalence of brucellosis was higher in communally grazed large herds of cattle. The same observation made by Maiga (1996) indicated that antibodies against *Brucella* were more prevalent among animals in rural concessionary than village herds of communal pen production systems. In general, the patchy distribution of brucellosis in the zone where some herds were free of the disease, while others had a high prevalence of brucellosis as observed in this study could be explained by the fact that some communal herds have been kept closed replacement animals from own herds whereas other cattle owners of the positive reactor herds may have purchased infected animals to replace their stock.

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

In this study, significantly higher seroprevalence was observed in older age category than younger age category. This observation is in agreement with that of Oloffs *et al*. (1998) in which

52 % of the seropositive cows were older than 6 years. Bekele *et al*. (2000) and Tesfaye (2003) also reported similar results that indicate a higher prevalence in animals above six years of age. It has been reported that susceptibility of cattle to *Br. abortus* infection is influenced by age of the individual animal (Radostits *et al*., 2000). Younger animals tend to be more resistant to infection and frequently clear infections, although latent infections do occur (Walker, 1999). Sexually mature and pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex (Radostits *et al.,* 2000).

In this study, seroprevalences were higher in larger herd sizes. This observation is in agreement with the previous findings of other authors. Asfaw *et al.* (1998) reported a higher prevalence of brucellosis in larger herds (9.1%) than smaller herds (3.3%). It was also indicated by Walker (1999) that herd size and animal density are directly related to prevalence of disease and difficulty in controlling infection in a population. In addition, Hellmann *et al.* (1984) indicated that herds of bigger size had higher seroprevalence of bovine brucellosis as compared to smaller herds. In larger herd sizes, the disease spreads by several modes of transfer, especially through contact with infected discharges from dam and its fetus. One possible explanation for the high prevalence of the disease in larger herds is that larger herd sizes are often maintained by the introduction replacement stock from outside sources and was proved to be a common practice by this study. It is also undeniable fact that the spread of the disease from one herd to another herd from one area to another is almost frequently due to the movement of an infected animal from an infected herd to a non-infected susceptible herd (Radostits *et al.,* 2000). Abela (1999) indicated that large herds and herds with small ruminants were most at risk to brucellosis infection. Thus, brucellosis should never be viewed as the disease of individual animals, but should be considered in the context of herd and also the animal population in the region.

An overall prevalence of 17.6 % was recorded for the occurrence of previous abortion in the study area. Cattle with history of abortion were also found to be at higher odds of being sero positive compared to those without history of abortion. Furthermore, the abortion rate in extensive management system, where there was a higher prevalence of the disease, was 19.6 %, which is higher than that of the intensive system. These all indicate the very close association between abortion and the prevalence of brucellosis. In highly susceptible non-vaccinated pregnant cattle, abortion after the 5^{th} month of pregnancy is cardinal feature of the disease (Radostits *et al.,* 2000). The finding regarding the prevalence of abortion in this study is higher

than that of Tesfay (1996) who reported a prevalence of 6.1% in Mekele dairy cattle and Yayeh (2003) who also reported a prevalence of 6.7% in North Gondar, Ethiopia.

An overall brucellosis prevalence of 2.4 % was recorded in human beings in this study. Animal health personnel and farmers accounted for 14.3% and 3.2%, respectively. It is not surprising to get people infected with brucellosis in an area where there are infected animals since the prevalence of brucellosis in man is largely influenced by the prevalence of disease among domestic animals around him (Weidmann, 1991; Omer *et al.,* 2002). Human beings become infected when there is indirect contact with cows at abortion, parturition, or in the post parturition period from splashing of infected droplets into the eyes (Sewell and Brocklesby, 1990) or drinking unpasteurized milk or milk products (Roberts, 1971). Brucellosis is an occupational disease, occurring most often in veterinarians, farmers, stock inspectors, abattoir workers, laboratory personnel, butchers (Sewell and Brocklesby, 1990; Staak, 1990; Bishop *et al.,* 1994). The disease is often treated as fever of unknown origin, and frequently misdiagnosed as other common febrile diseases such as malaria and typhoid fever. In present study, strong association was seen between *Brucella* seroprevalence and handling of parturient animals. A report from Saudi Arabia indicated that more cases of human brucellosis occurred in rural areas where most of the people are farmers or in close contact with animals (Alballa, 1995). In Ethiopia, so far there was no report on human brucellosis except fragments of information from Yirgu (1991) who reported a prevalence of 12.5 % from eight herdsmen at Abernosa using CFT. The possible explanation could be given from this finding is that both farmers and animal health personnel were infected while helping infected cows during parturition either through abrasions or the conjunctiva. Similar report from Walker (1999) indicated that humans acquire infections by handling tissues containing *Brucella* organisms. Similar observation was made by Staak (1990) that man contracts brucellosis either by handling infected animals or by living in a highly infected surrounding.

6. CONCLUSIONS AND RECOMMENDATIONS

Results of the present study revealed that bovine brucellosis is prevalent in the Jimma Zone of Oromia Region, Western Ethiopia, although it is much less than the figures of previous reports from southeastern and central part of the country. The finding of positive serological reactors does not only suggest the presence of the disease in the cattle population in the areas, but also indicates the presence of foci of infection that could serve as sources of infection for the spread of the disease into unaffected animals and herds. Besides, the study also showed that age and herd size are important risk factors associated with the prevalence of the infection. Moreover, the study indicated that the disease was also prevalent in human beings, who are among the high risk groups of contracting the infection. This emphasizes impact of brucellosis in public health and the need to control and prevent brucellosis in the study areas.

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

- Isolation of aborted animals and proper disposal of aborted fetuses and fetal membranes, preferably, by incineration.
- The isolation of calving animals' in separate calving pens.
- Replacement stock should be purchased from herd known to be free of brucellosis.
- Strict movement control of animal from one area to another in order to prevent the spread and transmission of the disease from infected cattle to the non-infected ones.
- Proper hygienic practices and good husbandry management should be exercised and these could in many situations minimize the spread of disease in the herd.
- Awareness creation among farmers, butchery men, abattoir workers and animal health workers about the nature and effect of the disease through informal educational channels is required.
- Unless and otherwise the reactor animals are removed from infected herds, greater percentage of the remaining animals in an infected herd and increasing number of herds in the population could acquire the infection.
- The implementation of test- and- slaughter policy with compensation payment to the farmers as the prevalence of the disease is low in the study area.

7. REFERENCES

Abela, B. (1999): Epidemiology and control of brucellosis in ruminants from 1968 to 1996 in Malta. *Revue Scientique et Technique de I` OIE,* v. **18** (3), Pp 684-659, 20 ref. office.

Alballa, R. S. (11995). Epidemiology of human brucellosis in southern Saudi Arabia. *Journal of Tropical Medicine and hygiene.* **98**, 185-189.

Asfaw, Y., Molla, B., Zessin Karl-Hans., Tegegn, A. (1998): The epidemiology of bovine brucellosis in intra and peri-urban dairy production systems in and around Addis Ababa, Ethiopia. *Tropical Animal Health and Production.* **46**, 217-224

Bakunzi, F.R., Zyambo, N.C.G., Morris, S.D. (1993): A serological survey of bovine brucellosis in the Molopo district of Botswana. *Journal of the South African Veterinary Association* **64**(4), 154-155.

Bekele, A., Molla, B., Asfaw, Y., Yigezu, L. (2000): Bovine brucellosis: sero-epidemiological study in selected farms and ranches in southeastern Ethiopia. *Bulletin of Animal Health and Production in Africa* **48**, 13-17.

Bekele, T., Kasali, O.B., Mukasa-Mugerwa, E., Scholtens, R.G., Yigzaw T. (1989): Prevalence of brucellosis in indigenous cattle in central Ethiopia. *Bulletin of Animal Health and Production in Africa* **37**, 97-98.

Bishop, G.C., Bosman, P.P., Herr, S. (1994): Bovine Brucellosis. In: Coetzer, Thomson and Tustin (eds.): Infectious Diseases of Livestock. Vol. **2.** Cape Town, RSA: Oxford University Press, pp 1053-1066.

Bureau of Planning and Economic Development for Oromia, the Office of Planning and Economic Development for Jimma Zone (2002): Statistical Abstract (1988-1992 E. C.). Jimma, Ethiopia.

Central Statistics Authority (1998): Ethiopian Livestock Estimates. Volume I. Bulletin 52. Addis Ababa, Ethiopia.

Chantal, J., and Bessiere, M.H. (1996): Serological survey of some zoonotic disease among abattoir personnel in Djibouti. *Buletin de la, societe de pathologic exotique* **89**(5), 353-357.

Chantal, J., Dorchies, PH., Legueno, B. (1994): A study on some zoonoses in Djibouti Republic. *Revue de Medicine Veterinaire* **145** (8-9), 633-640.

Chukwu, C.C. (1985): Brucellosis in Africa. Part I: The prevalence. *Bulletin of Animal Health and Production in Africa* **33**, 193-198.

Chukwu, C.C. (1987): Brucellosis in Africa. Part II: The importance. *Bulletin of Animal Health and Production in Africa* **35**, 92-98.

Dohoo, I. R., Wright, P. F., Ruckerbauer, G. M., Samagh, B. S., Robertson,F. J.,and Forbes, L. B. (1986): A comparison of five serological tests for bovine brucellosis. *Canadian Journal of Veterinary Research* **50**, 485- 493.

ESAP (1995): Ruminant livestock development strategy. *News Letter of the Ethiopia Society of Animal Production* Vol. **3**(1&2) 1995. Addis Ababa, Ethiopia.

Freeman, A.B. (1979): Burrows Text of Microbiology, 21st edition, London: W.B. Saunders .pp 671-573.

Gebre-mariam, K. (1985): The prevalence of bovine brucellosis in four different farms. FVM, AAU, Debre Zeit, DVM Thesis.

Hadigo, K. (1987): A survey of bovine brucellosis around Bahirdar. FVM, AAU, Debre Zeit, DVM Thesis.

Hellmann, E., Staak, C., Baumann, M. (1984): Bovine Brucellosis among two Different Cattle Populations in Bahr el Ghazal province of Southern Sudan. *Tropen Med.Parasit* **35**,123-126.

Hussein, S. A., Singh, S.S., Haji, H. (1978): A Survey of Bovine Brucellosis in Southern Parts of Somalia Democratic Republic. *Bulletin of Animal Health and production in Africa* **26**,150-153.

IDF. (1994): The Significance Pathogenic Microorganisms in Raw Milk. International Dairy Federation. Brussels (Belgium). 167-185.

Isselbacher, J. Kurt, Adams, D.R., Braunwald, G., Peter Sdorf, G.R., Wilson. A.J. (1980): Harrison's: Principles of Internal Medicine. 9th edition. Sydney: Graw-Hill International Book Company. 658-660.

Jiwa, S. F. H., Kazwala, R. R., Tungaraza, R., Kimera, I.S., Kalaye J. W. (1996): Bovine brucellosis serum agglutination test prevalence and breed deposition according to prevalent management systems in the Lake Victoria of Tanzania. *Preventive Veterinary Medicine* **26**,341-346.

Kagumba, M., and Nandokha, E. (1978): A survey of the prevalence of bovine brucellosis in East Africa. *Bulletin of Animal Health and Production in Africa* **26(**3), 224-229.

Kebede, F. (1999): An epidemiological survey of bovine brucellosis in Amhara National Regional State. Proceeding of 14th annual Ethiopian Veterinary Association Conference held at Addis Ababa 7th-10th June 2000.

Kubuafor, D. K., Awumbila, B., Akanmori, B. D. (2000): Sero prevalence of brucellosis in cattle and humans in the Akwapim-south distrit of Ghana: Public health implication. *ACTA TROPICA* **76** (1), 45-48.

Maiga, S., Traore, M. D., Niang, M., Troure, I. (1996): A Sero epidemiological investigation of bovine brucellosis in dairying belt of Bamako, Mali. In: Livestock production and disease of the Tropics: Livestock production and human welfare. Proceedings of the 8th international Conference, Zessin, K. H. (ed), Pp 289-289

Mekonnen, G. (2001): Sero-epidemiological investigation of bovine brucellosis in North-Eastern Ethiopia (unpublished Baherdar regional laboratory report).

Molla, B. (1989): Sero-epidemiological survey of bovine brucellosis in Arsi Region. FVM, AAU, Debre Zeit, DVM Thesis.

Nicoletti, P. (1984): The control of brucellosis in tropical and subtropical regions. *Preventive Veterinary Medicine* **2:** 193-196.

O.I.E (2000): Bovine brucellosis: Diagnostic Technique. Manual of Standard for Diagnosti Tests and Vaccines. 4th edition. Paris: Office International des Epizootics 1-37.

O.I.E. (1997): Disease risks to animal health from AI with bovine semen, bovine brucellosis. contamination of animal products: Prevention and risks for animal health. **16**(1), 271.

Oloffs,A., Baumann, O. P. M., Afema, J., Nakavuma, J. (1998): Experiences with strategy to investigate bovine brucellosis in rural area in the south west Uganda. *Revue d'Elevage et de Medicine Vétérinaire Des Pays Tropicaux* **51**(2) ,101-105.

Omer, K.M., Skjerve, E., Holstand, G., Woldehiwet, Z. (2000a): Prevalence of antibodies to *Brucella* species in cattle, sheep, goats, horses and camels in the State of Eritrea, influence of husbandry system, *Epidemol. Infect*. **125,447**-453

Omer, K. M., Skjerve, E., Woldehiwet, Z., Holstand, G. (2000b): Risk factors for *Brucella* species infection in dairy cattle farms in Asmara, State of Eritrea. *Preventive Veterinary Medicine* **46,** 257-265.

Omer, K .M., Assefaw, T., Skjerve, E., Tekleghiorghis, T., and Woldehiwet, Z. (2002): Prevalence of antibodies to *Brucella* species risk factor related to high risk occupational groups in Eritrea. *Epidemol. Infect***., 129**, 85-91.

PAHO/ WHO. (2001): Zoonoses and Communicable Diseases Common to Man and Animals. 3rd edition. V I. Bacteriosis and Mycosis. Scientific and Technical Publications. No 580. Pan American Health Organization Pan American Sanitary Bureau, Regional Office of the World Health Organization. Washington D.C. USA.

Radostits, O. M., Gay, C. C., Blood, C. D., Hinchcliff, K. W. (2000): Veterinary Medicine, Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses. 9th edition. New York: W.B. Saunders Company ltd, 867-882.

Rashid, M. (1993): Reproductive wastage in cattle due to bovine brucellosis. In: Institute of Agricultural Research (ed): Proceeding of the Fourth National Livestock Improvement Conference. Papers presented at Fourth National Livestock Improvement Conference held in Addis Ababa, Ethiopia, 13-15th November 1991. Addis Ababa, Ethiopia .270-272.

Roberts, J.S. (1971): Veterinary Obstetrics and Genital Diseases. 2nd edition. India: CBS Publisher and Distributors, 108-112.

Seifert, S. H. (1996): Tropical Animal Health. 2nd edition. Dordrechet: Kluwer Academic Publishers, 358.

Sewell, M. M. and Brocklebry, D. W. (1990): Handbook on Animal Diseases in Tropics. 4th edition. London: Bailliere Tindall. 41.

Shiferaw, A. (1987): The prevalence of bovine brucellosis under different management systems around Shoa based on serological test. FVM, AAU, Debre Zeit, DVM Thesis.

Silva, I., Dangolla, A., Kulachelvy, K. (2000): Sero epidemiology of *Brucilla abortus* infection in bovine in Sri Lanka. *Preventive veterinary medicine,* v. **46,** Pp 51-59. 22ref.

Sintaro, T. (1994): The impact of brucellosis on productivity in an improved dairy herd of Chaffa state farm, Ethiopia. Fachburg Veterinaemedizin, Frei Universitate, Berlin, Msc Thesis.

Staak, C. (1990): Serological techniques in brucellosis interpretation of results. First international conference on brucellosis. March 19-20, Mosul, Irak.

Staak, C., Salchow, F., Denzin, N. (2000): Practical Serology: From the Basic to the Testing. Bundesinstitut für Gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV): Deutsche Gesellschaft für Technische Zusammenarbeit(Gtz). München: Urban und Vogel.

Stata Corporation (2001): Intercooled Stata 7.0 for Windows 98/95NT. 702 University Drive East College Station, Texas, USA.

Tesfaye, A. (2003): Brucellosis in cattle and small ruminants in selected sites of Tigray Region, North Ethiopia. FVM, AAU, Debre Zeit, DVM Thesis.

Tesfay, G. (1996): Survey of major preparation and postpartum reproductive problems of dairy cattle in Mekele and its environ. FVM, AAU, Debre Zeit, DVM Thesis.

Thrusfield, M. V. (1995): Veterinary Epidemiology. 2nd edition. Oxford: Blackwell, Pp 183.

Walker, R. L. (1999): *Brucella*. In: Dwight C. Hirsh and Yuang Chung Zee (ED.): Veterinary Microbiology. USA: Blackwell Science Inc. 196-203.

Weidmann, H. (1991): Survey of means now available for combating brucellosis in cattle in tropics. Institute for Scientific Cooperation, Tubmgen, Georg Hauser, Metzingen, Germany. **33**, 98-111.

Weihaupl, I., Schopf, K. C., khachasi, D., Kapaga, A. M., Msami, H. M. (2000): Investigation of the prevalence of bovine tuberculosis and brucellosis in dairy cattle in Dares salaam region and zebu cattle in Lugoba area, Tanzania. *Tropical Animal health Production* **32** (3), 147-154.

Wernery, U., Karani A. A., Viertel, P. (1979): Bovine brucellosis in southern region of Somalia Democratic Republic. *Tropical Animal Health and Production* **11**, 31-35.

WHO (1986): Joint FAO/ WHO Committee on brucellosis. 6th Report. Geneva: WHO. (Technical Report Series. 740).

WHO (1997): Emerging and other communicable disease surveillance and control. The development of new/ improved brucellosis vaccines (Reports of the WHO Meetings, Geneva, December). 1-37.

Wondimu, A. (1989): The Epidemiology and economics of bovine brucellosis in the central highland of Ethiopia. VEERU, Reading University, Master's Thesis.

Yayeh, T. (2003): A survey of bovine brucellosis in selected areas of north Gondar zone, Ethiopia. FVM, AAU, Debre Zeit, DVM Thesis.

Yirgu, T. (1991): Sero prevalence study of bovine brucellosis at Abernosa. FVM, AAU, Debre Zeit, DVM Thesis.

Zewdu, E (1989): Sero-prevalence study of bovine brucellosis in selected sites of Sidamo region. FVM, AAU, Debre Zeit, DVM Thesis.

8. ANNEXES

Annex 1. Questionnaire format

Date…….. code no. ……

Farm structure

Farm owner……..

Occupation……

Educational status of the farm owner……

Address…….

Location ……..

PA …… Village……

Farm size……..ha.

Grazing land size ……. Crop land size……..

Is the farm fenced? Y/N

Annual farm income and expenses.

Annual incomes		Annual expenses	
Source	Amount in birr	Expenses for	Amount in birr

Do you have another job? Y/N
Specify job type and income……

How did you start dairy business?

a) inherited the enterprise
b) bought the enterprise
c) bought dairy animals
d) up grade the local breed
e) other……

How did you acquire skills to raise dairy cattle/farming?

a) Agricultural training (level) ……
b) From extension agents……
c) From parents……..
d) Others…….

What are your reasons for producing milk?

a) to earn a living
b) to supplement family food
c) to supplement family income
d) as a hobby
e) others

Herd size and composition of the farm.

Type of cattle	Number of animals	Remark
Lactating cows		
Pregnant cows		
Dry cows		
Heifers		
Bull		
Calves		

Which breed of cattle do you own? What is the number of cattle in each breed?

Breed of cattle	Number of cattle
Local	
25% cross	
50% cross	
More than 50%	

How is the parity status and the average calving interval of the dairy cows in your farm?

Cow identification	Parity status	Calving interval

What type of insemination do you use for your animals?

a) AI
b) Natural service
c) Both

How is the housing management?

a) Barn (Separately or mixed with other livestock)
b) Corral (Separately or mixed with other livestock)
c) Open field (Separately or mixed with other livestock)
d) Within the family house (Separately or mixed with other livestock)
e) Others

General farm hygiene and orderliness

a) Very good
b) Good
c) Satisfactory
d) Poor

Do you separate cows during parturition? Y/N
Is there separate parturition (maternity pen)? Y/N
Parturition pen are clean and dry and well bedded? Y/N

What do you do to the calving pen after the cow and calf left?

How is the feeding system of your cattle?

a) Only grazing (Separately or mixed with other livestock)
b) Grazing with stable feeding (Separately or mixed with other livestock)
c) Only stable feeding (Separately or mixed with other livestock)

What are the most common disease affecting your cattle, in order of priority

1)

2)

3)

4)

5)

6)

Are you aware of any disease that causes abortion? Y/N. If yes, specify the disease and its symptoms.

Was there any occurrence of abortion in your farm? Y/N

If your answer is yes, in which of the cows and at which time of pregnancy did it occur?

Cow identification	Time of abortion

What was the fate of the aborted cow (S)?

Cow identification	Fate

How many abortions have encountered in your farm for the last three years?……..

How do dispose off the after birth?

How do you dispose the aborted fetus?

Are dogs kept in the farm? Y/N

What are your culling criteria?

a) disease

b) old age

c) infertility
d) poor production
e) other

Where do you get your replacement stock?

Did the farm/ herd been tested for brucellosis since inception? Y/N, when?

Did vaccinations for brucellosis been carried since inception? Y/N, when?

Format to investigate occurrence of brucellosis in man

How frequent do you drink milk/ its products? Never Rarely Frequently

In what form (raw, boiled or processed) do you drink milk?

Has any member of the family, milkers, and other workers visited a health institution in the last six months? Y/N

If yes, for what was the health problem?.....

…….

Has any member of the family/ milker/ worker show symptoms of prolonged fever since starting being involved in dairy cattle management? for how long? Y/N

Has any member of the family/ milker/ worker show the following symptoms since starting being involved in dairy cattle management?

Symptoms	Yes	No
Headache		
Insomnia		
Pain over the spine		
Vague generalized pain/ aches		
Pain over the joint		
Pain over testes		
Nervous disorders		

Annex-2: Reagents and materials used for serological tests

2. 1. Reagents and materials required for RBT

Reagents:

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

Materials:

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

2.2. Reagents and materials required for CFT

Reagents:

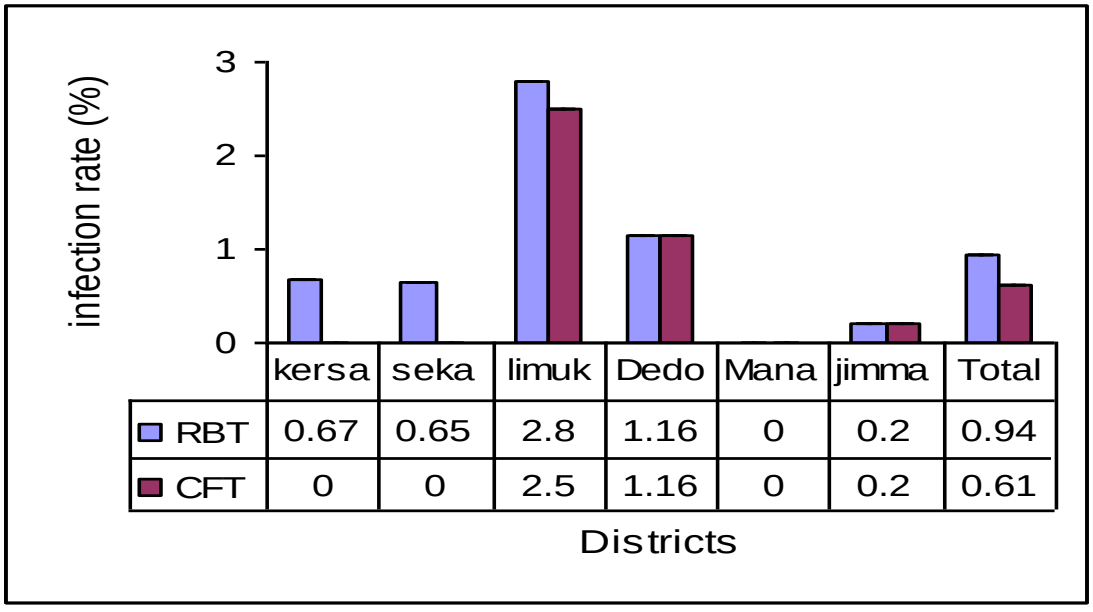
- Veronal buffered diluent (prepared by mixing 1vial of the constituents into 1 liter of distilled water (PH 7.25)).
- Working strength antigen
- Complement
- Ambocepter
- Alsever's solution
- Positive control sera
- Negative control sera (from previously tested

Materials:

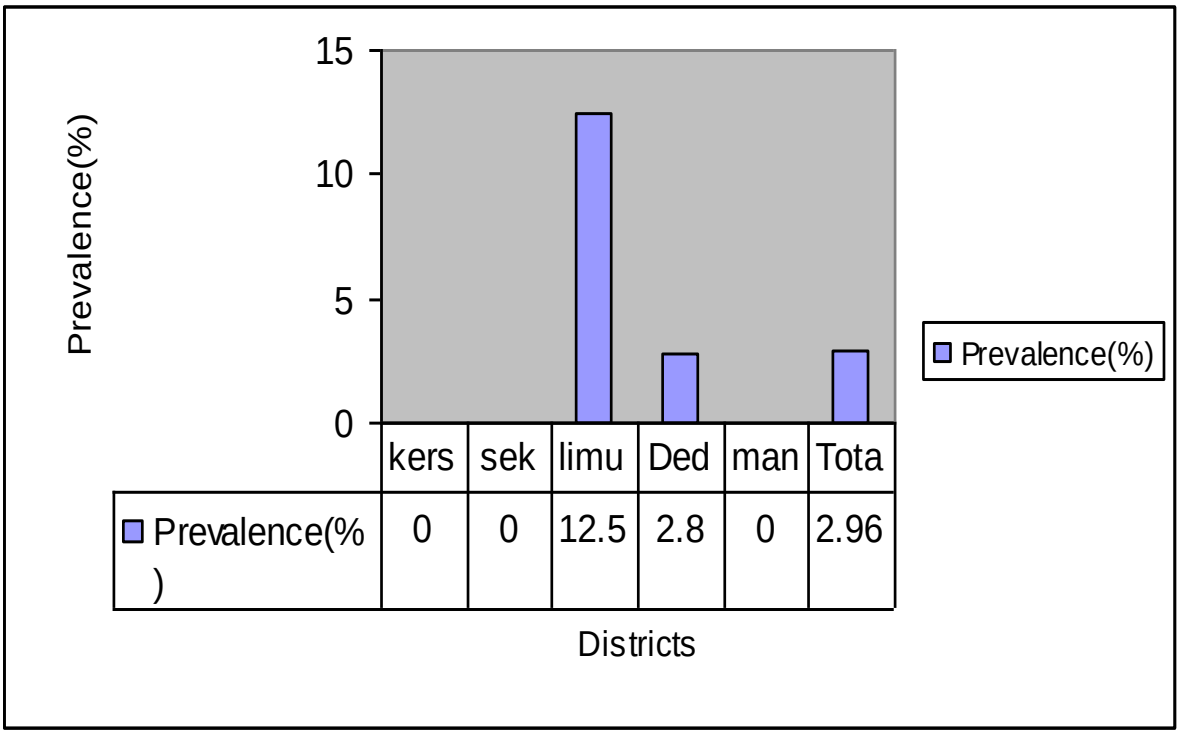
- Micro titer plate (U-shaped)

- Shaker housed in an incubator (37 ^{o}C)
- 0-100 µl adjustable single channel pipette
- 25 µl multi channel pipette
- 1000 µl adjustable single channel pipette
- Pipette tips
- Plastic troughs
- Traditional glass pipette
- Centrifuge tubes
- Measuring cylinders
- Glass and plastic beakers
- Water bath (58 ^{o}C)
- Centrifuge
- Refrigerator
- Rack

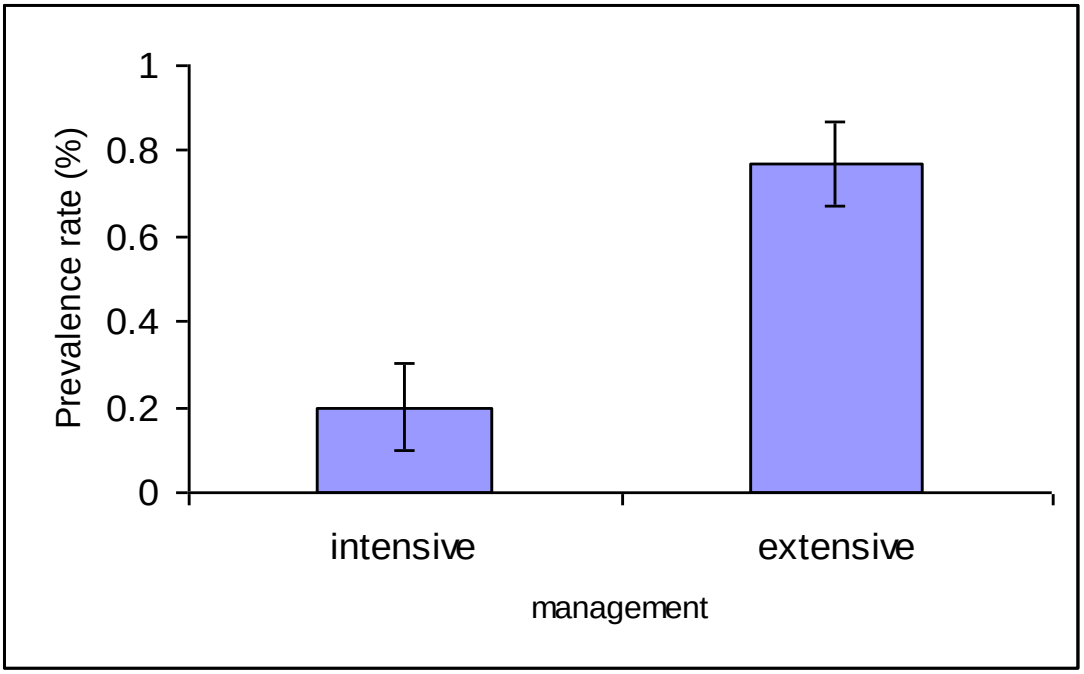
Annex-3: Results of RBT and CFT for brucellosis by districts



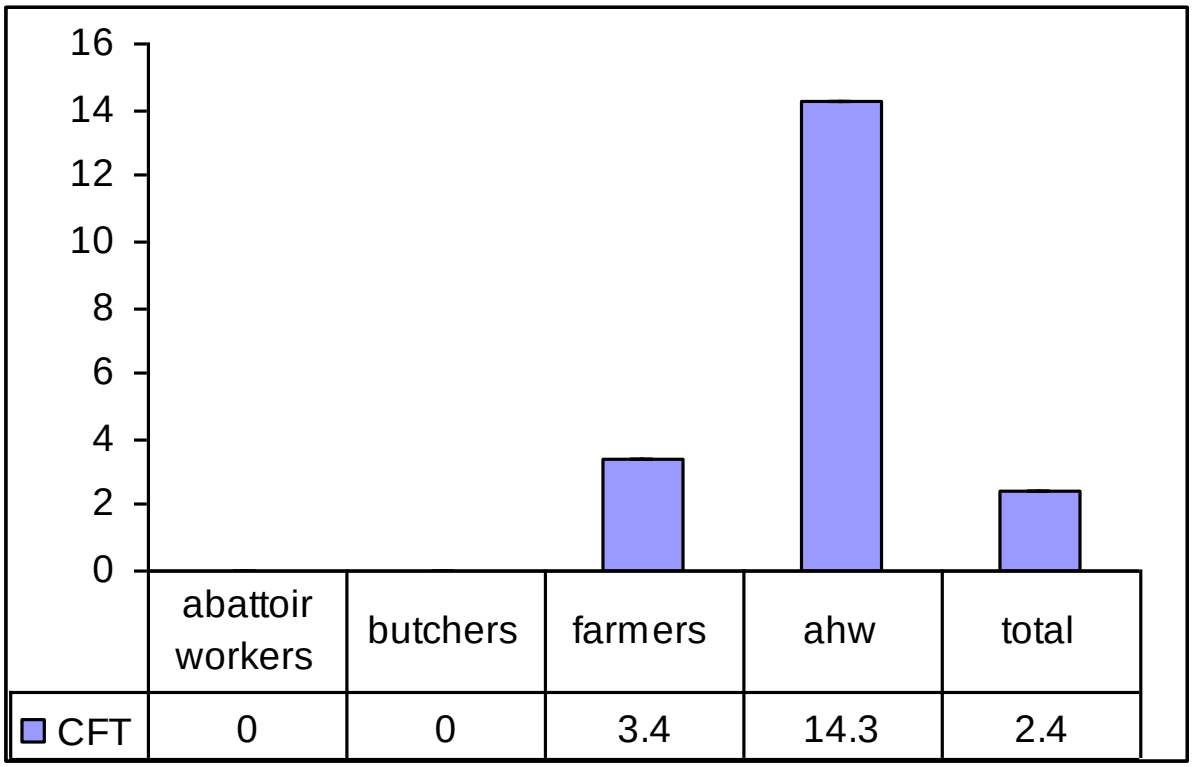
Annex-4: sero-prevalence of brucellosis at herd level in districts practicing only extensive system



Annex-5: Results of CFT for brucellosis by management systems in study areas



Annex-6: Results of CFT screening for brucellosis in different groups in the study areas



9. CURRICULUM VITAE

I. Personal data

Name: Tadele Tolosa

Sex: Male

Age: 37

Marital status: Married

Place of Birth: West Shoa, Oromia, Ethiopia.

Language (speaking and writing): Afan Oromo, Amharic, And English.

II. Educational Background

1972-1980: Primary and Junior Secondary school

- Bantu Primary and Junior school Promotion card

1981-July 1983: High school

- Sebeta Comprehensive secondary school Diploma in general Agriculture and Ethiopian school leaving certificate result card

Sep. 1983-July 1989: Higher education

- Addis Ababa University, Faculty of Veterinary Medicine
  A Degree in Doctor of Veterinary Medicine (DVM)

Oct. 2002- July 2004: Post Graduate studies

- Addis Ababa University, Faculty of Veterinary Medicine
  A Degree of Master of science in Tropical veterinary Medicine
  Specialization in food hygiene and Veterinary Public Health

III. Work Experience

Awraja veterinarian Sep. 1989- July 1993 Kafa, Southern Ethiopia

Forth Livestock Development Project Coordinator Aug. 1993- Dec. 1994 Jimma Zone, Oromia

Zonal veterinarian January 1995 ( ) Jimma Zone, Oromia

Consultant in Pestaloz (NGO) 1995 (full year) Jimma Zone, Oromia

Training Animal Health Technician Dec. 1998- Jun. 1999 Bako, Western Shoa, Oromia

National Livestock Project Coordinator Dec.1999 Jimma Zone, Oromia

IV. Research activities

Field study of Host resistance to ticks in cattle in Bedelle, Ethiopia (1989)

Civet cat management and health problems in Jimma zone, Western Ethiopia (Sep.2001-Aug. 2002).

Sero-prevalence study of bovine brucellosis and its public health significance in selected sites of Jimma zone, Western Ethiopia (Oct. 2003- Apr. 2004)

V. Special skills

Computer literate, good working knowledge with MS word, MS Access, MS, Excel, STATA and SPSS statistical soft ware.

VI. Research interest

Microbiology in the area of consumer food safety and public health (zoonotic diseases).

VII. Membership

Ethiopian Veterinary Association (EVA).

VIII. References

Dr. Bayleyegn Molla

Dr.Fekadu Regassa

Dr. Kelay Belhu

Faculty of Veterinary Medicine

POB 34, Debre zeit, Ethiopia

Bekele Mosisa

Facilitator Change Ethiopia

Addis Ababa

IX. Address

POX 896

E- mail: tadeletolosa @ yahoo. com

Jimma

Oromia

Ethiopia

10. SIGNED DECLARATION SHEET

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

Name Tadele Tolosa

Signature

Date of Submission

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

Dr. Fekadu Regassa

Dr. Kelay Belhu