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FACULTY OF VETERINARY MEDICINE

STUDY ON BOVINE BRUCELLOSIS IN CATTLE SLAUGHTERED AT ADDIS ABABA AND SULULTA ABATTOIRS WITH FOCUS ON OCCUPATIONAL HAZARD

A Thesis submitted to the School of Graduate Studies of Addis Ababa University, Faculty of Veterinary Medicine in partial fulfillment of the requirements for the Degree of Masters in Tropical Veterinary Medicine

BY

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Board of examiners **Signature**

DEDICATION

THIS PAPER IS DEDICATED TO MY BELOVED WIFE SERKALEM YESUF AND MY SONS LEUL AND NATHANEL MULUGETA

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LIST OF ABBREVIATIONS

A.A.	Addis Ababa
AAU	Addis Ababa University
AI	Artificial Insemination
CFT	Complement Fixation Test
CI	Confidence Interval
ELISA	Enzyme Linked Immuno Sorbent Assay
EC	European Commission
FAO	Food and Agriculture Organization
FPSR	False positive serological reaction
FVM	Faculty of Veterinary Medicine
ICFTU	International Complement Fixation Test Unit
Ig	Immunoglobulin
ILCA	International Livestock Center for Africa
IU	International Unit
μl	micro liter
masl	meter above sea level
ml	milliliter
mm	millimeter
MRT	Milk Ring Test
MZN	Modified Ziehl –Neelson
OIE	Office International des Epizooties
RBPT	Rose Bengal Plate Test
SAT	Serum Agglutination Test
SLPS	Smooth Lipo Poly Saccharide
US	United States
VBD	Veronal Buffer Diluent
WHO	World Health Organization

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ABSTRACT

A cross – sectional study was carried out at the Addis Ababa and Sululta abattoirs to investigate the status of bovine brucellosis and to assess the occupational hazard associated with brucellosis in abattoir workers. The work was conducted from September 2005 to March 2006.

The study methodology consisted of seroprevalence study on both cattle slaughtered at the abattoirs and also on humans working in the abattoirs. Risk factors were also assessed both in cattle and humans. Additionally, questionnaire surveys were conducted on abattoir workers.

A total of 1501 cattle, 759 from Addis Ababa abattoir and 742 from Sululta abattoir were included in this study. Blood samples were collected from 1304 male and 197 female cattle. Out of the total of 1501 cattle slaughtered in the abattoirs, 1398 were local and 103 crossbred cattle. In addition, 67 abattoir workers from Sululta abattoir were also participated in the study.

Serum samples collected from both cattle and abattoir workers were screened using the Rose Bengal Plate Test (RBPT). Positive sera were further subjected to the Complement Fixation Test (CFT) to maximize specificity and predictive value. All the subsequent test analyses were based on the sera that were positive to both the RBPT and CFT. The χ^2 tests and logistic regression were used to test the association between risk factors and Seropositivity. The χ^2 , and OR values were calculated using the computer software program STATA.

The overall individual animal prevalence of brucellosis in slaughtered cattle was 1.13%. (n = 17). The abattoir level seroprevalence was 1.19% (n = 9) and 1.08% (n = 8) for Addis Ababa and Sululta abattoirs respectively. Significant difference in seropositivity was not observed between the two abattoirs (P = 0.844).

A highly significant difference in seropositivity was observed between male and female (P = 0.011) and between local and crossbred (P = 0.012) cattle. Difference in seropsitivity between the two age categories was not observed (P = 1.00). Thus genotype (breed) and sex were identified as important risk factors associated with seropositivity in slaughtered cattle and may pose potential occupational hazard for abattoir workers.

All the sera (n = 67) obtained from Sululta abattoir workers were found negative for the presence of *Brucella* antibodies. On the basis of questionnaire surveys, consumption of raw meat, frequent cutting of hands and fingers at work and lack of knowledge about brucellosis and its zoonotic impact were identified as potential risk factors that may predispose the abattoir workers to the disease.

In conclusion, the present work generally showed low seroprevalence of brucellosis among cattle slaughtered in Addis Ababa and Sululta abattoirs and none in humans. However, in view of questionnaire survey and the associated potential risk factors, creation of awareness about the occupational hazard and public health significance of brucellosis, adoption of basic hygienic measures among abattoir workers, proper identification of animals brought for slaughter and the conduct of further studies, especially on export oriented abattoirs, are recommended.

Key words:

Brucellosis, Zoonosis, Occupational Hazard, Risk factors, RBPT, CFT, abattoir workers.

1. INTRODUCTION

Livestock play a crucial role in the livelihoods of the majority of Africans. In arid and semi-arid regions, livestock keeping is the main livelihood strategy, while in many crop–livestock systems in semi-arid, sub-humid and even humid zones, livestock are a critical resource, providing meat, milk, traction, nutrients for crops (e.g. manure) and cash income. Livestock also have important social roles in many cultural traditions (McDermott and Arimi, 2002). The proportion of people relying on livestock for some or all of their livelihoods is very high in Africa, ranging from 20 to 90% depending on the type of livestock production system and country (McDermott and Arimi, 2002).

Over the last decades, the population growth in sub-Saharan Africa, combined with rising per capita income has got rapid growth in food consumption, in particular of animal products. The World Bank has estimated that the demand for milk and dairy products in sub-Saharan Africa will increase by 5.5 million tones by the year 2025, an annual growth rate of 4%, questioning the supply side. Much of this increased demand will be concentrated in urban areas (World Bank, 1992).

Ethiopia is the third densely populated country in Africa next to Nigeria and Egypt, having an estimated population of about 67.2 million in July 2002 with annual growth rate of 2.9%. The total cattle population of Ethiopia is estimated to be 41,527,142; with the proportion of male to female of about 50.2% and 49.8%, respectively (CSA, 2003). According to recent data, Ethiopia has 41,527,142 heads of cattle, 28, 317, 128 sheep and goats, 4,421,297 equines and 42,915628 poultry. The livestock sector contributes about 30% of the agricultural GDP and 19% to the export earnings. In addition to this, about 6 million oxen provide draught power for the cultivation of grain crops (Tegegne and Gebre Wold, 1997).

Within sub-Saharan Africa, many of the known infectious diseases—bacterial, viral and parasitic, both directly and indirectly transmitted—occur commonly and are poorly controlled in both livestock and human populations (McDermott and Arimi, 2002).

These diseases have a great impact on economic development both in terms of direct losses (morbidity, mortality) and indirect losses, such as those due to the costs of ineffective control measures (Sachs, 1999; Perry *et al.*, 2001). However, despite their economic and social importance, programs to control infectious diseases in sub-Saharan Africa, such as brucellosis, have greatly declined over the past 20 years (Holden, 1999; McLeod and Wilsemore, 2002; McDermott and Arimi, 2002).

Good quality data on the impacts of different diseases and their control on animal and human populations in sub-Saharan Africa are usually lacking. However, a recent study has attempted to assemble expert advise on the potential impacts of animal diseases in the developing world (Perry *et al.*, 2002). One of the highest priority diseases, both in sub-Saharan Africa and other regions of the developing world was brucellosis and the importance of brucellosis reflects its widespread distribution and its impacts on multiple animal species and humans (McDermott and Arimi, 2002).

Brucellosis is a major zoonotic disease, widely distributed in both humans and animals, especially in the developing world. The occurrence of the disease in humans is largely dependent on the animal reservoir and high rates of brucellosis infection in sheep and goats usually cause the greatest incidence of infection in humans (WHO, 1997).

Brucellosis occurs worldwide in domestic animals such as cattle, sheep, goats, camels and pigs and creates a serious economic problem for both the intensive and extensive livestock production systems in the tropics (Schwabe, 1984; Seifert, 1996).

Brucellosis causes considerable economic losses in livestock production, due to abortion, lack of fertility and reduction in milk production; in addition, the zoonotic nature of the disease has a serious impact on public health (Anon, 1997; Garin-Bastuji *et al.*, 1998). In addition to abortion and loss of calves, bovine brucellosis results in an average loss of milk production of 20 %(Bishop *et al.*, 1994).

Due to its effects on multiple animal species and humans, the impact of brucellosis is considered great in sub-Saharan Africa (Perry *et al.*, 2002). However, valuing these impacts across species is complicated. Economic impacts of brucellosis on livestock species can be estimated, although data is lacking, because prices can be estimated for direct losses due to morbidity and mortality and indirect losses due to treatment costs (McDermott and Arimi, 2002)

When United States of America began bovine brucellosis eradication program in 1934, about 11.5% of adult cattle were infected with brucellosis and the estimated annual economic loss was U S $ 100 million; similarly, in Sweden prior to the eradication of brucellosis in 1957, the annual economic loss was estimated well above U.S $ 8.8 million (Schwabe, 1984).

Official estimates put annual economic losses due to bovine brucellosis in Latin America at approximately US $ 600 million (PAHO-WHO, 2001). In intensive dairy production system of the tropics, an incidence of infection up to 80% has been reported. The annual economic loss from bovine brucellosis of 150 million French Francs in Cote d'Ivore, 233.88 million US $ in Nigeria and 33.4 million US $ in Kenya, Tanzania and Uganda have been recorded (Chukwu; 1987; Seifert, 1996).

Bovine brucellosis (contagious abortion, Bang's disease) is a disease of cattle usually caused by *Brucella abortus*, less frequently by *Brucella melitensis* and rarely by *Brucella suis*. The disease is characterized by abortion in the last trimester or birth of unthrifty newborn in the female and orchitis and epydidimitis with frequent sterility in the male (Radostitis *et al.*, 2000; OIE, 2004).

Occasionally, other species of animals such as goats, sheep, pig, dog and horse may be infected by *Brucella abortus* (Nicoletti, 1998). Brucellosis has also been reported in one and two humped camels, domestic buffalo, American and European bison, yak, elk, African buffalo and antelopes (OIE, 2004).

In East Africa bovine brucellosis is a serious livestock health problem. Sero-prevalence of brucellosis in cattle that was recorded in some eastern African countries includes 11.9% in Somalia (Hussein *et al.*, 1978), 10% in Kenya (Kagumba and Nandoka, 1978), 34.9% in Rwanda (Akakpo *et al.*, 1978), 6.5-22.5% in Sudan (Hellmann *et al.*, 1984) and 1.8% in Uganda (Oloffs *et al.*, 1996).

In Ethiopia, prevalence of brucellosis in cattle 18.4% by Gebremariam (1985), 7.6% by Molla (1989), 38.7% by Rashid (1993), 22% by Sintaro (1994), 8.1% by Asfaw *et al.* (1998), 4.9% by Bekele *et al.* (2000), 1.92% by Asmare (2004), 0.61% by Tolosa (2004), 4.63% by Hailemelekot (2005) and 1.49% by Berhe (2005) have been reported.

Brucellosis is considered by the Food and Agricultural Organization, the World Health Organization and the Office International Des Epizooties as one of the most widespread zoonotic diseases (Schelling *et al.*, 2003). The importance of brucellosis lies on the severe public health hazard it causes and the economic impact on the animal industry resulting in effect on total animal protein supply (Mustofa and Nicoletti, 1993).

Each year, about half a million cases of brucellosis occur in humans around the world. The greatest prevalence in humans is found in those countries with high incidence of *B. melitensis* infection among goats, sheep, or both species. The Latin American countries, with the greatest number of recorded cases are Argentina, Mexico and Peru (PAHO-WHO, 2001).

Available evidence from sub-Saharan Africa supports the hypothesis that transmission to humans is primarily from livestock reservoirs of brucellosis. Thus, the risk to humans is a function of the risk in livestock and the human–livestock effective contact rate (Chukwu, 1985; Reichel *et al*., 1996; Muriuki *et al*., 1997; Kubuafor *et al*., 2000; Baba *et al*., 2001).

Beyond consumers and farmers, abattoir, animal health and other workers are at high risk of occupational exposure (McDermott and Arimi, 2002). Slaughterhouse workers, primarily those in the kill areas, become inoculated through aerosolization of fluids, contamination of skin abrasions and splashing of mucous membranes (Lisgaris, 2005).

In Africa, human brucellosis has been reported in various countries. Prevalence of 6.50% in Djibouti (Chantal *et al*., 1996), from 3 to 7.1% in Eritrea (Omer *et al*., 2002), from 5.55-29.80% in Nigeria (Chukwu, 1987), 0.60% in Somalia (Hussein *et al*., 1978), 22.6%, in Tanzania (Chukwu, 1987), 6.4% in Uganda (Mutanda, 1998), 10.4% in Kenya (Oomen and Waghela, 1974) and 13.3% in Cote d'Ivoire (Pilo-Maron *et al*., 1979) have been reported.

In Ethiopia, few attempts were made to study the prevalence of human brucellosis among veterinarians, farmers and slaughterhouse workers. In these studies, prevalence of 12.5% by Yirgu (1991), 2.4% by Tolosa (2004), 5.3% by Asmare (2004) and 3.78% by Hailemelekot (2005) have been recorded.

It is a well-known fact that abattoir workers are among the population group who are at the highest risk of brucellosis infection. Because of their occupation, they make frequent contact with the carcasses of animals and if an infected animal is slaughtered, then the chance of contracting the disease could be very high.

In Ethiopia, information about the status of bovine brucellosis in cattle slaughtered in abattoirs and the occupational hazard on abattoir workers is not available. All studies made on bovine and human brucellosis have focused on field investigations. To fill this information gap, the current study was proposed.

To undertake this study, the Addis Ababa and Sululta abattoirs were selected on the basis of the huge number of animals slaughtered and the large number of workers involved in the meat production processes.

Large number of cattle, sheep, goats and swine are slaughtered in Addis Ababa and Sululta abattoirs. Annually, about 153,000 cattle, 39,000 sheep, 3,200 goats and 750 swine are slaughtered in Addis Ababa abattoir alone and about 22,100 heads of cattle in Sululta abattoir. The Addis Ababa and Sululta abattoirs have more than 800 workers and this includes both the office and production line workers. On the production line, there are about 500 individuals. If infected cattle are slaughtered in these abattoirs, workers are at high risk of contracting the disease. To protect the abattoir workers as well as the public from the disease, investigating the status of brucellosis both in cattle and abattoir workers is of paramount importance. (A.A. Abattoir, 2004).

Therefore, the objectives of this study were:

- To determine the status of bovine brucellosis and the associated risk factors in cattle slaughtered in Addis Ababa and Sululta abattoirs through serological tests.

- To investigate the status of human brucellosis among abattoir workers and the risk factors associated with infection.

- To assess the clinical signs and symptoms and risk factors associated with human brucellosis through questionnaire survey and forward appropriate recommendation.

2. LITERATURE REVIEW

2.1. Etiology

In 1751, Cleghorn, a British army surgeon stationed on the Mediterranean island of Minorca, described cases of chronic, relapsing febrile illness and cited Hippocrates's description of a similar disease more than 2,000 years earlier (Cleghorn, 1751). In 1887, David Bruce, for whom the genus *Brucella* is named, isolated the causative organism from the spleens of five fatal cases and placed it within the genus *Micrococcus* (Bruce, 1887). In 1897, B. Bang, a Danish investigator, identified an organism, which he called the "Bacillus of abortion," in placentas and fetuses of cattle suffering from contagious abortion (Bang, 1897).

Brucella species are obligate parasites requiring an animal host for maintenance. They are small, gram negative, non-motile, non-spore-forming coccobacilli. At present they are classified into six species based on cultural, metabolic, antigenic properties and preferred natural host (Buxton and Fraser, 1977; Gillespie and Timoney, 1981; Walker, 1999).

Cattle are the preferential host for *B. abortus*, but can also be transmitted to buffalo, camels, deers, horses, pigs, sheep, goats, and man. *Brucella melitensis* primarily infects sheep and goats but can also infect cattle and man. *Brucella suis* is the causal organism of brucellosis in pigs, which can also be transmitted to man. *Brucella canis* is essentially an infection of dogs but is occasionally transmitted to man. *Brucella ovis* infections are limited to sheep and *Brucella neotomae has* only been found in desert wood rat (Corbel and Hendry, 1985; Quinn *et al.*, 1994; Walker, 1999).

Each *Brucella* species has preferred natural host that serves as a reservoir of infection. Secondary hosts play only a very small part if any in the maintenance or spread of a particular *Brucella* species (Quinn *et al.*, 1994).

Bovine brucellosis (contagious abortion, Bang's disease) is a disease of cattle usually caused by *Brucella abortus*, less frequently by *Brucella melitensis* and rarely by *Brucella suis*. *Brucella suis* does not appear to be contagious from cow to cow (Nicoletti, 1998). *Brucella abortus* has 7 biovars (1, 2, 3, 4, 5, 6, and 9); biovars 7 and 8 are no longer regarded as valid (OIE, 2004).

The recent isolation of distinctive *Brucella* strains, tentatively named *Brucella maris*, from marine animals in the United Kingdom and United States extends the ecologic range of the genus and, potentially, its scope as a zoonosis (Ross *et al*, 1994; Ewalt *et al*., 1994).

No proven differences in the pathogenecity or antigenecity among the field strain biotypes are observed (Radostitis *et al*., 2000). Sometimes multiple biotypes may be found within a herd (Nicolleti, 1980). *Brucella suis* has also 5 different biotypes. *Brucella melitensis* has got 3 biotypes (PAHO-WHO, 2001).

The biotypes within species are differentiated on the basis of specific combinations of characteristics. These include CO_2 requirement, H_2S production, urease activity, growth in the presence of dyes, serological specificity, phage typing, and oxidative metabolic profiles (Baron *et al*., 1994; Quinn *et al*., 1994). The characteristics of the different *Brucella* species are presented in Table 1.

Table 1.Characteristic of *Brucella* species.

Species	CO_2 required for growth	Time to Positive Urease	H_2S Production	Inhibition by Dye	
				Thionine	Fuchsine
B. abortus	+/-	2 hrs (rare 24hr)	+ (Most strains)	+	-
B. melitensis	-	2hr (rare 24hr)	+	-	-
B. suis	-	15 min	+/-	-	+ (Most)
B. ovis	+	$\geq$ 7 days	-	-	+ (Most)
B. neotomae	-	15 min	+	+	+
B. canis	-	15 min	-	-	+

Source: Baron *et al.* (1994)

2.2. Epidemiology

2.2.1. Occurrence

2.2.1.1. Global occurrence

Worldwide, brucellosis remains a major source of disease in humans and domesticated animals. Although reported incidence and prevalence of the disease vary widely from country to country, bovine brucellosis caused mainly by *B. abortus* is still the most widespread form (Corbel, 1997).

Brucellosis creates a serious economic problem for the intensive and extensive animal production systems of the tropics (Seifert; 1996). Bovine brucellosis has been eradicated from most industrial countries, especially in Europe, such as Finland, Norway, Sweden,

Denmark, the Netherlands, Belgium, Switzerland, Germany, Austria, Hungary, the former Czechoslovakia, Rumania, Bulgaria and some others (Fensterbank, 1986; PAHO-WHO, 2001). Canada, Japan, Israel, North Korea, Australia, and New Zealand have also eradicated bovine brucellosis (Corbel, 1997).

In other parts of the world the rates of brucellosis caused by *B. abortus* vary greatly from one country to another and between regions within a country (Seifert, 1996). The highest prevalence is seen in dairy cattle. The large meat producing countries such as France, UK, Australia, New Zealand, Canada and the United States are free of bovine brucellosis (Radostits *et al.*, 2000; PAHO-WHO, 2001).

2.2.1.2. Status of brucellosis in Africa

Published studies on the relative occurrence of brucellosis are largely confined to serological surveys, and are much more commonly conducted for bovine brucellosis, occasionally for shoats and rarely for pigs (Corbel, 1997). Brucellosis in cattle is prevalent and widespread in sub-Saharan Africa. In the field, *B. abortus* is the main causal organism, *B. melitensis* infections have been reported and *B. suis* infections suspected (Schutte, 1977; Domenech *et al.*, 1983; Chukwu, 1985).

In pastoral systems and livestock-subsistence crop systems in semi-arid areas, serological prevalence is almost always greater than 5% (range 4.8–41%) (Jiwa *et al.*, 1996; Kadohira *et al.*, 1997; Domingo, 2000). For crop–livestock systems in both the sub-humid and highland zones, prevalence tends to be lower than in pastoralist areas (Oomen and Wegener, 1982; Muriuki *et al.*, 1997; Baba *et al.*, 2001). However, prevalence varies even more widely than in pastoralist systems—ranging from high (25%) in large intensively managed herds (Mohan *et al.*, 1996) to low or non-existent in zero-grazing smallholdings in the east African highlands (Bekele *et al.*, 1989; Bedard *et al.*, 1993; Kadohira *et al.*, 1997).

Brucellosis is an important livestock disease entity in many African countries. According to Seifert (1996) an incidence of infection of up to 80% can be found in intensive dairy production systems of the tropics. In the extensive animal production systems of the Sahel, an average disease incidence of 25-30% has been calculated. In eastern Sudan, an infection rate in cattle of almost 22% and in sheep about 13.6% was found (Seifert, 1996). The prevalence of bovine brucellosis in some African countries is presented in table 2.

Table 2.Prevalence of bovine brucellosis in some African countries

Country	No.of animal examined	Prevalence (%)	Tests used	Authors and years
Cen.Afri.Repu.	8800	40.0	RBT, SAT,CFT	Domenech *et al.*, 1980
Chad Cameroon	14344	30.0	SAT	Domenech *et al.*, 1982
Somalia	2184	12.0	SAT	Hussein *et al.*, 1978
Tanzania	13078	10.8	SAT	Jiwa *et al.*, 1997
Kenya	640	15.0	ELISA	Kadohira *et al.*, 1997
Sudan	762	20.2	RBT	McDermott *et al.*,1987
Tanzania	17758	10.6	SAT	Msanga *et al.*, 1986
Kenya	1472	7.5	SAT	Oomen & Weneger *et al.*, 1982
Malawi	2017	0.3	SAT	Bedard *et al.*, 1993
South Africa	5059	1.5	RBT, CFT	Bishop, 1984
Zambia	1879	11.3	RBT, SAT,CFT	Gallanger, 1973
Kenya	1420	12.8	RBT, SAT,CFT	Kagumba and Nandokha, 1978
Tanzania	1167	1.8	RBT, SAT,CFT	Kagumba and Nandokha, 1978
Burundi	528	25.4	RBT	Merek and Schlitng, 1984

Kenya	46	4.3	SAT	Oomen and Wegener, 1982
Sierra Leone	2626	10.4	SAT	Optz, 1969
Guinea	1861	6.9	WSA,RBT,CFT	Sylla *et la*., 1982
Kenya	374	2.0	ELISA	Kadohira *et la*.,1997
Kenya	208	2.4	SAT	Oomen and Wegener, 1982
Kenya	99	16.2	RBT,SAT,CFT	Kagumba and Nandokha, 1978
Coted`Ivoire	1180	48.0	RBT	Camus, 1980
Uganda	169	2.4	RBT,SAT,CFT	Kagumba and Nandokha, 1978
Ghana	183	6.6	SAT	Kubuafor *et al*., 2000
Uganda	68	36.8	SAT	Thimm, 1972
Coted`Ivoire	12343	10.8	CFT, SAT,CAT	Pilo-Moron *et al*., 1979
Ghana	323	9.3	RBT	Turkson and Boadu, 1992
Togo	1056	41.0	RBT,SAT,CFT	Akakpo *et al*., 1978
Djibouti	499	4.0		Chantel *et al*., 1994

2.2.1.3.Status of brucellosis in Ethiopia

Different researchers have carried out prevalence studies of brucellosis in cattle in many parts of Ethiopia. These studies were conducted both in intensive and extensive livestock production systems and higher prevalence rates are recorded in intensive dairy farms (Mayer, 1980; Rashid, 1993; Sintaro, 1994). In these studies, seroprevalence ranging from 0.14% up to 38.7% are recorded. The results of seroprevalence studies of bovine brucellosis in Ethiopia are presented in Table 3.

Table 3.Seroprevalence of bovine brucellosis in Ethiopia

Location	No. animals examined	Prevalence (%)	Test used	Breed	Reference and year
Around A.A.	178	18.4	RBPT, SAT, CFT	Exotic, Zebu, cross	Gebremariam, 1985
Around Bahr Dar	678	9.8	RBPT, SAT, CFT	Zebu, cross	Hadgu, 1987
Shoa Region	3577	2.1	RBPT, SAT, CFT	Zebu, cross	Shiferaw, 1987
Central Ethiopia	1609	4.2	RBPT	Zebu	Bekele *et al.* 1989
Arsi Region	2178	7.62	RBPT, SAT	Exotic, Zebu, cross	Molla, 1989
Sidamo region	734	11.6	RBPT, SAT	Exotic, Zebu, cross	Zewdu, 1989
Abernosa	963	14.20	RBPT, SAT	Zebu, cross	Yirgu, 1991
Around Bahr Dar	1855	12.34	RBPT, SAT	Zebu, cross	Shiferaw, 1994
Chaffa farm	193	22.0	RBPT, SAT	Cross	Sintaro, 1994
Around AA	950	8.11	RBPT, CFT	Exotic, cross	Asfaw *et al.*, 1998
South east Ethiopia	4243	4.90	RBPT, CFT	Zebu, cross	Bekele *et al.*, 2000
Tigray Region	430	0.69	RBPT, CFT	Zebu, cross	Tesfaye, 2003
North Gondar	1447	0.14	RBPT, CFT	Zebu, cross	Yayeh, 2003
SidamaZone	2438	1.92	RBPT, CFT	Zebu, cross	Asmare, 2004
Jimma Zone	1813	0.61	RBPT, CFT	NA	Tolosa, 2004
IAR Farms	147	38.7	RBPT, SAT	Cross	Rashid, 1993
Bahir dar Milkshed	1944	4.63	RBPT, CFT	Zebu, cross	Hailemelekot, 2005
Tigray Region	1951	1.49	RBPT, CFT	Zebu	Berhe, 2005
Eastern Amhara	3644	1.8	RBT, CFT	Zebu	Fekadu, 1999

2.1.3. Transmission and source of infection

Brucellosis is transmitted by direct or indirect contact with infective excretors. Ingestion is the most common route of entry, although exposure through the conjunctival and genital mucosa, skin and respiratory routes occurs (Walker, 1999; Radostits *et al.*, 2000). The major source for exposure to *B. abortus* in cattle is through aborted fetuses, the placenta, and post abortion uterine fluids. Contaminated feed (hay) can spread the infection from infected pastures over large distances when it is traded. Insects and game animals may also carry the infection over long distances (Seifert, 1996; PAHO-WHO, 2001).

Ingestion of milk from infected cattle and goats is also another source for infection of calves and kids. Direct transfer in *utero* has also been documented. In closed environment, the infection may spread by aerosols; airborne infection has been demonstrated (Nicoletti, 1980; Walker, 1999; PAHO-WHO, 2001). The use of infected bulls for AI poses important risk and spreads the infection to many herds. Calves may be infected while in the uterus or by suckling infected milk of their mother. Venereal transmissions by infected bull to susceptible cows appear to be rare (Chukwu, 1987; Nicoletti, 1998).

2.1.3. Risk factors

2.1.4.1. Management

Once a herd is infected, the time required to become free from brucellosis is increased by large size, active abortions and loose housing (Radostits *et al.*, 2000). Herd size and animal density are directly related to prevalence of disease and difficulty in controlling infection in a population. Calving practices also play a major role in the spread of brucellosis. Separate calving pens allow for minimizing exposure of uninfected animals. Whether a herd raises its own replacement animals or purchases replacement animals affects the potential for introduction of the disease into the herd (Nicoletti, 1980; Walker, 1999).

There is a positive association between population density (number of animals to land area) and disease prevalence, which is attributed to increased contact between susceptible and infected animals (Omer *et al.*, 2000).

Unless the hygienic condition, measures to prevent the introduction of the disease and the resistance of the animals are improved altogether, the condition favors rapid spread of the disease and subsequently result in high prevalence of the disease in that particular herd (Nicoletti, 1980; Radostits *et al.*, 2000).

2.1.4.2. Host factor

Susceptibility to infection depends on age, sex, breed and pregnancy status of the animal. Younger animals tend to be more resistant to infection and frequently clear infections, although latent infection does occur; only 2.6% of animals infected at birth remain infected as adults (Radostits *et al.*, 2000). Sexually mature animals are much more susceptible to infection, regardless of gender. Most animals infected as adults remain infected for life.

After cattle reach sexual maturity, the state of pregnancy has a greater influence on the degree of susceptibility than does age (Manthei and Carter, 1949). Because of latent infections, calves borne of recently infected mothers often infect the herd where they join later in life (Nicoletti, 1984; Madsen, 1989). Bulls are more resistant than sexually mature heifers and less resistant than sexually immature heifers. Both venereal transmission and exposure to aborted fetuses and fetal membranes are important for maintaining infections in a herd (Walker, 1999; PAHO-WHO, 2001).

2.1.4.3. Environment

The environmental resistance of the pathogens depends on whether they are protected against the sunlight and high temperature. Neutral soil pH and a moist environment, which are rich in organic material, are favorable elements for survival. *Brucella* organisms can survive in the environment for long periods (Madsen, 1989; Seifert, 1996).

Brucella survive freezing and thawing. Under proper environmental conditions, they survive for up to 4 months in milk, urine, water and damp soil. In liquid manure the *Brucella* survive for months, in humid faces for 22 weeks, in aborted fetuses up to 4 months and after birth, in the dust of streets for 44 days, in tap water for 30 days, in sterile water for 51 days, in desert soil up to 2 months, and in frozen soil for 2 years. Contaminated straw remains infectious for longer than a month (Weidmann, 1991; Seifert, 1996). It is generally accepted that *Brucella* organisms do not multiply in the environment they merely persist and their viability is influenced by the existing environmental conditions (Radostits *et al.*, 2000)

2.2. Pathogenesis and Clinical Manifestations

The susceptibility of the animal depends significantly on their natural resistance, their age and their level of immunity and on environmental stress. If the infection is introduced into a non-infected herd in which all animals are immunologically naïve to brucellosis, the so-called abortion storms may occur and almost all pregnant cows will abort (Seifert, 1996).

Following exposure, *Brucella* penetrate intact mucosal surfaces. In the alimentary tract, the epithelium covering the ileal payer's patches are a preferred site for entry. Soon after entry into the host body, the *Brucella* are engulfed by phagocytic cells, notably the macrophages, in which they survive, multiply and are transported to the regional lymph nodes (Quinn *et al.*, 1994).

Various mechanisms are employed by *Brucella* to allow for survival inside the phagocytic cells. These include inhibition of phagolysosome fusion; super oxide dismutase and catalase production may play a role in defense against oxidative killing, stress proteins are thought to play a role in protecting the organisms from hydrolytic enzymes, oxygen radicals, and myeloperoxidase killing systems in the phagolysosome. The lipopolysaccharde of *Brucella* is directly associated with virulence and is thought to play a role in enhancing intracellular survival (Detilleux *et al.*, 1991; Walker, 1999).

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 (Walker, 1999; Radostits *et al.*, 2000).

A pyogranulomatous reaction occurs in affected placenta and abortion occurs from mid-gestation onwards. In cattle abortion commonly occurs in the fifth month of gestation or later and retained fetal membranes is a possible sequel (Radostits *et al.*, 2000; PAHO-WHO, 2001). Experimental infection studies have demonstrated that, at the cellular level, *Brucella* localize into the cisternae of the rough endoplasmic reticulum of trophoblasts of the placentome. Infection subsequently spreads to the fetus. The exact mechanism of abortion is unclear; however, likely possibilities are that abortion results from:

- Interference with fetal circulation due to the existing placentitis
- The direct effect of endotoxin, and/or
- Fetal stress resulting from the inflammatory response in the fetal tissue (Freeman, 1979; Quinn *et al.*, 1994; Walker, 1999).

The primary clinical manifestations of brucellosis are related to the reproductive tract. Abortion that occurs in the last trimester is the most obvious manifestation. The quantity of *Brucella* excreted during an abortion could be as much as 10^{12}-10^{13} capable of infecting 60,000- 600,000 pregnant heifers (Nicoletti, 1980; Fensterbank, 1986). Infection may also cause stillbirth or weak calves, retained fetal membrane, and reduced milk yield. Usually, general health is not impaired in uncomplicated abortion. Seminal vesicles, ampullae, testicles, and epydidimis may be infected in bulls. Testicular abscesses may occur; long-standing infections may result in arthritic joints and hygroma in some cattle (Seifert, 1996; Nicoletti, 1998). In general, animals do not exhibit overt systemic illness (Walker, 1999)

2.3. Diagnosis

The diagnostic alternatives for the recognition of brucellosis are more varied than with any other infectious disease. This depends on the reaction of the organisms on the chronic infection, which partly occurs intracellularly and which can be detected only incompletely with the usual serological techniques (Seifert, 1996).

Great care should be employed when working with infected tissues and cultures in the laboratory. All *Brucella* cultures should be handled following biosafety level 3 practices because of the potential for laboratory infection (Baron *et al*., 1994; Quinn *et al*., 1994). Diagnosis of brucellosis is the corner stone for any control and eradication program and is based on the following methods, bacteriological, immunological findings and molecular biology (Redkar *et al*., 2001).

2.3.1. Bacteriological methods

2.3.1.1. Demonstration by microscopic examination

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

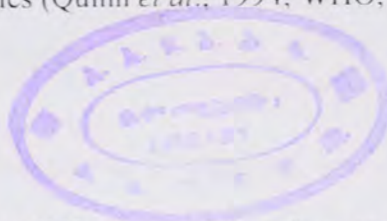
2.3.1.2. Isolation and identification of *Brucella*

Cultural examinations are very important in the epidemiology of bovine brucellosis, and positive results in culture are conclusive and should be used to evaluate all other diagnostic methods (Nicolleti, 1980). However, negative culture results are not always sufficient to rule out infection (Weidmann, 1991; Seifert, 1996)

Brucella grow well on 5-10% blood agar. However, other than fetal abomasal contents and colostrums, the specimens are likely to contain many contaminating bacteria and fungi, so selective media are usually required (Walker, 1999). 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 percent CO_2 for a minimum of 10 days and up to 21 days in highly suspicious cases (Quinn, *et al.*, 1994; PAHO-WHO, 2001).

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 (Freeman, 1979; Walker, 1999).

Preliminary identification of *Brucella* species require demonstrating pinpoint, smooth, glistening, bluish, translucent colonies. The *Brucella* are 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 identify the species (Quinn *et al.*, 1994; WHO, 1997).



2.3.2. Serological diagnosis

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

No single serological test and antigen combination showed 100% sensitivity and specificity simultaneously (Munoz *et al.*, 2005). However, such an assay is not available at present, although multiple tests increase the confidence in the diagnosis, and sequential testes over time also provide a better insight than single test (MacMillan, 1990).

Combinations of tests show a degree of sensitivity, which appear to be sufficient to detect infected animals. Frequently, highly sensitive but less specific tests are used for screening purposes and these then are followed by more specific tests for confirmation (Walker, 1999; Garrido-Abellan *et al.*, 2001).

The commonly used serological tests for the diagnosis of brucellosis are the RBPT, SAT, CFT and ELISA (Quinn *et al.*, 1999). The Rose-Bengal Plate Test (RBPT), the Serum Agglutination Test (SAT) and the Complement Fixation Test (CFT) are widely used for the detection of antibodies to *Brucella* in bovine serum, with the CFT internationally regarded as a confirmatory test (Alton *et al.*, 1975).

2.3.2.1. Rose Bengal Plate Test

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

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

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

2.3.2.2. Serum Agglutination Test (SAT)

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

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

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

2.3.2.3. Complement Fixation Test

The Complement-fixation test is recognized as the most reliable diagnostic test now in routine use for individual animals (FAO/WHO, 1986). CFT is a widely used and accepted confirmatory test although it is complex to perform, requiring good laboratory facilities and adequately trained staff to accurately titrate and maintain the reagents (OIE, 2004).

The work load resulting from the technical complexity of the complement-fixation test can be greatly reduced by using it only as a definitive test on samples that have been found positive in a preliminary screening test (FAO/WHO, 1986). CFT was found to be 92.9% sensitive and 100% specific in non-vaccinated populations (Dohoo *et al.*, 1986). The CFT detects IgG1 and IgM antibody classes (Fensterbank, 1986).

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

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

2.3.2.4. Enzyme Linked Immuno Sorbent Assay

The ELISA test has gained wide acceptance for serological diagnosis of bovine brucellosis because of its ability to detect antibody of all isotypes unlike other conventional testes (Nielsen *et al.*, 1996). The ELISA test can be useful in conjunction with the CFT during the latter stages of an eradication program when it is important to reduce the number of false negative serological reaction (Sutherland *et al.*, 1986). It is a useful test during an eradication program after vaccination has ceased for screening or as a supplementary test to the CFT (Radostitis *et al.*, 2000)

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

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

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

2.3.3. Non-culture detection methods

A number of non culture methods, including polymerase chain reaction (PCR), immunoperoxidase staining, DNA probes, and coagglutination, have been described for detection of *Brucella* in tissues and fluids (Walker, 1999; Redkar *et al.*, 2001; OIE, 2004).

2.4. Economic Significance

Brucellosis, occurring worldwide in domestic and game animals as well as in humans, creates a serious economic problem for the intensive and extensive livestock production systems. In addition to abortion in pregnant animals, outbreaks of bovine brucellosis result in an average loss of milk production of 20% (Schwabe, 1984; Bishop *et al.*, 1994).

Besides the reduction in milk production, there is also loss of calves, temporary or permanent infertility and interference with the breeding program due to culling of valuable cows. This is of greater importance in beef herds where calves represent the sole source of income (Radostits *et al.*, 2000). Abortion may lead to retained fetal membranes, metritis and a subsequent long period of infertility. Infertility increases the period between lactations and the average intercalving period may be prolonged by several months (OIE, 1997; Radostits *et al.*, 2000).

The economic losses due to bovine brucellosis have been generalized by Chukwu (1987) as follows:

- Loss of calves due to abortion,
- Reduced milk yield,
- Culling and condemnation of valuable cows because of breeding failure.
- Endangering animal export trading of a nation,
- Loss of man-hours and medical costs and
- Government costs incurred for research and eradication programs.

Using the term "infertility" in its widest sense, *Brucella abortus* is probably the most important single cause of breeding failure. Its effects are seen mainly as abortion in the second half of pregnancy. Infected cows usually abort once and seldom more than twice, although in subsequent pregnancies the uterus may be re-infected from the udder but the cow then carries the fetus to term (Arthur, 1975).

At the time when the American Bovine Brucellosis Eradication Program begun in 1934, 11.5% of adult cattle were infected with brucellosis and in 1947, the estimated annual economic loss was US $ 100 million. Similarly in Sweden, prior to its eradication in 1957, bovine brucellosis has caused annual economic loss of more than US $ 8.8 million (Schwabe, 1984). Likewise, the New Zealand brucellosis eradication scheme benefited a 10.3 % internal rate of return as a result of increased milk, decreased culling rate and market shares after successful eradication of the disease (FAO, 1998).

Available information indicates that brucellosis is one of the most serious diseases in cattle in Latin America as well as other developing areas. Official estimates put annual losses from bovine brucellosis in Latin America at approximately US $ 600 million (PAHO-WHO, 2001). A survey made in India in 1970 has tried to calculate the annual economic loss incurred by brucellosis in cattle and buffaloes. For calculation the annual loss of calves due to abortion, the reduction in breeding capacity, reduced milk yield and loss of man-days due to human infection were considered. In summary the survey pointed out that brucellosis costed India at least Rs.350 million annually in food, animals and man-days of labor (Schwabe, 1984).

The annual economic loss due to brucellosis in cattle was estimated to be 150 million French francs in Cote D'Ivore, US $ 233.88 million in Nigeria and US $ 33.4 million in Kenya, Tanzania and Uganda (Chukwu, 1987). In central Africa, an incidence of infection in cattle of above 30% has been found and economic losses of the yearly income of the animal holder have been calculated of up to 6% (Seifert, 1996).

2.5. Public Health Significance of Brucellosis

2.5.1. Global occurrence of human brucellosis

The Food and Agricultural Organization, the World Health Organization and the Office International Des Epizooties consider brucellosis as one of the most widespread zoonotic diseases (Schelling *et al.*, 2003). The importance of brucellosis lies on the severe public health hazard it causes and the economic impact on the animal industry (Mustofa and Nicoletti, 1993). Brucellosis in humans is a multisystemic, acute to chronic, disease characterized by fever, headache, joint pains, musculoskeletal pains, sweating, malaise and body wasting (McDermott and Arimi, 2002).

Brucellosis is a disease of animals in which man is infected as a terminal host, and the incidence of brucellosis in human is clearly correlated with the degree of incidence in the domestic animals around him (Weidmann, 1991). It is readily transmissible to humans, causing an acute febrile illness-undulant fever-which may progress to a more chronic form and can also produce serious complication affecting the musculoskeletal, cardiovascular and central nervous system (Hugh-Jones *et al.*, 1995; OIE, 1997).

Each year, about half a million cases of brucellosis occur in humans around the world .The greatest prevalence in humans is found in those countries with high incidence of *B. melitensis* infection among goats, sheep, or both species (WHO, 1997; PAHO-WHO, 2001;). The Latin American countries with the greatest number of recorded cases of human brucellosis are Argentina, Mexico and Peru. The same pattern holds true to the Mediterranean countries, Iran, the former Soviet Union, and Mongolia (PAHO-WHO, 2001). In the United States of America, brucellosis has become a rare disease due to the institution of veterinary control measures such as routine screening of domestic livestock, vaccination programs and since 1980, fewer than 200 cases annually were reported to the Center for Disease Control and Prevention (CDC) (Lisgaris, 2005). Table 4 shows cases of human brucellosis in some countries.

Table 4 Cases of human brucellosis in some countries.

Name of the country	Cases	Year
Saudi Arabia	7,893	1987
Iran	71,053	1988
Iran	80,000 per year	Since 1989
Turkey	5,003	1990
USA	6,321	1947
USA	224 per year	1972-1981
Denmark	500 per year	1931-1939
Spain	4,683	1988
Spain	3,041	1990

Source: PAHO-WHO (2001)

Brucellosis is usually an occupational disease occurring in veterinarians, farmers, stock inspectors, abattoir workers, laboratory personnel and butchers (Sewell and Brocklesby, 1990; Bishop *et al.*, 1994; Hugh-Jones *et al.*, 1995). *Brucella abortus* and *B. suis* infections usually affect occupational groups, while *B. melitensis* infections occur more frequently than the other types in the general population and are known to cause more severe clinical pathological effect (PAHO-WHO, 2001; Omer *et al.*, 2002).

The prolonged bacteremic phase of *B. suis* infection in swine poses a special risk for slaughterhouse workers handling infected tissues (Walker, 1999). Individuals who participate in the hunting of feral swine are also at risk for contracting *B. suis* infection (Walker, 1999; Radostits *et al.*, 2000). Relatively few cases of infections in humans due to *B. canis* have been reported (PAHO-WHO,2001).

Brucellosis is one of the most prevalent laboratory acquired infections. One report describes an outbreak involving eight microbiology laboratory workers who acquired *B. Melitensis* from aerosols produced during routine subcultures (on the bench) of a stock strain (Quinn *et al.*, 1994). The same isolate infected a CDC worker subsequently, although correct biosafety practices were followed (Quinn *et al.*, 1994). Thus laboratory manipulation of live cultures or contaminated materials from animals is hazardous and must be done under conditions of biohazard containment (OIE, 1997).

Brucellosis is also of special importance in African native communities where animals cohabit with their owners at night (Radostits *et al.*, 2000). Summary of previous reports on the prevalence of human brucellosis in some African countries is presented below in Table 5.

Table 5 Human brucellosis in some African countries

Country	No. examined	Prevalence(%)	Test used	Reference
Djibouti	108	6.50	CFT	Chantal *et al*., 1996
Coted`Ivoire	30	13.3	CFT, RBT	Pilo-Moron *et al*., 1979
Eritrea	130	7.1	CFT	Omer *et al*., 2002
Eritrea	21	4.6	CFT	Omer *et al*., 2002
Eritrea	105	3.0	CFT	Omer *et al*., 2002
Ethiopia	8	12.5	RBPT, CFT	Yirgu, 1991
Ethiopia	126	2.4	RBPT, CFT	Tolosa, 2004
Ethiopia	38	5.3	RBPT, CFT	Asmare, 2004
Ethiopia	238	3.78	RBPT, CFT	Hailemelekot, 2005
Ethiopia	2500	1.2	SAT	Seboxa, 1982
Ethiopia	49	24.5	RBPT	Gebreyesus, 2001
Kenya	452	10.4	RBT	Oomen and Waghela, 1974
Nigeria	13,999	7.6-29.80	SAT	Chukwu, 1987
Nigeria	738	5.55	SAT	Chukwu, 1987
Nigeria	500	5.2	RBT	Baba *et al*., 2001
Somalia	353	0.60	SAT	Hussien *et al*., 1987
Tanzania	540	22.60	SAT	Chukwu, 1987
Tanzania	80	20.00	SAT	Chukwu, 1987
Uganda	3164	6.40	SAT	Chukwu, 1987
Uganda	3164	6.4	AT	Mutanda, 1998

2.5.2. The disease in humans

Humans are susceptible to infection caused by *B. melitensis*, *B. suis, B. abortus, and B. canis*. No human cases caused by *B. ovis, B. neotomae, or B. suis biovar* 2 have been confirmed (Hugh-Jones *et al.*, 1995). The most pathogenic and invasive species for man is *B. melitensis*, followed in decreasing order by *B. suis, B. abortus, and B. canis* (Isselbacher *et al.*, 1980; Hugh-Jones *et al.*, 1995).

The incubation period of brucellosis in humans is highly variable and relatively long; it may range from one week to as long as four months. The case fatality rate is low and usually less than one percent (Freeman, 1979; Hugh-Jones *et al.*, 1995). The disease is septicemic with sudden or insidious onset, and is accompanied by continued, intermittent or irregular fever. The symptomatology of acute brucellosis, like that of many other febrile diseases, includes chills and profuse sweating. Weakness is an almost constant symptom, and any exercise produces pronounced fatigue (Freeman, 1979; Hugh-Jones *et al.*, 1995; PAHO-WHO, 2001; Omer *et al.*, 2002).

The common symptoms are insomnia, sexual impotence, constipation, anorexia, headache, arthralgia, and general malaise. The disease has a marked effect on the nervous system evidenced by irritation, nervousness, and depression. Hepatosplenomegaly is particularly frequent in patients infected by *B. melitensis* (Corbel, 1997; Lisgaris, 2005). Tissue reaction is granulomatous. The duration of the disease can vary from a few weeks or months to several years. Some times brucellosis produces serious complications, such as encephalitis, meningitis, peripheral neuritis, spondylitis, suppurative arthritis, vegetative endocarditis, Orchitis, seminal vesiculitis and prostatitis (Freeman, 1979; Walker, 1999; PAHO-WHO, 2001). For persons presenting with congestive heart failure from endocarditis, the prognosis is poor, with a mortality rate approaching 85% (Lisgaris, 2005). Without adequate and prompt antibiotic treatment, some patients develop chronic brucellosis syndrome with many features of 'chronic fatigue' syndrome (WHO, 1997).

2.5.3. Source of infection and mode of transmission in humans

Brucellosis, a bacterial zoonosis, is a disease that is caused by the bacterial genus *Brucella* and transmission to humans occur after occupational exposure or through ingestion of contaminated food products (Lisgaris, 2005).

Brucellosis in humans is probably always acquired from infected domestic animals through direct contact or indirectly by ingestion of animal products and by inhalation of airborne agents; man-to-man transmission is a possibility but rarely, if ever occurs (Freeman, 1979). Fresh cheese and raw milk from goats and sheep infected with *B. melitensis* are the most common vehicles of infection and can cause multiple cases of human brucellosis (Sewell and Brocklesby, 1990; WHO, 1997; PAHO-WHO, 2001). Slaughterhouse workers, primarily those in the kill areas, become inoculated through aerosolization of fluids, contamination of skin abrasions and splashing of mucous membranes (Lisgaris, 2005).

Cow's milk and milk products containing *B. abortus* may give rise to sporadic cases. The organisms rarely survive in sour milk, sour cream and butter, or fermented cheese (aged over three months). It is also possible for raw vegetables and water contaminated with the excreta of infected animals to serve as sources of infection (Hugh-Jones *et al.*, 1995; Seifert, 1996, Radostits *et al.*, 2000).

Transmission by contact predominates in areas where bovine and porcine brucellosis are enzootic. Humans may be infected by the handling of the tissues of diseased animals or by close contact with other infectious materials, presumably the *Brucella* enter through minute abrasions in the skin or possibly through the intact skin as well as through the conjunctiva by way of the hands. Employees of slaughterhouses, veterinarians, sausage-makers, and butchers are particularly exposed to infection by this means (Freeman 1979; PAHO-WHO, 2001). Table 6 shows occupation and the probable source of infection for different livestock handlers.

Airborne transmission has been proven by experimentation and research. In laboratories, centrifugation of *Brucella* suspension poses a special risk when done in centrifuges that are not hermetically sealed. An epidemic outbreak of 45 cases occurred among students at Michigan State University in 1938-1939(PAHO-WHO, 2001). Some cases of possible human -to-human transmission have been described. One of them occurred in Kuwait due to transmission of *B. melitensis* to a 30-day old girl through her mother's milk (PAHO-WHO, 2001).

Table 6.Human Brucellosis Cases (in the US, 1966): Occupation and probable source of infection.

Classification	Occupation	Probable source of infection							
		Swine	Cattle	Cattle and swine	Sheep or goats	Raw milk	Accidents	Others and unknown	Total
Animal industry employees	Packing house	48	17	25	1	-	-	10	101
	Rendering plant	-	1	2	-	-	-	1	4
	Stockyard	-	-	-	-	-	-	-	-
Farmers	Livestock	9	4	5	1	-	-	-	19
	Dairy	-	10	-	-	-	-	1	11
	Unspecified	-	-	-	-	-	-	-	-
Other categories	Housewives	3	1	-	-	8	-	6	18
	Children	1	1	-	-	3	-	6	11
	Veterinarians	-	-	2	-	-	2	1	5
	Other	3	6	10	2	4	2	11	38
	Unknown	-	-	-	1	4	-	7	12
Total		64	40	44	5	19	5	42	219

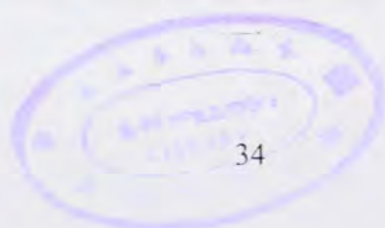
Source Schwabe (1984)

2.5.4. Diagnosis in humans

The clinical picture in human brucellosis can be misleading, and cases in which gastrointestinal, respiratory, dermal, or neurologic manifestations predominate are not uncommon (Santini *et al.*, 1994; Luzzi *et al.*, 1993). Because unusual cases with atypical lesions continue to be reported, diagnosis needs to be supported by laboratory tests (Young, 1995; McDermott and Arimi, 2002). Blood culture is still the standard method and is often effective during the acute phase; the lysis concentration method gives the best results (Kolman, 1991). Isolation and typing of the causal agent through culture is the definitive diagnosis and may also indicate the source of infection (PAHO-WHO, 2001; Corbel, 1997, Lisgaris, 2005). Sensitivity of blood cultures ranges from 17 – 85%, depending on the strain involved, disease stage, culture medium, quantity of circulating bacteria and the blood culture technique employed (Gotuzzo, 1986; Solomon and Jackson, 1992; Corbel, 1997; Lisgaris, 2005).

Although a number of serologic techniques have been developed and used for the diagnosis of human brucellosis, serum agglutination test (SAT) preferably in tubes is the simplest and most widely used standard test (Young, 1991; PAHO-WHO, 2001; Lisgaris, 2005). The serum tube agglutination test and the tray agglutination test (TAT) detect both immunoglobulin G (IgG) and immunoglobulin M (IgM) directed against smooth LPS. A prozone effect caused by hyperantigenemia can result in a negative titer. Prior to testing, routine dilution of serum beyond 1:320 can alleviate this problem. Cross-reactivity with *Afipia clevelandensis*, *Vibrio cholerae*, *Francisella tularensis*, *Salmonella* species, and *Yersinia enterocolitica* can cause a false-positive result because of the presence of LPS. (Lisgaris, 2005).

Other serological tests in use for the diagnosis of human brucellosis include Complement Fixation Test (CFT), 2- mercaptoethanol test (MET), the Rose Bengal Test (RBT), counterimmunoelectrophoresis and Enzyme Linked Immuno Sorbent Assay (ELISA)(Buchanan and Faber, 1980; PAHO-WHO, 2001; McDermott and Arimi, 2002).



PCR (Polymerase Chain Reaction) with random or selected primers gives promising results, but standardization and further evaluation are needed (Buchanan and Faber, 1980; Corbel, 1997; Lisgaris, 2005).

2.6. Prevention and Control

Approaches at control and prevention of brucellosis depend on the animal species involved, *Brucella* species, management practices, and availability and efficacy of vaccines.
Approaches used to control brucellosis include:

- Immunization alone
- Testing and removal of infected animals in conjunction with an immunization program
- Testing and removal of infected animals with out immunization (Walker, 1999).

2.6.1. Immunization

Vaccination with *B. abortus* strain 19 live vaccine is a valuable aid in bovine brucellosis control. Its main value is that it protects uninfected animals living in a contaminated environment, enabling infected animals to be disposed of gradually. The vaccine of choice for cattle is *B. abortus* strain 19 because of its worldwide use, the protection it gives for the useful life time of the animal, and its low cost; to avoid interference with diagnosis, it is recommended that vaccination be limited to young animals (female calves of 3 to 8 months). Usually a protection of 70% (65-75%) is obtained (Seifert, 1996; Radostits *et al.*, 2000).

Recently a new strain (*B. abortus* RB51) was developed and one vaccination with this strain is as effective as one vaccination with S-19 in protecting cattle against brucellosis (Cheville *et al.*, 1993, 1996). Vaccine strain RB51 (S-RB51) is attenuated, rough organism, which essentially lacks the O-side chain of the LPS. The lack of the O-side chain means

that this vaccine can be given single or multiple times without inducing antibodies that interfere with conventional diagnostic tests. Vaccine S-RB51 is now the official vaccine being used in the USA, Chile, Mexico, Venezuela, Colombia and Argentina (Schurig *et al*, 1991). *Brucella abortus* strain RB51 has shown no tendency to revert to virulent smooth organisms after many passages in vitro or in vivo. This is probably due to the nature and place of the mutations found in this strain (OIE, 2004).

2.6.2. Chemotherapy

As a general rule treatment of infected livestock is not attempted because of the high treatment failure rate, high cost and potential problems related to maintaining infected animals in the face of ongoing eradication programs (Radostits et al., 2000). Tetracycline and dihydrostreptomycin have been used to treat *B. ovis* infections in valuable rams with variable results (Freeman, 1979; Walker, 1999).

2.6.3. Prevention and control in humans

Prevention of brucellosis in humans still depends on the eradication or control of the disease in animal hosts, the exercise of hygienic precautions to limit exposure to infection through occupational activities, and the effective heating of dairy products and other potentially contaminated foods (Corbel, 1997).

The most rational approach for preventing human brucellosis is the control and elimination of the infection in animal reservoirs, as has been demonstrated in various countries in Europe and Americas. The direct human health benefits of the American bovine brucellosis eradication program during its early years may be a good example for this. Where as in 1947, 6321 cases of human brucellosis were reported for the country, this number had been reduced more than 95% to 252 in 1966 (Schwabe, 1984; PAHO-WHO, 2001). (Figure -1).

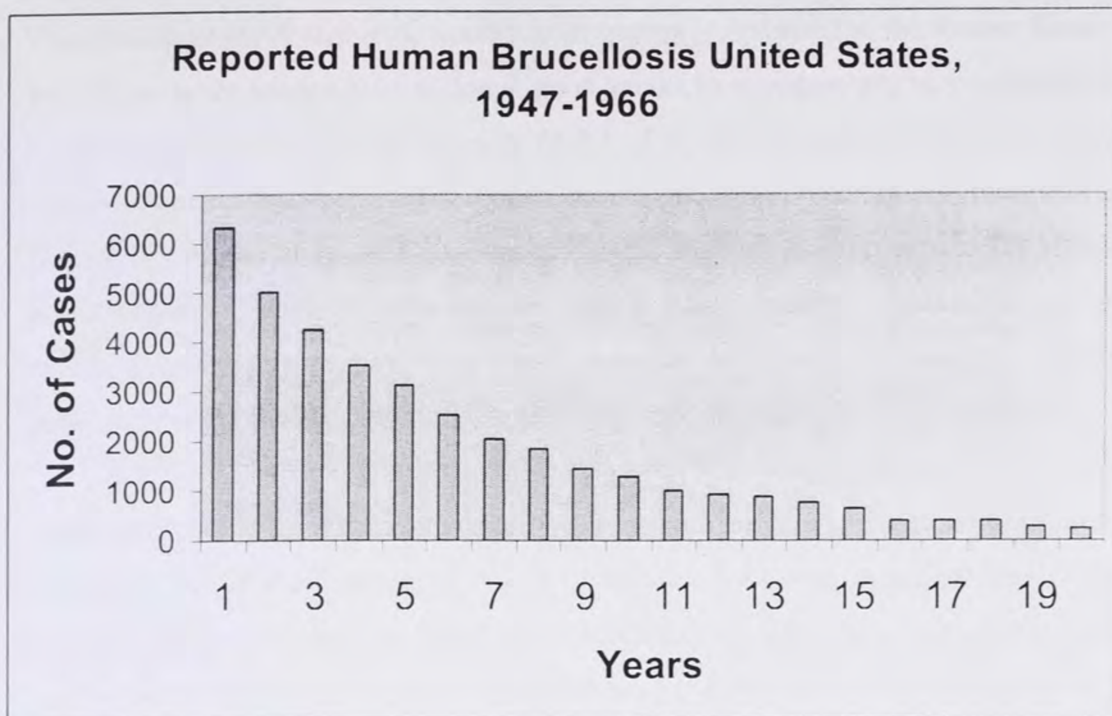


Figure 1.Rapid initial decrease in reported human brucellosis in USA as a result of brucellosis eradication effort in cattle.

Source: Schwabe (1984)

Some human population may be protected by mandatory milk pasteurization. In many goat- and sheep-rearing regions, pasteurization of milk is an unattainable goal for the time being. Prevention of infection in occupational groups (cattlemen, abattoir workers, veterinarians and other who come into contact with animals or their carcasses) is more difficult and should be based on health education, the use of protective clothing wherever possible, and medical supervision (PAHO-WHO, 2001).

Protecting refrigerator plant and slaughterhouse workers against brucellosis is particularly important because they constitute the occupational group at highest risk. Protection is achieved by separating the slaughter area from other sections and controlling air circulation. Employees should be instructed in personal hygiene and provided with disinfectants and protective clothing. (PAHO-WHO, 2001; Omer *et al.*, 2002).

The immunization of high-risk occupational groups is practiced in the former Soviet Union and China. In the former Soviet Union, good results have apparently been obtained with the use of a vaccine prepared from strain *19-BA of B. abortus*, applied by skin scarification. Annual revaccination is carried out for those individuals not reacting to serologic or allergic tests. In China, an attenuated live vaccine made from *B. abortus strain 104M* is applied percutaneously. These vaccines are not used in other countries because of possible side effects. Promising trials have also been conducted in France with antigenic fractions of *Brucella* (PAHO-WHO, 2001; WHO, 1997).

Great care should be exercised when working with infected tissues and cultures in the laboratory. All *Brucella* cultures should be handled following biosafety level 3 practices because of the potential for laboratory infection. All laboratory procedures should be performed in a manner that prevents aerosolization (Baron *et al.*, 1994; Quinn *et al.*, 1994; Walker, 1999).

3. MATERIALS AND METHODS

3.1. Study Area

A cross sectional study was conducted at the Addis Ababa and Sululta abattoirs, Ethiopia to determine the status of bovine brucellosis in cattle slaughtered in the abattoirs and also to assess the occurrence of human brucellosis among abattoir workers. The study period was from September 2005 to March 2006.

3.1.1. Addis Ababa abattoir

Addis Ababa is the capital city and administrative seat of the Federal Democratic Republic of Ethiopia, the African Union and other NGOs. The city lies in the central highlands of Ethiopia at an altitude of 2500 meters above sea level. The human population of Addis Ababa is estimated to be about 3 million. The average annual temperature and rainfall are 21°C and 1800mm respectively. Addis Ababa has a relative humidity that varies from 70% to 80% during the rainy season and 40% to 50 %during the dry season (NMSA, 1999). Addis Ababa and its peri-urban areas have 62,166 cattle, 22,647 sheep, 7,531 equines, 5,597 goats and 330,000 poultry (CSA, 2003).

The Addis Ababa municipal abattoir was established in 1956 as a private share company and was later on confiscated by the government during the derg regime. Now it is under the Addis Ababa Municipality.

The main activities of the abattoir are:

- Processing of livestock into fresh meat for human consumption
- Hygienic processing, storage and transportation of meat and other edible by-products

- Exercising close control and supervision over good environmental conditions at all stages of processing
- Prevention and breakdown of transmission of zoonotic meat-borne diseases through meat inspection
- Production and distribution /or selling of meat and bone meal, tallow, glue, horn, tail and skin and hide to different enterprises.

Cattle that are slaughtered in the Addis Ababa municipal abattoir originate from different parts of the country including Oromia, Amhara and Southern Nations Nationalities and Peoples Regional States. Cattle are usually brought to the abattoir individually or in groups on foot or by trucks. Animals are slaughtered after 24 hours of ante mortem inspection.

In the abattoir, cattle, sheep, goats and swine are slaughtered. On average 800 cattle, 250 sheep, and 75 goats are slaughtered per day. In addition to this, about 50 swine are slaughtered per week. The abattoir has separate slaughtering sections for cattle, small ruminants and swine. The Ministry of Agriculture and Rural Development (MOARD) conducts the meat inspection service in the abattoir. Annually about 153,000 cattle, 39,000 sheep, 3,200 goats and 750 swine are slaughtered in the abattoir. (A.A. Abattoir, 2004).

3.1.2. Sululta abattoir

Sululta abattoir is located in Oromia Regional State, Northern Showa Zone, Mullo- Sululta Wereda, and Sululta town. The Zone is situated between 9^0- 10^0 North latitude and 37^0 57' – 39^0 33' longitude (NSDAD, 2001). The total land coverage of the Zone is 11, 607 km^2 and the altitude ranges from 1000 – 3500 masl (CSA, 2003). The mean annual rainfall of the Zone is 1026 mm and the daily mean maximum and minimum temperature are 20.68^0 C and 11.23^0 C respectively. The human population of the Zone is estimated to be 1,420,571.

According to the result of CSA (2003), the total cattle population of the Zone is estimated to be 1,173, 543. The majority of the cattle population is indigenous breed (99.26%) and there are also 106,225(0.58%) crossbred and 27,220(0.15%) exotic breeds. The total cattle population of Mullo-Sululta Wereda is 186,136. There are about 6,593 crosses of Holstein and indigenous breed. Cattle to be slaughtered in this abattoir originate from Mullo-Sululta and the surrounding Weredas.

Sululta town is situated in the central highland of Ethiopia at a distance of 20 km from Addis Ababa in the northwest direction. On average, about 85 cattle are slaughtered per day (range 65 – 135) in the abattoir. The abattoir is a private limited cooperative administered by executive committee. There are about 81 abattoir workers and one accountant in the abattoir.

3.2. Study Population

The study population consisted of cattle that were slaughtered in the Addis Ababa and Sululta abattoirs during the study period and the people who are engaged in the meat production services in both abattoirs.

3.2.1. Cattle population

Important information such as age, sex and breed were collected for each animal before it was slaughtered. All the animals included in the sample were also observed for their body condition. Local animals were scored according to the guidelines established by Nicholson and Butterworth (1986) and scoring of crossbred animals was done according to the guidelines forwarded by Richard (1993).

A total of 1536 heads of cattle were sampled during the study period, 768 cattle from each abattoir. In both abattoirs, the number of female cattle that were slaughtered during the study period was very few compared to the males. Because of this reason, 73 females from Addis Ababa abattoir and 124 females from Sululta abattoir and totally 197 females were involved in this study. Of the total of 197 females, 91 were crossbred cattle.

3.2.2. Human population

The Addis Ababa municipal abattoir has about 700 workers and this includes both the office and production line workers. On the production line, there are about 420 individuals. Because of their working environment, the peoples that are engaged in meat production process are at high risk for contracting the disease. The Sululta abattoir has about 81 workers who are directly involved in the meat production process.

3.3. Study Design

A cross-sectional study was carried out in Addis Ababa and Sululta abattoirs from September 2005 to March 2006. The study subjects were cattle slaughtered in the abattoirs and individuals working in the production line with high risk. About 7 – 10 ml blood sample was collected from each cattle to determine the prevalence of bovine brucellosis in the abattoirs. Blood samples were collected twice in a week. 5 ml blood samples were also collected from the people working in meat production process in Sululta abattoir to investigate the status of brucellosis in humans.

Screening of sera was carried out using Rose Bengal Plate Test (RBPT). All sera testing positive to the RBPT were subjected to Complement Fixation Test (CFT) for confirmation. It is believed that such serial testing will maximize the specificity and predictive values of positive test result.

3.4. Sampling Method and Sample Size

All cattle that were slaughtered in both abattoirs during the study period were considered and had equal probability to be selected in the sample. Systematic random sampling technique was employed to select animals (units) that should be included in the sample.

Simple random sampling technique was used to calculate the required sample size for this study. To calculate the sample size, the following parameters were pre-determined. 95% level of confidence, 5% desired level of precision and 50% expected prevalence of brucellosis were considered to find the sample size (As bovine brucellosis is a low prevalence disease, 50% expected prevalence was used to calculate the minimum sample size so as to increase the precision of the sample estimate).

So, the relevant formula for a 95% confidence interval was (Thrusfield, 1995):

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

Where,

n = required sample size

Pexp = expected prevalence

d = desired absolute precision.

Using the above formula, the minimum sample size was about 384. But to increase the precision and to avoid sample bias in selecting male and female cattle sex and abattoir were considered as strata so that the total sample size that was collected during the study period was four fold of the formula output, which is 1536. 768 slaughtered cattle were sampled from each abattoir.

3.5. Public Health Survey

To assess the status of human brucellosis in the Sululta abattoir, convenience-sampling technique was used to collect blood samples from voluntary individuals working in the abattoir. The public health survey was conducted in collaboration with Choncho Health Center under close supervision of a physician.

Ethical consideration

Ethical issues were considered in human sampling. The purpose of the study was explained to the subjects involved in the study. Consent was obtained from the abattoir workers. Medical personnel from the nearby Chancho Health Center collected the blood specimens from the participants after consents were obtained. Participation was on voluntary basis and there was no inducement to encourage participation. Participants benefited from the free diagnostic support. Laboratory test results were reported to the participants.

3.6. Questionnaire Survey

A pre-tested structured questionnaire survey was also conducted to investigate the clinical signs and some risk factors associated with brucellosis in humans working in the abattoirs. The questionnaire format was properly designed and tested to evaluate its usefulness.

3.7. Serological Tests

Blood sample was collected from the jugular vein of each cattle using plain vacutainer tube. To assess the status of human brucellosis in the abattoirs, blood sample was collected from abattoir workers in collaboration with the nearby public health institution.

The blood samples thus collected were allowed to clot at room temperature. Then serum was separated from clotted blood by centrifugation. Separated sera were stored at $-20\ ^0C$ until to be tested by both RBPT and CFT.

Screening of sera was carried out using Rose Bengal Plate Test (RBPT). All sera testing positive to the RBPT were subjected to Complement Fixation Test (CFT) for confirmation. It is believed that such serial testing will maximize the specificity and predictive values of positive test result

3.7.1. Rose Bengal Plate Test (RBPT)

This test is a simple spot agglutination test using antigen stained with rose bengal and buffered to a low pH, usually 3.65 ± 0.05. Serum of 30µl was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone of approximately equal to 2 cm in diameter. The mixture was then rocked gently for four minutes at ambient temperature and then observed for agglutination. Any visible reaction was regarded as positive and otherwise negative (OIE, 2004).

All collected serum samples were screened using RBPT. The antigen consisted of a suspension of *Brucella abortus* obtained from Institut Purquier 326, Rue de la Galera 34097 MONTPELLIER CEDEX 5, France. The antigen was inactivated by heat and 0.5% phenol, adjusted to pH 3.65 and colored with Rose Bengal. The procedure described by Alton *et al.* (1975) was used. The degree of agglutination was visually graded from 0 (no agglutination) to 3 (coarse clumping), with corresponding RBPT scores of 0, 1, 2, or 3.

Materials required

The following materials are required to undertake serological tests:

A. For the Rose Bengal Plate Test (RBPT)

1. RBPT Antigen
2. Negative and positive control sera
3. Test serum
4. Plastic applicator
5. Enamel plate or glass slide
6. Rocking machine and centrifuge.

Test procedure

1. The serum samples and antigen were brought to room temperature (22 ± 4°C)
2. 30µl of each serum sample was placed on enamel plate
3. The antigen bottle was shaken gently, and equal volume of antigen was placed near each serum spot
4. Immediately after the last drop of antigen has been added to the plate, the serum and antigen were mixed thoroughly using a clean plastic rod for each test to produce a circular zone approximately 2 cm in diameter
5. The mixture was agitated gently for 4 minutes at ambient temperature on a rocker
6. Finally the plate was read for agglutination immediately after the 4 minutes period is completed. Any reaction was considered as positive and the degree of agglutination was visually graded from 0 (no agglutination) to 3 (coarse clumping), with corresponding RBPT scores of 0, 1, 2, or 3.

The RBPT is very sensitive. However, like all other serological tests, it could sometimes give a positive result due to S19 vaccination or due to false-positive serological reactions (FPSR). False-negative reactions occur rarely, mostly due to prozoning and sometimes may be detected by diluting the serum sample or retesting after a given time (OIE, 2004).

3.7.2. Complement Fixation Test (CFT)

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 (OIE, 2004). Numerous variations of the test occur, but each may be most conveniently carried out using micro titration plates (OIE, 2004). Sera that were positive to RBPT were further tested by CFT for confirmation.

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

Materials required

1. U-shaped microwell plates
2. Multi-channel and single-channel pipettes, pipette tips
3. Flasks and measuring cylinders
4. Digital beam balance
5. Incubator, water baths (37° C and 56 °C), deep freezer and centrifuge
6. Magnetic stirrer with hot plate
7. pH meter, reading mirror for microtitre plates
8. Test tubes, crownwall syringe 2ml and sealing tape
9. Veronal buffered diluent (VBD) and Alsever's solution
10. Complement, Amboceptors (anti-sheep hemolysin), control sera and sheep red blood cells (SRBC).
11. CFT Antigen
12. Test sera

Test procedure

The sera were pre-diluted to 1: 2.5 and incubated at 58^0C in a water bath for 30 minutes in order to inactivate the native complement. Using standard 96-well microtitre plates with round (U) bottoms, the test was performed as follows:

1. Volumes of 25µl of diluted inactivated test serum was placed in the wells of the first, second and third rows. The second row was an anti-complementary control for each serum. A volume of 25 µl of CFT buffer was added to the wells of the first row to compensate for lack of antigen. Volume of 25 µl of CFT buffer was added to all wells except those of the second row.
2. Serial doubling dilutions were then made by transferring 25µl volumes of serum from the third row onwards.
3. Volumes of 25 µl of antigen, diluted to working strength, and 25 µl of complement, diluted to the number of units required, was added to each well except in the first row.
4. Control wells containing diluent only, serum + complement + diluent, antigen + complement + diluent, complement + diluent, were set up to contain 75 µl total volume in each case. A control serum that gives a minimum positive reaction was tested in each set of tests to verify the sensitivity of test conditions.
5. The plates were incubated at 37° C for 30 minutes and a volume of 25 µl of sensitized SRBCs was added to each well. The plates were re-incubated at 37° C for 30 minutes.
6. The results were read after the plates were left to stand at 4° C for 2-3 hours to allow unlyzed cells to settle. The degree of hemolysis was compared with standards corresponding to 0, 25, 50, 75 and 100% lysis.
7. The plates were taken out from the refrigerator and left at room temperature for ten minutes. Positive reactions were indicated by the sedimentation of sheep red blood cells and absence of hemolysis. Negative reactions were indicated by hemolysis of the red blood cells.

The reading was as complete fixation (no hemolysis) was recorded as + + + +, partial hemolysis + + +, + + (50% hemolysis) or +. Complete lack of fixation (complete hemolysis) was recorded as 0. Prevalence was determined when both test results were positive.

3.6. Data Analysis

The mean prevalence for groups was compared using χ^2 test and P values of less than 0.05 was considered as statistically significant. Data obtained from both serological tests and questionnaires were stored in Microsoft Excel spreadsheet. The data were analyzed by descriptive statistic using SPSS 11.5 for window and analytical statistics using Intercooled Stata 7.0 soft wares. Cattle tested positive to both RBPT and CFT serially were considered as sero-positive.

The following descriptive statistics were employed:

- Frequency tables
- Quantification of disease events by rate, proportion, ratio
- Confidence interval for prevalence

Chi square tests were performed and OR and 95% confidence intervals were determined to estimate the magnitude of associations. Fisher's exact test was also used when found necessary.

4. RESULTS

4.1. Overall Prevalence in Cattle

A cross-sectional study was conducted to determine the status of bovine brucellosis in cattle slaughtered in Addis Ababa and Sululta abattoirs from September 2005 to March 2006. Serological tests were used to determine the prevalence of the disease in the two abattoirs and some risk factors associated with the occurrence of the disease were also investigated. In addition, attempts were made to investigate the status of human brucellosis among abattoir workers in Sululta abattoir. Questionnaire survey was also undertaken to assess the clinical signs and symptoms associated with human brucellosis and the risk factors for infection.

Serum samples were collected from 1536 cattle (above 6 months of age) that were slaughtered in the Addis Ababa and Sululta abattoirs, 768 samples from each abattoir. About 35 blood samples were perished during transportation and storage. The 1501 sera were subjected to RBPT. Twenty-two serum samples (n = 22) were found to be positive for RBPT and further tested with CFT for confirmation. Finally, 17 sera were positive for the CFT. Therefore, all the subsequent test analyses were based on the sera (n = 17) that were positive to both the RBPT and CFT. The overall individual level sero prevalence of bovine brucellosis in the two abattoirs was found to be 1.13 % (n = 17).

4.2. Abattoir Level Prevalence in Cattle

Table 7 shows the seroprevalence of brucellosis in slaughtered cattle investigated in Addis Ababa abattoir and Sululta abattoir. Comparison of seropositivity of slaughtered cattle was made between the two abattoirs to see the status of brucellosis.

The prevalence of brucellosis in the Addis Ababa and Sululta abattoirs was 1.19 % (n = 9) and 1.08 % (n = 8) respectively. The comparison revealed no significant difference between the Addis Ababa and Sululta abattoirs.

Table 7 Seroprevalence of bovine brucellosis among cattle slaughtered in Addis Ababa and Sululta abattoirs.

Abattoir	No animals tested	RBPT Positive	CFT Positive	Prevalence (%)	95 % C. I.
Addis Ababa	759	12 (1.58%)	9	1.19%	0.6 – 2.3
Sululta	742	10 (1.35%)	8	1.08%	0.5 – 2.1
Total	1501	22 (1.47%)	17	1.13%	0.7 – 1.8
Test statistics	$\chi^2 = 0.0388$	P = 0.844			

4.3. Risk Factors in Cattle

Susceptibility to brucellosis infection depends on the sex, age, breed and pregnancy status of the animal. So comparison was made on the seroprevalence of brucellosis with respect to sex, age and genotype.

4.3.1. Age

Age is assumed to have some association with seropositivity of brucellosis and is considered as a risk factor. The age of cattle that were slaughtered in the two abattoirs was classified into two categories.

The first age category included ages from 6 months to 5 years and the second category constituted age group above 5 years. The seroprevalence of brucellosis between the two age categories of cattle investigated in Addis Ababa and Sululta abattoirs is presented in Table 8. The seroprevalence of *Brucella* antibody in the two age categories was 1.09% (n = 3) and 1.14% (n = 14) for the first and second age categories respectively. Statistical analysis showed no significant difference between the two age categories (p = 1.00).

Table 8 Seroprevalence of brucellosis between the two age categories of cattle investigated in Addis Ababa and Sululta abattoirs.

Age category	No animals tested	RBPT Positive	CFT Positive	Prevalence (%)	95 % C. I.
6 months – 5 years	276	4(1.46%)	3	1.09	0.4 – 3.4
> 5 years	1225	18(1.47%)	14	1.14	0.7 – 1.9
Total	1501	22(1.47%)	17	1.13	0.7 – 1.8
Test statistics	Fisher's Exact = 1.00				

With respect to the prevalence of brucellosis in the two age categories in the two abattoirs, in the Addis Ababa abattoir, the two age categories had prevalence of 1.75% and 1.02% for 6 months – 5 years and > 5 years respectively. There was no statistically significant difference of seropositivity between the two age groups (P = 0.439). Similarly, in Sululta abattoir, the two age categories had seroprevalence of 0 % and 1.26% for 6 months – 5 years and > 5 years respectively with no significant difference in seropositivity between the two age categories (P = 0.609). On the whole, comparison was made on seropositivity of bovine brucellosis among the two age categories of cattle slaughtered in Addis Ababa and Sululta abattoirs and statistically significant differences were not observed between the two age categories. (Table 9)

Table 9 Comparison of seropositivity of brucellosis between the two age categories of cattle investigated in Addis Ababa and Sululta abattoirs.

Age category	No animals tested	RBPT Positive	CFT Positive	Prevalence (%)	95 % C. I.
A.A. abattoir					
6 months – 5 years	171	4 (2.33%)	3	1.75	0.6 - 5.4
> 5 years	588	8 (1.36%)	6	1.02	0.5 – 2.3
Total	759	12 (1.58%)	9	1.19	0.6 – 2.3
Test statistics	Fisher's Exact = 0.439				
Sululta abattoir					
6 months – 5 years	105	0	0	0	
> 5 years	637	10(1.57%)	8	1.26	0.6 – 2.5
Total	742	10(1.35%)	8	1.08	0.5 – 2.1
Test statistics	Fisher's Exact = 0.609				

4.3.2. Sex

Sex has some implications in the occurrence and spread of brucellosis, so comparison has been made between the two sexes. Table 10 shows seropositivity of bovine brucellosis in cattle slaughtered in Addis Ababa and Sululta abattoirs analyzed according to sex. The seroprevalence of brucellosis was found to be 0.84% and 3.05% in male and female cattle respectively. In this study, statistically significant difference was observed in seropositivity between the two sexes with P = 0.011 and OR of 3.7.

Table 10 Seropositivity of bovine brucellosis in cattle slaughtered in Addis Ababa and Sululta abattoirs analyzed according to sex.

Sex	No animals tested	RBPT Positive	CFT Positive	Prevalence (%)	95% C.I.
Male	1304	12(0.92%)	11	0.84	0.5 – 1.50
Female	197	10(5.08%)	6	3.05	1.4 – 6.7
Total	1501	22(1.47%)	17	1.13	0.7 – 1.8
Test statistics	$\chi^2 = 7.4118$				
	OR = 3.7	95% C.I. of OR = 1.35 –10.10 P = 0.011			

Comparison was also made on the seroposistivity of male and female cattle in the two abattoirs and the findings are presented in Table 11. The seroprevalence of male and female cattle slaughtered in Addis Ababa abattoir was 0.73% and 5.48% respectively. Statistically significant difference was observed between the two sexes with p value of 0.007 and OR 8. On the other hand, the seroprevalence of the two sexes in Sululta abattoir was 0.97% and 1.61% for male and female cattle respectively. There was no significant difference between the two sexes in this abattoir.

Table 11 Comparison of seropositivity of cattle to brucellosis based on sex and between the Addis Ababa and Sululta abattoirs.

Sex	No animals tested	RBPT positive	CFT Positive	Prevalence (%)	95% C. I.
A.A. abattoir					
Male	686	6(0.87%)	5	0.73	0.3 1.7
Female	73	6(8.22%)	4	5.48	2.1 14.2
Total	759	12(1.58%	9	1.19	0.6 – 2.3
Test statistics	Fisher's Exact = 0.007				
	OR = 8. 95% C.I. of OR = 2.07 – 30.09				
Sululta abattoir					
Male	618	6(0.97%)	6	0.97	0.4 – 2.2
Female	124	4(3.23%)	2	1.61	0.4 6.4
Total	742	10(1.35%)	8	1.08	0.5 2.1
Test statistics	Fisher's Exact = 0.627				

4.3.3. Genotype (Breed)

About 103 crossbred cattle were slaughtered in the two abattoirs during the study period. Comparison was made between the local and crossbred cattle to see the role of genotype in relation to seropositivity and the findings are presented in Table 12.

Seroprevalence of the local and crossbred cattle was 0.93% and 3.88% respectively. There was significant variation between the two genotypes with respect to seropositivity with P value of 0.025 and OR of 4.31.

Table12 Seropositivity to brucellosis in cattle slaughtered the both abattoirs examined according to genotype.

Genotype	No animals tested	RBPT Positive	CFT Positive	Prevalence (%)	95% C.I.
Local	1398	14(1.0%)	13	0.93	0.5 – 1.6
Cross	103	8(7.77%)	4	3.88	1.5 – 10.2
Total	1501	22(1.47%)	17	1.13	0.7 – 1.8
Test statistics	Fisher's Exact = 0.025				
	OR = 4.31. 95% C.I. of OR = 1.38 – 13.45				

Table 13 presents the seroprevalence of brucellosis in local and crossbred cattle slaughtered in Addis Ababa and Sululta abattoirs. Seroprevalence of 0.74% and 4.82% was found in Addis Ababa abattoir for the local and crossbred cattle respectively. Statistically significant difference of seropositivtiy was observed between the two genotypes with P = 0.011 and OR = 6.8. In Sululta abattoir, the genotype seroprevalence was 1.11% and 0% for the local and crossbred cattle and no significant difference was observed (P = 1.00).

Table 13 Seroprevalence of brucellosis in local and crossbred cattle slaughtered in Addis Ababa and Sululta abattoirs analyzed according to genotype

Breed	No animals tested	RBPT Positive	CFT Positive	Prevalence (%)	95% C.I.
A.A. abattoir					
Local	676	6(0.89%)	5	0.74	0.3 – 1.8
Crossbred	83	6(7.23%)	4	4.82	1.9 – 12.5
Total	759	12(1.58%)	9	1.19	0.6 – 2.3
Test statistics	Fisher's Exact = 0.011				
	OR = 6.8. 95% C.I. of OR = 1.79 – 25.83				
Sululta abattoir					
Local	722	8(1.10%)	8	1.11	0.6 – 2.2
Crossbred	20	2(10.0%)	0	0	
Total	742	10(1.35%)	8	1.08	0.5 – 2.1
Test statistics	Fisher's Exact = 1.00				

4.4. Study Finding in Abattoir Workers

In the present study, blood samples were collected from 67 voluntary abattoir workers by qualified laboratory technician to investigate the status of human brucellosis in Sululta abattoir. All sera (n = 67) were subjected to RBPT but none of them were found to be positive for the presence of *Brucella* antibodies.

4.5. Questionnaire Survey

In this study, 43 workers from Sululta abattoir were interviewed to assess some risk factors that may predispose them to infection. The results of this survey are presented below.

Concerning their educational status, 5 workers (11.6%) are illiterate, 35 (81.4%) of them attended elementary school and 3 (7.0%) workers attended high school. The abattoir workers were asked whether they consume raw meat, raw milk and fresh cheese and yoghurt. It was found that all (100%) of them consumed raw meat, 36 (83.7%) of them consume raw milk and 42 (97.7%) of them consume fresh cheese and yoghurt.

As *Brucella* organisms can easily enter into the body through skin abrasions, the workers were asked how frequent they cut their fingers or hand at work. Eight (18.6%) of the interviewed workers accidentally cut their fingers once in two months, 3 (7.0%) of them cut once in a month, 8 (18.6%) of them once in a week and 24 (55.8%) of them cut their fingers twice and above in a week In other words, about 81.4% of the interviewed workers cut their hands at least once in a month time. None of the interviewed workers use gloves for protection, especially during time of injury. They were also asked about whether they know about zoonotic diseases. 36 workers answered yes and mentioned anthrax and tuberculosis as examples but none of them had knowledge about brucellosis.

5. DISCUSSION

In this study, the status of bovine brucellosis in cattle, slaughtered in Addis Ababa and Sululta abattoirs, and the occupational hazard posed by the disease on abattoir workers have been investigated through serological test analysis and questionnaire survey.

5.1. Overall Seroprevalence in Cattle

In the present study, the overall seroprevalence of brucellosis among cattle slaughtered atthe Addis Ababa and Sululta abattoirs was found to be 1.13%. Previous seroprevalence studies in Ethiopia have reported variable prevalence rates in different parts of the country. High individual animal level seroprevalence of brucellosis was reported by many investigators including 38.7% by Rashid (1993) in the central highland of Ethiopia, 22% by Sintaro (1994) in Chaffa dairy farm, 19.5% by Yirgu (1991) in Abernosa cattle breeding ranch, 19.1% by Gebremariam (1985) in and around Addis Ababa, 12.34% by Shiferaw (1994) in and around Bahir Dar and 11.6% by Zewdu (1989).

Moderate level of seroprevalence rates of brucellosis were also reported in the country, 9.8% by Hadgu (1987) around Bahir Dar, 8.11% by Asfaw *et al*. (1998) in and around Addis Ababa, 8.2% by Gebreyesus (2001) North Western Amhara and 7.62% by Molla (1989). Lower seroprevalence rates were also reported, 4.9% by Bekele *et al*. (2000) in Arsi, 4.2% by Bekele *et al*. (1989) in Ghibe and Gobe area, 2.15% by Shiferaw(1987) in central highland of Ethiopia, 1.92% by Asmare (2004) in Sidama, 0.61% by Tolosa (2004) in Jimma, 4.63% by Hilemelekot(2005) in Awi, West Gojam and North Gondar and 1.49% by Berhe (2005) in Tigray Region.

In Ethiopia, information about the seroprevalence of brucellosis in cattle slaughtered in various abattoirs is generally lacking. In this study, the finding of 1.13 % seroprevalence appears generally low compared to most previous reports.

Various factors including location, breed (genotype), production system, management, abattoir business and settings, apart from technical issues involved in the serological diagnosis of brucellosis, may influence the prevalence of brucellosis. Therefore, in the present circumstances it is difficult to compare and contrast our findings with other similar studies in the country. So, attempts were made to compare our findings with the field studies made in the various parts of the country.

Nevertheless, the individual level seroprevalence obtained in this study (1.13%) was found to be well comparable with the reports of 1.92% by Asmare (2004), 0.61% by Tolosa (2004), 0.69% by Tesfaye (2003), 0.14% by Yayeh (2003), 2.1% by Shiferaw (1987), 1.8% by Fekadu (1999) and 1.49 by Berhe (2005). Our finding is however lower than the seroprevalence of 4.63% by Hilemelekot (2005), 4.2% by Bekele *et al.* (1989), 4.9% by Bekele *et al.* (2000). On the other hand, our finding was far lower than the findings that have reported higher seroprevalence such as 38.7% by Rashid (1993), 22% by Sintaro (1994), 18.4% by Kibru (1985), 14.2% by Yirgu (1991), 12.34%by Abeje (1994) and 11.2% by Zewdu (1989). This variation may be due to the type of serological tests employed and the number of animals examined. These researchers used RBT and SAT for the diagnosis of brucellosis and these tests are known to be less specific than CFT. These two factors could have contributed for the higher seroprevalence obtained in those studies.

5.2. Abattoir Level Seroprevalence in Cattle

The findings showed that the seroprevalence of the two abattoirs was 1.19% and 1.08% for the Addis Ababa and Sululta abattoirs respectively. There was slight difference of seropositivity between the two abattoirs but the difference was not statistically significant ($p = 0.844$).

This finding was quite comparable with and agreed to the reports of 1.92% by Asmare (2004) in Sidama Zone, 2.15% by Shiferaw (1987) in the central highland of Ethiopia, 0.61% by Tolosa (2004) in Jimma Zone, 1.85% by Fekadu (1999) in Amhara region, 0.14% by Yayeh (2003) in North Gondar, 0.69% by Tesfaye (2003) in Tigray Region and 1.49% by Berhe (2005) in Tigray Region.

But our finding differed from the reports with moderate to high prevalence by Asfaw *et al.* (1998), Molla (1989), Hadgu (1987), Shiferaw (1994), Sintaro (1994), Hilemelekot (2005), Rashid (1993), and others.

5.3. Risk Factors in Cattle

5.3.1. Age

In the present study, the overall prevalence of *Brucella* antibodies in the two age categories was 1.09% and 1.14% for the age category 6 months – 5 years and above 5 years respectively. Although there was no significant variation in seropositivity between the two age categories, the proportion of seroreactor cattle has increased with the increase in age. This finding was consistent with the report by Hilemelekot (2005) in the ranch and extensive production systems, in which significant difference of seropositivity was not observed among the three age categories.

Our finding was also in agreement with the result of Sintaro (1994) where by higher proportion of older cattle were infected than younger ones but the difference was not statistically significant. The result of this study was in variance with the report by Asfaw *et al* (1998) where by older cattle were twice more likely affected (4%) than younger ones (1.9%) and the variation was statistically significant.

Asmare (2004) had also reported that 97.87% of seroteactors were detected in the age group of above 2 years with statistically significant difference. Tolosa (2004) and Berhe (2005) had also reported that significantly higher seroprevalence of *Brucella* antibodies was found in mature cattle than immature ones.

In the present finding, about 82.4% of seroreactor cattle were in the age category of above 5 years old. This finding was in agreement with the report by Kubuafor *et al.* (2000) that older animals above 5 years of age are destined for slaughter resulting a potential occupational risk for abattoir workers.

5.3.2. Sex

In this study, highly significant association between sex and seropositivity was observed. The overall seroprevalence of *Brucella* antibodies in male and female was 0.84% and 3.05% respectively. Chi square analysis revealed strong association between sex and seropositivity with p value of 0.011 and OR of 3.7. This finding agreed with the report by Hilemelekot (2005) in which significant difference was observed between male (0.92%) and female (5.97%) with p value of 0.042. Our finding differed from the result of Asfaw *et al* (1998) in that female cattle were more infected (8%) than male cattle (0.11%), however, the difference was not statistically significant and this could be due to small number of males tested (10 males vs. 940 females). Tolosa (2004) reported that all male cattle tested were negative. According to Asmare (2004), 10 bulls in the intensive management were negative against *Brucella* antibodies, whereas in the extensive management system from 25 bulls examined, 1 positive reactor male was obtained (4%).

The sex difference of seropositivity is more significant in the Addis Ababa abattoir than in Sululta abattoir. In the Addis Ababa abattoir, the prevalence of antibodies of *Brucella* was 5.48% and 0.73% for female and male cattle respectively. This difference was statistically significant ($p = 0.002$) and with OR of 8. The implication of this is that the probability of slaughtering of infected female is 8 times more than that of males.

This finding was in agreement with the result in Eritrea by Omer *et al.* (2000) whereby seroprevalence was 8.5% and 1.61% for female and male cattle respectively. But our finding was in variance with the report by Kubuafor *et al.* (2000) in which the prevalence of *Brucella* antibodies was 8.5% (11/ 129) in cows and 1.9%(1/54) in bulls and the difference was not statistically significant.

5.3.3. Genotype (Breed)

Consensus has not been reached on the issue of breed susceptibility to brucellosis infection in cattle. In the present study, an attempt was made to compare the status of brucellosis in the local and crossbred cattle slaughtered in the study period. In our study, chi square analysis revealed significant difference in seropositivity between the two genotypes. The prevalence of *Brucella* antibodies of the two genotypes was 0.93% and 3.88% for the local and crossbred cattle respectively. This genotype difference in susceptibility was statistically significant with P value of 0.012 and OR of 4.31.

The genotype difference of seropositivity in the Addis Ababa abattoir was more significant than that of the Sululta abattoir. The seroprevalence of the two genotypes in Addis Ababa abattoir was 0.74% and 4.82% for the local and crossbred cattle respectively and the difference was statistically significant with P value of 0.005 and OR of 6.8. In Ethiopia, higher prevalence rates of 8.11% by Asfaw *et al.* (1998), 22% by Sintaro (1994), 22.86% by Shiferaw (1994), 5.88% by Hadgu (1987), 5.7% by Zewdu (1989) and as high as 38.7% by Rashid (1993) were recorded in crossbred cattle.

The present finding regarding genotype difference in Seropositivity was also in agreement with the result reported by Jiwa *et al.* (1996) in Victoria Province of Tanzania. In this study, four breeds of cattle were compared for susceptibility against brucellosis and significant variation in seropositivity was observed among Tanganyika shorthorn zebu (TSZ), Grade animals (zebu x exotic), Mpwapwa (MPW) and Exotic animals ($p < 0.001$).

On the other hand, our finding differed from the report by Hilemelekot (2005) in Andasa cattle breeding and multiplication center, where comparison was made between the seroprevalence of two breeds. The reported seroprevalence was 2.22% (1/45) and 3.91% (11/281) for crossbred and local cattle without significant difference in seropositivity between the two breeds.

Our finding was also not consistent with the result reported by Kubuafor *et al* (2000) in a study made on three cattle breeds in Ghana. The prevalence of brucellosis in Sanga, West African Shorthorn and White Fulani was 7.0%, 3.2% and 11.1% respectively but significant difference was not observed among the three breeds of cattle.

Madsen (1989) stated that variation of susceptibility to *Brucella* infection among the various breeds of cattle has not been reported. Similarly, Radostits *et al* (2000) indicated the absence of specific breed resistance against brucellosis and all breeds of cattle appear to be comparable in susceptibility. Nevertheless, from the present finding, it can be concluded that slaughtering of crossbred cattle in the Addis Ababa abattoir may be of potential occupational hazard for the abattoir workers, meat inspectors and butchers.

5.4. Study Finding in Abattoir Workers and the Associated Risk Factors

In the present study, an attempt was made to assess the occupational hazard posed by brucellosis to abattoir workers. Blood samples were collected from 67 workers in Sululta abattoir and subjected to RBPT but none of them were positive against *Brucella* antibodies. Tolosa (2004) reported that seroprevalence of 2.4% was obtained after testing 126 sera collected from occupational risk groups. In this study, 38 abattoir workers and 25 butchers were included but all of them were found to be negative.

Kubuafor *et al* (2000) in Akwapim- South District of Ghana tested blood samples collected from 44 people in high-risk groups and 30 people from control groups but none of them tested positive for the presence of antibodies against *Brucella*.

Based on the questionnaire survey and assessment of potential risk factors, it was evident that the majority of the abattoir workers experience frequent skin abrasion due to accidental cutting of hands, and also have the habit of consuming raw meat, milk and milk products such as cheese and yoghurt. Additionally, few had any knowledge of brucellosis.

6. CONCLUSIONS AND RECOMMENDATIONS

In the present study, relatively low numbers of seroreactor cattle were identified in both Addis Ababa and Sululta abattoirs without significant difference between them.

Sex and genotype (breed) of slaughtered cattle were found to be important risk factors associated with the occurrence of *Brucella* seropositivity in the abattoirs. Thus, slaughtering of female and crossbred cattle could impose potential occupational hazard to the abattoir workers.

In this study, the status of brucellosis among workers in Sululta abattoir was negative at least for those involved in the study. As seropositive cattle were identified from both abattoirs, brucellosis remains to be a potential hazard for the workers.Due to lack of cooperation and willingness for participation, it was not possible to assess the status of human brucellosis among abattoir workers in the Addis Ababa abattoir.

From the questionnaire answers, the low level of educational status of the workers, consumption of raw meat, frequent cutting of hands, lack of awareness about the disease brucellosis and absence of habit to use gloves or other protective dressings, especially in time of accident, were identified as potential risks which could create favorable condition for the entry and establishment of brucellosis.

Based on the above findings and conclusions, the following recommendations are forwarded:

1. Based on the interview findings, as almost none of the workers knew about brucellosis, awareness creation about the occupational hazard and zoonotic impact of brucellosis among abattoir workers and the public at large should be implemented.

2. Adoption of basic hygienic measures and the use of protective devices especially during slaughtering of female and crossbred cattle will avoid the risk of exposure and infection.

3. Animal identification with permanent numbers, registration and control of livestock movements nationwide using the recording system should be legalized and implemented by a decree in the country, as this is of paramount importance for the trace back of infected animals identified in abattoirs to their place of origin.

4. Further studies should be undertaken in other abattoirs, especially in export-oriented abattoirs, for the better understanding of bovine brucellosis in slaughtered cattle and its zoonotic impact to abattoir workers.

5. More generally, in places and situations where the prevalence of brucellosis is high, control programs against the disease through vaccination of females could bean important step in protecting the public at large and abattoir workers in particular from brucellosis.

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8. ANNEXES

Annex 1 Complement Fixation Test Procedure

Materials required for CFT

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

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

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

II. Amboceptor's (Hemolysin) titration:

1. Prepare 1:500 dilutions up to 1:8000.
    - 5 test tubes were prepared
    - 1m of VBD was added to test tubes 2-5

- 10μl amboceptor's was mixed with 4990μl VBD in the first tube
- 1ml was transferred from the first to the second up to the last tube and 1ml was discarded.

2. 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. The tubes were put in order of ascending dilution.

3. 0.5ml was transferred from each of the above test tubes to a second set of 10 tubes, starting from the 1:12,000 dilutions.
4. 1ml of VBD was added to each of the test tubes
5. 0.5ml of 2% sheep red blood cell was added to each of the test tubes and shacked well.
6. The test tubes were left on the bench for 10minutes.
7. 1ml of complement at working dilution was added.
8. The tubes were incubated for 30minutes in a water bath at 37^0 C
9. The last tube showing complete hemolysis, minimum hemolytic dose (MHD) was read and recorded. The working dilution of amboceptor's was four times the MHD.

III. Evaluation of complement.

Freeze dried complement was reconstituted according to its instruction.

A 1:100 complement was prepared

1) Complement was added into the 9 tubes increasing by 5 μl every time, starting with 10μl.
2) Diluent was added in to the 9 tubes in decreasing amount by 5μl, starting with 40μl.
3) 25μl of diluents was added into the tubes with the corn well syringe.
4) The test tubes were placed in a 37^0C water bath for 1hr.
5) 25μl of 2% sheep red blood cells was added in all tubes
6) 25μl of amboceptor's at working dilution 1:1000 was added in all tubes.

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

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

V. Titration of antigen

Micro titer plate I

a) 25 µl of VBD was added in all cups (wells).
b) 25µl prediluted antigen was added to all cups of row A.
c) By serial doubling (two fold) dilution, 25 µl of antigen was transferred from row A to B, and again from row B to C, etc, until row G by multi-channel pipette. 25-µl mixture was discarded from row G (row H contained only the diluent).

Micro titer plate II

a) 50 µl of VBD was added to all cups
b) 50 µl of prediluted (1:2.5) inactivated positive control serum was added to all wells of column 1.
c) 50 µl was serially transferred by two fold dilution, from column 1 to 2 and again from column 2, 3 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.
- 50 µl of complement in 1:40 dilution was added to all cups of plate I
- Plate I was kept in a refrigerator, covered with second empty plate (cold fixation)

- The following day, 50 μl of 2% sheep red blood cells, amboceptor's premixture, equal volume, i.e. 25 μl of sheep red blood cells and 25 μl of a 1:100 working dilution of amboceptor's, were added to all cups.
- The plate was covered with sealing tape, shaken well and kept in water bath at 37^0C for 30 minutes.
- The last cup with 50% sedimentation was read and recorded. The highest dilution of antigen with 50% sedimentation was the limiting antigen concentration or the right corner value.

The test proper, multiple sera technique

- The sera were predidiluted to 1: 2.5 and incubated at 58^0C in a water bath for 30 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
- 25μl of VBD was added to all wells except those of the first.
- Serial doubling dilution was 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 was added to all wells excluding those of the anti-complementary controls, which was received 25μl VBD instead.
- 25 μl of complement at working dilution (1.25) was added to all wells except control well.
- Control wells contained serum+ complement+ diluent, antigen control had antigen + complement + diluent, complement control has complement + diluent and hemolytic system had diluent, set up to 75μl total volume in each case before hemolytic system was added.
- The plates were sealed and incubated at 37^0C for 30minutes with agitation
- 25μl of sheep red blood cell suspension was added to each well.
- The plates were sealed and reincubated at 37^0C for 30 minutes with agitation. Before reading the result, the plates were left in the refrigerator at + 4^0C for 1 hour in order to allow none lysed cells to settle.

- Finally, the plate was taken out from refrigerator and left at room temperature for 10 minutes. Positive reactions were indicated by sedimentation of sheep red blood cell and absence of hemolysis. Negative reactions were indicated by heamolysis of sheep red blood cell.

Interpretation

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

Annex 2 Subject consent form

Participant's Name____________________

Sex____________________ Age________

Study No____________________

Date____________________

Researcher's /Physician's name ____________________

Abattoir's name____________________

The aim of this study is to investigate the status of human brucellosis in people working in Addis Ababa and Sululta abattoirs.

Human brucellosis is an infectious bacterial disease caused by the genus *Brucella*.The disease is septicemic with sudden or insidious onset, and accompanied by continued, intermittent or irregular fever. Chills, headache, arthralgia, profuse sweating, weakness,

insomnia, general malaise, sexual impotence irritation, nervousness and depression characterize the disease.

Human brucellosis is, for the most part, an occupational disease of stockyard and abattoir workers, workers, butchers and veterinarians. The disease is usually contracted by handling fetuses and afterbirth, or by contact with vaginal secretions, excreta and carcasses of infected animals.

In Ethiopia, researches made on the status of bovine brucellosis in cattle slaughtered abattoirs and its impact on abattoir workers is very few. Protecting abattoir workers against brucellosis is particularly important because they constitute the occupational group at highest risk.

For this reason, the Addis Ababa University, Faculty of Veterinary Medicine Research and Graduate Program wants to study the status of bovine brucellosis in cattle that are slaughtered in Addis Ababa and Sululta abattoirs with focus on occupational hazard.

The subjects in this study are abattoir workers who are believed to be occupationally exposed to the disease. The signs and symptoms of brucellosis in humans strongly mimic other febrile diseases such as malaria and typhoid, and thus brucellosis is often misdiagnosed. Thus, identification of the presence of the disease through serological tests will help prevent subjects suffering from further damages and complications which otherwise would be misdiagnosed and not properly treated.

Therefore, to carryout this research, you are asked for a good will to participate. While studying, the researcher will ask questions related to the disease. And about 5 ml blood specimens will be drawn from you for the diagnosis of the disease. For safety purposes, a qualified laboratory technician will take the blood with sterile disposable syringes and needles. There will be no harm to you except a minor bruise from vein puncture.

If positive cases are found, the researcher will advice and encourage these individuals to pursue medical treatment. All study related information will be kept confidential by the researcher and will not be given to others without your permission. The information that will be obtained from this study can be used as a baseline data for further studies in other parts of the country.

Taking part in this study will:

1. Enable you to know your status about the disease and help you to undertake immediate medical treatment;
2. Safe your time and money for laboratory diagnosis;
3. Make you contribute your part for the countrywide study of the disease and to formulate control and prevention schemes.

I have understood all that have been explained and I agree to participate in this study.

Participant

Name________________________ Signature________________________

Researcher's/Physician's

Name________________________ Signature________________________

Annex 3 Questionnaire survey format

ADDIS ABABA UNVERSITY

FACULTY OF VETERINARY MEDICINE

Questionnaire Survey for Investigation of Brucellosis in Abattoir Workers

1. Purpose of the questionnaire

The purpose of this questionnaire survey is to study the status of brucellosis in human working in Addis Ababa and Sululta abattoirs through investigation of the signs and symptoms associated with human brucellosis. And to assess risk factors for infection.

2. Consent of the respondent

Understanding the above-mentioned purpose of this questionnaire, are you willing to participate in this study? 1. Yes 2. No

3. Name of the abattoir ____________________

4. Respondent's Name ____________________

Sex ____________________. Age ____________________

5. Working area……………………….

6. Service year………………………..

7. Educational status 1. Illiterate 2. Elementary school
3. Secondary school 4. Higher education

8. Marital status 1. Single 2. Married

9. Family size ______________________

10. Wife's (husband's) occupation 1. Livestock rearing 2. Other

11. Do you have domestic animals? 1. Yes 2. No

12. If yes, which animal do you possess?

Sheep	1. Yes	2. No
Goat	1. Yes	2. No
Cattle	1. Yes	2. No
Equine	1. Yes	2. No
Dog	1. Yes	2. No

13. Do the animals have their own shelter? 1. Yes 2. No

14. Are the animals kept indoor? 1. Yes 2. No

15. Is there any animal that graze outside? 1. Yes 2. No

16. Do your animal make close contact with other animals? 1. Yes 2. No

17. Is there any problem during parturition? 1. Yes 2. No

18. If yes please mention the problem ______________________

19. With regard to the relationship with livestock, which activity do you perform?

Cleaning of livestock	1. Yes	2. No
Removing of dung	1. Yes	2. No
Milking cows	1. Yes	2. No
Assisting parturition	1. Yes	2. No
Slaughtering	1. Yes	2. No

Butter and cheese making 1. Yes 2. No

20. Do you consume raw meat? 1. Yes 2. No

21. Do you consume raw milk? 1. Yes 2. No

22. Do you consume cheese and yoghurt? 1. Yes 2. No

23. Do you accidentally cut your finger or hand at work? 1. Yes 2. No

24.If yes, frequency of cutting is:

1. Seldom 2. Once in a month

3. Once in a week 3. Twice and above in a week

25. Do you use gloves for protection? 1. Yes 2. No

26. Have you visited health institutions during the last 6 months?

1. Yes 2. No

27. If yes, what was the health problem? ____________________

28. What was the diagnosed disease? ____________________

29. Have you ever-experienced prolonged intermittent fever? 1. Yes 2. No

30. If yes, for how long did the fever continued? ____________________

31. Have you ever observed one of the following disease signs and symptoms?

S.No	Signs and symptoms	1. Yes	2. No
1	Headache		
2	Insomnia		
3	Back pain		
4	Prolonged intermittent fever		
5	Chills		
6	Profuse sweating		
7	Vague general pain		
8	Joint pain		
9	Pain in the testes		
10	Weakness		
11	Nervous disorder		
12	Depression		
13	Irritation		
14	Chest pain		
15	Coughing		

32. Are you aware of animal diseases that are transmitted to humans through handling of the carcasses of infected animals? 1. Yes 2. No

33. If yes, mention some

1 ____________________

2 ____________________

3 ____________________

4 ____________________

34. Do you know the disease brucellosis? 1. Yes 2. No

35. If yes, tell us some of the symptoms manifested by diseased animals.

1____________________

2____________________

3____________________

4____________________

36. Do you know how humans are infected by the disease brucellosis?____________

37. Do you know how the disease can be controlled and prevented in humans?__________

Thank you for your participation.

Filled by Name____________

Signature______________

Annex 4 Body condition scoring

A) For cross breed cattle

Lean

L_1 (0) - Animals are emaciated with spinous process, hipbones, tail head and ribs projected prominently. No fatty tissue can be detected; neural spine and transverse processes feel sharp.

L_2 (1) - Individual spinous processes are still sharp to the touch and there is no fat around tail, hipbones, tail head and ribs are still prominent, but appear less obvious.

Medium

M_1 (2) - Spinous processes can be identified individually when touched, but feel rounder rather than sharp. There is some tissue cover round the tail, over hip bones and flank. Individual ribs are no longer visually obvious.

M_2 (3) - Spinous processes can only be felt with firm pressure. Areas on either side on the tail head now have a degree of ft cover which can be easily felt.

Fat

F_1 (4) - Fat cover around tail head is evident as slight rounds soft to touch, spinous processes can not be felt even with firm pressure and folds of fat are beginning to develop over the ribs and thigh of the animal.

F_2 (5)- Bone structure is no longer noticeable and the animal presents a blocky appearance. Tail head and hipbone are almost completely buried in fatty tissue and folds of fat are apparent over ribs and thigh. Spinous processes are completely covered by fat and animal's mobility is impaired by large amount fat carried.

B) For local cattle

Lean

L_1 – Marked emaciation- the animal could be condemned ante mortem.

L_2 - Transverse processes project prominently, spines appear sharply.

L_3 - Individual dorsal spines are pointed to the touch, hips, tail head and ribs are prominent.

Medium

M_1- Ribs, hips and pins are clearly visible, muscle mass between hooks and pins are slightly concave

M_2 - Ribs are usually visible, little fat cover and dorsal spines are barely visible

M_3 - The animal is smooth, dorsal spines cannot be seen, but are easily felt.

Fat

F_1 - Animal is smooth and well covered but fat deposits are not marked

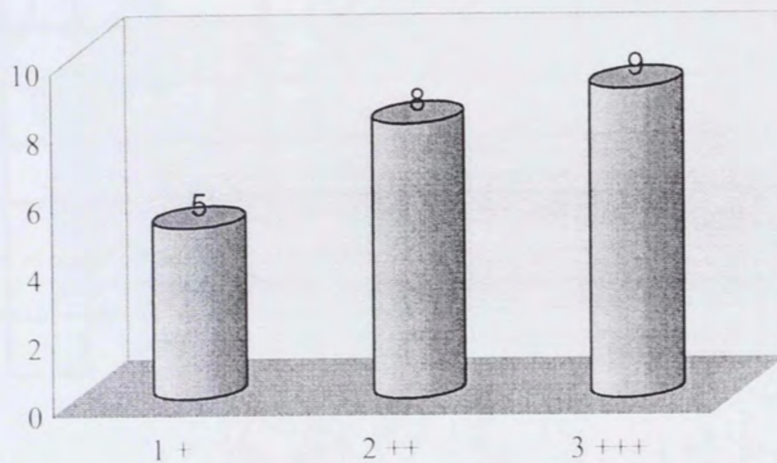
F_2 - Fat cover in critical areas can easily be seen and felt; transverse processes cannot be seen or felt.

F_3 - Heavy deposits of fat are clearly visible on the tail head, brisket, dorsal spines, ribs and hooks.

Annex 5 The proportion of CFT titers of *Brucella* sero-positive sera in cattle

CFT titer	ICFTU	Number of positive sera	Proportion (%)
1:10	50	6	35.3
1: 20	100	5	29.4
1: 40	200	4	23.5
1: 160	800	1	5.9
1:320	1600	1	5.9
Total		17	100.0

Annex 6 The degrees of serum agglutination in Rose Bengal Plate Test



Annex 7 Questionnaire format for blood sampling for cattle

Code	Sex	Age	Breed	Body condition	Origin	Name of abattoir	Tests		Remark
							RBPT	CFT	

9. CURRICULUM VITAE

1. Personal

Name	Mulugeta Tefera Yigeremu
Nationality	Ethiopian
Sex	Male
Date of birth	14 /09/ 1969 G.C.
Place of birth	Harar
Marital status	Married
No. of children	2

2. Education

2004 – 2006: Addis Ababa University, Faculty of Veterinary Medicine, debre Zeit, Ethiopia. MSc Degree in Tropical Veterinary Medicine.
Thesis Title: Study on Bovine Brucellosis in Cattle Slaughtered at Addis Ababa and Sululta Abattoirs with Focus on Occupational Hazard.

1988 –1993: Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit , Ethiopia. Degree of Doctor of Veterinary Medicine (DVM).
Thesis title: Prevalence and Economic Significance of Bovine Fascioliasis at the Sopral Kombolcha Meat Factory, South Wello, Ethiopia.

1985 – 1987: Secondary school and Ethiopian School Leaving Certificate Examination (ESLCE), Jijiga Senior Secondary School, Jijiga, Ethiopia.

1983 – 1984: Junior Secondary School, Jijiga, Ethiopia.

1977 – 1983: Elementary School, Weizero Yeshimebet School, Harar, Ethiopia.

3. Work Experience

2004 – 2006: 2 years as MSc student at Addis Ababa University, Faculty of Veterinary Meidcine.

1994 – 2004: 10 years as head of veterinary health services section of Soro Wereda Bureau of Agriculture, Hadiya Zone, SNNPRG. Responsibilities include planning and coordinating veterinary clinical and field activities and research works.

1999 – 2002: 3 years as partner with FARM Africa in the implementation of Dairy Goat Development Project in Soro Wereda, being responsible for selection of beneficiaries, purchasing and distributing local female goats, follow up of the health of goats, training of Community Animal Health Workers (paravets) and supervision of the activities of paravets.

1992 – 1993: one year, Kombolcha Zonal Veterinary Laboratory and Sopral Meat Factory, in veterinary clinical and loboratory works as well as meat inspection (External student).

4. Paper writings

1. " Economic Significance of Bovine fascioliasis in Ethiopia". A Review, May 1992, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

2. "Prevalence and Economic Significance of Bovine Fascioliasis at Sopral Kombolch Meat Factory, South Wello, Ethiopia". DVM thesis, June 1993, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

3. "Study on Bovine Brucellosis in Cattle Slaughtered at Addis Ababa and Sululta Abattoirs with Focus on Occupational Hazard, Ethiopia. ". MSc thesis, June 2006, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

5. Workshops and trainings attended

9 – 11 October 2000: Tsetse flies and trypanosomoses control and eradication, Southern Region Bureau of Agriculture, Yirgalem, Ethiopia.

21 – 25 August 2000: Advanced level Training Course on Goat health snd Management, FARM Africa Dairy Goat Development Project, Debre Zeit, Ethiopia.

28 Feb–1 March 2000: Introduction to the National Livestock Development Project, Hadiya Zone Bureau of Agriculture, Hossana, Ethiopia.

10 – 14 May 1999: Basic Introductory Course in Goat Production Imprevement under smallholder Farming, FARM Africa, Awassa, Ethiopia.

10 – 14 Feb. 1998: Tsetse flies and trypanosomiasis in Southern Ethiopia Region, Soddo Zonal Veterinary Laboratory, Soddo, Ethiopia.

27 – 30 Aug. 1996: Privatization of Veterinary Services Delivery, PARC, Debre Zeit, Ethiopia.

6. Languages

1. Amharic: Speaking writing and reading
2. English: Speaking writing and reading

3. Oromifa: Speaking and reading

4. Some Somali and Hadiya languages.

7. Additional Knowledge: Literate in computer manipulation.

8. Membership

Member of the Ethiopian Veterinary Association (EVA).

9. Personal References

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Mob. 0911052177

10. SIGNED DECLARATION SHEET

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

Name: Mulugeta Tefera Yigeremu

Signature: ________________

Date of submission: 19/06/2006

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

Professor Tesfu Kassa (DVM, FRVCS, MVSC) ________________

Dr. Kelay Belihu (DVM, PhD) ________________

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AUTHOR Mulugeta Tefera
TITLE Study On Bovine Brucell

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