

**ADDIS ABABA UNIVERSITY
SCHOOL OF VETERINARY MEDICINE**

**SEROLOGICAL AND CULTURAL DETECTION OF *BRUCELLA* INFECTION IN
CAMEL AND ITS PUBLIC HEALTH SIGNIFICANCE IN TWO SELECTED
DISTRICTS OF AFAR REGION, ETHIOPIA**

**BY
SISAY WELDEGEBRIEL ZEWOLD**

**JUNE 2011
DEBRE-ZEIT, ETHIOPIA**

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SISAY WELDEGEBRIEL ZEWOLO

**A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in
Partial Fulfilment of the Requirements for the Degree of Master of Science in Veterinary
Public Health**

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SISAY WELDEGEBRIEL ZEWOLD

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LIST OF ABBREVIATIONS

%	percent
&	and
+ ve	positive
CFT	Complement Fixation Test
CI	Confidence Intervals
CO ₂	Carbon dioxide
CSA	Central Statistics Agency
EDTA	Ethylene Diamine Tetra Acetic acid
ELISA	Enzyme-linked Immunosorbent Assay
H ₂ O ₂	Hydrogen Peroxide
H ₂ S	Hydrogen Sulphide
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
km ²	Square Kilometre
ml	millilitre
MoARD	Ministry of Agriculture and Rural Development
NB	Nota bene (Latin). notice well
NGO	Non-governmental Organization
No	Number
°C	Degree Celsius
OR	Odds Ratio
PA(s)	Peasant Association (s)
P-value	Probability of failure to detect positives
RBC	Red Blood Cell
RBPT	Rose Bengal Plate Test
RFM	Retained Fetal Membrane
SPSS	Statistical Package for the Social Sciences
v/v	Volume to Volume ratio
WHO	World Health Organization
µl	Micro litre (s)
χ ²	Chi-squared

ABSTRACT

A cross-sectional study was delineated to investigate the prevalence and risk factors of camel and human brucellosis in south Afar region, northeast Ethiopia. The study was carried out in two conveniently selected camel-rearing districts of Afar, Awash-Fentale and Amibara, in the period from October 2010 to May 2011. A total of 768 camel blood sera were randomly collected from eight pastoral and agro-pastoral residences of both districts, two PAs from Awash-Fentale and six from Amibara woreda, and human sera were collected from 200 purposely selected clinical patients in two health centers of the districts. The study with human subjects was performed according to the guidelines of the declaration of National Health Research Ethics Guideline of the Ethiopian Science and Technology Commission and consent was obtained from the institutional review board office of Addis Ababa University College of Health Sciences, Medical Faculty. Two serological tests, RBPT and CFT were used to screen all serum samples at the National Veterinary Institute (NVI) at Debre-Zeit. Descriptive statistics was used to depict the results in percentages and cross-tabulations and multivariate logistic regression was constructed to study the risk factors associated with *Brucella* sero-positive cases of humans and camels. The overall prevalence of camel brucellosis in the districts investigated was 11.9% by RBPT and 7.6% by CFT and the overall prevalence of human brucellosis was 16% by RBPT and 15% by CFT. Milk samples were collected from 34 (69.4%) sero-positive milking-camels to attempt cultural detection and identification of the *Brucella* organism and the isolation trial was made in the microbiology laboratory of School of Veterinary Medicine, Debre-Zeit, but the target organism was not successfully identified through the bacteriological approaches conducted. Structured questionnaires were administered to camel herdsmen to collect information on camel health and management practices and to human clinical patients to identify potential risk factors associated with the occurrence of human brucellosis. Multivariate logistic regression on animal level showed that age of the camels ($\chi^2 = 4.98$, $P = 0.026$), herd size ($\chi^2 = 8.47$, $P = 0.004$), contact or rearing with ruminants ($\chi^2 = 34.74$, $P = 0.0001$), and parity ($\chi^2 = 11.30$, $P = 0.004$) were statistically significant and were the potential risk factors associated with camel brucellosis. That is, adults were 1.70 times ($P = 0.026$, OR = 0.70, 95% C.I. = 1.45-1.95) more at risk of acquiring the disease than young camels while old age camels were 1.52 times ($P = 0.026$, OR = 1.52, 95% C.I. = 1.24-1.72) more at risk than young camels. However, geographical location of the study districts ($\chi^2 = 0.80$, $P = 0.373$), residence area ($\chi^2 = 0.96$, $P = 0.328$), sex ($\chi^2 = 1.10$, $P = 0.295$) and the pastoral and agro-pastoral farming systems ($\chi^2 = 1.35$, $P = 0.245$) were not statistically significant and not

considered as risk factors for the occurrence of the disease in the camel population. The ownership of milking camels, living within the pastoral and agro-pastoral communities, keeping of livestock in close contact, consumption of raw camel milk and milk products, assisting animals during parturition and grooming livestock were potential risk factors associated with human brucellosis. However, there was no statistically significant difference was observed in the prevalence of human brucellosis among the origin of the patients ($\chi^2 = 2.05$, $P = 0.360$), among the age groups ($\chi^2 = 2.15$, $P = 0.3411$) and between the urban and rural society ($\chi^2 = 0.12$, $P = 0.662$). The results of the present investigation indicate that human and camel brucellosis is widely distributed in study districts of Afar and hence, the author recommended further studies need to be done on animal and human brucellosis to determine which measure should be followed. Brucellosis should be included in disease education programs and control measures to mitigate the economic losses and public health hazard caused by the disease.

Keywords: Afar region/Camel brucellosis/Complement fixation test/Cross-sectional study/Ethiopia/Human brucellosis/Prevalence/Risk factors/Rose Bengal plate test

1. INTRODUCTION

Camels (*Camelus dromedarius*, one humped camel, dromedary) are vital domestic animal species that are best adapted to harsh environments and fluctuating nutritional conditions of arid and extreme arid zones. These animals are endowed with extra ordinary features that enable them to survive and perform in such hard conditions (Teka, 1991). Dromedaries are versatile living assets that ensure food security even during the dry periods and also serve as means of transportation and draught power (Yagil, 1985; Higgins *et al.*, 1992).

Africa hosts 80% of the world population of dromedary (16.5 million) of which 63% attributed to east Africa (Wilson, 1998). Camels are a subset of huge livestock resources in Ethiopia with the population estimated to be over one million. This number ranks the country third in Africa after Somalia and the Sudan and fourth in the world (India included). The arid and semi-arid areas of the country that constitutes more than 60% of the total area and home of 7.8 million pastoral and agro-pastoral communities (Abebe, 2000) are suitable for camel production. The eastern and southern parts of the country, namely Afar, Somale and Borena are the major areas where camel husbandry is widely practiced. In these areas, the livelihood of the pastoral communities is certainly ensured by dromedaries (Teka, 1991; Wossene, 1991).

Camels inhabit almost all peripheral drier lowlands that generally fall below 1,500 metres above sea level in Ethiopia, except the western areas where high humidity and the incidence of trypanosomiasis are common (Teka 1991; Wilson, 1998). These areas include the major parts of the Somali and Afar National Regional States and some parts of the Oromia National Regional State. Because of the outstanding performance of camels in the arid and semi-arid areas of eastern lowlands of Ethiopia where browse and water are limited, pastoralists rely mainly on camels for their livelihood. In these areas, camels are mainly kept for milk production and produce milk for a longer period of time even during the dry season when milk from cattle is scarce. In most pastoralists, camel milk is always consumed either fresh or in varying degrees of sourness in the raw state without heat treatment thus, can pose a health hazard to the consumer (Bekele *et al.*, 2002).

In spite of its vital importance particularly to the marginalized communities in the dry zones of tropics and subtropics, studies on the camels are very few. Due to the fact that camel production is in remote, migratory and poor infrastructure condition, available studies were based on small animal number, one time survey, interviewing, questionnaires, estimation and simulations (Schwartz and Dioli, 1992). From the last two to three decades onwards, scientific interests in different aspects of dromedary have shown progress (Baumann and Zessin, 1992).

Published information on diseases reveals that camels may be either carriers or suffering from a vast array of infectious and parasitic diseases (Kohler-Rollefson *et al.*, 2001). Trypanosomiasis caused by *T. evansi* is found to be the major health problems in the Afar and Borena areas. Other diseases such as pox infections, contagious skin necrosis, pneumonia and parasitic infestations are also known to affect the health of the camels in the camel-rearing areas (Richard, 1979; Demeke, 1998).

Some of the diseases such as brucellosis have considerable public health importance as camel milk is consumed raw (Abbas *et al.*, 1987; Gameel *et al.*, 1993). Brucellosis was reported in camel from different countries of Africa and Asia (Abbas and Agab, 2002; Wernery and Kaaden, 2002). Brucellosis is a widespread disease in camel producing areas. The infection rate is higher in intensive camel production system where large animals are kept in a farm. In countries with more of extensive form of husbandry, the rate is low (Abbas and Agab, 2002).

Brucellosis in human represents a major public health hazard, which affects social and economic development in various countries. Groups at high risk for brucellosis are animal health workers, butchers, farmers, and those who habitually consume raw milk and come in contact with animals (Chukwu, 1987). In man, transmission occurs as a result of ingestion of milk, contact via skin abrasion, mucous membranes and inhalation (Radostits *et al.*, 2000; Seifert, 1996). Masoumi *et al.* (1992) recorded higher prevalence rate among butchers and people who habitually consume raw milk. Camel keepers consume camel milk as well as liver without heat treatment. This is even considered as delicacy (Gameel *et al.*, 1993). There is also a close contact between herdsman and the animal during watering, grooming, riding, nursing sick ones and delivery assistance (Abbas *et al.*, 1987). The isolation of the two major pathogenic *Brucella* species: *B. melitensis* and *B. abortus*, from milk and other samples of camel origin (Gameel *et al.*, 1993; Agab *et al.*, 1994; Hamdy and Amin, 2002) clearly indicate the potential public health hazards of camel brucellosis.

The disease can generally cause significant loss of productivity in camels through late first calving age, long calving interval time, low herd fertility and comparatively low milk production (Wernery and Kaaden, 2002). Moreover, brucellosis in human represents a major public health hazard, which affects social and economic development in various countries. Camel milk as well as camel liver are consumed without heat treatment by camel keepers and even considered as delicacy (Gameel *et al.*, 1993). The isolation of *B. abortus* and *B. melitensis* (Radwan *et al.*, 1992; Gameel *et al.*, 1993; Agab *et al.*, 1994; Abou-Eisha, 2000) has certainly demonstrated the danger of camel milk to public health.

Previous serological surveys showed overall prevalence rates of 4.4% (Domenech, 1977), 5.5% (Richard, 1979) and 4.2% (Teshome *et al.*, 2003) in different camel-rearing areas of Ethiopia. Specifically in Borena area, Teshome and colleagues (2003) reported 4.2% where as Domenech (1977) reported 5.2% prevalence rate. A study on camel husbandry practice in eastern part of the country by Getahun and Kassa (2000) indicated abortion rates and stillbirths of 9% and 4.3%, respectively, for which brucellosis is more likely to be incriminated.

1.1 Scientific justifications

1.1.1 The impact of brucellosis on public health and livestock production

Ethiopia is introducing markets for exporting live animal and livestock products. Starting from 2007/08, privatized export abattoirs in central Ethiopia are exporting camel meat to the Middle East countries. The most important camel diseases endemic in rural areas include brucellosis, anthrax, tuberculosis, trypanosomosis and calf scour. The main factors of concern for the farmers of the camel-rearing areas are the low fertility of camels and the high mortality in young animals. Brucellosis is a major reproduction problem in Ethiopia. All the camel-rearing areas do not test for brucellosis regularly and even cannot afford to practice the system of culling positive animals despite their intimate companionship with their animals and lack of awareness of owners about the disease. Consequently, these animals become carriers of the *Brucella* organism and thus a potential source of infection which contributed to the significant health risks existent in these areas (Bekele, 2004). Food and water-borne illnesses are highly endemic throughout the country. The risk of acquiring brucellosis from direct animal contact is highest in rural areas where livestock is bred. However, the risk from contaminated meat and animal products is presented countrywide, including urban areas (Anonymous, 2003).

Brucellosis is prevalent in traditional pastoral communities of Ethiopia such as Afar, Borana and Hamar and the possible risk factors for brucellosis are living in close proximity of livestock, milking livestock and consumption of raw milk and milk products. Diagnosis can be improved by providing testing for brucellosis. Brucellosis should be included in disease education programs and control measures (Regassa *et al.*, 2009).

1.1.2 Significance of the study

The Ministry of Agriculture and Rural Development (MoARD) is currently encouraging the livestock sector to improve the production system and the bio-security of the animal rearing areas that is protecting introduction of new animals whose health status is unknown. Good farming practices and management are still problematic in the country as a whole and control measures, specific to brucellosis, are still far from the attention of all livestock rearing community and the prevalence of human and camel brucellosis is unknown in the pastoral and agro-pastoral livestock habitation areas. Moreover, available studies on camel brucellosis are so scanty and do not provide detail epidemiological information. In order to meet the government national policy, this study targets to measure the prevalence of brucellosis in the camel-rearing areas and consequently provide essential information for the authorities to control the zoonotic disease by means of vaccination and provide basis for future control, prevention and eradication programs in Ethiopia. Therefore, a survey on “serological and cultural detection of *brucella* infection in camel and its public health significance in selected districts of Afar region” was necessary.

1.2 Objectives

The present study was undertaken in the study areas with the following major objectives:

- To determine the seroprevalence of camel brucellosis and find out the potential risk factors associated with the occurrence of the disease in the camel herds of the pastoral and agro-pastoral farming systems.
- To study the prevalence of brucellosis in occupationally exposed human group.
- To assess the geographical distribution of camel brucellosis in relation to the type of farming systems of the study districts.
- To attempt the isolation of *Brucella* organism from raw camel milk.

2. LITERATURE REVIEW

Brucellosis is an infectious, contagious, and worldwide spread form of important anthroponozoonotic disease caused by bacteria of the genus *Brucella*. In animals, the disease primarily affects cattle, sheep, goats, camel, swine, and dogs, and is characterized by abortion or infertility and also affects people and other animal species (Ray and Steele, 1979). In human beings, the disease is characterized by intermittent fever, chills, sweating, headache, myalgia, arthralgia, and a diversity of nonspecific symptoms (Young and Corbel, 1989; Pal, 2007). In domestic animals, brucellosis has been commonly known as enzootic abortion or bovine contagious infection, epizootic abortion, infectious abortion, contagious abortion, sinking of calves, Bang's disease, and ram epididymitis. In the case of human brucellosis, it has been described by various names including undulant fever, Malta fever, Mediterranean fever, gastric fever, Mediterranean gastric fever, Gibraltar-Rock fever, Cyprus fever, Neapolitan fever, intermittent gastric fever or intermittent typhoid fever, pseudotyphus, febris typho-malariae, and fièvre sudorale (Ray and Steele, 1979; Pal, 2007).

2.1 Distribution of camels (*Camelus dromedarius*)

Camelid was probably among the last of major domestic species to be put to regular use by man. The most likely time of domestication is about 4000 years before present or slightly earlier. The presumed area of domestication is the southern Arabian Peninsula, probably the area of Yemen and Oman. From presumed center of domestication, dromedary has subsequently been distributed to almost the rest of the world (Schwartz and Dioli, 1992; Wilson, 1998).

Environmental, social and cultural factors have great influence on the distribution and production of camels. Arid and semi-arid zones of tropical and subtropical countries of Africa and Asia are found to be convenient ecology. The greatest cultural influences in recent distribution of camels was the advent of Islam, when Arabs spread their gospel, consolidating its ranges north and east wards in Asia, and along the Mediterranean littoral. There have been many attempts to introduce camels outside the "normal" ranges, in Brazil, Colombia, USA, Cuba, Spain, Italy and France.

Generally, there has been steady increase in camel population since about 1980s. However, decrease in numbers has been observed in some countries for instance, where oil is the principal commodity and the nomadic way of life is no longer the major one (Wilson, 1998). Eastern Africa is known to be the heartland for camel production as 80% and 63% of the Africa and world population, respectively produced in the region. Ethiopia ranks 4th in the world with total population estimated over one million (Table 1). Subsistence camel production is practiced in dry areas of Ethiopia that covers 61% to 65% of the total land area (Abebe, 2000). The eastern part of the country is considered as the heartland for camel production, which is the home of two- third of the nations camel population (Getahun and Bruckner, 2000).

Table 1: Camel population in some selected countries

Country	Number (,000)	Density (No per km ²)	Proportion to total national ruminants (%)
Djibouti	60	0.10	3.4
Egypt	170	0.16	5.8
Ethiopia	1030-1040	0.83	3.4
India	1100	0.33	0.4
Kenya	620-780	1.08	5.3
Niger	415	0.32	8.3
Saudi Arabia	165	0.00	14.9
Somalia	5800-63500	8.93	46.6
Sudan	2800-3100	0.99	11.1

Source: Wilson *et al.* (1990); Schwartz and Dioli (1992)

Afar region is the second important camel production area next to Borena rangeland of southern Ethiopia, the first and second being Somale. The Awash-Fentale district is the second important camel production areas of the Afar region next to Amibara woreda in the administrative zone 3 and the pastoral areas in zone 1 (Figure 1)

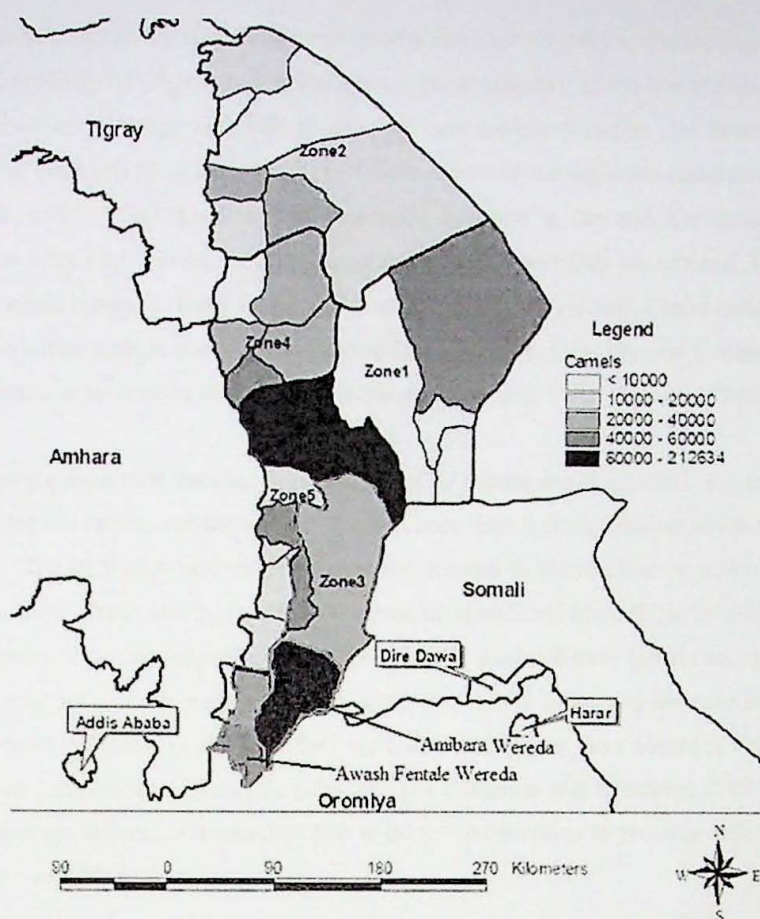


Figure 1: Camel population in camel rearing districts of Afar region

Source: MoARD, 2009

2.2 Potential importance of camels

Camels are primarily the domestic animals of pastoral communities that ensure food security. They produce milk, meat, hair and hides, and also serve as a draught animal for agriculture and transport people as well as goods (Schwartz and Dioli, 1992). Milk and meat are the important products that camels produce elsewhere. A study in eastern Ethiopia indicated 3 to 6 liters of daily milk yield over 13 to 15 months of lactation length (Getahun and Bruckner, 2000) while Tefera and Gebreab (2001) reported the average daily milk yield of 2.5 liters.

Long lactation and ability to maintain milk production over long dry spells are important facets of camel productivity. Apart from home consumption, majority of the households sell at least one-third of the produced milk sold to generate cash income (Getahun and Bruckner, 2000). Daily milk yield can be as high as 20 liters with improved management conditions (Schwartz and Dioli, 1992). Until the arrival of motorized transport in the arid and semi-arid zones, camels have been the sole means of transport in the areas where they are adapted. They are also used for wheel transport, water lifting and source of power for oil mill. Camel racing and other leisure activities such as camel safaris and trekking have recently become a tourist attraction and luxurious in some parts of the world (Schwartz and Dioli, 1992; Wilson, 1998).

From global perspective, the economic production of camels seems minimal. But the breaking-news is that the recent commencement of camel meat export from Ethiopia to the Middle East countries. The ELFORA modern slaughterhouse, located in Metehara town which is 170 kms far from Addis Ababa and the Hashim Ethiopian Livestock and Meat Exporter (HELIMEX) at Debre-Zeit have started slaughtering of camels brought from different camel-rearing districts of Afar, Somali and Oromia region. This is a good start to the country to increase the economic revenue from livestock. In Ethiopia, they are also the subset of huge livestock resource when considered from national economic point of view (Getahun and Bruckner, 2000). However, what makes the difference is its adaptation to harsh environments to produce milk from scanty and highly variable feed resources.

The most significant merits to perform in areas where other livestock species do not thrive and perhaps do not survive are attributed to the economic use of water in almost all metabolic functions and wide range of feed resource utilization (Yagil, 1985). In mixed species, the camel feeds on plants or part of plants that are not eaten by other conventional livestock due to its size to browse the highest strata, thus reducing competitions and enhancing complementarities (Ayan, 1984; Wilson *et al.*, 1990; Teka, 1991).

2.3 Constraints of camel production

Camels are produced by pastoral societies of the third world who dwell in dry marginal areas. Due to the fact that the production is usually a migratory system in remote areas with harsh living conditions and poor infrastructure, the animals are presumed to be inaccessible for

research. This affects the depth of our knowledge on the general aspects of camels (Schwartz and Dioli, 1992). Generally, there is negligence towards the promotion of camel health and production. It is only recently that the camel became the subject of more intensive and systematic interest (Baumann and Zessin, 1992). In Ethiopia until recently there has been very little systematic research and no development projects that feature the camel in any way.

Infectious and parasitic diseases are the primary constraints that hamper production. Trypanosomiasis is the most serious infectious disease of camels and widespread in camel rearing areas. Viral, bacterial, external parasites and helminthes are of clinical importance (Wossene, 1991; Higgins *et al.*, 1992; Köhler-Rollefson *et al.*, 2001). Trypanosomiasis caused by *T. evansi* is found to be the major health problems and other diseases such as pox infections, contagious skin necrosis; pneumonia parasitic infestations are the major diseases to affect the health of the camels in the camel-rearing areas (Richard, 1979; Demeke, 1998). Besides the prevailing diseases, camel keeping is relatively a recent event in Awash-Fentale and Amibara districts like the Borena pastoral system. Consequently, lack of appropriate traditional knowledge of husbandry practices, labor input requirement for its management and knowledge of its peculiar biology like inducible ovulation are perhaps the possible constraints of camel production in the area. Brucellosis is, therefore, one of the infectious diseases that affect both camels and camel owners (Wernery and Kaaden, 2002).

2.4 Camel brucellosis

2.4.1 Status of camel brucellosis in Ethiopia and other countries

Brucellosis is a widespread disease in camel producing areas. The infection rate is higher in intensive camel production system where large animals are kept in a farm. In countries with more of extensive form of husbandry, the rate is low (Wernery and Kaaden, 2002). Radwan *et al.* (1992) reported higher seroprevalence in camels raised under intensive management systems than those kept in backyards of houses in small groups in Saudi Arabia. Reports showed that the infection rates from camel-rearing areas of Ethiopia range from 4.2 to 5.5. Table 2 summarizes the prevalence rates of camel brucellosis in Ethiopia and different neighbouring countries.

Table 2: Prevalence status of brucellosis in Ethiopia and other countries

Country	Authors	Year	No. of camels tested	Prevalence (%)
Eritrea	Omer <i>et al.</i>	2000	98	3.1
	Azwai <i>et al.</i>	2001		3.5
Ethiopia	Domenech	1977	977	4.4
	Richard	1980	762	5.5
	Teshome <i>et al.</i>	2003	1442	4.2
	Bekele*	2004	3218	0.4-2.5
	Kebede*	2010	768	9.1
Kenya	Kagunya and Waiyaki	1978	174	4.6-10.3
	Waghela <i>et al.</i>	1978	172	14.0
	Wilson <i>et al.</i>	1990		6.0-38.0
Somalia	Ahmed and Ibrahim	1980	802	8.0-11.0
	Andreani <i>et al.</i>	1982	250	10.4
	Baumann <i>et al.</i>	1990		3.1
	Baumann and Zessin	1992	1039	0.3-1.9
	Bornstein	1984		8.5-11.5
	Elmi	1982	514	12.6
Sudan	Abbas <i>et al.</i>	1987	238	3.0
	Abudamir <i>et al.</i>	1984	740	4.9
	Agab <i>et al.</i>	1994	453	24.0
	Majid <i>et al.</i>	1999	805	13.9-43.9
	Osman and Adlam	1987	137	8.0

* Unpublished thesis papers

Source: Wernery and Kaaden, (2002) with slight modifications.

For comparison, the status of bovine brucellosis in the country varied with the type production systems and breed of cattle involved. Seroprevalence rates of 6.3% and 19.3% were reported from ranches of zebu cattle and mixed (Friesian and Zebu), respectively (Bekele *et al.*, 2000). Asfaw *et al.* (1998) recorded infection rate of 8.1% in dairy farms in and around Addis Ababa. Relatively low seroprevalence (1.8%) was reported from local cattle produced under extensive production system in northeastern Ethiopia (Kebede, 2000).

2.4.2 Epidemiology of camel brucellosis

i. Aetiology

There are six species that has so far been known in the genus *Brucella*: *B. abortus*, *B. melitensis*, *B. canis*, *B. suis*, *B. ovis* and *B. neotome* (Corbel and Morgan, 1984; Radostits *et al.*, 2000). But recently new species were discovered, in marine mammals (*B. pinnipedialis* and *B. ceti*), in the common vole *Microtus arvalis* (*B. microti*), and even in a breast implant (*B. inopinata*). However, the new taxonomy has named all *Brucella* species *Brucella melitensis*. They include *Brucella melitensis* 16M and 5 other biovars: *abortus*, *canis*, *neotomae*, *ovis*, and *suis* (Ray and Steele, 1979).

Brucellae are small, coccobacilli or short rod (measuring 0.5×0.7 to $0.6 \times 1.5\mu\text{m}$) occurring singly, in pairs or short chains. They are non-spore forming, non-motile, partially acid fast and gram-negative facultative intracellular bacteria. With modified Ziehl-Neelsen staining (0.5% acetic acid), *Brucella* appears as red staining coccobacilli (Quinn *et al.*, 2004). *Brucella* organisms are partially acid fast in that they are not decolorized by 0.5% acetic acid in modified Zeihl-Nelsons stain and thus appear pink in a blue background. Most strains are aerobic (some are micro-aerophilic) but many of them are carboxyphilic (capnophilic) and best grow in CO_2 enriched atmosphere. Growth is unlikely on an ordinary media. They are catalase and oxidase positive, produce H_2S , hydrolyse urea and reduce nitrate with some exceptions. The organisms neither produce indole, acetyl methyl carbinol nor utilize citrate (Corbel, 1990; Quinn *et al.*, 2004).

Brucellae are generally susceptible to heat, direct sun light, acidic conditions and common disinfectants (Radostits *et al.*, 2000). However, in favourable conditions the organisms may survive 4 to 6 days in urine, 6 weeks in dust, 4 to 10 weeks in water, 40 to 75 days in aborted fetus (Corbel, 1990). They also survive the production process of soft cheese up to 6 months, in butter up to 4 months, in milk up to 6 months and ice cream up to 30 days (Seifert, 1996). Variants of smooth colony are more virulent than non-smooth ones. This suggests the role of the O-chain of smooth lipopolysaccharide (LPS) in determining virulence. The A and M dominant surface antigens are also found in varying concentration among different smooth variants (Walker, 1999).

The disease in dromedary can be caused by *B. abortus*, *B. melitensis* and *B. ovis* (Seifert, 1996). Different study results showed that *B. abortus* and *B. melitensis* are the most frequent isolates (Table 3) (Radwan *et al.*, 1992; Gameel *et al.*, 1993; Agab *et al.*, 1994; Abou-Eisha, 2000; Hamdy and Amin, 2002). *Brucella melitensis* and *B. abortus* are capable of infecting a wide range of hosts including man (Walker, 1999).

Table 3: Summary of *Brucella* isolates from camels in different countries

Country	Authors	Organs or specimens	Species isolated
Egypt	Abou-Eisha (2000)	Milk	<i>B. melitensis</i> , biovar 3
Iran	Zowghi and Ebadi (1988)	Lymph node	<i>B. melitensis</i> , biovar 1
Kuwait	Zowghi and Ebadi (1988)	Lymph node	<i>B. melitensis</i> , biovar 3
	Al-Khalaf and El-Khaladi (1989)	Fetal stomach content	
Libya	Gameel <i>et al.</i> (1993)	Milk, aborted fetus, vaginal swab	<i>B. melitensis</i> , biovar 1
Saudi Arabia	Radwan <i>et al.</i> (1992)	Milk	<i>B. melitensis</i> , biovar 1, 2
	Radwan <i>et al.</i> (1995)	Milk	<i>B. melitensis</i> , biovar 1, 2, 3
	Ramadan <i>et al.</i> (1998)	Carpal hygroma	<i>B. melitensis</i>
Senegal	Verger (1985)	Milk	<i>B. abortus</i> biovar 1, 3
Sudan	Agab <i>et al.</i> (1994)	Lymph node, testes, vaginal swab	<i>B. abortus</i> biovar 3

Source: Abbas and Agab (2002); Wernary and Kaaden (2002)

ii. Transmission

Both vertical and horizontal transmissions exist in animal brucellosis. Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, via conjunctiva, inhalation and udder contamination during milking. Congenital infection that happens during parturition is frequently cleared and only few animals remained infected as adult (Radostits *et al.*, 2000). Spread of the disease is due to movement of infected animals to disease free herds.

Proximity of infected herd to clean herds happens at water points where a number of camels come together. Epidemiologically important risk factors are large herd size, poor managements, and active abortions, milking more animals by single person and herding with other ruminants. Survival of the organisms in the environment may also play a role in the epidemiology of the disease (Abbas *et al.*, 1987; Radwan *et al.*, 1992; Abuo-Eisha, 2000). Dafni *et al.* (1991) suggested that small ruminants act as extensive reservoir of *B. melitensis*, which constitutes a threat of infection to large ruminants including camels and man due to prolonged contact. The chance of transmission is higher during parturition and abortion when most of the *Brucella* contamination occurs (Abbas and Agab, 2002)

iii. Host factors

Infection may occur in animals of all age groups, but persists commonly in sexually mature animals (Radostits *et al.*, 2000). Generally, infection is acquired after three years of age with increase in the subsequent age groups (Majid *et al.*, 1999; Abou-Eisha, 2000). Some study results revealed the equal distribution of *Brucella* antibodies among males and females (Waghela *et al.*, 1978; Abu Damir *et al.*, 1984; Abbas *et al.*, 1987; Radwan *et al.*, 1992). In other findings it appeared that females are more susceptible to the disease than males (Agab, 1997; Ajogi and Adamu, 1998). Higher susceptibility in female animals is attributed to physiological stresses (Walker, 1999). Female animals have essential epidemiological importance not only in susceptibility but also in disseminating the disease via uterine discharge and milk. The role of males in the spread of disease under natural condition is not important (Radostits *et al.*, 2000).

The extent to which infection rate varies due to breed difference is not well known. Wernwry and Wernery (1990) reported that breeding camels had lower brucellosis infection rate than racing animals. This was justified as due to racing camels (but not breeding animals) utilizing unpasteurized cow milk.

iv. Environmental and climatic factors

Atmospheric conditions and seasons of the year may have influence on the management and contact of the infected and susceptible host. In dry areas, water resources are sparsely distributed (Helland, 1982). As a result, the congregation of a large number of mixed ruminants at water points facilitates disease spread.

The coincidence of parturition in wet season (Schwartz and Dioli, 1992) enhances the viability of the organisms in the environment, thus increasing the chance of infecting susceptible animals (Corbel, 1990). Baumann and Zessin (1992) reported higher brucellosis reactor rate in two wet seasons than dry seasons. The incidence of brucellosis in camel population appears to be related to breeding and husbandry practices. Herd sizes, density of animal population, and poor management are directly related to prevalence (Wernery and Kaaden, 2002).

2.4.3 Pathogenesis and pathology

Following exposure, the organisms penetrate intact mucosal surface. In the alimentary tract the epithelium covering the ileal Peyer's patches are the preferred sites of entry. After penetration the organisms may be engulfed by phagocytic cells and localized to regional lymph nodes (Walker, 1999). Then they proliferate, disseminate haemogenously and localize in the reticuloendothelial and reproductive tract (Radostits *et al.*, 1994). Various mechanisms are employed by *Brucella* organisms to survive inside the phagocytic cells: inhibiting phagolysosome fusion, blocking bactericidal action of phagocytes and suppressing the myeloperoxidase H_2O_2 halide system (Frenchick *et al.*, 1985; Harmon *et al.*, 1988; Tizard, 1992; Walker, 1999). Little is known about the pathological changes in camels. Gross lesion may be found in the predilection sites uterus, udder, testicles, lymph nodes, joint bursa and placenta. Hydrobursitis was often observed in brucellosis positive dromedaries causing swelling of the bursa (Wernery and Kaaden, 2002). The probable possibilities for the abortion in farm animals may be due to placentitis, direct effect of endotoxins or inflammatory response in fetal tissue (Walker, 1999).

2.4.4 Immune responses

i. Humoral immunity

Naturally infected and vaccinated animals can be serological reactors. After infection, the level of immunoglobulin isotypes: IgM, IgG and IgA will significantly increase in serum (Radostits *et al.*, 2000). IgM antibodies, which appear initially after infection and low levels of IgG, will cause complement-mediated lysis of *Brucella*. Secretory IgA is tend to be abundant in milk where as IgG is high in serum (Walker, 1999).

The O-chain of smooth lipopolysaccharide complex of the cell envelope together with the outer protein epitopes have contributory role as protective immunogens. On the other hand, the immunogenicity of the non-smooth variant is relatively low (Corbel, 1990; WHO, 1997). The O-chain specific antibodies play a major role in protective immunity, but don't eliminate the organisms as they are protected being intracellular (WHO, 1997). This indicates lack of correlation between protection and high antibody level (Walker, 1999).

ii. Cellular immunity

Characteristic chronic granulomatous lesions develop in infected tissue where macrophage, neutrophils and lymphocytes respond to *Brucella* antigens. As the organisms are facultative intracellular organisms, phagocytes play a key role in initiating T-cells by processing and presenting antigens. Sensitized T-cells release cytokines that activate macrophages which in turn combat *Brucella* by reactive oxygen intermediates. Both CD4 and CD8 subsets are involved in cell-mediated protection. Cytokines also play a role in controlling *Brucella* infections (WHO, 1997). Neutrophils effectively utilize the myeloperoxidase-hydrogen peroxide halide system in killing *Brucella*. However, the organisms inhibit degranulation and the respiratory oxidative burst, and able to survive in the cells (Riley and Robertson, 1984). Macrophages readily ingest *Brucella* when opsonized with either complement or specific antibodies. The survival of the organisms in macrophages may result from a failure of phagosome-lysosome fusion and resistance to oxidative killing by producing superoxide dismutase and catalase (Frenchick *et al.*, 1985; Harmon *et al.*, 1988; Quinn *et al.*, 2004). Tatum *et al.* (1992) suggested that anti-oxidant Cu-Zn superoxide dismutase plays a role in the survival of *Brucella* species in phagocytic cells.

2.4.5 Diagnostic methods

i. Isolation and identification of Brucella

Conventional method of bacterial isolation is still the only absolute method for establishing the infection status. Isolation from a single animal is sufficient evidence to establish the infection status of a herd and is considered to be the gold standard test. Great care should be employed during handling any material containing *Brucella* organisms.

Generally, precautions to be taken include use of safety cabinet in laboratory; wearing gloves, protective cloth and facemask, autoclaving materials in contact with the organism and disinfecting contaminated surfaces (Alton *et al.*, 1975). A wide range of selective media can be used for cultivation. The suitable media include *Brucella* agar medium (BAM) base, Farrell's medium, serum dextrose agar (SDA), castaneda's medium (OIE, 2004), serum dextrose (Agab *et al.*, 1994), serum tryptose agar, glycerol dextrose agar and trypticase soya agar (Alton *et al.*, 1975). Terzolo *et al.* (1991) suggested that Skirrow agar is a satisfactory medium for both *Brucella* species and *Campylobacter fetus*. Contamination is prevented by use of selective media containing actidione (30 mg/l), bacitracin (25mg/l), polymixin B (5mg/l) and vancomycin (20mg/l) (Walker, 1999; OIE, 2004).

Milk samples, vaginal swabs, semen and aborted fetus are useful for recovering the organisms during antemortem. Samples collected at necropsy include multiple lymph nodes, spleen, udder, pieces of uterus and testicular tissue (Agab *et al.*, 1994). Tissue specimens are directly cultured on solid media whereas milk cultures are performed by centrifuging milk at 5900 to 7700 x g for 15 minutes (Walker, 1999). Then cultures are incubated at 37°C with 5-10% CO₂ enrichment for three days and above (Alton *et al.*, 1975; Gameel *et al.*, 1993; Agab *et al.*, 1994). Characteristic colonies have small convex, smooth translucent appearance (Gameel *et al.*, 1993; Agab *et al.*, 1994). Demonstration of the bacteria is by staining with gram-negative stain or modified Ziehl-Neelsen staining. Animal inoculation (an old method) can also reveal characteristics lesion in liver, spleen and epididymis of a guinea pig (Walker, 1999). Further characterization is based on serotyping, phage typing, dye sensitivity, and biochemical tests. Fluorescent antibody test and polymerase chain reaction methods have been described for *Brucella* species identification (WHO, 1997; Walker, 1999; Quinn *et al.*, 2004).

ii. Serological methods

Isolation of *Brucella* organisms from patient is not always possible. Therefore, serological tests play a major role in the routine diagnoses of the disease (Alton *et al.*, 1975). Serum agglutination tests (slide or tube agglutination), card test and Rose Bengal plate test (RBPT) have been the principal serological methods used. RBPT has been found more efficient than other serum agglutination tests although antigens produced by different laboratories and working procedures may affect the sensitivity (Ajogi and Adamu, 1998; Quinn *et al.*, 2004). Accordingly, RBPT is considered as satisfactory screening test (Nicoletti, 1992; OIE, 2004).

Complement fixation test (CFT), on the other hand, is considered to be the most accurate one. Some researchers reported its superiority to the other mentioned tests (Mohammed *et al.*, 1981; Gameel *et al.*, 1993; Asfaw *et al.*, 1998). CFT detects predominately IgG antibodies as most of IgM destroyed during serum deactivation and so used as a confirmatory test (FAO, 2003). The test distinguishes reaction caused by other factors like vaccines and other bacteria infections. *Escherchia coli* O:157, *Yersinia enterocolitica* O:9, *Vibrio cholerae*, *Psuedomonas mallophilia* and *Salmonella* serotypes share common chain of LPS antigen with smooth *Brucella* strains and do cross react. *Francella tularensis* also cross reacts for unknown reason. Rough *Brucella* strains also cross-reacts with *Actinobacillus equuli*, *Pasteurella multocida* and *Pseudomonas aeruginosa* (Corbel, 1990; Cloeckart *et al.*, 1992; Garin-Bastuji *et al.*, 1999). These organisms contribute to false positive reactors for brucellosis in animal herds. Thus, the use of highly specific test such as monoclonal antibody based c-ELISA and CFT minimizes the risk of cross-serological reactions between *Brucella* and these groups of bacteria (Vizcaino *et al.*, 1991; OIE, 2004).

Several attempts have been made to use milk ring test for camel brucellosis. Camel milk, however, lacks agglutinating substances required to cluster fat globules (Straten *et al.*, 1997; Bastawrows, 2002). Straten *et al.* (1997) established a modified milk ring test in camel by adding *Brucella* negative cow milk to camel milk. Then after, the authors observed a typical colored creamy ring in Brucellosis positive samples. Recently, ELISA has been used not only to detect *Brucella* antibodies in sera but also in camel milk (Straten *et al.*, 1997; Azwai *et al.*, 2001). Besides its higher sensitivity than other conventional tests, ELISA is found to detect sera as positive about 2 to 4 weeks earlier (Gameel *et al.*, 1993). It can also be used both for screening and confirmatory tests (FAO, 2003).

Other tests such as 2- mercaptoethanol test, rivanol and Coomb's (antiglobulin) tests have been used for specific purposes (Alton *et al.*, 1975). The use of several tests to reliably detect brucellosis suggests shortcoming in each of these tests. Hence, consideration should be given to all factors that have impact on the relevance of test method and test result to a specific diagnostic interpretation and application (OIE, 2004).

2.4.6 Importance of camel brucellosis

i. Economic importance

Brucellosis is characterized by abortion, non-viable offspring birth in female, and orchitis and epididymitis in male animals (Seifert, 1996; Radostits *et al.*, 2000; Pal, 2007). Abortion is the major feature that is manifested in camels (Al-Khalaf and El-Khaladi, 1989). The disease is also associated with infertility and prolonged calving intervals, and has considerable impact on camel production. Chronic inflammation of epididymis, of the joints, tendon sheath and synovial bursae especially at the carpus may also occur in camels (Abbas and Agab, 2002; Wernery and Kaaden, 2002).

The disease can generally cause significant loss of productivity through late first calving age, long calving interval time, low herd fertility and comparatively low milk production, as in cattle may also happen in camels (Radostits *et al.*, 2000). The disease can also have an impact on export and import of animals constraining livestock trade. Afzal and Sakkir (1994) have suggested that sub clinical brucellosis can pose problems in racing camels by reducing the performance and productivity of these animals in the Arabian Peninsula where camel racing is highly popular.

ii. Public health importance

Brucellosis in human represents a major public health hazard, which affects social and economic development in various countries. Groups at high risk for brucellosis are animal health workers, butchers, farmers, and those who are habitually consume raw milk and come in contact with animals (Chukwu, 1987). In man, transmission occurs as a result of ingestion of milk, contact via skin abrasion, mucous membranes and inhalation (Radostits *et al.*, 2000; Seifert, 1996).

Masoumi *et al.* (1992) recorded higher prevalence rate among butchers and people who habitually consume raw milk. Camel keepers consume camel milk as well as liver without heat treatment. This is even considered as delicacy (Gameel *et al.*, 1993). There is also a close contact between herdsman and the animal during watering, grooming, riding, nursing sick ones and delivery assistance (Abbas *et al.*, 1987).

The isolation of the two major pathogenic *Brucella* species: *B. melitensis* and *B. abortus*, from milk and other samples of camel origin (Gameel *et al.*, 1993; Agab *et al.*, 1994; Hamdy and Amin, 2002) clearly indicate the potential public health hazards of camel brucellosis (Straten *et al.*, 1997). The disease in man may be misdiagnosed due to the prevailing malaria infections in dry areas (Abou-Eisha, 2000; El-Ansary *et al.*, 2001).

2.4.7 Control and prevention

The control and prevention of brucellosis in farm animals depend on animal species involved, *Brucella* species, management practices and availability and efficacy of vaccines. The options to control the disease include immunization, testing and removal, and improving management practices and movement control (Hunter, 1994; WHO, 1997; Wernery and Kaaden, 2002). Control of camel brucellosis should suite conditions in particular countries where camels are raised. In most of the developing countries where camels are raised by pastoralists, brucellosis prevalence is low. Thus control by herd immunization and vaccination of calves at 4 to 8 months of age is helpful. On the other hand, test and slaughter policy can be followed in counties where intensification is practiced (Abbas and Agab, 2002).

i. Immunization

The live attenuated *B. abortus* S19 and *B. melitensis* Rev-1 proved to be effective vaccine against the disease in camels and other ruminants. Both vaccines have disadvantages of causing abortion, being pathogenic to human beings and interference with serological tests (WHO, 1997; Wernery and Kaaden, 2002). The non-smooth strains of *B. abortus* RB51 and *B. melitensis* M111 have recently been introduced into some countries. These vaccines are said to be safe and do not interfere with serological tests (WHO, 1997).

ii. Management and movement control of herds

Improving management practices is one way of attempting to control brucellosis. This would aim to improve hygiene and reduce the chances of contact between infected and non-infected animals. Although it would not be easy under many circumstances, where resources are lacking and the movement of livestock is difficult to restrict. Public awareness, isolation of infected animals and female at parturition, proper disposal of aborted fetus, placental tissue and uterine

discharge and disinfecting of contaminated areas are important in successful control and prevention of brucellosis (Hunter, 1994; Radostits *et al.*, 2000; Pal, 2007).

2.5 *Brucella* infection in human beings

Brucellosis only rarely or slightly impairs animal health, but it may cause serious illnesses in the human body (FAO and WHO, 1999). Brucellosis can be transmitted to humans through contact with *Brucella* infected animals or their products. Statistics shows an increased incidence of human brucellosis in persons (substantial cases per 100,000 population, during 1997 - 2002) who are engaged in certain professions such as veterinarians, slaughterhouse employees, and dairy farmers and workers, livestock handlers, and laboratory personnel (Kozukeev *et al.*, 2003). The incidence of human brucellosis results from four primary routes: (1) ingestion of unpasteurized and contaminated milk or milk products; (2) inhalation; (3) penetration via ocular or oral mucosa, and (4) direct inoculation into the bloodstream through abrasions in the skin or vaccination (Forbes *et al.*, 2002; Pal, 2007).

2.5.1 Epidemiology

The epidemiology of human brucellosis, the commonest zoonotic infection worldwide, has drastically changed over the past decade because of various sanitary, socioeconomic, and political reasons, together with the evolution of international travel. Several areas traditionally considered being endemic, for example, France, Israel, and most of Latin America, have achieved control of the disease. On the other hand, new foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the near east, Syria for example is rapidly worsening. Furthermore, the disease is still present, in varying trends, both in European countries and in the USA. Awareness of this new global map of human brucellosis will allow for proper interventions from international public-health organisations (Georgios *et al.*, 2006).

Most of the human brucellosis cases are caused by *B. melitensis* but *B. abortus* also accounts for some. The occurrence of the disease in humans is largely dependent on the animal species, when brucellosis exists in sheep, goats and camels; it causes the greatest incidence of infection in humans (Shrestha, 2004).

In Europe, the highest prevalence of human brucellosis occurs in the countries of the Iberian Peninsula and the Mediterranean littoral or basin region (Portugal, Spain, Southern France, Italy, Greece, Turkey, North Africa), South and Central America, Eastern Europe, Asia, Africa, the Caribbean, and the Middle East (WHO, 1986).



Figure 2: Worldwide incidence of human brucellosis

Source: Georgios *et al.* (2006)

From a recent survey of the Arabic Peninsula, there was a serological evidence of exposure to *Brucella* of almost 20%. In Mediterranean and Middle East countries, the annual incidence of human brucellosis varies from 1-78 cases per 100,000. Certain communities in South European countries reported up to 77 cases per 100,000 people and 50-500 cases per 1,000,000 in Ethiopia (WHO, 1986; Mousa and Elhag, 1988). Although human brucellosis is a notifiable disease in many countries, official figures do not fully reflect the true incidence and it has been estimated to be between 10 and 25 times higher than the reported figures (Anonymous, 2001).

There was a study on acute human brucellosis in endemic areas of Saudi Arabia in 2003 and the active serological screening of 404 households' members of acute brucellosis cases detected 53 (13%) were seropositive (Almuneef *et al.*, 2004). In Ethiopia, brucellosis is prevalent in traditional pastoral communities such as Borana and Hamar. A study on human brucellosis in traditional pastoral communities of Borena and Hamar showed that 34.9% (33/88) and 29.4% (5/17), respectively (Genene *et al.*, 2009).

2.5.2 Pathogenesis

Brucellae invade the body via the alimentary tract, conjunctival mucosa, respiratory tract, or skin and localization within regional lymph nodes, followed by ingestion either by mononuclear or polymorphonuclear leucocytes. Virulent *Brucellae* have the ability to survive in both polymorphonuclear and mononuclear phagocytes and also can depress chemotaxis and phagocytosis by polymorphonuclear leucocytes (Ocon and Reguera, 1994). After phagocytosis, *Brucellae* probably multiply in the lymph nodes as parasites and then enter the blood and produce the bacteraemia followed by the acute febrile phase of the disease. From the blood, the organisms are distributed throughout the reticuloendothelial system and become present in large numbers in the liver and spleen. They also localize in many other sites such as the joints, heart, kidneys, the central nervous system and genital tract (Baldwin and Winter, 1994).

The response to infection may relate to the specific *Brucella* species, and the nature of the lesions produced depends upon the type of infecting organism; *B. abortus* has been related to the presence of granulomas in patient's livers (Spink *et al.*, 1982). Thus, *B. abortus* strains produce more typically a granulomatous reaction (Young, 1979).

2.5.3 Clinical manifestations

The clinical manifestations of human brucellosis are variable because of variables methods of exposure and nonspecific clinical signs. The main incubation period of brucellosis series cases of laboratory-acquired infections in people is determined to last about 3 weeks. In case of persons exposed to *B. abortus* strain 19, the onset of symptoms varied from 5-13 days.

In the re-exposure case the onset of symptoms was as short as 2 hours for a local reaction and as long as 8 hours for a systemic response (Young and Corbel, 1989). The clinical picture in human brucellosis can be mixed up with cases of gastrointestinal, respiratory, dermal, and neurologic manifestations predominantly, which are not uncommon (Shakir *et al.*, 1987). Acute or subacute diseases follow after fever and bacteremia, and may vary from 1 week to 6 weeks or several months. Acute brucellosis is characterized by an intermittent fever ranged from 38-41°C, but hyperpyrexia is present in a significant number of death cases. The undulant fever is typical in untreated cases in which the disease has persisted for some time. Death may occur from hyperpyrexia, severe toxemia, endocarditis, and meningo-encephalitis (Dalrymple-Champneys, 1960).

Chronic brucellosis may result from untreated patients when the acute or subacute disease has persisted for 6 months or longer with or without complications. In that case, the disease is regarded as chronic, but in practice such type of evidence is difficult to obtain. The symptoms are generally related to the state of hypersensitivity of the patient and then the illness may persist for years (Araj and Lulu, 1986).

2.5.4 Diagnosis

Because of variable symptoms, non-distinctive clinical signs, and subclinical and atypical infections; the clinical diagnosis of brucellosis in humans is particularly difficult. A wide variety of symptoms were revealed by persons acquiring the disease in a slaughter plant, on a farm or ranch, or from the consumption of raw milk or milk products made from raw milk, many of which did not result in an initial diagnosis of brucellosis (Young, 1983). The confirmation of the diagnosis came mostly after detecting *Brucella* organism and its antibodies by bacteriological and serological tests. However, the results remain unsatisfactory in the chronic phase of the disease. Bacteriological tests in a laboratory found *Brucella* organisms in blood or bone marrow, lymph nodes, cerebrospinal fluid, synovial fluid, urine and abscesses and were occasionally isolated from sputum, placenta, mother's milk, vaginal discharge and seminal fluid (Castaneda, 1947; Finegold and White, 1969; Gotuzzo and Carillo, 1986; Pal, 2007).

Numerous serological tests have been tried in the diagnosis of human brucellosis to detect *Brucella* antibodies. Until recently, the most widely used were the standard tube agglutination test (STA), which measures total antibody (both IgM and IgG), and the 2-mercaptoethanol tube test (2-ME test), which measures only IgG antibody. Other useful tests used in the diagnosis of human brucellosis include: the Rose Bengal plate test (RBPT), enzyme-linked immunosorbent assay (ELISA), the complement fixation test (CFT), the Coombs anti-human globulin test (AHG, Coomb's), counter-immuno-electrophoresis test (CIEP), radioimmunoassay test (RIA), and the agglutinating complement fixation test (Pal, 2007). The intradermal test or skin test is only a complementary test in the diagnosis of brucellosis, and a positive intradermal test in healthy persons indicates a state of specific allergy for brucellosis with a past exposure to infection (Diz *et al.*, 1989)

2.5.5 Prophylaxis

The ideal method to prevent the spread of brucellosis in humans is the removal of the disease from animals. In addition, control measures among animals have a beneficial effect on the incidence of the disease in humans. The incidence of human brucellosis in any region mainly depends on the spread and density of the infection, and direct or indirect contacts between humans and animals or their excreta or products. No satisfactory vaccine exists for the prevention of human brucellosis but the live *B. melitensis* Rev.1 vaccine is a useful tool to decrease infections with *B. melitensis* in humans. In whole flock or herd vaccination programmes of animals greatly decreases the prevalence of brucellosis in animals as well as human populations (European Commission Report, 2001).

Vaccinating people in high risk agricultural occupations has been attempted in Russia and China (WHO, 1986). The vaccine is given by the intradermal route and immunity is produced in a short duration where annual booster doses are required. Repeated doses of vaccines are increasing the risk of hypersensitivity reactions. Epidemiological evidences indicate that even attacks on clinical brucellosis leads to an incomplete protection against reinfection. For this reason, a safe and effective vaccine against human brucellosis may be difficult to produce (Lopez-Merino and Asselineau, 1976).

2.5.6 Treatment

The fact that *Brucella* is localized within the cells of the reticuloendothelial system presents a difficulty since many antibiotics cannot cross the cell walls. *Brucella* strains are susceptible to a wide range of antibiotics, but combinations of streptomycin and tetracycline have been the preferred treatment for over 30 years-old persons (Young and Corbel, 1989). The World Health Organization recommended that rifampicin (600 - 900 mg) and doxycycline (200 mg) need to be taken as a single dose for at least 6 weeks (WHO, 1986). Longer established is the combination of intramuscular streptomycin with an oral tetracycline, which results in fewer relapses (Ariza *et al.*, 1985). Also a combination of rifampicin with quinolones seems as effective as either of these regimens (Akova, 1993). Rifampicin has shown a strong inhibitory and bactericidal activity against *Brucella* strains in both vitro (Corbel and Bracewell, 1976) and experimental infections (Philippon and Plommet, 1977). It has proved very useful against brucellosis in young children and pregnant woman (Llorens-Terol and Busquets, 1980).

The response of chronic brucellosis to treatment is much slower than that of the acute disease and immunostimulant therapy with levamisole, which brought benefits to patients (Thornes, 1977). Antigen therapy is potentially hazardous and is not recommended (WHO, 1986).

3. MATERIAL AND STUDY METHODS

3.1 Description of the study areas

Afar tribal land is located in the Great Rift Valley, comprising rangeland in northeast Ethiopia and it has 5 administrative zones, which are further subdivided into 29 woredas and one special woreda currently called as Argoba. The population estimate of the region is estimated at 1.2 million of which 90% are pastoralists and 10% agro-pastoral. Gewane is the Administrative center of Zone 3. The administrative zone 3 consists of six districts (woredas) predominantly occupied by pastoral and agro-pastoral communities (Asnake *et al.*, 2005). The altitude of the region ranges from 120 - 1500 metres above sea level.

Awash-Fentale district

Awash-Fentale is part of the Administrative Zone 3. It is located at about 170 kms east of Addis Ababa and lies at 8° 54' North latitude and 36° 23' to 39° 54' East longitude. There are six woredas (Amibara, Argoba, Awash-Fentale, Bure-Modaitu, Dulecha and Gewane) in the Administrative Zone 3. It is bordered on the south by the Oromia Region, on the west by the Amhara Region, on the north by Dulecha, and on the east by Amibara. The woreda has four rural peasant associations (PAs) (Boloita, Dudub, Kebena and Doho) and two urban PAs or kebeles (Awash-Sebat and Sabure). PA is the lowest administrative unit within the district that is considered during the survey. In agro-climatic terms, about 85% of the area is classified as arid and the remaining as semi-arid. The district is a lowland area with an altitude of 650 - 1010 meters above sea level. The average annual rainfall of the district is 486 mm. It has a mean minimum and maximum temperature of 36°C and 42°C, respectively (FARM-Africa-Report, 2005-2007).

The predominant agricultural practice in Doho and kebena is pastoralism whereas the agricultural practice in Dudub, Sabure and Boloita is agro-pastoral. The livestock population is about 365,802 cattle, 347,050 sheep, 549,993 goats, 13,809 horses, 8,282 mules, 194,083 donkeys and 68,331 camels. Camels, goats and cattle are the most common livestock. Migration to the border areas south east Amhara region, Argoba special, Dulecha and Amibara districts is common in years of low rainfall. The camel population of the district share for pasture and water with the livestock herds of the different habitation areas of the Amibara woreda through the route of Awash River Valley in the dry seasons (PCDP, 2004).

Amibara district

Amibara woreda is part of the Administrative Zone 3. It is found 250 km from Addis Ababa on the main road to Djibouti port. In terms of geographical reference, the district is located between 8°9' - 8°10' North latitude and 39°45' - 40°30' East longitude. The average altitude of the district is 740 meter above sea level with mean annual temperature of 25°C. The average mean annual rainfall is about 575 mm (Kassa, 2001). The district is bordered on the south by Awash-Fentale, on the west by the Awash River which separates it from Dulecha to the southwest then on the northwest by the Administrative Zone 5, on the north by Gewane, and on the east by the Oromia Region. The woreda has four urban and fourteen rural PAs. The Urban PAs in Amibara are Awash-Arba, Awash-Sheleko, Melka-Sedi and Melka-Werer and the rural are Andidu, Gedamytu, Buri, Kala'at, Rakba, Hasoba, Badeham, Wele'ese, Ambash, Ga'as, Halaydeghi, Algeta, Angelile and Kurkura. In four of the eleven rural PAs of the district, namely Andidu, Gedamytu, Halaydeghi, Algeta, Angelile, Kala'at and Rakba, the predominant agricultural practice is pastoralism whereas the agricultural practice in Hasoba, Buri, Badeham, Wele'ese, Ambash, Ga'as and Kurkura is agro-pastoral. The livestock population is about 275,106 cattle, 187,000 sheep, 895,697 goats, 3,809 horses, 2,085 mules, 243,252 donkeys, 164,684 camels and 80,623 poultry. Camels, goats and cattle are the most common livestock (PCDP, 2004).

Amibara woreda is a pathway for pastoralists during their migrations for pasture and water and this woreda is endowed with several irrigation points as it is divided by the Awash River Valley and hence, non-governmental cotton farms are found which attracts the pastoralists to feed their camels after the cotton is being harvested (Senait *et al.*, 2009). The Melka-Werer Agricultural Research Centre is found in Melka-Werer of Amibara district. The research centre has researched various agriculture related studies and advocates that rivers, lakes, springs and ground water resources of zone 3 facilitate both small and large scale irrigated agriculture. FARM Africa is working in Afar region, zone 3 and 5, since 1997. FARM is involved in community animal health programmes, small scale irrigation, women development initiatives and livestock marketing (Asnake *et al.*, 2005).

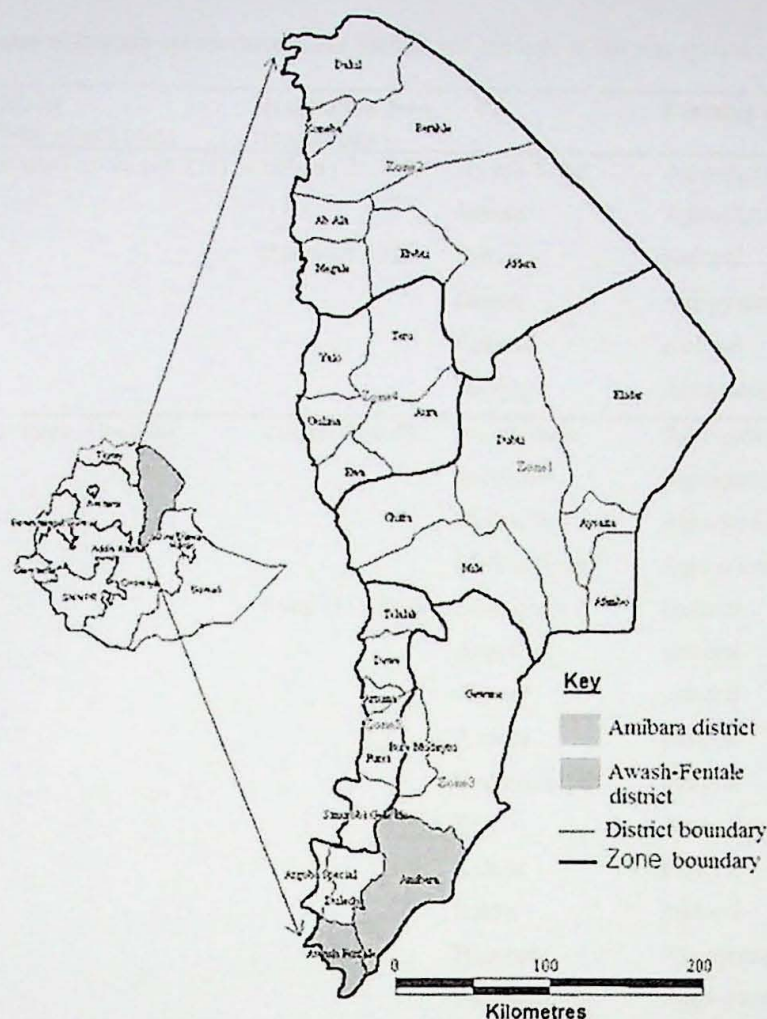


Figure 3: Map of the study districts (administrative zone 3)

Source: Asnake *et al.* (2005)

3.2 Study population

The camel populations of Awash-Fentale and Amibara districts were about 68,331 and 164,684, respectively, which were considered as study population. Among which 70% are found in the rural community and 30% in the urban dwellers. Herdsmen or patients who attended the health centers for diagnosis with clinical symptoms similar to malaria were considered as the study human population in each study districts.

Table 4: Peasant associations in the districts and the type of farming system

District (Total camel ppn)	Habitation area (camel ppn)	PA	Farming system
Awash-Fentale (68,331)	Urban (20,499)	Awash-Sebat	Agro-pastoral
		Sabure*	Agro-pastoral
	Rural (47,832)	Doho*	pastoral
		Dudub	Agro-pastoral
		Kebena	pastoral
		Boloyta	Agro-pastoral
Amibara (164,684)	Urban (49,495)	Awash Arba	Agro-pastoral
		Sheleko*	Agro-pastoral
		Melka Sedi	Agro-pastoral
		Melka-Werer*	Agro-pastoral
	Rural (115,279)	Halaydegghi *	pastoral
		Angelile	pastoral
		Algeta*	pastoral
		Andidu	pastoral
		Gedamytu	pastoral
		Buri	Agro-pastoral
		Kala'at	pastoral
		Rakba	pastoral
		Hasoba*	Agro-pastoral
		Badeham	Agro-pastoral
		Wele'ese	Agro-pastoral
		Ambash*	Agro-pastoral
		Ga'as	Agro-pastoral
		Kurkura	Agro-pastoral

PA = peasant association, ppn = population. * randomly selected study PAs

Source: FARM-Africa-Report (2005-2007); PCDP (2004)

3.3 Study design

A cross-sectional study design was conducted from October 2010 to May 2011, to determine the prevalence of *Brucella* infection in camel and human beings in selected pastoral and agro-pastoral residences of the Awash-Fentale and Amibara districts and to identify potential risk factors associated with the disease. The study was conducted in two randomly selected PAs (Doho and Sabure) of Awash-Fentale and in six PAs of the Amibara district (Table 4). Samples were collected and questionnaires were administered to each randomly selected camel owner and to all human clinical patients.

3.4 Sampling method

First, two districts/woredas were selected based on their accessibility (non-probability: convenience sampling). At present, there are 6 peasant associations (PAs) in Awash-Fentale and 18 in Amibara district and 30% of the PAs (proportionalized) in each of the districts were included in the investigation on the basis of feasibility and affordability or cost. In this study, PA indicates the lowest administrative unit within a district that was considered during the survey. Hence, two PAs from Awash-Fentale and six from Amibara were selected randomly. Camel herds in the selected PAs were sampled during the study and herds (clusters) were sampled by cluster sampling. Cluster sampling was the suitable method for this study as constructing sample frame for random sampling was not possible in pastoral production system. The formula to determine cluster size is as follows (Thrusfield, 2005):

$$g = \frac{1.96^2 (n \times V_c + p(1-p))}{nd^2}, \text{ then total sample size (TS)} = g \times n$$

Whereas g = Number of cluster, p = prevalence rate, n = number of animal per cluster or herd, V_c = between cluster variation and d = is precision level. However, to apply the formula there was no information about the between cluster variation (V_c) in the study area. Therefore, it was necessary to look for other alternative that is modification of simple random sampling. Information about the prevalence rate of the disease is needed from the area when simple random sampling is to be used. But there was no previous year's prevalence of brucellosis in the districts. Hence, the average expected prevalence rate was assumed to be 50% for the area within 95% Confidence Intervals (CI) at 5% desired accuracy.

Hence, the formula used by Thrusfield (2005) to calculate sample size (n) was as follows:

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

Where n = sample size, d = desired absolute precision (0.05), P_{ex} = expected prevalence (50%), thus the desired sample size for $P_{ex} = 0.5$ is $n = 384$. Cluster sampling can lead to an increased sampling variance and a large sample size would be required to reduce variance to acceptable levels (Thrusfield, 2005). Therefore, it was safe to inflate the sample size by two-folds as far as practically possible. Hence, $n = 384 \times 2 = 768$. Demeke (1998) reported the average herd size to be 14 animals, $768/14 = 55$ herds (clusters). Therefore, it was planned to sample a total of 768 camels using *two-stage cluster sampling* method. In order to get this number of camels, it was obligatory to go to the Awash River valley.

Since the study was started in the late days of October 2010, it was unnecessary to visit all the vacant PAs because the camel population from the agro-pastoral PAs were moved to the Awash River valley for search of pasture and water. Camel herds were mixed together with other camel population of the pastoral PAs which usually localize the watering point almost throughout the year. Hence, it was obligatory to route a long distance along the Awash River valley to find the target animals and administration of the questionnaire to the herdsman. The number of camel herds (clusters) from the selected PAs was determined on-site along the Awash River valley and the number of individual camels in each clusters was obtained from the herdsman and then, individual camels were randomly selected on proportional basis from each cluster (*two-stage cluster sampling*). Individual camels above six months of age were included and blood samples were collected from individual camels at random.

Purposive sampling (non-probability sampling) method was used in the survey of human brucellosis. Clinical patients who have attended the health centers in seek of medical service were included in the study and herdsman with previous and current disease history and willing ones were advised to visit the Awash-Sebat or Werer health center and give blood sample. All human patients with febrile illness presenting with clinical signs and symptoms resembling brucellosis but malaria negative within specified time duration of 90 days (from first November, 2010 to 29 January, 2011) were included in the study (purposive sampling) to determine the prevalence of human brucellosis and 200 sera samples of both sexes were

collected during the study period at the health centers of Awash-Sebat and Melka-Werer of the Awash-Fentale and Amibara districts, respectively. A special medical and ethical consideration was given to under-age (<18 years old) clinical patients.

After the informed consent approach has made: no refusal to participate was recorded and then blood samples were collected and tested for the presence of antibody against *Brucella* infection using serological tests (RBPT and CFT). Proportional number of herdsmen were randomly selected from each of the 55 camel herds and approached for the administration of the questionnaire which result a total of 113 respondents (two translators added) and all of them were adult males of above 18 years of age (Table 7).

Table 5: Number of camels sampled from each PAs of the study districts

Woreda	PA	No sampled	Farming system
Awash-Fentale	Sabure	97	Agro-pastoral
	Doho	127	Pastoral
	Subtotal	224	
Amibara	Sheleko	132	Agro-pastoral
	Halaydeghi	123	Pastoral
	Melka-Werer	82	Agro-pastoral
	Algeta	78	pastoral
	Hasoba	68	Agro-pastoral
	Ambash	61	Agro-pastoral
	Subtotal	544	
	Total	768	

PA = peasant association. No = number

3.5 Study methodology

3.5.1 Questionnaire survey

A structured questionnaire (Annex C) was administered to camel herders. This questionnaire was designed for a survey of the potential risk factors associated with the disease in camel herds and to gather relevant information on the overall camel herd management practices.

The information was based on the main risk factors, such as the introduction of infected animals into the herds, the type of farming system and the exposure of the herds to an infectious disease. Age groups of camels were classified as young (6 months - 3 years), adult ([3-7] years) and old aged camels (> 7 years) or as immature (before puberty, < 3 years old) and matured (age of puberty and above, i.e. ≥ 3 years) (Annex H).

Camel calves below 6 months of age were not included in the study and actually owners were not willing even to lay a hand on their camel calves. Herd size was classified into three categories as small (14-20 camels in it), medium (21-40 camels in it) and large (more than 40 camels in the herd). Parity was also categorized into three classes as 'no parturition', 'single parity' and 'more than one'. It was also concerned with the general characteristics of the herds and the management system. All possible risk factors have been collected using a standard questionnaire format to record information such as follows (Table 6).

Table 6: The summary of questionnaires and checklist

Risk factors	Checklist	Description
General characteristics	Herd owner	Name, address, sex, age, educational level, district/woreda/, PA/kebele/, types of livestock kept, raw milk, cheese and meat consumption, assist at parturition, milking animals, slaughter livestock, grooming animals, visit health after any health problems
	Herd features	Herd size/number of males, camel calves and breeding females/, sex, age, source of animals, parity, identification number or particular name of animal, Occurrence of abortion and RFM, contact with other animal species,
Introduction of infected animals into the herd	Disease monitoring system	Breeding season, milking and production systems, traditional therapeutic approach.
Management system of camel herds	Management system	Farming system/pastoral, agro-pastoral/, watering, feeding.

There were more than 3,000 individual camels observed within 10 kilometres distance along the route of the Awash River during the investigation period and these camels were from more than 30 PAs of the Amibara, Awash-Fentale, Argoba special woreda, Dulecha, Gele'alo and Bure Mudaytu districts. But random sampling of individual camels was only from the selected PAs of the study woredas (Table 5). Camel herders as well as those with previous and current disease history were requested for their willingness and strongly recommended to visit the health centers at Awash-Sebat and Melka-Werer as the medical staffs were not willing and even demand costly payment to go to the distant sampling areas. Herdsmen from each camel-herd were randomly selected for the administration of questionnaire. Children, old men and women were not included in the questionnaire survey. They are usually remain at their residence as their labour division is to keep mainly small ruminants near the permanent dwelling areas in case of the agro-pastoral PAs and temporary residential territory in case of the pastoral people.

Table 7: Number of respondents involved in the study districts

Woreda	PA	No of Interviewed (all males)	No of willing respondents and with disease history
Awash-Fentale	Sabure	14	6
	Doho	21	9
	Subtotal	35	15
Amibara	Sheleko	21	7
	Melka-Werer	12	3
	Halaydegghi	13	4
	Algeta	12	2
	Hasoba	10	2
	Ambash	10	3
	Subtotal	78	21
Total		113	36

Likewise, a structured questionnaire (Annex D) was administered to clinical patients with malaria like symptoms by the medical persons at the health centers. Before the start of this study and the beginning of blood sampling for brucellosis medical staff at the health centers were unaware of the existence of this disease and even none of the medical staffs knew such a disease condition named as 'brucellosis'.

In fact there were no senior medical professionals in these health centers. Hence, medical staff/nurses and laboratory technologists/ involved in the study received an update on brucellosis. It was to collect information on potential risk factors for the occurrence of brucellosis in humans. The information collected include the area where the patients came from, sex, age (less than 18 years old, 18 up to 40 years old and greater than 40 years old), duration or stage of illness as acute (<6 months of illness), subacute (6-12 months) and persistent (>12 months), ownership of camels and other livestock, raw camel milk, cow milk and/or goat milk consumption, raw meat consumption, assist at parturition, involved in milking, slaughtering livestock, grooming animals and other relevant queries were administered.

3.5.2 Serology

During the collection of human and camel blood samples, shaking of the tubes was avoided during transport to prevent the distraction of the RBCs or hemolysis. The blood samples were allowed to clot at room temperature. The tubes were placed vertically at room temperature for overnight and then sera were separated from clotted blood by decanting into plastic cryovials and were transported in icebox (assumed to be +4°C) and then kept in a freezer (-20°C) in the immunology laboratory of the National Veterinary Institute at Debre-Zeit until testing with the serological tests in target. Repeated freezing and thawing were avoided as that might have an effect on the protein structure of the serum.

Camel blood samples

About 5-10 ml of blood samples were collected from jugular vein of each camel using plain vacutainer tubes (without EDTA) after proper restraining. All she-camels which were included in the study were numerically marked deliberately on their lower neck and tail area with a liquid marking pink ink obtained from Werer Agricultural Research Center. Camel owners were told details of the study and hence, not to remove or wash the marking-ink until the end of the chores with their animals.

Application of the plastic-card ear tag was tried but owners become unwilling when they saw a drop of blood oozing out of the pinna after the procedure. Hence, an ink was used for marking of target animals which was used by the community service providers of the FARM Africa as

this NGO was involved in community animal health programmes in zone 3 and 5 in collaboration with the research center. This temporary marking ink (made from iron oxide and ferricyanide) can persist for more than three months on their fur/skin/ which of course neither cause any harm nor create cumulative or residual effect on the animals and the environment but it causes slight alopecia on the painted area of the skin which can be recovered within 2-3 weeks. Off course, such side effect of the marking ink on their animals was creating discomfort on the camel owners. This marking was to easily identify CFT confirmed seropositive she-camels upon revisiting the herds for milk samples. Recapturing of these seropositive she-camels was based on the critically collected information (district, PA, owner name, clan name, name of territory where the camels inhabit for pasture and water) during the blood sample collection. Therefore, a total of 768, 159 males + 609 females, camel sera samples were collected and analysed for brucellosis using RBPT and CFT at the National Veterinary Institute (NVI) at Debre-Zeit.

Human blood samples

Human blood samples (about 5ml) were collected from two public health centers at the two study districts namely: Awash-Sebat and Melka-Werer health centers. Patients with either of the clinical history or findings of acute or insidious onset of fever ($>38^{\circ}\text{C}$), chills, night sweating, undue fatigue (weakness), anorexia, weight loss, severe or persistent headache, joint pains (arthralgia), muscle pains (myalgia), bone pain, back pain, nausea/vomiting and thirsty were critically approached. The patients were first diagnosed for malaria (plasmodium in blood) and then only malaria negative patients were included in the study for blood/serum/ samples and 164 sera samples were obtained from patients of different districts and peasant associations practicing different farming systems. After administration of questionnaires, herdsmen were requested to visit local health centers to give about 5ml blood for the target of the study. Among the 113 interviewed camel herders (all males), 36 were willing (Table 7) and blood samples were taken from them and 164 clinical patients were recorded at Awash-Sebat and Melka-Werer health centers who came with malaria like symptoms. Therefore, a total of 200 (36 + 164) human sera samples were collected and analysed for brucellosis using RBPT and CFT at the National Veterinary Institute at Debre-Zeit.

Rose Bengal Plate Test (RBPT)

According to the Office International des Epizooties (OIE) approved tests, Rose Bengal plate test (RBPT) is sensitive and rapid screening test and the RB antigen is a suspension of smooth cells of *Brucella* organisms stained with the Rose Bengal dye, buffered to pH 3.6 and is commercially available. The RBPT is a simple test for the diagnosis of human and animal brucellosis. The basic principle is the buffered acid antigen stained with Rose Bengal used for the early detection of *Brucella* specific agglutinins (Annex F: A). The RBT is a rapid agglutination test based on mixing a drop of serum and a drop of antigen together (ECR, 2002). Rose Bengal plate test was conducted at the National Veterinary Institute and it was a suspension of smooth cells of *B. abortus*, antigen S99 which was commercially available. The RB antigen was shaken up gently before it was used. It was not frozen, and was stored at 4°C in a dark place. Sera samples were kept in refrigerator at +4°C before testing. Sera and antigen were left at room temperature for half an hour before testing to maintain to room temperature (18-25°C). All sera samples collected were initially screened and the test procedure recommended by Alton *et al.* (1975) was followed.

One drop (30µl) of serum was mixed with an equal volume of antigen on an enamel plate to produce a zone approximately 2 cm in diameter. After that, both drops were mixed by the disposable stirring stick, spreading them over the full surface of the circle. Afterwards, the serum and antigen were thoroughly mixed. The mixture plate was then rotated manually for 4 minutes at room temperature. The results were then analysed according to the presence or absence of any degree of agglutination. The assessment was carried out exactly 4 minutes after the beginning of the shaking. Then any visible agglutination reaction was considered to be positive. As a screen test, the degree of agglutination reactions was read and recorded as: + + ++ (coarse clumping and clearing), + + + (clumping and some clearing), + + (visible fine agglutination), + (weak fine agglutinations using magnifying glass) in case of positive reactions and 0 (no agglutinations) in case of negative reactions.

Complement Fixation Test (CFT)

The standardisation of antigen and sheep erythrocytes was performed as described in the literature. Sera that tested positive to the Rose Bengal plate test (RBPT) were further confirmed using complement fixation test (CFT) for confirmation using Standard *Brucella abortus* antigen. CFT was conducted at the National Veterinary Institute, at Debre Zeit.

Preparation of the reagent was evaluated by titration and performed according to protocols recommended (Annex F; B). Standard *B. abortus* antigen S99 was used as an antigen (CVL, New Haw Weybridge, and Surry KT15 3NB, UK). The antigen, control sera and complement were obtained from Germany. The 2% sheep red blood cells suspension was prepared before the beginning of the test. Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive (OIE, 2004). The test was examined by the eye and interpretation was the sedimentation of the sensitized RBC was positive result and the complete lysis of the sensitized RBC was negative result.

3.5.3 Bacteriological examination of milk samples

Milk samples were collected through aseptic sample collection procedure from the udder of each seropositive milking-camel using sterile universal bottles. The udder of each seropositive milking-camel was thoroughly disinfected with savlon, cleaned and dried with sterile cotton and then teat openings were disinfected with 70% of ethyl alcohol. After discarding few drops of milk, about 10 ml of milk was collected and transported in ice box to the laboratory and stored in a freezer (-20°C) until processing at later day. Dried or non-lactating she-camels were excluded despite their seropositivity. Bacteriological processing of milk samples was conducted at the microbiology laboratory of the School of Veterinary Medicine, Debre Zeit. In every procedure of the isolation trial, biohazard safety cabinet level II was used and all obligatory laboratory safety measures were followed. Bacterial isolation trials from milk samples were made on *Brucella* Medium Base (Oxoid) supplemented with 5% v/v horse blood. *Brucella* medium was prepared and cooled to 50°C and 5% horse blood which was incubated at 35°C for 10 minutes were incorporated immediately to the *Brucella* medium and mixed well and poured into sterile petri dishes.

The milk samples were centrifuged at 4000 gravity for 10 minutes in conditions that avoid the risk of aerosol contamination and the cream was spread on the prepared *Brucella* medium. Cultures were incubated in duplicate for 7-10 days in a 5% and without 5% CO₂ tension at 37°C. Bacterial isolates were identified according to the standard procedures (Quinn *et al.*, 2004; OIE, 2004) (Annex G).

The plates were observed at every 24 hours for colony growth. The suspected colonies of *Brucella* were picked up and transferred to other *Brucella* agar medium plates and incubated under 5% CO₂ tension to obtain pure culture. Colony characteristics were seen on the pure bacterial colonies on *Brucella* medium according to the procedures of Moriyon *et al.* (2004). The isolates so obtained were streaked on plates of blood agar (7% v/v sheep blood) and MacConkey agar. The isolates producing haemolysis on blood agar and colonies grown on MacConkey agar were eliminated considering non *Brucella*. Suspected isolates were subjected to Gram's staining and modified Ziehl-Neelsen (MZN) staining for checking the purity of cultures and morphological characters. The isolates suspected to be of *Brucella* were subjected to agglutination and biochemical tests: oxidase, catalase, motility (SIM medium), urease production (urea agar slants), H₂S production (SIM medium) and Oxidation-Fermentation test (OF medium) (with sterile 10% dextrose, 10% sucrose and 10% lactose sugars).

3.6 Ethical considerations

All experiments in this study were conducted fulfilling the guidelines of the declaration of National Health Research Ethics Guideline of the Ethiopian Science and Technology Commission, National Health Science and Technology Council and hence, consent was obtained from the institutional review board (IRB) office Addis Ababa University College of Health Sciences, Medical Faculty (Ethical clearance, Annex E). All participants were informed about the aim and procedure of the study and were asked for their consent to be clinically examined and for the collection of blood sample and no refusal to participate was recorded. Special attention was given when clearing proposals involving vulnerable groups such as children, pregnant women, mentally disabled, subordinates, communities unfamiliar with clinical concepts, etc. Herders were asked for participation one point in time in this one year study. Herdsmen and/or clinical patients were advised to visit the health centers for diagnosis and giving blood sample. Written informed consent was obtained from all participants in the study (Annex E). The consent forms were typed in English and Amharic versions but were translated to the local language, Afarigna, during the administration of the information to target groups.

3.7 Data management and processing

The data were summarized and compiled by summing up the laboratory findings of human and camel study population. An animal or human was said to be positive if it tested positive to both RBPT and CFT. Individual camel or human level seroprevalence was calculated on the bases of RBPT and CFT positive results divided by total number of camel/human tested. Coded data were stored in Microsoft Excel 2007 spread sheet and transferred to SPSS* Version 15 for statistical analysis. Descriptive and analytic statistics were computed using software SPSS* V15. Logistic regression and Chi-square test (χ^2) were employed to see the association of risk factors with that of seropositivity to *Brucella* antibody; the degree of association was computed using Odds ratio (OR) and 95% confidence interval (CI). Odd ratio (OR) was used to indicate the degree of risk factor association with the disease occurrence signified by 95% confidence intervals. Odd ratio is the ratio of the odds of disease occurring among individuals exposed to a variable and the odds of the disease occurring among individuals not so exposed (Thrusfield, 2005).

4. RESULTS

4.1 Camel brucellosis

Results of the serological diagnosis of brucellosis in humans and camels at the study districts and peasant associations (PAs) and milk cultural detection trial are summarized in Tables (8-25). The study districts, Awash-Fentale and Amibara, were practicing pastoral and agro-pastoral farming systems. The 224 camels from the PAs of Awash-Fentale belonged to Sabure and Doho (Table 8) with agro-pastoral and pastoral farming systems, respectively, and owned by the same clan who were moving together and were driven to the Amibara woreda along the route of the Awash river in search of pasture and water in the dry seasons. On the other hand, the 544 camels examined in Amibara woreda belonged to Sheleko, Melka-Werer, Hasoba and Ambash with agro-pastoral and Halaydeghi and Algeta with pastoral farming systems (Table 8).

4.1.1 Serosurvey of brucellosis in camels using RBPT and CFT

A total of 768 camels were sampled and serum samples were collected from camels above six months of age. RBPT identified 91 (11.9%) positive reactors out of 768 serum samples. Further confirmation by CFT showed 58 (63.7%) positive cases out of 91 (11.9%) (Table 8). In this study at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were considered as positive for *Brucella* antibodies and hence, approximately 64% (n = 37) gave a result of at least 2+ and 36% (n = 21) tested at 3+ and 4+.

Table 8: Serosurvey of brucellosis in camels using RBPT and CFT

Woreda	No of PA	No examined	RBPT +ve (%)	CFT +ve out of RBPT +ve (%)
Awash-Fentale	Two	224	26 (11.6%)	14 (53.8%)
Amibara	Six	544	65 (11.9%)	44 (67.7%)
Total		768	91 (11.9%)	58 (63.7%)

4.1.2 Overall seroprevalence of camel brucellosis

Camel brucellosis was detected in both of the study districts with district level prevalence rates ranging from 6.3% (n=224) in Awash-Fentale to 8.1% (n=544) in Amibara district and the overall prevalence of brucellosis in the camel population of the study districts was 7.6% (n=768). It was also shown that antibodies were detected in camels of all the peasant associations studied within each district. The PA level prevalence rates ranged from 4.1% (n=97) to 7.9% (n=127) in Awash-Fentale with higher prevalence in Doho and from 6.6% (n=61) to 9.8% (n=123) in Amibara woreda with higher prevalence in Halaydegghi (Table 9). However, no statistically significant difference ($\chi^2 = 3.20$, $P = 0.872$) was observed in the prevalence of camel brucellosis among the peasant associations of Amibara and Awash-Fentale districts (Table 9).

Table 9: Distribution of seropositivity to camel brucellosis on the basis of PAs of the districts

Woreda	PA	No examined	CFT +ve (%)	χ^2	P-value
Awash-Fentale	Sabure	97	4 (4.1%)	3.20*	0.872
	Doho	127	10 (7.9%)		
	Subtotal	224	14 (6.3%)		
Amibara	Sheleko	132	9 (6.8%)		
	Halaydegghi	123	12 (9.8%)		
	Melka-Werer	82	7 (8.5%)		
	Algeta	78	7 (9.0%)		
	Hasoba	68	5 (7.4%)		
	Ambash	61	4 (6.6%)		
	Subtotal	544	44 (8.1%)		
Total		768	58 (7.6%)		

NB: One cell had expected count less than 5 & hence, it was calculated with Fisher's exact χ^2 test *

4.1.3 Risk factors and seroprevalence

There was no statistically significant difference in the seroprevalence of camel brucellosis between the study districts ($\chi^2 = 0.80$, $P = 0.373$), habitation area ($\chi^2 = 0.96$, $P = 0.328$) and the pastoral and agro-pastoral farming systems ($\chi^2 = 1.35$, $P = 0.245$).

Table 10: Risk factors for the occurrence of seropositivity to camel brucellosis

Risk factors	Category	No examined	CFT positives	% positives	χ^2	P-value	OR (95% CI)
District	Awash-Fentale	224	14	6.3	0.80	0.373	
	Amibara	544	44	8.1			
Habitation area	Urban	311	20	6.4	0.96	0.328	
	Rural	457	38	8.3			
Farming system	Pastoral	328	29	8.8	1.35	0.245	
	Agro-pastoral	440	29	6.6			
Sex	Male	159	9	5.7	1.10	0.295	
	Female	609	49	8.1			
Age	Young	226	-	-	4.98	0.026*	1
	Adult	355	49	13.8			1.70 (1.45-1.95)
	Old age	187	9	4.8			1.52 (1.24-1.72)
Herd size	14-20 (small)	282	-	-	8.47	0.004*	1
	21-40 (medium)	353	54	15.3			1.58 (1.40-1.84)
	>40 (large)	133	4	3.0			1.20 (1.06-1.50)
Contact	With cattle	230	5	2.2	34.74	0.0001*	1
	With goats	219	8	3.7			1.23 (1.11-1.50)
	With cattle & goats	319	45	14.1			1.71 (1.55-5.30)
Parity	No parturition	102	-	-	11.30	0.004*	1
	Single parity	200	17	8.5			1.25 (1.08-2.32)
	More than one	307	32	10.4			3.26 (1.53-6.96)

*Significant at 95% level of significance, NB. Zero values of CFT +ves were replaced with 1 to calculate the OR.

Likewise, there was no statistically significant association ($\chi^2 = 1.10$, $P = 0.295$) between the occurrence of the disease and sex of the study animals (Table 10). Out of 159 male camels examined 9 (5.7%) were seropositive for brucellosis and 121 (76.1%) of them were at puberty age (that is three years and above) at which age they can mate and used for breeding in the herds and none of the immature males (less than three years of age) were positive for the disease.

Out of 609 she-camels examined, 49 (8.0%) were seropositive and 421 (69.1%) of them were at the age of puberty (Table 11). This indicated that the presence of seropositive breeding males and she-camels were considered as risk factors playing a role in the transmission of the disease in the study districts. There was statistically significant difference ($\chi^2 = 4.98$, $P = 0.026$) in seropositivity among the different age groups of camels and significantly higher seroprevalence (13.8%) was recorded in adults of 3 to 7 years of age than in young (0%) and old age camels (4.8%) (Table 10). That is, adults were 1.70 times ($P = 0.026$, $OR = 0.70$, 95% $C.I. = 1.45-1.95$) more at risk of acquiring the disease than youngs and old age camels were 1.52 times ($P = 0.026$, $OR = 1.52$, 95% $C.I. = 1.24-1.72$) more at risk of being seropositive than young camels.

Table 11: Status of sero-positivity to camel brucellosis before and after age of puberty

Age	No examined		Total	CFT +ve (% per sex)		Total +ve (%)
	M	F		M	F	
-Before puberty (< 3 years)	38	188	226	-	-	-
-After puberty (≥ 3 years)	121	421	542	9 (7.4%)	49 (11.6%)	58 (10.7%)
Total	159	609	768	9 (5.7%)	49 (8.0%)	58 (7.6%)

M= male, F = female

Age was also classified as 'before and after puberty' in order to see the distribution of the disease in immature and sexually matured camels. There were no seropositive camels below the age of puberty (< 3 years). All positive animals were above three years of age (Table 11) and hence, age was one of the potential risk factor for the occurrence of the disease (Table 10). Herd size was classified into three categories (small: 14 - 20, medium: 21- 40 and large: > 40 animals) as judged with respect to personal wealth of the herdsman and local herding condition context. Relatively small numbers of individual camels were included in the sample size from large herd sizes and there was statistically significant difference ($\chi^2 = 8.47$, $P = 0.004$) in the occurrence of the disease among the different categories of herd size.

The camels within medium herd size (21-40 individual camels) were 1.58 times ($P = 0.004$, $OR = 1.58$, 95% C.I. = 1.40-1.84) more at risk of being seropositive to brucellosis than the camels in small herd size (14-20) while individual camels within large herd size (> 40 camels) were 1.2 times ($P = 0.004$, $OR = 1.20$, 95% C.I. = 1.06-1.50) more at risk than camels within small herd size. The seroprevalence of the medium and large herd size was 15.3% and 3%, respectively.

There was also highly statistically significant difference ($\chi^2 = 34.74$, $P = 0.0001$) in the prevalence of the disease in these camel population which made contact with ruminants. Those camel herds which usually made close contact on pasture with goats and cattle were 1.71 times ($P = 0.0001$, $OR = 1.71$, 95% C.I. = 1.55-5.30) more at risk of being seropositive to the disease than those which made contacts with cattle while those camel herds which made contacts or rearing with goats were 1.23 times ($P = 0.0001$, $OR = 1.23$, 95% C.I. = 1.11-1.50) more at risk of acquiring the infection than those camel herds which made contacts or rearing with cattle. Higher seroprevalence was observed in the camel population which made close contact with cattle and goats (14.1%) on pasture than either with goats (3.7%) or with cattle (2.2%) (Table 10).

Likewise, there was statistically significant association ($\chi^2 = 11.30$, $P = 0.004$) between parity and the seroprevalence of the disease. Those she-camels with the history of more than one parity were 3.26 times ($P = 0.004$, $OR = 3.26$, 95% C.I. = 1.53-6.96) more at risk of being seropositive to *Brucella* infection than those with no parturition and those she-camels with single parity were 1.25 times ($P = 0.004$, $OR = 1.25$, 95% C.I. = 1.08-2.32) more at risk of being seropositive than those did not give birth (with no history of parturition). Higher prevalence was recorded in the she-camels which gave birth to more than one calf (10.4%) than those with single parity (8.5%) and no seropositivity was recorded in those she-camels with no-parturition (0%) (Table 10).

4.1.4 Frequency of abortion and seroprevalence

During the data collection, female camels were found with a history of abortion at the second half and last stage of pregnancy (gestation period 365-395 days). Of the total 432 she-camels, 46 (10.6%) had aborted and among which 10 (21.7%) were positive for brucellosis.

There was higher seropositive and aborted she-camels in Awash-Fentale district than in Amibara with 7 (41.2%) and 10 (21.7%), respectively. The highest seropositive aborted she-camels was recorded in Doho and followed by Sabure and Ambash with equal infection rate of 28.6% (Table 12).

Of the 159 breeding males in the herds of the camel population of the study areas, 9 (5.7%) were found seropositive to brucellosis. Among which, 3 (6.4%) and 6 (5.4%) were found in Awash-Fentale and Amibara districts, respectively. Higher seropositive breeding males were observed in Melka-Werer and Halaydeghi which logically contribute to the occurrence of the disease.

Table 12: Distribution of seropositive breeding males and aborted she-camels

Location		Males		Females		Aborted females	
District	PA	Total No	+ve (%)	Total No	+ve (%)	Total No	+ve (%)
Awash-Fentale	Sabure	20	1 (5.0%)	77	3 (3.9%)	7	2 (28.6%)
	Doho	27	2 (7.4%)	100	8 (8.0%)	10	5 (50.0%)
	Subtotal	47(21%)	3 (6.4%)	177	11 (6.2%)	17(9.6%)	7 (41.2%)
Amibara	Sheleko	27	1 (3.7%)	105	8 (7.6%)	11	2 (18.2%)
	Halaydeghi	25	2 (8.0%)	98	10 (10.2%)	12	3 (25.0%)
	Melka-Werer	17	2 (11.8%)	65	5 (7.7%)	5	1 (20.0%)
	Algeta	16	1 (6.3%)	62	6 (9.7%)	5	1 (20.0%)
	Hasoba	14	0	54	5 (9.3%)	6	1 (16.7%)
	Ambash	13	0	48	4 (8.3%)	7	2 (28.6%)
	Subtotal	112	6 (5.4%)	432	38 (8.8%)	46(10.6%)	10 (21.7%)
Total		159	9 (5.7%)	609	49 (8.0%)	63(10.3%)	17 (27.0%)

The frequency of abortion was recorded as once and twice (Table 13). Among the 17 aborted she-camels in Awash-Fentale district, 12 (70.6%) were aborted once and 5 (29.4%) were cases of she-camels aborted twice. Similarly, out of the 46 aborted she-camels in Amibara woreda, 38 (82.6%) were aborted once and 8 (17.4%) were aborted twice. Among the 63 (10.3%) of aborted cases of she-camels in the study districts (Table 12), 50 (79.4%) aborted once and 13 (20.6%) aborted twice (Table 13). Highest abortion rate was observed in Awash-Fentale district despite its camel population included in the study.

Table 13: Frequency of abortion of she-camels in the study peasant associations

Location		Aborted females	Frequency of abortion	
District	PA	Total No	No aborted once (%)	No aborted twice (%)
Awash-Fentale	Sabure	7	5 (71.4%)	2 (28.6%)
	Doho	10	7 (70%)	3 (30%)
	Subtotal	17	12 (70.6%)	5 (29.4%)
Amibara	Sheleko	11	9 (81.8%)	2 (18.2%)
	Halaydegghi	12	10 (83.3%)	2 (16.7%)
	Melka-Werer	5	4 (80%)	1 (20%)
	Algeta	5	4 (80%)	1 (20%)
	Hasoba	6	6 (100%)	0 (0%)
	Ambash	7	5 (71.4%)	2 (28.6%)
	Subtotal	46	38 (82.6%)	8 (17.4%)
Total		63	50 (79.4%)	13 (20.6%)

4.1.5 Geographical distribution of camel brucellosis

The secondary objective of this study was to assess the geographical distribution of camel brucellosis in relation to the farming systems of the peasant associations of the districts. As can be seen from Table 14, relatively higher percentages of seropositivity to camel brucellosis were observed in those locations with pastoral type of farming system.

The infection rates of camel brucellosis in the pastoral peasant associations were 12 (9.8%) and 7 (9.0%) in Halaydeghi and Algeta of the Amibara district, respectively, and 10 (7.9%) in Doho of the Awash-Fentale district (Table 14). Therefore, relatively higher seropositivity to the disease was observed in that livestock-owning community which was practicing pastoral type of farming system.

Table 14: Geographical distribution of camel brucellosis in relation to the type of farming system

Woreda /district/	PA	No of camels sampled	Farming system	CFT +ve (%)
Awash-Fentale	Sabure	97	Agro-pastoral	4 (4.1%)
	Doho	127	pastoral	10 (7.9%)
	Subtotal	224		14 (6.3%)
Amibara	Sheleko	132	Agro-pastoral	9 (6.8%)
	Halaydeghi	123	pastoral	12 (9.8%)
	Melka-Werer	82	Agro-pastoral	7 (8.5%)
	Algeta	78	pastoral	7 (9.0%)
	Hasoba	68	Agro-pastoral	5 (7.4%)
	Ambash	61	Agro-pastoral	4 (6.6%)
	Subtotal	544		44 (8.1%)
Overall Total		768		58 (7.6%)

4.2 Prevalence of brucellosis in human

During the study period a total of 200 humans; 97 from Awash-Fentale, 82 from Amibara and 21 from Dulecha woredas have been attended the Awash-Sebat and Melka-Werer health centers. Patients with febrile illness presenting with clinical signs and symptoms resembling brucellosis were included in the study. After the purpose of the study was explained and consent (Annex E) to participate in the study was obtained from the patients or their parents, they were interviewed by the medical staff using a structured questionnaire (Annex D) to collect demographic, epidemiological and clinical data and blood samples were obtained.

Screening of serum samples by RBPT identified 32 (16%) positive reactors out of 200 sera samples. Further confirmation by CFT showed 30 (15%) positive cases out of 32 (16%) (Table 15). In this study at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were considered as positive for *Brucella* antibodies. The district level prevalence rates of human brucellosis were: 18 (18.6%), 9 (11%) and 3 (14.3%) in Awash-Fentale, Amibara and Dulecha, respectively. Therefore, out of the 200 human blood samples, 30 were seropositive to *Brucella* antibodies and hence, the overall prevalence rate of human brucellosis was 15% (Table 15).

Table 15: Serosurvey in human patients of different districts using RBPT and CFT

Patient origin	No examined	RBPT +ve (%)	CFT +ve (%)
Awash-Fentale	97	19 (19.6%)	18 (18.6%)
Amibara	82	9 (11%)	9 (11%)
Dulecha	21	4 (19.1%)	3 (14.3%)
Total	200	32 (16%)	30 (15.0%)

$\chi^2 = 2.05$, P-value = 0.360

No statistically significant difference was observed in the prevalence of human brucellosis among the origin of the patients ($\chi^2 = 2.05$, P = 0.360). This means that there was no association between the occurrence of the disease and the origin of the patients from different districts of different geographical location. Relatively higher distribution was observed in Awash-Fentale and followed by Dulecha district. Patients coming from different peasant associations of different pastoral districts had about the same chance of acquiring the infection.

Table 16: Distribution of seropositivity on the basis of sex and patient origin

Patient origin	No examined	Sex		RBPT +ve		CFT +ve	
		Male	Female	Male	Female	Male	Female
Awash-Fentale	97	38	59	8	11	8 (8.3%)	10 (10.3%)
Amibara	82	44	38	8	1	8 (9.8%)	1 (1.2%)
Dulecha	21	14	7	3	1	2 (9.5%)	1 (4.8%)
Total	200	96	104	19	13	18 (9.0%)	12 (6.0%)

Patients were stratified according to the duration of illness in acute (<6 months of illness), subacute (6-12 months) and persistent (>12 months). Of the 30 (15%) seropositive patients, 9 (30%) were acute, 4 (13.3%) subacute and 17 (56.7%) had persistent disease (Table 18).

Table 18: Seropositivity to brucellosis in patients according to stage of illness and patient origin

Patient origin	No of seropositive patients in each stage of illness (%)			Total
	Acute	Subacute	Persistent	
Awash-Fentale	6 (33.3%)	3 (16.7%)	9 (50.0%)	18
Amibara	2 (22.2%)	-	7 (77.8%)	9
Dulecha	1 (33.3%)	1 (33.3%)	1 (33.3%)	3
Total	9 (30%)	4 (13.3%)	17 (56.7%)	30

Among the 200 patients, 60 (30%) were considered acute, 27 (13.5%) subacute and 113 (56.5%) had persistent disease (Table 19). All seropositive patients from the study districts suffered from headache, weakness, joint pains (arthralgia) and back pain. Muscle pains (myalgia) and bone pain were other prominent symptoms but of this only back pain was observed slightly more often among seropositive patients compared with seronegative patients. However, different clinical findings were recorded among the stages of illness of seropositive and seronegative patients.

Table 19: Clinical cases of non-brucellosis and seropositivity to brucellosis in patients according to stage of illness

Test result	No of patients in each stage of illness (%)			Total
	Acute	Subacute	Persistent	
Seropositive	9 (4.5%)	4 (2%)	17 (8.5%)	30 (15%)
Seronegative	51 (25.5%)	23 (11.5%)	96 (48%)	170 (85%)
Total	60 (30%)	27 (13.5%)	113 (56.5%)	200 (100%)

Majority of the patients were suffering from persistent and acute clinical ailments with overall proportion of 113 (56.5%) and 60 (30%), respectively (Table 19). This means that many people were suffering from a disease of unknown etiology and health effects.

There was statistically significant difference ($\chi^2 = 28.41$, $P < 0.0001$) in the prevalence of human brucellosis between the pastoral and agro-pastoral communities where the patients came from. Those patients who came from agro-pastoral areas were 2.1 times ($P < 0.0001$, OR = 2.1, 95% C.I. = 1.70-6.30) more at risk of being seropositive to *Brucella* antibodies than those from pastoral areas. Of the 30 (15%) seropositive patients, 4 (13.3%) were from pastoral and 26 (86.7%) were from agro-pastoral areas (Table 20).

On the other hand, there was no statistically significant difference ($\chi^2 = 0.12$, $P = 0.662$) in the prevalence of human brucellosis between the urban and rural society (Table 20). This means that there was no difference in the occurrence of the infection in the human population of the urban and rural residential areas of the study districts.

Table 20: Seropositivity to human brucellosis and spatial risk factors

Risk factors	Test result		Total (%)	χ^2	P-value	OR (95% CI)
	Positive	Negative				
<i>Farming system</i>						
-Patients came from pastoral areas	4	41	45 (22,5%)	28,41	<0,0001	2.1 (1.70-6.30)
-Patients came from agro-pastoral areas	26	129	155 (77.5%)			1
<i>Residence area</i>						
- Urban	17	89	106 (53%)	0.12	0.662	
- Rural	13	81	94 (47%)			

The occurrence of human brucellosis in those with the ownership of livestock or milking camels was 2.3 times ($P = 0.001$, OR = 2.3, 95% C.I. = 1.78 -8.35) more at risk of being seropositive to the disease than in those who don't have milking camels or other animals at all.

The likelihood of occurrence of seropositivity to human brucellosis was 1.2 times (0.0003, OR = 1.20, 95% C.I. = 1.58 -2.24) more in those patients who consumed raw milk, cheese and meat than did not (Table 21).

Table 21: Analysis of potential risk factors from questionnaire survey of qualitative nominal categories related to human brucellosis

Risk factors	No of Non-brucellosis cases (%) (N= 200)	No of Seropositive to Brucellosis (%)	P-value	OR (95% CI)
Ownership of livestock or milking camels	145 (72.5%)	28 (14%)	0.001*	2.30 (1.78 -8.35)
Keep animals at yard	46 (23%)	9 (4.5%)	0.115	1.28 (0.61-2.64)
Keep animals in pasture	143 (71.5%)	23 (11.5%)	0.588	1.21 (0.55 -2.75)
Cleaning animals	109 (54.5%)	19 (9.5%)	0.441	0.77 (0.37 -1.59)
Grooming livestock	109 (54.5%)	23 (11.5%)	0.003*	1.17 (1.58 -2.32)
Milking animals	107 (53.5%)	23 (11.5%)	0.062	1.04 (0.51 -2.11)
Assisting parturition	100 (50%)	19 (9.5%)	0.0001*	1.20 (1.60 -2.29)
Slaughtering livestock	72 (36%)	16 (8%)	0.264	1.00 (0.48-2.50)
Consumption of raw camel milk	73 (36.5%)	21 (10.5%)	0.030*	1.1 (1.51 -2.12)
Consumption of raw cattle/goat milk	132 (66%)	25 (12.5%)	0.025*	1.1 (1.47 -2.88)
Consumption of fresh cheese	71 (35.5%)	13 (6.5%)	0.0032*	1.40 (1.68-2.76)
Consumption of raw meat	103 (51.5%)	22 (11%)	0.0347*	1.03 (1.50 -2.08)
Consumption of raw milk, cheese and meat	160 (80%)	30 (15%)	0.0003*	1.20 (1.58 -2.24)

* Statistically significant association at 95% CI

Keeping of livestock in close contact at yard and in pasture, consumption of raw camel milk, assisting animals during parturition and grooming livestock were potential risk factors which contributed to the occurrence of human brucellosis in the camel or livestock rearing communities of the study districts. In general, the human population in the study districts were exposed to the risk of acquiring the disease due to the presence of the explanatory 'yes' or 'no' type categorical variables given in Table 21.

4.3 Results of questionnaire survey on camel herders

The objective of the questionnaire survey was to determine the camel management practices and feeding habit of the herdsman and identify the potential risk factors which can inevitably associated with the occurrence of the disease in humans and animals within the society of the pastoral and agro-pastoral areas. In order to achieve a full understanding of the distribution of the disease, the questionnaire survey was conducted to assess the potential risk factors by interviewing camel herders. A total of 113 respondents (all males) were included in the survey from the study districts. The data collected from the questionnaire survey were analyzed by using descriptive statistics (percentages).

The results of the questionnaire survey are summarized in Table 22-24. The results of questionnaire investigation included possible risk factors (Table 23) related to the occurrence of the disease. Out of the 113 respondents, 36 (32%) had previous and current disease history and were willing to visit the health centers and were serologically tested for *Brucella* antibodies and 8 (7.1%) were seropositive to human brucellosis (Table 22).

Table 22: Number of respondents in each PAs of the study districts and seropositivity to human brucellosis

District /woreda/	PA	No respondents	No of willing & with disease history	RBPT +ve	CFT +ve (%)
Awash-Fentale	Sabure	14	6	1	0
	Doho	21	9	2	1 (7.1%)
	Subtotal	35	15	3	1 (2.9%)
Amibara	Sheleko	21	7	3	2 (9.5%)
	Halay deghi	13	3	2	1 (7.7%)
	Melka-Werer	12	4	1	1 (8.3%)
	Algeta	12	2	2	1 (8.3%)
	Hasoba	10	2	1	1 (10%)
	Ambash	10	3	1	1 (10%)
	Subtotal	78	21	10	7 (9%)
	Total	113	36 (32%)	13	8 (7.1%)

These seropositive herders were in fact summed up with the 200 clinical patients in the health centers and their overall percentages and distribution of human brucellosis in each of the study districts is given above in 6.2 under the 'Prevalence of brucellosis in human'. Table 22 is only to show the proportion of seropositives among the respondents who had previous disease history and willing to get the diagnosis in relation to the total number of respondents or camel herders involved in the questionnaire survey.

Table 23: Seropositivity of respondents on the basis of different categories

Categories	No respondents (% from N= 113)	Seropositives (%)
District/woreda		
Awash-Fentale	35 (31%)	1 (2.9%)
Amibara	78 (69%)	7 (9%)
Farming system		
Pastoral	46 (41%)	3 (6.5%)
Agro-pastoral	67 (59%)	5 (7.5%)
Types of livestock kept		
Camels	32 (28%)	2 (6.3%)
Camels and goats	51 (45%)	3 (5.9%)
Camels, goats and cattle	30 (27%)	3 (10%)
Herd size		
14-20	29 (26%)	1 (3.5%)
21-40	60 (53%)	4 (6.7%)
>40	24 (21%)	3 (1.3%)
Age		
18-40 years	86 (76%)	7 (8.1%)
>40 years	27 (24%)	1 (3.7%)
Education level		
Literate	19 (17%)	-
Illiterate	94 (83%)	8 (8.5%)

Most (59%) of the respondents were from agro-pastoral areas and majority (45%) of them owned camels and goats and 28% of them owned only camels while the remaining 27% owned mixed livestock (camels, cattle and small ruminants) (Table 23).

Potential risk factors commonly practiced by the study population such as assisting with parturition, slaughtering of animals, involving in milking of animals and consumption raw milk and meat were the major points of interest which have got positive response by 93-98% of the respondents (Table 24).

Table 24: Major risk factors related to seropositivity to human brucellosis

Risk factors	No of respondents (N= 113)	%
Raw milk consumption	111*	98%
Assist animals during delivery	105	93%
Slaughtering livestock	107	94.7%
Raw meat consumption	107	94.7%
Involved in milking of animals	111*	98%

N = total number of respondents

Of the 113 respondents, only two persons* were not drinking raw camel milk and never involved in milking of animals starting from few years after their marriage. Both of them were in the same age group, > 40 years, and clan named as 'Ga'as'. There is a surprising social custom of this clan to completely cease drinking camel milk and even milking of camels if an adult man is found sterile. They are considered as cursed persons who received strong punishment from Allah and hence, they can rear camels but never drink the milk and milk products. List of *Brucella* seropositive patients were sent to their corresponding health centers with their personal information to immediately start the therapeutic recommendation of the medical persons.

4.4 Isolation and identification of *Brucella*

All she-camels (n = 609) which were included in the study were numerically marked deliberately on their lower neck and tail area with a liquid marking pink ink which was helpful to specifically know the seropositive she-camels in the herds. In order to attempt the isolation, milk samples were collected from seropositive milking-camels by revisiting the pastoral and agro-pastoral camel herds. Dried or non-lactating she-camels were excluded despite their seropositivity. Out of 49 seropositive she-camels, 34 (69.4%) were milking camels whereas 15 (30.6%) were non-lactating she-camels (Table 25).

Table 25: Number of milk samples among the seropositive she-camels of the study districts

District	Seropositive & milking camels	Seropositive but Non-lactating	Total seropositive she-camels
Awash-Fentale	8	3	11
Amibara	26	12	38
Total	34	15	49

Milk samples were, therefore, taken only from the lactating ones. The milk samples were centrifuged at 4000 gravity for 10 minutes and the cream was spread on *Brucella* medium. Thirty four cultures were incubated in duplicate for 7-10 days with and without 5% CO₂ tension at 37°C. Growth of bacterial colonies was observed in all of the 34 plates in both of the incubators, with and without 5% CO₂.

Five plates (2 within the 5% CO₂ tension and 3 in normal incubator) were showing heavy contamination and were discarded. Colonies from 29 plates (11 from normal and 18 from 5% CO₂ incubators) were sub cultured onto other *Brucella* media and growth of pure bacterial colonies were observed. In the presence of sufficient light source: pinpoint, smooth, glistening and translucent colonies were observed in 17 plates (7 from normal and 10 from 5% CO₂ incubators) which were incubated with and without 5% CO₂ tension and streaked on plates of blood agar and MacConkey agar. The isolates (13 plates) producing haemolysis on blood agar and colonies grown on MacConkey agar were eliminated considering non *Brucella* (4 from normal and 9 from 5% CO₂ incubators). The isolates (4 plates: 1 from normal and 3 from 5% CO₂ incubators) suspected for *Brucella* (non-haemolytic on blood agar and failed to grow on MacConkey agar) were subjected to Gram's stain and modified Ziehl-Neelsen (MZN) staining for checking the purity of cultures and morphological characters. Small rods, red-staining Gram-negative organisms were observed under the oil immersion magnification power.

The isolates suspected to be *Brucella* (4 plates) were subjected to agglutination and biochemical tests. The pure colonies from *Brucella* media were found oxidase-positive (*B. ovis* and *B. neotomae* are oxidase negative), catalase-positive, non-motile, purple/pink colour was observed in urea agar slants indicating ability of the isolates to produce urease enzyme.

The SIM media was used to see H_2S production by the isolates but there were no blackening of the strip and hence, inability of the isolates to produce H_2S was confirmed. The Oxidation-Fermentation (OF) test was used to determine the ability of the isolates to metabolize carbohydrates (sterile 10% dextrose, 10% sucrose and 10% lactose) and oxidative and fermentative reactions were observed in the open and paraffin-sealed tubes. *Brucella* is unreactive and does not produce acid on agar containing carbohydrate, and does not ferment lactose and hence, the remaining isolates were then eliminated considering non *Brucella*. In fact the microbes such as *Haemophilus*, *Campylobacter* and *Pasteurella* are unreactive to OF-test but further investigation of these microbes was not the objective of this study. Bacteriological approaches were conducted to attempt the isolation and identification but the target organism was not successfully identified in the seropositive camel milk samples.

5. DISCUSSION

In the waning years of the twentieth century, zoonotic diseases remained uncontrolled. At the threshold of the twenty-first century and today, the emergence or re-emergence of zoonoses takes place worldwide. One step towards their destruction is the killing of infected reservoir animals. The contamination of their products may infect people from a direct or indirect exposure to zoonotic agents. The prevention of human exposure to zoonoses has been the main task of the veterinarian and the livestock rearing society. The control of human and animal brucellosis should be a major national and international priority for medical and veterinary doctors (Beran and Steele, 1994).

Elimination of infected animals by test-and-slaughter to obtain brucellosis free flock/herds and regions is usually accepted that a programme of eliminating brucellosis by test-and-slaughter policy is justified on economic grounds only when the prevalence of infected animals in an area is about 2% or less. For the implementation of such a program it is essential that the flocks are under strict surveillance and movement control. Animals must be individually identified and an efficient and well organised veterinary service for surveillance and laboratory testing must be in place (Alton, 1990; Nicoletti, 1990).

Brucellosis, caused by *Brucella abortus* and *B. melitensis*, remains a worldwide public health problem. These are considered a public health problem largely in countries like Ethiopia where people of all ages are likely to consume milk and milk products. A study of ruminant brucellosis has been done previously in some limited areas but the camel and human prevalence of brucellosis in the country particularly in the camel-rearing pastoral and agro-pastoral areas was unknown. The results obtained in the present study are discussed as follows.

5.1 Camel brucellosis

The study's main objective was to determine the prevalence of camel and human brucellosis in a total sample size of 768 camels and 200 human patients in Awash-Fentale and Amibara districts of the zone 3 of the Afar region, during the study period.

5.1.1 Serosurvey of camel brucellosis using RBPT and CFT

The RBPT, which is based on *B. abortus* antigen, is less sensitive in detecting antibodies against *B. melitensis* (Garin-Bastuji *et al.*, 2006). This study demonstrated that the overall seroprevalence proportion of camel brucellosis in the study districts was 11.9% by the RBPT and 7.6% by CFT. Of the camels that tested positive by CFT, 64% gave reactions of at least 2+ and 36 % gave very strong reactions (3+ and 4+).

Since none of the animals under study was vaccinated, this seems to reveal a moderate prevalence and natural transmission of *Brucella* organisms in the study areas. Less than half of the sera which tested positive for anti-*Brucella* antibodies by RBPT, tested negative by CFT. This could be due to cross-reactions between *Brucella* and other bacteria which share similar epitopes. Cross-reacting bacteria such as *Escherchia coli*, *Yersinia enterocolitica* and *Salmonella serotypes* (Cloeckaert *et al.*, 1992; Garin-Bastuji *et al.*, 1999) have potential to affect serological findings when tests of low specificity are used.

On the other hand, the immuno-suppressive effects of trypanosomiasis, often prevalent in where camels are raised were reported in vaccinated cattle and goats implying possible impact on serological findings (Chukwu, 1985). Ouma *et al.* (1997) have observed classical pass way hemolytic complement activity was negatively correlated with parasitaemia, indicating immuno-suppression by trypanosomiasis. Sample selection bias also might affect serological findings (Ajogi and Adamu, 1998). It is important to note that slide agglutination test and tube agglutination tests have been shown to have poor diagnostic sensitivity compared to RBPT or card test (Alton *et al.*, 1975; Quinn *et al.*, 2004). Accordingly, RBPT is considered as satisfactory screening test (Nicoletti, 1992; OIE, 2004; Quinn *et al.*, 2004).

The highest specificity of CFT permits one to use the test as confirmatory test in serial testing (OIE, 2004). Therefore, the use of serial testing procedure initially screened all samples by RBPT, and then applying CFT on positive reactors as employed in the current test improves the efficiency of detecting brucellosis. Improvement of test diagnostic specificity is particularly useful in control programs when test and slaughter policy is adopted (Dohoo *et al.*, 1985; Teshome *et al.*, 2003).

5.1.2 Overall seroprevalence of camel brucellosis

The 7.6% prevalence of brucellosis in camels observed by CFT in the study is in accord with the results of many previous observations of different countries, including studies in Kenya by Kagunya and Waiyaki (1978) and by Wilson *et al.* (1990) who reported prevalence rates of 4.6-10.3 and 6.0-38.0, respectively, and in Sudan by Osman and Adlam (1987) who reported a prevalence of 8.0% (Table 2). However, the result in this study is greater than the result recorded by Bekele (2004), Teshome *et al.* (2003) and Domenech (1977) in Borena, Oromia region with prevalence rates of 0.4-2.5%, 4.2% and 4.4%, respectively, and by Rechard (1980) who reported prevalence rates of 5.5% in Afar region and in other camel-rearing areas of Ethiopia.

On the other hand, the result of this study is less than the result recorded by Kebede (2010) in Fentale district of the Oromia region with prevalence rate of 9.1%, in Somalia by Andreani *et al.* (1982) and Elmi (1982) who reported prevalence rates of 10.4% and 12.6%, respectively. This difference could be due to differences in the sample size and the tests used. Elmi (1982) used the enzyme-linked immunosorbent assay (ELISA) to detect antibodies against *Brucella*. The author of the present study used the RBPT as a screening test and the CFT as the confirmatory test. The RBPT, which is based on *B. abortus* antigen, is less sensitive in detecting antibodies against *B. melitensis* (Garin-Bastuji *et al.*, 2006). The difference could also be due to variations in animal management and production systems. Kenya and Sudan are characterised by mixed farming (Wilson *et al.*, 1990; Schwartz and Dioli, 1992) in which fewer animals are raised and they are raised separately, whereas in the camel-rearing areas of Ethiopia, large numbers of different species of animals are raised on communal pastures and watering areas.

5.1.3 Risk factors and seroprevalence

There was no statistically significant difference in the infection rate of camel brucellosis between the study districts ($\chi^2 = 0.80$, $P = 0.373$) and the pastoral and agro-pastoral farming systems ($\chi^2 = 1.35$, $P = 0.245$) (Table 10). Hence, the difference in districts and type of farming system used in the camel-rearing areas were not potential risk factors for the occurrence of the disease.

The seroprevalence of brucellosis in camels is lower in extensively kept agro-pastoralist camels than camels kept by pastoralists. Thus, prevalence ranging from 2 to 8.5% were reported from most countries where camels are produced by agro-pastoralists (Abbas and Agab, 2002; Wernery and Kaaden, 2002). This is therefore, in agreement with the prevalence rates of the disease, ranging from 4.1 to 8.5%, in the agro-pastoral areas of the present study (Table 14). On the other hand, it was reported as high as 8 to 15% in intensively kept camels especially in Saudi Arabia (Radwan *et al.*, 1992) and Kuwait (Al-Khalaf and El-Khaladi, 1989).

Even though there was no statistically significant difference, there was slight observable variation in the prevalence rates between the farming systems. The present study showed that prevalence rate of 7.9 to 9.8% in the pastoral areas of the study districts (Table 14) and hence, it is in accord with these previous studies in Saudi Arabia and Kuwait. In most previous studies, pastoralists are of the same clan and move in group in search of pasture and water. In such farming system, large herd size together with overcrowding in restricted watering area provide more chances of contact between animals leading to increased likelihood of infection.

Of the male camels examined 9 (5.7%) were seropositive for brucellosis and 121 (76.1%) of them were at puberty age (that is three years and above) at which age they can mate and used for breeding in the herds and none of the immature males (less than three years of age) were positive for the disease. Likewise, out of 609 she-camels examined 49 (8.0%) were seropositive and 421 (69.1%) of them were at the age of puberty (Table 11). This indicated that the presence of seropositive breeding males and she-camels were considered as risk factors playing a role in the transmission of the disease in the study districts. Seropositivity to camel brucellosis was seen in adults than in young camels as it is a disease of sexually matured animals.

No significant difference was observed in the prevalence of brucellosis between sex (Table 10). Larger proportions of females in herds of both districts indicate a strong desire of herdsmen to maximize their herd size. The number of breeding males in the camel herds of the present study was very small on which random sampling method was applied and which inevitably bias the statistical analysis. Even though it is difficult to draw a firm conclusion, due to the smaller sample of males, the lack of difference between the two sexes observed in this study contradicts the established facts. Hirsh and Zee (1999) have reported that male animals are less susceptible to *Brucella* infection, due to the absence of erythritol.

However, in support of the present findings, other authors reported equal distribution of *Brucella* antibodies between both sexes (Waghela *et al.*, 1978; Abbas *et al.*, 1987; Abu Damir *et al.*, 1984). Moreover, Ashenafi *et al.* (2007) has also reported no observable difference in the prevalence of brucellosis between male and female small ruminants in the Afar region.

On the contrary, reports from Ethiopia (Borena) (Bekele, 2004), Sudan (Yagoub *et al.*, 1990; Agab *et al.*, 1994) and Nigeria (Ajogi and Adamu, 1998) revealed the likelihood of occurrence of infection is higher in females than male animals. Relatively higher susceptibility of she-camels could be due to the fact that they have more physiological stresses (Walker, 1999). Okoh (1979) reported that male animals had higher infection rate but the sample size of females was too small to make the comparison. There was statistically significant difference ($\chi^2 = 4.98$, $P = 0.026$) in seropositivity among the different age groups of camels and significantly higher infection rate (13.8%) was recorded in adults of 3 to 7 years of age than in young (0%) and old age camels (4.8%) (Table 10). This result is, therefore, in consistent with the study by Bekele (2004) that revealed higher seropositivity to camel brucellosis in adults than in young animals. It has been reported that brucellosis is essentially a disease of sexually mature animals (Quinn *et al.*, 2004).

Sexually mature and pregnant animals are more prone to *Brucella* infection and brucellosis than sexually immature animals of either sex (Radostits *et al.*, 2000). On the other hand, it is also true that younger animals tend to be more resistant to infection and frequently clear an established infection, although latent infections can occur (Walker, 1999). This may result from the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity (Radostits *et al.*, 2000). The presence of growth factors such as erythritol and hormones favour infection in mature animals (Walker, 1999; Quinn *et al.*, 2004).

Camels produced under extensive production system reach maturity at 3 - 4 years of age (Wilson, 1998). Tefera and Gebreab (2001) recorded age at puberty and first calving to be 4 and 5 years, respectively for females whereas males had age of 5 years at puberty in eastern Ethiopia. Wossene (1991) also reported the same age for puberty and first calving in Ogaden female dromedaries. Accordingly, age was classified as 'before and after puberty' in order to see the distribution of the disease in immature and sexually matured camels and camels 3 years and above considered mature (adult) and less than 3 years considered sexually immature for

this study. Statistically significant difference was observed between young, adult and old aged camels ($\chi^2 = 4.98$, $P = 0.026$); the probability of disease occurrence being 1.70 times more in adults than in young camels and old age camels were 1.52 times more at risk than young camels (Table 10). There were no seropositive camels below the age of puberty (< 3 years). All positive animals were above three years of age (Table 11) and hence, age was one of the potential risk factor for the occurrence of the disease.

In the recent study herd size was classified into three categories (small: 14 - 20, medium: 21-40 and large: > 40 animals) on the basis of personal wealth of the herdsman and local herding condition context. Some camel herders replied 'I am too poor!' to the question 'how many camels do you have?' and this indicated that they consider as if they have nothing if they have 14 to 20 camels and hence, this was taken as the basis for the further stratification of herd size as small, medium and large. Small number of herds were containing large herd size (> 40 camels) and this is because of the presence of sudden camel mortalities due to unknown causes and hence, relatively small number of individual camels were included in the sample size and there was statistically significant difference ($\chi^2 = 8.47$, $P = 0.004$) in the occurrence of the disease among the different categories of herd size. The seroprevalence of the medium and large herd size was 15.3% and 3%, respectively (Table 10).

Bekele (2004) reported that the within herd seroprevalence of brucellosis in large herd size was 2.7% which is comparable with the present study. Teshome *et al.* (2003) recorded lower prevalence, 1.2%, in small herd size from Borena which seem in disagreement with the current finding which is 0% in small herd size and 15.3% in the medium herd size. As herd size increases, the chance of contact between animals increases leading to more chances of infection (Abbas and Agab, 2002), which is particularly more important during calving or abortion when most of brucellosis contamination occur (Gameel *et al.*, 1993; Agab *et al.*, 1994). Thus, herd size and density of animal population together with poor management are directly related to infection rate (Abbas *et al.*, 1987; Abu-Eisha, 2000; Wernery and Kaaden, 2002).

High number of camels, cattle and goat diversification were observed in the study districts. Such animal species distribution and diversification is common to other areas and has economic and ecological advantages (Wilson *et al.*, 1990; Getahun and Kassa, 2000).

However, it increases the chance of brucellosis and other disease transmission from other infected ruminants to dromedaries (Andreani *et al.*, 1982; Radwan *et al.*, 1992). In the present study, there was highly statistically significant difference ($\chi^2 = 34.74$, $P = 0.0001$) in the prevalence of the disease in the camel population which made contact with other livestock. Those camel herds which usually made close contact on pasture with goats and cattle were 1.71 times more at risk of being seropositive to the disease than those which made contacts with cattle while those camel herds which made contacts or rearing with goats were 1.23 times more at risk of acquiring the infection than those camel herds which made contacts or rearing with cattle (Table 10). Higher seroprevalence was observed in the camel population which made close contact with cattle and goats (14.1%) on pasture than either with goats (3.7%) or with cattle (2.2%). It can be concluded that ruminants had a role in the transmission of the disease to the camel population.

Amibara district is a pathway for pastoralists during their migrations for pasture and water to their animals and this woreda is endowed with several irrigation points as it is divided by the Awash River Valley and non-governmental cotton farms are found which attracts the pastoralists to feed their camels after the cotton is being harvested (Senait *et al.*, 2009) and hence, a large number of livestock herds are congregated at this cotton farm and other water points which facilitates the spread of disease. Traditional wells, ponds and Awash River are major permanent water sources in the area where animals come to close each other. Mixed herding and frequent contact with small ruminants and cattle are contributing factors to infection rate. Herders in Afar and elsewhere invariably keep small ruminants and cattle alongside with camels. There is high chance of brucellosis transmission from these ruminants to dromedaries as they live in free range in contact in the bush and at water points (Andreani *et al.*, 1982).

Specially, contact between dromedaries and small ruminants were more incriminated for the transmission of brucellosis to camels (Radwan *et al.*, 1992). Abou-Eisha (2000) also observed higher seroprevalence in camels that were in contact with sheep and goat. Moreover, higher frequencies of *B. melitensis* isolation from camels perhaps magnify the role of small ruminants in the transmission of brucellosis to camels. The seroprevalence status of brucellosis in Afar goats was reported to be 5.8% (Ashenafi, 2007) from Afar pastoral and agro-pastoral areas. This indicates the potential role of goats, and cattle possibly sheep in the transmission of the disease to camels though there is no information on cattle brucellosis status in the area.

There was statistically significant association ($\chi^2 = 11.30$, $P = 0.004$) between parity and the seroprevalence of the disease. Those she-camels with the history of more than one parity were 3.26 times more at risk of being seropositive to *Brucella* infection than those with no parturition. Those she-camels with single parity were 1.25 times more at risk of being seropositive than those with no history of parturition. Higher infection rate was recorded in the she-camels which gave birth to more than one calf (10.4%) than those with single parity (8.5%) and there was no record of seropositivity in those she-camels with no parity (0%) (Table 10). The present study is therefore, in consistent with the previous study by Bekele (2004) where higher reactor rate was recorded in camels with more than one parity, compared to other group of camels. On the other hand, this study is in disagreement with the findings in Borena low lands (Bekele, 2004) where with single parity had slightly lower reactors than without parturition, where the reverse was expected.

5.1.4 Frequency of abortion and seroprevalence

The clinical manifestations of brucellosis in camels are similar to those in cattle and small ruminants in regard to abortion, stillbirth and reproductive failure. Thus this disease causes heavy economic losses in animal production resulting from abortions, sterility, decreased milk production, and the costs of replacer animals (Ariza *et al.*, 1992; Radostits *et al.*, 2000). During the data collection, female camels were found with a history of abortion at the second half and last stage of pregnancy (gestation period 365-395 days). In the present study, the seropositivity in aborted she-camels of the Awash-Fentale and Amibara districts were 41.2% and 21.7%, respectively.

The frequency of abortion was recorded as 'once' and 'twice'. In general, there was statistically significant association ($\chi^2 = 17.92$, $P = 0.001$) between the presence of seropositive breeding males and the occurrence of abortion in she-camels in the herds of the study districts (Table 12). Higher abortion rate was observed among seropositive breeding female camels by Teshome (2003) and Bekelle (2004) in the low land pastoral areas of Borena and it is in concurrence with the present study which showed 27% were seropositive among the 63 (10.3%) cases of abortions.

Due to the higher percentage of seropositives in aborted cases in this study, it may be possible to suggest that those clinical manifestations or abortions were due to brucellosis. Getahun and Kassa (2000) reported annual abortion and stillbirth rates of 9% and 4.3%, respectively in camels kept under similar production systems in eastern Ethiopia.

Brucellosis in camelids occurs in all known forms whereby abortion is the most obvious manifestations (Al-Khalaf and El-Khaladi, 1989; Agab *et al.*, 1994; Wernery and Kaaden, 2002). Radwan *et al.* (1992) recorded abortion rate of 12% with 8% seroprevalence. Low seroprevalence report in Somalia by Baumann and Zessin (1992) did not indicate the presence of brucellosis in camels with abortion as clinical manifestations. In extensively produced animals other diseases such as trypanosomiasis may also cause abortion (Wilson, 1998). Brucellosis can generally cause significant economic loss through abortion, late first calving age, long calving interval time, low herd fertility and comparatively low milk production (Chukwu, 1987; Wernery and Kaaden, 2002).

5.1.5 Geographical distribution of camel brucellosis

Camel brucellosis was found to be distributed among the two study districts of the Afar pastoral and agro-pastoral region, and there was no significant difference in prevalence rate among the districts. The disease was detected in all peasant associations investigated. Brucellosis is, therefore, well entrenched across the entire districts. This might be well attributable to the use of similar animal production and management systems of similar agro-ecological conditions throughout the peasant associations of the districts of the Afar region.

Moreover, unrestricted animal movements and contact with other livestock may have enhanced the spread of infection, such as movements of animals in search of pasture and water, trade within and between zones and districts, the mixing of animals at marketplaces, pastures and watering points, such as the Awash River, especially during the dry season. Accordingly, the results of this study indicated that the prevalence of camel brucellosis was higher (7.6%) in the districts of the Afar region where there was frequent mixing of flocks, than in the Somali region where clan-based flock/herd segregation was usual. In this regard, Baumann *et al.* (1990) and Baumann and Zessin (1992) showed that prevalence rates of 3.1% and 0.3-1.9%, respectively.

The absence of hygiene measures, such as the use of isolated calving areas, appropriate disposal of aborted materials and removal of foci of infection, can aggravate the spread of infection as pastures and water become contaminated.

5.2 Prevalence of brucellosis in human

At the beginning of this study, the medical staffs at the health centers were unaware of the existence of this disease in these parts of Afar districts and even none of the medical staffs were knowing such a disease named as 'brucellosis'. In fact there were no senior medical professional in these health centers. After the purpose of the study was explained to the medical staffs, blood samples were collected from 200 clinical patients on the basis of clinical manifestations and 30 were seropositive to *Brucella* antibodies with a confirmatory serological test. Although no single test provides 100% sensitivity and specificity, RBPT and CFT still remains the test of choice in the diagnosis of the disease. In the presence of appropriate signs and symptoms, a presumptive diagnosis of brucellosis was performed serologically and hence, the overall prevalence rate of human brucellosis was 15% (Table 15).

Data from developing countries in the Mediterranean basin, particularly the Middle East, reported seroprevalence rates ranging from 8% in Jordan (Fox and Kaufman, 1977) to 12% in Lebanon and Kuwait (Araj and Azzam, 1996). Even higher seroprevalence rates have been reported in sub-Saharan countries, with percentages of 18% in Uganda (Ndyabahinduka and Chu, 1984) and 13% in Nigeria (Six *et al.*, 1987). The present study is, therefore, about in consistent with these findings in the different countries.

Since there is close contact between humans and their livestock, which sometimes share the same housing enclosures, brucellosis is a significant health risk for the entire community of the study areas. As per the information from medical persons of the health centers at the study districts, there has been high human morbidity and less mortality due to diseases with symptoms such as fever, aches and pains, fatigue and debility. These symptoms were generally considered as malaria, even without any laboratory confirmation, but in fact brucellosis may also be a possible additional cause. Reproductive problems, such as abortion, stillbirth and retained foetal membranes, are the most common reproductive wastage in camels, cattle and small ruminants in the study districts and humans may become contaminated while handling

the infected material. Either brucellosis or malaria could be the cause of human illness in these situations. In brucellosis endemic areas, identifying the population at risk is important to detect unrecognized cases. Different epidemiological and diagnostic strategies have been used to identify cases and have led to a higher incidence of the disease than has been reported (Abramson *et al.*, 1991). In the present study, no statistically significant ($\chi^2 = 2.05$, $P = 0.360$) difference was observed in the prevalence of human brucellosis among the origin of the patients, this means that there was no association between the occurrence of the disease and the origin of the patients from different districts of different geographical location. But the proportion of seropositivity to brucellosis was higher in women and the male-to-female ratio of seropositive patients was 1.5. This is about in agreement with the results of Genene *et al.* (2009) that the male-to-female ratio of seropositive patients of Borena and Hamar was 1.4 but no difference in seropositivity was observed between the two sexes.

In this study, majority of the seropositive females were non-afar tribes. In fact, Afar females of 15-40 years of age are culturally constrained not to drink the camel milk as it is believed to make them sexily, rude and cheeky in behaviour. Nowadays, camel milk is supplied by Afar pastoralist and sold in retailer shops and used by different non-Afar tribes such as Amhara, Argoba, Oromo, Welayta, Tigrean and Hadiya which developed custom of consuming raw camel milk as a substitute of cow milk and as traditional treatment. Therefore, about half of the seropositive females were non-Afar tribes. At present, some of Afar females are changing their customs in spite of their cultural obligations and started consuming raw camel milk.

There was no statistically significant difference ($\chi^2 = 2.15$, $P = 0.3411$) in the seroprevalence of the disease among the age groups. In contrast to this study, observations made in Iran, Jordan, Lebanon, and Kuwait (Araj and Azzam, 1996) shown that the prevalence rate of brucellosis increases with age. All seropositive patients from the study districts suffered from headache, weakness, joint pains (arthralgia) and back pain. Muscle pains (myalgia) and bone pain were other prominent symptoms but only back pain was observed slightly more often among seropositive patients compared with seronegative patients and this finding is similar to that reported by Genene *et al.* (2009). However, different clinical findings were recorded among the stages of illness of seropositive and seronegative patients.

The principle of the test is that IgG fixes the complement well whereas IgM does not. In early stages of the disease when only IgM is present, CFT is usually negative. In acute brucellosis, CFT titres appear in the second month and reach the maximum at the fourth month of the disease. CFT is usually positive in subacute and chronic brucellosis. High titres remain about 12 months (Taleski, 2010). On the basis of this fact, stages of illness was classified as acute (<6 months of illness), subacute (6-12 months) and persistent (>12 months). Majority of the patients were suffering from persistent (chronic) and acute clinical ailments with overall proportion of 113 (56.5%) and 60 (30%), respectively. This means that many people were suffering from a disease of unknown etiology and health effect. There was statistically significant difference ($\chi^2 = 28.41$, $P < 0.0001$) in the prevalence of human brucellosis between the pastoral and agro-pastoral communities where the patients were come from. Those patients who came from agro-pastoral areas were 2.1 times more at risk of being seropositive to *Brucella* antibodies than those from pastoral areas. Of the 30 (15%) seropositive patients, 4 (13.3%) were from pastoral and 26 (86.7%) were from agro-pastoral areas (Table 20).

The occurrence of human brucellosis in those with the ownership of livestock or milking camels was 2.3 times more at risk of being seropositive to the disease than in those who don't have milking camels or other animals at all. There are very few reports of indigenously acquired human cases of brucellosis, while acute imported human infections continue to occur, often associated with the consumption of raw cheese or milk (Taylor and Perdue, 1989). The consumption of contaminated food and an occupational contact remain the major sources of an infection. Small number of incidences reported in brucellosis-endemic areas may reflect a poor standard of surveillance and reporting, even though other factors such as the methods of food preparation, heat treatment of dairy products, and direct contact with animals also influence the risk to the population (Mantur and Mulimani, 1996).

The likelihood of occurrence of seropositivity to human brucellosis in the present study was 1.2 times more in those patients who consumed raw milk, cheese and meat than those who did not. According to the present findings most of patients had a history of raw milk and cheese consumption or direct contact with camels, goats and cattle. Almuneef *et al.* (2004) reported that ingestion of raw milk was considered the likely source of infection among family members and the present study is in accord with it. Keeping of livestock in close contact at yard and in pasture, consumption of raw camel milk, assisting animals during parturition and grooming livestock were potential risk factors which contributed to the occurrence of human brucellosis

in the camel or livestock rearing communities of the present study districts. The medical staff at the health center knew many of these patients for commonly occurring infectious disease such as malaria or had not been able to help these patients. After treatment at these earlier occasions patients either had not shown improvement or had become ill again. Frequent changes and/or periodical absence of medical staff together with poor record keeping however did not allow the investigator to trace back the exact medical history of these patients.

After CFT analysis of the sera samples of humans, the investigator has contacted the medical persons in the health centers of the study districts and submitted the list of all seropositive patients to start the treatment as per the recommendation of medical persons. As per the information from the health centers, those patients were contacted personally, not all, started the treatment and showed significant improvement after antibiotic therapy. The medical persons notified that the available drug for treatment was doxycycline (4 mg/kg per day; maximum, 200 mg/d in two divided doses) orally and this need to be continued for 6 weeks as prolonged therapy is imperative for achieving a cure.

5.3 Results of questionnaires survey on camel herders

The questionnaire surveys were conducted to assess the potential risk factors by interviewing the 113 camel herders of both the pastoral and agro-pastoral areas. As a result of the questionnaire surveys essential risk factors were identified which are associated with the spread of the disease to the animal and human population. The objective of the questionnaire survey was to determine the potential risk factors of the management practices and relate the occurrence of the disease with the social custom of drinking raw milk and relate it with the occurrence of camel and human brucellosis. The camel brucellosis can be a health hazard to human beings particularly to pastoral households who in many ways are exposed to the disease (Abbas and Agab, 2002). Camel owners of the study area consume raw milk and milk products and meat, and do delivery assistance, clean newborns, assist suckling and carry the young from field to home without any protection. The knowledge about brucellosis is nil among herdsmen. These can put the public health of the area at risk. Abou-Eisha (2000) reported 1% (3 out of 330) brucellosis seroprevalence among nomadic people. The disease in man may be misdiagnosed due to the prevailing malaria infections in dry areas (Abou-Eisha, 2000; El-Ansary *et al.*, 2001). Hence, the same phenomenon is observed in the present study that the

disease is misdiagnosed by the available health professionals and even the usual occurrence of malaria infections together with the absolute absence of awareness of this disease. Most of the respondents were from agro-pastoral areas and majority of them owned camels and goats and 28% of them owned only camels while the remaining 27% owned mixed livestock (camels, cattle and small ruminants) (Table 23). Potential risk factors commonly practiced by the study population such as assisting with parturition, slaughtering of animals, involving in milking of animals and consumption raw milk and meat were the major points of interest which have got positive response by the respondents (Table 24).

Well known risk factors including living in close proximity of livestock, keeping and attending to livestock and the consumption of raw milk and fresh cheese were identified as risk factors for having brucellosis by the study areas' people. All the respondents were males which indicate that only adult males are moving long distances in dry seasons with their animals and contacts of animals may pose a higher risk of infection than females, kids and aged ones who usually stay either in their temporary (pastoralist) or permanent (agro-pastoralist) shelter. Precise knowledge of the different risk factors is important for the development of control and preventive measures. For instance, El-Sherbini *et al.* (2007) found that in villages in Egypt keeping sheep but not the consumption of fresh dairy products was associated with brucellosis and suggested that this was related to the relatively low prevalence of the infection.

The questionnaire in this study also, included questions related to knowledge of brucellosis but as expected none of the herders had any knowledge about the cause, prevention and treatment of brucellosis. Health education could help to prevent brucellosis but given the living conditions and dietary habits of these pastoralist communities, control of brucellosis in the livestock population may be the main option to prevent disease. The control of brucellosis in rural and pastoral Ethiopia will be challenging given the mobile lifestyle of the pastoral population, the diversity of farming practices and husbandry systems and the high number and diversity of animals involved. Most of the Awash-Fentale and Amibara districts own camels, cattle, goats and/or sheep, each of which may be infected and transmit brucellosis. For the prevention of zoonoses such as brucellosis and to control other diseases in their livestock improvement of the veterinary services is essential as well. With the growing population pressure to improve the agricultural system is increasing, diseases such as brucellosis are impediments for export and economic growth.

Experience in developed countries has shown that it takes decades to control brucellosis through a control strategy based on vaccination and that the presence of a test and slaughter policy of infected animals could be crucial. In Ethiopia vaccination may become available and the availability of laboratory testing for brucellosis at medical health facilities in rural areas in Ethiopia could help to reduce the risk of severe disease and disability.

5.4 Isolation and identification of *Brucella*

To declare the consumption of raw camel-milk and milk products as the most important risk factors for human brucellosis over the other routes of infection, isolation trial was attempted from milk samples. Brucellosis is diagnosed either by isolation of *Brucella* organism in culture, or by a combination of serological tests and clinical findings consistent with brucellosis. Isolation of the *Brucella* organism is the definitive means of diagnosis, but in practice it is difficult due to the early tissue localization and the exacting culture requirements of the organism. Isolation of *Brucella* species is usually difficult, cultural examinations are time consuming, impractical, hazardous and not sensitive, however the isolation of *Brucella* is considered to be the only unequivocal method for the confirmation (Alton *et al.*, 1988). Thus, clinicians often rely on the indirect proof of infection.

The bacteriological diagnosis of *Brucella* can be made by microscopic examination of stained smears from milk, vaginal swabs, placentas or aborted fetuses. However, morphologically related organisms such as *Chlamydophila abortus* or *Coxiella burnetii* can cause misleading diagnoses. Therefore, isolation of *Brucella* on appropriate culture media is recommended for accurate diagnosis (AL-Garadi *et al.*, 2011). In fact, the cultural detection trial was attempted using *Brucella* medium base supplemented with 5% horse serum, 10% sterile dextrose solution and antibiotics and made to selective medium agar. But the antibiotics available were out-of-date allowing the growth of fungus and some other bacterial colonies. In the present study, the absence of *Brucella* selective media and uptodate antibiotic supplements together with the fact that the bacteria are excreted intermittently in small numbers in milk throughout the lactation and the likelihood of the organism to find in the milk samples taken in one point in time made the isolation trial impossible.

6. CONCLUSION AND RECOMMENDATIONS

Brucella contaminated milk presents a potential threat to the new born camel-calves and human beings as it can spread through ingestion and causes infectious abortion and undulant fever. The present study was undertaken to detect the *Brucella* antibodies in camel and human sera and to isolate *Brucella* organisms in camel milk. The result of the present serosurvey of brucellosis showed that brucellosis is an important cause of ailment in camel and human and well entrenched infection across the selected districts of the south Afar region. Comparatively higher seropositivity (15%) of *Brucella* antibodies was observed in human than in camels (7.6%) by CFT. The traditional husbandry and animal management practices together with the habit of raw camel milk consumption of the Afar tribe support the spread of human and camel brucellosis in the areas. This shows that brucellosis is becoming an impediment to the exploitation of the huge camel population of the pastoral and agro-pastoral districts of the Afar region. Based on the present cross-sectional study, the sero-reactor camels and humans were found widely distributed.

Age, herd size, parity and contact of camels with ruminants were found important risk factors associated with the episode of brucellosis in camels. Lack of awareness about brucellosis, close proximity/contact with livestock/milking camels, habit of consumption of raw milk and milk products, assisting animals during parturition and milking of animals were the potential risk factors associated with human brucellosis. The importance and achievements of this research is made clear by the fact that it records the animal health situation and the prevalence of brucellosis for the first time, thus enabling the authorities to control the disease by providing them with the data of distribution of brucellosis in camels and humans in the south Afar region.

Based on the study results, the following recommendations have been made to reduce the risk of human and animal brucellosis:

- The overall prevalence rate of camel brucellosis observed in the study area may suggest the absence of implementation of test-and- slaughter policy. Therefore, improving management practices can assist in reducing the spread and maintenance of the infection in camel herds.

- Pastoralists are often marginalized far from public services, facilities and information. Thus, awareness (public health education) through extensive extension service on modern animal husbandry and disease prevention is quite necessary.
- Public health centers and local intellectuals should put maximum efforts to gradually change the risky habit of consumption of raw camel-milk. Hence, camel-milk should not be consumed raw despite the social custom of the Afar tribes to consume the camel milk as fresh.
- Diagnosis of brucellosis is often difficult to establish, because of its similarity with clinical presentations of other infections such as malaria. Therefore laboratory testing should be an absolute prerequisite for a proper diagnosis of brucellosis in humans.
- The screening of camel herd owners and family members should be mandatory in order to start early treatment which is likely to decrease the rate of complication and relapse.
- The national animal health department should be strengthened and animal health professionals should be available in the distant pastoral districts and hence, assist and educating local extension personnel's as to the control of the transmission of disease.
- Further epidemiological research work should be conducted that intended on isolation and identification of the biotypes of *Brucella* responsible for infection in Ethiopia. Such investigations have important implications for the type of vaccine that should be used. Vaccination of animals at areas of high prevalence could reduce the incidence of this disease to a significantly low level.

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Annex C: Questionnaire for the survey of camel management practices by camel owners (Questionnaires distributed to 113 camel herders)

Note: This questionnaire is designed for a survey on the potential risk factors to camel brucellosis associated with camel management practices. The data are collected for study purposes only.

Date...../...../.....

I. General information

District PA Village.....

Name of respondent Age Sex.....

Family size.....

Educational level:

Primary School _____ High School _____ Secondary School _____ Graduate _____

Professional training _____ Uneducated _____

Number of experienced years in camel keeping _____ years

II. Comparative importance of camels and its products

1. Types of livestock kept and purpose

Species	More liked (1 st , 2 nd , 3 rd , ...)	Why? (Purpose)	Relative importance	Count
Cattle				
Goats				
Sheep				
Camels				
Equines				

2. Information regarding the study animals in target (camels)

(a) Initial camel origin/Name of the area where from (Location) _____

(b) How many camels are there in a herd? (Herd size) _____ heads.

Female _____ heads.

Male/Bull _____ heads.

Camel calves' _____ heads

Milking she-camels _____ heads.

(c) Do they have an identification number? Yes _____ No _____

(d) If no, do they have a particular name? Yes _____ No _____

3. What is the purpose of camel production?

a. High milk production

b. Drought mitigation

c. Bush encroachment control

d. Herd accumulation e. mixed purposes

4. Rank the use of camels:

a. Milk production

b. Transportations

c. Draught power

d. Cash income by sale

e. Meat consumption

5. Herd composition or Herd inventory

- Breeding females dry (non pregnant).....

- pregnant..... - Lactating.....

- Breeding bulls.....

- Castrated male.....

- Non-breeding males (below 5 years).....

- Non-breeding females

- Unweaned males

- Unweaned females

5. What is the amount of camel milk used for?

5.1 home consumption? (% from total)

a. (100%), b. 75%, c. 50%, d. 25%

5.2 cash income by selling?

a. (100%), b. 75%, c. 50%, d. 25%

6. Milk consumption and preservation means

6.1 Frequency of milk consumption

a) Frequently consumed

b) Rarely consumed

c) Delicacy/more liked

6.2 How to drink?

a) Freshly or when it is raw

b) After boiling

c) When it is becoming sour (days

after)

6.3 What is the shelf life of camel milk (for how many days can be kept)?

7. Do you slaughter camel at home? (Yes / No)..... if yes for what reason?

a. for home consumption,

b. group share,

c. ceremony,

d. emergency slaughtering.

8. How do you consume camel meat?

a. cooked,

b. raw,

c. other treatment

III. Herd dynamism

9. Animal entered the herd (born, purchased, gift in) or left the herd (sold, dead, gift out, slaughtered or eaten by predator)

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

10. Do you do delivery assistance? Yes ☐ No ☐

If yes how do you do? a. Hand pulling b. other means.....

11. How do you take care for new born?

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

IV. Herd management and health care

12. Activities and labour divisions

Activities	Youngsters (13-25 years)		Adult (> 25 years)	
	Male	Female	Male	Female
Herding				
Watering				
Milking				
Delivery assistance				
Mating assistance				

13. Water points in different seasons

Season	Water sources				Frequency of watering
	River	Ponds	Traditional wells	Others	
Dry season					
Wet season					

14. Do you know if your camel gets sick? Yes ____ No ____

If yes, how? _____

15. What is the main means of health care for your camels?

- a. Traditional healer b. Self-administered Vet drugs c. Vet clinic d. Left untreated

15.1 What are the commonly encountered diseases affected your camel in order of importance?
(List and prioritize major camel diseases)

A).....B).....C).....D).....E).....

15. Reproductive disease events in the herd (indicators of brucellosis)

Events in the camel herd	Since one year		Since three years		Season
	Yes/No	Number			
Abortion					
Still birth					
Retained fetal membrane					
Bull with swollen tests and joints					

16. Do you know any diseases that cause abortion in camels? Yes ____ No ____

If yes, what are the local names of these diseases?

17. Was any event of abortion in your herd? Yes ____ No ____

If yes, which animals and at what time (month) does it occurred?

17.1 What do you do with camels that frequently abort?

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

17.2 How do you manage aborted fetus/fetal membrane?

- a. Leave in the field b. Disposing c. Give to dog d. others.....

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

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

18. Where is the source of bull from?

- a. From own herd b. village bull c. others.....

19. How do you herd Camels?

- a. Separately. b. with village herd. c. with cattle. d. with small ruminants

19.1 How is night resting?

- a. Separate. b. share with cattle. c. Share with small ruminants

19.2. Have you ever sold breeding females? (Yes/No).....

If yes what was the reason of selling

- a. Disease b. Infertility c. Shortage of money d. Others.....

V. Breeding aspects

20. Comparative age of male and female (years)

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

20.1 Age at first calving (Years)..... Gestation length (Month).....

Caving interval (Month)..... Lactation length (Month).....

21 Seasonal variations of reproductive traits

Parameters	Dry season	Wet season	Both seasons
Calving (Yes/No)			
Mating (Yes/No)			
Milking frequency			
Milk off take per day (litres)			

Annex D: Questionnaires to human clinical patients
(Questions to be administered by the physician/clinician)

Note: This questionnaire is designed for a survey on the potential risk factors associated with human health problems. The data are collected for study purposes only.

Date...../...../.....

A. Patient demographic data

- (a) Name _____
- (b) District _____ PA/kebele _____ village _____
- (c) Sex: Male _____ Female _____
- (d) Age _____ years
- (e) Educational level:
 Primary School _____ High School _____ Secondary School _____ Graduate _____
 Professional training _____ Uneducated _____
- (f) The number of household members _____

B. Patient history

1. Clinical symptoms

Sign/symptom	Yes	No	Unsure	If Yes, Date of onset
Fever ($>38^{\circ}\text{C}$)				
Chills				
Sweating/night sweating/				
Severe or persistent headache				
Joint pains (Arthralgia)				
Muscle pains (Myalgia)				
Fatigue (weakness)				
Unintended weight loss				
Bone pain				
Back Pain				

2. Do you have camels and/or other animals? Yes _____ No _____
3. Have you ever assisted animals during delivery? Yes _____ No _____
 To which animals? Camels _____ Cow _____ Sheep & Goats _____
4. Do you have milking camels? Yes _____ No _____

5. How do you consume camel milk? As fresh/raw _____ after boiling _____
6. How do you consume goat milk? As fresh/raw _____ after boiling _____
7. Consume fresh cheese? Yes _____ No _____
8. Where do you keep your animals? at yard _____ in pasture _____
9. Do you clean or groom your animals? Yes _____ No _____
10. Who is the one involved in milking animals in your family?
Your self _____ Family member _____
11. Do you slaughter animals? Yes _____ No _____; If yes, when? On holidays

12. How do you consume meat? As fresh/raw _____ after cooking _____
13. Have you visited any health center before? Yes _____ No _____

Annex E: Consent and assent forms (English & Amharic versions) and Ethical clearance

A) Consent for participation in this research work

Human Brucellosis

Awash-Fentale and Amibara Districts

Invitation to Participate and Description of Research Work

You are invited to participate in a study designed to gather information about Human Brucellosis in Awash Fentale and Amibara districts. The results of the study will be used to better understand the disease brucellosis and to help the camel-rearing pastoral community of the Afar region who decide to quit consumption of raw camel milk. The study will also used to know whether the disease causing bacterium can be released via milk or not. You are invited to participate because you are rearing camels and because you have indicated that you currently consume raw camel milk and assisted animals during delivery. Your participation in this study will last only one week (from date of blood sampling to the time of giving feedback to you about your test result), and it will require approximately 10 minutes of your time to give about 3-5 ml of blood.

In order to decide whether to participate in this study, you should know enough about the risks and benefits to make an informed decision. This consent form gives you detailed information about the study which a member of the research team (the researcher and the human health professional at the public health centers) will discuss with you. Once you understand the study, you will be asked if you wish to participate, and, if you do, you will be asked to sign this form.

Description of the Procedures

If you decide to participate in this study, you will be asked to complete questionnaires about your camel rearing and management practices and other related activities. These questionnaires are expected to take approximately 10 minutes of your time. In addition, you may be asked to speak to a health care professional for approximately 15 minutes about your feeding behavior and beliefs. During this meeting, you may be asked to give a 5 ml of blood which involves venepuncture of radial vein of your arm and a tube for about 15 seconds and you will be subjected to the procedure only once under most possible minimum discomfort and risk. One week after, we will ask you to come back and know your test result and then you will be given recommendations accordingly.

Risks and Inconveniences

There are no risks or discomforts anticipated for persons who volunteer to take part in this study. In the unlikely event that you experience any discomfort from your participation, you may discuss this with the health care professional who may meet with you, or you may contact Sisay Weldegebriel at +251 912 89 13 04.

One potential benefit of this study is that you may get better information about the hazards associated with the disease and public health consequences and hence, quit consuming fresh or raw camel and goat milk should you choose to do so. However, you may not receive any direct benefits from participating in this study. Generally, the result of the study could help to increase food security and improve the value for trade in livestock and livestock products.

Economic Considerations

If you choose to participate in this study you will receive free medical consultation services and treatment and recommendations to the result of your diagnosis.

Confidentiality

The information you provide will be strictly confidential. A file number will be assigned to all the information you provide in this study so that your rights to privacy and confidentiality are protected. All questionnaires will be kept in a locked filing cabinet that only the researchers will be able to access. Your name will be kept in a separate filing cabinet and will be accessed only to schedule follow-up appointments. Your identifying information will never be connected to your questionnaire responses.

Voluntary Participation

You are free to decide to participate in this study and free to withdraw at any time without giving the blood sample or economic incentive promised for the session during which you decide to withdraw.

Questions

Please ask for an explanation of any aspect of this consent form that you do not understand.

Authorization:

Do you agree to participate in this study (please circle one)? Yes No

I, _____, have read this form and decided that I will participate in the project described above. Its general purposes, the nature of my involvement, and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I am 18 years of age or older and that I received a copy of this consent form.

Signature

Date

If you have further questions about this research work, please contact Sisay Weldegebriel at +251 912 89 13 04.

B) Solicitation letter to parents of child participants (For children of <18 years old)

Dear Parent:

My name is Sisay Weldegebrail and I am a post-graduate student at the School of Veterinary Medicine of the Addis Ababa University. I am currently conducting my research work on the **“Serological and cultural detection of *Brucella* infection in humans and camels in selected pastoral & agro-pastoral residences of the Awash-Fentale and Amibara districts of the Afar region, Ethiopia”**. I hope that this research will be useful to the pastoral communities of the afar region by protecting the society from the potentially damaging disease called Brucellosis which can be acquired from the direct consumption of raw camel milk and close contact with animals of the pastoral community (camels and goats). I am writing to describe this research and to request you to give permission for your child to participate in a one-time 15-minute session.

The study will take place this time at the Awash Sebat and Werer health centers at a time that is convenient for you and your child. In this session, your child will participate in this study individually to give about 3-5ml of blood and get diagnosed for the disease in target and will be given positive feedback about the result of the diagnosis. Children will be asked questions about their motivation for the puzzles and for school more generally. Children will be participated twice in the study period (during blood collection and during provision of the feedback about the result of the diagnosis. Participation in this study is voluntary, and you have the right to withdraw your consent at any time without consequences, and your child can refuse to participate in the study. Your permission in no way obligates your child to participate in the study if s/he is unwilling.

If you agree to allow your child to participate in this study, please sign and tell your child to return the attached permission slip to me or health professional in the health centers of your area as soon as possible. Either I or the medical person, Mr. Moges, will contact you at the beginning of the January 2011 to arrange a convenient time for your child to come to the health center. Once your child arrives at the health center, s/he will subsequently be asked if s/he would like to participate. If so, s/he will be asked to sign a separate consent form. *Please keep this letter in a secure location to ensure that certain information about the study is protected until after your child has completed the study (from date of blood sample collection up to the time of result notification).*

If you have any questions about any aspect of the study, please do not hesitate to contact me for further information or clarification at +251 912 89 10 04. I would be happy to talk with you! Please remember that the success of this study rests on children not knowing the goals of the study in advance. Thank you for your valuable time, kind helps and esteemed cooperation.

Sincerely,

1) Parent consent form

Please only return this page
Keep the letter for your information

My signature indicates that **I AGREE** to allow my child to participate in the research study of Sisay Weldegebriel previously described.

Child's Name: _____

Child's Birth Month/Year _____

Parents' Names _____

Family Address _____

Telephone Number _____

To schedule the procedure session at the health center, what would be the best time of day for us to call you? _____

Parent's Signature _____ Date _____

Thank you!

2) Child Assent Form

- We would like you to be in our research study about the investigation of very damaging diseases in human and camels which make your society and friends interested in knowing and preventing themselves from acquiring diseases from your animals. Your parents have already said that it would be fine for you to participate, but we want to make sure that you would like to be in the study.
- If you agree to be in this study, we will ask you some questions about camels, other animals which you and your families have and about consumption of raw camel milk. Think about your regular activities and relationship with your animals. Then you will donate only about 3-5ml of blood. These are all "opinion questions" and there are no right or wrong answers. The whole procedure will take about 15 minutes and you will be back after few days to know your test result and get recommendations or treatment accordingly.
- Everything you work on today will be kept confidential. This means that we will not tell anyone else what you think about the study. We won't tell your teachers, parents, or friends, even if they ask us to. We will write a code number, not your name, on all the questionnaire forms and everything that you work on today.
- Remember, being in this study is your choice. No one will be upset if you don't want to participate or even if you change your mind later after the questions and want to stop in the middle before donating your blood. No matter what, you will still get the morals and positive smiles from us. If you want to skip a question, that's alright too. Just say, "I don't want to answer that question".
- You can ask any questions about the study now or at any time. Signing your name at the bottom means that you agree to be in this study.

(Name of child)

(Signature of child)

(Date)

ሀ) በምርምር ሥራው ውስጥ ለመሳተፍ የፈቃደኝነት ሃሳብ መቀበያ ቅጽ**በአዋሽ ፈንታሌና በአሜባራ ወረዳዎች፣ አፋር ክልል**

እርስዎን በዚህ ከአንስሳ ወደ ሰው ተላላፊ በሆነው የሰዎች በሽታ ላይ በሚደረገው የምርምር ሥራ ውስጥ ለመሳተፍ እስፈላጊ ሆኗል። የምርምር ሥራው በአዋሽ ፈንታሌና በአሜባራ ወረዳዎች ይካሄዳል። የዚህ ጥናት ውጤት ለአርብቶ አደሩ እንዲሁም የግመል ወተትና የወተት ተዋጽኦ ተጠቃሚ ለሆነው ህብረተሰብ ይጠቅማል። ስለዚህ የእርስዎ ተሳትፎ በይበልጥ የሚጠቅመው ለእርስዎና በዚህ ወረዳ ለሚገኙ የአርብቶ አደር ህዝብ ነው። እርስዎ በዚህ የምርምር ሥራ ውስጥ እንዲካተቱ የተፈለገዎት ዋና ምክንያት በግመል አርብቶ አደርነት ሥራ መስክ ላይ ስላሉ ነው። በዚህ የምርምር ሥራ ውስጥ የሚቆዩት ደግሞ ለአንድ ሳምንት ብቻ ነው። ከእርስዎ የሚጠበቀው 5ሲ.ሲ. ያህል ደምና የምርመራ ውጤቶች ሲያውቁ ደግሞ ከሐኪሞች የሚሰጠው የምክር አገልግሎት መቀበል ነው። ይህ የደም መጠን ከእርስዎ ሲወሰድ ምንም ዓይነት የኤክስ እና የመንፈስ ጤንነት መጓደል እንደማያስከትልብዎት ከወዲሁ እገልጽልዎታለሁ። ለዚህ የደም መውሰድ ተግባርና ተጓዳኝ ጥያቄዎች 10 ደቂቃ ብቻ ይፈጅብዎታል።

በዚህ የምርምር ሥራ ውስጥ ለመሳተፍ እና የሚቀርብልዎትን ጥያቄ ለመመለስ የ30 ደቂቃ ጊዜ ብቻ ይሆናል። በዚህ ጥናት ውስጥ ለመሳተፍ ፍቃደኛ መሆንዎንና አለመሆንዎን የመጀመሪያው ጥያቄ ይሆናል። ቀጥሎም ስለ ግመሎች እያያዝና የእርስዎንና የቤተሰብዎን ከግመል ተዋጽኦ ጋር ተያያዥኝነት ያለው የአመጋገብ ሁኔታ ይሆናል።

አሁንም በድጋሚ መጥቀስ የምፈልገው ነገር ቢኖር በእኩልነትና በጤንነትም የሚደርስ መቃወስ አይኖርም። ለበለጠ መረጃ ተመራማሪውን ወይም በሥራ ላይ ያለ የጤና ባለሙያ ማማከር ይችላሉ። በተረፈ ከዚህ የምርምር ሥራ ቀጥተኛ የሆነ የገንዘብ ወይም የቁሳቁስ ጥቅማጥቅም እንደሌለው ከወዲሁ መግለጽ እፈልጋለሁ። በእርስዎ የደም ናሙና ውስጥ ጥናቱ የሚመረምረውን በሽታ አምጪ ባክቴሪያ ከተገኘ በወቅቱ የነጻ መድኃኒት እንዲጀምሩ ያደርጋል። የደም ምርመራ ውጤትም ለእርስዎ ብቻ ካልሆነ ለሌላ ወገን አይነገርም።

በዚህ የምርምር ሥራ ላይ ለመሳተፍ ፍቃደኛ የመሆንዎንና አለመሆንዎን የእርስዎ ውሳኔ ነው።

ግልጽ ያልሆንልዎና እንዲብራራ የሚፈልጉት ጉዳይ ካለ ፊት ለፊት ይጠይቁኝ።

አመሰግናለሁ።

እሺታ

በዚህ የምርምር ሥራ ውስጥ ለመሳተፍ ፍቃደኛ ኖት? 1. አዎን ☐ 2. አይደለም ☐

እኔ _____ የተባልኩ ግለሰብ የቀረበልኝን የምርምር ሃሳብ ተቀብሮ በምርምር ሥራው ውስጥ እንደሳተፍና የእኔ ተሳትፎ ለአርብቶ አደሩ ህብረተሰብ እንደሚጠቅም ተገንዝቤ እንዲሁም በጥናቱ ወቅት ሊደርስብኝ የሚችለውን ተብራርቶልኝ ሙሉ በሙሉ ሃሳቡን ተቀብሮለሁ። ስለዚህ በጥናቱ ውስጥ ለመሳተፍና ከ18 ዓመት በላይ መሆኔን በመግለጽ የዚህ የእሺታ መመያ ቅጽ እንደወሰድኩ በፊርማዬ አረጋግጣለሁ።

ፊርማ _____

ቀን _____

ለ) ልጆችን በምርምር ሥራ ላይ ለማሳተፍ ለወላጆች የሚቀርብ ማሳመኛ ቅጽ

ክቡር ወላጅ /አሳዳጊ/

ስሜ ሲሳይ ወ/ተብርኤል እባላለሁ። በአዲስ አበባ ዩኒቨርሲቲ ሼተርናሪ ፐብሊክ ኸልዝ የ2ኛ ዲግሪ ተማሪ ነኝ። በአሁኑ ወቅት የምርምር ሥራዬን ከእንሰላት ወደ ሰዎች በሚተላለፈው ብሩሲላ በተባለ በሽታ አምጪ ህዋስ ላይ ጥናት ለማድረግ ነው። ይህ ጥናት በአፋር ክልል ለሚገኘው የአርብቶ አደር ህብረተሰብ እንደሚጠቅም መግለጽ እወዳለሁ። የጥናቱ ውጤት ከዚህ አደገኛ በሽታ አምጪ ህዋስ በተለይ ለግመል አርብቶ አደር ህብረተሰብ ጠቀሜታው የጎላ ነው። ይህ ህዋስ ወይም ባክቲሪያ ከግመሎች ጋር ቅርብ የሆነ ግንኙነት፣ ጥሬ የግመል ወተትና የወተት ተዋጽኦ እንዲሁም የግመል ጥሬ ሥጋ በመመገብ የሚተላለፍ ነው። ይህ የጥናት ማብራሪያ የምሰጣችሁ ልጃችሁ በዚህ ጥናት ውስጥ ለአንድ ጊዜ የ15 ደቂቃ ያህል የፈቃደኝነትዎን ሃሳብ እንዲሰጡ ነው። ይህ ጥናት የሚካሄደው በአዋሽ ሰባት እና በመልካ ወረር ጤና ጣቢያዎች ውስጥ ነው። ለእርስዎና ለልጅዎ ወደሚቀርበው ጤና ጣቢያ በመቅረብ ወይም ለተመራማሪው ልጅዎን በጥናቱ ለማሳተፍ ፍቃደኝነትዎን መስጠት ይቻላል። ልጅዎ ለጥናቱ ሂደት ከ3 እስከ 5 ሲሲ ደም በመስጠት ለሚፈለገው የህዋስ ምርመራ እንዲሰግስና የምርምሩ ውጤት እንደ አስፈላጊነቱ የምክርና የክትትል አገልግሎት ይሰጣል። የእርስዎ ልጅ በጥናቱ ላይ ለሁለት ጊዜ ብቻ ይሳተፋል (ደም ሲሰግስና የደም ምርመራ ውጤቱን ሲያውቅ)። የጥናቱ ግብ ላልሆነ በሽታ አምጪ ምርመራ አይካሄድም። ልጅዎ ደግሞ ሲጠራ የሚችለው ጥናቱ የሚፈለገውን ህዋስ ካገኘና ህክምናውን ለመስጠት ብቻ ይሆናል። ምንም እንኳን እርስዎ ልጅዎ እንዲሳተፍ ቢፈቅዱም ልጅዎ ፍቃደኛ ካልሆነ አይገደድም።

ልጅዎ ጥናቱ ውስጥ ለመሳተፍ ፍቃደኛ ሆኖ በምርምር ሥራ ውስጥ እስካለ ድረስ ይህ ቅጽ ከእርስዎ አይለይ። ክቡር ወላጅ ማንኛውም ዓይነት ጥያቄ ካልዎት እኔን ከመጠየቅ ወደኋላ አይበሉ።

አመሰግናለሁ።

1) የወላጅ እሺታ ቃል መቀበያ ቅጽ

(ይህን ገጽ ብቻ ይመልሱ)

ተመራማሪው በሰጠኝ ማብራሪያ መሠረት ልጄ በምርምር ሥራው ውስጥ እንዲሳተፍ/እንደትሳተፍ በፈርማዬ ተስማምቻለሁ።

የልጅ ስም _____ ጾታ _____

የትውልድ ዘመን _____

የወላጅ / የአሳዳጊ ስም _____

አድራሻ: _____

ስ.ቁ _____

ለአርስዎና ለልጅዎ የሚመች ቀን እና ሰዓት ይጥቀሱ _____

የወላጅ ፊርማ _____

ቀን _____

አመሰግናለሁ።

2) የልጆች የእሺታ ቃል መቀበያ ቅጽ

አንተ/ቺ በምርምር ሥራው ተሳታፊ እንድትሆን/ህ ተመርጠዋል/ሻል። ወላጆችህም/ሽም አንተ/ቺ በጥናቱ ተሳታፊ እንድትሆን/ህ ፈቅደዋል። ስለዚህ እኛም የአንተን/የአንቺን የፈቃደኝነት ቃልህ/ሽ እንድትሰጠን/ትሰጩን እንፈልጋለን።

በዚህ ጥናት ላይ ፍቃደኛ ከሆንክ/ሽ እንዳንድ ጥያቄዎች እንትሉ መልስልኚ ከ3-5ሊሊ ደም እንድትሰጡ/ሽ እንፈልጋለን። ደም ሲወሰድ ምንም ዓይነት ህመምም ሆነ ጉዳት አይኖረም። ለዚህ ተግባር የ15 ደቂቃ ጊዜ ብቻ ይወስዳል። የምርምር ውጤቱም ሆነ የጥያቄዎቹ መልስ ሚስታፊራዊጽ የተቷበቀ ጅሆኑል። የማይመለከትህ/ሽ ወይም መመለስ የማትፈልገው/ገው ጥያቄ ካለ መዝለል ይቻላል።

መጠየቅ የምትፈልገው/ገው ጉዳይ ካለ ለመግለጽ ዝግጁ ነኝ። ስለዚህ ፍቃደኛ ከሆንክ/ሽ ከታች በተሰጠው ቦታ ላይ ስምህን/ሽን ጻፍ/ፊ።

የልጅ ስም _____ ቀን _____
ፊርማ _____

አመሰግናለሁ።

	Addis Ababa University Medical Faculty Institutional Review Board	SOP# AAUMF 008 Version 2.0 Effective date: 1 Feb. 2009 Page 14 of 13
	Title: 3.2. Use of Study Assessment Form	

ANNEX 3
Form AAUMF 03-008

IRB's Decision

Meeting No: 035/2011

Date (D/M/Y): June 01/2011

Protocol number: 003/11/Ext.

Assigned No.....

Protocol Title: Serological, Cultural and molecular Detection of Brucella infection in Camel and its public health significance in selected pastoral residences of the Awash fenatal and Amibara districts, Awash sebat, Afar region, Ethiopia.	
Principal Investigators:	Sisay W/Gebriel
Institute:	AAU- School of Veterinary Medicine
Elements Reviewed (AAUMF 01-008):	☒ Attached ☐ Not attached
Review of Revised Application ☐ Yes ☐ No	Date of Previous review: February 09/2011
Decision of the meeting:	☒ Approved ☐ Approved with Recommendation ☐ Resubmission ☐ Disapproved

- I. Elements approved-
1. Protocol Version No.
 2. Protocol Version Date.....
 3. Informed consent Version No.
 4. Informed Consent Version Date

II. Obligations of the PI-

1. Should comply with the standard international & national scientific and ethical guidelines
2. All amendments and changes made in protocol and consent form needs IRB approval
3. The PI should report SAE within 10 days of the event
4. End of the study, including manuscripts and thesis works should be reported to the IRB

III. TO ESTM ☐

Institution Review Board (IRB) Approval: Period from 07/06/2010 to 06/06/2013

Follow up report expected in

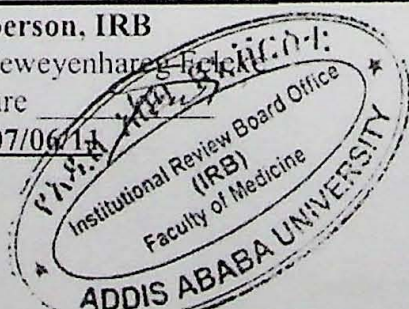
3 Months ____ 6 months ☒ 9 months ____ one year ____

Chairperson, IRB

Prof. Yeweyenhareg Felleke

Signature _____

Date: 07/06/11



Associate Dean for

Postgraduate and Research

Signature _____

Date _____

Annex F: Procedures, list of equipments, chemicals and reagents used for *Brucella* diagnosis

A) Rose Bengal plate test (RBPT)

A rapid agglutination slide test for the detection of *Brucella* specific agglutinins

Intended use

Brucella antigens are bacterial suspensions for use in slide agglutination tests to detect the presence of bacterial agglutinins associated with bacterial infection or previous exposure to a related organism. This slide test is recommended as a screening procedure only to establish the presence or absence of homologous antibody.

Principle of the test

In the course of *Brucella* infection with any pathogenic microbiological agent, a variety of antibodies are formed. Among these antibodies are the agglutinins. An agglutinin when combined with homologous antigen (agglutigen) under the properly controlled conditions is capable of causing agglutination. A suspension of *Brucella* possessing active antigen agglutinates when exposed to homologous *Brucella* antibody. This agglutination forms clumps of bacteria which become macroscopically visible. The Rose Bengal stained *Brucella* antigen is used for the early detection of *Brucella* agglutinins (*Brucella Abortus*, *B. Melitensis* and *B. Suis*).

Reagents

- Rose Bengal *Brucella* Antigen (0.5% phenol)
- Positive Control (0.01% sodium azide)
- Negative Control (0.01% sodium azide)
- Test sera

Materials

- *Brucella* Antigen, Rose Bengal Stained
- Positive Control.
- Negative Control.
- White Glass slide (or enamel plate).
- Stirring Sticks/applicator stick
- Micropipettes for dispensing 30-40µl serum.

Procedure

- 1- Allow reagents and serum samples to reach room temperature for testing.
- 2- Shake the antigen bottle gently to insure a uniform suspension.
- 3- Place 30µl sample serum onto the selected ring of the White Glass slide (or enamel plate).
- 4- Place one drop (30µl) of the Rose Bengal antigen onto serum sample.
- 5- Mix serum sample with Rose Bengal antigen using Stirring stick.
- 6- Repeat these steps using the positive and negative controls instead of serum sample.
- 7- Gently shake the slide for 2 to 4 minutes (automatic rotator can also be used).
- 8- Observe for agglutination after 2 to 4 minutes from beginning of shaking (this is the optimum time limit).

Results

Negative: No agglutination

Positive: (Presence of Specific Antibodies): Agglutination

Interpretation of results

This test is a screening test only for the detection of *Brucella* agglutinins. If result is positive it must be confirmed by CFT or other serological tests for Brucellosis.

Reaction was identified as 0, +, ++, +++, according to Nielsen and Dunken (1990).

0 = No agglutination

+ = barely visible agglutination, using magnified glass

++ = fine agglutination some clearing

+++ = Coarse clumping definite clearing

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

B) Complement Fixation Test (CFT)

Material preparation, evaluation and titration of Complement Fixation Test.

Materials required

I. Reagents

1. **Veronal buffer:** diluents (prepared by mixing 1-vial of the constituents into 1 liter of distilled water ($\text{pH} = 7.25$). Commercially available or could be prepared as follows and stored in a refrigerator

- NaCl 85.00g
- 5,5 diethyl barbituric acid 5.75g
- Na 5= 5diethyl barbiturate 2.00g
- $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1.68g
- CaCl_2 0.28g
- Dissolve the barbituric acid in 500 ml hot deionized or distilled water and then add other ingredients.
- Make up to 2:1 with deionized water
- Autoclave at 121°C for 15 mins
- For use, dilute 1/5 in deionized water
- Adjust pH 7.25

2. Sheep RBC

A. Collect blood from jugular vein of a sheep into Alsever's solution at a ratio of 1:1

✓ Alsever's solution

- D-glucose (Dextrose) 0.05g
- Sodium citrate 0.8g
- Sodium chloride 0.420g
- Citric acid 0.005g
- Distilled water 100ml
- Autoclave at 121°C for 15 mins. Store $+4^\circ\text{C}$
- Wash erythrocytes in the blood 3 times in Alsever's solution
- Centrifuge collected blood in a tube at 5000 rpm for 10 mins
- Discard the supernatant
- Centrifuge at 1500rpm for 10 mins
- Discard the supernatant

- Continue the washing once more
- This can be used fresh or can be stored at 4°C for 2 weeks.

B. preparation of 4% sheep red blood cell (RBC)

- Transfer an aliquot of the stock erythrocytes in Alsever's solution to a centrifuge tube
- Add veronal buffer and wash cells by gentle pipetting
- Centrifuge at 3000 rpm for 15 mins
- Remove supernatant fluid and discard and add an equal volume of veronal buffer
- Repeat centrifugation until the supernatant fluid is clear
- Finally remove sufficient packed cells to give a 4% suspension in veronal buffer.

3. Haemolysin (available commercially)

4. Standard antigens

- Standard antigens are prepared from appropriate infected mice, embryonated eggs or cell culture etc.
- Some antigens exhibit non-specific hemolytic activity which can be removed by treatment overnight with chloroform, fluorocarbon or absorption with RBC

If necessary in some cases:

- Add 2% chloroform to the antigen suspension and shake vigorously by hand for 2 minutes. And then leave overnight at +4°C.
- Some antigens are also anti-complementary. They are treated by heating at 56°C for 30 minutes or by adding 5-10% inactivated guinea pig serum and incubating the mixture for one hour at 37°C

5. Complement: commercially available or collect fresh guinea pig serum which could be stored in sealed glass ampoules at -70°C in small aliquots or can also be preserved by Richardson's methods and stored at 4°C for 6 months.

A) Preparation of complement

- Starve guinea pig for 24 hours
- Bleed guinea pigs and collect blood in sterile container
- Hold collected blood at room temperature for 1-2 hours

- Carefully separate the clot from the wall of the tube with an applicator stick.
- Store at 4°C overnight
- Decant the fluid and centrifuge at 3000rpm for 15-30 minutes.
- Remove serum

B) Richardson's method for preserving complement

- | | |
|---|--------|
| • Borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) | 0.57g |
| • Sodium azide (NaN_3) | 0.81g |
| • Sorbitol ($\text{C}_6\text{H}_{14}\text{O}_6 \cdot 12\text{H}_2\text{O}$) | 11.74g |

Dissolve in saturated NaCl solution and make up to 100ml.

To preserve complement mix 8 parts of guinea pig serum with one part of solution A, and then add one part of solution B, and store at 4°C for up to 6 months.

To use in CFT test dilute one part of the preserved serum with 7 parts of distilled water. The effective dilution is 1/10.

6. Standard antisera

- Standard positive and negative sera collected from the same species are used.
- Incubate all standard sera at 56°C for 30 minutes to inactivate the complement.
- Many sera are anti-complementary. Reduction of anti-complementary activity is sometimes achieved by mixing equal parts of serum and 1/10 complement and holding the mixture overnight in a refrigerator.

Standardization of reagents

- One unit of a complement (HD50) is the dilution giving 50% lysis with the optimal sensitizing dose (OSD) of haemolysin
- One unit of a complement (HD100) is the dilution giving 100% lysis with the optimal sensitizing dose (OSD) of haemolysin
- One unit of haemolysin (OSD) is the dilution giving most lysis with highest dilution of complement
- Units are assessed simultaneously in a chessboard titration using dilution of complement down the board and dilutions of haemolysin across the board.

Materials

- Preserved guinea pig complement
- Distilled water
- Veronal buffer (VB)
- 4% sheep RBC in VB
- 15 test tubes and rack
- 4x1 ml pipettes
- Syringe
- Refrigerator
- Incubator
- Agglutination plate

Haemolysin Dilution

Prepare the two fold serial dilution starting with 1/100 in test tube

0.1 haemolysin into	9.9 ml VB = 1/100
1.0 ml 1/100	1.0 ml VB = 1/200
1.0 ml 1/200	1.0 ml VB = 1/400
1.0 ml 1/400	1.0 ml VB = 1/800
1.0 ml 1/800	1.0 ml VB = 1/1600
1.0 ml 1/1600	1.0 ml VB = 1/3200
1.0 ml 1/3200	1.0 ml VB = 1/6400

Control 1.0 ml VB

- Add 1.0 ml 4% sheep RBC in VB to each test tube including the control tube.
- Hold at room temperature for 30 minutes.

Complement dilution

Prepare a dilution 1/50 of the complement

3.0 ml 1/50 complement into	1.0 ml VB = 1/67 --- 1/70
3.0 ml 1/67	1.0 ml VB = 1/89 --- 1/90
3.0 ml 1/89	1.0 ml VB = 1/119 --- 1/120
3.0 ml 1/119	1.0 ml VB = 1/159 --- 1/160
3.0 ml 1/159	1.0 ml VB = 1/212 --- 1/120
3.0 ml 1/212	1.0 ml VB = 1/283 --- 1/280

Position the WHO standard agglutination plate to have 8 columns and 10 rows

- Add 0.2 ml VB into all wells in rows 1-8 and columns 1-7
- Add 0.3 ml of VB into 8 wells of columns 8

- Add 0.1 ml volumes of the complement dilutions. The 8 wells of each column receive the same complement dilution. Start with the highest dilution in column 7, column 6, and column 5 etc.
- Add 0.1 ml of the sensitized erythrocytes. The 8 wells of each row receive the same haemolytic dilution; start with the highest haemolysin dilution in row 7.
- Add 0.1 ml of the RBC control into the 8 wells of row 8.
- Incubate the plate at 37°C for 45 minutes shaking at 5, 30 and 45 minutes.
- Hold for 2 to 4 hours at 4°C until the cells have settled.

OSD (optimal sensitizing dose) = haemolysin dilution giving most lysis with highest dilution of complement showing activity = 1/400 in the example.

HD 50 (median haemolytic dose) = one unit of complement is the dilution giving 50% lysis with OSD is 1/1600.

In the CFT test proper 4 units of complement are used i.e. 1/400

Standardization of antisera

Materials	4x1 ml pipettes
Antiserum	4x 1 pasteur pipettes
Distill water	1x5 ml pipettes
Haemolysin	Veronal buffer
14 tests tubes and rack	4% sheep RBC in VB
Preserved guinea pig complement	water bath at 56°C
Refrigerator	plate
Water bath 37°C	incubator

Procedure

Inactivate the antiserum at 56°C for 30mins

Prepare a 2 fold dilution series of the standard anti serum in the test tube blanks containing 1.0 ml VB starting with 1/8.

0.3 ml antiserum into 2.1 ml VB	= 1/8
1.0 ml 1/8 " 1.0 ml "	= 1/16
1.0 ml 1/16 " 1.0 ml "	= 1/32
1.0 ml 1/32 " 1.0 ml "	= 1/64

1.0 ml 1/64	“	1.0 ml	“	= 1/128
1.0 ml 1/128	“	1.0 ml	“	= 1/256
1.0 ml 1/256	“	1.0 ml	“	= 1/512

Prepare a 2 – fold dilution series of the standard antigen in test tube blanks containing 1.0 ml VB from $\frac{1}{2}$ to 1/128.

Position the agglutination plate to have 8 columns and 10 rows

Add 0.1 ml volumes of the antigen dilutions. The 8 wells of each row receive the same dilution starting with the highest dilution in row 7

Add 0.1 ml VB to wells 1-7 in column 8 and row 8

Add 0.3 ml VB into the 8th wells of column 8 or row 8 and into 2nd, 4th, and 5th well of row 9.

- Add 0.4 ml VB into the 1st well of row 9.
- Add 0.1 ml complement diluted in VB to contain 4 units to the 1st well in row 9 mixes and transfer 0.3 ml through to well 5 in rows 9. Discard 0.3 ml of the mixture from the 5th wells.

Incubate for 30 min. at 37°C

- Prepare 4% sheep RBC and sensitize by mixing with an equal volume of haemolysin diluted to give the OSD (Add haemolysin dilution to the cells)
- Incubate the mixture in a water bath at 37°C for 10 min.
- Add 0.1 ml sensitized erythrocytes to all wells
- Shake and incubate the plate at 37°C for 10 min.
- Add 0.1 ml sensitized erythrocytes to all wells
- Shake and incubate the plate at 37°C for 40 min. shaking at 50, 30 and 45 minutes.
- Hold at 4°C for 2-4 hours to allow the cell to settle

Antigen detection by complement fixation

Materials

- | | |
|-----------------------------------|-------------------------|
| ➤ Standard positive serum | ➤ Incubator |
| ➤ Standard negative serum | ➤ 4% sheep RBC |
| ➤ Verona buffer | ➤ water bath at 56°C |
| ➤ Standard antigen | ➤ 2x1 ml pipettes |
| ➤ Suspect antigen | ➤ refrigerator |
| ➤ Distill water | ➤ Haemolysin |
| ➤ Preserved guinea pig complement | ➤ WHO plate and cover |
| | ➤ 2 test tubes and rack |

Procedure

- Inactivate the standard positive and negative antisera by heating at 56°C for 30mins
- Dispense 0.1 ml VB into the 8 wells of rows 1, 2, 5, and 6.
- Dispense 0.2 ml VB into the 1st 7 wells of rows 3 and 7.
- Dispense 0.4 ml VB into the first well of row 9 and 0.3 ml VB into wells 2,3,4,5, and 8 and row 9
- Prepare 2-fold dilution of the standard antigen in the well of row 1 up to the 7th well using a transfer volume of 0.1 ml. Discard 0.1ml from the 7th well
- Prepare 2-fold dilution of the standard antigen in the well of row 3 up to the 7th well using a transfer volume of 0.1ml.
- Discard 0.1 ml from 7th well and row.
- Prepare 2-fold dilutions of the suspect antigen in the well of row 7 up to the 7th well using a transfer volume of 0.2 ml
- Add 0.1 ml of the inactivated standard positive antiserum diluted to contain 4 units of the optimal dilution to the entire well in rows 1 and 5 including the 8th well.
- Add 0.1 ml complement diluted in VB to contain 4 units to all wells in rows 1,2,3,4,5,6 and 7 except the 8th wells in row 3 and 7
- Add 0.2 ml complement diluted in VB to contain 4 into the 1st well of row 9. Mix and transfer 0.3 ml through to well 5 in row 9. Discard 0.3 ml of the mixture from the 5th well. Incubate at 37°C for 45mins shaking at 15, 30, and 45 mins. Hold the plate for 2-4 hrs to allow the cells to settle

Interpretation:

Sedimentation of the sensitized RBC = Positive result.

Complete lysis of the sensitized RBC = Negative result.

The Proper Test Steps of Complement Fixation Test

		1	2	3	4	5	6	7	8	9	10	11	12
A	+ ve Control												
B	- ve Control												
C	Sample 1												
D	Sample 2												
E	Sample 3												
F	Sample 4												
G	Sample 5												
H	Sample 6												
		(1:5)	(1:10)	(1:20)	(1:40)	(1:80)	(1:160)	(1:320)	(1:640)	(1:1280)	(1:2560)	(1:5)	(1:10)

50 µl Sample(S)

25 µl VBD

50µl S 25µl S

Transfer 25 µl of sample from col. 1 - 10 & discard 25 µl from col. 10

25 µl of diluted antigen

25 µl VBD

50 µl of diluted complement

50 µl of hemolytic system

Transfer 25µl of sample from col. 11-12 & discard 25µl from col. 12

The results are read after the plates have been left incubated in a waterbath at 37 °C for 30 minutes or at 4 °C for 14-18 hours.

The reading is:

- As complete fixation (no hemolysis) with water clear supernatant = recorded as + + + +
- Nearly complete fixation (75% clearing) as + + +
- Partial hemolysis (50%) + +
- Some fixation (25% clearing) as +
- Complete lack of fixation (complete hemolysis) was recorded as 0.

The Proper Test Steps of Complement Fixation Test

		1	2	3	4	5	6	7	8	9	10	11	12
A	+ ve Control												
B	- ve Control												
C	Sample 1												
D	Sample 2												
E	Sample 3												
F	Sample 4												
G	Sample 5												
H	Sample 6												

(1:5) (1:10) (1:20) (1:40) (1:80) (1:160) (1:320) (1:640) (1:1280) (1:2560) (1:5) (1:10)

50 μ l Sample(S) 25 μ l VBD 50 μ l S 25 μ l S

Transfer 25 μ l of sample from col. 1 - 10 & discard 25 μ l from col. 10

Transfer 25 μ l of sample from col. 11-12 & discard 25 μ l from col. 12

25 μ l of diluted antigen 25 μ l VBD

50 μ l of diluted complement

50 μ l of hemolytic system

The results are read after the plates have been left incubated in a waterbath at 37 °C for 30 minutes or at 4 °C for 14-18 hours.

The reading is:

- As complete fixation (no hemolysis) with water clear supernatant = recorded as + + + +
- Nearly complete fixation (75% clearing) as + + +
- Partial hemolysis (50%) + +
- Some fixation (25% clearing) as +
- Complete lack of fixation (complete hemolysis) was recorded as 0.

Annex G: Differential characteristics of species and biotypes of the genus *Brucella*

Species	Colony morphology	Oxidase	Biotype	CO ₂ required	H ₂ S production	Urease activity (hours)	Growth in presence of dyes		Agglutination in monospecific sera			Lysis by phage Tbilisi		Main host (s)
							Thionin (20µg/ml)	Basic fuchsin (20µg/ml)	A	M	R	RTD	10 ⁴ xRTD	
<i>B. abortus</i>	S	+ ^c	1	(+)	+	1-2+	-	+	+	-	-	+	+	Cattle, camel
			2	(+)	+	1-2+	-	-	+	-	-	+	+	Cattle, camel
			3	(+)	+	1-2+	+	+	+	-	-	+	+	Cattle, camel
			4	(+)	+	1-2+	-	(+)	-	+	-	+	+	Cattle, camel
			5	-	-	1-2+	+*	+	-	+	-	+	+	Cattle, camel
			6	-	(+)	1-2+	+*	+	+	-	-	+	+	Cattle, camel
			7 ^a	(-)	+	1-2+	+*	+	-	+	-	+	+	Cattle, camel
			Strain 19	-	+	1-2+	-	+	+	-	-	+	+	Vaccine
<i>B. melitensis</i>	S	+	1	-	-	v	+*	+	-	+	-	-	-	Goats, sheep, camels
			2	-	-	v	+*	+	-	-	-	-	-	Goats, sheep, camels
			3	-	-	v	+*	+	+	+	-	-	-	Goats, sheep, camels
<i>B. suis</i>	S	+	1	-	+	0-0.5+	+	(-)	+	-	-	-	+	Pigs
			2	-	-	0-0.5+	+*	-	+	-	-	-	+	Pigs, hares
			3	-	-	0-0.5+	+	+	+	-	-	-	+	Pigs
			4	-	-	0-0.5+	+	(-)	+	+	-	-	+	Reindeer, caribou
			5	-	-	0-0.5+	+	-	+	-	-	-	+	Rodents
<i>B. neotomae</i>	S	-		-	+	0-0.5+	-	-	+	-	-	-	+	Desert wood rat
<i>B. ovis</i>	R	-		+	-	-	+*	(-)	-	-	+	-	-	sheep
<i>B. canis</i>	R	+		-	-	0-0.5+	+	-	-	-	+	-	-	Dog

+ = positive, (+) = most strains positive, (-) = most strains negative, - = negative, v = variable reactions, * = inhibited by 40µg/ml thionin.

a = formerly *B. abortus* biotype 9, biotype 7 and 8 were deleted by International Committee on Bacterial Taxonomy.

A = *B. abortus* antigen, M = *B. melitensis* antigen, S = smooth, R = rough, RTD = routine test dilution, c = Some African isolates of *B. abortus* biova 3 are negative

Annex H: Ageing camels by the teeth

The camel has 22 milk teeth and 32 permanent teeth. It is different to other ruminants in having two front teeth in the upper jaw. Camels also have a pair of canine (dog teeth) in both the upper and lower jaws which are used to crush woody plants for food. The first pair of permanent cheek teeth is separate from the other teeth and are dark in color.

A) The milk teeth of the camel

The camel has 22 milk teeth arranged as:

Upper jaw	one front tooth on each side	2
	one canine (dog) tooth on each side	2
	three cheek teeth on each side	6
Lower jaw	three front teeth on each side	6
	one canine (dog) tooth on each side	2
	two cheek teeth on each side	4

Ageing camels from the milk teeth

(1) New born:	There are no teeth.	
(2) One month:	Upper jaw:	2 cheek teeth on each side
	Lower jaw:	one cheek tooth on each side 2 front teeth
(3) Three months:	Upper jaw:	1 canine, 3 cheek teeth on each side
	Lower jaw:	3 front teeth. 1 canine, 2 cheek teeth on each side
(4) Six months:	Upper jaw:	1 front, 1 canine, 3 cheek teeth on each side
	Lower jaw:	3 front, 1 canine, 2 cheek teeth on each side

B) The permanent teeth

There are 34 permanent teeth. These are larger than the milk teeth and are arranged as follows:

Upper jaw	one front tooth on each side	2
	one canine on each side	2
	six cheek teeth on each side	12
Lower jaw	three front teeth on each side	6
	one canine on each side	2
	five cheek teeth on each side	10

Ageing camels after 1 year of age

1 year	Upper jaw:	4 cheek teeth on each side
	Lower jaw:	3 cheek teeth on each side
2.5 years	Upper jaw:	4 to 5 cheek teeth on each side
	Lower jaw:	3 to 4 cheek teeth on each side
3 years	Upper jaw:	5 cheek teeth on each side
	Lower jaw:	4 cheek teeth on each side
4.5 years	First permanent front teeth are showing	
5 years	Milk cheek teeth replaced by permanent teeth, 2 replaced on each side of upper jaw, 1 on each side of lower jaw.	
5.5 years	2 more permanent front teeth on lower jaw	
	Upper jaw:	6 cheek teeth on each side
	Lower jaw:	5 cheek teeth on each side
6 years	Upper jaw:	1 front tooth and permanent canine through on each side
	Lower jaw:	permanent canine through
7 years	Full set of permanent teeth	
	<i>First cheek teeth on both jaws are black</i>	

The canines appear at 6 years of age and by the age of 7 are very large. These teeth in the upper jaw can be 4 centimeters long. The lower ones may be cut off by some camel owners. Camels can live to around 40 years of age but from 15 years of age they will have difficulty with hard feedstuffs as the front teeth wear and begin to spread.

9. SIGNED DECLARATION SHEET

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

Name: **Sisay Weldegebriel Zewold**

Signature _____

Date of submission _____

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

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