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ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

SEROEPIDEMIOLOGICAL STUDY OF BOVINE BRUCELLOSIS IN TIGRAY REGION, NORTHERN ETHIOPIA

BY
GEBRETSADIK BERHE GEBREMEDHIN

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DEBRE ZEIT, ETHIOPIA

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SEROEPIDEMIOLOGICAL STUDY OF BOVINE BRUCELLOSIS IN TIGRAY REGION, NORTHERN ETHIOPIA

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

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LIST OF ABBREVIATIONS

BgVV	Bundesinstitut fur Gesundheitlichen Verbaucherschutz und Veterinarmedizin
BoANR	Bureau of Agriculture and Natural Resources
BoPED	Bureau of Planning and Economic Development
CFT	Complement Fixation Test
CI	Confidence Interval
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
ICFTU	International Complement Fixation Test Unit
IFN gamma	Interferon gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IU	International Unit
MAb	Monoclonal Antibody
masl	meter above sea level
µl	micro liter
MCH	Major Histocompatibility Complex
MRT	Milk Ring Test
OIE	Office International des Epizooties
OIEISS	OIE International Standard Serum
O-PS	O-Chain Polysaccharide
OR	Odds Ratio
RBPT	Rose Bengal Plate Test
R-LPS	Rough Lipopolysaccharide
S-LPS	Smooth Lipopolysaccharide
S-RB51	Strain Rough *Brucella* 51 vaccine
VBD	Veronal Buffer Diluents
VCM	Veronal Buffer with Calcium and Magnesium
WHO	World Health Organization

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ABSTRACT

A cross-sectional epidemiological study was carried out from September 2004 to March 2005 to determine the seroprevalence of bovine brucellosis in the intensive and extensive cattle production systems of Tigray Region, to identify the risk factors that contribute to the occurrence of brucellosis and assess the public health significance of the disease.

The study populations were comprised of indigenous and crossbred cattle in the Region and samples were selected by two-stage cluster sampling. Serum samples collected from 1,951 cattle above six months of age, of which 1,135 from the intensive farms and 816 from the extensively managed herds were screened for *Brucella* antibodies by the Rose Bengal Plate Test (RBPT) and the reactor sera were further tested by the Complement Fixation Test (CFT). Moreover, information was gathered on age, sex, herd size, management, and agro-ecology of each sampled animal to determine the risk factors for brucellosis seropositivity. Likewise, data on farm or herd-level risk factors and other farm characteristics were obtained using a questionnaire survey, which was administered in person to 112 intensive farms and members of animal owners in the 26 extensive herds. Furthermore, in order to assess the zoonotic importance of the disease, 185 human serum samples were also screened for brucellosis.

In this study, the overall seroprevalence of *Brucella* antibodies in cattle was 1.49%. However, individual animal seroprevalence was found to vary from 0.26% in the intensive farms to 3.19% in animals reared under the extensive management system. Statistically significant difference was observed in seroprevalence values between the intensive and extensive management systems ($p<0.05$). The overall herd-level prevalence varied from 2.68 % in the intensive farms to 42.31% in the extensively managed herds whereas within-herd prevalence differed from 0% - 33% in the intensive farms to 0% - 11% in the extensive herds based on CFT. However, no antibodies against *Brucella* were detected in the human sera.

The results of univariate logistic regression analysis revealed that age, herd size and parity number of animals had no statistically significant effect on individual animal seropositivity to brucellosis in the intensive management system. However, in the extensive management system, seropositivity to brucellosis was significantly higher in animals kept under the transhumance management sub-system than animals in the sedentary sub-system ($p<0.001$). The results also indicated that there was a statistically significant increase in seroprevalence to

brucellosis with increasing age ($p<0.01$) but not parity ($p>0.05$). Significant increment of seropositivity was also observed as herd size increases from small to medium ($p<0.05$) and then to large sizes ($p<0.001$). In addition, a significantly higher seroprevalence was found in animals in the lowland than those in the highland agro-climatic zones. Nevertheless, in the multivariate logistic regression analysis, systemic factor (OR = 10.6%, 95% CI = 2.3 – 49.3, $p<0.01$) and age (OR = 4.2, 95% CI = 2.3 – 49.3, $p<0.01$) were identified as the major risk factor for individual animal seroprevalence in the extensive management system. Transhumance management sub-system was also significantly associated with increased herd seroprevalence ($p<0.01$) but not herd size ($p>0.05$) in the extensive management system. Furthermore, there was a statistically significant association between seropositivity to brucellosis and history of previous abortions (Fisher's exact $p<0.001$) and stillbirths (Fisher's exact $p<0.05$) in the extensive herds but only of abortion (Fisher's exact $p<0.01$) in the intensive farms.

The results of this study showed that brucellosis is an endemic and widely distributed disease in Tigray Region with relatively high seroprevalence in the extensive than the intensively managed cattle. The implementations of better management practices are recommended to prevent further spread of the disease.

Keywords: Bovine, brucellosis, seroepidemiology, seroprevalence, Tigray Region, Northern Ethiopia

1. INTRODUCTION

Ethiopia is a country whose agricultural sector is the biggest contributor to its Gross Domestic Product (GDP) and major contributor to its export earnings. Currently the contribution of this sector to the GDP and export earrings is 48% and 90%, respectively (Beyene, 1997). Livestock production is a major component of the agricultural economy of the country that contributes 30% to the agricultural GDP and 17% to the export earnings (Tegegn and Gebre Wold, 1997). In Ethiopia, livestock supports rural and urban population with milk, meat, employment, investment opportunities, and draft power for crop production (Demeke and Tesfaye, 1997).

The livestock population in Ethiopia is estimated to be about 41.5 million cattle, 14.6 million sheep, 13.6 million goats, 5.8 million equines, 0.44 million camels, and 42.9 million poultry (CACC, 2003). Despite these huge livestock resources, productivity of animals is poor even lower than the African averages. Total herd off take is estimated at 7% annually for cattle and 33% and 37% for sheep and goats, respectively. Live weight gains are low at about 20 kg per annum and mortality is high at about 29%. Cows do not reach maturity until 4 years of age, they calve every second year and produce only 1.5 - 2.1 liters of milk per day over a 150 - 180 days of lactation period (Beyene, 1997; Demeke and Tesfaye, 1997). As a result, the contribution of livestock sector to the national economy in general and to the improved living conditions of farmers in particular is minimal.

One major constraint to low productivity and mortality of animals in Ethiopia is the presence of different widespread animal diseases in the country. Devastating epizootic diseases like rinderpest, contagious bovine pleuro-pneumonia, lumpy skin disease and endemic bacterial diseases such as pasteurellosis, anthrax and blackleg cause high mortality in animals but are to some extent in a better control by prophylactic vaccinations. However, other diseases like brucellosis and tuberculosis which have serious economic and public health impact but low mortality, have not received the attention they deserve. In Ethiopia, the current trend of intensification of livestock production in the urban and peri-urban areas of the country, and the increasing international animal trade with increasing movements of animals is creating ground for the increasing importance of bovine brucellosis in the intensive herds particularly in dairy farms. Several reports have established the growing importance of brucellosis in the

country (Asfaw *et al.*, 1998, Bekele *et al.*, 2000). Moreover, the large herd size in the intensive production system is often maintained by introduction of replacement cattle from outside sources, however, these animals may originate from multiple sources increasing the probability of introducing cattle with incubative infectious disease like brucellosis. Therefore, among others, brucellosis is becoming a threat to the intensification of livestock production from a smallholder subsistence farming to a market oriented commercial farming and health hazard to the human population in Ethiopia.

Brucellosis is an infectious and contagious disease caused by organisms of the genus *Brucella* and affects domestic animals, man and wildlife (Chukwu, 1985). Bovine brucellosis (also called contagious abortion) is caused by *Brucella abortus*, less frequently by *B. melitensis* and rarely by *B. suis* (OIE, 2004).

In animals, brucellosis mainly affects reproduction and fertility, reduces survival of newborns, and reduces milk-yield, however, mortality of adult animals is insignificant (Swell and Brocklesby, 1990). The disease is also frequently associated with retention of fetal membranes after calving and occasionally infection may result in development of metritis. In addition, localization of *Brucella* organisms in the udder may cause mastitis. Brucellosis is also responsible for many cases of hygromas, bursitis, arthritis and subcutaneous abscessation in cattle. In the bull, *B. abortus* is known to produce orchitis and sterility (Chukwu, 1987; Radostits *et al.*, 2000). However, the disease in humans is characterized by a multitude of somatic complaints, including fever, night sweats, anorexia, fatigue, malaise, weight loss, and depression (WHO, 1997).

Brucellosis has a considerable impact on animal and human health, as well as wide socio-economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products (Roth *et al.*, 2001). According to Nicoletti *et al.* (1984), brucellosis is perhaps the most widespread and economically important disease in tropical and subtropical regions. In cattle, the disease causes losses due to abortion, diminished milk production, condemnation of animals failing to breed, affects the animal export trade of a nation, and losses in financial investment and government cost on research and eradication schemes. The direct loss of meat in infected herds of cattle was estimated to be 15% and for milk at 20% per infected cow. In addition, human brucellosis also causes loss of man-hours and medical cost (Chukwu, 1987).

Bovine brucellosis is found worldwide, but several countries in Northern and Central Europe, Canada, Japan, Australia and New Zealand are believed to be free from the agent. However, its occurrence is increasing in developing countries (Acha and Szyfres, 2001; OIE, 2004). In Africa, bovine brucellosis is widespread and a high prevalence is recorded in many countries. However, it is difficult to compute national prevalence figure for each country as most surveys have been conducted on selected herds or areas (Chukwu, 1985).

In Ethiopia, generally, prevalence of brucellosis was found to be high in the more intensive management systems like dairy farms and ranches than in the extensively managed animals. Seroprevalence of bovine brucellosis in Ethiopia was reported to vary from 0.14% to 38.7% depending on the intensive or extensive cattle management systems. On the other hand, information on the epidemiology of brucellosis in animals and humans in the study area i.e., Tigray Region, is non-existent or scant. However, considering the size and diversity of cattle population in Tigray Region, the rapid development of intensive livestock farming and the increasing livestock movement and trade in the Region could be conducive for the maintenance and spread of infectious diseases like brucellosis which has significant economic and zoonotic importance. In Tigray, Tesfay (2003) reported a seroprevalence of 0.7% (3/430) in cattle in selected sites of the Region. However, the survey was not conducted using sufficient and representative samples. Thus, a well-designed, systematic and extensive survey that can thoroughly examine the prevalence and distribution of brucellosis both in the extensive and intensive cattle production systems of the Region is required. Understanding the epidemiological features of this disease in the Region in turn will enable to formulate sound control measures. Therefore, the objectives of this study were:

- Establish the seroprevalence of bovine brucellosis in the extensive and intensive cattle production systems of Tigray Region.
- Identify risk factors for occurrence of brucellosis
- Assess the public health significance of brucellosis.

2. LITERTURE REVIEW

2.1 Epidemiology

The maintenance, spread and control of brucellosis are related to the animal population, biology of the disease and the type of management in the herd (Salman and Meyer, 1984). According to Crawford *et al.* (1990), the factors influencing the epidemiology of *Brucella* infections in cattle in any geographical region can be classified into factors associated with the transmission of the disease between herds and factors influencing the maintenance and spread of infection within herds.

2.1.1. Geographical distribution and occurrence

Bovine brucellosis is found worldwide. However, most of the European countries are free from the disease. Brucellosis in cattle has been eradicated from Finland, Norway, Sweden, Denmark, the Netherlands, Belgium, Switzerland, Germany, Austria, Czechoslovakia, Rumania, Hungary and Bulgaria as well as other countries (Fensterbank, 1986; Acha and Szyfres, 2001). Furthermore, New Zealand, Canada, and the United Sates, among the others, are free from bovine brucellosis or close to being so. Three important cattle-raising countries, Argentina, Brazil, and Mexico still have limited control programs (Acha and Szyfres, 2001). In the rest of the world, seroprevalence figures vary greatly from one country to another and between regions within a country and the highest prevalence have been recorded in dairy cattle. In many countries including most of the Latin American countries that have no control programs, the data are unreliable. Nevertheless, the available information indicates that brucellosis is still one of the most important diseases of cattle in Latin America as well as in other developing countries (Acha and Szyfres, 2001).

Chukwu (1985) summarized the extensive surveys carried out on bovine brucellosis in Africa and reached to a conclusion that the high prevalence of bovine brucellosis in Africa showed that many countries have not yet started control or eradication programs. Furthermore, most or all of the surveys carried out on bovine brucellosis were conducted on selected herds or persons in the country; therefore, it is difficult to have a national average for each country.

The following were some of the surveys conducted in East African countries.

- Hellmann *et al.* (1984) found seropositivity of 6.5% from 5,982 randomly selected cattle in Bahr el Ghazal Province of Southern Sudan. On the other hand, McDermott *et al.* (1987) reported a prevalence of 15.45% in cows in Kongor Rural Council of Southern Sudan.
- In Eritrea, Omer *et al.* (2000b) screened 2,427 cattle for brucellosis using RBPT and CFT. They obtained a higher seroprevalence in dairy cattle (8.2%), followed by pastoral western lowlands (5%) and 0.3% in the mixed-crop livestock system in the southern highlands.
- Kagumba and Nandokha (1978) carried a survey of bovine brucellosis in Kenya, Uganda and Tanzania. The prevalence of brucellosis on the basis of CFT was 9.2% (10% in RBPT) in Kenya, 4.6% (5% in RBPT) in Uganda and 5% (5.8% in RBPT) in Tanzania.
- Jiwa *et al.* (1996) found a prevalence of 4.3% - 15% in Lake Victoria Zone of Tanzania. Recently, Weinhaupl *et al.* (2000) recorded seropositivity of 14.1% and 12.3% in crossbred dairy animals in Dar es Salaam and zebu cattle in Lugoba area of Tanzania, respectively, using serum agglutination test.
- In Somalia, Hussein *et al.* (1978) reported seroprevalence of 2.7% to 11% in cattle whereas Andreani *et al.* (1982) found a prevalence of 15.45%.
- Chantl *et al.* (1994) documented a prevalence of 4% in Zebu cattle in Djibouti by testing 499 animals.

Other works in some other African countries showed prevalence of 2.1% in Molopo district of Bophuthatswana (Bakunzi *et al.*, 1993), 6.6% in Ghana (Kubufor *et al.*, 2000) and 7% in Chad (Schelling *et al.*, 2003).

In Ethiopia, there is very little information on how and when bovine brucellosis was introduced and established. However, in the last two decades, different researchers have carried out several studies on prevalence of the disease. These surveys have showed that bovine brucellosis is an endemic and widespread disease with high prevalence in organized farms than extensively managed herds. Results of bovine brucellosis seroprevalence surveys conducted in Ethiopia are summarized in Annex 1.

Among the studies carried out in the intensive farms, Meyer (1980) reported a seroprevalence of 39% from 1,010 cattle at the Institute of Agricultural Research Centre, Bako. Sintaro (1994) had reported a prevalence of 22% in Chaffa State Dairy Farm in northeastern Ethiopia. Asfaw *et al.* (1998) found an overall prevalence of 8.1% of the 1,114 dairy animals tested in and around Addis Ababa. Bekele *et al.* (2000), in their study in southwestern Ethiopia, recorded seropositivity of 7.3%, 10.8% and 10.4% in Didatiura, Agarfa and Abernosa ranches, respectively. In this study, a total of 4,094 animals were tested using MRT, RBPT, CFT and ELISA. Moreover, Tolosa (2004) and Asmare (2004) documented prevalence of 0.2% and 2.4% in Jimma and Sidama zones, respectively.

On the other hand, in the extensive cattle production system, Kebede (2000) and Mekonen (2001) carried out surveys in indigenous breed cattle managed under the extensive system of northeastern and northwestern Ethiopia and reported seroprevalence of 1.8% and 8.3%, respectively. Recently, Tolosa (2004) reported a prevalence of 0.77% in Jimma zone from 1,305 sampled animals while Asmare (2004) showed a seroprevalence of 1.66%, out of 1,627 tested study animals, in Sidama zone. Both workers used RBPT and CFT as screening and confirmatory tests, respectively.

2.1.2. Factors related to the agent

2.1.2.1. Etiology

Brucellosis is caused by *Brucellae* which are Gram-negative, facultative intracellular bacteria that can infect many species of animals and humans. Six species are recognized within the genus *Brucella*: *B. abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae* (WHO, 1997; OIE, 2004). However, recently, strains of *Brucella* have been isolated in the last decade from marine mammals that cannot be ascribed to any of the above-recognised species. Investigations are continuing to establish their correct position in the taxonomy of the genus and it is proposed that they could be classified into two new species, *B. cetaceae* and *B. pinnipediae* (Foster *et al.*, 2002). *Brucella* species and their different biotypes are currently distinguished by differential tests based on sero-typing, phage typing, dye sensitivity, CO_2 requirement, H_2S production and metabolic properties (WHO, 1986; WHO, 1997; Quinn *et al.*, 1999; OIE, 2004). Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and rarely by *B. suis* (OIE, 2004).

Natural infections in cattle with *Brucella* species other than *B. abortus* are rare. Infection of cattle with *B. melitensis* occurs in regions where the organism is enzootic in sheep and goats (Waghela *et al.*, 1980). However, abortion in cows due to *B. melitensis* is rare although some may become carriers and excrete organisms in their milk (Nicoletti, 1980).

2.1.1.2. Morphology, staining and biochemical properties

Brucella species are small (0.6x 0.6 to 1.5μm), non-motile, non-spore forming, aerobic, non-fermenting, coccobacilli or short rods, Gram-negative bacteria and partially acid fast in that they are not decolorized by 0.5 per cent acetic acid in the modified Ziehl- Neelsen (MZN) stain. In MZN stained smears of body fluids or tissues, they characteristically appear as clusters of red cocobacilli. The *Brucellae* are aerobic and capnophilic, catalase positive, oxidase positive (except *B. ovis* and *B. neotomae*), urease positive (except *B. ovis*) and will not grow on MacConkey agar (Quinn *et al.*, 2002; OIE, 2004).

2.1.2.3. Antigenic structure

B. abortus, B. melitensis, B. neotomae or B. suis may occur as either smooth or rough strains expressing smooth-lipopolysaccharide (S-LPS) or rough-lipopolysaccharide (R-LPS) as major surface antigen. *B. ovis and B. canis* are two naturally occurring species, expressing R-LPS as major surface antigen (WHO, 1997). All *Brucella* species possesses A-antigen (abortus) and M-antigen (melitensis) but in varying amounts. *B. abortus* is said to have 20 times more A-antigen than M-antigen. Based on the immunoelectrophoresis and immunodiffusion analysis of soluble extracts of smooth (S) and rough (R) cells, the knowledge of the antigenic structure of *Brucellae* is improved. This analysis indicated that the main antigens so far identified include the smooth and the rough lipopolysaccharides (S-LPS and R-LPS) and two related polysaccharide: native hapten (NH) and B polysaccharide (poly B), and at least 20 protein or glycoprotein antigens. The lipopolysaccharide (LPS) antigens are all located on the surface, whereas most of the protein antigens occur within the *Brucella* cell. The LPS and some protein antigens are involved in diagnostic tests and the protective activity of vaccines. S-LPS is the major antigen involved in the standard diagnostic tests such as agglutination, complement fixation, rose bengal plate and milk ring tests. The NH and poly B haptens, which are S-LPS related, are used in the radial-immunodiffussion test for differentiating infected from immunized animals. The intracellular A_2 antigen used in immunodiffusion and

immunoelectrophoresis test is also able to distinguish infected from immunized animals under some circumstances (WHO, 1986; WHO, 1997). The first antigen to be clearly identified as protective against smooth *B. abortus* was the S-LPS even though other protein antigens must be involved (WHO, 1997).

Serological coss-reactions have been demonstrated between smooth *Brucella* species and *E. coli* O:116 and O:157, *Francisella tularensis*, Salmonella group N (O:30), *Pseudomonas maltophila*, *Vibreo cholerae* and *Yersinia enterocolitica* serogroup O:9. Moreover, non-specific agglutinins towards *Brucella* sometimes appear in the sera of certain animals, which cannot be attributed to conventional antibodies arising from infection (WHO, 1986).

2.1.2.4. Resistance

Brucella organisms can survive in the environment for long periods at low temperature (under appropriate conditions). Their ability to withstand inactivation under natural condition is relatively high compared with most other groups or non-sporing pathogenic bacteria (WHO, 1986). Moreover, under proper environmental conditions, they survive for up to 4 months in milk, urine, water and damp soil. However, pasteurization effectively kills *Brucella* in milk. The most commonly used disinfectants also kill *Brucella* (Walker, 1999).

2.1.3. Factors related to the host

2.1.3.1. Susceptibility and latency

Susceptibility of the animal depends on the animal's natural resistance, age, sex, level of immunity and environmental stress (Seifert, 1996; Acha and Szyfres, 2001). Moreover, susceptibility is determined by number and virulence of the organisms required to produce infection (Nicoletti, 1980; WHO, 1986). Furthermore, sexual development and pregnancy status, exposure to infection and vaccination status are also factors that affect susceptibility (WHO, 1986; Madsen, 1989). Latent infection does occur in calves since 2 per cent of animals infected at birth remain infected as adults (Walker, 1999). However, according to WHO (1986), about 5% of calves born from infected dams remain latently infected with the clinical signs and the serological response becoming apparent during their first pregnancy and causes abortion.

2.1.3.2. Age

Cattle are more resistant to brucellosis before maturity and become increasingly susceptible as they approach breeding age (Radostits *et al.*, 2000). Young cattle are less susceptible to *B. abortus* than older sexually mature cattle. Calves that ingest *Brucella* contaminated milk excrete the organisms in their faeces, but rarely develop a persistent infection (WHO, 1986). Susceptibility appears to be more commonly associated with sexual maturity and risk of infection increases with pregnancy and as the stage of pregnancy increases (Crawford *et al.*, 1990). However, after sexual maturity, apparently, there are no differences in susceptibility among non-vaccinated pregnant cattle (Radostits *et al.*, 2000).

2.1.3.3. Sex

It appears that no controlled studies have been conducted on the relative susceptibility of female and male cattle to brucellosis. Based upon 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).

2.1.3.4. Breed

Apparently no breed-resistance to brucellosis is known and all breeds of cattle appear to be comparable in susceptibility to brucellosis (Madsen, 1989; Radostits *et al.*, 2000).

2.1.4. Factors related to the management and environment

The system of husbandry as well as the environmental conditions will greatly influence the spread of infection (WHO, 1986).

2.1.4.1. Management

Management factors exert a significant influence on prevalence of the disease. It is not merely the herd size that affects prevalence but the intensity of cattle contact within and between herds and with infected pasture and water. This shows that incidence of brucellosis increases with change from a purely extensive system to a more intensive form of cattle management

(Thimm and Wundt, 1976). However, if once brucellosis is introduced into large herds, a higher proportion of the animals in the herd become infected and the disease persists and is more difficult to eradicate (Crawford *et al.*, 1990; Radostits *et al.*, 2000). Likewise, vaccination level, population density, method of housing and use of maternity pens influence the probability of exposure of cattle to infection (Crawford *et al.*, 1990). Moreover, poor sanitation condition can favor rapid spread and subsequently high prevalence of the disease in the herd (Nicoletti, 1980). In addition to this, calving in dark crowded enclosures is more favorable in spreading the disease than calving in the open air in a dry environment (WHO, 1986).

2.1.4.2. Environment

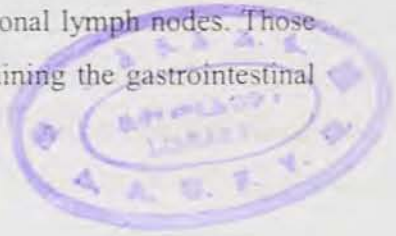
The environment in which the host and the agent interact determines the epidemiology of bovine brucellosis. The existing environmental condition influences the viability of the organism outside the host. *Brucella* organisms merely persist but do not multiply in the environment. Favorable climatic condition (cool agro-climatic condition) increases the survival of *Brucella* organisms in the environment for a long time and helps to maintain endemicity in an enzootic area. Moreover, change of climatic condition in the same environment in the different seasons of the year will affect management systems, hence, affects spread of the disease between infected and susceptible individuals (Radostits *et al.*, 2000).

2.1.5. Source of infection

The main sources of infection for cattle are aborted fetuses, afterbirth, and vaginal discharge containing large numbers of *Brucella*. To a lesser extent, calves fed on contaminated milk can contaminate farm areas by their fecal matter (WHO, 1986; Acha and Szyfres, 2001). Contaminated pastures, fodder, or water are also important source of infection (Radostits *et al.*, 2000). Ingestion of contaminated milk from infected cattle is another source of infection in calves (Walker, 1999).

After abortion, uterine infection normally declines within months. The animal may not abort during the subsequent pregnancy, but it will continue to discharge the *Brucella* organisms. Udder and milk infection lasts for several months or years and may be a source of uterine

infection during subsequent pregnancies (Mukasa-Mugrewa, 1989). Persistent infection of mammary glands and supra-mammary lymph nodes is common, with constant or intermittent shedding of the organisms in the milk in succeeding lactations. Some viable calves born to infected dams have infections that may persist in the lungs and regional lymph nodes. Those fed with infected milk may have infections of the lymph nodes draining the gastrointestinal tract and may shed *Brucella* organisms in the faeces (WHO, 1986).



2.1.6. Mode of transmission and route of infection

Brucellosis can be transmitted horizontally by the introduction of acutely or latently infected animals into a herd, or vertically in utero to calves born from infected dams (Radostits *et al.*, 2000). Infection occurs when animals make direct or indirect contact with infective excretions (Quinn *et al.*, 1999; Walker, 1999). Although brucellosis is acquired most often by ingestion, infection can occasionally follow venereal contact, penetration through skin abrasions, conjunctivae or inhalation (Acha and Szyfres, 2001; Quinn *et al.*, 2002).

A controlled experimental study indicated that a large number of *Brucella* organisms are necessary to infect a cow via the vaginal route. However, there is no doubt that the intrauterine route used in artificial insemination is very important in transmitting infection. Thus, the use of infected bulls for artificial insemination poses risk since the infection can spread to several herds (Chukwu, 1987; Seifert, 1996). Cow's habit of licking the genital organs of other cows also contributes to the transmission of infection (Radostits *et al.*, 2000).

2.1.7. Reservoir of infection

There are two principal epidemiological situations in which *Brucella* infection may be prevalent in wildlife (WHO, 1986). The first situation involves various species of wild and domestic animals, birds, insects, and ticks to which the infection is transmitted from the principal carriers of *Brucella* (farm animals), disappearing again after eradication of the foci of infection among the latter. The second situation involves a number of animal species among which brucellosis exists independently, e.g. hares infected with *B. suis* biovar 2, wild reindeer, bison, feral swine and certain species of rodents. Dogs and wild canids commonly ingest the product of parturition and it is likely that they will ingest *Brucella* containing fetal membranes or post- parturient lochia and get infected (WHO, 1986; Detilleux *et al.*, 1991).

2.2. Pathogenesis and clinical signs

Following exposure, *Brucella* penetrates intact mucosal surfaces. After penetrating mucosal barriers, organisms may be engulfed by phagocytic cells (Walker, 1999). Inability of leucocytes to effectively kill virulent organisms at the primary site of infection results in dissemination of the organisms to regional lymph nodes, reticuloendothelial system, gravid uterus, udder, joint, bursa, testicles, and accessory sex glands (Walker, 1999; Radostits *et al.*, 2000; Quinn *et al.*, 2002)).

After infection of the regional lymph nodes, bacteraemia occurs which can last for 1-3 weeks and distribute to the lymphatic system, other organs and tissues. In pregnant animals, the uterus is preferred site of infection where it leads to a necrotizing placentitis. In non-pregnant animals, the first infection often occurs in the udder followed by the infection of the uterus after the onset of pregnancy. In cattle, the uterus is the central site of multiplication of the pathogen; the enhanced virulence of the *Brucellae* inside the reproductive system is supposed to be the consequence of the increased level of the sugar alcohol erythritol (Seifert, 1996). According to Seifert (1996), the variable facets of clinical signs are the consequence of the individual level of host defense, level of immunity, age of the animal, productivity, condition, and environmental influences as well as virulence of the pathogen. The primary clinical manifestation of brucellosis is related to the reproductive tract. In general, animals do not exhibit overt systemic illness (Walker, 1999). The following complexes of symptoms are typical consequence to brucellosis infection (Chukwu, 1987; Seifert, 1996; Walker, 1999; Acha and Szyfres 2001).

- Early embryonic death and thus symptomless infection
- Abortion after the fifth month of pregnancy
- Birth of weak calves
- Retention of fetal membranes
- Metritis and mastitis
- Chronic inflammation of the joints, tendon sheaths, synovial bursae (hygroma) especially in the cows.
- Epididymitis and orchitis are the most common in males; however, inflammation of the seminal vesicle and vesicular gland also occurs.

2.3. Diagnosis

Unequivocal diagnosis of brucellosis infection in animals can be made only by the isolation and identification of *Brucella*, but in situations where bacteriological examination is not practicable, diagnosis must be based on serological methods. There is no single test by which a bacterium can be identified as *Brucella*. A combination of growth characteristics, serological and bacteriological methods is usually needed (WHO, 1986; OIE, 2004).

2.3.1. Demonstration by microscopic examination

This is a useful procedure for the examination of aborted material. Smears of placental cotyledon, fetal stomach contents, or uterine exudates should be heat-fixed and stained by a differential method such as Koster's, Macchiavello's, or Stamp's modification of the Ziehl-Neelsen stain (Alton *et al.*, 1975; OIE, 2004). *Brucella* organisms resist decolorization by weak acids and a diagnosis can often be based solely on microscopic examination. *Brucella* appears as small, red-staining cocobacilli in clamps because of their intracellular growth (Quinn *et al.*, 1999). The method will not differentiate between *Brucella* and *Coxiella burnetii* or *Chlamydia*. However, the fluorescent antibody method has been advocated to increase specificity. In practice, interpretation of the results of this test is difficult and it offers few advantages over the modified acid-fast stains (OIE, 2004).

2.3.2. Isolation and identification

Direct isolation and culture of *Brucella* are usually performed on solid media. This is generally the most satisfactory method as it enables the developing colonies to be isolated and recognized clearly. Such media also limit the establishment of non-smooth mutants and excessive development of contaminants. However, the use of liquid media may be recommended for voluminous samples or for enrichment purpose. Isolation and identification of *B. abortus* is confirmatory (Alton *et al.*, 1975; WHO, 1986; Quinn *et al.*, 2002; OIE, 2004). The *Brucellae* grow well on 5 - 10 per cent blood agar. However, other than fetal abomasal contents and colostrum, the specimens are likely to contain many contaminating bacteria and fungi, so selective media are usually required. The selective media contain nutritive blood agar base with 5 per cent sterile sero-negative equine or bovine serum and an antibiotic supplement (Quinn *et al.*, 1999). Commonly used media include serum dextrose, tryptose, and

Brucella (Albimi) agars. If contamination is likely to be a problem, isolation attempts should be made using media containing actidione (30mg/L), bacitracin (7500 IU/L), and polymyxin B (1800IU/L). Selective media are used both with and without the incorporation of ethyl violet (1:800,000). Cultures should be incubated at 37°C in 5% - 10% CO_2 for a minimum of 10 days and up to 21 days in highly suspicious cases (Walker, 1999).

Animal inoculation is the most sensitive method for detection of *Brucella* and is sometimes necessary when very low numbers of organisms are present. Guinea pigs are the most sensitive laboratory animals for this purpose. Two guinea pigs are inoculated and sacrificed at 3 and 6 weeks post - inoculation (Alton *et al.*, 1975).

2.3.3. Serology

An ideal serological test would establish an early diagnosis, identifies chronic infections, and distinguishes between antibodies of vaccination and those of infection. A test also needs to be economical, simple and possible to repeat on numerous occasions. However, no serological test possesses all these qualities (Fensterbank, 1986).

Antibody detection is commonly used for diagnosing brucellosis and in control programs. For routine tests, anti-*Brucella* antibodies are detected in serum, milk and sometimes in vaginal mucus or semen (Walker, 1999). *Brucella abortus* strain 99 or 1119-3 usually is used for preparation of antigen. The antigen primarily involved in either case is the surface lipopolysaccharide (OIE, 2004). Although, several variety of serological tests are employed for the diagnosis of bovine brucellosis in different countries; it has been limited to those methods that were used in this study.

2.3.3.1. Rose Bengal Plate Test (RBPT)

This is a simple spot agglutination test using stained antigens and buffered to a low pH. of 3.65 or 4.0. The Rose Bengal Plate test has found wide application as a screening test for individual diagnosis of herds of cattle. It is generally considered to be oversensitive, especially in cattle immunized with strain 19 (Alton *et al.*, 1975; WHO, 1986). Serum samples may be screened using the RBPT. Serum (20 - 30µl) is mixed with an equal volume of antigen on a white enamel plate to produce a zone approximately 2 cm in diameter.

The mixture is rocked gently for 4 minutes at ambient temperature and then observed for agglutination. Any visible agglutination is considered to be positive. The test is very sensitive, especially in vaccinated animals, and positive samples should be retested by a confirmatory test such as the CFT or ELISA. False-negative reactions may occur and can be detected by retesting animals at intervals over a period of at least 3 months (OIE, 2004).

2.3.3.2. Complement Fixation Test (CFT)

Activation of the classical complement system by antibody to antigen results in the generation of membrane attack complexes capable of disrupting cell membranes. If the antibody is bound to erythrocyte surfaces, the erythrocyte membranes are disrupted and haemolysis occurs. It is possible to use this reaction to measure serum antibody levels and this test is known as Complement Fixation Test. The principle of CFT is that if complement is fixed by antigen-antibody immune complex, it is unavailable to lyses the target cells in the indicator system. In the absence of antibody, the complement remains free to lyses the target cells in the indicator system (Tizard, 1992).

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. There are numerous variations of the CFT in use, but this test is most conveniently carried out in a microtitre format. 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 (Alton *et al.*, 1975; OIE, 2004).

Reference sera: Primary bovine reference standards are those against which all other standards are compared and calibrated. For RBT and CFT, the OIE International Standard Serum (OIEISS, previously the WHO Second International anti-*Brucella abortus* Serum) that contains 1000 IU and ICFTU (international complement fixation test units) is used. These sera have been developed and designated by the OIE as International Standard Sera. The use of these promotes international harmonisation of diagnostic testing and antigen standardisation (OIE, 2004).

Standardization of the results of CFT: there is a unit system that is based on the OIEISS. This serum contains 1000 ICFTU (international complement fixation test units) per ml. If this serum is tested in a given method and gives a titre of, for example 200 (50% haemolysis), then the factor for an unknown serum tested by that method can be found from the formula: 1000 x 1/200 x titre of test serum = number of ICFTU of antibody in the test serum per ml. The OIEISS contains specific IgG; national standard sera should also depend on this isotype for their specific complement-fixing activity. Difficulties in standardisation arise because different techniques selectively favor CF by different immunoglobulin isotypes (OIE, 2004).

Interpretation of the results: Sera giving a titre equivalent to 20 ICFTU/ml or more are considered to be positive

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. Females that have been vaccinated with *Brucella abortus* S19 between 3 and 6 months are usually considered to be positive if the sera give positive fixation at a titre of 30 or greater ICFTU/ml when the animals are tested at an age of 18 months or older (OIE, 2004).

2.3.3.3. Other tests

A number of immunodiagnostic tests have been developed in cattle. These tests detect different classes and types of antibodies and vary in their sensitivity and specificity (Walker, 1999). The most commonly used tests in individual animals are: the serum agglutination test, buffered *Brucella* agglutination plate test (BBAPT) and the ELISAs. The performance of ELISAs is comparable with that of the CFT, and as they are technically simpler to perform and more robust, their use may be preferred in many laboratories as screening or confirmatory tests (Nielsen *et al.*, 1995). The Milk Ring Test (herd level test) is also efficient means of screening dairy herds by testing milk from bulk tank (OIE, 2004). In addition, a number of other methods have been described for detection of *Brucella* in tissues and fluids including PCR, immunoperoxidase staining, DNA probes and Brucellin test (Walker, 1999; Redkar *et al.*, 2001; OIE, 2004).

2.4. Immunity to brucellosis

Infection with *Brucella* usually results in the induction of both humoral and cell-mediated immune responses. The magnitude and duration of these responses can be affected by many factors including virulence of the infecting strain, size of incoulum, age, sex, pregnancy, species, and immune status of the host (WHO, 1986). Although humoral immune response plays an important role in immunity to *Brucella*, it is the cell-mediated response that is most important in providing protection (WHO, 1997).

2.4.1. Humoral immunity

IgG_1, IgG_2, IgM, and IgA are the immunoglobulin isotypes present in serologically significant concentrations in bovine serum. Similar isotypes at different relative concentrations occur in milk, although most of the IgA is present in the secretary form. Although secretary IgA in milk does play an important role in the Milk Ring Test, IgM also participates in this reaction, whereas IgG_1 will produce an agglutinate at the bottom of the tube and may interfere with ring formation by other isotypes (WHO, 1986).

The first immunoglobulin produced after an initial heavy infection or strain 19 immunization is IgM. This can usually be detected in the first or second week following the initial antigenic stimulus, but is soon followed by IgG antibody. IgG_1 immunoglobulin is the most abundant in serum and exceeds the concentration of IgG_2. The magnitude and duration of the antibody response following immunization is directly related to the age at immunization and the number of organisms administered. Following immunization with the standard dose of strain 19 during calf hood, IgG antibody concentrations usually decline to diagnostically insignificant levels over 3 - 6 months. Residual antibody, if present, is usually predominantly of the IgM class (WHO, 1986).

Exposure to a relatively large dose usually produces a significant agglutinin titre within 2 to 4 weeks. With a minimum dose, the time required for development of "reactor" titres may vary from 2 to 7 months after exposure. Under natural conditions, the majority of infected cattle will probably have developed a diagnostic agglutinin titre 30 to 60 days after exposure (Manthei and Deyoe, 1977). According to WHO (1986), following exposure to virulent *Brucella abortus*, antibody may appear in 4-10 weeks or longer, depending on the size and

route of entry of the inoculum and the stage of pregnancy of the animal, but even under controlled experimental conditions there is a great variation in response from animal to animal. In infected environments, animals exposed to low doses may develop transient low antibody titres, but show no clinical or bacteriological evidence of infection. A disturbing number of infected animals do not develop antibody of the IgG class until parturition, or 1-3 weeks after parturition. These animals may have low IgM titres a few weeks earlier, but in a vaccinated population they cannot be differentiated from non infected vaccinated animals.

Antibodies of the IgA, IgM, IgG_1, and IgG_2 isotypes can all react in the tube-agglutination test, but those of the IgM class are by far the most efficient. Antibodies of the IgG_1 isotype produced in some sera, at least, have the capacity to block agglutination by other isotypes particularly IgM. The agglutinating and precipitating activity of IgG_1 antibodies is enhanced at high salt concentrations or under acid conditions and this isotype is reactive in the card and Rose Bengal tests. The reactivity of IgM in this type of test is dependent on the precise method of preparation of the antigen and the procedures used (WHO, 1986).

2.4.2. Cell-mediated immunity

Brucella is a facultative intracellular bacterium that survives and replicate in both phagocytic and non-phagocytic cells. Phagocytes play a key role in initiating T-cell responses by processing and presenting antigens (WHO, 1997). Studies carried out in mice (Araya *et al.* 1989) indicated that acquired resistance to infection with *B. abortus* is the result of independent, and probably interactive, effects of antibodies and effector T-cells of both CD4 and CD8 phenotypes. Further studies shown that CD8 T-cells (MHC Class I restricted) play an important role in clearance of *Brucellae* following the peak of infection, probably by lysing infected macrophages (Olivera and Splitter, 1995).

The role of cytokines in the control of *Brucella* infection has been investigated by injection of recombinant cytokines or by inhibition of their activity using specific MAbs. Macrophage-derived cytokines such as the interleukin-1 (IL-1), IL-12 and tumor necrosis factor-alpha contribute to the control of early *Brucella* infection; IL-12 by stimulating natural killer (NK) cells and T-cells to produce IFN-gamma and TNF-alpha via an IFN-gamma independent pathway, probably by recruiting macrophages and promoting granuloma formation (WHO, 1997).

2.5. Economic and public health importance

Brucellosis affecting a wide range of animal hosts is recognized worldwide for its great economic losses in livestock industry and its serious public health hazard (Chukwu, 1987; Weidmann, 1991; WHO, 1997).

2.5.1. Economic importance

The highest prevalence of bovine brucellosis is seen in dairy cattle (Acha and Szyfres, 2001), as a result, serious economic losses are encountered in dairy farms as described below.

- *Losses due to abortions*: abortions reduce financial returns because of losses in calf-crop, in replacement and sales, prolonged intercalving interval increase the period between lactation (Chukwu, 1987; Radostits *et al.*, 2000). Literature values ranged between 10% to 50% incidence of abortion in infected animals (Murillo, 1989; Bernues *et al.*, 1997).
- *Reduced milk production*: the direct loss in milk production was estimated to be 20% per infected cow (Nicoletti *et al.*, 1984). Other workers showed ranges between 10% to 25% reduction of total yield (Murillo, 1989; Bernues *et al.*, 1997).
- *Infertility and culling of animals due to breeding failure*: breeding failure is common in *Brucella* infected cows and frequently three or more services are needed per conception. The average delay in conception following an infected calving is estimated to be about 63 days (Chukwu, 1987). Moreover, it causes sterility in aborted cows to the extent of 20% (Murillo, 1989).
- *Meat losses*: the loss in meat production was estimated at 5% both in cows and in infected calves (Murillo, 1989; Bernues *et al.*, 1997). Other estimate showed a value of 15% (Nicoletti *et al.*, 1984).
- *Perinatal mortality*: 5% to 20% incidence of perinatal mortality was observed in calves born from infected cows (Murillo, 1989; Bernues *et al.*, 1997).
- *Mortality*: a mortality risk of 1% among aborted cows was estimated (Bernues *et al.*, 1997).
- *Replacement requirements*: the increase in replacement requirements is 15% of the positive tested animals (Bernues *et al.*, 1997). This increase was due to abortions, temporary infertility and decrease in milk production.

- *Endangering animal export trade of a nation*: brucellosis could endanger the animal export trade of a country, particularly when other countries purchase only *Brucella*-free animals (Chukwu, 1987).
- *Losses associated in financial investments and government research and eradication schemes* are also important.
- *Human brucellosis causes loses of man-hours and medical costs* (Chukwu, 1987).
- *Adverse effects on the reproductive potential of African wildlife* (Chukwu, 1987).

2.5.2. Public health importance

The Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Office International des Epizooties (OIE) consider brucellosis as one of the most widespread zoonoses in the world. Human brucellosis caused by *B. abortus* and *B. melitensis* is an occupational hazard affecting primarily farmers, butchers, meatpacking employees, and veterinarians. In countries with endemic brucellosis, serological prevalence of 50% to 60% in veterinary practitioners is common (Chukwu, 1987). Each year about half a million cases of brucellosis occur in humans around the world (WHO, 1986).

Brucellosis in humans due to *B. melitensis* and *B. abortus* causes an acute febrile disease with undulant fever, which can progress to a more chronic form. There are evidences of serious complications affecting the musculo-skeletal, cardio-vascular and central nervous system (Radostits *et al.*, 2000; WHO, 1997). The modes of infection are ingestion, direct contact, inhalation, and accidental inoculation. The most common source of infection is by ingestion of milk or its products (Alton *et al.*, 1975). Brucellosis is an occupational disease in veterinarians, abattoir workers, farmers, and others who contract infection when they work with animals or consuming their products. The incidence of human brucellosis can sometimes be related to climatic conditions, if animals are brought close to, or within, the home environment for protection (WHO, 1997). Manure, soil, and pasture can be a source of viable organisms for several months after contamination. Some cases of human-to-human transmission of brucellosis have been described in Kuwait due to breast milk (Acha and Szyfres, 2001).

Several surveys carried on human brucellosis showed zoonotic importance of the disease. In Chad, seroprevalence of 3% was recorded (Schelling *et al.*, 2003). Hussein *et al.* (1978)

recorded seropositivity 0.6% in Somalia. A prevalence figure as high as 30% have been reported in Kenya, while in other African countries like the Sudan, Uganda and Tanzania prevalence within the range of 1% - 25% was reported (Chukwu, 1985). Omer *et al.* (2002) found seroprevalence of 3.0% - 7.1% in Eritrea. Staak (1990) recorded seroprevalence of 10% in Iraq where people prefer to consume fresh or sour milk. In Ethiopia, limited survey has been conducted on human brucellosis. Tolosa (2004) reported seroprevalence of 2.4% in Jimma zone while Asmare (2004) found 5.3% in Sidama zone, southern Ethiopia. Both conducted the study in occupational risk groups and thus don't represent the prevalence in the population.

2.6. Prevention and control of bovine brucellosis

According to Nicoletti (1980), the justification for the prevention and control of bovine brucellosis are usually divided into two major categories-economic and public health. Three major forms of control and prevention are usually recommended. These are: Immunization, test-and-slaughter of seropositive cattle and management practices (Nicoletti, 1980; WHO, 1986; Weidmann, 1991; Walker, 1999).

2.6.1. Immunization

Vaccination is recommended for control of bovine brucellosis in enzootic areas with high prevalence. The choice of vaccine is *B. abortus* strain 19, confirmed by its worldwide use, the protection it gives for the useful lifetime of the animal, and its low cost. To avoid interference with diagnosis, it is recommended that vaccination be limited (by legislation) to young animals (calves of 3 to 8 months old), as these animals rapidly lose the antibodies produced in response to vaccine. It is estimated that 65% to 80% of vaccinated animals remain protected against the infection. In a systematic vaccination program, the best results are obtained with 70% to 90 % annual coverage in calves of the proper age of vaccination. Male calves and females over 8 months of age should not be vaccinated (Acha and Szyfres, 2001). The drawbacks of *B. abortus* S19 vaccine are: it causes abortion in a proportion of pregnant animals; pathogenic for humans and induction of O-PS specific antibodies that interfere with the widely used serological tests which employ S-LPS as antigen (WHO, 1997).

On the other hand, *B. abortus* strain RB51 vaccine is attenuated, devoid of the O-chain, rough, and with little or no abortifacient activity. When used in single vaccination, its protective effect in cattle is similar to that of S 19. After RB51 vaccination, all species tested have remained serologically negative in all conventional serological tests for brucellosis (WHO, 1997). Moreover, *Brucella suis* S2 is a live vaccine developed in China, which is administered orally, and a relatively new choice in the means of combating brucellosis. For beef cattle, sheep, goats and pigs, protection rates of 75%, 83%, 82%, and 72% is attained with a single application, respectively. It allows administering the vaccine via the watering point without negative effect on pregnant animals. The vaccine is also suitable for mass immunization in extensive husbandry systems (Weidmann, 1991).

The fundamental disadvantage of killed vaccines (45/20 and H 38) is their relatively short and weaker prophylactic effect comparable to live vaccines, several vaccinations usually have to be performed, with a consequence of strong production of antibodies. But male animals can be vaccinated and handling the vaccine involves no risk to self-vaccination (infection). Killed vaccines are used where eradication is already at a highly advanced level, or where a quick prophylaxis of an entire herd is to be obtained without risking abortion (Weidmann, 1991).

The rough inactivated strain 45/20 vaccine causes few diagnostic problems except with the CFT. Two doses administered 6-12 weeks apart produce resistance comparable to strain 19 vaccinations (Nicoletti, 1980). However, the H 38 vaccine provides a higher level of protection after a single application than the 45/20 vaccine, but it produces very high titres in all immunoglobulin classes. Animals in all age groups and thus entire herds can be vaccinated with it at one time without risk (WHO, 1997).

2.6.2. Test-and-slaughter

In regions or countries with a low prevalence of the disease, an eradication program can be carried out by repeated serologic diagnostic tests applied to the entire herd, and elimination of reactors until all foci of infection have disappeared. This procedure can be used alone (in countries with a low prevalence of brucellosis) or in combination with vaccination of calves. Epidemiological surveillance and control of animal movement are very important in such program (Acha and Szyfres, 2001). According to Weidmann (1991), test and slaughter can be started when the rate of infection is reduced to an acceptable level, about 1% - 2%.

2.6.3. Management practices

Good husbandry and management systems help to reduce the within-herd prevalence (Salman and Meyer, 1984). The management practices that should be implemented at an early stage of the disease include: the isolation of calving animals in separate calving pens which are subsequently disinfected, the burning or burials of fetal membranes, aborted fetuses, etc., testing and quarantine of newly arrived animals, regular examination of the herd, and slaughtering of positive reactor animals. If one's own herd is disease free, contact with neighboring herds must be prevented. If the disease is enzootic, certain group sizes should not be exceeded and unnecessary new groupings should be avoided (Nicoletti, 1980; WHO, 1986; Weidmann, 1991; Asfaw *et al.*, 1998). The impact of newly introduced infection can be minimized by maintenance of a resistant herd and early detection. Cattle identification is also essential in modern cattle commerce (Nicoletti, 1980).

3. MATERIALS AND METHODS

3.1 Study areas

The study area, Tigray Region, is located in the northern part of Ethiopia (Figure1). The Region is situated between 12° 15' N and 14° 57' N latitude and 36° 27' E and 39° 59' E longitude. The land surface area is approximately 53,638 square kilometers with an average population density of about 65-persons/km^2 and population growth rate of 3% (BoPED, 1998).

The topography of Tigray Region is mainly the extension of the central Ethiopian highlands and the associated western lowlands. A few portion of it lies in the escarpment of the Rift Valley (BoPED, 1998). The altitude also varies from below 500 masl in eastern Erob to the highest peak at Tsibet Mountain, which is above 3500 masl.

The mean annual rainfall ranges from 990mm in the southwestern parts of the Region to less than 200mm in the Dankil depression (BoANR, 1997). Rainy season occurs mainly between June to September, although quasi-double rainfall pattern within a small peak in April and maximum in August is observed in the eastern and most parts of the southern zone of Tigray Region (BoPED, 1998).

The Region is divided into six main agro-ecological zones. These include: the hot to warm semi-arid (SA1) lowlands in the northwestern part; tepid to cool sub-moist (SM2) mid-highlands in the central part of the Region (this covers above 50% of the Region); the hot to warm sub-moist (SM1) lowlands consisting valleys, escarpments and river gorges; the cold to very cold sub-moist (SM3) zone found at the peaks of Alaje Mountain area; the hot to warm moist (M1) lowlands of Welkaite, Tsegede and Tselemti *Weredas*, and the tepid to cool moist (M2) mid-highlands of Welkaite and Tsegede *Weredas* (BoANR, 1996). Currently, the Region is divided into five zones and one metropolitan sub-zone. A total of 34 *Weredas*, 544 rural and 59 urban *Tabias* (equivalent to PA's) are found in the Region (BoPED, 1998).

The study was carried out at 26 *Tabias* found in 12 *Weredas*. These *Weredas* represent four major agro-ecological zones that possess more than 95% of the livestock population in the

Region. The *Weredas* covered in this survey include: Tahtay Adiabo, Alamata, Raya Azebo and Tselemti from the transhumance cattle husbandry system and Offla, Endamehoni, Adwa, Laelay Maichew, Tahtay Koraro, Enderta, Saesea TsaidaEmba and Mereblehe from the sedentary cattle husbandry system (Figure 1).

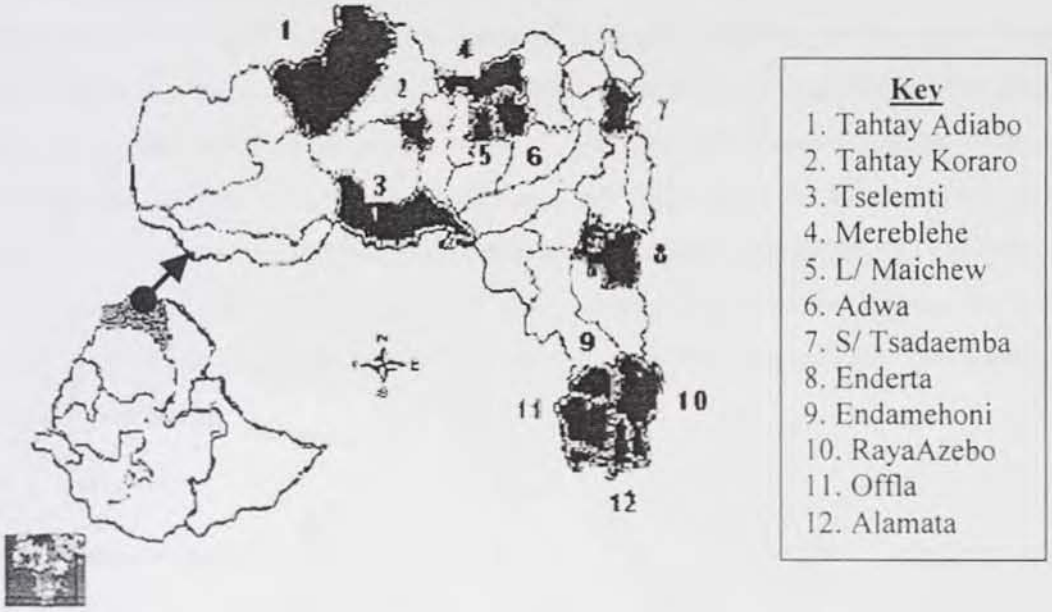


Figure 1. Map of the study areas in Tigray Region

3.2. Study animals

Agriculture is the means of livelihood for more than 85% of the Tigray population. Livestock production is one of the major components of agricultural activities that play an important role in the economy of the Region (BoANR, 1997).

According to the Central Statistical Census Commission (2003), Tigray Region has about 2.6 million heads of cattle, 0.68 million sheep, 1.75 million goats, 0.43 million equines, and 0.037 million camels. In 1995, there were a total of 11,162 crossbred animals with 50% or more exotic blood level in the Region. Out of these, 5,158 were cows, 1,527 heifers, 1,366 bulls, 1,262 male calves and 1,849 female calves. Currently, the projected estimated number of crossbred animals in the Region is far greater than this figure (Gebregziabher, personal communication, 2004).

Cattle production in the Region is mainly characterized by extensive type of management system, which includes sedentary and transhumance cattle husbandry systems. Sedentary farming is a feature of the highlands while transhumance prevails in the north-western and south-eastern lowlands. Moreover, an intensive cattle farming is practiced in the urban and peri-urban areas of the Region. The study animals were comprised of indigenous and cross-bred (Friesian x local) animals. Six kinds of indigenous cattle breed/types are recognized in the Region and these are: the Arado, the Abyssinian zebu, the Raya/Sanga, the Barka/Begait, the Medenenes and the Abergelle breed/type. The first two breeds are found in the highlands and the rest four exist in the low land areas. All these types are known to be multipurpose animals, however, the Barka/Begait is considered as dairy type cattle. On the other hand, the crossbred cattle are found in the urban and peri-urban areas of the Region and are used for dairy production (BoANR, 1997). All the cattle in the Region have not been vaccinated against brucellosis.

3.3. Study design

A cross-sectional epidemiological study was carried out on bovine brucellosis using serological (RBPT and CFT) and questionnaire survey from September 2004 to March 2005 in Tigray Region, Northern Ethiopia. The serological survey of bovine brucellosis was intended to determine the individual animal, herd and within-herd prevalence and also to identify individual animal risk factors associated with seropositivity to brucellosis both in the intensive and extensive cattle production systems of the Region. Also, questionnaire was administered at the same time to assess the role of different management related farm or herd-level risk factors in influencing brucellosis seroprevalence. Likewise, cross-sectional seroprevalence study was conducted on human brucellosis.

3.4. Sample size determination

Sample size was determined using a method recommended by Thrusfield (1995) for two - stage cluster sampling for both intensive (a system of livestock raising based on zero grazing) and extensive management system (a system of livestock keeping mainly based on natural pasture). Sample size calculation was based on the following formula.

$$T_s = \frac{(1.96)^2 * g * P_{exp}(1 - P_{exp})}{g * d^2 - (1.96)^2 * Vc}$$

Where;

g = number of clusters to be sampled

P_{exp} = expected prevalence

d = desired absolute precision

T_s = total number of animals to be sampled

Vc = between - cluster variance

The following estimates were used to calculate the sample size for the intensive system:

- Expected prevalence of 11% (Bekele *et al.*, 2000)
- Error of 2%
- Between - cluster variance estimate of 0.00014641 (This was estimated from an overall mean cluster prevalence of 11%)
- 95% confidence level
- Number of clusters to be sampled 80.

Accordingly, the estimated sample size for the intensive system was 957 animals; however, to increase the precision, 1135 animals belonging to 112 farms were actually sampled.

The following estimates were used to calculate the sample size for the extensive system:

- Estimated prevalence of 8% (Mekonen, 2001)
- Error of 2%
- Between - cluster variance estimate of 0.00004096 (This was estimated from an overall mean cluster prevalence of 8 %).
- 95% Confidence level
- Number of clusters to be sampled 21.

Accordingly, the number of animals to be sampled from the extensive system was computed as 718, but, 816 animals belonging to 26 herds were sampled to increase the precision.

3.5. Sampling procedures

3.5.1 Sampling procedure for the extensive system

In this study, two-stage cluster sampling technique was used where *Tabias* and herds were the established clusters. First, the study areas were stratified by cattle husbandry system into sedentary (sub-system of the extensive management system where livestock owners and their livestock remain permanently settled in one area without practicing seasonal migration to other areas in search of feed and/or water) and transhumance systems (sub-system of the extensive management system where there is seasonal movement of herds following precise routes and repeated each year), subsequently; the total sample size was proportionally allocated to the size of cattle population in the two systems (Table 1). The following procedure was followed during sampling.

- From each cattle husbandry system, random sample of *Tabias* were selected (10 from the transhumance and 16 from the sedentary system) using random number method.
- In each *Tabia*, one herd was randomly selected by a lottery method.
- From each herd, at least 30 animals above six months of age were sampled. A herd, in this study, was defined as group of animals sharing the same grazing area and/or watering point.

Table 1. Proportion and number of animals sampled from each cattle husbandry system

Husbandry systems	Cattle population	Percentage of sample	Calculated sample size	Actually sampled
Transhumance	1,004,997	38	273	312
Sedentary	1,663,081	62	445	504
Total	**2,668,078**	**100**	**718**	**816**

3.5.2. Sampling procedure for the intensive system

Two-stage cluster sampling was used as a sampling strategy. Accordingly, towns with at least 100 crossbred cattle and farms were selected as primary and secondary sampling units, respectively. Thereafter, sampling was conducted as follows:

- Random sample of five towns, out of 8, were selected for the study by a lottery method. Then, the total sample size was proportionally allocated to each town according to its cattle population (Table 2).
- In each town, random sample of farms were selected using random number method from a list of farm owners.
- In each farm, all animals above six months of age were sampled.

Table 2. Proportion and number of animals sampled from each selected town

City	Cattle population	Percentage of sample	Calculated sample size	Actually sampled
Maichew	433	8	80	85
Mekelle	2381	45	450	562
Adigrat	1280	24	240	245
Axum	619	12	120	126
Shire	553	11	110	117
Total	**5266**	**100**	**1000**	**1135**

3.6 Data collection

3.6.1. Blood sample collection

About 10 ml of blood was collected form the jugular or coccygeal vein of each selected animal using plain vacutainer tubes. Blood samples were left overnight to clot at room temperature and then separated serum was temporarily stored in a portable electrical/gas refrigerator during the serum collection period. Sera samples were transferred in iceboxes to Mekelle Veterinary Research and Diagnostic Laboratory, Tigray, and stored at -20°C until testing.

3.6.2 Serological tests

According to WHO (1986) and OIE (2004), RBPT is recommended as a screening test with positive samples being retested by the complement fixation test (CFT). In this study, the RBPT was used as screening test and the CFT as a confirmatory test. The RBPT was reported to have a sensitivity of 98.3% and specificity of 68.8% (Dajer *et al.*, 1999). Complement fixation test has also a specificity of 100% in non-vaccinated cattle (Dohoo *et al.*, 1986) and sensitivity of 95.2% (Uzal *et al.*, 1995).

3.6.2.1. Rose Bengal Plate Test (RBPT)

Antigen and sera required for each day for serological testing was taken out form the cold storage and brought to room temperature before testing was undertaken. The RBPT test was carried out according to the method recommended by Alton *et al.* (1975). The antigen was obtained from Institut Pourquer, 3409 Montpellier Cedex 5, France. The RBPT test was performed at Mekelle Veterinary Research and Diagnostic Laboratory using the following procedures:

a. Equal volume (30µl) of the test sera and antigen was taken by a pipette and placed next to each other on a white enamel plate.
b. The antigen and serum was mixed with a stick and then the plates were gently rotated by hand for 3 - 4 minutes.
c. Final reading was taken as positive or negative by examining the degree of agglutination.

Interpretation: Any observed agglutination was considered as positive. Depending on the degree of agglutination, results were recorded as 4+, 3+, 2+, 1+ and doubtful.

3.6.2.2. Complement Fixation Test (CFT)

Sera positive to RBPT were retested by complement fixation test. The CFT test was done at National Veterinary Institute, Debre Zeit, Ethiopia. Preparation of the reagents and the CFT test proper was done according to the protocols recommended by OIE (2004). Antigen,

control sera and complement were obtained from the BgVV, Berlin, Germany. Materials, preparation and titration of reagents for CFT are shown in Annex 2.

The test proper: Multiple sera technique

Using standard 96-well microtitre plates with round (U) bottoms, the technique is usually performed as follows:

a. Volumes of 25 µl of diluted inactivated test serum are placed in the well of the first, second and third rows. The second row is an anti-complementary control for each serum. Volumes of 25 µl of CFT buffer are added to the wells of the first row (anti-complementary controls) to compensate for lack of antigen. Volumes of 25 µl of CFT buffer are added to all other wells except those of the second row. Serial doubling dilutions are then made by transferring 25 µl volumes of serum from the third row onwards.
b. Volumes of 25 µl of antigen, diluted to working strength, and 25 µl of complement, diluted to the number of units required, are added to each well except in the first row.
c. Control wells containing diluent only, serum + complement + diluent, antigen + complement + diluent, complement + diluent, are set up to contain 75 µl total volume in each case. A control serum that gives a minimum positive reaction should be tested in each set of tests to verify the sensitivity of test conditions.
d. The plates are incubated at 37°C for 30 minutes or at 4°C overnight, and a volume (25 or 50 µl according to the technique) of sensitised SRBCs is added to each well. The plates are re-incubated at 37°C for 30 minutes.
e. The results are read after the plates have been centrifuged at 1000 g for 10 minutes at 4°C or left to stand at 4°C for 2 – 3 hours to allow unlysed cells to settle. The degree of haemolysis is compared with standards corresponding to 0, 25, 50, 75 and 100% lysis. The absence of anti-complementary activity is checked for each serum in the first row.

The reading of results for the CFT was carried out as follows: When there was complete fixation (no haemolysis) with clear water supernatant, result was recorded as + + + +, nearly complete fixation (75% clearing) as + + +, partial haemolysis (50%) as + + and some fixation

(25% clearing) as +. Complete lack of fixation (complete haemolysis) was recorded as 0. For positive reactions final titrations was registered.

Interpretation: sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 (Dohoo *et al.*, 1986) or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive (Alton *et al.*, 1975; OIE, 2004).

Together with the collection of blood samples, the following data were collected on individual animal basis (Annex 3): breed, sex, age, herd size, presence of swollen joints, animal management and agro-ecology of the area. In addition, number of calves produced, age at first calving, interval since last calving, occurrence and number of abortions and stillbirths were taken from cows. Abortion was explained to each farmer, as being premature calving from 3-8 months of age while a stillbirth is expulsion of dead fetus that has reached a full term (McDermott *et al.*, 1987).

3.6.3. Questionnaire survey

A structured questionnaire was prepared and applied to 112 and 26 farm owners belonging to the intensive (Annex 4) and extensive cattle production system (Annex 5), respectively. The questionnaire was pre-tested in the field and adjusted as required. The objective was to characterize the production system and identify risk factors for occurrence of brucellosis. With regard to sampling methodology, for the intensive system, all farms selected for serological survey were also selected for the questionnaire interview; however, in the extensive system, judgment sampling was used to select the key informants. The questionnaire was administered in a single visit personal interview to individual farm owners in case of intensive farms but in the extensive system to a group of 3-5 farmers. The questions studied in the questionnaire for the intensive system include: general farm information, herd structure, management and hygiene factors that are believed to influence the spread and maintenance of brucellosis, history of major diseases in the farm, occurrence of abortion or stillbirths, and retention of fetal membranes. The information gathered for the extensive system was: general farm data, cattle husbandry system, grazing and watering system, movement of animals and disease and abortion history. One hundred per cent (100%) response rate was obtained.

3.7. Blood sample collection for human brucellosis

The examination of human blood samples was to determine the seroprevalence of brucellosis in human population in the capital city of Tigray Region, Mekelle, where dairy farming is becoming a well-established practice. Sample size was determined by standard procedure (Thrusfield, 1995).

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

Where; n = required sample size, P_{exp} = expected prevalence, d = desired absolute precision.

An expected prevalence of 2% and 2% desired absolute precision was substituted in the formula. Accordingly, sample size was calculated as 188 persons. Sampling was conducted by convenience sampling method. Serum samples were collected from people who submitted their blood samples to Mekelle hospital form January to March 2005. A total of 185 sera were collected from the population and tested for brucellosis using RBPT. However, it was not possible to collect blood samples from the high-risk groups.

3.8 Data analysis

Data was stored in Microsoft (MS) Excel Spread Sheet program and analysis was done using MS Excel (Version 6.0, 2000), EpiInfo Version 6 (Dean *et al.*, 1994) and Intercooled STATA 7.0 for windows (Stata Corporation, Texas, USA, 2001) programs. The total prevalence was calculated by dividing the number of RBPT and CFT positive animals by the total number of animals tested. Herd prevalence was calculated by dividing the number of herds with at least one reactor in RBPT and CFT by the number of all herds tested. The within-herd prevalence was calculated by dividing the number of RBPT and CFT reactors within a herd by the number of serum samples tested in the herd (Thrusfield, 1995).

MS Excel 6.0 software programs served to analyze descriptive statistics of the questionnaire and serological results. EpiInfo Version 6 program was used to calculate confidence interval for proportion. Fisher's exact test in Stata 7.0 program was utilized to test if statistically significant associations exist between parity number, abortion, stillbirth or hygroma with

brucellosis seropositivity status. Odds Ratio (OR) in Stata program was also utilized to measure the degree of association between risk factors such as age, herd size, parity number, systemic factor, agro-ecology and farm or herd-level risk factors with brucellosis seroprevalence. All risk factors that had p value ≤ 0.20 in the univariate logistic regression analysis were subjected to multivariate logistic regression analysis to identify major risk factors that have true effect on seropositivity to brucellosis. When two independent variables were highly correlated ($r>0.5$) only one of such variables with more biological relevance was included in the multivariate analysis (Rao and Richard, 2003).

4. RESULTS

4.1. Individual animal seroprevalence

4.1.1. Individual animal seroprevalence in the study areas

In this study, a total of 1,951 cattle serum samples, 1,135 from the intensive and 816 from the extensively managed animals were tested using RBPT and CFT. The RBPT and CFT results are shown in Table 3. The overall seroprevalence of bovine brucellosis in the study area was 1.49% using CFT. Among the 12 Weredas included in the study, *Brucella* antibodies were detected in 6 Weredas which are dispersedly located in the Region. In the study area, seroprevalence was significantly higher in the extensively managed animals as compared to animals in the intensive management system (Figure 2).

Table 3. Results of RBPT and CFT by management system in Tigray Region

Management system	Animals tested	Number (percentage) positive samples	
		RBPT	CFT
Intensive system	1135	4 (0.35%)	3 (0.26)
Extensive system	816	27 (3.43%)	26 (3.19%)
Total	**1951**	**31 (1.59%)**	**29 (1.49%)**

Results on the degrees of agglutination in the RBPT and the intensity of titre in the CFT were recorded. Nineteen of the serum samples (70.3%) examined by the CFT showed a titre of 1:320. The remaining samples depicted a titre of 1:160(2 samples), 1:80 (3 samples), 1:40 (3 samples) and 1:20 (2 samples), respectively. In contrast, 6 (19.35%), 11 (35.48%), 5 (16.13%), 7 (22.58%) and 2 (6.45%) sera samples tested by RBPT had +4, +3, +2, +1 and ± reaction of agglutination, respectively.

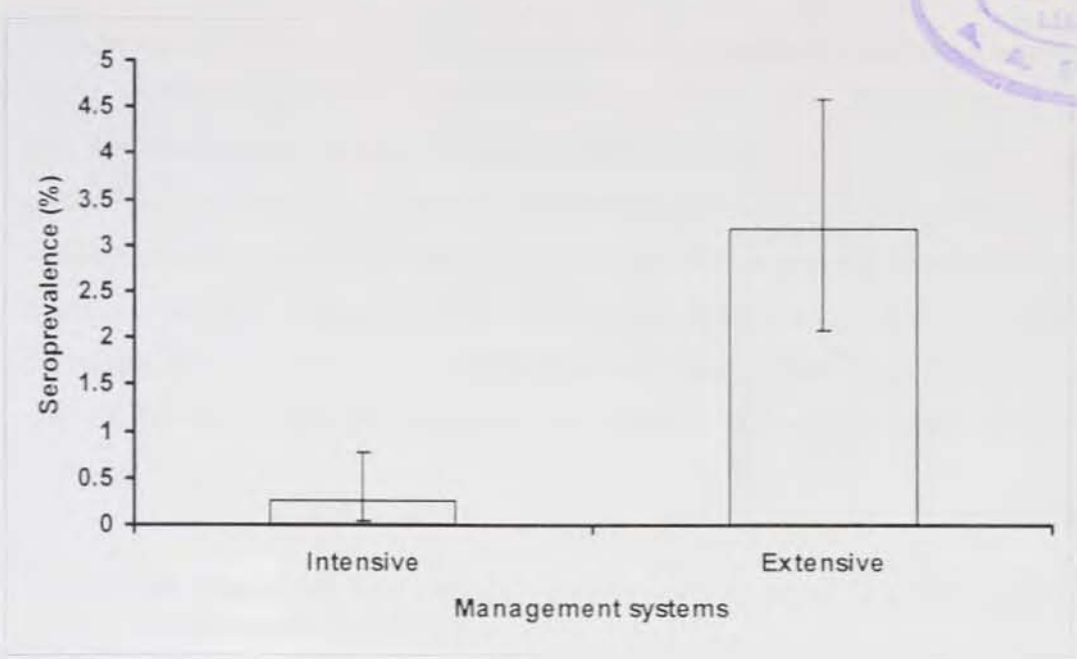


Figure 2. Seroprevalence of bovine brucellosis by cattle management system in Tigray Region

4.1.2. Individual animal seroprevalence in the intensive farms

In cattle under the intensive management system a comparatively low individual animal seroprevalence was found. Out of the 1,135 serum samples tested, 4 (0.35%) were proved to be positive by RBPT from which 3 (0.26%) samples were positive reactors with a titre of 1:320 when retested with CFT.

In the intensive system, age, herd size and parity number were studied as risk factors for brucellosis seropositivity at the individual animal-level using univariate logistic regression (Table 4). An apparently higher seroprevalence (0.31%) was observed in animals greater than 3 years of age than those 0.6 - 3 years of age (0.20%). However, the effect of age was not statistically significant ($p>0.05$). On the other hand, in reference to the effect of sex on seropositivity, it was found that the proportion of positive rectors were 0.27% in females. However, no *Brucella* antibodies were detected in samples collected from the male animals.

The effect of herd size on seroprevalence to brucellosis was also examined in this study. A higher prevalence was observed in farms that kept between 21 - 50 animals (1.56%) followed by farms that had 1 - 20 (0.30%) animals and no *Brucella* antibodies were detected in farms

with greater than 50 animals. However, statistically significant variation was not observed among the herd size groups ($p>0.05$). Moreover, difference in seropositivity to brucellosis was also investigated among the different parity groups in female animals. The highest prevalence was observed in animals with single parturition status (0.53%) followed by multiple parturitions (0.39%) and none in animals with no parturition status. There was no significant variation among the three parity groups (Fisher's exact $p=0.402$). Furthermore, the results of Fisher's exact test revealed that individual animal seropositivity to brucellosis was independently associated ($p<0.01$) with history of previous abortions but not with stillbirths ($p>0.05$).

Table 4. The effect of risk factors on individual animal seropositivity to brucellosis in the intensive management system

Variables	N	Number (%) positives	Univariate Logistic Regression		
			OR	P-value	95% CI
Age (year)					
0.6 - 3	496	1 (0.20%)			
>3	639	2 (0.31%)	1.5	0.719	0.1 – 172
Herd size					
1 - 20	664	2 (0.30%)			
21 - 50	164	1 (0.61%)	2.0	0.564	0.2 - 57.3
> 50	307	0 (0.00%)			

N= number of observations

4.1.3. Individual animal seroprevalence in the extensive herds

In this study, the highest individual animal seroprevalence was recorded in cattle in the extensive cattle production system. Of the 816 sera examined, 27 (3.3%) were seropositive to RBPT. However, test results of CFT revealed a prevalence of 3.2% (26/816) with a titre of 1:20 and above. Variation was observed in the seroprevalence of antibody to *Brucella* in the male and female animals kept under the extensive system. A seroprevalence of 3.2% was detected in female animals while males were seronegative.

A univariate and multivariate logistic regression was run to determine association of risk factors such as systemic factor, age, herd size, agro-climate and parity number with brucellosis seroprevalence status (Table 5). In the extensive cattle production system, the 95% confidence interval calculation for proportion illustrated that cattle in the transhumance management sub-system (7.37%) had a significantly higher seroprevalence as compared to cattle in the sedentary system (0.60%) (Figure 3). Moreover univariate results depicted that animals in the transhumance management sub-system were approximately 13 times more likely to develop brucellosis than animals in the sedentary system ($p<0.001$).

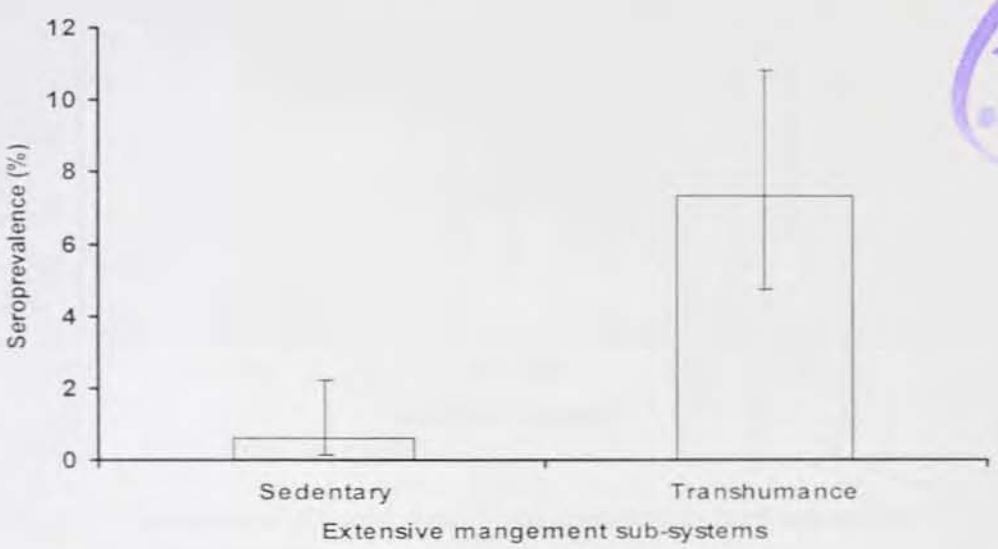


Figure 3. Seroprevalence of bovine brucellosis in sedentary and transhumance management sub-systems in Tigray Region

Analysis of the effect of age on the individual animal seroprevalence using univariate logistic regression indicated that animals above 5 years of age had significantly higher prevalence (5.18%) than those between 0.6 - 5 years of age (1.02%) ($p<0.001$). The univariate analysis estimated that older animals were about 5 times more likely to develop brucellosis than younger animals. There was also a trend of increment in individual animal seroprevalence with the increment of herd size which was statistically significant (Figure 4). The risk of seropositivity was 8.5 and 4.3 times higher in the large and medium size herds, respectively, in comparison to the small herd size. Furthermore, a significant variation in individual animal seroprevalence was observed between the two agro-climatic zones ($p<0.001$). The higher seroprevalence was recorded in the lowlands (6.08%) than in the highland agro-climatic zones (0.68%). Odds Ratio indicated that animals in the lowlands were 9.4 times more likely to be

seropositive to brucellosis than those in highland agro-climatic zone. Regarding parity number, a comparatively higher seropositivity was found in animals with single parturition status (4.07%), followed by multiple parturitions (3.6%) status and least in animals with no parturition status (0.69%). However, significant difference in seropositivity was not observed among the three parity groups ($p>0.05$).

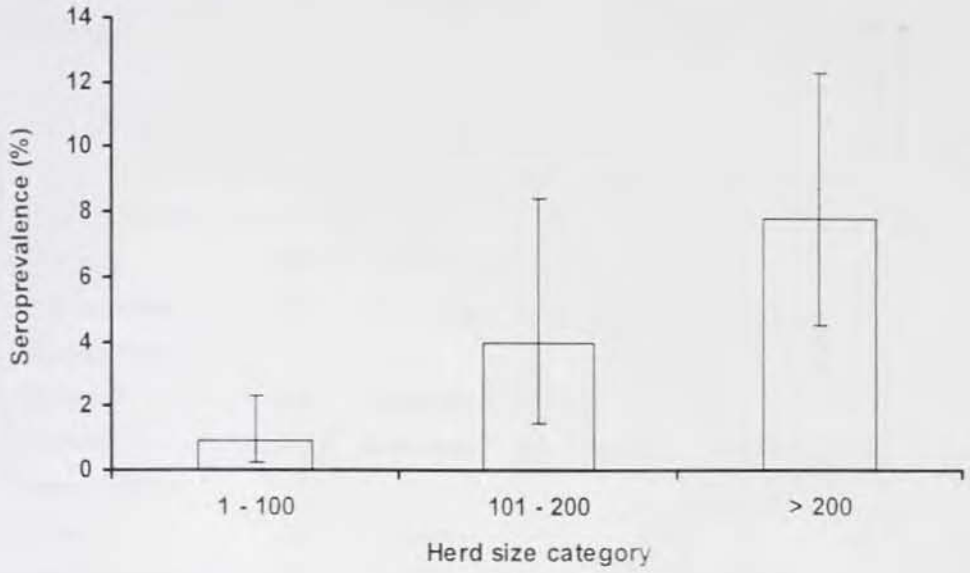


Figure 4. Seroprevalence of bovine brucellosis according to herd size groups in the extensive management system

Risk factors that showed significant effect in the univariate logistic regression were fitted in a model for multivariate logistic regression except those which were thought to be confounding with age and system effect (Table 5). The result revealed that system effect was the major risk factor that was found to be significantly associated with individual animal seroprevalence to brucellosis ($p<0.001$). The OR value showed that animals in the transhumance system were about 11 times at risk to brucellosis than those in the sedentary system. In the multivariate analysis, age also exerted a significant effect on individual animal seroprevalence to brucellosis ($p<0.01$). Older animals were approximately 4 times more likely to be affected by brucellosis than younger ones. However, the effect of herd size on brucellosis seropositivity was not appreciated in the multivariable logistic regression analysis.

Table 5. The effect of risk factors on individual animal seropositivity to brucellosis in the extensive management system

Variables	N	Number (%) positives	Univariate			Multivariate		
			OR	P-value	95% CI	OR	P-value	95% CI
Age (year)								
0.6 - 5	391	4 (1.02%)						
>5	425	22 (5.18%)	5.3	0.002	1.8-15.5	4.2	0.009	2.3 - 49.3
Herd size								
1 - 100	438	4 (0.91%)						
101 - 200	151	6 (3.97%)	4.3	0.025	1.2-15.5	1.5	0.607	0.3 - 6.3
> 200	205	16 (7.80%)	8.5	0.000	2.8-25.6	1.2	0.835	0.3 - 4.8
Systemic factor								
Sedentary	504	3 (0.60%)						
Transhumance	312	23 (7.37%)	13.3	0.000	3.9-44.8	10.6	0.003	2.3 - 49.3
Agro-climate								
Highland*	438	3 (0.68%)						
Lowland**	378	23 (6.08%)	9.4	0.000	2.8-31.5			
Parity number								
No parturition	144	1 (0.69%)						
Single parturition	172	7 (4.07%)	6.1	0.094	0.7-49.9			
Multiple parturition	500	18 (3.6%)	5.3	0.104	0.7-40.3			
≥ 1 parturition	672	25 (3.72%)	5.5	0.095	0.7-41.1			

N= number of observations, * = >1500 masl, ** = < 1500 masl

In the extensive cattle production system, seroprevalence was studied in relation to agro-ecology of the area. Results showed that seroprevalence of animals in the three warm agro - ecological zones (semi-arid 5.79%, warm moist 14.29%, and warm sub-moist 5.51%) are significantly higher than seroprevalence of animals in the cool sub-moist (0.59%) agro-ecological zone (Figure 5).

Breed based analysis of seroprevalence results was not carried out as there were no comparable sizes of different breeds of animals in similar management system. However, crude seroprevalence values of bovine brucellosis by breeds of animals in the study area are 0.27% Cross, 5.6% Barka, 5.5% Raya and 2.1% Arado (Annex 6).

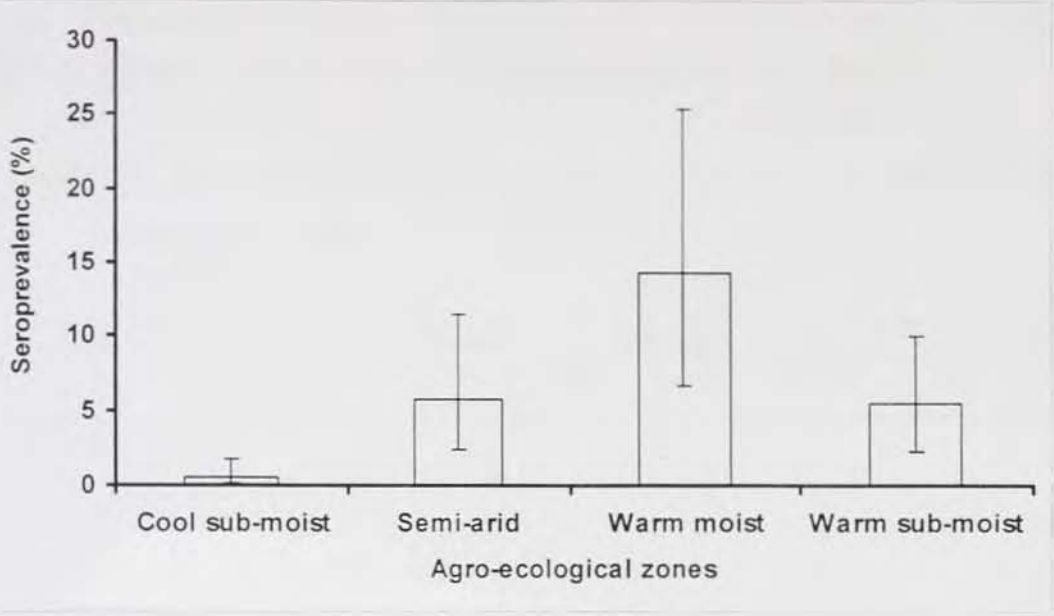


Figure 5. Seroprevalence of bovine brucellosis by agro-ecological zone in Tigray Region

Statistical association of outcome variables such as abortions or stillbirths was examined with brucellosis seroprevalence status in the extensively managed herds. Results have shown that history of previous abortions (Fisher's exact $p<0.001$) and stillbirths (Fisher's exact $p<0.05$) in the individual animal were significantly associated with brucellosis seropositivity.

4.2. Herd-level seroprevalence

4.2.1. Herd-level seroprevalence in the intensive farms

Among the 112 intensive farms investigated in this study, 4 (3.6%) farms had at least one reactor using RBPT and 3 (2.8%) by CFT. Within-herd prevalence varied from absence of reactor animals in the herd to presence of one reactor out of 3 animals (33.3%).

In the intensive management system, seven farm-level risk factors were identified and examined by logistic regression for presence of any association with farm-level seropositivity to brucellosis. None of the risk factors considered in the univariate analysis had significant effect on farm-level seroprevalence to brucellosis ($p>0.05$) except loose fencing condition that had marginal p-value ($p=0.057$). In addition, factors with p-values less than or equal to 0.20

were fitted into the multivariate logistic regression model. Nevertheless, none of the farm-level risk factors were also found statistically significant (p>0.05) (Table 6).

Table 6. The effect of risk factors on farm-level seropositivity to brucellosis in the intensive management system

Variables	N	Number (%) positives	Univariate			Multivariate	
			OR	P-value	95% CI	OR	P-value
Herd size factor							
1 - 20	107	2 (1087%)					
21 - 50	7	1 (14.29%)	0.3	0.102	0.6-105.4	1.6	0.800
> 50	3	0 (0.00%)					
Livestock species intermix							
Absence	67	1 (1.49%)					
Presence	45	2 (4.44%)	0.8	0.831	0.1 - 8.7		
Fencing condition							
Intact	93	1 (1.08%)					
Loose	19	2 (10.53%)	10.9	0.057	0.9 - 126.1	16.8	0.084
Replacement strategy							
Own farm	88	1 (1.14%)					
Outside source	26	2 (7.69%)	7.5	0.107	0.6 - 86.1	15.8	0.154
Mating practice							
Natural	23	1(4.35%)					
Artificial insemination	89	2 (2.25%)	0.5	0.585	0.04 - 5.8		
Hygienic condition							
Poor*	104	2 (1.92%)					
Good	6	1 (16.67%)	10.4	0.073	0.8 - 134.9	2.3	0.698
Awareness on brucellosis							
No	105	2 (1.90%)					
Yes	7	1 (14.29%)	8.5	0.097	0.7 - 108.6	12.6	0.151

N= number of observations, *=farms practicing less than three hygienic measures (separation of cows during parturition, proper disposal of fetal membranes and/or aborted fetus, cleaning and disinfection of stables and proper waste and manure disposal)

4.2.2. Herd-level seroprevalence in the extensive herds

In the extensively managed herds, 11 (42.31%) herds were detected seropositive using CFT among the 26 examined. Moreover, the range for within-herd prevalence differed between none to 15.15% (5/33) based on CFT.

Univariate logistic regression analysis results revealed that herd-level seroprevalence in the transhumance management sub-system (80%) was significantly higher than prevalence in the sedentary system ($p<0.01$). The values of OR indicated that transhumance herds were about 17 times more likely to be seropositive than herds in the sedentary system. However, herd size was not associated with herd-level seropositivity to brucellosis ($p>0.05$) (Table 7).

Table 7. Herd-level risk factors to brucellosis seropositivity in the extensive management system

Variables	N	Number (%) positives	Univariate analysis		
			OR	p value	95% CI
Systemic factor					
Sedentary	16	3 (18.75%)			
Transhumance	10	8 (80%)	17.3	0.005	2.3 - 127.3
Herd size factor					
1 - 100	14	4 (28.57%)			
101 - 200	6	3 (50.00%)	2.5	0.368	0.3 - 18.0
> 200	6	4 (66.67%)	5	0.125	0.6 - 39.0

N= number of observations

4.3. Results of the questionnaire survey

4.2.1. Results of the questionnaire survey in the intensive farms

The mean herd size in the studied 112 farms was 13.67 ranging from 1 to 174 animals. With respect to the herd structure, the results revealed that the proportion of cows in the herd was 50 %, heifers 21%, bulls 3% and calves 26% (Annex 7). The breeds of animals in the

intensive farms comprises of 1113 crosses (Friesian x local), 15 Holstein-Friesian and 7 indigenous (1 Raya, 4 Barka and 2 Arado) breed animals. Of the 112 farm owners questioned, most farms (44%) were established in the last ten years and 34% of the farm owners attended high school or college education. On the other hand, for 20% of the farm owners, dairy farming is their major occupation and in the remaining, dairy farming is a sideline business. With regard to provision of veterinary services, only 14% of the farms had service on regular basis. About 42% of the farms used hired labor. Of all the farm owners questioned, none of them had a maternity pen or separate cows during parturition. Only 3% of the farms practiced cleaning and disinfection; likewise, 3% of the farms properly disposed any aborted fetus and/or fetal membranes. The responses of 112 farm owners to questions related to different farm characteristics are illustrated in Annex 8.

Concerning disease problems, the following diseases were reported by the farm owners in the order of importance: mastitis, anthrax, pneumonia, abortion, lumpy skin disease, and FMD. The other questions were related with knowledge of farm owners on diseases that cause abortion in cattle and about brucellosis. Only 8 (7%) farm owners replied that they knew brucellosis as well as other diseases that cause abortion in cattle. Descriptive statistics of reproductive performance of animals in the intensive management system is presented in Table 8.

Table 8. Descriptive statistics of reproductive performance of animals in the intensive management system in Tigray Region

Description	N	Range	Mean	Std. Deviation
Age (year)	1135	0.6 -18	4.73	2.91
Age at first calving (year)	705	1.9 - 7	4.31	0.64
Interval since last calving (months)	666	1 - 24	6.46	4.44
No. of calving	705	1 - 15	2.95	1.87

N = number of observations

4.3.2. Results of the questionnaire survey in the extensive herds

Of the 26 visited herds, the proportion of herds practising sedentary husbandry system was 62% compared to 38% for the transhumance sub-system. The animals in the extensive

management system comprises of 127 Raya, 121 Barka and 568 Arado breed. With regard to animal movement to other areas, all the herds in the transhumance sub-system move from place to place seasonally in search of feed and/or water but not in the sedentary sub-system. With reference to water sources in the dry season, 19 herds used rivers, 6 wells and 1 lake. On the other hand, knowledge of farmers to diseases that cause abortion in cattle and about brucellosis was in the order of 4% and 0%, respectively. Farmers claimed spoiled feed and febrile conditions as causes of abortion when they were asked to mention specific diseases. However, farmers in almost all herds (90%) mentioned physical injury and mating as the main causes of abortion in cattle. Farmers were also asked to describe the most important cattle diseases in their herds in the preceding year. The answer was variable, however, on the average: septicemic diseases (anthrax, pasteurellosis), parasitism, FMD, trypanosomosis and blackleg were described in that order of importance. The proportion of herds that had a history of abortion or stillbirths and hygroma were 96% and 19%, respectively. Descriptive statistics of reproductive performance of animals in the extensive management system is depicted in Table 9.

Table 9. Descriptive statistics of reproductive performance of animals in the extensive management system in Tigray Region

Description	N	Range	Mean	Std. Deviation
Age (year)	816	1.1 - 18	6.72	2.76
Age at first calving (year)	669	2.11 - 9	5.15	0.92
Interval since last calving (months)	652	1 - 50	7.97	6.12
No. of calving	672	1 - 11	2.71	1.63

N = number of observations

4.4. Results of human brucellosis survey

None of the 185 human sera samples tested by RBPT gave positive reaction.

5. DISCUSSION

5.1. Individual animal seroprevalence in the intensive farms

The 0.26% seroprevalence of bovine brucellosis in the intensively managed animals was considerably lower than most of the previous reports so far in Ethiopia and other African countries. Many reports recorded a higher prevalence of brucellosis in the intensively managed cattle herds as compared to those in the extensive management system. In Ethiopia, seroprevalence reports of 39% at the Institute of Agricultural Research in western Ethiopia (Meyer, 1980), 8.2% in Arsi area (Molla, 1989), 22% in Chaffa State Dairy Farm in northeastern Ethiopia (Sintaro, 1994), 8.1% in dairy farms in and around Addis Ababa (Asfaw *et al.*, 1998) and 11% - 15% in dairy farms and ranches in southwestern Ethiopia (Bekele *et al.*, 2000), respectively, are among others who reported relatively high seroprevalence figures in different areas of the country. In other African countries, seroprevalence of 8.2% was found in dairy cattle in Eritrea (Omer *et al.*, 2000b). In the Sudan, McDermott *et al.* (1987) have also reported a prevalence of 15.45% in Kongor Rural Council. Using slow agglutination test in Dar es Salaam, Tanzania, 14.1% of the tested samples showed positive results (Weinhaupl *et al.*, 2000). Jiwa *et al.* (1996) also obtained a prevalence of 6.3% and 15% in dairy and ranch animals, respectively, in contrast to 4.3% in extensively managed indigenous breed of animals in Tanzania.

In the present study, the finding of low individual animal seroprevalence in the intensive farms is similar to observations made by Tolosa (2004); however, it was slightly higher than the results reported by Yayeh (2003). Yayeh (2003) observed a prevalence of 0.14% by screening 853 crossbred dairy cattle in north Gondar zone. On the other hand, the proportions of positive reactors reported by Tolosa (2004) were 0.2%. According to Tolosa (2004), the reasons for the low prevalence of bovine brucellosis in his study area were: better hygienic practices, the use of maternity pen and/or separation of cows during parturition, cleaning and disinfection activities, culling of infected animals, depending on own herds for replacing stock, and farm owners had better knowledge on brucellosis in the intensive farms than in the extensive system.

Unlike to previous reports, Belihu (2002) and Tesfay (2003) had observed negative results. Belihu (2002) found no reactor animals to RBPT after screening 747 animals at Selale,

Mulo-Selale and Degem areas of central Ethiopia. Likewise, Tesfay (2003) reported similar result by testing 100 crossbred animals in selected sites of Tigray Region. Belihu (2003) attributed the negative results to a small herd size nature of the farms he studied and probably to the awareness created by previous studies to farm owners who could have selectively eliminated positive reactors.

The present observation of low seroprevalence in the intensive dairy farms of this study might have arisen from a number of reasons: It could be associated to the relatively younger age of the intensive dairy system in the Region. Because of the long-existed civil unrest in the Tigray Region, commercial dairy farms in cities and towns of the Region were not introduced and developed at the same time and rate as in other areas of the country. It was in the last decade that the local breed animals managed under the traditional system were upgraded by intensive artificial insemination service and changed into intensive dairy farms. The result of the questionnaire survey also revealed that 44% of the farms were established in less than ten years period and 39% were in the last 20 years. The other reason might be related to the small herd size nature of most farms and the fact that replacement was made mainly from own farms. Thus, the introduction of animals from multiple sources that harbor brucellosis was low. This result was supported by the results of the questionnaire survey that showed about 78.57% of the farms used to replace animals from their own herds. In the study area, artificial insemination is being practiced and semen is supposed to be collected and processed from *Brucella* free breeding bulls. Contact between farms was also negligible as more than 84% of the intensive farms had no contact with the neighboring herds.

5.2. Individual animal seroprevalence in the extensive herds

The 3.19% seroprevalence result of brucellosis in cattle in the extensive management system is in agreement with figures reported from other areas of the country and countries with similar cattle husbandry systems (Bekele *et al.*, 1989; Chantl *et al.*, 1994, in Djibouti; Jiwa *et al.*, 1996, in Tanzania; Kebede, 2000; Omer *et al.*, 2000b, in Eritrea; Tolosa, 2004). In general, in the extensive cattle management system in Ethiopia, prevalence figures within the range of 0.77% (Tolosa, 2004) to 8.2% (Mekonen, 2001) were reported.

In the extensive cattle production system, significantly higher seroprevalence of *Brucella* antibodies was detected among cattle in the transhumance management system. This could be attributed to the mobility of herds and the large herd size of cattle in this system. The higher prevalence observed in large herd size is similar to observations made by Kagumba and Nandokha (1978), Hellmann *et al.* (1984), Miga *et al.* (1996), Asfaw *et al.* (1998), Bekele *et al.* (2000), Asmare (2004) and Tolosa (2004). According to Hellmann *et al.* (1984), large herd size enhances the exposure potential, especially following abortions through increased contact and common feeding and watering points promoting transmission of *Brucella* organisms. Moreover, Omer *et al.* (2000b) explained that mobile herds have greater opportunity to come into contact with other potentially infected herds during their movement into the different areas. Furthermore, migration increases the chance of coming into contact with geographically limited or seasonally abundant diseases and also increases the opportunity for interactions of domestic and wild animals (MacPherson, 1995).

On the contrary, the finding of low brucellosis prevalence in the sedentary husbandry system is consistent with several previous reports (Thimm and Wundt, 1976; Hellmann *et al.*, 1984; Miga *et al.*, 1996; McDermott and Arimi, 2003). It was observed that cattle in this system are small in size and sedentary with little potential of contact with other infected herds, thus, there was less risk of acquiring the disease. McDermott and Arimi (2003) described, in general, the incidence of brucellosis is high in pastoral production systems, and decreases as herd size and size of land holding decreases. Similarly, Hellmann *et al.* (1984) stated that herds of bigger size were found to be more frequently infected than smaller herds.

In the extensive management system, the significantly higher seroprevalence of *Brucella* antibodies in mature than in immature animals is in accordance with many reports. According to Walker (1999) and Radostits *et al.* (2000), cattle become increasingly susceptible as they approach breeding age. Our finding is also consistent with the findings of Hellmann *et al.* (1984), Asfaw *et al.* (1998), Bekele *et al.* (2000), and Kubuafor *et al.* (2000) who reported significantly higher proportion of positive reactors in older animals.

The absence of male reactor animals in this study could probably be due to the smaller number of male (n=47) animals studied as compared to females (n=1904). It was also reported that serological response of male animals to *Brucella* infection is limited. Crawford *et al.* (1990) indicated that the testis of infected male animals were usually observed to be

non-reactors or showed low antibody titres. Similarly, Nicoletti (1980) stated that male cattle are more resistant than females. However, the apparently high seroprevalence figure in female animals than males in this study agrees with the findings of Asfaw *et al.* (1998), Bekele *et al.* (2000), Kubuafor *et al.* (2000), and Tolosa (2004).

The significantly higher seropostivity result in the large herd size categories is in consonance with several authors. Large herd size was reported as one of the major risk factor for occurrence and higher prevalence of bovine brucellosis (Hellmann *et al.*, 1984; Miga *et al.*, 1996; Asfaw *et al.*, 1998; Bekele *et al.*, 2000; McDermott and Arimi, 2003; Tolosa, 2004). Moreover, in the extensive system, a significantly higher seropositivity to brucellosis in animals in the hotter lowland agro-climate, which is unsuitable for survival of *Brucella* organisms is unexpected (Radostits *et al.*, 2000). However, this could probably show that agro-climate is not crucial risk factor for occurrence of brucellosis but may have a confounding effect with management system.

5.3. Herd-level seroprevalence

Due to the low prevalence of brucellosis in the intensive farms, the strength of associations between most of the farm-level management related risk factors and brucellosis seroprevalence status was not significant. Though, none of the factors considered for herd-level prevalence in the intensive system were significant, the influences of management related risk factors and characteristics of the population for occurrence of infection in a herd are reported to have an important role (Salman and Meyer, 1984; Crawford *et al.*, 1990). On the other hand, in the extensive system, transhumance management system showed significantly higher risk of seropositivity to brucellosis at herd-level when compared to the sedentary system. The consistent significant finding of this risk factor at the individual animal and herd-level seroprevalence showed the importance of the management of herds in brucellosis epidemiology. Overall, in addition to prevalence estimate, knowledge of herd or farm-level risk factors for cattle brucellosis was considered essential for introduction of a cost-effective and efficient brucellosis control program.

The high herd-level seroprevalence in the extensive management system might show the mobility and interaction of the herds, difference in herd size, animal husbandry and

movement patterns. However, the wide range figures in the within-herd prevalence could probably be the result of presence or absence of potential foci of infection within the herd for disease transmission among members of the herds and to management and hygienic measures practiced in the farms.

A history of previous abortions or stillbirths was significantly associated with brucellosis seropositivity both in the intensive and extensive cattle production systems. This could be explained by the fact that abortions or stillbirths and retained placenta are typical outcomes of brucellosis infection (Swell *et al.*, 1990; Radostits *et al.*, 2000). Similar results were also obtained by Kubuafor *et al.* (2000), McDermott and Arimi (2002) and Schelling *et al.* (2003).

5.4. Questionnaire results

The questionnaire survey results showed that in the intensively managed animals, most farms were of small herd size (mean = 14, range = 1 - 174) with little or no contact among them and replacement was usually made mainly from own farms. These factors were not probably conducive for the introduction and transmission of brucellosis from one farm to the other. However, if once brucellosis was introduced into any of the farms, the disease might have disseminated easily among members of the same herd since the management and hygienic practices were poor. This is justified by the absence of maternity pen or separation of cows during parturition, lack of proper disposal of aborted fetus and/or fetal membranes and poor farm hygienic status. Similarly, in the extensive system, lack of proper disposal of fetal membranes and /or aborted fetus could facilitate contamination of pasture or watering point, thereby, resulting in rapid spread of the disease. On the other hand, awareness of farmers both in the intensive and extensive production system of the Region on diseases that cause abortion in cattle in general and brucellosis in particular was almost poor. As a result, this lack of knowledge could prevent farmers from taking all management and hygienic measures necessary for preventing the introduction and establishment of brucellosis. The poor management and hygienic condition and lack of awareness on brucellosis both in the intensive and extensively managed animals is similar to the findings of Tolosa (2004), however, there was a discrepancy with regard to stock replacement strategy. In the study made by Tolosa (2004), most of the farmers were relying on outside sources for stock replacement contrary to this observation.

5.5. Human brucellosis

Absence of *Brucella* antibodies in human serum samples in Mekelle area doesn't mean absence of human brucellosis in Tigray Region. However, the absence of *Brucella* antibodies in human samples in the present study could be associated with the low seroprevalence of brucellosis in cattle in Mekelle area. Weidmann (1991) and WHO (1986) indicated that prevalence of brucellosis in humans is largely associated with the prevalence of the disease among domestic animals around him. The other reason could be lack of sampling people who are at high risk of acquiring brucellosis. According to WHO (1986) and Swell and Brocklesby (1990), brucellosis is an occupational disease occurring most often in veterinarians, farmers, stock inspectors, abattoir workers, laboratory personnel and butchers. It was suggested also that more cases of human brucellosis occurs in rural areas where most of the people are farmers or in close contact with animals (Alballa, 1995). It is also recognized that samples taken from hospital are usually biased and unrepresentative. Elsewhere in Ethiopia, seroprevalence of 2.4% (Tolosa, 2004) and 5.3% (Asmare, 2004) were reported in Jimma and Sidama zone, respectively. However, in both cases serum samples were taken from occupational groups and thus do not represent the prevalence in the population. Omer *et al.* (2002) recorded also a prevalence of 3% in humans in Eritrea.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

In this study, it can be concluded that:

- Bovine brucellosis is a wide spread and an endemic disease in Tigray Region with relatively higher seroprevalence in the extensive than in the intensive management system.
- Transhumance management system and age of animals were identified as major risk factors for seropositivity to brucellosis in the extensive management system.
- The current management practices are inadequate to prevent further introduction and establishment of brucellosis in the intensive management system and aggravation of the situation in the extensive management system.
- Awareness among the livestock owners on brucellosis is very poor.
- In the present study, public health importance of brucellosis could not be established in the human population examined.

6.2. Recommendations

The control of bovine brucellosis is of paramount importance considering the economic impact of the disease to the livestock industry and its repercussion on human health. However, control methods can vary on the basis of the epidemiological situation of the disease and economic development of the country. The present finding of low prevalence in the intensive dairy farms, theoretically, could justify the application of test-and-slaughter policy, however, practically; this couldn't be implemented for a number of reasons: the prevailing uncontrolled animal movement in the Region, the high cost for compensation, infrastructure, diagnosis and surveillance activities is unaffordable in the presence of other priority epizootic diseases, and stockowners cooperation may not be reliable since their knowledge on the disease and its impact is low. However, there is a need to design and implement control measures aiming at preventing further spread of the disease in the Region through the use of better management practices, which are cheap and practical options.

Accordingly, the following recommendations are made:

- Implementation of better management practices like replacement of animals from own sources or introducing brucellosis free animals, the use of maternity pen or separation of cows during parturition, proper disposal of fetal membranes and/or aborted fetus and cleaning and disinfection activity.
- Movement of dairy animals in Tigray Region or importation of animals should be allowed after the animals are certified "brucellosis free".
- Education of farmers on economic and public health importance of brucellosis is needed.
- Continuous surveillance of brucellosis in dairy herds is necessary to follow the status of brucellosis.
- Further study on human brucellosis is required particularly in high-risk occupational groups.

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8. ANNEXES

Annex 1. Summary of seroprevalence surveys of bovine brucellosis in Ethiopia

Sources	Prev. (%)	System	Breed	Location	Sample	Tests
Meyer (1980)	39	Intensive	Cross	Central Ethiopia	1010	RBPT
Hadgo (1987)	9.8	Intensive	Mixed	Bahir Dar	678	CFT
Shiferaw (1987)	2.1	Mixed	Mixed	Central Ethiopia	3577	SAT, CFT
Molla (1989)	8.86	Intensive	Cross	Arsi	2178	RBPT
	7.62	Intensive	Cross		2178	SAT
Wendimu (1989)	15	Intensive	Cross	Central Ethiopia	NA	RBPT
	3	Extensive	Zebu	Central Ethiopia		SAT
Zewdu (1989)	15.8	Intensive	Cross	Sidamo	734	RBPT
	11.6	Intensive	Cross			SAT
Yirgu (1991)	14.2	Intensive	Mixed	Aberonsa	3577	
Rashid (1993)	38.7	Intensive	Cross	Bako area	NA	RBPT,CFT
Sintaro (1994)	22	Intensive	Cross	Chaffa State farm	182	RBPT,SAT
Shiferaw (1994)	16.9	Mixed	Mixed	Bahir Dar	1855	RBPT
	12.3					SAT
Asfaw *et al.* (1998)	8.1	Intensive	Friesian Cross	In and around Addis Ababa	1114	RBPT, CFT
Bekele *et al.* (2000)	4.9	Intensive	Mixed	Southeastern Ethiopia	4243	RBPT,CFT
Kebede (2000)	1.8	Extensive	Local	Northeastern Ethiopia	3644	RBPT,CFT
Mekonen (2001)	8.3	Extensive	Mixed	Northwestern Ethiopia	NA	RBPT
Yayeh (2003)	0.14	Intensive	Cross	Gondar zone	853	RBPT,CFT
Tesfay (2003)	0	Intensive	Cross	Tigray Region	100	RBPT,CFT
	0.69	Extensive	Local	Tigray Region	300	RBPT,CFT
Asmare (2004)	2.5	Intensive	Cross	Sidama zone	811	RBPT,CFT
	1.7	Extensive	Local	Sidama zone	1627	RBPT,CFT
Tolosa (2004)	0.8	Intensive	Cross	Jimma zone	508	RBPT,CFT
	0.2	Extensive	Local	Jimma zone	1305	RBPT,CFT

Prev. = Prevalence, NA= Not available, Mixed for breed = when two or more breeds were involved, Mixed for systems = when surveys were carried out in both intensive and extensive management systems.

Annex 2. Materials, preparation, evaluation and titration of reactives for CFT

I. Materials for CFT

- Micro titer plates (U-shaped), multi channel and single channel micropipettes, pipette tips, universal bottles for preparation of solutions, stirrers (magnetic), plate sealer tape, test tubes, measuring cylinders, weighing balance, thermometers, pH indicator, incubator, water bath, deep freezer, centrifuge, etc.
- Standard *Brucella abortus* antigen (CVL, New Haw, Weybridge, Surrey KT15 3NB, UK) with 1:10 working strength, complement (Biome'rieux, France at 1:20 strength), hemolysin or amboceptor (Inttitut Pourquier, France at 1:700 working dilution), positive and negative control sera (BgVV, Berlin Germany).
- Sheep RBC (bled from live animal at the National Veterinary Institute, Ethiopia), Veronal buffer and Alsever's solutions (prepared at the NVI, Ethiopia).

II. Preparation of sheep red blood cells (SRBC) for the hemolytic system

- 7.5 ml of sheep red blood cells was drawn into 12.5 ml of in Alsever's solutions and centrifuged at 2500 rpm for 5 minutes.
- The supernatant was discarded and replaced by Veronal buffer in calcium and magnesium (VCM).
- The sheep red blood cells were re-suspended in diluents completely and 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 until the meniscus of SRBS was reached.
- SRBC was diluted in VCM to 2% suspension and stored at 4^0C. SRBC for CFT should be at least two days old.

III. Amboceptor's (Hemolysin) titration

A. 1:500 to 1:8000 serial dilutions of amboceptor was prepared

- 5 test tubes were prepared and 1m of VCM was added to tubes 2 to 5

- 10 µl amboceptor was mixed with 4990 µl VCM in the first tubes and 1ml was transferred up to the fifth tube

B. 1:750 amboceptor was prepared and serially diluted serially up to 1:12000

- 5 test tubes were prepared and 1ml of VCM was added to test tubes 2 to 5
- 10 µl amboceptor was mixed with 7490 µl VCM in the first tube and 1ml was transferred up to the fifth tube in order of ascending dilution

1st	1:500	1:1000	1:2000	1:4000	1:8000
2nd	1:750	1:1500	1:3000	1:600	1:1200

C. 0.5 ml from each of these test tubes was transferred to a second set of 10 tubes Start with the 1:12000 dilution (working from lower to the higher concentrations)

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

E. 0.5 ml 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 10 minutes

G. 1ml of complement at working dilution was added

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

I. SRBC sensitized by mixing equal volume of 2% erythrocytes with diluted amboceptor and allow standing for 30 minutes at room temperature or with agitation

J. Result was read and the last tube showing minimum haemolytic dose (MHD) was recorded.

IV. Evaluation of complement

- Freeze dried complement was reconstituted with diluent according to its instruction.
- 1:100 complement was prepared
- Complement was added into the 9 wells increasing by 5 µl every time, starting with 10µl
- Diluent was added in to the 9 wells in decreasing amount by 5µl, starting with 40µl.
- 25 µl of antigen was added into the wells
- The plate was placed in water bath a 37^0C for 1hr
- 25µl of hemolytic system was added to all the wells
- The plate was shacked and put again in the water bath at 37^0Cfor 30 minutes
- The test was read by recording the minimum hemolytic dose of complement (MHD). which was represented by the first well showing complete haemolysis. The next well

contains the full hemolytic dose (FHD). The complement dilution = 2FHD/dilution of complement i.e. 2FHD/100.

V. Titration of antigen

Micro titer plate I

- 25 µl of VCM was added to every well of U-plate
- 25 µl pre-diluted antigen was added to wells of row A, B, C ..., G.
- By serial doubling 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 VCM was added to all the wells of U-plate
- 50 µl of pre-diluted 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
- 25 µl of complement at working dilution was added to all wells of plate I
- Plate I was incubated at 37^0C for 30 minutes (covered with second empty plate)
- 25 µl of 2% sheep red blood cells, amboceptor pre-mixed, equal volume, i.e. 25 µl of sheep red blood cells and 25 µl of working dilution of amboceptor's was added to all wells.
- 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.

Annex 3. Serum sampling format

Annex 4. Questionnaire format for intensive farms

CROSS-SECTIONAL STUDY ON THE EPIDEMIOLOGY OF BOVINE BRUCELLOSIS IN TIGRAY REGION

1. General Information **Date** ----------

1.1. Wereda _____ Tabia _____Owner name _____

1.2. What is your major occupation? (1 = dairy farming, 2 = other)

1.3. What is your educational status? (1 = illiterate, 2 = elementary, 3 = high school, 4 = College)

2. Farm and management information

2.1. When did you start farming dairy?

2.2. What is the size of your farm?

2.3. Is your farm fenced? (0 = no, 1=yes)

2.4. What type of labor do you use for the farm? (1 = family, 2 = hired, 3 = both)

2.5. How do you get veterinary service? (1 = regularly, 2 = on call bases, 3 = both)

2.6. What is the structure and composition of your herd?

Category of animals		Breed of animals			Total
		Local	Holstein-Friesian	Cross	
Cows	Pregnant				
	Lactating				
	Dry				
Heifers	1 ≤ 2 yrs				
	2 ≤ 4 yrs				
Bulls	Mature (>3yrs)				
	Young (1≤3yrs)				
Calves	Female (<1yr)				
	Male (<1yr)				
Total					

2.7. Do you keep any of the following animals? (1 = small ruminants, 2 = equines, 3 = camels, 4 = others)

If so, is there a direct contact of cattle with these animals? (0 = no, 1 = yes)

2.8. Is there contact among your animals and other herds? (1 = no contact, 2 = fence contact, 3 = pasture contact, 4 = mixed entirely)

2.9. Do you have your own bull or bulls? (0 = no, 1 = yes)

2.10. Where do you get replacement stock? (1 = buy in, 2 = own farm, 3 = both)

2.11. How do you breed your animals? (1 = artificial insemination, 2 = natural service, 3 = both)

2.12. What are your culling criteria? (1 = disease, 2 = other reasons)

2.13. Do your cattle receive any supplementary feeding? (0 = no, 1=yes)

If so, what _____, _____, _____.

2.14. Do you separate cows during parturition? (0 = no, 1=yes)

2.15. Do you have a maternity pen in your farm? (0 = no, 1=yes)

If yes, how is it maintained? (1 = strictly guarded, 2 = partially guarded, 3 = connected with other pens)

2.16. What do you do to the aborted fetus and/or after birth? (1 = bury, 2 = throw, 3 = doing nothing, 4 = other)

2.17. What do you do to a floor or straw contaminated with a cow birth discharges? (1 = cleaning and disinfection, 2 = only cleaning, 3 = doing nothing)

2.18. Do you feed pooled milk to calves? (0 = no, 1=yes) if yes, how?

3. Disease information

3.1. What were the most important cattle diseases encountered at your farm in the previous year?

Local name	Major symptoms	Scientific name
_____	_____	_____
_____	_____	_____
_____	_____	_____

3.2. Do you know any disease that causes abortion in cattle? (0 = no, 1 = yes)

If yes, what are they?

Local name	Scientific name
_____	_____
_____	_____
_____	_____

3.3. Do you know a disease characterized by abortion and retained placenta in first - calf heifers frequently after five month of pregnancy? (0 = no, 1 = yes)
If yes, what is the name of the disease?

3.4. Have you encountered any abortion or stillbirth in your farm last year? (0 = no, 1= yes)

3.5. Have you seen swelling on knee or testicle in your animals? (0 = no, 1 = yes)

3.6. Have you come across placental retention for more than 24 hrs in your farm last year? (0 = no, 1 = yes)

3.7. Have you seen birth of weak calves at your farm? (0 = no, 1=yes)

4. Farm inspection (supported by observation)

4.1. What type of animal house do you have? (1 = pen, 2 = barn, 3 = free stall)

4.2. What is the type of flooring in the animal house? (1 = concrete, 2 = earth, 3 = other)

4.3. Do you tie the animals or keep loose? (1 = tie, 2 = loose)

4.4. Does the building allow sunshine into the animal house? (0 = no, 1 = yes)

4.5. Have you provided adequate floor space to each animal (220x110cm)? (0 = no, 1 = yes)

4.6. How do you dispose animal waste like dung, urine and left over feed/fodder? (1 = poor, 2 = good, 3 = very good)

Annex 5. Questionnaire format for extensive herds

CROSS-SECTIONAL STUDY ON THE EPIDEMIOLOGY OF BOVINE BRUCELLOSIS IN TIGRAY REGION

1. General Information **Date** ----------

Wereda _____ Tabia _____ Village _____ Herd _____

Name of respondents _____, _____, _____

2. Management information

2.1. What is your cattle husbandry system? (1 = sedentary, 2 = transhumance)

2.2. What types of livestock species are kept in the area? (1 = cattle, 2 = sheep, 3 = goats, 4 = equines, 5 = camels, 6 = others)

2.3. Do you move your animals to other areas in search of feed and/or water? (0 = no, 1 = yes)

2.4. What is your grazing system? (1 = communal, 2 = individual, 3 = both)

2.5. What is your water source in the dry season? (1 = river, 2 = spring, 3 = pond, 4 = well, 5 = borehole, 6 = other)

2.6. What type of house do you use for your animals? (1 = only fenced, 2 = fenced with shed, 3 = other)

2.7. What type of mating do you use? (1 = random natural, 2 = natural with selection, 3 = artificial insemination, 4 = other)

3. Disease information

3.1. What were the most important cattle diseases encountered in your herd during the previous year?

Local name	major symptoms	scientific name
_____	_____	_____
_____	_____	_____
_____	_____	_____

3.2. Do you know any disease that causes abortion in cattle? (0 = no, 1 = yes)

If yes, what are they?

Local name	scientific name
_____	_____
_____	_____
_____	_____

3.3. Do you know a disease characterized by abortion and retained placenta in first - calf heifers frequently after five month of pregnancy? (0 = no, 1 = yes)

If yes, what is the name of the disease?

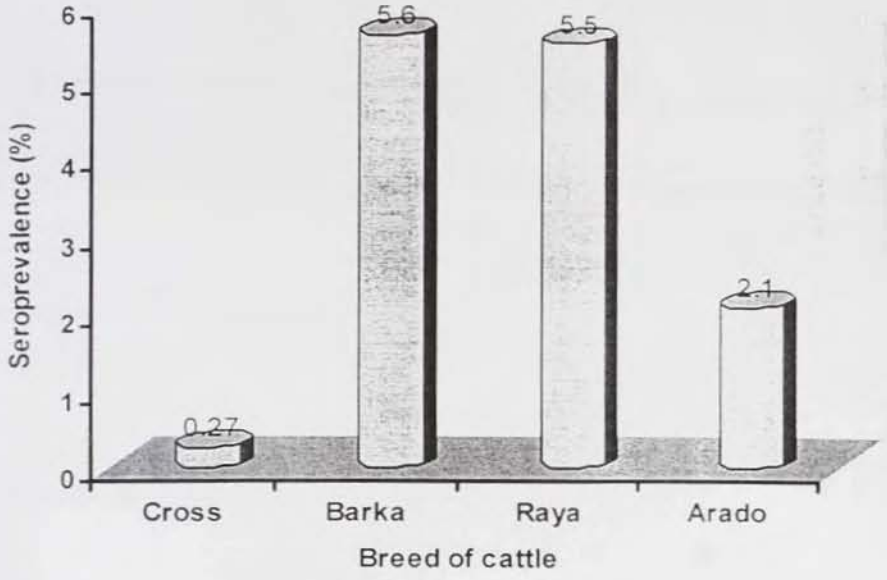
3.4. Have you observed abortions or stillbirths in your herd last year? (0 = no, 1 = yes)

3.5. Do you separate cows during parturition? (0 = no, 1 = yes)

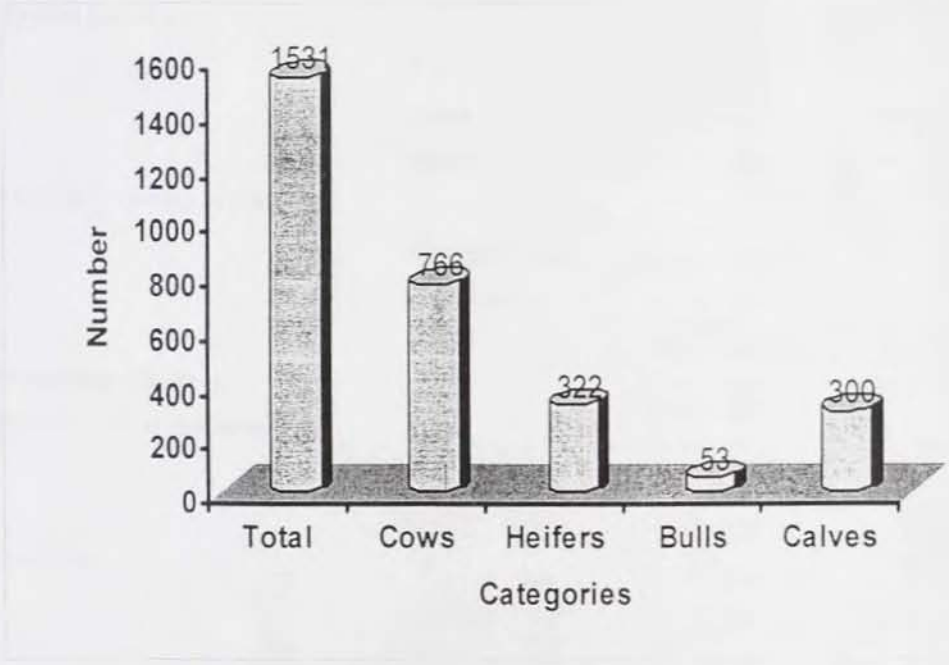
3.6. What do you do to the aborted fetus and/or after birth? (1 = bury, 2 = throw, 3 = doing nothing, 4 = other

3.7. Have you observed swelling on knee or testicle in your animals? (0 = no, 1 = yes)

Annex 6. Seroprevalence of bovine brucellosis by breed of cattle in Tigray Region



Annex 7. Composition of cattle herds in the intensive farms of Tigray Region



Annex 8. Summary of farm characteristics in the intensive farms of Tigray Region

Variable description	Categories	Responses of farm owners (n = 112)	
		No. of yes responses	% of responses
Establishment period of the farm			
	≤ 10 yrs	49	44
	10 ≤ 20 yrs	44	39
	> 20 yrs	19	17
Educational status of farm owners			
	Illiterate	25	22
	Elementary	48	43
	High school	28	25
	College	11	9
Major occupation of farm owners			
	Dairy farming	22	20
	Other	90	80
Type of labour use			
	Family	40	36
	Hired	47	42
	Both	25	22
Veterinary service provision			
	On regular basis	16	14
	On call basis	92	82
	Both	4	4
Ownership of bulls		27	24
Housing type of the farm			
	Pen	45	40
	Barn	67	60
Floor type			
	Concrete	82	73
	Earth	30	27
Feeding of pooled milk to calves		27	24
Occurrence of abortions or stillbirths		25	22
Presence of hygroma		21	19
Occurrence of placental retention		55	49
Birth of weak calves		22	20
Farms with good milking hygiene		6	5

9. CURRICULUM VITAE

1. Personal data

Name: Gebretsadik Berhe

Date of birth: February 1968

Place of birth: Tigray, Ethiopia

Marital status: Married with two children

Nationality: Ethiopian

Profession: Veterinarian

Occupation: PACE Project, Mekelle Branch Coordinator

2. Educational background

Year	Institution	Award
1976 - 1880	Hibret Elementary School	Certificate
1981 - 1986	Fassiledes Comp. Secondary School	ESLCE certificate
1986 - 1992	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit	Doctor of Veterinary Medicine (DVM)
16 May - 24 June 1996	International Centre of Insect Physiology and Ecology, Kenya	Certificate in Tsetse Trapping Technology
22 June - 11 Sept. 1998	University of Edinburgh, Edinburgh	Certificate in Project Planning and Management
2003 - 2005	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit	MSc in Tropical Veterinary Epidemiology

3. Work experience

Year	Institution	Responsibility
January 1993 - April 1993	Private Enterprise	Provision of curative and prophylactic veterinary service
May 1994 - June 1996	Tigray Bureau of Agriculture and Natural Resources	Overall planning and management of animals health activities in a zone
July 1996 – To date	Tigray Bureau of Agriculture and Natural Resources	Overall planning and management of rinderpest control activities

4. **Special skills**

- Computer skill in MS DOS, MS Windows. MS Excel and MS Access
- 3rd grade driving license

5. **Languages**

Tigrigna: speaking and writing

Amharic: speaking and writing

English: speaking and writing

6. **Publications**

- Some morphometric data of the Donkeys of the Debre Zeit area (student scientific journey program)
- Economic importance of small ruminants in Ethiopia (Seminar paper)
- The effect of elevated testicular temperature on semen quality of Ethiopian Menz and Horro rams (DVM thesis).
- Ticks distribution in western Zone of Tigray Region (Unpublished).
- Rinderpest eradication verification survey in Alamata and Raya Azebo *Weredas* of southern zone of Tigray Region, Ethiopia. In: Proceedings of the 16th Ethiopian Veterinary Association Annual Conference. June 2002, Addis Ababa, Ethiopia.
- The Seroepidemiological study of bovine brucellosis in Tigray Region, Northern Ethiopia (MSc thesis)

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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.

Name: Gebretsadik Berhe

Signature: _____

Date of submission: _____

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

Dr Kelay Belihu: _____

Dr Yilkal Asfaw: _____

Seroepidemiological Study Of Bovine Brucellosis In Tigray Region, Northern Ethiopia

Gebretsadik Berhe