ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

SEROPREVALENCE STUDY OF BRUCELLOSIS IN CATTLE AND HUMAN IN BAHIRDAR MILKSHED

BY

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DEBRE ZEIT, ETHIOPIA

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ABBREVIATIONS

AAIPUDPS	Addis Ababa Intra Peri-Urban Dairy Production System
AAU	Addis Ababa University
AI	Artificial inSemination
BgVV	Bundesinnsstitut Gesundheitlichen Verbaucherschutz und Veterianarmedizin
CFT	Complement Fixation Test
CHE	Central Highland of Ethiopia
χ^2	Chi square
CI	Confidence Interval
Cfu	Colony forming units
CSA	Central Statistic Authority
CSF	Chafa State Farm
^{O} C	Degree centigrade
Df	Degree of freedom
EANRS	East Amhara National Regional State
ELIS	Enzyme Linked Immunosorbent Assay
EC	European Commission
FAO	Food and Agriculture Organization
FPSR	False positive serological reaction
>	Greater than
IFAT	Immunofluorescent Assay Test
ICFTU	International complement fixation test unit
IFA	Immuno fluorescent Antibody
Ig	Immunoglobulin
ILCA	International Livestock Center for Africa
ILRI	International Livestock Research Institute
IU	International Unit
IM	Intra Muscular
Km	Kilo meter
<	Less than

≤	Less than or equal
µm	micrometer
masl	meter above sea level
ML	Milli liter
mm	milli metre
MRT	Milk Ring Test
MZN	Modified Zeihl -Neelson
OIE	Office International des Epizootics
P	Probability
PA	Peasant Association
RBPT	Rose Bengal Plate Test
SAT	Serum Agglutination Test
SD	Standard Deviation
SE	Standard Error
SEE	South East Ethiopia
SLPS	Smooth Lipo Lipopolysacchride
US	United States
WANRS	West Amhara National Regional State
WHO	World Health Organization

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ABSTRACT

A cross-sectional study was conducted in South Gondar, West Gojjam and Awi zones of Amhara Regional State, between October 2004 and March 2005, to determine the sero-prevalence of brucellosis in cattle and humans as well as to identify the likely potential risk factors. Two stage cluster-sampling was employed, by which 11 towns are identified as primary units and 195 farms as secondary units in semi-intensive production system. Similarly, 29 Peasant Associations as primary units and 145 individual cattle owners as secondary units were selected for the extensive production system. In the government cattle breeding ranch all eligible cattle were sampled. Human risk groups were sampled using purposive sampling method. The study methods involved collection serum sample, data, questionnaire surveys and serological tests conducted in laboratories. Accordingly, 1944 cattle (341 clusters in the three production systems) were sampled. The sampling involved all cattle above 6 months of age with no history of previous vaccination against brucellosis. The number of cattle sampled in extensive, semi-intensive and ranch were 864, 754 and 326, respectively. The types of tests used to detect the presence of *Brucella* antibodies were Rose Bengal Plate Test (RBPT) as screening test, and Complement Fixation Test (CFT) as confirmatory test. Results of the two tests were interpreted serially. A cluster was said to be positive if at least one animal reacts positively by both RBPT and CFT.

The overall individual and cluster level sero-prevalence of cattle in the extensive, semi-intensive and ranch were 4.63 % (n=90) and 14.96 % (n=51), respectively. The within cluster sero-prevalence varied between 0% and 100%, with an overall mean (±SD) of 24.27% (±17.68). For semi-intensive production system, the sero-prevalence varied between 0% and 100% the mean (±SD) sero- prevalence was 26.82 % (±22.52), and in the extensive production system the within cluster sero-prevalence varied between 0% and 50% and an average (±SD) sero- prevalence of 25.44% (±10.45).

There was a highly significant difference in sero-prevalence to *Brucella* antibody among Awi, West Gojjam, South Gondar at both individual level (p=0.000) and cluster level (p=0.000). However, Awi and West Gojjam zones did not vary significantly at both individual and cluster

levels. In the midland and highland *Brucella* sero-reactor varied significantly at both individual level (p=0.000) and cluster level (p=0.000), respectively. Apparently there was no significant difference among the ranch, extensive and semi-intensive production systems, at both individual and cluster levels (p=0.082) and (p=0.255), respectively.

Sero-reactor female and male were found to vary significantly in the semi-intensive production (p=0.042) as opposed to extensive (p=0.115) and ranch (p=0.50). Generally, females were found more affected. Significant difference in sero- prevalence to *Brucella* antibody among age groups (0.5-1,>1- 3, >3 years) was observed in the semi-intensive production (p=0.001), yet in extensive production and ranch the variation was not significant. Virtually, the sero-porevalences of the three herd categories having $\leq$ 5 cattle, 5- 10 cattle and those with > 10 cattle were found significantly different in semi-intensive production (p=0.000); however, herd size had not been crucially important in extensive production. Our current finding revealed no significant variation between the two breeds of cattle, Fogera (indigenous zebu) and cross bred (Fogera X Friesian).

Abortion and retained fetal membrane were found to be significantly related to brucellosis in the semi-intensive production (p=0.021) and (p=0.001), respectively; however, the two clinical signs were not found significantly associated in the extensive production. None of the management and husbandry related factors from the questionnaires were found significantly associated with sero-positivity, though a few of them had some association.

Of the total 238 human serum samples tested, 3.78% (n=9) sera were found positive; both sexes were almost equally affected. Although not statistical significant, middle and old age groups were more affected than younger ones. In conclusion, the present study revealed a wider distribution of sero-reactor cattle in the study area; similarly the zoonotic importance of the disease was established, hence warranting future detailed study in the area.

Key words: Sero-prevalence, sero-reactor, sero-positive, semi-intensve, extensive, ranch, cattle, *Brucella*, Awi, West Gojjam, South Gondar production system.

1. INTRODUCTION

Meeting the food needs of urban populations is of growing concern in developing countries. Demand for milk and dairy products exceeds supply in most parts of sub-Sahara Africa (ILCA, 1990). Over the last decades, the population growth in sub-Sahara Africa, combined with rising per capita income has got rapid growth in food consumption, in particular of dairy products. The World Bank (1992) has estimated that demand for milk and dairy products in sub-Saharan Africa will increase by 5.5 million tones by the year 2025, an annual growth rate of 4%, questioning the supply side. Much of this increased demand will be concentrated in urban areas. The population in sub-Sahara Africa is expected to increase by 2.75% a year between 1990 and 2025, resulting in an additional 800 million people to feed. Of these, over 500 million will be in cities and large towns.

In the past, much on this demand for milk and milk products was met by cheap imports from the European Union. But with recent falls in the "Milk Lake" and consequent increases in world market prices, most developing world governments can no longer afford this option. This has created opportunities to develop local production (ILRI, 1995).

Although inadequate to meet the increase in demand, marketed dairy production is already increasing in most African countries as a direct response to consumer demand, to change in the infrastructure in rural areas (better road system, input markets) or as a result of development; important examples are efforts to promote smallholder dairy production. Despite the dynamic changes market-oriented dairy production is facing several constraints in its sustainable development; feed resource shortage, genotype upgrading, management of reproduction and diseases of intensification (ILCA, 1990).

In Africa as a whole, smallholder dairying generates more regular income than any other rural enterprise. Dairying obviously contributes enormously towards alleviating poverty and improving food security (ILRI, 1997).

The low-level livestock productivity is also reflected in the very low percapita consumption of animal protein in general. The per capita of animal product consumption in developing countries represents 15-30% of that of developed countries. An example is the case of milk consumption where an average milk consumption of 200 kg per person per year in the developed countries, as opposed to 27.7 kg for Africa and 20 kg for Ethiopia (Tegegne and Gebre Wold, 1997).

According to CSA (2003) Ethiopia is the third most populous countries in Africa next to Nigeria and Egypt, having an estimated population of about 67.2 million in July 2002 with annual growth rate of 2.9%. The total cattle population of Ethiopia is estimated to be 41,527,142; with the proportion of male to female of about 50.2% and 49.8%, respectively.

Livestock contribute about 30% of the agricultural GDP and 19% to the export earnings. Besides, about 6 million oxen provide draught power for the cultivation of grain crops (Tegegne and Gebre Wold, 1997).

Cattle population of Amhara national regional state is estimated to be 10, 512,777 (CSA, 2003), of which 1,805,759 are found in our eleven study *weredas* with a proportion of 1,799,275 local breeds and 5,703 crossbreeds (Addisu and Zelalem, 2003). The total milk production in Amhara national regional state is estimated to be 492.964,417 liters, of which 40.96% used for household consumption, 0.74% for sell 0,75% for wage and the rest 57.54% for other purposes (production of butter and cheese)(CSA, 2003). The comparative huge resource that the country possesses and the economic return gained from this sub-sector does not seem to coincide. The reasons that are attributed to these are under nutrition, malnutrition, low productivity, age-old traditional management and diseases. One of the infectious diseases, which are major constraint for animal productivity is brucellosis (Tamirat, 1985).

Brucellosis is one of the old zoonotic diseases tracing back to the date of Hippocrates 450 A.D. It is a major problem confronting food production in tropical and sub-tropical regions of the world (Nicoletti, 1984). David Bruce, for whom the genus *Brucella* is named isolated the causative organism in 1887, from the spleens of five fatal cases and placed it with in the genus Micrococcus (Corbel, 1990). Fredrick Bang of Copenhagen discovered *Brucella abortus* in 1897 (Corbel, 1990).

Bovine brucellosis (contagious abortion, Bang's disease) is a disease of cattle usually caused by *Brucella abortus*, less frequently by *Brucella melitensis* and rarely by *Brucella suis*. The disease is characterized by abortion in the last trimester or birth of unthrifty newborn in the female and orchitis and epydidimitis with frequent sterility in the male (Radostitis *et al*., 2000; OIE, 2004). Occasionally, other species of animals such as goats, sheep, pig, dog and horse may be infected by *Brucella abortus* (Nicoletti, 1998). *Bruella* has also been reported in one and two humped camels, domestic buffalo, American and European bison, yak, elk, African buffalo and antelopes (OIE, 2004).

Brucellosis is an important global zoonotic problem; the public health significance of brucellosis includes not only the direct or indirect transmission of disease from animal to man and consequent illness, physical incapacity and loss of human resource, but also serious diminution of much needed food stuffs, especially animal proteins which are essential for human health and well being (Rana *et al.*, 1985).

The distribution of the different species of *Brucella* and their biovars varies with geographic areas. *Brucella abortus* is the most widespread species whereas *Brucella melitensis* and *Brucella suis* are irregularly distributed (Quinn *et al*., 1999). Brucellosis is perhaps the most widespread and economically important of the zoonotic diseases in tropical and sub-tropical regions. Ancient practices such as nomadism, communal grazing and modern changes towards intensification and increased international trade caused additional problem (Nicoletti, 1984). High commercial structures in the international cattle trade, changing forms of husbandry, more extensive cattle movements, and an increasing intensification of cattle raising have increased the significance of brucellosis as a typical contagious disease in many countries in recent years (Weidman, 1991).

In East Africa the disease is a serious livestock health problem. The sero-prevalence in cattle is found to be 11.9% in Somalia (Hussein *et al*., 1978), 10% in Kenya (Kagumba and Nandoka, 1978), 34.9% in Rwanda (Akakpo *et al.*, 1978), 6.5-22.5% in Sudan (Hellmann *et al.*, 1984), 1.8% in Uganda (Oloffs *et al*., 1996). The situation in Ethiopia is not that different; prevalence of 18.4% by Kibru (1985), 7.6% by Bayleyegn (1989), 38.7% by Muktar (1993), 22% by Tariku

(1994), 8.1% by Yilkal *et a*l. (1998), 4.9% by Abay *et al.* (2000), 2.46% in intensive and 1.66% in extensive by Kassahun (2004) and 0.77% in intensive and 0.2% in extensive by Tadele (2004) have been reported.

Brucellosis is considered by FAO/WHO/OIE as the most wide spread zoonoses. The importance of brucellosis lies on the severe public health hazard it causes and the economic impact on the animal industry resulting in effect on total animal protein supply (Mustofa and Nicoletti, 1993).

The objectives of this work were therefore:

To determine the prevalence of brucellosis in cattle;

To investigate potential risk factors associated with brucellosis; and

To investigate the presence of human sero-reactors.

2. LITERATURE REVIEW

2.1. Etiology

The different species of the genus *Brucella* are able to infect a broad range of hosts (Seifert, 1996). The six-*Brucella* species are *Brucella abortus* (cattle), *Brucella melitensis* (goats/sheep), *Brucella suis* (pig), *Brucella canis* (dog), *Brucella ovis* (ram), and *Brucella neotoma*e (desert wood rat) (Alton *et al.,* 1975; Walker, 1999), and, more recently, types infecting marine mammals (Corbel, 1997). *Brucella* species isolated from the sea mammals are divided into two groups based on their carbon dioxide-dependency and the host species. All the seal and the otter isolates required co_2 where as the cetacean isolates did not (Foster *et al*., 1996). Marine mammal isolates appear to comprise several new species of *Brucella* corresponding to diverse marine mammal hosts, and some species may contain more than one subtype (Bricker *et al*., 2000).

Each *Brucella* species has preferred natural host that serves as a reservoir of infection. Secondary hosts play only a very small part if any in the maintenance or spread of a particular *Brucella* species (Quinn *et al.,* 1999). Bovine brucellosis (contagious abortion, Bang's disease) is a disease of cattle usually caused by *Brucella abortus*, less frequently by *Brucella melitensis* and rarely by *Brucella suis*. *Brucella suis* does not appear to be contagious from cow to cow (Nicoletti, 1998). *Brucella abortus* has 7 biovars (1, 2, 3, 4, 5, 6, and 9); biovars 7 and 8 are no longer regarded as valid (Corbel, 1990; OIE, 2004). No proven differences in the pathogenecity or antigenecity among the field strain biotypes are observed (Radostitis *et al*., 2000). Sometimes multiple biotypes may be found within a herd (Nicolleti, 1980).

Occasionally other species of animals such as goats, sheep, pig, dog and horse may be infected by *Brucella abortus*. In horses suppurative bursitis, most commonly recognized as "fistulous wither" or "poll evil" is most common condition associated with brucellosis (Nicoletti, 1998). It is unlikely that infected horses are a source of the disease for other horses, other animal species, or man (Nicoletti, 1998).

2.2. Morphology and Staining

Brucella species are cocci, coccobacilli or short rods, 0.5-0.7 µm in diameter and 0.6-1.5 µm in length (Corbel, 1990). They are arranged singly and, less frequently in pairs, short chains or small groups (Corbel, 1990). It does not possess capsule, spore and flagella; however, an external envelope has been demonstrated by electron microscope (Walker, 1999).

Brucella are partially acid fast in that they are not decolorized by 0.5% acetic acid in modified Zeihl - Neelson and, thus appear pink in a blue background. *Brucella* are Gram negative by Gram staining (Walker, 1999; Quinn *et al.*, 1999; Alton *et al.*, 1975).

2.3. Epidemiology

The factors influencing the epidemiology of brucellosis infection in any geographic location can be classified into factors associated with the transmission of the disease between herds and factors influencing the maintenance and spread of infection within the herd (Crawford *et al.*, 1990). Factors associated with brucellosis include host factor (age, sex, and breed), agent factor and extrinsic factors (environmental factors) including management and ecology (Nicoletti, 1980).

2.3.1. Distribution

Although reported incidence and prevalence of the disease vary widely from country to country, bovine brucellosis caused mainly by *Brucella abortus* is still the most widespread form (Corbel, 1997). Brucellosis is wide spread and of major economic important in most countries of the world, particularly amongst dairy cattle. The prevalence varies considerably between herd, between areas and between countries (Nicoletti, 1998). In 1986, bovine brucellosis was recorded in 120 of 175 countries, 33 countries did not record the disease, and data were not available from the remaining (Crawford *et al*., 1990). Despite being controlled in many developed countries, the disease remains endemic in many parts of the world, including Latin America, the Middle East,

Spain, parts of Africa, and Western Asia (Memish and Balkhy, 2004). Few developed countries (Australia, Canada, Czechoslovakia, Finland, German Democratic Republic, Ireland, Japan, Norway, Poland, Romania, Switzerland and United Kingdom) obtained complete eradication of the disease (Fensterbank, 1987). Brucellosis is the most widespread and economically important zoonotic diseases in tropical and sub-tropical regions (Nicoletti, 1984).

Table 1: Sero-prevalence of *Brucella* reactor cattle in Africa

Country	Sero-prevalence (%)	**AUTHORS AND YEARS**
Djibouti	4%	Chntel *et al*.,1994
Eritrea	8.5%	Omer *et al*.,2000
Ghana	6.6%	Kubuafor and Nandokha, 2000
Guinea	6.9%	Chukwu, 1985
Kenya	10%	Kagumba and Nandokha,1978
Kenya	7.73%	Chukwu, 1985
Kenya	9.2%	Chukwu, 1985
Kenya	3.74%	Chukwu, 1985
Nigeria	1.5%-14.3% private 79.7% government ranch	Esuruoso, 1980
Rwanda	35.1%	Akakpo *et al.*,1978
Somalia	9.5%	Wernery *et al*.,1979
Somalia	2.8% farm 11.9% nomadic	Hussien *et al.*,1978
South Africa	2.1%	Bakunzi *et al.*, 1993
Sudan	6.5%- 22.5%	Hellmann *et al.*,1984
Sudan	25.3%	McDermott *et al*.,1987
Tanzania	4.3% Local management 6.3% dairy farm 15.8% ranch	Jiwa *et al.*,1996
Uganda	3%	Oloffs *et al.*, 1996

Table 2: Summary of sero-prevalence of cattle brucellosis in Ethiopia

Author and years	Location	Breed	No tested	% sero-prevalence	Type of test
Kibru, 1985	CHE	Cross	178	18.4	RBT, CFT
Kassaye, 1985	Bahir dar	Mixed	678	9.8	CFT
Asegid, 1987	CHE	Mixed	3577	2.1	SAT, CFT
Tekleye *et al*., 1989	Ghibe, Gobe	Zebu	1606	4.2	RBPT
Abebe, 1989	CHE	Cross Zebu	no data no data	15 3	RBPT, SAT RBPT, SAT
Bayleyegn, 1989	Arsi	Mixed	2178 (28herd)	8.86 7.62	RBPT SAT
Endrias, 1989	Sidamo	Cross	736 (5herd)	15.8 11.6	RBPT SAT
Muktar, 1993	CHE	Cross	N	38.7	RBPT, SAT
Tariku, 1994	CSF	Cross	182	22	RBT, SAT
Abeje, 1994	Bahir dar	Mixed	1855	16.92 16.55	RBT SAT
Yilkal *et al.*, 1998	AAIPUDPS	Cross	950	8.1	RBT, CFT
Fekadu, 1999	EANRS	Zebu	3644	1.8	RBT, SAT
Abay *et al*., 2000	SEE	Mixed	4243	4.9	RBT, CFT
Gebreyesus, 2001	WANRS	Zebu	N	8.2	RBT
Kassahun, 2004	Sidama	Intensive Extensive	811 1627	2.46 1.66	RBPT, CFT
Tadele, 2004	Jimma	Intensive Extensive	508 1305	0.77 0.2	RBPT, CFT

2.3.2. Age

It is widely accepted that susceptibility increases with sexual development and pregnancy (Nicoletti, 1980; Morgan and McKinnon, 1979; Roberts, 1971). Young sexually immature cattle generally do not become infected following exposure or recover quickly (Radostitis *et al*., 2000). Numerous reports now confirm that a small but important percentage of heifer calves which are infected in early life are negative to serologic tests, and abort or have an infected calving during the first pregnancy (Nicoletti, 1980). It is estimated that 2.52% of heifer calves born to serologically positive dams reacted in early adulthood and constitute a risk to the herd (Nicoletti, 1980). Little is known about the factors which may affect latent infection such as severity of infection in the dam, number of organisms in the milk and antibody content, time of waning, and vaccine administration. It is certain that latent infections are more frequent than previously believed and present serious difficulties in elimination of brucellosis in cattle herds (Nicoletti, 1980).

2.3.3. Sex

No control studies have been conducted on the relative susceptibility of female and male cattle to brucellosis; nonetheless, based on reactor rates it is probable that bulls are more resistant than sexually mature heifers and cows, and less resistant than sexually immature heifers (Nicoletti, 1980). There is a tendency for bulls to become infected at younger age than females and they may acquire infection during calf hood and retain it into adult life (Nicoletti, 1980).

2.3.4. Breed

All breeds of cattle appear to be comparable in susceptibility to brucellosis and apparently no specific breed resistance to brucellosis is known (Radostitis *et al*., 2000). Susceptibility between breeds has not been reported (Madsen, 1989). Rather there are varying degrees of individual resistance to infection, which are dependent up on gestation, exposure dose, age, vaccination and unknown host-resistance factors (Nicoletti, 1980).

2.3.5. Agent Survival

Brucella are facultative intracellular bacteria, hence has protection from the innate host defense and from therapeutics; moreover, in quiescent state does not cause formation of humoral antibodies; it is unknown if aberrant forms of the bacteria are produced and play a role in the host parasite relationship. Nevertheless, this cytoplasmal survival results in many epidemiological problems (Nicoletti, 1980).

It is generally accepted that the organism does not multiply in the environment but merely persist and its viability out side the host is influenced by the existing environmental conditions (Radostitis *et al*., 2000). Temperature, humidity, presence of other microorganisms, presence of nutrition, autolytic enzymes and pH of the environment influence the survival of *Brucella* (Radostitis *et al*., 2000). The organism is sensitive to direct sunlight, disinfectant and pasteurization (FAO/WHO, 1986; Seifert, 1996). *Brucella* survive for up to 4 months in aborted fetuses and placenta, 22 weeks in humid faeces, 44 days in dust of street, 30 days in tap water, 51 days in sterile water, 2 months in desert soil, up to 2 years in frozen soil. *Brucella* survive up to 6 months in soft cheese, up to 4 months in butter, up to 6 weeks in milk, for 14 days in cooled meat and in ice cream up to 30 days (Weidman, 1991). Disinfectants like caustic soda, formalin 2% and Lysol 1% destroy *Brucella* (Seifert, 1996). Exposure to sunlight kills the organisms within a few hours (Nicolleti, 1998).

2.3.6. Management

There is a close correlation between the kind and intensity of the husbandry system and the rate of infection. In general increased animal concentration and contacts favor the spread of brucellosis (Weidman, 1991). Management factors for high incidence of infection are high stocking densities, unhygienic housing and calving, lack of sanitary measures (cleaning and disinfection) and large herd size (Crawford *et al*., 1990; Nicoletti, 1980; Morgan and McKinnon, 1979). Frequent farm disinfection is an effective method of control of brucellosis because of low resistance of *Brucella* (FAO/WHO, 1986).

2.4. Transmission, Pathogenesis and Clinical Sign

The primary route of infection is through ingestion of contaminated feed and water, inhalation during over crowding, contact through intact skin and conjunctiva; calves may be infected while in the uterus or by suckling infected milk of their mother (Morgan and McKinnon, 1979; Nicoletti, 1998). Venereal transmissions by infected bull to susceptible cows appear to be rare. Transmission may occur by artificial insemination (Nicoletti, 1998).

Following entry into the host, *Brucella* is either free in the extracellular environment or in phagocytes localized to the regional lymph nodes (Quinn *et al*., 1999). In the phagocytes, the organism is capable of surviving and multiplying. Intracellular survival in the macrophages and to lesser extent neutrophils is enhanced by suppression of the myeloperoxidase H_2O_2 halide system and production of super oxidase dismutase and catalase against oxidative killing. Stress protein protects the organism from hydrolytic enzyme and oxygen radicals (Walker, 1999).

Seronegative infections prior to sexual maturity and first parturition (latency) perpetuate the disease (Nicoletti, 1998). The predilection site for *Brucella* is the reproductive tract of males and females, especially pregnant uterus and reticuloendothelial system (Morgan and McKinnon, 1979; Quinn *et al*., 1999). Allantoic factors, erythritol and steroid hormones which are present in the placenta and male genital tract of cattle, sheep, goats and swine, but not human, stimulate growth of *Brucella* (Quinn *et al*., 1999). *Brucella* possesses an endotoxin that contributes to the pathogenesis (Walker, 1999). The biggest problem of *Brucella* infection is the uncertain incubation period, which an infected animal can conceal under certain conditions for months and years (Weidman, 1991); the period varies considerably and is affected by factors such as gestation, exposure dose, age, vaccination, and other unknown host-resistance influences (Nicoletti, 1980).

The primary clinical manifestations of brucellosis are related to the reproductive tract. Abortion that occurs in the last trimester is the most obvious manifestation. The quantity of *Brucella* excreted during an abortion could be as much as 10^{12}-10^{13} capable of infecting 60,000- 600,000 pregnant heifers (Nicoletti, 1980; Fensterbank, 1986). Infection may also cause stillbirth or weak

calves, retained fetal membrane, and reduced milk yield. Usually, general health is not impaired in uncomplicated abortion. Seminal vesicles, ampullae, testicles, and epydidimis may be infected in bulls. Testicular abscesses may occur; long-standing infections may result in arthritic joints and hygroma in some cattle (Nicoletti, 1998; Seifert, 1996).

2.7. Immunology

2.7.1. Humoral Immunity

Infection with *Brucella* usually results in the induction of both humoral and cell mediated immunity. The magnitude and duration of these responses can be affected by many factors including virulence of the strain, size of inoculum, age, sex, pregnancy, species and immune status of the host (FAO/WHO, 1986). The immunoglobulin isotypes present in serologically significant concentrations in serum are IgG1, IgG2, IgM, and IgA; the first isotype produced after an initial heavy infection or immunization is IgM usually detected in the first or second week but is soon followed by IgG antibody, which is the most abundant in serum (FAO/WHO, 1986).

It is clear that *Brucella abortus* infected cattle contains high level of IgM, IgG1, IgG2 and IgA isotypes of antibodies; however, it should be noted that IgG2 and IgA antibody levels are considerably lower than the IgM and IgG1 antibody level (Nielsen and Duncan, 1988). Synthesis of IgG2 is essential component of the antibody response required to eliminate *Brucella* abortus S19 or field infection and interestingly IgG2 was noted to be the only antibody isotype to be effective in antibody dependent cell mediated cytotoxicity (Nielsen and Duncan, 1988). Humoral factors probably have a role in bovine resistance to brucellosis by mediating extracellular killing of bacteria. However, the role of humoral substances in bovine resistance to brucellosis ill defined (Hoffmann *et al*., 1990), IgM is efficient in normal agglutination, rose bengal and complement fixation tests. IgG1 is in active in normal agglutination but efficient in agglutination at pH 3.6 and fixes complement and IgG2 is active in normal agglutination but not at pH 3.6 and does not fix complement (Nicolleti, 1980).

2.7.2. Cell Mediated Immunity

Brucella species are facultative intracellular pathogens. They are readily phagocytised by macrophages and polymorphonuclear leucocytes and are capable of surviving within these cells (FAO/WHO, 1986). Effective immunity is primarily cellular in nature; specifically sensitized T-lymphocytes release cytokines that activate macrophages, which in turn control *Brucella* by reactive oxygen intermediates (Walker, 1999).

2.8. Diagnosis

Diagnosis of brucellosis is made possible by direct demonstration of the causal organism using staining, immunofluorescent antibody, culture, animal inoculation and polymerase chain reaction (PCR) and indirectly by demonstration of antibodies using serological techniques (Alton *et al.* , 1975; Weidman, 1991; Corbel, 1997; Quinn *et al*., 1999).

2.8.1. Microscopic Examination

Gram staining of fetal stomach contents from aborted fetus, placenta, vaginal discharge, and semen reveal gram-negative cocci, coccobacilli and small rods. In the modified Ziehl-Neelsen method smears are made from fetal membranes, fetal stomach contents, vaginal swabs and semen; the smear is dried and fixed over a flame, stained with carbol fuchsin for 10 minutes, washed in tap water, flooded with 0.5 % acetic acid for 30 seconds, washed thoroughly and counter stained using 1% methylene blue for 20 second, *Brucella* stain red with a blue background (Alton *et al*., 1975).

2.8.2. Immunofluorescent Antibody Method

An anti-*Brucella* serum conjugated with two-resicin isothiocyanate fluorescent antibody is used. This is then applied on a smear on a thin microscope slide, which is fixed by acetone for 10 minutes. The preparation is allowed to react for 30 minutes to 1 hour at 37 ^{o}C in closed humidity container. If *Brucella* is present in the smear a green-yellow fluorescent appears (Alton *et al*., 1975).

2.8.3. Culture and Biochemical Method

Cultural examinations are very important in the epidemiology of bovine brucellosis, in which positive results are conclusive and should be the basis of evaluation of all other diagnostic methods. Biotype identification is sometimes useful for investigation on possible sources of infection (Nicolleti, 1980).

Materials for laboratory examination should be cooled immediately or frozen and transported to the laboratory as quickly as possible in leak-proof containers (Alton *et al*., 1975). Appropriate samples for culture are stomach contents, lung, spleen, and meconium of aborted fetus, fetal membrane, vaginal swabs, blood, milk and lymph nodes (Supramammary, submaxillary, internal iliac). Isolation and identification of the *Brucella* is the only diagnostic procedure not subject to equivocation; however, negative culture result is not sufficient to rule out infection (Weidman, 1991).

Culture media are classified into primary media and selective media; primary media in turn are divided into solid and liquid media (FAO/WHO, 1986). Solid medias include nutrient agar, serum dextrose blood agar and commercial media (tryptose agar, trypticase Soya) and liquid media either trypticase Soya broth, tryptose broth, or other commercial selective media prepared by addition of antibiotics such as cycloheximide, bacitracin, polymixin B, and chemical ethyl violet on serum-dextrose or serum-potato agar or any of the basal media (Alton et *al*., 1975; FAO/WHO, 1986; Corbel, 1990).

Brucella abortus requires supplementation of 5-10% Co_2 for growth, but *Brucella melitensis* does not require supplementation of Co_2 (Quinn *et al*., 1999). Optimum temperature and pH for growth of *Brucella* are 37^{o}C and 6.6-7.4, respectively, but growth can occur between 20^{o}C – 40^{o}C; after 3-5 days incubation on selective serum agar colonies are pinpoint, smooth, glistening, bluish, translucent about 3-4mm in diameter (FAO/WHO, 1986; Quinn *et al*.,1999).

The organism is catalase and oxidase positive, (*Brucalla ovis* and *Brucella neotomae* are oxidase negative) reduce nitrate to nitrite (except *Brucella ovis*), rapid urease activity, some strains *Brucella abortus* produce H_2S while others do not. *Brucella melitensis* does not produce H_2S, *Brucella* does not cause haemolysis on blood agar, does not produce acid on agar containing glucose, and does not ferment lactose; usually grow in the presence of basic fuchsin and thionin at standard concentration (Corbel and Morgan, 1984; Quinn *et al*.,1999).

Generally, *Brucella* usually hydrolyze urea but some strains may not, after three to four days of cultivation, examination of the media /culture/ for growth is required; since *Brucella* is fastidious, most recovery is made within 7-14 days and the media should be kept at least for 35 days before discarding as negative (FAO/WHO, 1986; Quinn *et al*., 1999).

2.8.4. Animal Inoculation

Animal inoculation is the most sensitive method for detection of *Brucella* and is sometimes necessary when very low number of organisms is present. Guinea pigs are the most sensitive laboratory animals, two guinea pigs are inoculated IM 0.5-1.0 ml of suspected tissue homogenate and are sacrificed at 3 and 6 weeks post inoculation and serum is taken along with spleen and other abnormal tissue for serology and bacteriological examination, respectively (Walker, 1999).

2.8.5. Serological Examination

Diagnosis of brucellosis in cattle is frequently difficult to achieve because the serologic response in cattle infected with *Brucella abortus* is influenced by several factors, including long and variable incubation period, cattle vaccination status, type of exposure (Vaccinated, infected or challenged) and stage of gestation at the time of exposure to infection (Lord *et al*., 1989).

The perfect serological test is the one, which would detect infection early in the incubation stage of the disease, discriminate antibodies due to vaccination and field infection and not subject to cross reaction due to non specific antibodies. However, such an assay is not available at present, although multiple tests increase the confidence in the diagnosis, and sequential testes over time also provide a better insight than single test (MacMillan, 1990).

No single serological test and antigen combination showed 100% sensitivity and specificity simultaneously (Munoz *et al*., 2005). A battery of serological tests has been developed for the diagnosis of *Brucella*. Combinations of tests show a degree of sensitivity, which appear to be sufficient to detect infected animals. Frequently, highly sensitive but less specific tests are used for screening purposes and this then are followed by more specific tests for confirmation (Walker, 1999; Garrido-Abellan *et al*., 2001). The commonly used serological tests are the RBPT, SAT, CFT, ELISA (Quinn *et al*., 1999).

The Rose-Bengal test (RBPT), the serum agglutination test (SAT) and the complement fixation test (CFT) are widely used for the detection of antibodies to *Brucella abortus* in bovine serum, with the CFT internationally regarded as a confirmatory test (Alton *et al*., 1975).

Although successful eradication programs have been carried out using SAT or CFT, there has been a concern that these assays may only measure some of the isotopes of antibody (Nielsen *et al*., 1988). This led to the adoption of the RBT (which has a high sensitivity but a low specificity) as a screening test followed by a confirmatory test such as the CFT with a high specificity but lower sensitivity (Nielsen *et al*., 1996).

Various authors have compared conventional serological tests used in the diagnosis of *Brucella abortus* infections to each other or compared the performance of the conventional tests with ELISA (Dohoo *et al*., 1986). It is desirable that the specificity and sensitivity of each test be determined and compared to standard tests before its use for diagnostic purposes (Dohoo *et al*., 1986).

2.8.5.1. Rose Bengal Plate Test

The RBPT is very sensitive test; however, like all other serological tests it could sometimes give a positive result due to S19 vaccination or due to false-positive serological reactions (FPSR). Therefore positive reactions should be investigated using suitable confirmatory strategies and epidemiological investigation (OIE, 2004).

The Rose Bengal plate test has a wide application as a screening test for individual diagnosis in herds of cattle. It is generally considered to be oversensitive, especially in cattle immunized with strain –19. For these reasons sera positive in the Rose Bengal test are usually retested by a definitive test, such as the complement fixation test (FAO/WHO, 1986). False-negative reactions occur rarely, mostly due to prozoning and can be avoided by diluting the serum sample or retesting after a given time (OIE, 2004). Nevertheless RBPT appears to be adequate as a screening test for detecting infected herds or to guarantee the absence of infection in brucellosis free herds (OIE, 2004).

The antigen consists of *Brucella* stained with Rose Bengal and suspended in buffer at pH 3.65±0.05. The test is conducted on ruled enamel strips, on a glass or ceramic tile or WHO hemagglutination plate. Equal amounts of serum and antigen (0.03ml) are put on white enamel or plastic trays, mixed, and read after a 4-minutes rocking (Alton *et a*l., 1975; MacMillan, 1990). The test is usually interpreted as positive if any agglutination is apparent, and the reaction may be graded according to its rapidity and appearance (MacMillan, 1990). The RBPT is economical, simple and rapid, and gives few false negative and false positive results **(**Fensterbank, 1986).

2.8.5.2. Serum Agglutination Test

Serum agglutination test which is the ancestor of all serological tests is still widely used often in conjunction with the CFT and ring tests (Fensterbank, 1986). While not recognized as a prescribed or alternative test, the SAT has been used with success for many years in surveillance and control programmes for bovine brucellosis (OIE, 2004).

The antigen represents a bacterial suspension in phenol saline (NaCl 0.85 % [w/v] and phenol at 0.5 % [v/v]). Formaldehyde must not be used; EDTA may be added to the antigen suspension to 5 m M final test dilution to reduce the level of false-positive results. Subsequently the pH of 7.2 must be readjusted in the antigen (OIE, 2004). Normally conducted by making doubling dilution of the serum in phenol saline in a round-bottomed tube and adding an equal amount of (volume) of standard antigen. After mixing, the tubes are incubated overnight at 37ºC, and the degree of agglutination is read by comparing the opacity against standards representing various degrees of agglutination (Alton *et al*., 1975; Macmillan, 1990). The OIEISS contains 1000 IUs of agglutination. The mixture of antigen and serum dilutions should be incubated for 16–24 hours at 37°C. If the test is carried out in microplates, the incubation time can be shortened to 6 hours. At least three dilutions must be prepared for each serum in order to refute prozone negative responders (OIE, 2004).

Although the SAT is widely used it has the following limitations, detects non-specific antibodies, and does not reach diagnostically significant level during the incubation stage of the disease, inability to detect chronic stage of the disease and inability to differentiate antibodies resulting from natural infection and vaccination (Radostitis *et al.*, 2000). SAT detects antibodies of the classes IgG2 and IgM (Fensterbank, 1986).

The EC antibody level of less than 30 IU in brucellosis-free herds and less than 80 IU in vaccinated are classified as negative, whereas in the US for vaccinated herds antibody level of 200 IU or more is positive and less than 50 IU negative, for non-vaccinated 100 IU or more is positive and less than 25 IU considered negative (MacMillan, 1990; OIE, 2004). It should be

stressed that the serum agglutination test (SAT) is generally regarded as being unsatisfactory for the purposes of international trade (OIE, 2004).

2.8.5.3. Complement Fixation Test

The Complement-fixation test is recognized as the most reliable diagnostic test now in routine use for individual animals (FAO/WHO, 1986). CFT is a widely used and accepted confirmatory test although it is complex to perform, requiring good laboratory facilities and adequately trained staff to accurately titrate and maintain the reagents (OIE, 2004). The work load resulting from the technical complexity of the complement-fixation test can be greatly reduced by using it only as a definitive test on samples that have been found positive in a preliminary screening test (FAO/WHO, 1986). CFT was found to be 92.9% sensitive and 100% specific in non-vaccinated populations (Dohoo *et al.,* 1986). The CFT detects IgG1 and IgM antibody classes (Fensterbank, 1986).

There are numerous variations of the CFT in use, but this test is most conveniently carried out in a microtitre format (OIE, 2004). Either warm or cold fixation may be used for the incubation of serum, antigen and complement either 37°C for 30 minutes or 4°C for 14–18 hours). A number of factors affect the choice of the method: anti-complementary activity in serum samples of poor quality is more evident with cold fixation, while fixation at 37°C increases the frequency and intensity of prozones, and a number of dilutions must be tested for each sample (OIE, 2004).

The CFT is very specific. However, like all other serological tests, it could sometimes give a positive result due to S19 vaccination or due to FPSR. Therefore positive reactions should be investigated using suitable confirmatory strategies. The CFT is also particularly useful to differentiate between young hood vaccination and infection, as antibodies produced by vaccination react negative six months post vaccination (Morgan and McKinnon, 1979). The EU considers any serum containing 20 ICFTU, or more as positive (Macmillan, 1990; OIE, 2004).

2.8.5.4. Enzyme Linked Immuno Sorbent Assey

The ELISA test has gained wide acceptance for serological diagnosis of bovine brucellosis because of its ability to detect antibody of all isotypes unlike other conventional testes (Nielsen *et al.,* 1988). The ELISA test can be useful in conjunction with the CFT during the latter stages of an eradication program when it is important to reduce the number of false negative serological reaction (Sutherland *et al*., 1986). It is a useful test during an eradication program after vaccination has ceased for screening or as a supplementary test to the CFT (Radostitis *et al*., 2000)

The large majority of ELISAs in use in brucellosis diagnosis are indirect ELISA (iELISA). Antigen is bound to polystyrene micro titer plate so that antibody, if present in a sample, binds to the antigen and may be detected by anti-globulin-enzyme conjugate combined with chromatogenic substrate giving a color reaction indicative of the presence of antibody in sample (Garrido-Abellan *et al*., 2001). The I-ELISA provides a rapid, simple, highly sensitive (100%) and specific (99.8%) diagnostic system for large-scale detection of antibodies against *Brucella abortu* (Paweska *et al*., 2002).

The indirect ELISA, while more sensitive than the conventional tests, has been less specific, even using highly specific monoclonal antibodies to bovine immunoglobulins as detection reagents. Similarly, the indirect ELISA could not distinguish antibody arising either from vaccinal or infection and the cost per test is considerably high (Nielsen *et al*., 1996).

A compititive ELISA more specific than the indirect ELISA and which could discriminate vaccinal antibody from antibody induced by infection is also available. This assay use either O-polysaccharide prepared from lipopolysaccharide or lipopolysaccharide from *Brucella abortus* as the antigen and a monoclonal antibody specific for an O-polysaccharide epitope for competition. Lipopolysaccharide, because of the hydrophobic lipid A portion of the core region, attaches readily to polystyrene (Nielsen *et al*., 1996).

2.8.6. Milk Ring Test (MRT)

An efficient means of screening dairy herds is by testing milk form the bulk tank. Milk from these sources can be obtained cheaply and more frequently than blood. When positive test result is obtained, all cows contributing milk should be blood tested (OIE, 2004). If necessary samples could be pretreated with preservative (0.1% formalin or 0.02% bronopol) for 2-3 days at 4^0C prior to be tested (OIE, 2004).

The test is carried out by adding one drop (0.03ml) of antigen, (haematoxylin or tetrazolium stained) to 1ml of milk (Alton *et al*, 1975). The milk/ antigen mixtures are normally incubated at 37^0C for one hour, together with positive and negative working standards. Overnight incubation at 4^0C increases the sensitivity of the test and allows for easier reading (OIE, 2004). A strongly positive reaction is indicated by formation of a dark blue ring above a white milk column. Any blue layer at the interface of milk and cream should be considered to be positive. The milk is negative if the color of the underlying milk exceeds that of cream layer (OIE, 2004).

In large herds (>100 lactating cows), the sensitivity of the test becomes less reliable. False-positive reactions may occur in cattle vaccinated less than 4 months prior to testing, in samples containing abnormal milk (such as colostrum) or in cases of mastitis. Therefore, it is not recommended to use this test in very small farms where these problems have a greater impact on the test results (MacMillan, 1990; OIE, 2004).

2.8.7. Allergic Test

An alternative immunological test, the brucellin skin test, can be used for screening unvaccinated herds. Brucellin preparations should not contain smooth lipo polysaccharide antigen to avoid nonspecific inflammatory reaction or interfere with subsequent serological tests and be standardized, one such is bruellin INRA prepared from a rough strain of *Brucella melitensis* and standardized antigen preparation (brucellin INRA) is used (OIE, 2004).

The delayed type hypersensitivity reaction, which is mediated by sensitized T-lymphocytes, is more widely used in the diagnosis of brucellosis in sheep and goats than cattle (Morgan and McKinnon, 1979). The technique involves inoculation of 0.1 or 0.2 ml of *Brucella* allergen into the caudal fold or dorsal surface of the ear of goat intradermally. It can also be given at a dose of 0.5 ml subcutaneous or 0.1 ml intradermally in to the upper or lower eyelid (Alton *et al*, 1975).

The test is well suited to large scale testing and avoids collection of numerous blood samples and it may aid in the interpretation of serological reactions thought to be due to infection with other cross-reacting bacteria and hence highly specific (Alton *et al.*, 1975; Fensterbank, 1986). The allergic test seems to give positive results earlier than serological tests; also, serum antibodies increase titer after the test (FAO/WHO, 1986; Alton *et al.*, 1975).

The drawbacks of allergic test is that it does not differentiate infection from vaccination; animals recovered from infection may be detected as reactor, animals shedding the organism in the milk or genital secretions may be negative, hence less sensitive (Alton *et al*., 1975; Morgan and McKinnon, 1979; FAO/WHO, 1986)

The use of dermal hypersensitivity test in brucellosis has been suggested for several reasons including clarification of serological results, earlier detection of infection and for survey (Nicoletti, 1983). The skin test gave valuable information, in combination with the serological tests, in both acute and chronic brucellosis. The skin test discriminated brucellosis clearly from false positive serological reactions due to infections with Yersinia enterocolitica O9 (Saegerman *et al*., 1999; OIE, 2004).

The allergic test is recommended in France for diagnosing ovine brucellosis as part of official prevention policy; three countries Cyprus, Switzerland and the former USSR used the test in their control strategy (Fensterbank, 1986; FAO/WHO, 1986).

2.8.8. Molecular Diagnosis

Because of their potential to detect very small numbers of organisms, PCR-based assays have been applied recently to diagnose many infectious diseases (Al-Attas *et al*., 2000). Direct proof of *Brucella* infection is now done by identification of bacteria with well-established PCR (polymerase chain reaction). The high sensitivity and specificity of PCR-ELISA, together with its speed, versatility in sample handling, and risk reduction for laboratory personnel, makes this technique a very useful tool for the diagnosis of brucellosis (Morata *et al*., 2003).

2.9. Treatment

Treatment of infected cattle with antibiotics is not recommended because of the high treatment failure rate, cost, potential risk of maintaining infected animals, and antibiotic residues in cheese production (Walker, 1999). However, tetracycline and didihydrostreptomycin have been used to treat *Brucella* (Walker, 1999).

2.5. Public Health Importance

Human brucellosis is widely distributed all over the world, with high endemicity in the Mediterranean, Middle East, Latin America, and parts of Asia (Corbel, 1997). The true incidence of human brucellosis is unknown (Garrido-Abellan *et al*., 2001). More than 500,000 new cases are reported each year, and according to the World Health Organization, this figure underestimates the magnitude of the problem (WHO, 1997). In Saudi Arabia 7,893 human cases of brucellosis were recorded 74 per 100, 000 inhabitants (Acha and Szyfres, 2001). In Iran 71,051 cases 13 per 100,000 were recorded in 1988, and it is estimated that 80,000 cases have occurred each year since 1989. In Turkey 5,003 cases 9 per 100,000 were recorded in 1990 (Acha and Szyfres, 2001). The incidence in humans ranges widely between different regions, with values of

up to 200 cases per 100,000 populations (Orduña *et al*., 2000). Human brucellosis as geomedical survey show is known to exist in 37 of 49 (75.5%) African countries (Thimm, 1982).

Table 3: Human sero-reactors to *Brucella* antibody in Africa

Country	Number tested	Prevalence%	Serology	Author and year
Djibouti	108	6.5	CFT	Chantal *et al.*, 1994
Eritrea	130	7.1	CFT	Omer *et al*., 2000
Eritrea	21	4.6	CFT	Omer *et al*., 2000
Eritrea	105	3	CFT	Omer *et al*., 2000
Nigeria	13,999	7.6 – 29.8	SAT	Chukwu, 1985
Nigeria	738	5.55	SAT	Chukwu, 1985
Somalia	353	0.6	SAT	Hussein *et al.*, 1987
Tanzania	540	22.6	SAT	Chukwu, 1985
Uganda	3,164	6.4	SAT	Chukwu, 1985

The occurrence of human brucellosis in Ethiopia to our Knowledge was documented as early as 1972-1974 after the isolation of *Brucella* organisms from 3 blood culture (Efrem, 1981). The same author had reported serological and clinical brucellosis in a 12 year old girl from the highland area of Bale who suffered from fever, chills, sweat, headache, backache, epistaxis and weakness. The patient had been drinking raw milk and her sister had similar illness and died afterwards upon physical examination the spleen and liver were enlarged, fortunately the patient recovered after 3 weeks of treatment (Efrem, 1981).

Table 4: Human *Brucella* antibody sero-reactors in Ethiopia

Author and year	Total tested	Number positive	Prevalence%	Serology
Taye, 1991	8	1	12.5%	RBPT,CFT
Gebreyesus, 2001	49	12	24.5	RBPT
Kassahun, 2004	38	2	5.8%	RBPT, CFT
Tadele, 2004	126	3	3.4%	RBPT, CFT

The significance of brucellosis as a zoonosis has ever increased in recent times, as a result of expansion of international commerce in animals and animal products, increase urbanization with growing numbers of animals in closest proximity to people, increasing tourism (consumption of local animal products), and new methods of cattle production involving higher animal concentration (Weidman, 1991). Brucellosis is most common in rural areas and among those involved in animal husbandry it also occurs in urban settings when animals are kept in compounds around houses and among meat-packers, dairy workers and veterinarians (Smits *et al*., 1999).

Each human case originates from animals (Mustofa and Nicoletti, 1993). Human infection is frequently the first indication of brucellosis occurrence in the animals (Weidman, 1991). Brucellosis is a true zoonosis and the incidence of *Brucella abortus* in humans is directly influenced by the incidence in animal species (Nicolleti, 1980).

Human beings can be infected by *Brucella abortus, Brucella melitensis, Brucella suis, Brucella canis* and recently new types reported in marine mammals eventually pathogenic to man and unofficially designated "B. maris" (Aleixo *et al*., 1999). Human infection is acquired by contact with diseased animals and their excretion and by ingestion of animal products containing the pathogenic agent especially raw milk, curds and soft cheese (Weidman, 1991). Human infections also occur during laboratory manipulation of culture for vaccine or antigen production or diagnosis and infection due to accidental self-inoculation during the administration of live

vaccine (Morgan and McKinnon, 1979). Humans are accidental and almost always dead end hosts of *Brucella* infections; human-to-human transmission is very rare and no human to lower animal transmission has been reported (Nicoletti, 1980).

In human beings the onsets of clinical signs occur within 2-3 weeks of exposure; Clinical signs include recurrent fever, chills with night sweats, fatigue, muscle and joint pain and backache, depression and insomnia are common (Walker, 1999; Weidman, 1991). Symptoms and signs are nonspecific, and may simulate several other febrile illnesses such as glandular fever, influenza, malaria, and enteric infections (Smits *et al*., 1999).

Due to its heterogeneous and poorly specific clinical symptoms, the diagnosis of brucellosis always requires laboratory confirmation, either by isolation of the pathogen or by demonstration of specific antibodies (Morata *et al.*, 2003). On the other hand, blood culture sensitivity is often low, ranging from 50 to 90% depending on disease stage, *Brucella* species, culture medium, quantity of circulating bacteria, and the blood culture technique employed **(**Yagupsky, 1999).

At present there is a battery of serological tests, which can be used for diagnosis of human brucellosis, although each has its own important limitations. Their sensitivity is poor in the early stage of the disease, during which the levels of antibodies can still be low, and their specificity is reduced in areas where the disease is highly endemic, in exposed professionals, and in the frequent relapses of the disease (Morata *et al*., 2003).

Many serological tests have been used for the diagnosis of human brucellosis. The most commonly used tests are the serum agglutination test (SAT), the Coombs anti-*Brucella* test, the Rose Bengal test, and complement fixation (Orduña *et al*., 2000); the detection of *Brucella*-specific immunoglobulin M (IgM) antibodies allows the diagnosis of patients with brucellosis at an early stage or acute disease and also may help to discriminate between patients in the early phase of brucellosis and those with chronic brucellosis. In countries where the disease is highly endemic, a large proportion of the population may have persistent *Brucella*-specific IgG antibodies. Under such conditions, the detection of specific IgM antibodies is important to make the laboratory diagnosis of brucellosis in the early phase of the disease. Specific IgM antibodies

can be detected by SAT performed in the presence of either 2-mercaptoethanol (2-ME test) or dithiothreitol (SAT-DTT) and by ELISA (Smits *et al*., 1999).

The ELISA IgM and IgG tests achieved a specificity and sensitivity of 100% and 96% respectively, while the positive and negative predictive values were 100% and 94% respectively. The *Brucella* ELISA is a reliable and sensitive test in the diagnosis of brucellosis. The test is rapid, easy to perform and can be automated (Osoba *et al*., 2001).

In situations where appropriate diagnostic facilities are lacking, a colorimetric test so called dipstick assay with two horizontal bands an antigen band consisting of reactive *Brucella* antigen (lower band) and an internal control (upper band) could be employed (Smits *et al*., 1999). It is an easy-to-perform method for the quick serodiagnosis of acute human brucellosis. Due to its robustness and simplicity, the assay is highly suitable for application under field conditions (Smits *et al*., 1999). Ideally, application of two dipsticks, one for the detection of specific IgM antibodies and another for the detection of specific IgG antibodies, would be needed to cover the possibility of both acute or recent and chronic brucellosis (Smits *et al*., 1999). The sensitivity of the dipstick assay was 93.5% when only blood culture proven brucellosis patients were considered and the specificity was calculated to be 98.6 %(Smits *et al.*, 1999).

The PCR-based assay has several advantages over the current microbiological methods for the diagnosis of brucellosis, including speed, safety, high sensitivity (100%) and specificity (88%), therefore, it should be considered (Al-Attas *et al*.,2000). For evaluation of asymptomatic, occupationally exposed persons, the traditional methods might be superior, especially if the cost is taken into consideration. Nevertheless, the information provided by PCR should be considered complementary to the results of conventional methods for the time being (Al-Attas *et al*., 2000).

The best drug recommended by WHO is rifampicin at a dosage of 600-900 mg daily combined with doxicycline at 200mg daily, both drugs are given in the morning as a single dose and relapse is unusual after a course of treatment continued for at least 6 weeks (FAO/WHO, 1986).

2.6. Economic Importance

It is estimated that brucellosis causes heavy economic losses in livestock producers. The economic losses stem from abortion, diminished milk production, cull and condemnation of animals due to breeding failure, endangering animal export trade of a nation particularly when other countries purchase only *Brucella* free animals as West Germany does and EU regulations require that all animals above six months of age be tested, human brucellosis causing loss of man hours and medical costs and government costs on research and eradication schemes (Chukwu, 1987 ; Mustofa and Nicoletti, 1993)

Official estimates put annual losses from bovine brucellosis in Latin America at approximately US $ 600 million (Acha and Szyfres, 2001). The New Zealand brucellosis eradication scheme found a 10.3% internal rate of return as a result of increased milk yield decreased culling rate and market shares after successful eradication of the disease (FAO, 1998).

Unfortunately, only few controlled studies have been conducted to evaluate the effect of brucellosis on reproductive efficacy and the cost of various control methods. The inflammatory changes in the infected mammary gland reduce milk production by an estimated 10% (FAO/WHO, 1986).

2.10. Control and Prevention

The probability of success in any zoonotic disease control/eradication program considers the following pre-requisites; effective method for stopping or reducing agent transmission, high socio-economic importance, epidemiological features that allow good case detection and good surveillance for measuring progress and providing information (Robinson, 2003). The control and prevention measures to be adopted on brucellosis should be realistically based on thorough understanding of local and regional variations in animal husbandry practices, social customs, infrastructure and epidemiological patterns of the disease (Mustofa and Nicoletti, 1993).

2.10.1. Surveillance

Surveillance of brucellosis is achieved through herd screening using RBPT or card test, MRT and allergic skin test and confirmation using more specific tests CFT or ELISA (Musutofa and Nicoletti, 1993; Bayleyegn, 2004).

2.10.2. Immunization

2.10.2.1. Strain 19 (S 19)

Despite more than a half-century of research S-19 vaccines remains the most widely accepted immunizing vaccine against bovine brucellosis, which remains also the reference vaccine to which any other vaccines are compared (Nicolleti, 1980; OIE, 2004). It is used as a live vaccine and is normally given to female calves aged between 3 and 6 months as a single subcutaneous dose of 5–8 x 10^{10} viable organisms. A reduced dose from 3 x 10^{8} to 3 x 10^{9} organisms can be administered subcutaneously to adult cattle, but some animals will develop persistent antibody titers and may abort and excrete the vaccine strain in the milk. Alternatively, it can be administered to cattle of any age as two doses of 5–10 x 10^{9} viable organisms, given by the conjunctival route; this produces protection without a persistent antibody response and reduces the risks of abortion and excretion in milk (OIE, 2004).

The often quoted protection rate of S-19 is about 65 - 70% is largely based upon individual cattle challenge with standardized strains and doses. The protection on a herd basis is much greater due to reduction of clinical symptoms and increased herd resistance (Nicoletti, 1980). Whenever an unclear epizootiological situation exists, in the extensive animal production systems in Africa, vaccination with S 19 ought to be the best alternative (Seifert, 1996).

2.10.2.2. 45/20 (Dyphavac)

This vaccine is prepared from killed rough *Brucella abortus* strain 45/20 in oil adjuvant; it lacks the smooth LPS antigen that interferes with serological diagnosis. It can be used to animals of all age and also to pregnant cows (FAO/WHO, 1986). In Australia, the vaccine is applied for the anamnestic test in order to identify carrier animals in which case simple test methods do not detect incomplete antibodies. After application of 45/20, such hidden reactors produce agglutinating antibodies (FAO/WHO, 1986; Seifert, 1996). Usually two doses of 45/20 vaccines 6-12 weeks apart are considered to be necessary to induce good immunity, and an annual booster is generally recommended (FAO/WHO, 1986). The duration of immunity by 45/20 vaccine has not yet been established (FAO/WHO, 1986).

2.10.2.3. RB51

Recently, a new vaccine strain (B. abortus RB51) was developed and one vaccination with this strain is as effective as one vaccination with S-19 in protecting cattle against brucellosis (Cheville *et al*., 1993, 1996). Vaccine strain RB51 (S-RB51) is an attenuated, rough organism, which essentially lacks the O-side chain of the LPS and if given singly or multiple times does not induce antibodies that interfere with conventional diagnostic tests (Schurig *et al*., 1991).

Vaccine S-RB51 is now the official vaccine being used in the USA (replacing S-19), and its use has been approved and initiated in Chile, Mexico, Venezuela, Colombia and Argentina. In the USA, the official recommendation is calf hood vaccination with $1x10^{10}$ to $3 - 4x10^{10}$ colony-forming units (cfu) of RB51 and the suggested dose for adult animals is $1–10^{9}$ organisms. Presently studies confirm that vaccination of adult cattle with S-RB51 does not induce detectable antibodies when using the conventional serological tests used for the diagnosis of brucellosis (BPAT, RBT, 2-ME and CFT) or the new indirect and competitive ELISA tests designed to detect specific antibodies to the O-chain of the LPS (Stevens *et al,* 1994; Nielsen *et al*., 1995).
Several studies have reported that RB51 vaccine has proven effective for the vaccination of cattle and compatible with a control strategy based on sanitary measures alone (Cheville *et al*., 1993; 1996). *Brucella abortus* strain RB51 has shown no tendency to revert to virulent smooth

organisms after many passages in vitro or in vivo. This is probably due to the nature and place of the mutations found in this strain (OIE, 2004).

2.10.3. Test and slaughter of positive reactors

This is made possible when the sero-prevalence is reduced to less than 2% (Walker, 1999; Weidmann, 1991). Animals, which are positive to both RBPT and CFT, are slaughtered.

2.10.4. Management and hygienic measures

These include safe disposal of aborted fetus, disinfection of contaminated areas and education of the public not to drink raw milk or eat products made from unpasteurized or otherwise untreated milk (Weidmann, 1991; Mustafa and Necolitte 1993; Bayleyegn, 2004)

3. MATERIAL AND METHODS

3.1. Study Area

A cross sectional study was conducted in eleven milk-shed *weredas* that are located in South Gondar, West Gojjam and Awi administrative zones. All the *weredas* are located within 150kms radius from Bahir dar town. Eighty-five peasant associations and town kebeles in the 11 *weredas* were studied to supply milk to milk processing plant yet to be established at Bahirdar town (Addisu and Zelalem, 2003). From the 85 peasant associations and town kebeles only 29 Peasant associations and 11 towns were selected for our study.

The study area is characterized by different landscapes, highland (Farta, Banja- Shikudad and Fagita-Lekoma), Medium altitude (Dangila, Achefer, Mecha, Bahir dar zuria and Yilmana Densa) and medium altitude with rolling plain (Fogera, Dera and some parts of Libo Kemkem) (Table 5). The climate of the study area is marked by two distinct seasons; dry season, which prevails from December to May "Bega", and rainy "Kiremt" running from June to October. There is a small rainy season in late February and March. The climate of the study area is traditionally classified into three major categories dry land ("Kolla") having an altitude of <1500 masl representing 5.8% of the study area, midland ("Weynadega") with an altitude ranging between 1500-2400 masl and Highland ("Dega") having >2400 masl, respectively, constitute 67.1% and 27.1% of the total area (Table 5). Three types of soil predominate in the study areas with the proportion of 31.85% black soil, 47.6% clay soil and 20.6% loam soil. The farming system practiced in the area is of traditional small holding mixed crop-livestock type. Urban and peri-urban people exercise some semi-intensive livestock farming. There has been one government cattle breeding ranch established to disseminate Frisian and Fogera cross (F1) heifers to farmers with the view to augment milk production. The largest river and Lake of Ethiopia, Abay and Tana, respectively, are found in this area.

Andasa ranch (currently renamed as cattle breeding and multiplication center)

Andasa ranch is found in Bahir dar zuria *wereda* about 22km South east of Bahirdar town, on the way to Tis Abay falls, situated in an area having an altitude of 1730 m asl and located between 11^0 29^1 latitude north and $37^0 29^1$ longitude east. The total area of the ranch is 360 ha, nearly 110 ha is natural grassland mainly used for grazing and 115 ha is water logged and is covered with natural grass vegetation during the rainy season and utilized for hay making and natural grazing during the dry season. The remaining, 10 ha is cultivated for improved forage production and the other covered with forest and river basin (Gidey, 2001).

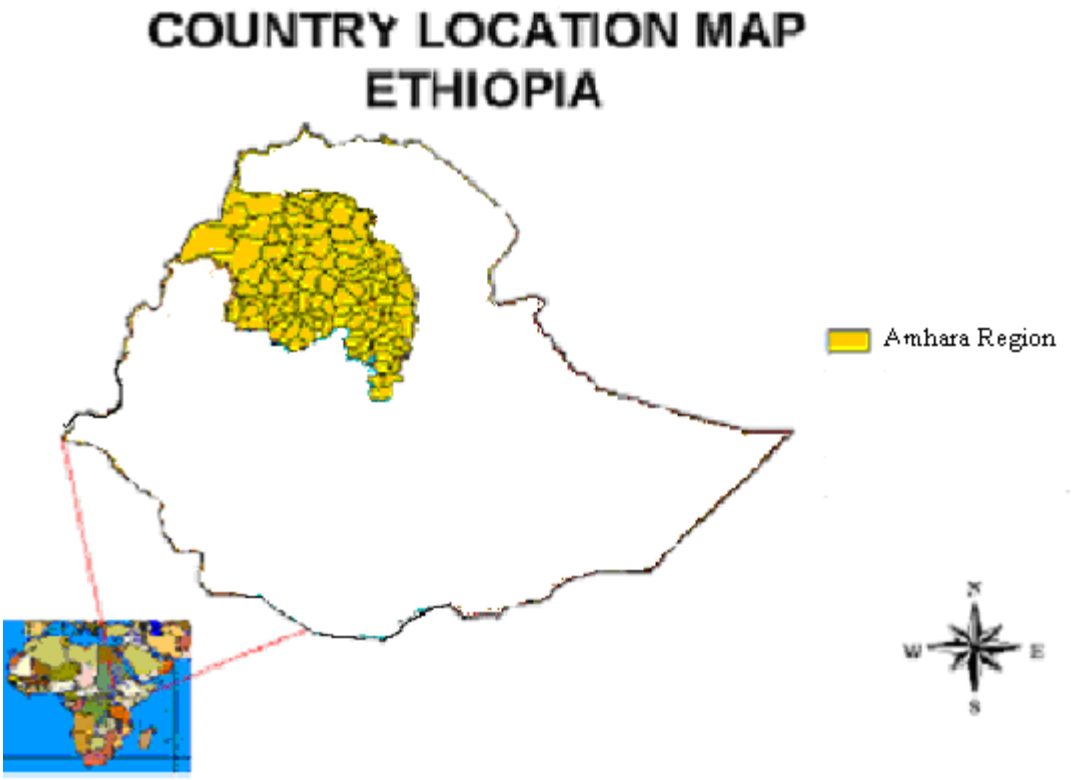

Figure 1: Map of Ethiopia

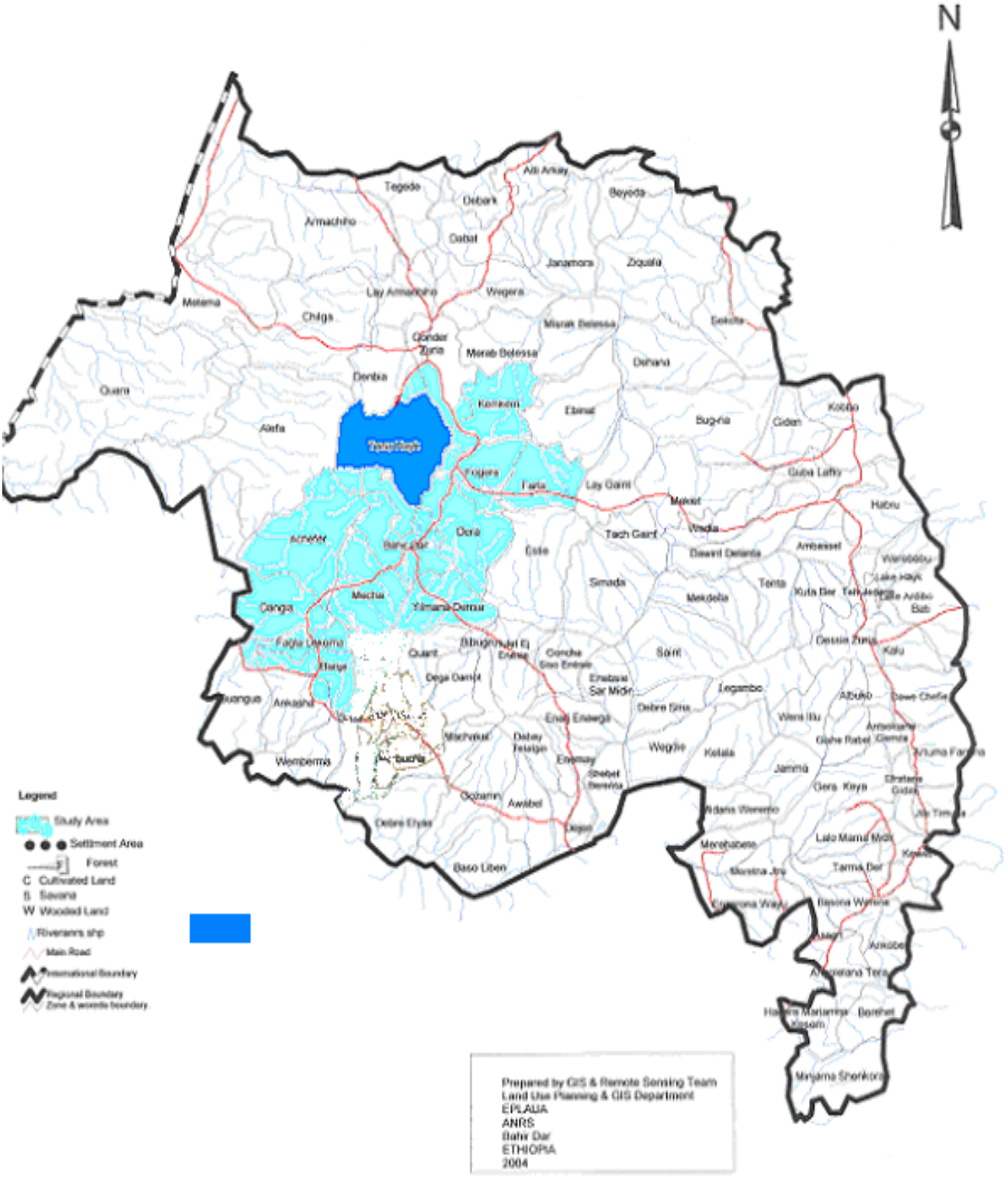

Figure 2: Map of the study area

Table 5: The eleven milk-shed *weredas*, Pas and agro-climate (Awi, West Gojjam and South Gondar)

Name of zone	Name of *Wereda*	Area in square km.	Number of Target PAs	Number of study PAs	Temperature in ^{0}C	Annul rainfall in mm	Altitude masl
Awi	Banja shikudad	566.55	5	1	16-26	2000-2120	1900-2700
Awi	Fagta Lekoma	579.52	3	2	20-26	2370-2372	1800-2952
Awi	Dangila	4,191.68	9	4	16-38	1481.25	600-2300
W.Gojjam	Achefer	-	6	3	25-29	1450-2500	1500-2300
W.Gojjam	Mecha	1,598.99	6	3	24-27	1500	1800-2500
W.Gojjam	Bahirdar zuria	1,511.19	23 7 rural 16 urban	3 rural	10-32	800-1250	1730-2300
W.Gojjam	Yilma and Densa	1,309.92	2	-	-	-	-
S. Gondar	Dera	1,691.88	8	4	16-22	900-1600	1560-2600
S. Gondar	Fogera	1,041.12	9	3	20-22	900-1200	1850
South Gondar	Libo Kemkem	832.19	6	2	13-24	900-1400	1800-2800
South Gondar	Farta	1,137.79	8	4	9.9-21	1250-1599	1920-4135
Total		14,460.83	85	29	9.9- 32	600-2500	600- 4135

Source: Office of agriculture of the respective *weredas.*

3.2. Target and study populations

Cattle

The total cattle population in the 11 *weredas* was estimated to be 1,805,759, of which 1,799,275 were local breeds and the crossbreeds amounted to 5,703. The selected 85 rural PAs and town *kebeles* harbor a total of 484,089 cattle with 477,729 being local and 5,703 cross breeds, and this was our target population (Table 6). From the 85 Peasant associations and town *kebeles* only 29 peasant associations and 11 towns were selected, and these were supposed to represent the extensive and semi-intensive production systems, respectively, and thus were our study area having a total 144,080 local and 5,703 cross breed cattle (Table 6). There was one government cattle breeding ranch in the study area established to supply Fogera and Friesian cross F1 heifers to farmers in order to augment milk production in the area. Almost all the three hundred and twenty six eligible cattle were possibly sampled from the ranch.

The types of cattle breeds that are raised in the study area are local (Fogera and non-descript zebu) and crosses of Fogera x Friesian. Fogera cattle, which is the dominant cattle breed in the study site is one of the five distinguishable main cattle breed types identified in Ethiopia. The breed belongs to intermediate zebu-sanga type originated around Lake Tana in Gondar and Gojjam administrative zones, and is characterized by having straight profile with medium head size. Horns are stumpy, small and pointed up and out and many are pooled. The dewlap is long and folded, the hump is small in size and cervical and cervico-thoracic in position. The navel flap in females and perpetual sheath in males is very long and pendulous. From the wither, the back goes straight up to the beginning of the rump, and the rump slops down very steeply from the pins to hocks. They are generally of larger size, being tall with long legs. Their coat colors varies, white with black spots or pure white; muzzle and hooves are almost always black (Alberro and Hailemariam, 1982).

Table 6: Eleven milk-shed weredas and their livestock population in (Awi, West Gojjam, and South Gondar zones)

Zone	Wereda	Target population		Study population		Sample population	
		Local breed	Cross breed	Local breed	Cross breed	Local breed	Cross breed
Awi	Fagita Lekoma	119,525	71	13,829	71	57	8
Awi	Banja Shikudad	122,512	89	1,791	89	27	12
Awi	Dangila	191,963	229	15,991	229	116	30
W.Gojjam	Achefer	218,944	41	25,758	41	86	6
W.Gojjam	Mecha	193,636	14	18,976	14	96	-
W.Gojjam	Yilma and Densa	186,671	183	-	183	-	23
W.Gojjam	Bahirdar Zuria	176,075	2,133	13,965	2,133	78	284
S.Gondar	Farta	182,728	2,272	10,043	2,272	115	298
S.Gondar	Libo Kemkem	113,633	415	3,075	415	58	59
S.Gondar	Fogera	189,199	195	20,419	195	91	26
S.Gondar	Dera	104,389	61	17,209	61	140	8
Total	Eleven *weredas*	1,799,275	5703	144,080	5703	864	754

Source: Office of agriculture of the respective weredas

Human Population

According to Addisu and Zelalem (2003) the total human population of the study area is estimated to 2,716,527 of which 2,367,446 were rural and 349,081 were urban dwellers. The number of livestock development and animal health service providers in the study area was such that, 114 animal health professionals, 9 AI technicians, 28 breeding and feed development experts.

3.3. Study Design

3.3.1. Prevalence Study

Cross-sectional study was conducted to determine overall prevalence, cluster (herd) prevalence and within cluster prevalence.

3.3.2. Sampling method and Sample size

Three types of cattle production systems were classified as ranch, semi-intensive and extensive. The semi-intensive production system is mainly composed of cross bred cattle and depends highly on feed supplementation at home; occasionally they are allowed to graze around the homestead. The extensive production system consisted of local Fogera and non-descriptive breeds of cattle which depend for feed on grazing at the field with minor supplementation at night when they come back home. The ranch consisted of both indigenous and cross breed cattle.

The sampling method employed was two-stage cluster sampling. The primary and secondary units in the extensive production system were PAs and individual cattle owners, respectively; accordingly, primary units (PAs) were selected following random procedure while secondary units(cattle owners) did not have sampling frame and hence were sampled by selecting any 5 cattle owners encountered at the beginning of the study. In the case of semi-intensive production

systems the primary units were towns and secondary units were farms (individual cattle owner) where at each stage sampling units were selected using simple random sampling method. Owing to the small number of cattle kept in the government cattle breeding ranch all-eligible cattle were sampled.

Sample size for extensive and semi-intensive production systems was determined using cluster sample formula (Thrusfield, 1995).

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

Where,

Ts = Total sample size

p= prevalence

Q= 1- prevalence

g= cluster

d= desired absolute precision

Vc= between cluster variance

The average number of cattle kept in the extensive production system was assumed to be (n= 6) cattle, a total of 145 clusters from (29 PAs) and 5 cattle owners(secondary units) from each PAs were pre-determined to be selected, with 95% confidence level, 10% expected prevalence 8.2% (Gebreyesus, 2001), and 2% desired absolute precision (d^2). The between cluster variance (Vc) for each cluster was not available and thus, determined by guessing the standard deviation (i.e. the average difference expected between an individual cluster prevalence and the overall mean cluster prevalence). Squaring the standard deviation helps to estimate the variance component between clusters (Thrusfield, 1995). Therefore the standard deviation was 0.10^2 and squaring it gave the variance 0.0001; thus, giving 870 cattle. Yet due to various technical inconveniences in the course of the work only 864 serum samples were made available for laboratory testing and further analysis.

The sample size in the semi-intensive system was determined following the same formula while the average cluster size was guessed to be 4 cattle. At the outset 11 towns (primary units) and 195

(g) clusters (farms) from the 11 towns were pre- determined to be sampled with 95% confidence level, 10% expected prevalence 8.1% (Yilkal *et al*., 1998), 2% desired absolute precision and the between cluster variance was guessed to be 0.0001. Thus, giving sample size (n) of 868. However, as the calculated sample size was more than 10% of the population size which was 13.38% an adjustment was made using the formula (Thrusfield, 1995).

$$n_{adj} = \frac{N \times n}{N + n}$$

Where N= is total population

n= is calculated sample size

$$\text{Thus } n_{adj} = \frac{5783 \times 868}{5783 + 868}$$

Therefore adjusted sample size was 754 cattle

In the government owned breeding ranch all eligible cattle were sampled (n=326).

The sample sizes for human risk groups was calculated using simple random formula (Thrusfield, 1995).The prevalence was predetermined to be 20% . Ninety five percent confidence level and 5% absolute precision were used during the calculation. The sampling technique employed for human risk groups was purposive sampling technique which included veterinarians, AI technicians and farmers. For the professionals sampling was undertaken at their respective working sites while for the farmers serum samples were collected at Bahirdar Felegehiwot hospital.

$$N = \frac{1.96^2 \times p\,(q)}{d^2}$$

Where, p is prevalence (20%)

q is 1- prevalence

d absolute desired precision

Thus the calculated sample size was 246 human risk groups; however, due to inconveniences during transportation, 8 samples were perished and, hence, only 238 samples were made available for testing and subsequent analysis.

3.3.3. Serological tests

Blood sample was collected from jugular vein of each cattle greater than 6 months of age with no history of vaccination from the selected cattle using plain vacutainer tubes. The blood samples were allowed to clot at room temperature. Then serum was separated from clotted blood by centrifugation. The Separated sera were stored at –20 ^{0}C until tested by both RBPT and CFT.

3.3.3.1. Rose Bengal Plate Test

Materials used

RBPT *Brucella* antigen
Positive control serum
Negative control serum from previously tested negative serum
Glass slide
Micropipette
Micropipette tip
Mixing applicator

Test procedure

All serum samples collected were screened using RBPT. The antigen consisted of a suspension of *Brucella abortus* obtained from (Institut Purquier 326, Rue de la Galera 34097 MONTPELLIER CEDEX 5, France) inactivated by heat and 0.5% phenol, adjusted to pH 3.65 and colored with Rose Bengal. The procedure described by Alton *et al*. (1975) was used.

Briefly, test sera and antigen were left at room temperature for half an hour before the test.

- 30 μl of each test serum was taken and placed on a clean glass slide,
- 30 μl of RBPT antigen was added to the side of each test serum using a dropper,
- Then, the antigen and test serum mixed thoroughly by an applicator,
- The slide was shacked by hand for 4 minutes and,

The results were read by examining the degree of agglutination in good light source and when deemed necessary using magnifying glass.
Interpretation: After four minutes rocking, any visible agglutination was considered as positive.

3.3.3.2. Complement Fixation Test

The CFT is a widely used and accepted confirmatory test although it is complex to perform, requiring good laboratory facilities, and adequately trained staff to accurately titrate and maintain the reagents. Numerous variations of the test occur, but each may be most conveniently carried out using micro titration plates (OIE, 2004). Sera that are positive to RBPT were further tested by CFT for confirmation.

Complement fixation test had been undertaken at the National Veterinary Institute Department of Immunology, and preparation of the reagents was performed according to the protocols recommended. Standard *Brucella abortus* antigen S 99 (CVL, New Haw Weybridg, and Surry KT15 3NB, UK) was used. Antigen, control sera and complement were obtained from the BgVV, Berlin, Germany.

Interpretation: Serum with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1: 5 and at least with 50% fixation of complement (2%) at a dilution of 1:10 and at dilution of 1:20 were classified as positive (Alton , *et al*., 1975; OIE, 2004).

3.3.4. Questionnaire survey

Fifty randomly selected livestock owners from the extensive production system (n=145) were interviewed using pre-tested structured questionnaire to determine management and husbandry risk factors, which are known or thought to influence the spread and maintenance of brucellosis. Productive and reproductive parameters presumed to be affected by brucellosis were also incorporated in the questionnaire. The respondent recall memory was used as a data source. This result was then compared with serological results.

3.3.4. Public health survey

Blood sample was collected from 238 risk groups (222 farmers, 14 veterinarian and 2 AI technicians) in collaboration with *wereda* health offices and Bahir dar Felegehiwot hospital. Relevant risk factors(removal of retained fetal membrane, drinking raw milk and eating raw meat) and clinical manifestations (recurrent fever, chills with night sweats, fatigue, muscle and joint pain, headache, weakness, and anorexia) pertaining to brucellosis were also gathered during serum collection. The separation and storage of human sera followed similar procedure. The serological tests employed were RBPT for screening and sera tested positive to RBPT were further tested with CFT. Only serum samples that were positive to both RBPT and CFT were considered positive and used for the subsequent analysis.

4. DATA ANALYSIS

Data obtained from both serological tests and questionnaire were stored in Microsoft excel spreadsheet. These data then were analyzed by descriptive statistic using SPSS 11.5 for window and analytical statistics using Intercooled Stata 7.0 soft wares. Cattle and humans tested positive to both RBPT and CFT serially were said to be sero-positive. Clusters (herds) having at least one sero-positive cattle were considered to be positive. The individual animal and human level sero-prevalence was calculated on the basis of RBPT and CFT positive results divided by total number of animals and humans tested. Similarly, herd (clusters) level sero-prevalence was computed as the number of clusters (herds) with at least one positive animal divided by the total number of clusters (herds) tested. The within cluster (herd) sero-prevalence was calculated by dividing the number of positive reactors in the cluster (herd) by total number of animals tested in that cluster (herd) (Thrusfield, 1995).

Questionnaire data was expected to provide some information about the existence of brucellosis. Accordingly, the data included risk factors associated with management, husbandry, presence or absence of dogs, Culling of frequently aborting cow, reasons for sell of breeding cow, method of disposal of fetal membrane, dry season watering and those reproductive parameters thought to be influenced by the disease like calf crop, age at first calving and calving interval were also administered. The results obtained from questionnaire were compared with that of serological results.

Generally, the kinds of descriptive statistics used were frequency Tables, bar charts and graph plotting; quantification of the disease was accomplished by rate, proportion, ratio, mean, variance and standard deviation. Kinds of analytical statistics employed were univariate logistic regression analysis, Pearson's chi-square test (χ^2) and when deemed necessary Fisher's exact test. These tests determine the association of risk factors with the sero-prevalence of brucellosis. The degree of association between potential risk factors and sero-prevalence was computed using odds ratio. During analysis of the data sero-prevalence was considered as dependent variable and was designated as (1) for sero-positivity and (0) for sero-negativity. And risk factors that would likely

predict the outcome variable in this case sero-prevalence were considered as independent variables.

5. RESULTS

Overall

Serum samples were collected from 341clusters (herds) having (1944) cattle above 6 months of age with no history of previous vaccination against brucellosis. The sampling involved the three-production systems semi-intensive consisting of 195 clusters (n=754), extensive 145 clusters (n=864) and ranch only 1 cluster (n=326). Ninety-six (n=96) sera were found to be positive to RBPT; upon further testing of the (n=96) RBPT positive sera with CFT only 90 sera became positive. Thus, the subsequent analyses were based on the ninety (n=90) sera that were positive to both the RBPT and CFT (serial interpretation) test results.

The overall individual and cluster (farm) level cattle sero-prevalence of the study area were 4.63% (n=90), and 14.96%. (n=51), respectively (Table 7). The overall within cluster sero-prevalence ranged between 0% and 100% with an overall mean (±SD) of 24.27% and (±17.68%).

Administrative zone

Comparison of the sero-prevalence to *Brucella* antibody in the three administrative zones was conducted to ascertain its status in different localities. The overall individual cattle sero-prevalences among the three administrative zones: Awi, West Gojjam and South Gondar were in the order of 9.6%, 6.12% and 1.38% (Table 7), respectively; there was a statistically significant difference among them (P = 0.000) (Table 10). Pair with comparison between zones revealed significant difference between Awi and South Gondar zones (P= 0.000) and between West Gojjam and South Gondar zones (P=0.000), however, Awi and West Gojjam zones were not significantly different (P=0.054) (Table 10).

Cluster (farm) level sero-prevalence of 32.07%, 32.88% and 4.65% were found, respectively, in Awi, West Gojjam and South Gondar administrative zones (Table 7); and there was significant difference among the three zones (p= 0.000). Pair wise comparison between the zones was such that South Gondar against Awi and South Gondar against West Gojjam zones revealed statistical

significance difference (p= 0.000) and (p= 0.000), respectively. However, Awi and West Gojjam zones did not show statistical difference (p= 0.499) (Table10)

Table 7: Overall individual and cluster level *Brucella* antibody sero-prevalence in Awi, West Gojjam and South Gondar zones

Zone	Number tested	Number and (%) positive	95% confidence interval	Number of cluster	Number and (%) positive cluster	95% confidence interval
South Gondar	795	11 (1.38%)	0.57%- 2.19%	215	10 (4.65%)	1.84% - 7.46%
West Gojjam	899	55 (6.12%)	4.55%- 7.69%	73	24 (32.88%)	22.1% - 43.66%
Awi	250	24 (9.6%)	5.95%-13.25%	53	17 (32.07%)	19.5% - 44.64%
Total	1944	90 (4.63%)	3.96% - 5.56%	341	51 (14.96%)	11.23% - 18.69%

Agro climate

Based on altitude differences the target area was broadly classified in to midland or "Weynadega" (1500-2400 masl), highland or "Dega" (> 2400 masl) and lowland "Kola" (< 1500). The study had excluded the lowland as the lowland PAs were out side the milk-shed areas. Thus, comparison was made on the sero-prevalences of the Highland ("Dega") having 1.97% and Midland ("Weynadega") with 5.61% (Table 8). There was a highly significant variation in sero-prevalence between the two agro climates at both individual level (p=0.000) and cluster level (p= 0.000) (Table 10).

Table 8: Comparison of *Brucella* antibody sero-reactor cattle between midland and highland (in Awi, West Gojjam and South Gondar)

Agro climate	Number tested	Number and (%) positive	95% confidence interval	Number of clusters	Number and (%) positive cluster	95% confidence interval
Highland ("Dega")	517	10(1.97%)	1.24% - 2.70%	158	10 (6.33%)	2.53-10.13%
Midland ("Weynadega")	1427	80(5.61%)	4.42% - 6.80%	183	41(22.4%)	16.36-28.44%
Total	1944	90(4.63%)	3.96%- 5.56%	341	51(14.96%)	11.23-18.69%

Production systems

The study was based on three cattle production systems. These include extensive which consisted of local Fogera breeds of cattle which depend for feed on grazing at the field with minor supplementation at night when they come back home, and semi-intensive mainly composed of cross bred cattle and depend highly on feed supplementation at home, occasionally they are allowed to graze around the homestead. There was also a ranch having both cross and local breeds of cattle with the local breeds predominating.

Individual level positive reactors to *Brucella* antibody of the three production systems were 3.82% for extensive, 5.97% for semi-intensive and 3.68% ranch (Table 9). The overall individual sero-prevalence among the three production systems did not vary significantly (p=0.082) (Table 10).

Cluster level sero-prevalence of the ranch was 100%, owing to a single ranch being sampled and hence was excluded during comparison to avoid over estimation of cluster level sero-prevalence. Therefore, cluster level sero-prevalence was computed for extensive and semi-intensive productions with prevalences of 17.25% and 12.82%, respectively (Table 9). However, their difference was not significant (p=0.255) (Table 10).

Table 9: *Brucella* antibody sero- reactor cattle in extensive, semi-intensive and ranch (Awi, West Gojjam and South Gondar)

Production System	Number tested	Number and (%) positive	95% confidence interval	Number of clusters tested	Number and (%) positive cluster	95% confidence interval
Extensive	864	33 (3.82%)	2.54%- 5.09%	145	25 (17.24%)	11.09%-23.39%
Semi-intensive	754	45 (5.97%)	4.92% -7.66%	195	25 (12.82%)	8.13% - 17.51%
Ranch	326	12 (3.68%)	1.62%- 5.72%	1	1 (100%)	100%
Total	1944	90 (4.63%)	3.96%- 5.56%	341	51 (14.96%)	11.23% -18.69%

The within cluster sero-prevalence

The within cluster sero-prevalence of extensive production system ranged between 0% and 50% with an average (±SD) sero-prevalence of 25.44% (±10.45).

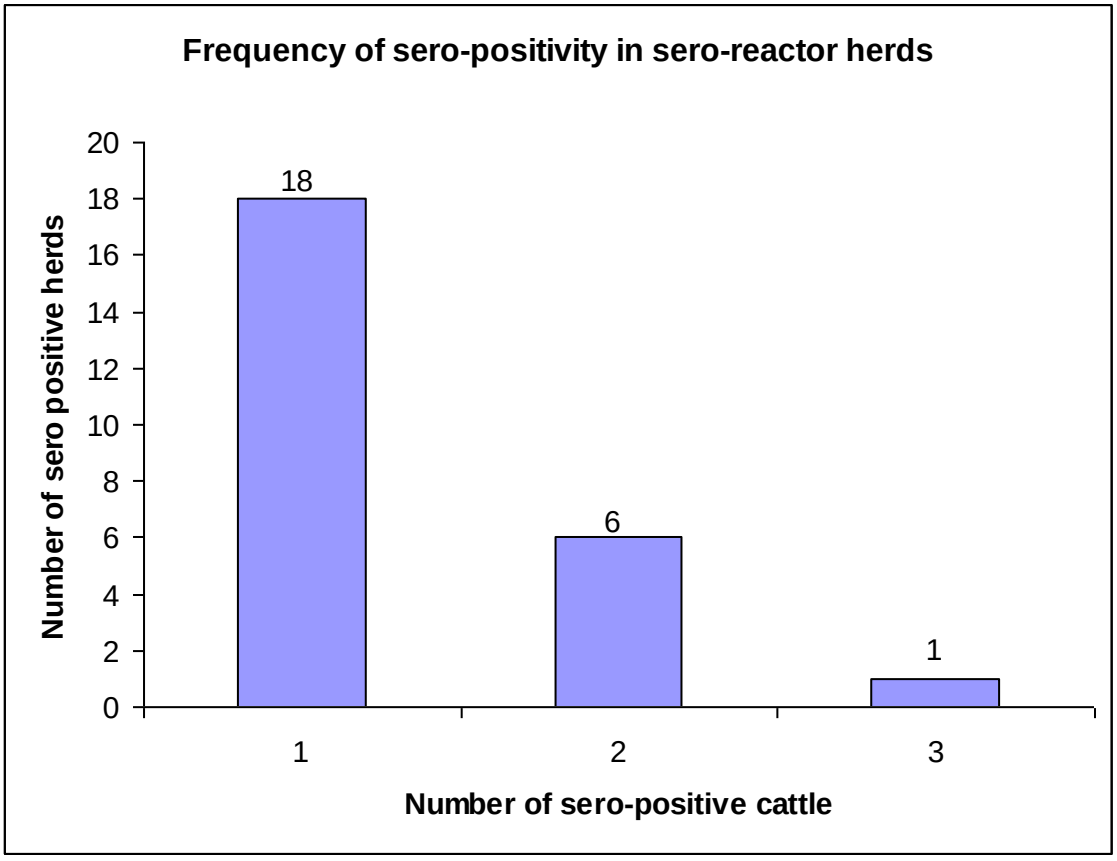

Figure 3: Frequency of sero-positive herds in respect to the number of positive cattle in the extensive production system

At the same time the within cluster sero-prevalence of semi-intensive production varied between 0% and 100% with a mean (±SD) sero-prevalence of 26.82 % (±22.52).

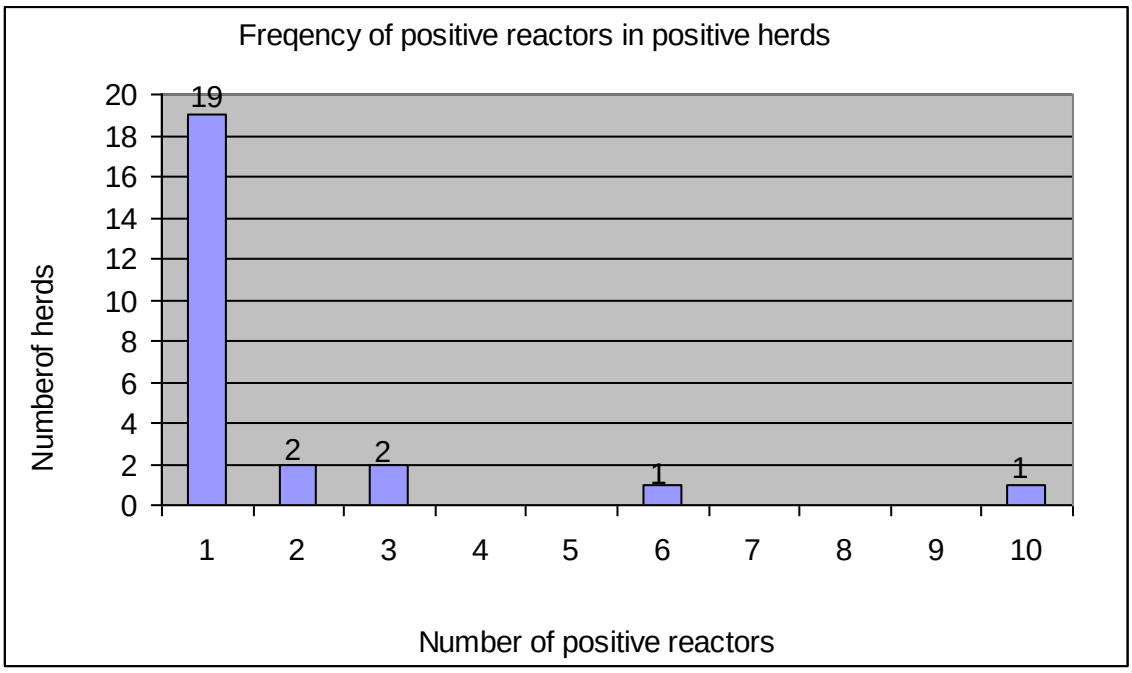

Figure 4: Distribution of positive reactor (cluster) herds against the number of sero-positive cattle in the semi-intensive production system

Table 10: Statistical analysis results of individual and cluster level *Brucella* antibody sero-reactors in Awi, West Gojjam and South Gondar zones, two agro climates (midland and highland) and three production systems (extensive, semi-intensive and ranch)

Variables	Individual sero-prevalence			Cluster sero-prevalence		
	DF	χ^2	P-value	Df	$\chi 2$	P-value
Zones Awi West Gojjam South Gondar	2	37.47	0.000	2	49.37	0.000
Agro climate High land("Dega") Midland("Weynadega")	1	11.5890	0.000	1	19.37	0.000
Production systems Extensive Semi-intensive Ranch	2	5.006	0.082	1	1.29	0.255

Df =degree of freedom $\chi 2$= chi square p-value= probability value

Potential Risk factors

Sex

Sex has some implication in the epidemiology of brucellosis; accordingly, comparison was made on the sero-prevalence of female and male. The sero-prevalence of females was 4.47%, 6.82% and 3.99% for extensive, semi-intensive and ranch, respectively, and of males 2.11% for extensive, 0.92% for semi-intensive and 2% for the ranch (Table11). In the extensive production and ranch there was no statistical difference between the two sexes. However, in both productions systems it was found out that sero-reactors were at higher proportions in females than males. The semi-intensive production showed a different scenario where there was significant difference between females and males with (OR=7.9, P=0.042). However, the findings should be seen in the light of the fewer males tested.

Age

Age is supposed to have some association with the recovery of antibodies against brucellosis, as sexual maturity is important for the multiplication of *Brucella.* Therefore, based on its biological relevance age was classified into three categories. In the extensive production sero-prevalences of *Brucella* antibody in the three age categories were in the order of 2.70%, 4.22% and 4.23% with age groups of 0.5-1 year, >2- 3 years and > 3 years (Table 11.), respectively. In the semi-intensive production system sero-prevalences of 2.94%, 2.30% and 9.31%, respectively, were found in the three age categories of 0.5- 1year, >1- 3 and > 3 years %(Table 11). In the ranch age groups of 0.5- 1 years, >2- 3 years and > 3 years, respectively, had sero-prevalences of 0%, 3.85% and 4.04% (Table 11). Highly statistically significant difference was observed in the three age categories of the semi-intensive production system with (OR=2.64, P= 0.001). However, the three age categories were not significantly different in the ranch and extensive production systems despite the increase in the proportion of sero reactors with age.

Herd size

Herd size is one of the risk factors that affect the occurrence of brucellosis. Thus, based on the prevailing livestock holding of the study area the herd was categorized in to three groups. Sero-prevalences of 12.16%, 24.14%and 15.38% were, respectively, found from herd categories of ≤ 5 cattle, > 5- 10 cattle and > 10 cattle in the three productions (Table 11). In the semi-intensive production the three herd categories, ≤ 5 cattle, 5- 10 cattle and > 10 cattle had sero-prevalences of 6.47%, 40% and 80%, respectively (Table 11.). The ranch was not statistically computed as it is a single ranch sampled, yet it was an infected ranch. Highly significant variation between the three herd categories (OR= 8.16, P= 0.000) was the case in semi-intensive production whereas extensive production system revealed no significant association among the three herd categories, yet it was evident that larger sized herds were found at higher proportion than smaller herds.

Breed

There is discrepancy among different authors on the issue of breed susceptibility to brucellosis. However; Radostitis *et al*. (2000) clearly stated that there is no breed difference. Based on this fact we have attempted to look into the association of breed to that of sero-positivity to *Brucella* antibodies, the comparison was undertaken in the ranch where both indigenous and crosses (Friesian and indigenous) were kept together. Sero-prevalences of 2.22% and 3.91% were, respectively, found in crossbred and indigenous cattle, yet there was no significant variation between them (Table 11).

Table 11: Descriptive and analytic results of potential risk factors with *Brucella* antibody sero-reactor cattle in extensive, semi-intensive and ranch

	Extensive production			Semi-intensive production			Ranch		
Risk factors	No tested	No positive	Sero-prevalence	No tested	No positive	Sero-prevalence	No tested	No positive	Sero-prevalence
Sex									
Female (1)	627	28	4.47%	645	44	6.82%	276	11	3.99%
Male (0)	237	5	2.11%	109	1	0.92%	50	1	2%
Total	864	33	3.82%	754	45	5.97%	326	12	3.68%
Test statistics		P=0.115	OR 95% CI [0.83-5.68]	OR(7.9)	P=0.042	OR 95% CI [1.08-57.99]		P=0.50	OR 95% CI [0.26-16.11]
Age(years)									
0.5- 1 (1)	37	1	2.70%	102	3	2.94%	24	0	0%
>1- 3 (2)	273	10	4.22%	261	6	2.30%	104	4	3.85%
> 3 (3)	590	22	4.23%	391	36	9.21%	198	8	4.04%
Total	864	33	3.82%	754	45	5.97%	326	12	3.68%
Test statistics		p=0.75	OR 95% CI [0.50-1.64]	OR(2.64)	p=0.001	OR 95% CI [1.47- 4.74]		P=0.46	OR 95% CI [0.52-4.21]
Herd size									
< 5 (1)	74	9	12.16%	170	11	6.47%	0	0	0%
>5- 10 (2)	58	14	24.14%	15	6	40%	0	0	0%
>10 (3)	13	2	15.38%	10	8	80%	1	1	100%
Total	145	25	17.25%	195	25	13.02%	1	1	100%
Test statistics		p=0.24	OR 95% CI [0.78- 2.78]	OR(8.16)	P=0.000	OR 95% CI [3.81-17.47]		No	
Breed									
Cross (1)	0	0	0%	754	45	5.97%	45	1	2.22%
Local (2)	864	33	3.82%	0	0	0	281	11	3.91
Total	864	33	3.82%	754	45	5.97%	326	12	3.68%
Test statistics		No			No			P=0.58	OR 95%CI [0.07-4.42]

Reproductive status versus sero-prevalence

In the present study the reproductive status of females was categorized in to four groups: pregnant, lactating, dry and heifer. Virtually, no significant difference in sero-positivity among the four groups was observed in the three production systems: extensive, semi-intensive and ranch (Table 12).

Pregnant cattle were again grouped in to two on the basis of their stage of gestation where one group consisted of cattle below 5 months of pregnancy (1) and the other group having cattle with equal to and above 5 months of pregnancy (2). Incidentally, there was significant difference between the two groups in the semi-intensive production ($p=0.039$). But there was no significant difference between the two groups in the extensive production and ranch (Table 12).

To assess the sero-prevalence of cattle at different parity number three categories were created: those with no calving, those that calved once and those with two and more calvings. Accordingly, the semi-intensive production revealed significant variation at the different parity number statuses ($p=0.018$). However, no significant difference was obtained in the extensive production and ranch (Table13)

Table 12: *Brucella* sero-reactor cattle at different reproductive statuses in the extensive, semi-intensive and ranch (Awi, West Gojjam and South Gondar)

	Extensive production			Semi-intensive production			Ranch		
Reproductive status	No tested	No positive	Sero-prevalence	No tested	No positive	Sero-prevalence	No tested	No positive	Sero-prevalence
Pregnant (1)	117	6	5.13%	196	9	4.59%	80	4	5%
Lactation(2)	187	4	2.14%	230	25	10.87%	64	2	3.13%
Dry (3)	108	8	7.41%	14	1	7.14%	26	2	7.69%
Heifer (4)	199	8	4.02%	140	6	4.29%	87	3	3.45%
Total	611	26	4.26%	580	41	7.07%	257	11	4.28%
Test statistics		P=0.97	OR 95% CI [0.71-1.40]		P=0.179	OR 95% CI [0.51-38.61]		P=0.78	OR 95% CI [0.57-1.52]
Duration of pregnancy									
< 5 months(0)	56	1	1.79%	85	1	1.18%	40	3	7.50%
> 5 months(1)	61	6	9.85%	100	5	5%	40	1	2.50%
Total	117	7	5.98%	185	6	3.24%	80	4	5%
Test statistics		P=0.102	OR 95% CI [0.70-51.49]	OR(0.71)	P=0.039	OR 95% CI [0.51-0.98]		P=0.33	OR 95% CI [0.03-3.18]
Parity No									
No parity(0)	277	13	4.69%	228	8	3.51%	107	5	4.67%
once(1)	128	6	4.69%	106	10	9.43%	44	1	2.27%
> once(2)	217	9	4.15%	149	23	15.44%	103	5	4.58%
Total	522	28	5.36%				254	11	4.33%
Test statistics		P=0.82	OR 95% CI [0.61-1.47]	OR(1.58)	P=0.018	OR 95% CI [1.01-2.31]		P=0.79	OR 95%CI [0.48 -1.74]

Sero-positivity to *Brucella* antibody and clinical signs

In the ranch there were no records of abortion and retained fetal membrane and hence was excluded in the study. In the semi-intensive production abortion was significantly related to *Brucella* antibody sero-positivity (p=0.021), however, this was not true for extensive production where abortion was not associated with *Brucella* antibody sero-reaction (Table 13).

Retained fetal membrane had been significantly related to sero-reactor cows in the semi-intensive production (p=0.001). Whereas in the extensive production retain ion of fetal membrane had no significant relation with sero-positivity (Table 13).

To compare the difference in seropositivity with respect to abortion frequency, two categories were produced: the first category with a single abortion and the second category having more than one abortion frequencies. The two production systems showed no significant variation with respect to the frequencies of abortion (Table 13).

Comparison between two parity groups one having only one parity and the other with more than one parity revealed no statistical difference in both extensive and semi-intensive production systems(Table13).

Table 13: Univariate logistic regression and Fisher's test results of *Brucella* sero-positives and clinical signs of cattle in the extensive& semi- sntensive

	Extensive production			Semi-intensive production		
Clinical sign	No tested	No positive	Sero-prevalence	No tested	No positive	Sero-prevalence
Abortion						
Present (1)	23	1	4.35%	35	6	17.14%
Absent (0)	389	18	4.63%	548	35	6.38%
Total	412	19	4.61%	583	41	7.03%
Test statistic		P=0.95	OR 95%CI [0.12-7.34]	OR(3.03)	P=0.021	OR 95% CI [1.18– 7.79]
Retained fetal membrane						
Present (1)	9	1	11.11%	24	7	29.16%
Absent (0)	403	18	4.47%	559	34	6.08%
Total	412	19	4.61%	583	41	7.03%
Test statistic		P=0.38	OR 95% CI [0.30-21.7]	OR(6.35)	P=0.001	OR 95% CI [2.47- 16.38]
Abortion frequency						
Only once (1)	20	1	5%	29	5	17.24%
> once(2)	3	0	0%	6	1	16.66%
Total	23	1	4.35%	35	6	17.14%
Test statistic		Fisher's exact=1.00			P=0.97	OR 95% CI [0.09-10.1]
Abortion month						
< 5 (0)	2	0	0%	3	0	0%
> 5 (1)	21	1	4.76%	32	6	18.75%
Total	23	1	4.35%	35	6	17.14%
Test statistic		Fisher's exact= 1.00			Fisher's exact= 1.00	
Abortion parity						
First parity (1)	9	1	11.11%	8	0	0%
Second & more(0)	14	0	0%	27	6	22.22
Total	23	1	4.35%	35	6	17.14%
Test statistic		Fisher's exact =0.391			Fisher's exact=0.30	

Management and husbandry risk factors

Fifty randomly selected livestock owners were interviewed in the extensive production system with the view to see the association of various management and husbandry risk factors and this then was compared with serological analysis result. The finding revealed that 12 % (n=6) of the respondents had sero-positive herds.

Table 14: Comparison between serological and questionnaire survey results of the three administrative zones (Awi, West Gojjam and South Gondar zones)

Zone	Total number of respondents	Number and (%)sero-positive	95%CI
Awi	12	5 (41.67%)	13.76% - 69.56%
S/Gondar	22	0 (0%)	0%
W/Gojjam	16	1 (6.25%)	6.91% -19.41%
Total	50	6 (12%)	2.99% - 21%

Fisher's Exact (0.001)

From the questionnaire result it was observed that 12% (n=6) of the respondents had sero-positive cattle with higher proportion in Awi zone (n=5) to be followed by West Gojjam zone. In South Gondar zone none of the respondents had sero-positive cattle (Table 15). The variation among the three administrative zones was highly significant (p=0.001)

Table 15: Univariate and Fisher's exact test result of management and husbandry risk factors obtained from the questionnaire survey and serological analysis (Awi, West Gojjam and South Gondar zones)

Management and husbandry risk factors	Number of respondent	Number Positive	Sero-prevalence %	P-value	OR 95% CI
Presence of dog				0.41	0.28-24.19
Yes (1)	16	1	6.5%		
No (0)	34	5	14.70%		
Disposal of fetal membrane				Fisher's	
Burring (0)	7	0	0%	exact	
Throwing to field, river, giving to dog, (1)	43	6	13.95%	0.58	
Culling of cattle frequently aborting				0.32	0.42-13.40
Sell & slaughter (0)	34	3	8.82%		
Keeping (plough)(1)	16	3	18.75%		
Reason for selling of breeding female				0.54	0.04-5.60
Infertility (0)	5	1	20%		
Old age & money (1)	29	3	10.34%		
Dry season watering				0.82	0.13-12.42
Wells (0)	10	1	10%		
River, pond, spring (1)	40	5	12.5%		

Reproductive and productive parameters

An attempt was made to assess the reproductive and productive parameters of cattle in the extensive production system using questionnaire survey. The parameters of interest were age at first calving, calving interval and number of calf crops per production life. Accordingly, the mean age at first calving of the local cows kept in the extensive production system was 4.49 years while the minimum age was 3 years and the maximum was 7 years, and the standard error was 0.12. Tesfaye (1995) reported that the age at first calving for indigenous zebu is 4.42 years (53 months). However, Mucassa-Mugerwa (1989) had found age at first calving of 3.67 years (44 months) in zebu cattle. Similarly, for the indigenous breed of cattle in the study area the calving interval ranged between 1 and 4 years with an average of 2.23 years and standard error of 0.075. Tesfaye (1995) reported the calving interval of zebu to 2.08 years (25 months). It was also found that the number of calf crops produce per cow per production life varied between 2 and 9 calves with an average of 4.62 calves and standard error 0.21. Based on the difference in the mean number of calves produced between RBT-positive and RBT-negative groups, the best estimate was that brucellosis decreases the number of calves produced by approximately 10% (McDermott, 1987).

According to Seifert (1996) brucellosis causes significant impact which lead to a significant reduction in productivity; these include late first calving, long intercalving time, herd fertility below 60% and comparatively low milk production.

Comparison was made on the impact of sero-positivity and sero-negativity on the three reproductive and productive parameters. The result was that sero-negative herds had their first calf at an age range of 3 and 7 years with an average of 4.36 years and standard error of 0.30 where as the sero-positive counter parts had their first calves between the age of 4 and 6 years with a mean age of 5.42 years and standard error of 0.12. Our result revealed significant variation between brucellosis sero-positives and sero-negatives with respect to age at first calving ($p=0.014$). The calving interval of sero-negative cattle varied between 1 and 4 years with an average of 2.17 years and standard error of 0.20, while the calving interval for sero-positive reactors ranged between 1.8 and maximum of 3 years, the average being 2.63 years and with a

standard error of 0.08. The calving interval between sero-positive and negative herds did not vary significantly, yet sero-positives had a protracted intercalving interval.

The number of calf crops produced per cow per production life in the sero-negative cows ranged between 2.5 and 9 calves with a mean of 4.8 and standard error of 0.33.The sero- positive cows produced a minimum of 2 calf crops and maximum of 4 calves with a mean of 3.33 calves and a standard error of 0.22 during their production life. The number of calves produced per production life per cow between sero-positive and sero-negative cows significantly varied ($p= 0.036$)

Table 16: Impact of *Brucella* antibody sero-positivity on herd reproductive performance by comparing serological result with questionnaire data (Awi, West Gojjam and South Gondar zone**s)**

Reproductive parameters	Mean (Years)	Range (Years)	Standard Error	Odds Ratio	P-value	OR 95% CI
Age at first calving						
Sero-positive (1)	5.42	4 - 6	0.3	4.02	0.014	1.33 - 12.13
Sero-negative (0)	4.36	3 – 7	0.119			
Total	4.49	3 - 7	0.12			
Calving interval						
Sero-positive (1)	2.63	1.8 - 3	0.194		0.065	0.91-24.07
Sero-negative (0)	2.17	1 – 4	0.08			
Total	2.23	1 - 4	0.075			
Number of calf crop/ cow/ production life (number)				0.24	0.034	0.06-0.80
Sero-positive (0)	3.33	2 - 4	0.33			
Sero-negative (1)	4.8	2.5 – 9	0.22			
Total	4.62	2 - 9	0.21			

Study findings in human

Out of 238 human serum samples tested, 3.78% (n=9) were found positive by both the RBPT and CFT, with a CFT titer ranging between 20 and 640. All the positive reactors had the habit of eating raw meat and drinking raw milk. Signs of fever, headache, night sweat, anorexia and weakness were seen in all (n=9) of the positive reactors. Additional signs of backache, chills and fatigue were observed in 88.89% (n=8) of the sero-reactors. About 66.67% (n=6) of the positive-reactors had joint pain. All (n=9) of the positive reactors were farmers, none of the animal health professionals and AI technicians had *Brucella* antibody in their serum.

Table 17: Distribution of human sero-rectors according to different occupation (Awi, West Gojjam and South Gondar)

Occupation	Number and (%) tested	Number and (%) positive	95% confidence interval
Farmers	222 (93.28%)	9 (4.05%)	1.46% - 6.64%
Veterinary assistants and technician	14 (5.88%)	0 (0%)	0
AI technician	2 (0.84%)	0 (0%)	0
Total	238 (100%)	9 (3.78%)	1.36% - 6.20%

To see the distribution of human sero-reactors at different age groups, three categories were created based on the prevailing local condition (Table 18). From our finding it was apparent that sero-positivity increases with increasing age. The highest sero-prevalence was at age group

greater than 40 years with a sero-prevalence of 6.60%, followed by age group of 20- 40 years with sero-prevalence of (4.08%) and the least being age group less than 20 years having a sero-prevalence of 1.72% (Table 18). Nonetheless, there was no significant variation in age amongst the three age groups (p=0.28). Sex specific sero-prevalence of human was computed between females and males; however no significant difference had been observed (Table 18).

Table 18: Univariate logistic regression analysis result of human *Brucella* antibody sero-reactors according to age and sex Categories (Awi, West Gojjam and South Gondar zones)

Risk factors	Number tested	Number positive	Percent positive	95% confidence interval	P-value	OR 95% confidence interval
Sex					0.31	0.32- 4.73
Female	120	5	4.17%	0.59 - 7.75%		
Male	118	4	3.39%	0.12 - 6.66%		
Total	138	9	3.78%	1.36 - 6.20%		
Age group (years)					0.28	0.21- 3.08
< 20	58	1	1.72%	1.63% - 5.07%		
21- 40	147	6	4.08%	0.82% - 7.28%		
> 41	33	2	6.06%	0% -14.20%		
Total	238	9	3.78%	1.36- 6.2%		

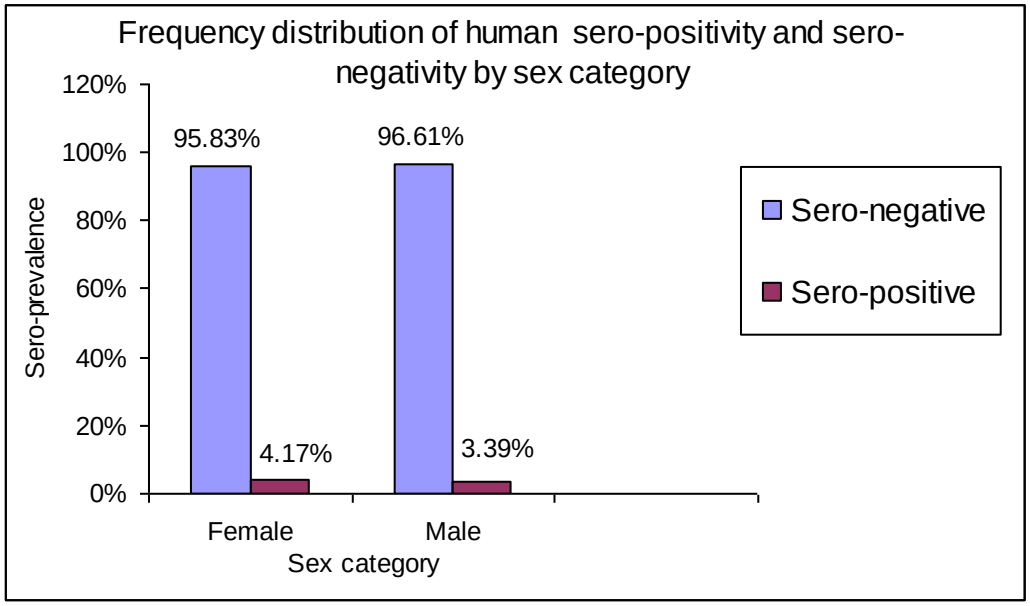

Figure 5: Frequency distribution of human positive reactors according to sex category

6. DISCUSSION

In the present study the occurrence of *Brucella* sero-reactors in the three administrative zones (Awi, West Gojjam and South Gondar) has been investigated in both cattle and humans applying field study, serological analysis and questionnaire surveys.

Over all sero-prevalence

The occurrence of sero-reactor cattle in Ethiopia had been reported by Meyer as early as 1980s. However, until the last two decades the disease was not substantially surveyed. Sero-prevalences as large as 38.7% in the central high lands of Ethiopia by Muktar (1991), 22% in Chafa state farm by Tariku (1994), 19.5% in Abernosa cattle breeding ranch by Taye (1991), 19.1% in and around Addis Ababa by Kibru (1985), 16.65 % by Abeje in and around Bahirdar (1994) and 15.8% in Sidamo by Endrias (1989) were reported. In Ethiopia, again there was moderate sero-prevalence occurrences reported: 8.2% by Bayleyegn (1989) in Arsi, 8.2 % in North Western Amhara on local indigenous zebu by Gebreyesus (2001) and 8.11% by Yilkal *et al*. (1998) in urban and peri-urban areas around Addis Ababa. Similarly, lower sero-prevalences were also reported, 4.9% in Arsi by Abay *et al*. (2002), 4.2% in Ghibe and Gobe by Tekleye *et al*. (1987), 2.15% in central highland of Ethiopia by Assegid (1987), 1.92% in Sidama by Kassahun (2004), 0.61% in Jimma by Tadele (2004) and almost none around Selale and Addis Ababa by Kelay (2002). Summary of sero-prevalences of cattle at different times in different corners of the country have been established (Table 2).

In the present investigation the overall individual and cluster level sero-prevalences of *Brucella* sero-reactor cattle in the three administrative zones (Awi, West Gojjam and South Gondar) and the three production systems, respectively, were 4.63% and 14.96%. This finding agrees well with the finding of 4.9 % by Abay *et al*.(2002), 4.2% by Tekleye *et al*. (1989), 2.15% by Assegid (1987), 1.92% by Kassahun (2004) and 1.8% by Fekadu (1999) at individual level. Previous studies conducted in the same areas were moderately higher, 8.2% by Gebreyesus (2001) in which he had used only RBPT and 9.8% reported by Kassaye (1987) in which case he

sampled only dairy farms and a ranch. Abeje (1994) has also reported higher sero-prevalence (16.65%); however, the higher sero-prevalence could be due to the type of test he used (RBPT and SAT) which are known to be less specific than CFT. Comparable sero-prevalences were also reported in Africa: 4% in Djibouti (Chantel *et al*., 1994), 5% in Uganda by (Kagumba and Nadokha, 1978), 3% in Uganda by (Oloffs *et al*., 1996), 4.3% under local management conditions, 6.3% in dairy farms and 15.8% in ranch in Tanzania (Jiwa *et al*., 1996) and also 4.2% in nomadic/ pastoral cattle (Omer *et al*., 2000). According to Acha and Szyfres (2001) the rates of infection vary greatly from one country to another and also between regions within a country.

Cluster level sero-prevalence

The overall cluster level sero-prevalence of the present study was 14.96%. Tadele (2004) has also reported a herd level sero-prevalence of 14.5% in the extensive production system. Similarly Kassahun reported herd level sero-prevalence of 13.7% in the extensive system. However, Yilkal *et al.* (1998) reported higher herd level sero-prevalence (33.3%). Omer *et al*. (2000) reported herd sero-prevalence of 35.9% which was higher than our finding.

Zonal sero-prevalence

The present findings also showed significantly higher variation both at individual and cluster level sero-prevalences among the three zones, Awi, West Gojjam, and South Gondar, with individual sero prevalences of 9.6%, 6.12%, 1.38% and cluster sero-prevalence of 32.07%, 32.88%, and 4.65%, respectively (Table 7). However, upon pair-wise comparisons, Awi and West Gojjam zones did not show significant variation at both individual and cluster level. Gebreyesus (2001) had found larger number of sero-positive cattle in Awi zone (16.19%) and West Gojjam (8.69%), which are relatively comparable to the result in this study; however, the sero-prevalence in South Gondar was slightly higher than our finding (11.11%).

Agro climate

Comparatively higher sero-prevalences were found in the midland with rates of 5.61% at individual and 22.4% at cluster level; while at the same time sero-prevalences of 1.97% at individual and 6.33% at cluster level were found in the highland (Table 8). The difference in seropositivity between the two agro climates was found to vary significantly at both individual and cluster level (Tables 10 and 11). The possible explanation that can be given for higher level of sero-reaction in the midland than the highland is the relatively larger number of cattle kept in the midland with the consequent higher stocking density and the other possibility is the favorability of the environment for the survival and multiplication of the organism and its subsequent infection. Incidentally, closer finding had been reported by Fekadu (1999) in the North Eastern Amhara region where he found 0.2% in the highland and 3% in the lowland. On the other hand, Tekleye *et al.* (1989) reported almost equivalent sero-prevalences in Gibe which represent the lowland (4.3%) and Gobe representing highland (4.1%).

Production system

extensive, semi-intensive and ranch were the three production systems studied; the sero-prevalences among the three production systems were 3.82%, 5.97% and 3.68%, respectively. However, the variation was found insignificant. Previous workers in the same area reported 21.13% in dairy farms around Bahirdar and 8.34% in traditionally managed zebu (Abeje, 1994) and these were comparatively higher prevalences. Kassaye (1987) had also reported sero-prevalence of 2.15% in Tis Abay producers cooperative farm, 7.16% in Andasa cattle breeding ranch, 7.7% in Bahirdar teachers college dairy farm and 28.79% in Jigna producers cooperative farm. Incidentally, some of the farms were included in this study for instance Andasa cattle breeding ranch, where the sero-prevalence was relatively lower than the previous study; the latter may be due to a better management practices like the introduction of cattle after being tested by RBPT and the daily cleansing of the houses. Similarly, Bahirdar teachers training college was found in this study to have no sero-reactor cattle. Furthermore, Abay *et al*. (2000) had reported a sero-prevalence of 2% in extensive grazing and 3.4% in intensive management, which was closer to our finding. Similar findings were also reported by Kassahun (2004) with sero-prevalence of

1.66% in the extensive and 2.45% in the Intensive. On the contrary, Bakunzi *et al.* (1993) reported a relatively higher sero-prevalence of 10.3% using RBPT in peri-urban privately and state owned farms than extensive rural village with prevalences of 0.54%. At the same time in Lake Victoria zones of Tanzania, Jiwa *et al*. (1996) reported the existence of significant difference among local management (4.3%), dairy farm intermediate (6.3%) and ranch (15.8%). There are reports that indicate the rise of infection with change from extensive to intensive management whether they have indigenous cattle or introduced (FAO/WHO, 1986). Weidman (1991) reported the close correlation between the kind and intensity of husbandry system and rate of infection. Crawford *et al*. (1990) had also described on the influence of methods of management and characteristics of the population on the occurrence of infection in a herd.

Risk factors

Generally, the susceptibility of cattle to *Brucella abortus* infection is influenced by age, sex, breed and reproductive status of the individual animal (Radostitis *et al*., 2000). Below, an attempt has been made to discuss the findings of this study along these lines:

Sex

The present study revealed significant association (p=0.042) between female (5.97%) and male (0.92%) in the semi-intensive production. However, in the ranch and extensive production systems despite the higher proportion of female reactors, the difference between the two sexes was not found significant (Table 11). This result is consistent with the finding of Yilkal *et al*. (1998) where he found higher proportion of female (8%) infected than male (0.11%), though the difference was not significant. Tadele (2004) has also reported higher proportion of female reactors than males in both extensive and intensive production systems, though the difference was again not significant. According to Kassahun (2004) the proportion of sero-reactor female cattle was 2.49%, whereas 10 of the bulls tested were found negative in the intensive management; on the other hand, the results on extensive production system was found different from the reports so far, where the proportion of males (4%) outweighed that of the females

(1.62%). Similarly, Kubuafor *et al.*, (2000) had reported a sero-prevalence of 8.5% in females and 1.9% in males in Akwapim-Southern district of Ghana, yet the difference was not significant. Although no controlled study has been conducted on the relative susceptibility of female and male cattle to brucellosis, based on reactor rates it is probable that bulls are more resistant than sexually mature heifers and cows, however are less resistant than sexually immature heifers (Nicoletti, 1980). It is important to note that serological data may underestimate *Brucella abortus* infection in males as infected bulls tested were generally nonreactors or only had low antibody levels (Crawford *et al*., 1990).

Age

In the semi-intensive production system the present finding revealed a statistically significant variation (p=001) among the three age categories, ≤ 1 year, > 1-3 year and > 3 year; however, in the ranch and extensive production systems, the three age categories did not differ significantly, despite the increment in sero-positivity with age (Table11). This finding is consistent with that reported by Yilkal *et al*. (1998) where older cattle were twice more likely affected (4%) by brucellosis than younger ones (1.9%). Tariku (1994) had also found higher proportion of old cattle being affected, but the difference among age groups was not found significant. Additionally, Kassahun (2004) reported that the majority (97.87%) of sero-reactors were detected in the age strata above 2 years in both the extensive and intensive management systems. The value in the different age groups was (2.29%) for > 4years, (1.89%) for 2-4 years and (0.29%) for age group < 2 years. Tadele (2004) too has reported significant variation among age groups in extensive production systems with almost zero cases in the age group 0.5-<3 years, 1.1% in the age group >3-6 years and 1.6% in the age group > 6 years. In dairy farms in Asmara highest sero-prevalence was recorded in cattle 2-4 years old as reported by Omer *et al*. (2000). Furthermore, Kubuafor *et al*. (2000) from Ghana reported the significant increase in sero-positivity with respect to age. Chukwu (1987) pointed out that sexually mature cows and bulls were very susceptible to brucellosis. Walker (1999) has described that younger animas tend to be more resistant to infection and frequently clear infections, although latent infections do occur.

Herd size

In this study highly significant variation (p=0.000) among the three herd categories: <5 cattle, >5 -10 cattle and > 10 cattle in the semi-intensive production were observed; however the difference was not statistically significant in the extensive production, though comparatively higher proportion was found in the herd category having > 5 -10 cattle and > 10 cattle than those herds with < 5 cattle (Table 11). The ranch was not analyzed because its number was single. Our finding is in accordance with the result of Yilkal *et al*. (1998) in which he found significant association between *Brucella* infection and herd size. Kassahun (2004) as well has also reported that in both extensive and intensive systems established that infections increased together with herd size from small to large, despite the absence of significant difference in both age categories (0.5- 2 years, 2-4 years and >4 years). On the other hand, Tadele (2004) reported highly significant variation between herds having 1-5 cattle and those with >5 cattle, with higher sero-prevalence in groups having >5 cattle (the extensive production system) but the difference was not significant in the intensive system. In Rakungiri district of Uganda the majority of reactors were detected only in large and medium sized herds (Oloffs, 1996). It is generally accepted that an increase in herd size is usually accompanied by an increase in stocking density and increase in risk of exposure to infection especially following abortion (Nicoletti, 1980; Salman and Meyer, 1984). Stocking density is an important determinant of the potential between susceptible and infected animals (Crawford *et al*., 1990; Omer *et al*., 2000).

Breed

In the present study an attempt has been made to compare the susceptibility of the indigenous (Fogera) and crossbred (Fogera x Frisian) breeds of cattle raised in the same management system (Andasa cattle breeding and multiplication center). Apparently, 2.2% of crossbred and 3.91% of indigenous Fogera breeds of cattle were found sero-positive (Table11). However, the difference between the two of breeds was not significant despite minor variation in their proportions. This study is consistent with the result found in Ghana by Kubuafor *et al*., (2000) where he found no significant difference among 3 types of breeds, Sanga, West African shorthorn and White Fulani. On the other hand, Jiwa *et al*. (1996) had reported a significant variation among Tanganyika

shorthorn zebu (TSZ) and Grade animal (zebu and exotic), Mpwapwa and exotic animals ($p<0.001$) in Victoria province of Tanzania with sero-prevalences of TSZ< Grade<Exotic< MPW. Radostitis *et al*. (2000) clearly stated that all breeds of cattle appear to be comparable in susceptibility to brucellosis and apparently no specific breed resistance to brucellosis is known. Madsen (1989) had also mentioned that susceptibility between breeds has not been reported.

Reproductive status and sero-positivity

The four reproductive status lactating, pregnant and dry and heifer did not show significant difference in sero-prevalence among the three production systems (Table12). Comparable finding was reported by Omer *et al*. (2000) where high sero-prevalence was found in adult cows irrespective of their lactating or pregnancy status although the proportion of heifers was relatively small (3.8%). However, Sexual mature and pregnant cattle are more susceptible to infection with the organism than sexual immature cattle of either sex (Radostitis *et* a*l*., 2000). This is because of the localization of *Brucella* in the uterus mostly associated with pregnancy and the production of erythritol, a carbohydrate found in fetal tissue and possibly to steroid hormones (Nicoletti, 1980; Quinn *et al*., 1999).

An attempt was made to see the difference in sero-reaction between pregnant cows less than 5 months of pregnancy and those with greater than 5 months of pregnancy, significant association ($p=0.039$) was observed in the semi-intensive productions, however, there was no significant variation in the two pregnancy groups, despite higher proportion of cows in the former than the latter in the extensive production system. In the case of the ranch higher proportion of sero reactors were pregnant cows less than 5 months (Table 12). Nicoletti (1980) described the rapid multiplication of the bacteria during the 2^{nd} and 3^{rd} trimester of pregnancy and excretion of large number of organisms at the time of abortion and parturition.

Three parity levels (no parity, only one parturition and more than one parturitions) were compared to ascertain the association of parity number with that of sero-positivity to *Brucella* antibody. There was significant association between parity number and sero-reaction in semi-intensive production ($p=0.018$). However, significant association was not observed in the extensive production and ranch. Yilkal *et al*. (1998) had also found a significant association

between animals having at least one parturition and those not parturated. Radostitis *et al*. (2000) had shown that significant differences exist between animals with no birth and those with at least one parturition.

Sero-prevalence and clinical sings

Abortion

Abortion is the most obvious manifestations of brucellosis. There have not been records of abortion and retained fetal membrane in the ranch. However, in semi-intensive production abortion was found to be significantly associated with sero-prevalence (p=0.021). Contrary to this in the extensive production system abortion was independently associated with sero-positivity (Table 14). The cause of abortion in the extensive production system might be due to other prevalent diseases in the area like trypanosomosis, anthrax, black leg and pasteurellosis. Similarly, Tadele (2004) reported the significant association between sero-positivity to abortion (p=0.000) in the intensive production. Nonetheless, Yilkal *et al*. (1998) had also found significant association between abortion and sero-reaction to *Brucella* at herd level (p=0.009), however, no significant association between sero-prevalence and abortion was observed at individual level (p=0.94). According to Acha and Zyphres (2001) the predominant symptom in pregnant female is abortion or premature or full term birth of dead or weak calves. However, according to Weidman (1991) the main clinical symptoms in the tropics are joint inflammations and hygromas.

Retained fetal membrane

The present finding disclosed significant association (p=0.001) between retained fetal membrane and sero-reactor cattle in the semi-intensive production system. However, the association was not significant in the extensive production system (Table13). Brucellosis is frequently associated with retention of fetal membrane and metritis (Chukwu, 1985).

Management and husbandry risk factors

Fifty randomly selected cattle owners in the extensive production system were interviewed with the view to identify relevant management and husbandry factors associated to the occurrence of the disease under investigation and at the same time to assess the impact of the disease on some reproductive and productive parameters.

None of the management and husbandry risk factors considered (presence of dog, way of disposal of fetal membranes, culling of frequently aborting cows, reason for selling of breeding female and dry season watering site) were found significantly associated to sero-prevalence of *Brucella* antibody. However, those farmers that kept frequently aborting cows were found to have relatively higher proportion of sero-reactors. Similarly farmers that dispose the fetal membranes to the field, river and giving to dog and those that use rivers, pond and spring as watering point were found to have higher proportion of sero-reactors than farmers that bury the fetal membrane and use their own well as watering point. The presence of dog and selling of breeding females due to infertility were not important risk factors (Table15). This is in agreement with Yilkal *et al*. (1998) who reported absence of significant association between sero-reactors and disposal of fetal membrane and culling of infected animals.

Reproductive and productive parameters and sero-prevalence

Age at first calving and the number of calves born per cow per production life were found significantly associated with sero-positivity with values of p=0.014 and p=0.034, respectively. It was also found out that sero-positive cows had their first calf protracted by four fold than their sero-negative counter part. The finding of odds ratio of 0.24 does mean that sero-positivity would reduce the number of calves to be delivered. Although not significant, the calving interval of sero-positive cows was relatively longer than the sero-negative counter parts. On the other hand, Yilkal *et al*. (1998) found no significant association between sero-positivity and age at first calving and calving interval. Tariku (1994) also reported that no significant difference in age at first calving between animals tested positive or negative in SAT or RBPT; however, a wider range of age for SAT positive group were found with prolonged age at first calving. This could

be due to abortion in the first pregnancy or delay in reaching puberty. Similarly intercalving interval between sero-positive and sero-negative groups were not significantly different (Tariku, 1994).

Study finding in Human

The present study on human sera revealed 3.78% (n=9) sero-prevalence to *Brucella* antibody in the three occupational groups: farmers, veterinarians and AI technicians sampled. All of the sero-reactors were farmers. This result agrees with previous work in Sidama area by Kassahun (2004) in which he found sero-prevalence of 5.8%. Similarly, Tadele (2004) has reported a sero-prevalence of 3.4% in Jimma. In Africa similar findings have been reported: in Nigeria 5.5% (Chukwu, 1985), 4.6%and 3% in Eritrea (Omer *et al*., 2002), in Uganda 6.4% (Chukwu, 1985) and 6.5% in Djibouti (Chantel *et al.*, 1994). On the contrary to the present finding, Gebreyesus (2001) reported a very high sero-prevalence of 24.5% in North Western Amhara Region, the higher reported sero-prevalence could be associated to the type of serological test utilized which was only RBPT.

In the current study, females and males were found almost equally susceptible to brucellosis with sero-prevalences of 4.17% and 3.39%, respectively. This may reflect the social and cultural behavior where both female and male are almost equally exposed to infected livestock. Cooper (1991) had reported comparable findings in Saudi Arabia where the proportion was 5.4 per 1000 per year in females and 6.34% per 1000 per year in males with no significant difference. However, Alballa (1995) reported higher proportions of females (22.1%) being sero-reactor than males (15.3%). Additionally, it was apparent that the age group > 40 years was found to have a relatively higher proportion (6.06%) followed by the age group 21-40 years (4.08%), the least being age the group < 20 years (1.72%). Comparable findings have been reported by Hadad and Smith (1986) where they found a peak incidence of brucellosis in the age group of 20-45. Similarly, Cooper (1991) had found a marked trend of increasing incidence of brucellosis with increasing age having significant variation among them ($p<0.001$). Rana (1985) also found maximum sero prevalence of 37.3% in the age group ranging from 31 to 40 years followed by the

age group ranging from 41 to 50 years. It seems that the sero prevalence is higher in old and middle age groups suggesting the frequent contact of this age categories with infected livestock.

7. CONCLUSIONS AND RECOMMENDATIONS

The current cross-sectional study revealed a low to moderate number of *Brucella* antibody sero-reactor cattle. These sero-reactor cattle were found widely distributed in the three zones of Amhara National Regional State (Awi, West Gojjam and South Gondar zones). However, there was significant variation in sero-prevalence among the three zones and the two agro climatic zones (midland and highland). The three production systems: extensive, semi-intensive and ranch did not show significant variation in sero-prevalence despite the different management and husbandry systems prevalent in the production systems. Sex, age and herd size were found important risk factors associated for the occurrence of *Brucella* reactor cattle. However, breed was found less important factor associated to sero-positivity. Although there were minor variations among cattle at different reproductive statuses (pregnant, lactating, dry and heifer) their difference in respect to sero-reaction was not found significant. The two main clinical signs of brucellosis, abortion and retained fetal membrane were significantly associated with sero-prevalence to *Brucella* in the semi-intensive production; however, in the extensive production system they were not found significantly associated, probably other diseases prevalent in the area could have contributed to the condition. The zoonotic importance of brucellosis has been established in the study area. In the study it was noted that females and males were nearly equally affected, however, middle and old age groups were more affected than younger age groups.

Based on the present study and findings, the following recommendations are put forward.

- Proper hygienic practices need to be introduced and adhered to (proper disposal of aborted fetus, fetal membrane, cleansing and disinfection.

- Take measures to prevent the spread and transmission of the disease (use of separate parturition pen, purchasing from disease free herds).

- Institute vaccination in farms where the sero-prevalence is high.

- Brucellosis should be sought as a disease of public health importance and hence, collaboration between veterinarians and human health personnel is utmost important.

- Creation of public awareness on the economic and public health importance of the disease by all medias available (transmission through drinking raw milk and contact of infected retained fetal membrane or aborted fetus).

- Further study on the infection, lesion and microorganism is warranted.

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Annex 1: Questionnaire format for serum sampling

Date------

Region----------

Zone----------

District----------

PA----------

Village (herd)

Code no	Sex	Age	Status (bull, heifer, cow pregnant, lactating, dry)	Herd size	Parity no	Type of in Semi-nation AI, natural or both	History of abortion	Presence of other species	Production system

Annex 2: Questionnaire format for risk groups

Wereda	Sex	Age	Raw milk con.	Raw meat con.	Reml.of plac.	Fever	Head ach.	Night sweat	Back ache	Chills	Fatigue	Anorexia	Weakness	Joint pain

Annex 3: Questionnaires for individual owners

Date……..

I. General information

Region …… Zone …… District…… PA. ……

Village. ……

Name of respondent……. Age……..Sex…….

Herd size: …… (a. Small < 5 b. Middle 5-10 c. Large > 10)

Owner experience (Qualification) …… Family size…….

Herd composition or herd inventory

- Breeding females dry (non pregnant)
- Pregnant
- Lactating
- Breeding bulls
- Castrated male
- Non-breeding males (below 5 years
- Non-breeding females
- Un weaned males
- Un weaned females

What do you do to milk?

- Home consumption
- Cash income by selling
- Both

Milk consumption and preservation means

Descriptions	Fresh	Boil	Sour	Smoked	Other treatment
Preservation					
Shelf life (days)					
Usually consumed					
Delicacy / more liked					

Do you slaughter animals at home? (Y / N). If yes for what reason?

a. For home consumption, b. group share,

c. Ceremony, d. Emergency slaughtering,

How do you consume meat?

a. Cooked

b. Raw

c. Other treatment

Animal acquisition (born, purchased, gift) and disposed (Sold, dead, gift)

Animal types	Animals entered the herd				Animals left the herd				Remarks
	This year		Last year		This year		Last year		
	No.	reason	No.	reason	No.	reason	No.	reason	
Breeding female									
Young (F)									
Young (M)									
Adult (M)									
Calf (M)									
Calf (F)									

Activities and labor divisions

Activities	Young		Adult	
	Male	Female	Male	Female
Herding				
Watering				
Milking				
Delivery assistance				
Mating assistance				

Watering points in different seasons

Seasons	Water sources				Frequencies days
	River	Ponds	Traditional wells	Springs	
Dry					
Wet					

How do you treat your diseased animals

a. Traditional healer
b. Self-administered vet drugs
c. Vet clinic

What type of insemination do you use?

-AI

-Natural mating

- Both

Are there separate parturition pen?

Yes no

How do you assist delivery?

a. Hand pulling b. other c. no assistance

How do you take care for new born?

a. Cleaning newborn b. Hand feeding of weak calves c. Carrying weak ones to home

How do you dispose of the fetal membrane?

a. Burring b offering to dogs c. leaving in the field

Where do you get your replacement stock?

a. Owen stock b. village bull c. both

Are dogs kept in the farm?

a. Yes b. no

List five top diseases……..

……

Disease events in the herd (indicators of brucellosis)

Events in the l herd	Since three years		Before three years		Remarks
	Yes or no	Number	Yes or no	Number	
Abortion					
Still birth					
Birth to weak calf					
Retained placenta					
Cycling female					
Bull with swollen tests and joints					

What do you think that cause abortion? …….

What do you do to animals that frequently abort?

a. Sell b. slaughter c. keeping d. other ……

What do you do with female that doesn't conceive?

a. Sell b. slaughter c. keeping d. other

How do you herd?

a. Separately b. with village herd

How is night resting?

a. Separate b. Mixed

Do you sell breeding female? (Y/N)…….. If yes what is the reason of selling

a. Disease b. Infertility c. shortage of money d. others……..

Comparative age of male and female

Parameters	Male	Female
Weaning age		
Age at first mating		
Reproductive life		
Life span		

Gestation length (Mon)…….. Caving interval (Mon)……..

Lactation length (Mon)……

Seasonal variations

Parameters	Dry season	Wet season	Both seasons
Calving season			
Mating			
Milking frequencies			
Milk off take per day			
Fixing breeding time			

House type (floor)?

a. Concrete b. soil c. other

Feed type?

a. Concentrates b. only grazing c. grazing and concentrate d. other

Annex 4: CFT test procedure

Materials for CFT

Micro titer plates (U-shaped), multi channel and single channel micropipettes, pipette tips Universal bottles for preparation of solutions, stirrers (magnetic), Cylinders weighing balance thermometers, pH indicator, Incubator, water bath, deep freezer, centrifugator, Antigen, complement, hemolysin (amboceptor's), control sera, sheep RBC, veronal buffer, Alsever`s solutions and washing buffer

I. Preparation of sheep red blood cells for the hemolytic system:

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

II. Amboceptor's (Hemolysin) titration:

Prepare 1:500 dilutions up to 1:8000.

- 5 test tubes were prepared
- 1m of VBD was added to test tubes 2-5
- 10µl amboceptor's was mixed with 4990µl VBD in the first tube
- 1ml was transferred from the first to the second up to the last tube and 1ml was discarded.

B. Prepare 1:750 amboceptor's and dilute serially up to 1:12000

- 5 test tubes were prepared and 1ml of VBD was added to test tubes 2 to 5
- 10µl amboceptor's was mixed with 7490µl VBD in the first tube
- 1ml was transferred from first tube to second up to fifth tube and 1ml was discarded, put tubes in order of ascending dilution

C. 0.5ml was transferred from each of these test tubes to a second set of 10 tubes. Start with the 1:12,000 dilution

D. 1ml of VBD was added to each of the test tubes

E. 0.5ml of 2% sheep red blood cell was added to each of the test tubes and shacked well.

F. The test tubes were left on the bench for 10minutes.

G. 1ml of complement at working dilution was added.

H. The tubes were incubated for 30minutes in a water bath at 37^0

I. Result read and recorded, the last tube showing complete hemolysis (MHD)

The working dilution of amboceptor's is four times the MHD

III. Evaluation of complement.

Freeze dried complement was reconstituted according to its instruction.

A 1:100 complement was prepared

Complement was added into the 9 tubes increasing by 5 µl every time, starting with 10µl.

- Diluent was added in to the 9 tubes in decreasing amount by 5µl, starting with 40µl.
- 25µl of diluents was added into the tubes with the corn well siring.
- The test tubes were placed in a 37^0C water bath for 1hr.
- 25µl of 2% sheep red blood cells was added in all tubes
- 25µl of amboceptor's at working dilution 1:1000 was added in all tubes.

The tubes were properly mixed and put again in the water bath of 37^0C for another 30 minutes

The test was read by recording the minimum hemolytic dose of complement (MHD), which was represented by the first tube showing complement hemolysis. The next tube contains the full hemolytic dose (FHD). The complement dilution = 2FHD/dilution of complement.

V. Titration of antigen

Micro titer plate I

- 25 µl of VBD was added in all wells.
- 25µl prediluted antigen was added to all wells of row A.

By serial doubling (two fold) dilution 25 µl of antigen was transferred from row A to B, and again from row B to C, etc. Until row G by multi-channel pipette. 25 µl mixture was discarded from row G (row H will only contain the diluent)

Micro titer plate II

- 50 µl of VBD was added to all wells
- 50 µl of prediluted (1:2.5) in activated positive control serum was added to all wells of column1.
- 50 µl was serially transferred by two-fold dilution, from column 1 to 2 and again from column 2 to3 etc. until column 11. 50 µl was discarded from column 11.

Mix plate I and II

- 25 µl was transferred from plate II to Plate I.
- 25µl of complement in 1:40 dilution was added to all wells of plate I
- Plate I was kept in a refrigerator, covered with second empty plate (cold fixation)

The following day, 50 µl of 2% sheep red blood cells, amboceptor premature, equal volume, i.e. 25 µl of sheep red blood cells 25 µl of a 1:100 working dilution of amboceptor was added to all cups.

The plate was covered with sealing tape, shacked well and kept in water bath at 37^0C for 30 minutes.

The last well with 50% sedimentation was read and recorded. The highest dilution of antigen with 50% sedimentation is the limiting antigen concentration or the right corner value. 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 2 units.

The test proper, multiple sera technique

The sera were predidiluted to 1: 2.5 and incubated at 58^0c in a water bath for 3 minutes inorder to inactivate the native complement.

- 25 µl of diluted test sera were placed in wells of first and second rows of U- bottom plates and
- 25µl of VBD was added to all except those of the first.
- Serial doubling dilution were then made by transferring 25µl of serum from the second row onwards continuing for at least four dilutions
- 25µl of antigen diluted to working dilution excluding those of the anti complementary controls, which received 25µl VBD instead.
- 25 µl of complement at working dilution (1.25) was added to all wells except control well.

Control wells containing serum+ complement+ diluent, antigen control has antigen+ complement+ diluent, complement control has complement+ diluent and hemolytic system has diluent set up to 75µl total volume in each case before hemolytic system was added.

- The plate were sealed, incubated at 37^0C for 30minutes with agitation
- 25µl of sheep red blood cell suspension was added to each well .The plates were sealed and reincubated at 37^0c for 30 minutes with agitation. Before reading the result the plates were left in the refrigerator at $+4^0$c for 1-hour inorder to allow none lysed cells to settle.

Plates were taken out from refrigerator and left at room temperature for 10 minutes

Positive reactions were indicated by sedimentation of sheep red blood cell and absence of hemolysis. Negative reactions by heamolysis of sheep red blood cell.

Annex 5: Proportions of individual and cluster RBPT positive versus CFT positive in cattle in extensive, semi-intensive and ranch (Awi, West Gojjam and South Gondar Zones)

Production system	Individual		Cluster	
	RBPT positive	CFT positive	RBTP positive	CFT positive
Extensive	37	33	28	25
Semi-intensive	46	45	26	25
Ranch	13	12	1	1
Total	96	90	55	51

Annex 6: The proportion of CFT titers of *Brucella* sero-positive sera in cattle

CFT titer	ICFTU	Number of positive sera	Proportion
1: 5	25	9	10%
1:10	50	16	17.78%
1: 20	100	36	40%
1: 40	200	24	26.67%
1: 80	400	4	4.44%
1:320	1600	1	1.11%

Annex 7: The proportion of CFT titers of *Brucella* sero-positive human sera

CFT titer	ICFTU	Number positive sera	Proportion (%)
1: 20	100	1	11.11%
1: 40	200	1	11.11%
1: 320	1600	6	66.67%
1: 640	3200	1	11.11%

Annex 8: The within cluster sero-prevalence in extensive production

Wereda	Cluster size	No of positive sera	RBPT	CFT	With in herd sero-prevalence (%)
Mecha	7	1	1	1	14.29
Mecha	8	1	1	1	12.5
Mecha	6	2	1	1	33.33
Mecha	4	1	1	1	25
Mecha	4	1	1	1	25
Achefer	5	1	1	1	20
Achefer	6	2	1	1	33.33
Achefer	2	1	1	1	50
Banja	9	1	1	1	11.11
Fagta	5	1	1	1	20
Dangila	5	1	1	1	20
Dangila	10	1	1	1	10
Dangila	6	1	1	1	16.67
Dangila	7	2	1	1	28.57
Dangila	11	1	1	1	9.09
Dangila	3	1	1	1	33.33
Dangila	8	2	1	1	20
Dangila	9	3	1	1	33.33
Dangila	3	1	1	1	33.33
Dangila	3	1	1	1	33.33
Dangila	7	2	1	1	28.57
Fogera	6	2	1	1	33.33
Libo Kemkem	17	1	1	1	5.88
Farta	6	1	1	1	16.67
Farta	6	1	1	1	16.67

Annex 9: The within cluster sero-prevalence in semi-intensive production

Wereda	Cluster size	No of positive sera	RBPT	CFT	With in cluster sero-prevalence (%)
Bahirdar	32	1	1	1	3.12
Bahirdar	25	2	1	1	8
Bahirdar	15	3	1	1	20
Bahirdar	35	6	1	1	17.14
Bahirdar	15	10	1	1	66.67
Bahirdar	5	2	1	1	40
Bahirdar	10	3	1	1	30
Bahirdar	6	1	1	1	16.67
Bahirdar	9	1	1	1	11.11
Bahirdar	15	1	1	1	5.88
Bahirdar	35	1	1	1	2.86
Achefer	4	1	1	1	25
Adiet	6	1	1	1	16.67
Dangila	1	1	1	1	100
Dangila	2	1	1	1	50
Dangila	5	1	1	1	20
Dangila	2	1	1	1	50
Dangila	4	1	1	1	25
Tillily	6	1	1	1	16.67
Wereta	9	1	1	1	11.11
Debretabor	18	1	1	1	5.56
Debretabor	3	1	1	1	33.33
Debretabor	5	1	1	1	20
Debretabor	4	1	1	1	25
Debretabor	2	1	1	1	50

9. **CURRICULUM VITAE**

Name: Mussie Hailemelekot Demsash

Address: Metema Office of Agricultural and Rural Development
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Nationality: Ethiopian

Date of birth: May 4 1972

Place of birth: Addis Ababa

Marital status: Single

Education: 1977 – 1983, elementary education at Addis Zemen primary school
1984 – 1989, Junior and High School at Addis Zemen
secondary school, Addis Zemen
1990 – 1995, Addis Ababa University, Faculty of Veterinary Medicine

Qualification: DVM from Addis Ababa University in 1995

Work experience: -As district Veterinarian in Amhara National Regional state, North Gondar,
From 1996 - 2002

As desk head of animal production development and health department from 2002- 2003

Current duties and responsibilities: Animal Production Development and Health department
Desk head (Metema *Wereda*)

Research output/Technical paper

1. Seminar on the use of embryo transfer in cattle infectious disease control program.

2. Bovine hydatid disease an assessment trail of its prevalence and economic importance at Bahirdar slaughterhouse.

Language skills

Amharic; fluent written and spoken

English: fluent written and spoken

10. SIGNED DECLARATION SHEET

I the undersigned, declare that the thesis is my original work and has not been presented for a degree in any university and that all sources of material used have been duly acknowledged.

Name: _____

Signature: _____

Date of submission. _____

This thesis has been submitted for examination with my approval as University Advisor

Dr. Yilkal Asfaw. _____.

Professor Tesfu Kassa. _____.