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A STUDY ON BOVINE AND HUMAN BRUCELLOSIS IN URBAN AREAS AND IN CATTLE BREEDING CENTERS, NORTH WESTERN AMHARA REGION, ETHIOPIA

BY

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DEBRE ZEIT, ETHIOPIA

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LIST OF ABBREVIATIONS

AI	Artificial Insemination
CSA	Central Statistical Authority
CFT	Complement Fixation Test
FHD	Full Hemolytic Dose
iELISA	indirect Enzyme Linked Immuno Sorbet Assay
kms	kilometers
masl	meter above sea level
µl	microliter
mm	millimeter
ml	milliliter
MHD	Minimum Hemolytic Dose
OIE	Organization International des Epizootics
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
SAT	Serum Agglutination Test
sRBC	sheep Red Blood Cell
UNDP	United Nations Development Programmame
VBD	Veronal Buffer Diluent
WHO	World Health Organization

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ABSTRACT

A cross-sectional study was conducted from October 2007 to March 2008 in urban areas (Bahir Dar and Chagni towns) and in cattle breeding centers (Andassa Livestock Research Center and Metekel Cattle Breeding and Improvement Center) to determine the seroprevalence and to identify potential risk factors associated with bovine brucellosis, to find out its association with reproductive disorders and to assess the public health hazard. Sampling units were selected by one-stage cluster sampling method. Samples were tested serologically for brucellosis and questionnaire data was collected. In cattle breeding centers, all eligible cattle were sampled. Sera of 2,224 cattle from 183 herds were subjected to Rose Bengal Plate Test and those positives were further tested by Complement Fixation Test. An overall individual and herd level seroprevalences of 0.49% (11 of 2,224) and 4.9% (9 of 183), respectively were found. Statistically significant difference in seroprevalence was found between urban areas 1.1% (5 of 443) and cattle breeding centers 0.3% (6 of 1,781) ($P< 0.05$). The potential risk factors such as sex, age, breed, parity, herd size, pregnancy status and source of replacement stock were not significantly associated with brucellosis seroprevalence. Among the reproductive disorders, abortion was significantly associated with seroprevalence of brucellosis ($P<0.05$). Furthermore, management and husbandry risk factors were not significantly associated with brucellosis seropositivity. Out of 79 individuals tested for human brucellosis, 6.3% (5) were positive seroreactors. However, there were no statistical associations between brucellosis and potential risk factors (occupation, sex, age, consumption of raw milk and raw meat as well as contact with fetal membrane). In conclusion, the seroprevalence of bovine brucellosis in urban areas and in cattle breeding centers was very low. Thus, slaughter of positive reactors and enhancing the management and husbandry practices were recommended.

Key Words: Bovine brucellosis, Cattle breeding centers, Human brucellosis, Urban areas

1. INTRODUCTION

Brucellosis in cattle, Bang's disease, is characterized primarily by abortion late in pregnancy, frequently followed by fetal membrane retention and endometritis, which may be the cause of infertility in subsequent pregnancies. In bulls, the disease usually causes orchitis, epididymitis, seminal vesculitis and sterility (Swell and Brocklesby, 1990; Aiello, 1998; Radostits *et al*., 1994). It is an important zoonosis, causing undulant fever in humans characterized by an acute septicemic phase followed by a chronic stage, which may extend over many years (Buxton and Fraser, 1977).

The disease in cattle is caused almost exclusively by *Brucella abortus,* which has seven biovars (Acha and Szyfers, 2001). Approximately 85% of infections are from biotype 1 (Aiello, 1998; Radostits *et al*., 1994). Phenotypic characterization of *Brucella* strains isolated from livestock in Nigeria has indicated that all strains belonged to one species, *Brucella abortus* biovar 1 (Ocholi *et al*., 2004). The pathogen can be killed by heating to 60^{0}C for 10 minutes and is susceptible to an acid pH, disinfectants and direct sun light but remains viable for long periods of time at low temperature (Buxton and Fraser, 1977).

The infected pregnant cow or heifer is the most important source of *B. abortus*. The fetus, fetal membranes and vaginal discharges from an aborting animal may contain 10^{12} to 10^{14} organisms, which contaminate the vulva, tail and legs of the animal and the surrounding environment (Radostits *et al*., 1994). To a lesser extent, farm areas can be contaminated by feces of calves fed on contaminated milk, since not all organisms are destroyed in the gastrointestinal tract (Acha and Szyfres, 2001). Infection usually occurs through ingestion of contaminated pasture, water, or by licking the discharge of an infected animal, newborn calf or retained fetal membrane. Transmission can also occur via penetration of intact skin, the conjunctiva or by inhalation. Calves can be infected in utero or by suckling of infected dams (Swell and Brocklesby, 1990; Aiello, 1998). Bulls that are infected and discharge semen containing organisms are most unlikely to transmit the disease, but the chance of spread from the bull is very great, if the semen is used for artificial insemination (Radostits *et al*., 1994).

Brucellosis is essentially a disease of sexually mature animals, the predilection sites being the reproductive tracts of males and females. If the animal is not pregnant, a chronic disease results without symptoms and perhaps the animal becomes serologically negative. However, if such an animal becomes pregnant the production of simple carbohydrate erythritol in the fetus and its membranes causes enormous multiplication of bacteria in the uterus and this is likely to end up in abortion (Alton, 1981; Quinn *et al*., 1994). Infection spreads rapidly and causes many abortions in unvaccinated infected herds. However, in a herd in which disease is endemic, an infected cow aborts only once after which a degree of immunity develops and the animal remain infected and excrete large number of *Brucellae* in fetal fluids at subsequent parturitions (Aiello, 1998; Quinn *et al*., 1994).

Brucellosis is wide spread and of a serious economic problem particularly amongst dairy cattle in most countries of the world. The prevalence varies greatly from one country to another and between regions within a country (Radostits *et al*., 1994; Acha and Szyfres, 2001). The occurrence of brucellosis is increasing in tropical and subtropical regions because of practices such as nomadism, communal grazing, and modern changes towards larger animal populations and increased commerce (Nicoletti, 1984). Importation of high producing livestock due to demands for additional animal protein and the trend towards intensification of animal production favors the spread and transmission of infection (Seifert, 1996).

The incidence of human brucellosis is clearly correlated with the incidence in the domestic animals (Weidmann, 1991). The infection is usually contracted by handling fetuses and fetal membranes, or by contact with vaginal secretions and carcass of infected animals, as well as consumption of raw animal products. The disease is septicemic, with sudden or insidious onset, and is accompanied by continued intermittent fever (Acha and Szyfers, 2001). Seroprevalences of human brucellosis have been reported to be higher in Africa (Chukwu, 1987; Omar *et al.*, 2002; Schelling *et al.*, 2003). In Ethiopia, few studies have shown the public health importance of zoonotic brucellosis (Yirgu, 1991; Tolosa, 2004; Asmare, 2004; Hailemelekot, 2005).

Many countries have made considerable progress in eradicating brucellosis but in countries found in Africa, it is considered to be one of the most serious disease problems facing the veterinary profession (Radostits *et al*., 1994). In Ethiopia, the prevalence of bovine brucellosis ranges from

0.14% (Yayeh, 2003) to 22.0% (Sintaro, 1994). However, current information concerning brucellosis both in animals and humans is scarce, especially in urban areas and in cattle breeding centers. Hence, this cross-sectional survey of brucellosis was undertaken in urban areas and in cattle breeding centers of Northwestern part of Amhara National Regional State of Ethiopia, with the objectives:

- To determine the seroprevalence and to identify potential risk factors of bovine brucellosis in urban areas and in cattle breeding centers.
- To find out whether there is association between bovine brucellosis and reproductive disorders.
- To assess the public health hazard of bovine brucellosis.

2. LITERATURE REVIEW

2.1. Etiology

At present, six species are known in the genus *Brucella*: *B. melitensis, B. suis, B. abortus, B. canis, B. ovis,* and *B. neotomae.* These species of *Brucella* are obligate intracellular parasites requiring an animal host for maintenance. They are small, gram negative, non-motile, non-spore-forming coccobacilli. Each *Brucella* species has preferred natural host that serves as a reservoir of infection. Secondary hosts play only a very small part in the maintenance or spread of the disease (Quinn *et al.,* 1994; Acha and Szyfers, 2001).

Cattle are the preferential hosts for *B. abortus*, but can also be transmitted to buffalos, camels, deer, horses, pigs, sheep, goats, and man. *Brucella abortus* has seven biovars with different geographic distributions. Biovar 1 is the universal and predominant among the seven that occur in the world (Walker, 1999; Acha and Szyfers, 2001).

2.2. Epidemiology

2.2.1. Occurrence

Bovine brucellosis is found worldwide, but has been eradicated in Finland, Norway, Sweden, Denmark, the Netherlands, Belgium, Switzerland, Germany, Austria, Hungary, the former Czechoslovakia, Rumania and Bulgaria (Acha and Szyfers, 2001). In other parts of the world the rates of brucellosis caused by *B. abortus* vary greatly within and among countries. The highest prevalence has been observed in dairy cattle (Radostits *et al*., 1994; Seifert, 1996; Acha and Szyfers, 2001).

Brucellosis is perhaps the most widespread and economically important zoonotic disease in tropical and subtropical regions. The prevalence of the disease in many African countries ranges from 4% (Chantel *et al*., 1994) up to 35.1 % (Akakpo *et al.,* 1978; Table 1).

Table 1: Seroprevalence of bovine brucellosis in some African countries

Country	Prevalence (%)	Authors and year
Rwanda	35.1	Akakpo *et al.,* 1978
Somalia	9.5	Wernery *et al.,* 1979
Nigeria	1.5 - 14.3	Esuruoso, 1980
Guinea	6.9	Chukwu, 1985
Kenya	7.7	Chukwu, 1985
Sudan	25.3	McDermott *et al.,* 1987
South Africa	2.1	Bakunzi *et al.,* 1993
Djibouti	4.0	Chantel *et al.,* 1994
Tanzania	15.8	Jiwa *et al.,* 1996
Uganda	3.0	Oloffs *et al.,* 1996
Eritrea	8.5	Omer *et al.,* 2000

Different investigators have carried out studies on the prevalence of bovine brucellosis in many parts of Ethiopia, both in intensive and extensive livestock production systems. High prevalences were recorded in intensive dairy farms (Shiferaw, 1994; Sintaro, 1994; Asfaw *et al.,* 1998) and the seroprevalence ranged from 0.14% (Yayeh, 2003) to 22.0% (Sintaro, 1994; Table 2).

Table 2: Seroprevalence of bovine brucellosis in Ethiopia

Study area	Anls exam	Prevalence (%)	Test method used	Source
Around AA	178	18.4	RBPT, SAT, CFT	Gebremariam, 1985
Around BD	678	9.8	RBPT, SAT, CFT	Hadgu, 1987
Shoa region	3,577	2.1	RBPT, SAT, CFT	Shiferaw, 1987
Cent. Eth.	1,609	4.2	RBPT	Bekele *et al.,* 1989
Arsi reg.	2,178	7.62	RBPT, SAT	Molla, 1989
Sidamo reg.	734	11.6	RBPT, SAT	Zewdu, 1989
Abernosa	963	14.20	RBPT, SAT	Yirgu, 1991
Around BD	1,855	16.92	RBPT, SAT	Shiferaw, 1994
Chaffa State Farm	193	22.0	RBPT, SAT	Sintaro, 1994
Around AA	950	8.11	RBPT, CFT	Asfaw *et al.,* 1998
Southeast Eth.	4,243	4.90	RBPT, CFT	Bekele *et al.,* 2000
Tigray	430	0.69	RBPT, CFT	Tesfaye, 2003
Gondar	1,447	0.14	RBPT, CFT	Yayeh, 2003
Jimma	1,813	0.61	RBPT, CFT	Tolosa, 2004
Tigray reg.	1,951	1.49	RBPT, CFT	Berhe, 2005
BD	1,944	4.63	RBPT, CFT	Hailemelekot, 2005
Cent. Orom.	1,238	2.99	RBPT, CFT	Jergefa, 2006
AA and Sululta	1,501	1.13	RBPT, CFT	Tefera, 2006

* BD= Bahir Dar * Cent.= Centeral *Prev.= prevalence

* AA= Addis Ababa * Orom.= Oromia * Als exam= animals examined

* Eth.= Ethiopia

*reg.= region

2.2.2. Source of infection and mode of transmission

The major sources of infection are aborted fetuses, the placenta, and post abortion uterine fluids from diseased cattle. Infected lactating cow is also another source of infection for calves. Brucellosis is transmitted by direct or indirect contact with infective excretors. Although exposure through the conjunctival and genital mucosa, skin and respiratory routes can occur, ingestion is the most common route of entry (Radostits *et al*., 1994; Walker, 1999). Contaminated feed can spread the infection from infected pastures over long distances during purchasing and selling activities (Seifert, 1996; Acha and Szyfers, 2001). Airborne infection has also been documented in closed environment (Nicoletti, 1980; Walker, 1999; Acha and Szyfers, 2001). The use of infected bulls for artificial insemination poses an important risk and spreads the infection to many herds. Calves may be infected while in the uterus or by suckling contaminated milk. Venereal transmissions by infected bull to susceptible cows appear to be rare (Chukwu, 1987; Aiello, 1998).

2.2.3. Risk factors

Management

Once infected, the time required to become free from brucellosis is prolonged by large herd size, active abortions and loose housing (Radostits *et al*., 1994). Increase in herd size and animal density are directly related to the high prevalence of the disease and difficulty in controlling infection in a population (Walker, 1999). Calving practices also play major roles in the spread of brucellosis. Separate calving pens allow for minimizing exposure of uninfected animals. Replacement animals from other sources increases the potential for the introduction of the disease into herds (Nicoletti, 1980; Walker, 1999). Poor hygienic measures and susceptible animals favor the rapid spread of the disease and subsequently resulting in high prevalence of the disease in that particular herd (Nicoletti, 1980; Radostits *et al*., 1994).

Host

Susceptibility to infection depends on age, sex, breed and pregnancy status of the animal. Male and female calves up to six months of age are not very susceptible and generally experience only transient infection. Sexually mature animals are much more susceptible to infection, regardless of

gender. After sexual maturity, the state of pregnancy has a greater influence on the degree of susceptibility. Bulls are more resistant than sexually mature heifers and less resistant than sexually immature heifers. Some animals, even the most susceptible groups, never become infected, or if they do, their infection is transient, and this accounts for individual difference in disease resistance (Walker, 1999; Acha and Szyfers; 2001).

Environment

The environmental resistance of the pathogens depends on whether they are protected against the sunlight and high temperature. Survival is prolonged by low temperatures and the organism remains viable for many years in frozen tissues. Neutral soil pH and a moist protected environment, which are rich in organic material, are favorable elements for survival of *Brucella* (WHO, 1986; Madsen, 1989; Seifert, 1996). It is generally accepted that *Brucella* organisms do not multiply in the environment, instead they merely persist and their viability is influenced by the existing environmental conditions (Radostits *et al.*, 1994)

2.3. Pathogenesis

Following exposure, *Brucella* penetrates intact mucosal surfaces. In the alimentary tract, the epithelium covering the ileal payer's patches is a preferred site of entry. Soon after entry into the host body, *Brucella* is engulfed by phagocytic cells, notably the macrophages, in which they survive, multiply and are transported to the regional lymph nodes (Quinn *et al*., 1994). From the regional lymph nodes, *Brucella* organisms disseminate hematogenously and localize in the reticuloendothelial system and reproductive tract. In pregnant animals, the uterus is a 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 later after the onset of pregnancy (Seifert, 1996). Unknown factors in the gravid uterus, collectively referred to as allantoic fluid factors, stimulate the growth of *Brucella*. Erythritol, a four-carbon alcohol, is considered to be one of these factors (Radostits *et al*., 1994; Walker, 1999). A pyogranulomatous reaction occurs in affected placenta and abortion occurs from mid-gestation on wards. In cattle, abortion commonly occurs in the fifth month of gestation or later and retained fetal membrane is a possible sequel (Radostits *et al*., 1994; Acha and Szyfers, 2001).

2.4. Diagnosis

2.4.1. Bacteriological methods

Microscopic examination

Specimens of fetal stomach contents from an aborted fetus, placental cotyledon, vaginal discharges, fetal lung and liver are stained with Gram or modified Ziehl-Neelsen stains. *Brucella* appears as small red-colored coccobacilli in clumps (Quinn *et al*., 1994; Walker, 1999).

Isolation and identification

Brucella grows well on 5-10% 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. Commonly used media include serum dextrose, tryptose, and *Brucella* (Albimi) agars with the addition of antibiotic such as actidion, bacitracin or Polymyxin B. The inoculated plates are incubated at 37^0C under 5-10 % CO_2 for a minimum of 10 days and up to 21 days in highly suspicious cases (Quinn, *et al*., 1994; Acha and Szyfers, 2001). Preliminary identification of *Brucella* species requires demonstrating pinpoint, smooth, glistening, bluish, translucent colonies. *Brucella* is non-hemolytic, catalase-positive, oxidase positive (except *B. ovis* and *B. neotomae*). A fluorescent Antibody Test is used for rapid identification. *Brucella* biotyping is usually carried out in reference laboratories and the tests involved definitively identifying the species (Quinn *et al*., 1994; WHO, 1997). Animal inoculation is the most sensitive method for *Brucella* detection and is sometimes necessary when very low numbers of organisms are present. Two guinea pigs are inoculated and at 3 and 6 weeks post inoculation an animal is scarified. Serum is examined for antibodies and tissues are cultured for organisms (Walker, 1999).

2.4.2. Serological methods

Serological diagnosis utilizes basic tests suitable for large scale testing. The tests detect antibodies, which are evidence of infection, and make their appearance after a variable incubation period, after which they tend to persist. The relative proportion of antibodies in the immunoglobulin classes IgG1, IgG2, IgM differ according to the stage of evolution of the

disease. In addition, vaccination is also responsible for inducing antibodies of the same classes. An ideal serological test would establish an early diagnosis, identify chronic infections and distinguish between the antibodies of vaccination and those of infection (Fensterbank, 1986).

Serological tests are used for diagnosis more often than are cultures, but only a positive culture yields a definitive diagnosis (Baron *et al*., 1994). It was realized that serological diagnosis was susceptible to false positive reactions resulting from, for instance, exposure to cross-reacting microorganisms. It was also realized that this test format was inexpensive, simple and could be rapid; also results were subjectively scored. Therefore, a number of modifications were developed along with other types of tests. This served two purposes: one was to establish a rapid screening test with high sensitivity and perhaps less specificity and a confirmatory test, usually more complicated but also more specific, to be used on sera that reacted positively in screening tests (Nielsen, 2002). Samples tested include serum, milk, and occasionally semen. A number of immunodiagnostic tests have been developed. These tests detect different classes and types of antibodies and vary in their sensitivity and specificity. Pooled milk samples can be tested using Milk Ring Test whereas serum samples are tested by agglutination tests, Complement Fixation Test, precipitation tests and primary binding tests (Seifert, 1996; Walker, 1999; Acha and Szyfers, 2001; Nielsen, 2002; OIE, 2004).

2.4.3. Molecular method

Numerous Polymerase Chain Reaction (PCR) based assays have been developed for the identification of *Brucella* to improve diagnostic capabilities. Polymerase Chain Reaction is highly sensitive, very specific, inexpensive and easily adapted to high volume demands. The process is rapid, simple, and requires little manual labor. As long as careful attention is given to avoid contamination, the method is very reliable and usually highly reproducible at any properly equipped laboratory (Bricker, 2002). It has been demonstrated that PCR has high potential for rapid identification of strains (Klevezas *et al.,* 2000).

2.5. Economic significance

Brucellosis creates a serious economic problem for the intensive and extensive livestock production systems. Direct losses are due to abortion in pregnant animals, loss of milk

production, and calf mortality with subsequent temporary or permanent infertility and interference with the breeding program due to culling of valuable cows (Schwabe, 1984; Bishop *et al*., 1994; Radostits *et al.*, 1994). This is of great importance in beef herds where calves represent the sole source of income. In addition to the direct losses, brucellosis incurs indirect economic losses resulting from expenditure spent on research, eradication schemes, losses impeding the export trade and human brucellosis causing loss of man-hours as well as medical costs (Chukwu, 1987).

2.6. Public health significance

Humans are susceptible to infection caused by *B. melitensis*, *B. suis, B. abortus,* and *B. canis* with decreasing order of invasiveness and pathogenisty (Acha and Szyfers, 2001). Human brucellosis is acquired from infected domestic animals through direct contact or indirectly by ingestion of raw animal products or by inhalation of airborne agents. Milk contaminated with *B. melitensis* and *B. suis* produce outbreaks of epidemic proportions. Milk and milk products containing *B. abortus* may give rise to sporadic cases. The organisms rarely survive in sour milk, sour cream, butter and fermented cheese (Radostits *et al*., 1994; Acha and Szyfers, 2001).

Human brucellosis is, for the most part, an occupational disease of stockyard and slaughterhouse workers, butchers and veterinarians. A study in Delhi, India, has shown that the highest sero-positivity was recorded in veterinary personnels (Rana *et al.*, 1985). The infection is usually contracted by handling fetuses and after birth, or by contact with vaginal secretions, excreta and carcasses of infected animals (Acha and Szyfers, 2001). Alballa (1995) has indicated that direct contact with domestic animals and consumption of raw animal products are the main risk factors. The disease is readily transmissible to humans, causing an acute febrile illness, which may progress to a more chronic form. The common symptoms are insomnia, sexual impotence, constipation, anorexia, headache, arthralgia, and general malaise (Acha and Szyfers, 2001).

High prevalence in human is found in those countries with the high incidence of *B. melitensis* infection among goats, sheep, or both species (Acha and Szyfers, 2001). Brucellosis has a special importance in African native communities where animals cohabit with owners at night (Radostits *et al.*, 1994; Table 3).

Table 3: Seroprevalence of human brucellosis in Africa

Country	Number tested	Prevalence (%)	Test method used	Source
Somalia	353	0.6	SAT	Hussein *et al.,* 1978
Nigeria	738	5.55	SAT	Chukwu, 1987
Tanzania	540	22.6	SAT	Chukwu, 1987
Uganda	3,164	6.4	SAT	Chukwu, 1987
Djibouti	108	6.5	CFT	Chantal *et al.,* 1994
Eritera	273	6.2%	RBPT, CFT	Omar *et al.,* 2002
Chad	860	3.8%	iELISA	Schelling *et al.,* 2003

In Ethiopia, the public health importance of bovine brucellosis has been documented by few studies. In these studies, prevalences of 12.50%, 3.40%, 5.30% and 3.78% have been reported by Yirgu (1991), Tolosa (2004), Asmare (2004) and Hailemelekot (2005), respectively.

2.7. Control and prevention

Control and prevention of brucellosis depend on the animal species involved, *Brucella spp*, management practices, availability and efficacy of vaccines. Approaches used to control brucellosis are immunization, testing and removal of reactors (Walker, 1999). The most rational approach for preventing brucellosis is the control and elimination of the infection in reactors and animal reservoirs, as has been demonstrated in various countries in Europe and America (Schwabe, 1984; Acha and Szyfers, 2001). However, vaccination is a valuable aid in bovine brucellosis control in developing countries. The first effective *Brucella* vaccine was based on live *B. abortus* strain 19. This induces protective immunity against *B. abortus*, but at the expense of persistence of serological positivity. This problem has been overcome by the development of the rifampicin-resistant mutant *B. abortus* RB51 strain. This strain has proved safe and effective in the field against bovine brucellosis and exhibits negligible interference with diagnostic serology (Schurig *et al*., 2002).

3. MATERIALS AND METHODS

3.1. Study areas

The study was conducted in urban areas, Bahir Dar and Chagni towns, as well as in two government cattle breeding centers: Andassa Livestock Research Center and Metekel Cattle Breeding and Improvement Center. Bahir Dar is located at 11°36′N and 37°23′E, 578 kms Northwest from the capital of Ethiopia, Addis Ababa. Bahir Dar has altitude of 1,840 meters above sea level (masl). It has an annual rainfall and temperature varies between 800 to 1,250 mm and 10 to 32°C, respectively. Metekel Cattle Breeding and Improvement Center is located near Chagni town, 10°57′N and 36°30′E. It is 505 kms from Addis Ababa. It has an altitude of 1,583 masl. The annual rainfall and temperature varies from 2,370 to 2,378 mm and 20 to 26°C, respectively. Andassa Livestock Research Center is located 578 kms Northwest from Addis Ababa. It has an altitude of 1,780 masl, the annual rainfall and temperature varies from 900 to 1,500 mm and 9 to 34°C, respectively (CSA, 2003). The study areas are shown in figure 1

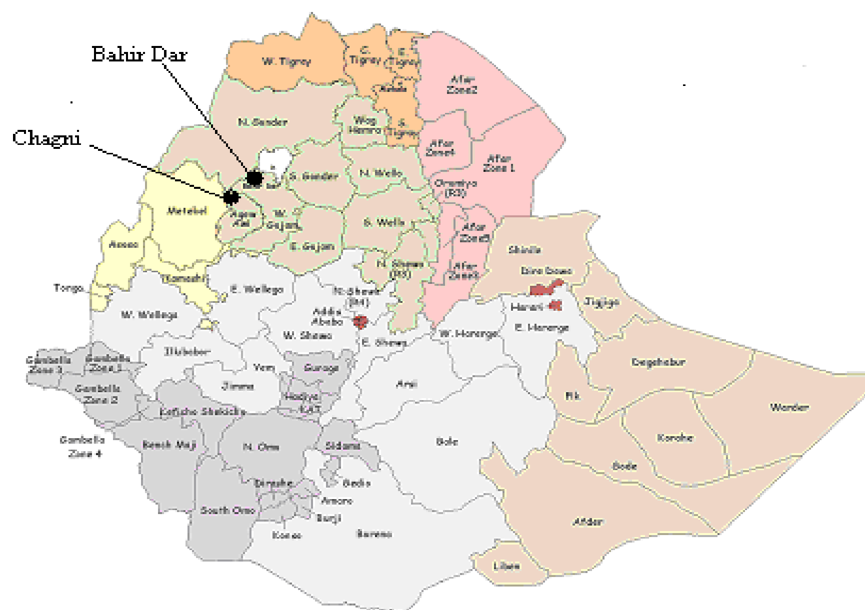

Figure 1: Map of Ethiopia showing the study areas

3.2. Study population

The study populations were cattle found in urban areas and in cattle breeding centers. Cows, replacement heifers, and breeding bulls of local breeds (*Fogera* and *Zebu*) and Holstein-Friesian crossbreeds above six months of age with no history of vaccination against brucellosis were studied. In addition, individuals who had close contact with cattle such as animal health workers, Artificial Insemination (AI) technicians and cattle owners were included in the study.

3.3. Study design, sample size determination and sampling procedure

A cross-sectional survey was carried out between October 2007 and March 2008 in urban areas and in cattle breeding centers. One stage cluster sampling method was employed in urban areas. Dairy herds found in Chagni and Bahir Dar towns were considered as clusters. The numbers of herds to be sampled were calculated according to Thrusfield (2005) for one-stage cluster sampling using 95% confidence level as follows:

$$g = \frac{1.96^2\{nV_c + P_{exp}(1-P_{exp})\}}{nd^2}$$

Where,

g= number of clusters

n= predicted average number of cattle per dairy herd

P_{exp} =expected prevalence

d= desired absolute precision

V_c= between cluster variance

The average number of cattle per dairy herd was four.

The between cluster variance was assumed be large (0.04).

Therefore, using 4.63% (Hilemelekot, 2005) expected prevalence of bovine brucellosis, the number of dairy herds was 156.

Registration of dairy herds available in Bahir Dar and Chagni towns was made. From the registered dairy herds (sampling frame), 156 herds were selected by systematic random sampling

method. However, 18 cattle owners did not participate in the study. Hence, only 138 herds comprising 443 cattle were studied.

In cattle breeding centers, all cattle above six months of age were considered. Metekel cattle Breeding and Improvement Center has 2,500 cattle population, of which 1,570 were sampled. Similarly, from 559 cattle population of Andassa Livestock Research Center, 211 of them were sampled during the study. Hence, a total of 1,781 cattle were bled from cattle breeding centers.

3.4. Questionnaire survey

Information on potential risk factors of bovine brucellosis was collected using a pre-tested questionnaire. Individual cattle details such as identification number (name), sex, age, breed, parity, pregnancy status and source of replacement stock together with the reproductive disorders such as history of abortion, fetal membrane retention, stillbirth, and birth to weak calves were recorded. In addition, management risk factors like herd size, breeding method, calving pen, disposal of fetal membranes as well as level of awareness and regular veterinary services were also considered.

3.5. Collection of blood samples

Approximately 10 ml of blood was taken either from the jugular vein or coccygeal vein of each animal using plain vaccutainer tubes and needles. The identities of each animal were labeled on each corresponding vaccutainer tube. The tubes were kept tilted over night at room temperature to allow clotting. On the following day, the serum was removed from the clot (unretracted blood centrifuged) by siphoning into another sterile test tube of similar label with the animal identity. Finally, serum samples were kept at -20^{0}C in Bahir Dar Regional Veterinary Laboratory until assayed.

3.6. Serology

3.6.1. Rose Bengal Plate Test

The Rose Bengal Plate Test (RBPT) was done in Bahir Dar Regional Veterinary Laboratory in order to screen positive samples. Positive sera were then retested using Complement Fixation Test (CFT) at the National Veterinary Institute (NVI), Debre Zeit. Samples were considered positive for brucellosis if they were positive for RBPT and CFT.

For the RBPT, the procedure described by Nielsen and Dunkan (1990) was followed. The sera and antigen were taken out from the refrigerator and left at room temperature for at least 30 minutes before the test was commenced. Then, 30 µl of test sera were dispensed on each of the 22 circles of the plate. The antigen bottle was gently shaked and a drop of RBPT antigen (30 µl) was placed alongside the serum. Negative and positive controls were included in the remaining two circles of the plate. The antigen and serum were mixed thoroughly using applicator stick and the plate was rocked manually for about 4 minutes. Finally, results were read in a good light source or using magnifying glass when micro-agglutinations were suspected.

Reactions were categorized as 0, +, ++, +++, according to Nielsen and Dunkan (1990), where: 0 = means no agglutination, + = barely perceptible agglutination (using magnifying glass), ++ = fine agglutination, some clearing, and +++ = clumping, definite clearing. Those samples identified with no agglutination (0) were regarded as negative, while those with +, ++ and +++ were regarded as positive.

3.6.2. Complement Fixation Test

All the immunoreactants required for CFT were evaluated by titration. A 2% sheep Red Blood Cell (sRBC) suspension was prepared before being used in the test. The preparation of reagents and CFT procedure were performed according to the protocols of the Federal Institute for Consumer Protection and Veterinary Medicine Service Laboratory, Berlin, Germany and Nielsen and Dunkan (1990).

Interpretation:

A serum with at least 50% fixation of a complement at a dilution of 1:10 was taken as positive. A hemolytic reaction of 75% or less at a dilution of 1:5 was considered as the minimum sera positive threshold (Dohoo *et al*., 1985; OIE, 2004).

3.7. Survey on public health hazard

To assess the potential public health hazard of bovine brucellosis in the study areas, individuals who had close contact with cattle were considered. During the study period, four volunteer animal health workers and four AI technicians from Metekel Cattle Breeding and Improvement Center together with three volunteer assistant veterinarians from Chagni Veterinary Clinic were included. In addition, sixty-eight cattle owners from Bahir Dar who visited Felege Hiot Hospital (in Bahir Dar) for different health problems were also included in the study. Blood was collected by laboratory technicians at Felege Hiot Hospital, and sera were separated and subjected to RBPT and CFT as described in subsections 3.6.1 and 3.6.2. Relevant potential risk factors such as sex, age, raw milk consumption, raw meat consumption, contact with fetal membranes and clinical manifestations pertaining to brucellosis were collected using pre-tested questionnaire.

3.8. Data analysis

The results of serological tests and questionnaire survey were stored in Microsoft Excel spreadsheet. Data were exported to Statistical Package for Social Science (SPSS) version 11.5.0 for windows for analysis (SPSS, 2002). Descriptive statistics were done to determine the prevalence of both bovine and human brucellosis. In addition, the relationships of potential risk factors with brucellosis seropositvity were done by using chi-square tests. Moreover, the associations of reproductive disorders with brucellosis seropositivity were also determined.

4. RESULTS

4.1. Data layout

Table 4: Descriptive statistics of potential risk factors of bovine brucellosis in urban areas and in cattle breeding centers.

Potential risk factors	Urban areas		Cattle breeding centers	
	Number	Proportion	Number	Proportion
Sex				
Female	440	99.3%	1,738	97.6%
Male	3	0.7%	43	2.4%
Age				
0.5 - ≤3 years	115	26%	255	14.3%
3 – 6 years	112	25.3%	382	21.4%
≥6years	216	48.8%	1,144	64.2%
Breed				
Cross	369	83.3%	521	29.3%
Local	74	16.7%	1,260	70.7%
Parity				
No parity	91	20.7%	604	35%
With parity	349	79.3%	1,134	65%
Pregnancy status				
Non-pregnant	307	69.8%	1,415	81.4%
Pregnant	133	30.2%	323	18.6%
Herd size				
< 10 cattle	268	60.5%	10	0.6%
≥10 cattle	175	39.5%	1,771	99.4%
Source				
Purchased	186	42%	248	13.9%
Homebred	257	58%	1,533	86.1%
Abortion				
Absent	333	95.4%	1,111	98%
Present	16	4.6%	23	2%
Fetal membrane retention				
Absent	326	93.4%	1,133	99.9%
Present	23	6.6%	1	0.1%
Stillbirth				
Absent	347	99.4%	1,133	99.9%
Present	2	0.6%	1	0.1%
Birth to weak calf				
Absent	340	97.4%	1,133	99.9%
Present	9	2.6%	1	0.1%

4.2. Seroprevalence

4.2.1. Overall seroprevalence

A total of 2,224 cattle sera were collected from 183 herds with no history of previous vaccination against bovine brucellosis. Fifteen (15) sera were found positive with RBPT. Out of these eleven (11) were positive with CFT. Thus, the subsequent data analyses were based on the eleven sera that were positive to both the RBPT and CFT test results. An overall individual level of 0.49% (11 of 2,224) and herd level of 4.9% (9 of 183) seroprevalences of bovine brucellosis were found in the study areas (Table 5).

Statistically significant difference in individual level seroprevalence was found between urban areas with 1.1% (5 of 443) and cattle breeding centers with 0.3% (6 of 1,781) ($P < 0.05$; Table 5). Herd level seroprevalence was 2.9% (4 of 138) in urban areas and 11.1% (5 of 45) in cattle breeding centers ($P<0.05$).

Table 5: Overall individual and herd level seroprevalence in the study areas

Study areas	Individual level			Herd level		
	Number tested	Number (%) Positive	P-value	Number tested	Number (%) Positive	P- value
Urban areas	443	5 (1.1%)		138	4 (2.9%)	
Cattle breeding centers	1,781	6 (0.3%)	0.049	45	5 (11.1)	0.042
Total	2,224	11 (0.49%)		183	9 (4.9%)	

4.2.2. Seroprevalence in urban areas and in cattle breeding centers

Seroprevalences in Chagni and Bahir Dar towns were 2.1% (1 of 48) and 1% (4 of 395), respectively. Metekel Cattle Breeding and Improvement Center had a seroprevalence of 0.4% (6 of 1,570). However, no positive reactors were detected in Andassa Livestock Research Center (Table 6). Herd level seroprevalence was 14.3% (1 of 7) in Chagni town; 2.3% (3 of 131) in Bahir Dar town and 13.9% (5 of 36) in Metekel Cattle Breeding and Improvement Center (Table 7).

Table 6: Individual level seroprevalence of bovine brucellosis in urban areas and in cattle breeding centers

Individual level	Number tested	Number (%)positive	P-value
Urban areas			
Bahir Dar town	395	4 (1.0%)	
Chagni town	48	1 (2.1%)	0.438
Total	443	5 (1.1%)	
Cattle breeding centers			
ALRC	211	0	
MCBIC	1,570	6 (0.4%)	1.000
Total	1,781	6 (0.3%)	

*ALRC = Andassa Livestock Research Center
*MCBIC = Metekel Cattle Breeding and Improvement Center

Table 7: Herd level seroprevalence of bovine brucellosis in urban areas and in cattle breeding centers

Herd level	Number tested	Number (%)Positive	P-value
Urban areas			
Bahir Dar town	131	3 (2.3%)	
Chagni town	7	1 (14.3%)	0.190
Total	138	4 (2.9%)	
Cattle breeding centers			
ALRC	9	0	
MCBIC	36	5 (13.9%)	0.566
Total	45	5 (11.1%)	

4.3. Potential risk factors

When age was considered as a risk factor, seroprevalences of 1.8% (2 of 112) and 0.5% (6 of 1,144) were found in age category of 3 to 6 years and ≥6 years in urban areas and in cattle breeding centers, respectively. The seroprevelence in large herd size was 1.7% (3 of 175) and 0.3%(6 of 1,771) in urban areas and cattle breeding centers, respectively; in crossbreeds 1.4% (5 of 369) in urban areas and 0.4% (2 of 521) in cattle breeding centers; in pregnant females 1.5% (2 of 133) and purchased cattle 2.2% (4 of 186) in urban areas. No positive reactors were detected in breeding bulls and non-parturient females in both urban areas and cattle breeding centers. However, there were no statistical associations between the potential risk factors and *Brucella* seroprevalence (Table 8).

Table 8: Seroprevalence and chi-square test results of potential risk factors of bovine brucellosis in urban areas and in cattle breeding centers.

Potential risk factors	Urban areas			Cattle breeding centers		
	Number tested	Number (%) positive	P-value	Number tested	Number (%) positive	P-value
Sex						
Female	440	5 (1.1%)		1,738	6 (0.3%)	
Male	3	0	1.000	43	0	1.000
Total	443	5 (1.1%)		1,781	6 (0.3%)	
Age category in years						
0.5 – ≤3	115	0		255	0	
3 – 6	112	2 (1.8%)	0.391	382	0	0.187
≥ 6	216	3 (1.4%)		1,144	6 (0.5%)	
Total	443	5 (1.1%)		1,781	6 (0.3%)	
Breed						
Cross	369	5 (1.4%)		521	2 (0.4%)	
Local	74	0	0.595	1,260	4 (0.3%)	1.000
Total	443	5 (1.1%)		1,781	6 (0.3%)	
Parity category						
No parity	91	0		604	0	
With parity	349	5 (1.4%)	0.588	1,134	6 (0.5%)	0.980
Total	440	5 (1.1%)		1,738	6 (0.3%)	
Pregnancy status						
Non pregnant	307	3 (1%)		1415	5 (0.4%)	
Pregnant	133	2 (1.5%)	0.641	323	1 (0.3%)	1.000
Total	440	5 (1.1%)		1,738	6 (0.3%)	
Herd size						
≤10	268	2 (0.7%)		10	0	
>10	175	3 (1.7%)	0.308	1,771	6 (0.3%)	1.000
Total	443	5 (1.1%)		1,781	6 (0.3%)	
Source						
Purchased	186	4 (2.2%)		248	0	
Homebred	257	1 (0.4%)	0.167	1,533	6 (0.4%)	1.000
Total	443	5 (1.1%)		1,781	6 (0.3%)	

4.4. Reproductive disorders

Statistically significant difference in seroprevalence was found in cows that had history of abortions 5.1% (2 of 39) when compared to non-aborted 0.6% (9 of 1,144) cows ($P < 0.05$). The odds ratio result indicated that aborted cows were almost 9 times more likely to be seropositive in contrast to non- aborted cows (Table 9). Seroprevalence was 10% (1 of 10) in cows that showed birth to weak calf and 0.7% (10 of 1,473) in case of normal birth. None of the cows with fetal membrane retentions or those that gave stillbirths were positive reactors (Table 9).

Table 9: Chi-square test results of reproductive disorders with bovine brucellosis seropositivity in urban areas and in cattle breeding centers.

	Number tested	Number (%) Seropositive	P-value
Abortion			
Abscent*	1,444	9 (0.6%)	0.032
Present	39	2 (5.1%)	OR=8.6, CI (1.8 – 41.2)
Fetal membrane retention			
Abscent	1,459	11(0.8%)	
Present	24	0	1.000
Stillbirth			
Abscent	1,480	11 (0.7%)	
Present	3	0	1.000
Birth to weak calf			
Abscent	1,473	10 (0.7%)	0.072
Present	10	1 (10%)	

*refrerence category

4.5. Questionnaire survey

All dairy herd owners in urban areas were asked about the method of breeding, way of fetal membrane disposal, presence of separate calving pen, availability of regular veterinary service and level of awareness to see the association of management and husbandry risk factors with serological test result. Most of the dairy herd owners 81 (58.7%) used natural breeding method, 39 (28.3%) opted for AI and 18 (13%) used both artificial insemination and natural breeding methods. The questionnaire survey also showed that 120 (87%) dairy herd owners were not aware of bovine brucellosis and 133 (96.4%), 131(94.9%), 109 (79%) had no separate calving pen, no regular veterinary service and didn't practice safe disposal of fetal membranes, respectively. However, these management and husbandry risk factors were not significantly associated with seroprevalence of *Brucella* antibody (Table 10).

In cattle breeding centers, both artificial insemination and natural breeding methods were used. In addition, both breeding centers purchased "*Fogera*" cattle breed from local market after they screened against bovine brucellosis using Rose Bengal Plate Test. In Andassa Livestock Research Center, a relatively better sanitation, safe disposal of fetal membranes and good veterinary services were observed when compared to Metekel Cattle Breeding and Improvement

Center where there was poor sanitation, poor veterinary services and absence of safe disposal of fetal membranes.

Table 10: Chi-square test results of management and husbandry risk factors with bovine brucellosis seropositivity in urban areas

Management and husbandry risk factors	Number (%) of respondents	Number (%) Positive	P-value
Breeding method			
Natural	81(58.7%)	2 (2.5%)	0.771
AI	39 (28.3%)	1 (2.6%)	
Both	18 (13%)	1 (5.6%)	
Safe disposal of fetal membrane			
Absent	109 (79%)	4 (3.7%)	0.579
Present	29 (21%)	0	
Separate calving pen			
Absent	133 (96.4%)	4 (3%)	1.000
Present	5 (3.6%)	0	
Awareness about brucellosis			
Absent	120 (87%)	4 (3.3%)	1.000
Present	18 (13%)	0	
Regular veterinary service			
Absent	131 (94.9%)	4 (3.1%)	1.000
Present	7 (5.1%)	0	

4.6. Human brucellosis

Serological results showed that out of 79 individuals tested, 5 (6.3%) showed seropositivity to *Brucella* antibody (Table 11). Seroprevalence was 7.3% (5 of 68) in cattle owners; 8% (4 of 50) in females; 9% (4 of 44) in age groups between 15 to 30 years old; 7.1% (4 of 56) in those who consumed raw milk; 10.5% (2 of 19) in those who did not consume raw meat and 7% (4 of 57) in individuals who have made contact with fetal membranes. However, no statistical associations were found between these risk factors and brucellosis seropositivity (Table 11).

Table 11: Chi-square test results of potential risk factors with human brucellosis seropositivity in urban areas and in cattle breeding centers

Risk factors	Number tested	Number (%) Seropositive	P-value
Occupation			
AI technician	4	0	
Animal health workers	7	0	0.649
Cattle owners	68	5 (7.3%)	
Total	79	5 (6.3%)	
Sex			
Female	50	4 (8%)	0.647
Male	29	1 (3.4%)	
Age category in years			
15-30	44	4 (9%)	0.376
≥30	35	1 (2.8%)	
Raw milk consumption			
No	23	1 (4.3%)	1.000
Yes	56	4 (7.1%)	
Raw meat consumption			
No	19	2 (10.5%)	0.589
Yes	60	3 (5%)	
Contact with fetal membrane			
No	22	1 (4.5%)	1.000
Yes	57	4 (7%)	

Humans were also asked to the symptoms of illnesses they experienced in the past such as headache, fever, joint pain, fatigue, backache and chill. Out of 79 individuals interviewed during blood sampling, 62 (78.4%) of respondents mentioned at least three of the symptoms. From five seropositives, two individuals had complained all disease symptoms and one adult female reported only sterility. However, the two seropositive individuals didn’t mention any of the symptoms.

5. DISCUSSION

The present study revealed that the seroprevalences of bovine brucellosis in urban areas and in cattle breeding centers was very low. The very low seroprevalence (1.1%) of brucellosis obtained in urban areas was in agreement with seroprevalences reported by Asmare (2004) in Sidama Zone (1.9%); Tolosa (2004) in selected sites of Jimma Zone (0.6%); Berhe (2005) in Tigray Region (1.5%) and Jergefa (2006) in selected three agro ecologies of central Oromia (3%). On the other hand, higher seroprevalences were reported by Asfaw *et al.* (1998) in urban and peri-urban production systems in and around Addis Ababa (8.1%); Molla (1989) in cooperative dairy farmers in Arsi Region (8.9%) and Bekele *et al.* (2000) in Agarfa Farm (10.8%). Compared to the present study, moderate level of seroprevalence (6%) was reported by Hailemelekot (2005) in semi-intensively managed cattle in Awi, Western Gojjam and South Gondar. Unlike many reports, Belihu (2002) has reported the absence of bovine brucellosis around Selale. Belihu (2002) attributed the absence of positive reactors at Selale to the small herd size nature of the farms and the awareness of farmers.

The very low seroprevalence of bovine brucellosis in our study in urban areas could probably be due to the small numbers of cattle kept in most of the dairy herds and the limited contact between herds. This is justified by the questionnaire survey that the majority (89.1%) of cattle owners kept herds with less than ten cattle. In addition, dairy cows were kept indoor where there was limited contact between herds. Herd size and animal density are directly related to the presence of the disease and difficulty in controlling infection in a population (Radostits *et al.,* 1994; Walker, 1999). The relatively high seroprevalence observed in Chagni town (2.1%) compared to Bahir Dar town (1%) might be due to the small numbers of cattle sampled in Chagni.

Seroprevalence of bovine brucellosis in our study in cattle breeding centers was also very low (0.3%). This contrasts previous reports of bovine brucellosis in cattle raised under ranch production systems in Ethiopia. Higher seroprevalences were reported by Molla (1989) in Gobe Ranch (6.81%), Bekele *et al.* (2000) in Abernossa Ranch (18.9%) and Dida Tuyura Ranch (6.3%), Shiferaw (1994) in Andassa Ranch (10%) and Metekel Ranch (22.84%). It was clear that in Andassa and Metekel Ranch, recently named as Andassa Livestock Research Center and

Metekel Cattle Breeding and Improvement Center, revealed high seroprevalences of bovine brucellosis.

The very low seroprevalence of bovine brucellosis found in cattle breeding centers when compared to the previous report was attributed to the elimination of positive reactors based on previous report by Shiferaw (1994). In addition, the previous report has also alerted the responsible bodies of both cattle breeding centers to take immediate preventive measures. Starting from 1994 up to 2004, screening of cattle against bovine brucellosis had never been conducted in both breeding centers. However, Hailemelekot (2005) found 12 positive reactors out of 326 tested cattle in Andassa Livestock Research Center, which could have contributed to the absence of any positive reactors in this study. The presence of six positive reactors, detected in the present study, in Metekel Cattle Breeding and Improvement Center indicates existence of foci of infection; which is the danger of spread in the future unless the poor management and husbandry systems prevailing in the breeding center are improved. Purchasing of cattle from local market after testing for brucellosis using Rose Bengal Plate Test also contributes to the reduction of brucellosis to very low level in the breeding and improvement center. Purchasing of animals tested for brucellosis is one of the factors, which reduce inter-herd transmission of the disease (Radostits *et al*., 1994).

The other possible explanation for the very low seroprevalence in breeding centers may be the limited contacts between herds kept in breeding centers. In cattle breeding centers, herds were kept separately during day and night. In addition, the hot environment where cattle were kept for grazing could not have been a favorable condition for the bacterium (*Brucella abortus*) to survive, multiply and induce infection in susceptible populations. The spread of brucellosis under traditional methods of cattle husbandry is low due to intense solar radiation and less contacts between different herds (Wernery *et al*, 1979). The other point, which was not mentioned above but might have contributed to the low prevalence was the absence of contact between herds found in the surroundings and herds kept in cattle breeding centers. The grazing areas of cattle breeding centers are separated from neighbouring herds kept by shepards. Thus, there were no entrances of cattle from the surrounding area to cattle breeding centers. It has been indicated that contacts at fence lines, grazing together and intrusion of infected animals into clean herds are common methods by which transmission occurs to adjacent herds (Radostits *et al*., 1994). In this study, the

seroprevalence of bovine brucellosis was higher in urban areas (1.1%) when compared to cattle in breeding centers (0.3%). This difference in seroprevalence could be attributed to the purchasing of cattle after screening against bovine brucellosis in breeding centers.

Susceptibility to *Brucella* infection in cattle depends on potential risk factors such as age, sex, breed, and pregnancy status (Walker, 1999). Herd size and source of replacement cattle are among the variables that contribute significantly to brucellosis seropositivity (Radostits *et al.*, 1994).

Because of the low power of the present study in urban areas, as the numbers of samples were small, none of the potential risk factors were significantly associated with brucellosis seropositivity. However, higher seroprevalence was found in cattle above three years old and no infection in less than or equal to three years old. This finding is in consistent with the report of Asmare (2004) where higher prevalence was found in cattle above four years old and none in cattle below two years old. Berhe (2005) has reported higher proportions of seropositives in cattle above three years old and no positive reactors in cattle aged six months to three years. Furthermore, Asfaw *et al*. (1998), Tolosa (2004) and Hailemelekot (2005) have reported significant increase in seropositivity with respect to age. Chukwu (1987) pointed out that sexually mature cows and bulls were very susceptible to brucellosis. Animals, which are sexually mature, are more susceptible to *Brucella* infection with the organism than sexually immature cattle of either sex (Radostitis *et* a*l*., 1994). This is because, although latent infections do occur, young animas tend to be more resistant and frequently clear infections of brucellossis (Walker, 1999).

In the present study, none of the forty-six breeding bulls tested were seropositive for brucellosis. This was inline with that of Asmare (2004) who has reported the absence of seropositivity in ten bulls tested for brucellosis in Sidama Zone, Southern Ethiopia. Similarly, Tolosa (2004) and Berhe (2005) didn’t get male reactors in their study. However, bulls are susceptible to brucellosis (Acha and Szyfers, 2001) despite the fact that some researchers are of the opinion that bulls are more resistant to infection of brucellosis than females (Walker, 1999). Therefore, the absence of male seroreactors in the present study could be due to the small numbers of bulls sampled (46) as compared to females (2178).

This study has indicated that higher proportions of crossbreds were seropositive when compared to proportions of local breeds. However, the difference between the two breeds was not statistically significant. This is in agreement with the report of Hailemelekot (2005) in Bahir Dar milk shed on similar breeds of cattle. All breeds of cattle appear to be comparable in susceptibility to brucellosis and apparently no specific breed resistance to brucellosis is known (Radostitis *et al*., 1994). However, Hellmann (1984) reported presence of statistically significant difference in seropositivity between *Fellata* and *Dinka* cattle in Bahir el Ghazal province of Southern Sudan. According to that study, almost four times as many *Fellata* cattle were positive (22.5%) compared to *Dinka* cattle (6.5%). The possible explanations established by Hellmann (1984) for the significant differences between the two breeds were herd contacts and the high *Trypanosoma* infection rate in *Fellata* cattle (14.4%) than in *Dinka* cattle (3%).

Higher prevalence was observed in pregnant cows when compared to non-pregnant ones. However, no significant difference in seroprevalence was observed between pregnant and non-pregnant cows. This was in agreement with the reports of Tolosa (2004) who has obtained the absence of significant difference in seropositivity between pregnant and non-pregnant females. Cows, especially when pregnant, are the most susceptible (Radostits *et* a*l*., 1994; Acha and Szyfers, 2001). The status of parity (no parturition versus parturition) were compared to ascertain the association of parity with seropositivity to *Brucella* antibody, however, seroreactors were not detected in animals that didn't gave birth in contrast to those, which gave birth. This is in agreement with the report of Berhe (2005) who conducted bovine brucellosis in Tigray Region, Northern Ethiopia.

Though no statistically significant difference in prevalence was observed between purchased and homebred cattle, higher proportions of reactors were noted in purchased cattle when compared to homebred ones in urban areas unlike in breeding centers, where seroreactors were not detected in purchased cattle. It appeared that those herds whose owners made frequent purchase of cattle had an increased risk of acquiring brucellosis, particularly those who made purchases from other herds or from cattle dealers (Kellar *et al.,* 1976). In this study, the proportions of seropositives were higher in herds with more than ten cattle compared to herds with ten or less animals. However, this difference was not statistically significant. This finding is in agreement with the study result of Asmare (2004) and Tolosa (2004) who have reported increase in seropositivity as

herd size increases. Herd size and source of replacement cattle are among the variables, which significantly affect the occurrence of brucellosis (Radostits *et al.,* 1994).

The relationship of reproductive disorders such as abortions, fetal membrane retentions, stillbirths, birth to weak calves and seropositivity were calculated. Among the reproductive disorders, abortion had a statistically significant association with brucellosis. That is, aborted cows were almost nine times more likely to be seropositive when compared to non-aborted cows. This finding is in consistent with Tolosa (2004) and Hailemelekot (2005) who have reported significant association between abortion and seropositvity in intensive and semi-intensive management systems, respectively. The predominant symptom in pregnant females is abortion during the second half of pregnancy, often with fetal membrane retention or premature or full term birth of dead or weak calves (Acha and Szyfers, 2001).

The management and husbandry risk factors considered (use of breeding method, disposal of fetal membranes, use of separate calving pen, level of awareness and regular veterinary service) were not significantly associated with seroprevalence of *Brucella* antibody. However, individuals who were not disposing fetal membranes safely, those who didn't use separate calving pen and those who were not aware of brucellosis and didn't get regular veterinary service had positive seroreactors. This was in consistent with Hailemelekot (2005) who didn't observe any significant association of management and husbandry risk factors with seroprevalence to *Brucella* antibody. Lack of statistical associations between husbandry risk factors and brucellosis seropositivity could be due to the small numbers (4) of infected herds.

The overall seroprevalence of *Brucella* in the present study from human sera of three occupational groups namely cattle owners, animal health workers and AI technicians was 6.3%. This is almost is in agreement with the report of Asmare (2004) for human sera in Sidama Zone of 5.8%, Southern Ethiopia despite that all seropositives were cattle owners in the present study. Tolosa (2004) and Hailemelekot (2005) have also reported seroprevalences of 3.4% and 3.78% from human sera in Jimma and Bahir Dar, respectively. Similar findings were observed in Eritrea where a higher prevalence (7.1%) was observed in dairy farm workers than in veterinary personnel (4.6%) (Omar *et al.,* 2002). The absence of seropositive in animal health workers and AI technicians might be due to the high level of awareness about brucellosis for they might have

used protective gloves and maintained good sanitation when they anticipated the risk of brucellosis.

Relatively comparable levels of seroprevalences were observed in humans in relation to some potential risk factors. Brucella seroprevalences were 8%, 9%, 7.1%, 10.5% and 7% in females, in age group of 15 to 30 years, in those who drank raw milk, in those who did not consume raw meat and in those who had contact with fetal membranes, respectively. However, there was no significant associations between these potential risk factors with human brucellosis seropositivity, which could be due to the low power of the present study, as the numbers of individuals sampled were small. Seroprevalence of *Brucella* in individuals who consumed raw milk is in agreement with the report of Alballa (1995) and Omar *et al.* (2002) concerning the associations of risk of brucellosis and raw milk consumption. The prevalence of *Brucella* in females is in line with the report of Cooper (1991) who found higher risk in young and middle aged women than men in his study of human brucellosis in a well defined urban population in Saudi Arabia. The high seroprevalences observed in females and in the age group 15 to 30 years old could probably due to their close contact with livestock as they might have more frequently involved in the management and husbandry practices. The relatively high seroprevalence in individuals who didn't consume raw meat when compared to those who ate raw meat could probably be due to the small sample size. However, uncooked meat consumption is one of the risk factors for the occurrence of human brucellosis (Acha and Szyfers, 2001).

Individuals were also asked about the symptoms of illness they experienced in the past. Out of five seroreactors, only two persons reported all symptoms, the other two didn't mention any of the symptoms and one reported on sterility. The reason for no complains of illness in the two seropositive individuals could be due to the absence of active disease. However, the majority, 60 (75.9%) of seronegative individuals reported at least three of the symptoms such as headache, fever, joint pain, fatigue and backache. This might be due to the presence of malaria in the area so that respondents were not able to clearly identify between symptoms of brucellosis and symptoms of malaria.

6. CONCLUSION AND RECOMMENDATIONS

The current study revealed that the seroprevalence of bovine brucellosis in urban areas and in cattle breeding centers in Northwestern Amhara Region is very low. Due to the very low seroprevalence of bovine brucellosis and relatively small sample size in urban areas, none of the potential risk factors were associated with the occurrence of *Brucella* reactor cattle. Among reproductive disorders, abortion has strong association with *Brucella* infection in cattle. Seroprevalence of bovine brucellosis is high when there is poor management and husbandry practices such as absence of regular veterinary service, lack of safe disposal of fetal membranes and absence of separate calving pen. Seroprevalence of bovine brucellosis is also influenced by level of awareness about brucellosis and breeding method.

The presence of seropositive individuals in this study may indicate a high public health risk of bovine brucellosis in the study area. However, the present study indicated differences in probability of risk depending on occupation, sex, age, contact with fetal membrane, consumption of uncooked meat and raw milk.

Based on the above conclusion, the following recommendations are forwarded:

- Positive cattle reactors should be slaughtered.
- Cattle breeding centers should continue screening for brucellosis before purchasing replacement stock and better if they implement regular test and slaughter policy to eradicate the disease.
- Management and husbandry practices should be enhanced in urban areas and in cattle breeding centers.
- The public should be made aware about the methods of acquiring brucellosis.
- Further study covering a wide area with a higher sample size should be conducted in human and bovine brucellosis to identify potential risk factors in the region.

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8. ANNEXES

Annex 1: CFT test procedure

Materials and reagents for CFT

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

I. Preparation of sRBC for the hemolytic system:

- Ten (10) ml of sRBC in Alsever's was centrifuged at 2500 rpm for 5 minutes.
- The supernatant was discarded and replaced by Veronal Buffer Diluents (VBD).
- The sRBC were resuspended in diluents completely.
- This procedure was repeated three times. Before discarding the supernatant after the last washing, the packed cell volume was measured by placing an identical tube next to the blood containing tube filled up to the level of blood by a measured amount of water.

By addition of calculated amount of water, a 2% sRBC suspension was prepared.

II. Amboceptor's (Hemolysin) titration:

A. Prepare 1:500 dilutions up to 1:8000.

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

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

- 5 test tubes were prepared and 1ml of VBD was added to test tubes 2 to 5
- 10µl amboceptor's was mixed with 7490µl VBD in the first tube

-1ml was transferred from first tube to second up to fifth tube and 1ml was discarded, put tubes in order of ascending dilution.

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

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

E. 0.5ml of 2% sRBC was added to each of the test tubes and shacked well.

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

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

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

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

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

III. Evaluation of complement.

Freeze dried complement was reconstituted according to its instruction.

A 1:100 complement was prepared

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

- Diluent was added into the 9 tubes in decreasing amount by 5µl, starting with 40µl.
- 25µl of diluents was added into the tubes
- The test tubes were placed at 37 ^{0}C water bath for 1hr.
- 25µl of 2% sRBC was added in all tubes
- 25µl of amboceptor at working dilution 1:1,000 was added in all tubes.

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

The test was read by recording the Minimum Hemolytic Dose of complement (MHD), which was represented by the first tube showing complete hemolysis. The next tube contains the Full Hemolytic Dose (FHD). The complement dilution = 2FHD/dilution of complement.

V. Titration of antigen

Micro titer plate I

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

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

Micro titer plate II

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

Mix plate I and II

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

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

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

The last well with 50% sedimentation was read and recorded. The highest dilution of antigen with 50% sedimentation is the limiting antigen concentration or the right corner value. In this case the corner value was 1:25 dilution and was used throughout the test. The 50% sedimentation was taken as one unit and the working dilution of the antigen was 2units.

The test proper, multiple sera technique:

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

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

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

- The plate were sealed, incubated at 37 ^{0}C for 30minutes with agitation
- 25µl of sRBC suspension was added to each well .The plates were sealed and reincubated at 37 ^{0}C for 30 minutes with agitation. Before reading the result on the plates were left in the refrigerator at +4 ^{0}C for 1-hour in order to allow none lysed cells to settle.

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

Positive reactions were indicated by sedimentation of sRBC and absence of hemolysis. Negative reactions by heamolysis of sRBC.

Annex 2: Questionnaire format for bovine serum sampling

Bovine brucellosis survey questionnaire form 1

Town ------------------- Kebele -------------------- Owner name --------------------- Date ----------------

.No.	Tag no. (Name)	Breed	Sex	Age	Source	Pregnancy status			Parity/ies	Stillbirth	Birth to weak calf
						Pregnant		Non-pregnant			
						Yes	Month				

Bovine brucellosis survey questionnaire form 2

Town ------------------- Kebele -------------------- Owner name --------------------- Date ----------------

No	Tag no (Name)	History of abortion					History of fetal membrane retention			
		Y/N	Cause	Rec. date	Frequency	Parity	Y/N	Rec. date	Frequency	Parity/ies

Annex 3: Questionnaire on management and husbandry risk factors

1. How many cattle do you keep in your home?
2. Are there separate calving pen for cows?

   a. Yes b. No

3. How do you dispose of fetal membranes?

   a. Burring b. leaving on the ground c. offering to dogs

4. Do you have regular veterinary service?

   a. Yes b. No

5. Do you have awareness about brucellosis?

   a. Yes b. No

6. What type of breeding method do you use?

   a. AI b. natural c. both

Annex 4:Questionnaire format for human risk groups

Residence	Name	Sex	Age	Raw milk consumption	Raw meat consumption	Removal RFM	Sign of illness						Sterility
							Back ache	Joint pain	Fever	Chill	Head ache	Fatigue	

*RFM= Retained Fetal membrane

Annex 5: Curriculum Vitae

Personal information

Name: Taddesse Yayeh Yihun

Date of birth: June 26th, 1976

Place of birth: W/Gojjam

Nationality: Ethiopian

Martial status: Single

Religion: Christian/ Orthodox/

Educationl Background

1986/87 – 1993/94: Primary education

-Dembecha Elementary and Junior Secondary School

1994/95 – 1997/98: Secondary education

-Dembecha Senior Secondary School awarded with Ethiopian School Leaving Certificate Examination (ESLCE)

1998 – 2002/03: Addis Ababa University, Faculty of Natural Science and Veterinary Medicine awarded with Doctor of Veterinary Medicine (DVM)

2003 – 2006/07: Working in Gode Agricultural, Technical, Vocational, Education and Training (ATVET) College, Somali Region.

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9. SIGNED DECLARATION SHEET

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

Taddesse Yayeh Yihun

Signature ……………………………

Date of submission. ……………………………..

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

Girma Zewde (DVM, PhD)

Signature…………………………..

Ermias W/mariam (DVM, MSc)

Signature………………………..