

**ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE**

**CAMEL MANAGEMENT AND STATUS OF CAMEL BRUCELLOSIS IN JIJIGA ZONE
SOUTH EAST LOWLAND AREAS SOMALI NATIONAL REGIONAL STATE ,
EASTERN ETHIOPIA**

BY

BERHANU TILAHUN CHEFEK

**JUNE 2006
DEBREZEITE, ETHIOPIA**

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

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ABBREVIATIONS

AAU	Addis Ababa University
SAT	Serum Agglutination Test
RBPT	Rose Bengal Plate Test
CFT	Compliment Fixation Test
ELISA	Enzyme Linked Immuno Sorbent Assay
H ₂ O ₂	Hydrogen Peroxide
UAE	United Arab Emirates
MZN	Modified Zeihl-Neelsen
MSc	Masters of Science
FVM	Faculty of Veterinary Medicine
FAO	Food and Agricultural Organization
LPO	Lipopolysacharide
OIE	Office Internationale des Epizootics
IgM	Immunoglobulin M
IgG	Immunoglobulin G
IgA	Immunoglobulin A
SNRS	Somali National Regional State
LCNRDB	Livestock, Crop and Natural Resource Development Beuro
MRT	Milk Ring Test
ELISA	Enzyme Linked Immuno Sorbent Assay

ACKNOWLEDGMENTS

First and foremost, I would like to thank the Gursum district Agricultural and Rural Development Office and Gursum Administration's for signing sponsorship agreement for post graduate programme studies at Addis Ababa University FVM Debrezeit.

I am gratefully indebted to my advisors Dr. Merga Bekana and Dr. Kelay Belihu for unreserved help, encouragement and correction of the paper.

My thanks goes to Jijiga Veterinary Laboratory Staffs specially to Mr. Eshetu Zewde, Mr. Tamiru Yimer and the rest former SERP staffs for their unreserved professional support during my field work at Jijiga area.

I wish to extend my heart felt thanks to my work mates of Gursum District Veterinary Staffs particularly to Mr. Behaylu Tefera for his assistant and timely transformation of my monthly salary. I am also indebted to Mr. Tsegaye Kebede, Mr. Afework Abebe and the rest staffs for their assistance in several issues related to my daily life and encouragement.

My thanks also goes to Mr. Tilahun Abera Babile District Veterinary staff and Mr. Dereje Abikke Gursum Health Center for their professional support during my fieldwork at Babile.

My special thanks goes to Dr. Bashahwured Shiferaw for his technical support, provision of office facilities and vehicle during my fieldwork activities.

My deep hearted thanks goes to my lovely sister Frezewed Tilahun for her unreserved over all support, advice and encouragement during the course and research work.

Last but not least, I would like to extend my thanks to W/ro. Ayelech G/Michael for her moral encouragement and care during my studies. I am also indebted to Mr. Abera Kumsa and Mr. Abdi Hadji for language translation and other supports during my fieldwork at Jijiga.

ABSTRACT

A cross-sectional epidemiological study was carried out from October 2005 to March 2006 to determine the seroprevalence of camel brucellosis in south east low land areas in Jijiga zone, Somali National Regional State (SNRS), to characterize the management system, determine the seroprevalence of camel brucellosis and identify the risk factors that contribute to the occurrence of brucellosis. A simple random sampling method was used to select 822 camel and 185 herds (households). 594 camels and 133 herds were selected from Jijiga area while 228 camels and 52 herds were selected from Babile area. Serum samples were collected from all camels (822) 2 years old or above. The blood samples were screened by RBPT and all RBPT positive reactors were further tested by the CFT for confirmation. Additionally, a structured questionnaire format was prepared and administered to hundred of the 185 selected households to collect information about animal attributes and farm attributes. The results showed that significant proportions of the households kept camel together with cattle and shoats (40%) and only with cattle (32%). The camel herd was dominated by pregnant animals (21.8%), lactating animals (21.1%) and mature non-lactating animals (19.3%). The most important diseases affecting camels in the area as mentioned by the households were trypanosomosis (93%), anthrax (80%), pneumonia (70%), bent neck (59%), abscess (59%), endoparasites (54%) and ectoparasites (51%). Camel management practices like herding and watering, milking and delivery and mating assistance were mainly the responsibilities of adult and young males. In this study, the overall seroprevalence of brucella antibodies in camels was 2.43% (95% CI=1.6-3.8). Among the potential risk factors considered (sex, age, herd size and parity), none of them had significant effect on individual animal seroprevalence ($p>0.05$). The herd level seroprevalence was 10.3% (95% CI= 6.7-15.7). Although the herd level seroprevalence was higher in Jijiga (12%) than in Babile (5.8%), the difference was not statistically significant. The herd level seroprevalence was significantly increasing with the increment of the herd size ($p<0.01$). It can concluded thus camel brucellosis had low prevalence in the areas and herd size was the important risk factor for the occurrence of the disease at herd level.

Key words: camel, brucellosis, seroprevalence, management, risk factor, Jijiga, Babile

1. INTRODUCTION

Ethiopia is a country whose agricultural sector is the biggest contributor to its gross domestic product (GDP) and major contributor to its export earnings. Currently the contribution of this sector to the GDP and export earnings is 48% and 90% respectively (Beyene 1997). In Ethiopia, livestock supports rural and urban population with employment and investment opportunities and draft power for crop production (Demeke and kumsa, 1997). Among the huge livestock resources camels (*Camelus dromedarius*) are the important livestock species uniquely adopted to hot and arid environments. Camels provide milk, meat, wool, hair, hides and serves for riding and use as a draft animal for transport. Camels have been known to have particular or peculiar physiological features, by which they regulate body temperature to changes in ambient temperatures, which enable them to survive and adapt to such hard conditions (Yagil, 1985; Wilson, 1984; Higgins *et al.*, 1992). The liveliest ecological zones for camels are the tropical, sub tropical arid areas of Africa, and Asia. In Africa, camel production dominantly exercised in east Africa has been reported to be consist 11.5 million heads of animals representing 80% of the African and two thirds of the world population of camels, which consists 16.5 million (Schwartz and Dioli, 1992; Wilson, 1998). Camels are kept in the arid and semi arid lowlands of Ethiopia, which constitutes 61- 65% of the total area and home range of 7-8 million, mainly pastoral and agro pastoral communities (Abebe, 2000). Even though camels played a vital role in the livelihoods of the pastoralists, communities in the arid and semi arid areas/ zones/, scarce or no studies on camel brucellosis have been conducted. Limited published information on the diseases of the camels indicates that camels may be carriers of or are susceptible to or suffer from a vast array of infectious and parasitic diseases (Wilson, 1998). Among some of the diseases affected camels like brucellosis caused a major constraint to low productivity and mortality in camels and have considerable public health importance as camel milk is consumed raw in the pastoral areas (Abbas *et al.*, 1987). In camels, *B. abortus*, *B. ovis* and *B. melitensis* have been known to cause brucellosis (Seifert, 1996). The infection has been reported from various countries of Africa and Asia (Wernery and Kaaden, 2002). In general, the disease causes abortion, non-viable offspring, retention of fetal membrane in female, orchitis and epididymitis in male animals and infertility in both sex (Straten *et al.*, 1997) Moreover, brucellosis in human represents a major public health hazards, which affects social and economic development in various countries. The isolation of

the two major pathogenic Brucellae species: *B. abortus* and *B. melitensis* from milk and other samples of camel origin and a wide range consumption of raw camel milk by camel producers even considered a delicacy, clearly indicates the potential public health hazards of camel brucellosis, which affects social and economic development in various countries (Gameel *et al.*, 1993; Straten *et al* 1997; Agab *et al.*, 1994).

Generally the previous serological surveys showed an over all sero prevalence rates of 4.4% (Dominech 1997), 5.5% (Richard, 1980) and 4.16% (Teshome *et al*, 2003) in Ethiopia specifically in Jijiga areas Teshome (2001) reported 2.84 Jijiga areas seroprevalence, but the survey was not conducted extensively in the study areas. Hence a well-designed systematic and extensive survey is required that can thoroughly examine the prevalence of the disease. There for the objective of this study were:

- To describe the camel management practices.
- To determined the seroprevalence of camel brucellosis.
- To identify risk factors for occurrence of brucellosis in Jijiga low land areas of Somali National Regional State.

2.LITRETURE REVIEW

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” range, in Brazil, Colombia, USA, Cuba, Spain, Italy and France (Wilson, 1998). 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. Subsistence camel production is practiced in dry areas of Ethiopia that cover 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).

2.2. Potential importance of camels

Camels are primarily the domestic animals of pastoral communities that ensure their food security (Wilson, 1998). 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 - 6 liters of daily milk yield over 13 - 15 months of lactation length. 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 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; Wilson, 1998). 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 for deriving oil mill. Camel racing and other leisure activities such as camel safaris and trekking have recently become a tourist attraction and luxurious in some part of the world (Schwartz and Dioli, 1992; Higgins *et al.*, 1992; Wilson, 1998).

From global perspective, the economic production of camels seems minimal. 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 environment 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; Wilson, 1998). 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 (Wilson, 1998).

2.3. Constraints of camel productions

Camels are produced by pastoral societies of the developing countries who dwell in dry marginal areas. Because 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. Only recently that 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 (Schwartz and Dioli, 1992).

Beside management and nutritional factors, Infectious and parasitic diseases are the primary constraints that hamper production. Trypanosomosis is the most serious infectious disease of camels and wide spread in camel rearing areas. Viral, bacterial, external parasites and helminthes are of clinical importance (Higgins *et al.*, 1992; Wilson, 1998). Brucellosis is one of the infectious diseases that affect both camels and camel owners (Wernery and Kaaden, 2002).

2.4. Camel brucellosis

2.4.1. Importance of camel brucellosis

Brucellosis is a contagious disease associated with abortion, birth of non-viable offspring, prolonged calving intervals in female, orchitis and epididimitis in male and sterility in both sexes has considerable impact on camel productivity (Radostits *et al.*, 1994; Straten *et al.*, 1997; Bauman and Zessin,1992). The isolation of the two major pathogenic Brucellae species: *B. abortus* and *B. melitensis* from milk and other samples of camel origin and a wide range consumption of raw camel milk by camel producers even considered as delicacy, clearly indicates the potential public health hazards of camel brucellosis, which affects social and economic development in various countries (Gameel *et al.*, 1993; Straten *et al* 1997; Agab *et al.*, 1994).

According to Kyebambe, 2005 there are four species of *Brucella* pathogenic to humans and each of them has a specific types of animal reservoir: *B. abortus* (cattle, buffalo), *B. melitensi* (goats, sheep, camels), *B. suis* (pigs) and *B. canis* (dogs). Humans are infected when they are exposed to body fluids from an infected animal. Brucellosis is a disease of economic importance as it adversely affects the product and productive potential of the affected animals in terms of loss of calves, infertility, as well as a reduction or complete loss of milk yield after the abortion (Seifert 1996). However, it is reported that the progeny of suffering animals might catch the infection from affected parents, although the rate of seroconversion among such progenies is not significant among cattle. Indeed, the different species of *Brucellae* are not strictly host specific, but *Brucellae* are potentially pathogenic and insidious in nature and a human health hazard, causing a variety of disease syndromes varying from symptom less carrier stage to undulant fever, anorexia, nocturnal perspiration, malaise, depression, fatigue, loss of body weight and muscle aches etc (Chahota *et al.*, 2003)

2.4.2. Factors influencing camel brucellosis

Host factor

Susceptibility to infection is influenced by age, sex, and reproductive status of the individual animal. Sexually mature pregnant female are more susceptible to infection, whereas sexually immature animals generally do not become infected (Radostits *et al.*, 1994).

Agent factors

Brucellae organisms survive, freezing, thawing and under proper condition they survive up to 4 months in milk, water, urine and damp soil (Seifert, 1996). This may increase the chance of infection in camels.

Environmental factors

The incidence of parturition in wet season enhances the viability of the organisms in the environment, thus increasing the chance of infecting susceptible animals (Schwarz and Dioli, 1992). The spread of the disease from one herd to another and from one area to another is always due to unrestricted movement of an infected animal to non-infected susceptible herd. Once infected, the time required to become free of Brucellosis was increased by large herd size, by active abortion and poor discarding practices (Radostits *et al.*, 1994). Herd size and animal density are directly related to prevalence of disease and difficulty in controlling infection in a population (Walker, 1999). The author has also shown that calving practices also play a major role in the spread of Brucellosis, whereas separate calving pens allow for minimizing exposure of uninfected animals.

2.4.3. Epidemiology of camel brucellosis

Common sources for infection are aborted fetuses, placentas and post abortion uterine fluids; infected animals shed and organisms in milk (Seifert, 1996). Humans acquire infection by handling tissue containing Brucellae organisms and ingestion of contaminated raw milk. Seroprevalence in camels raised under intensive management systems has been found to be higher than those kept in backyard of houses in small groups in Saudi Arabia (Radwan *et al.*, 1992). According Abbas and Agab, 2002. The seroprevalence of brucellosis in camels appears to follow two distinct patterns: a low (2-5%) prevalence in nomadic or extensively kept camels and a high (8-15%) prevalence in camels kept intensively or semi- intensively. The infection is caused by different biotypes of *B. abortus* and *B. melitensis*. Large and Small ruminants, camels, horses, buffalos and humans have been found to be susceptible to Brucellae spp (Seifert, 1996)

Etiology

Brucellosis has been known as an infectious bacterial disease caused by members of the genus Brucella. There are six Brucella species; *B. abortus*, *B. melitensis*, *B. ovis*, *B. canis*, *B. Suis* and *B. neotomae* (Dwight and Yuan, 1999). According to Walker (1999) Brucellae are small gram

negative coccobacilli measuring 0.6 to 1.5µ m x 0.5 to 0.7µ m size. They are typically arranged singly but also occur in pairs or clusters with, no capsules, flagella or spores are produced.

Brucellae survive freezing and thawing, under proper environmental condition, they survive for up to 4 months in milk, urine, water and damp soil. It is killed by most of the disinfectants. There are smooth or non-smooth colonies that are determined by the polysaccharide side O - chain in the lipopolysaccharide /LPS/. A and M are the dominant surface antigens and that also found in varying concentration among different smooth variants. The causes of brucellosis in camels are *B. abortus*, *B. melitensis* and *B. ovis* (Seifert, 1996) and the first two have been documented to be most frequent isolates.

Table 1. Summary of Brucella isolates from camels (*C. dromedarius*) in different countries

Country	Organs or specimens	Species isolated	Source
Egypt	Milk	<i>B. melitensis</i> biovar-3	Abou-Eisha, (2000)
Iran	Lymph node	<i>B. melitensis</i> biovar- 1	Zowghi and Ebadi (1988)
Kuwait	Lymph node Fetal stomach content	<i>B. melitensis</i> , biovar-3	Zowghi and Ebadi (1988) Al-Khalaf and El-Khaladi (1989)
Libya	Milk, Aborted fetus, Vaginal swab	<i>B. melitensis</i> biovar-1	Gameel <i>et al.</i> (1993)
Saudi Arabia	Milk Milk Carpal hygroma	<i>B. melitensis</i> biovar- 1,2 <i>B. melitensis</i> biovar-1, 2,3 <i>B. melitensis</i>	Radwan <i>et al.</i> (1992) Radwan <i>et al.</i> (1995) Radwan <i>et al.</i> (1998)
Senegal	Milk	<i>B. abortus</i> biovar-1, 3	Verger <i>et al.</i> (1979)
Sudan	L.node, testes and vaginal swab	<i>B. abortus</i> biovar 3	Agab <i>et al.</i> (1994)

Source: Adapted from Megersa (2004)

Transmission

Transmission of infection has been found to occur by direct contact of healthy animals with infected ones and indirectly through ingestion of contaminated feed, damaged skin, conjunctiva, and genital mucous and respiratory routes (Blood and Radostits, 1989). The main direct transmission happens through aborted fetuses, the placenta and post abortion uterine fluids (Walker 1999). Ingestion of milk from infected animals is also another source for infection to healthy animals and human. Infected bulls and discharge of semen containing organisms are most unlikely to transmit the disease if the semen is used for artificial insemination (Blood and Radostits, 1989). Spread of the disease has been linked to movement of infected animals to disease free herds, proximity of infected herd to clean herds happens at water points when a number of camels come together (Abbas *et al.*, 1987). Epidemiologically important risk factors are large herd size, poor managements and active abortions, milking more animals by single person and herding with other ruminants, as well as survival of the organisms in the epidemiology of the disease (Radwan *et al.*, 1992; Abou-Eisha, 2000).

Distribution

The distribution of camel Brucellosis has been reported in several countries including Egypt, Somalia, Ethiopia, Kenya, Chad, Tunisia, Nigeria, Niger, Russia, Mongolia, Libya, India, Iran, Saudi Arabia, Kuwait and the U.A.E. A serological prevalence of 2-23% was found (Wernery and Kaaden, 1994).

2.4.4. Pathogenesis and pathology

After getting entrance to the host the *Brucella* organism get localized to the regional lymph nodes, where they proliferate and disseminate haematogenously and localize in the reticuloendotelial system and other reproductive organs (Blood and Radostits, 1989). The facultative intracellular organisms may infect all organs and tissues. In pregnant animals the uterus is a preferred site of infection where it leads to a necrotizing placentitis (Seifert, 1996). In adult non-pregnant female animals, the organism localised in the mammary gland and later spreads to the uterus when pregnancy occurs (Kendrisk and Howarth.1974) probably causing

abortion due to placentitis, direct effect of endotoxines or inflammatory response in fetal tissue (Walker, 1999). The alanthoic factors have been suggested to stimulate the growth of most brucellae (Quinn *et al.*, 2002). These factors include erythritol, possibly steroid, hormones and other substances. Brucella organism has been reported to survive inside the phagocytic cells: this is maintained by inhibiting phagolysosome fusion and enhanced by suppressing the myeloperoxidase H₂O₂ halide system and production of stress proteins protecting the Brucella organisms from hydrolytic enzymes. The authors postulated that macrophages serve both as a means of avoiding killing by antibodies and complement and as a vehicle for systemic spread of infection. The enhanced virulence of the Brucella inside the reproductive system is supposed to be the consequence of the increased level of the sugar erythritol, a four-carbon alcohol, which is maintained in the reproductive system (Dwight and Yuan, 1999). Brucella has been known to cause intercotyledonary thickening with gelatinous fluid, the cotyledons are frequently necrotic covered with thick brown exudates, and aborted fetus is edematous with various degree inflammation of the organs (Blood and Radostits, 1989; Seifert,1996; Wernery and Kadden,2002). In male camels, inflammation and enlargement of the epididymis, characterized by hyperplasia, degeneration of tubular epithelium, orchitis and inflammation of other accessory sex organs are common (Dwight and Yuan, 1999; Seifert, 1996; Wernery and Kaaden, 2002).

2.4.5. Immune response

Once the Brucella organism enters to the body of the animal and causes infection, it has been known to enhance the humoral and cell mediated immune responses in the host (FAO, 1989; Seifert 1996; Wernery and Kaaden, 2002). As the organisms are facultative intracellular organisms, phagocytes play a key role in initiating T-lymphocytes releases cytokines that activate macrophages, which in turn control Brucella by reactive oxygen intermediate. IgM antibodies, which appear initially after infection and low levels of IgG will cause complement- mediated lysis of Brucella. However, elevated levels of IgG appear to act as blocking antibodies that modulate the ability of the complement membrane attack complex to lyse cells. This may account for resistance to complement mediated lysis in the face of high specific antibody levels and the lack of correlation between protection and high antibody titers (Walker, 1999)

The O chain of smooth lipopolysaccharide complex of the cell envelope together with the outer protein epitopes have contribute role as protective immunogens. On the other hands, the immunogenesity of the non-smooth variant is relatively low. The O chain specific antibodies play a major role in protective immunity, but do not eliminate the organisms as they are protected being intracellular (Corbel, 1990). The level and duration of the immune response has been reported to depend on many factors such as virulence of the strain, age, sex, species, pregnancy and the immune status of the host. Both antibody and cell- mediated immune responses are diagnostically useful, but the formers have lent themselves most readily to quantitative measurement (WHO. 1986).

2.4.6. Diagnostic methods

Bacteriological methods

According to Walker (1999), appropriate samples for diagnosis of Brucellosis depend on animal species affected, species of Brucella involved, and clinical presentation, absces material, semen, vaginal fluids associated with recent abortions are useful for recovering organisms antimortem. Milk samples for antmortem isolation attempts and for immunodiagnostic evaluation. Smears are made from specimens and stained by the modified Zeihl- Neelsen (MZN) stain Brucellae appear as small, red- staining coccobacilli in clumps because of their intracellular growth. After 3-5 days incubation on selective serum agar pinpoint, smooth, glistening, bluish, translucent colonies appear (Quinn *et al.*, 2002).

Serological Method

In the recent investigation, titers of 1:100 or higher has been taken as diagnostic evidence for camel brucellosis and full correlation between all sera has showed 1:100 or higher titers in the U.S plate agglutination test and positive reaction with the Rose Bengal Test (O.I.E, 1992). The test procedure outlined for the diagnosis of bovine brucellosis can be applied for camels (OIE, 2000). To what extent biochemical and physiological peculiarities of camels could contribute to the test result variability is not well defined. Lacking of agglutinating substances (that required to cluster globules) in the camel milk affected the application of conventional milk ring test in these

animals (Straten *et al.*, 1997). Like wise, unlike other animals, camels uniquely possess a type of dimeric immunoglobulins that consists of two heavy chains only, lacking the light chains (Pilstrom, 2002; Su *et al.*, 2002), where its effects is not clear.

According to Seifert (1996), the use of serological diagnosis is directed to the detection of immunological response to the agent or the presence of antibodies using various serological techniques and he cited that, RBPT, CFT, MRT, ELISA, SAT etc, are some of serological tests are widely used in diagnosing Brucellosis.

2.4.7. Prevalence of camel brucellosis in Ethiopia and its neighboring countries

Brucellosis is a widely spread disease in camel producing horn of African countries such as Ethiopia, Eritrea, Somalia and Sudan. According to Radwan *et al.* (1992), higher seroprevalence in camels raised under intensive management systems than those kept in backyards of houses in small groups in Saudi Arabia.

2.4.8. Control and prevention

The strategies for preventing Brucellosis have suggested to adopt to the animal with two alternative (Seifert, 1996, Abbas, B and Agab,H, 2002): Test and slaughter principle i.e. recognition of all animals immunologically responded to *Brucella* infection and subsequent culling of the reactors, as well as vaccination of exposed herds with inactivated or live vaccine. Attenuated live vaccines have been found to give the best protection against Brucellosis are: strain 19 (Buck 19, S 19), strain Rev 1 attenuated *B.melitensis* strain used for vaccination of small ruminants and also for beef cattle. This vaccine used parentally with out causing side reactions in pregnant animals. Inactivated vaccines with *Brucella* antigen provide a short- living comparatively low immuno protection effect. 45/20 (Dyphavac), H 38 and B112 – H105 are some of the inactivated vaccines under use (Seifert, 1996). Recent studies (Schuring *et al.*, 1994) has shown that a stable rough variant of virulent *B. abortus* 2308 that has been designated as RB51.is known to diminish virulence in comparison with strain 2308 and 19 with no formation of

OPS specific antibodies. Strain RB51 may serve as an alternative vaccine for cattle because of the close genetic relationship among species of the genus *Brucella*.

Table 2. A summary of Camel brucellosis prevalence in Ethiopia and its neighboring countries

Country	No. Tested	Prevalence (%)	Source
Eritrea	98	3.1	Omer <i>et al.</i> (2000)
	-	3.5	Azwai <i>et al.</i> (2001)
Ethiopia	977	4.4	Domenech (1977)
	762	5.5	Richard. (1980)
	1442	4.2	Teshome <i>et al.</i> (2003)
Somalia	802	8.0-11.0	Ahmed and Ibrahim (1980)
	250	10.4	Andreani <i>et al.</i> (1982)
	-	3.1	Bauman <i>et al.</i> (1990)
	1039	0.3-1.9	Bauman and Zessin (1992)
	-	8.5-11.5	Bornstein <i>et al.</i> (1984)
	514	12.6	Elmi (1982)
Sudan	238	3.0	Abbas <i>et al.</i> (1987)
	740	4.9	Abudamir <i>et al.</i> (1984)
	453	24.0	Agab <i>et al.</i> (1993)
	805	13.9-43.9	Majid <i>et al.</i> (1999)
	137	8.0	Osman and Adam (1987)

Source: Adapted from Megersa (2004)

3. MATERIALS AND METHODS

3.1. Study area and population

This study was conducted in two districts (Jijiga and Babile) of the Jijiga Zone of the Somali National Regional State (Figure 1). Jijiga and Babile districts are located east of Addis Ababa at 630km and 562km. Generally, the Jijiga zone represents a vast lowland area of SNRS and is bordering to the west with Oromia region, to the north with Shinile zone, to the east with north Somalia and to the southeast with Fik and Degehabur zone. The climate is generally semi arid and arid with annual average rainfall records 560mm the annual daily minimum and maximum temperature ranges from 13 - 27⁰C (Teshome, 2001). The total camel population in Jijiga zone consists about 29,000 (LCNRDB, 2006) (Table 5). The area is inhabited by different tribes of Somali communities, of which the Yebere, Abskul, Gedebursi, Malingur, Bertire, Giri, Hawya and Jarso are known to be camel owning tribes in the area. These camel herders, unlike other pastoralists living out of the Jijiga zone are not migrating or walking long distances in search of pasture and water to other area. They are shifting their animals within the Jijiga zone to areas where good grazing pasture and watering is available.

Table 3. Livestock population in Jijiga Zone

District	Cattle	Sheep	Goat	Camel	Equine	Total
Jijiga*	139882	375970	156629	8403	12116	693000
Kebribeyah	66625	179385	74709	3967	5739	330465
Awbere	66504	178976	75328	3944	5739	330464
Harshin	58191	156604	67470	3451	4998	290714
Babile*	97772	262674	109381	5789	8384	484000
Gursum	59986	162391	64483	3446	5051	295357

* Study districts (areas)

Source: LCNRDB, 2006

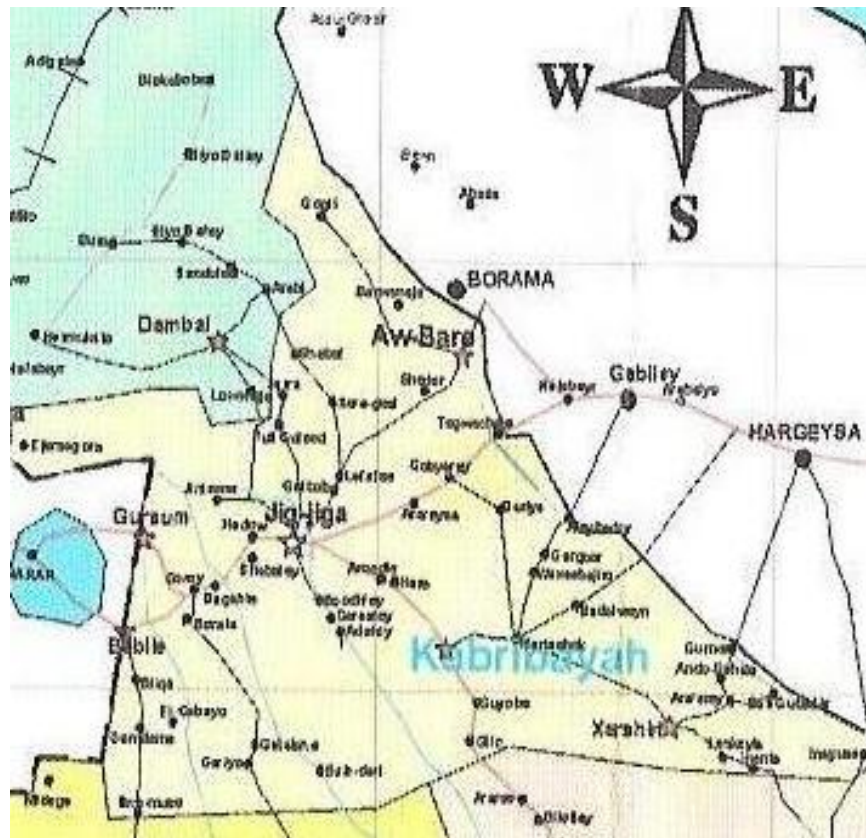


Figure 1. Map of Jijiga Zone east of Addis Ababa

3.2. Study design

A cross-sectional epidemiological study and questionnaire survey was carried out on camel management practices and the status of camel brucellosis from October 2005 to March 2006 in Jijiga lowlands, Eastern Ethiopia.

3.2.1. Sample size determination

Sample size was determined using a method recommended by Thrusfield (1995) for simple random sampling. Sample size calculation was based on the following formula:

$$N = \frac{1.96^2 p(1-p)}{d^2}$$

Where $d = 0.02$, $P = 0.03(3\%)$, $n =$ desired sample size

Accordingly, 279 animals were supposed to be sampled. However, to increase the precision 822 animals were selected from the two districts.

3.2.2. Sampling methodology

The two districts were purposively selected based on accessibility and camel population. 26 settlement areas were selected from Jijiga district while 10 were selected from Babile district randomly. This was followed by again a systematic random sampling of individual animals found in the settlements. Accordingly, 185 herds owning the selected animals were included in the study. For the questionnaire survey on management practices herders (households) from the 185 selected farms were selected by simple random sampling.

3.3. Data Collection

3.3.1. Questionnaire survey

A structured questionnaire was prepared and 100 camel owners were interviewed (Annex 1). The questionnaire was pre-tested in the field, adjusted as required to characterize the production system and to identify risk factors for occurrence of brucellosis. In the questionnaire survey herd structure, management and hygiene factors that are believed to influence the spread and maintenance of brucellosis, history of major diseases in camels, occurrence of abortion or stillbirths, camel husbandry system, grazing and watering system were considered.

3.3.2. Serological survey

Blood samples were collected once from jugular vein of each selected camel using plain vacutainer tubes. The blood samples were allowed to clot at room temperature. Then, serum was recovered in separated plastic tubes. Whenever, serum was not separated from clotted blood, centrifuge was used for serum separation. The separated serum was stored at -20°C until both RBPT and CFT were performed. Meanwhile, individual animal based data regarding age, sex, herd size, number of calves produced, occurrence and number of abortions and stillbirths were collected from breeding camels (Annex 2).

Screening test by Rose Bengal Plate Test (RBPT)

Antigen and sera required for each day for serological testing was taken out from the cold storage and brought to room temperature before testing was undertaken. The RBPT test was carried out according to the method described by Alton *et al.* (1975). The antigen was obtained from Institute Pourquer, 3409 Montpellier Cedex 5, France. The RBPT was performed at the Microbiology Laboratory, Faculty of Veterinary Medicine of Addis Ababa University, Debre Zeit. The details of the procedures of RBPT are presented in Annex 3.

Complement Fixation Test (CFT)

Positive sera to RBPT were subjected to CFT, which was performed at National Veterinary Institute, Debre Zeit, Ethiopia. Preparation of the reagents and the CFT test were performed according to the protocols recommended by OIE (2004). Antigen, control sera and complement were obtained from the BgVV, Berlin, Germany. Preparation, titration of reagents for CFT and multiple sera technique are shown in Annex 3.

3.4. Data analysis

Data was stored in Microsoft (MS) Excel Spread Sheet program, and were analyzed using Win-episcopy 2.0 (Thrusfield *et al.*, 2001) and Intercooled Stata 7.0 for windows (2001) to determine prevalence and analyze the association with risk factors. The statistical methods used include

descriptive statistics and univariate logistic regression. Sex, age, herd size and parity were considered as potential risk factors for individual animal seroprevalence while district and herd size were considered for herd level seroprevalence.

4. RESULTS

4.1. Questioner survey

4.1.1. Camel herd composition

According to the result of questionnaire survey, the members of the community in the study area kept different types of animals together with camel for various purposes. Camels are found to be the main production animals the areas. The highest proportion of the farms (40 %) had both cattle and shoats as supplementary to the predominant camel herd while very few farms (4 %) had equines in addition to cattle together with the camel herd (Table 4).

Table 4. Proportions of herds with different compositions of livestock species

Livestock (species)	No. of camel herds	% from total
Camels only	16	16
Camels & cattle	32	32
Camels,cattle & shoats	40	40
Camels,cattle & equi.	4	4
Camel, sheep & goat	8	8

The mean camel herd size was 21.7 with the maximum and minimum values being 100 and 4, respectively. The camel herd in the study areas was dominated by pregnant camels (21.8%) followed by lactating (21%) and non-lactating camels (19.3%) in that order (Table 5). Camel bulls constituted only 12.4% of the herd. Females in general constituted about 74.6 % of the total herd while immature animals made 25.4% of the herd. As to the experience of camel herding, respondents described that they had from 4 years minimum to 50 years maximum camel raring practice in the area. Mean value of experience 23.97 years.

Table 5. Camel herd composition or structure

Camel by reproductive status	Number of camels	% from the total
Dry camel	426	19,3
Pregnant camel	482	21.8
Lactating camel	466	21.1
Bull camel	274	12.4
None breeding male < 5 years	197	8.9
Non breed Females < 5 years	287	12.9
Unweaned male	30	1.4
Unweaned female	48	2.2

All respondents (100%) were camel herders and described the purpose of camel production to be mainly for milk (84%) and to some extent (10%) draught mitigation and herd accumulation (6%). Cattle were mainly kept for milk production while sheep and goats were used as the sources of meat for home consumption and immediate cash income by sells. There are some indications, which suggest that now a day the number of shoats decreased from the livestock herds. Donkeys are kept for transportation of water and other materials for home usage.

According to 87% of the respondents, 75% of the total milk production in the household was sold to the near by urban dwellers (mainly in Jijiga town) to generate income. The remaining 25% said they use milk only for home consumption. All the herders (100%) consumed fresh raw milk without any heat treatment but mixed with boiled tea. Came meat was consumed in the area cooked but some (18%) also consumed liver and hump of camel as raw.

4.1.2. Camel management and health care practices

In all the sampled households, activities like herding and watering were done by young and adult male. On the other hand, milking was done by adult males (56%), young males (31 %) and young and adult females (13 %). According to the herders the main water sources during dry season were traditional wells (59%) while the rest (41%) used also ponds. Camels in the area were

staying without drinking water for 5-20 days in the dry season. In the wet season, camels stayed longer (for more than 24 days) without water due to the fact that they also get water indirectly from the green feed available particularly cactus. In some cases (7%), camels were allowed to drink water from rivers in 20-24 days interval. The main water points in wet season for camels in the area include Jerer, Fafen and Daketa rivers, which originate from the highlands of Oromia Region and the surrounding areas of the Jijiga lowland. During the prolonged dry season, the shortage of water and pasture become severe and hence camels move about 30 to 40 km in search of water and grazing pasture.

According to the respondents, the most important camel diseases prevalent in their herd include trypanosomosis (93%), anthrax (80%), pneumonia (70%), bent-neck (59%), abscess (59%), endoparasites (54%), ectoparasites (51%), abortion (30%), wound (23%) and paralysis (5%) (Figure 2).

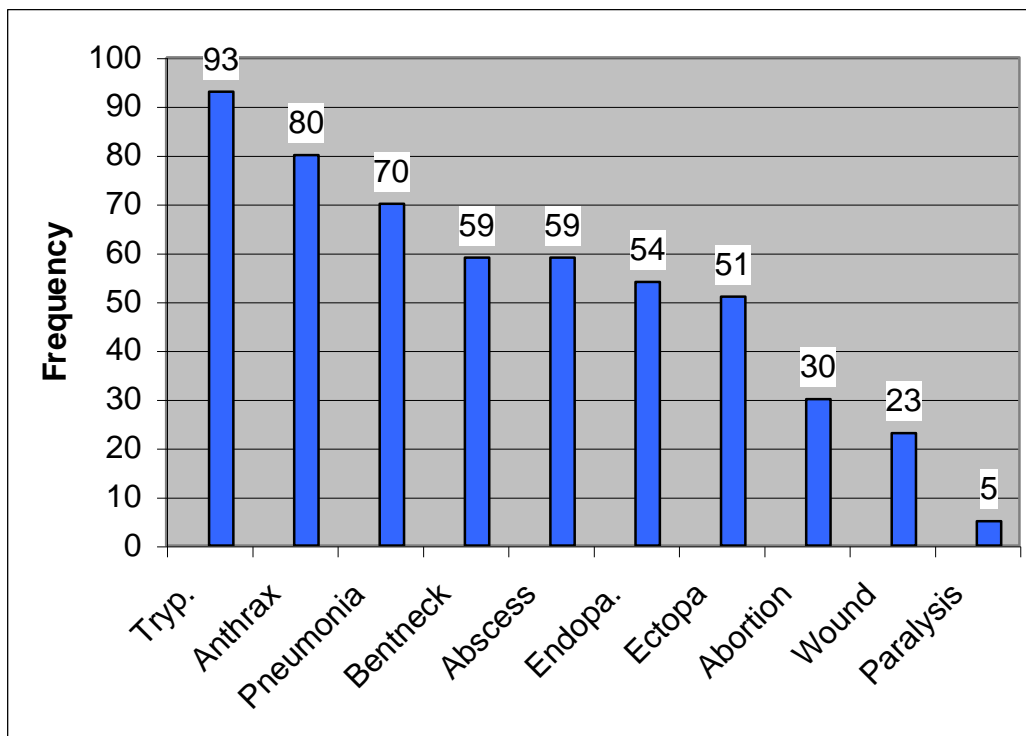


Figure 2. Camel diseases prevalent in the study area according to herders

The most important diseases mentioned as causes of abortion were trypanosomosis (54%), anthrax (20%), endoparasite (9%), toxic plants (8%), sunstroke (5%) and pneumonia (4%). Brucellosis was never mentioned as a cause of abortion (Figure 3).

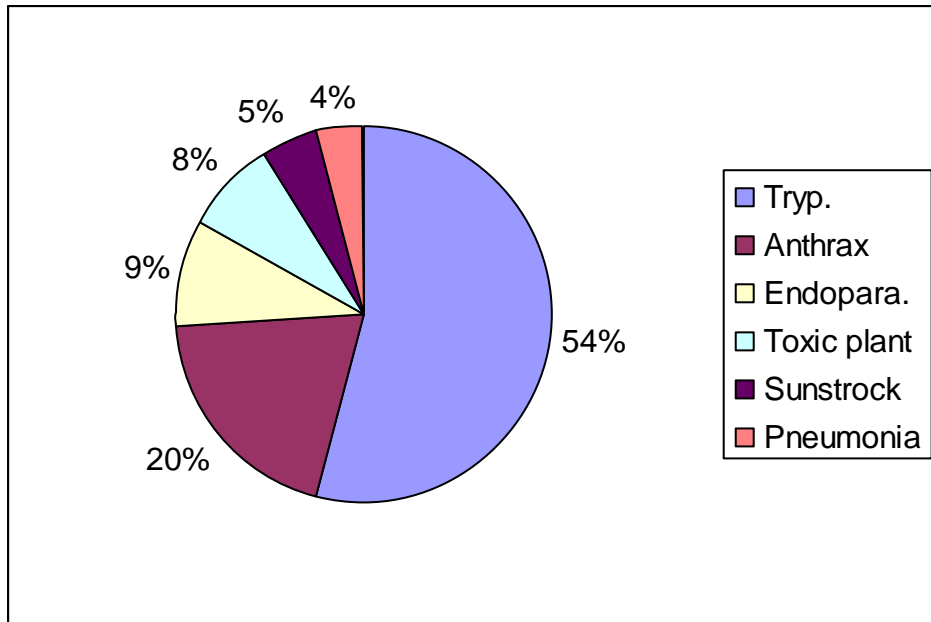


Figure 3. Diseases suggested as causes of abortion

Delivery and mating assistance to camels were strictly the job of adult males (99%) and young males had very limited role in these aspects (1%). Most of the farmers (65%) got animal health service in public veterinary clinics, while a significant proportion (30%) administered veterinary drugs by themselves. Small proportions were totally dependent on traditional healers (5%).

The farmers also mentioned disease symptoms, which are suggestive of brucellosis including abortion, stillbirth, and swollen joints, which occurred in 64%, 35% and 27% of the herds in a year, respectively. As described by the herders, aborted camels are culled or removed from the herd by selling and aborted fetus and other materials are left on the ground. Most of the herders used breeding bull from their own herd (90%) while the remaining proportion (10%) used village bull. The majority of the farmers herd their camels separately (61%) and about 34% of the

farmers practiced mixed herding. Almost all of the camel herds (98%) had separate night resting area and only 2% were sharing with cattle and small ruminants.

4.2. Serological survey

4.2.1. Individual animal seroprevalence

According to the result of RBPT and CFT, the overall seroprevalence of camel brucellosis in the study area was 2.43 % (20/ 822). Among the 36 settlement areas included in the study, brucellosis was detected in 11, which are dispersedly located in the study area. Higher prevalence was found in Jijiga area than in Babile (Table 6). The titration ranged from 1:10 to 1:320.

Table 6. Seroprevalence of camel brucellosis at animal level the two districts

District	N	Number of positive animals (Prevalence)	
		RBPT	CFT
Jijiga	594	23 (3.87)	17 (2.86)
Babile	228	5 (2.19)	3 (1.32)
Total	822	28 (3.41)	20 (2.43)

N= number of animals examined

Age, sex, parity and herd size were considered as potential risk factors to the seroprevalence of camel brucellosis at individual animal level were fitted in univariate logistic regression model. The results revealed that all the factors had no significant effect ($p>0.05$). However, high seroprevalence was observed in camels greater than 4 years of age (2.6%) than in those under 4 years of age (1.7%). The prevalence was also higher in male (2.8%) animals than females (2.3%) and the prevalence increased with parity number and herd size (Table 7).

Table 7. The effects of potential risk factors on individual animal seroprevalence of camel brucellosis

Risk factor	Category	N	Number positive (Prevalence)	95% CI	P-value
Sex	Male	181	5(2.8)	0.012-0.066	0.497
	Female	641	15(2.3)	0.014-0.039	
Age	2-4	174	3(1.7)	0.009-0.061	0.497
	>4	648	17(2.6)	0.016-0.042	
Herd size	4-20	208	3 (1.4)	0.005-0.044	0.419
	21-34	325	8 (2.5)	0.012-0.049	
	>34	289	9(3.1)	0.016-0.059	
Parity	Heifers	192	3 (1.6)	0.05-0.048	0.500
	Single parity	158	4 (2.5)	0.010-0.066	
	Multiple parity	291	8 (2.7)	0.014-0.054	

N= number of observations

4.2.2. Herd-level seroprevalence

Among the 185 herds investigated in this study, 28 herds had at least one positive reactor after RBPT and 20 by CFT. The result revealed an overall herd level seroprevalence of 10.3% (19/185). Within herd prevalence varied from absence of reactor animals to presence of one reactor out of the herd (0 to 7.7%). Herd size and district were considered as a potential risk factor and tested by univariate logistic regression. The result showed that only herd size had significant effect on herd-level seroprevalence ($p < 0.01$) (Table 8). The herd level seroprevalence increases with the increment of herd size. The OR indicated that the chance of getting a farm infected with brucellosis was 2.69 times higher in larger herd size than smaller ones.

Table 8. Risk factors associated with seroprevalence at herd level

Risk factor	Category	N	Number positive (Prevalence)	95% CI	P-value	OR
Herd size	4-20	75	3 (4.0)	0.013-0.121	0.003	2.69
	21-34	70	7(10.0)	0.050-0.202		
	>34	40	9(22.5)	0.127-0.400		
District	Jijiga	133	16(12.0)	0.076-0.190	0.218	
	Babile	52	3(5.8)	0.019-0.173		

N=number of observations

5. DISCUSSION

In the present study, the overall camel seroprevalence is low (2.43%) with variations from 1.3 % and 2.86% in Jijiga and Babile districts, respectively. At the herd and within herd level seroprevalence was 10.3% and 0-7.7%, respectively, which is about in accordance with the previous reports (Teshome, 2001), but higher than that of Megersa (2004) in southern part of the country. In different parts of Ethiopia, higher camel seroprevalence ranging from 3.1 to 5.5% 4.4% were reported (Dominech, 1977; Richared, 1980; Teshome *et al.*, 2003; Megersa, 2004). Variations in camel seroprevalence have been reported in several African countries such as 3.1% in Eritrea (Omer *et al.*, 2000), in Somalia 0.3%-1.9 % (Baumann and Zessin, 1992), and 4.9%, 24%, 13.9-43.9%, in the Sudan (Abudamir *et al.*, 1984; Agab *et al.*, 1993; Majid *et al.*; 1999; In our studies, the low prevalence is possibly due to the low density of camels population comparing to the wide and extended grazing land, the presence of many water points in the river path of the valleys and the restricted movement of camels with in the Jijiga zone. This is further potentiated with the good habit of the herder in timely culling practice of aborted females, as well as non-conceiving females from the herds. However, the isolation of *B. abortus* and *B. melitensis* from milk and a wide range consumption of raw camel milk by the producers by previous workers indicates the potential public health hazards of camel brucellosis, which is in accordance with previous reports (Gameel *et al.*, 1993; Straten *et al.*, 1997; Agab *et al.*, 1994).

In the current study, the highest complement fixations titration (1:320) was exactly similar to the recent report by Megersa (2004) in southern part of pastoral Ethiopia but lower than the 1: 640 titration reported by Teshome *et al.* (2003).

Although not significant, there was a slightly higher seroprevalence of camel brucellosis in older animals which is in line with previous reports. Radostits *et al.* (1994) indicated that infection may occur in animals of all age groups but persists commonly in sexually mature animals. This could be due to the fact that hormones such as erythritol might stimulate the growth and multiplication of *Brucella* organisms which tend to increase the concentration with age and sexual maturity as has been suggested earlier (Radostitis *et.al.*, 1994). The insignificant effects of age groups and sex on individual level Seroprevalence are in agreement with the previous reports in Ethiopia

(Teshome, 2001) and Saudi Arabia (Radwan *et al.*, 1992) suggesting a similar susceptibility to brucellosis exist among male and female camels of different age groups.

The herd level seroprevalence was significantly affected by herd size in this study ($p < 0.01$), suggesting that it was the major risk factor for to determine the occurrence brucellosis at herd level. This is probably due to the fact as herd size increases, the chance of contact between animals increases leading to more chances of infection as has been earlier reported (Abbas and Agab, 2002). Thus, herd size and density of animal population together with poor management are closely related to infection rate (Abbas *et al.*, 1987; Abu-Eisha, 2000; Wernery and Kaaden, 2002). In the area, camels are herding mainly in extended common grazing land separated from other species, but accumulation of camels are observed at water points like traditional wells and ponds during dry seasons. This might facilitates the spread of diseases including brucellosis, as the animals have a contact with each other and a direct access to water points which they contaminated by their discharge and increases the infection rates as has also been reported previously (Radwan *et al.*, 1992; Majid *et al.*, 1999; Abbas and Agab, 2002).

The management of camels along with cattle and small ruminants in the current study areas was also reported by Megersa (2004) for Borena area. The proportion of pregnant (21.8%) and lactating animals (21.1%) found in this study is very close to the reports of Megersa (2004). The similarities between the current study and that of Megersa (2004) could be possibly due to ethnic and agroecological similarities in the two areas. The herd level occurrence rates of abortion (64%) and stillbirth (35%) found in this study are by far higher than the reports of Megersa (2004). This difference could be may be due to the variation in the prevalence of other infectious diseases, which can also cause abortion. Wilson (1998) suggested that other diseases such as trypanosomosis might also cause abortion in extensively managed animals. The response of the herders in this study also confirms this in that the most important diseases identified as the cause of abortion in camels were trypanosomosis (54%) and anthrax (20%).

6. CONCLUSIONS AND RECOMMENDATIONS

From the present study it can be concluded that the seroprevalence of camel brucellosis in Jijiga Zone, south eastern part of Ethiopia is low at individual animal level, where as relatively high at herd. Herd size was the major risk factor for the occurrence of seroprevalence at herd level with high risk to human beings (herders) and due to a close contact with the infected herds. The camel management practices in the study areas were not so different from other areas about which reports are available.

Based on these, the following recommendations are suggested:

- ✓ Create public awareness among the community as to the extent of the disease prevalence in relation to the habit of consuming raw camel milk to avoid the real public health hazard;
- ✓ Create public awareness on the major risk factors and better management system which can help reduce the spread and maintenance of infection in camel herds;
- ✓ Brucellosis must be combated by collaborations of veterinarians and human health professionals together with the public;
- ✓ Further study on isolation of the causative agent and identification of the biotype is important in prevention and control of Brucella infection.

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8. ANNEXES

Annex 1. Questionnaire format for management practices

Date.....

Questionnaires for individual camel owners

Region Zone District..

PA. Village.

Name of respondent.....Age.....Sex.....

Herd size: (a. Small, b. Middle, c. Large)

Owner experience (years) Family size.....

II. Comparative importance of camels and its products

1. Types of livestock kept and purpose

Species	Purpose	More liked	Relative importance	Count
Cattle				
Goats				
Sheep				
Camels				
Equines				

2.What is the purpose of camel production?

- a. High milk production
- b. Drought mitigation
- c. Bush encroachment control
- d. Herd accumulation

- 3.Rank the use of camels:
- a. Milk production
 - b. Transportations
 - c. Draught power
 - d. Cash income by sale.....
 - e. Meat consumption

4. Herd composition or Herd inventory

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

5. What amount of camel milk used for?

5.1 home consumption? (Percent 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

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

7. Do you slaughter camel at home? (Y / N).....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

9. Activities and labor divisions

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

10.. Water points in different seasons

Seasons	Water sources				Frequencies days
	River	Ponds	Traditional wells	Flood water	
Dry					
Wet					

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

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

12.1 List ten top camel diseases.....

13. Disease events in the herd (indicators of brucellosis)

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

13.1. What do think that cause abortion in camels?

.....

13.2 What do you do with camels that frequently abort?

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

Annex 2. Serum sampling format for individual camels

Region Zone..... District PA..... Date.....

Code No.	Sex	Age	Herd size	Herding experience (year)	Breeding female history in the herd			
					Calving	Abortion	Stillbirth	Retained placenta

Annex 3. Materials used and procedures of RBPT

Materials used:

RBPT brucella antigen

Known positive control serum

Known negative control serum,

Glass slide

Micropipette

Micropipette tip

Mixing applicator

Procedure:

- The test sera and the antigen were left at a room temperature for half an hour every time before the test is started
- 30 µl of test serum was taken and placed on a clean glass slide
- 30 µl of RBPT antigen was added to the side of each test serum
- Then the antigen and the test serum were mixed thoroughly by an applicator,
- The glass slide was shaken by hand for 4 minutes and
- Finally the result of each test was read by looking the presence or absence of agglutination and the degree of agglutination was also appreciated in a very good light source and when necessary magnifying glass was used.

Annex 4. Materials used and procedures of CFT

Materials used

1. Micro well plates (U-shaped), Multi channel and single channel micropipettes, pipette tips
2. Flasks and measuring cylinders
3. Beam balance (Digital balance)
4. Incubator, water bath, deep freezer, Centrifuge
5. Veronal buffer, Alsever solution,
6. Complement, Hemolysin (Amboceptor), Control Serra, Sheep RBC
7. CFT Antigen

Preparation of sheep red blood cells for the hemolytic system

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

Amboceptor titration

1. Pre-dilution of amboceptor was made in jumping dilution.
2. Prepared 1:500 dilution was made up to 1:8000.
3. From these dilutions 500 μ l was transferred to a set of tubes, always starting with the dilution 1:12000 dilutions.
4. 1 μ l of diluents was added to each of the test tubes.
5. 0.5 μ l Of 2% sheep red blood cells was added, and shaken well.
6. The tubes were kept on the bench for ten minutes.
7. 1 μ l of complement at a dilution of 1:40 is was added.
8. The last tube showing complete hemolysis, minimum hemolytic dose (MHD) was read and recorded.

Evaluation of complement

1. Set up of 3 rows of 9 tubes each was prepared.
2. A 1:40 complement was prepared.
3. Complement was added into the 9 tubes increasing by 0.05ml every time, starting with 0.1ml.
4. Diluent was added into the 9 tubes in decreasing amount by 0.05ml, starting with 0.4ml.
5. 1.5 μ l of diluents was added into the tubes with the corn well syringe.
6. The set tubes were placed in a 37⁰c water bath for 1hr.
7. 0.5 μ l of 2% sheep red blood cell was added in all tubes
8. 0.5 μ l of amboceptor at working dilution 1:1000 was added in all tubes.
9. The tubes were properly mixed and put again in the water bath of 37⁰c for another 30 minutes.
10. The test was read by recording the minimum hemolytic dose of complement (MHD), which was represented by the first tube showing complement hemolysis. The next tube contains the full hemolytic dose (FHD).

The complement dilution=2FHD/dilution of complement.

Titration of antigen

❖ Microtiter plate I

1. 25 µl of VBD was added in all cups (wells).
2. 25 µl pre-diluted antigen was added to all cups of row A.
3. By serial doubling (two fold) dilution 25µl of antigen was transferred from row A to B, and again from row B to C, etc; until row G by multichannel pipette. 25µl mixture was discarded from row G (row H will only contain the diluents)

❖ Microtiter plate II

1. 50 µl of VBD was added to all cups.
2. 50 µl of pre-diluted (1:2.5) in activated positive control serum was to all wells of col.1
3. 50 µl was serially transferred by two-fold dilution from col.1 to col.2, and again from col. 2 col. 3 etc. until col.11. 50µl is to be discarded from col. 11.

❖ Mix plate I and II

1. 25 µl was transferred from plate II to Plate I.
2. 50 µl of complement in 1:40 dilution was added to all cups of plate I
3. Plate I is to be kept in a refrigerator, covered with second empty plate (cold fixation)
4. The following day, 50µl of 2% sheep red blood cells, amboceptor's premixture, equal volume (i.e.25µl) of sheep red blood cells, 25µl of a 1:100 working dilution of amboceptor was added to all cups.
5. The plate was covered with sealing tape, shaken well and was kept in water bath at 37⁰c for 30 min.
6. The last cup with 50+ sedimentation was read and recorded. The highest dilution of antigen with 50+ sedimentation is the limiting antigen concentration or the right corner value.

The test proper: Multiple sera technique

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

1. 25 μ l of diluted inactivated test serum are placed in the well of the first, second and third rows. The second row is an anti-complementary control for each serum. Volumes of 25 μ l of CFT buffer are added to the wells of the first row (anti-complementary controls) to compensate for lack of antigen. Volumes of 25 μ l of CFT buffer are added to all other wells except those of the second row. Serial doubling dilutions are then made by transferring 25 μ l volumes of serum from the third row onwards.

2. 25 μ l of antigen, diluted to working strength, and 25 μ l of complement, diluted to the number of units required, added to each well except in the first row.

3. Control wells containing diluent only, serum + complement + diluent, antigen + complement + diluent, complement + diluent, are set up to contain 75 μ l total volume in each case. A control serum that gives a minimum positive reaction should be tested in each set of tests to verify the sensitivity of test conditions.

4. The plates are incubated at 37°C for 30 minutes or at 4°C overnight, and a volume (25 or 50 μ l according to the technique) of sensitized SRBCs is added to each well. The plates are re-incubated at 37°C for 30 minutes.

5. The results are read after the plates have been centrifuged at 1000 g for 10 minutes at 4°C or left to stand at 4°C for 2 – 3 hours to allow unlysed cells to settle. The degree of haemolysis is compared with standards corresponding to 0, 25, 50, 75 and 100% lysis. The absence of anti-complementary activity is checked for each serum in the first row. The reading of results for the CFT was carried out as follows: When there was complete fixation (no haemolysis) with clear water supernatant, result was recorded as + + + +, nearly complete fixation (75% clearing) as + +

+, partial haemolysis (50%) as ++ and some fixation (25% clearing) as +, or complete lack of fixation (complete hemolysis) recorded as 0. Prevalence rate was determined when both test results are positive.

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

9. CURRICULUM VITAE

1. Bio data

Name: Berhanu Tilahun Chefek
Nationality: Ethiopian
Sex: Male
Date of birth: Nov. 27, 1959
Place of birth: Harar
Marital status: No Married

2. Educational background

2.1. Elementary and High school education

1967-1971 Elenentary education completed at Harar 2nd Model school
1972-1973 Junior secondary school completed at Harar Hailesilasie 1st school
now it is named Arbegnoch school.
1974-1978 High school completed at Harar secondary school.

2.2 Higher education

1979-1980 Awarded Diploma in Animal health from Debrezeite
Assistant Veterinarian Training College.
1983-1989 Awarded Degree of Doctor of Veterinary medicine / DVM/ from
Kishinov Agricultural institute of the former USSR /MSSR/.

3. Work experience

1980-1983

- Worked as Assistant Veterinarian in various provinces under the Ministry of Agriculture taking the following responsibilities .
- Organizing Veterinary activities at field, assessing outbreaks, reporting and participating in disease controlling program.

1990-1994

- served as zonal head of Animal health section in Gursum South East Range land project /SERP/.
- Organizing and supervising field veterinary activities and planning and controlling program.
- Controlling and supervising the proper utilization of Medical instruments and drugs.
- Participating in treating sick animals in the clinic and field.

1994-1996

- Worked at the position of zonal project coordinator in addition to the responsibility of head of Animal health

2001-2003

- Served as district head of veterinary team under Gursum MoARD office
Sep.2003 – sep. 2005
- Served as the coordinator in the PACE Harar branch office.

4 . Others

- Attended the advanced level training course in goat medicine at Diredawa veterinary laboratory organized by FARM Africa dairy goat project unit from Nov.29 – Dec.14,1995.
- Attended a participatory rural appraisal (PRA) training in Jijiga organized by SCF,UK and SERP from August 23 – Sep. 1, 1995.
- Attended a training program on participatory disease search (PDS) organized by PACE in Dolo-odo from March 19 – 25, 2004

5. Language ability

Amharic, English and Russian languages – Reading, writing and Speaking

6. References

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SIGNED DECLARATION

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

Name BERHANU TILAHUN

Signature _____

Date of submission _____

The thesis has been submitted for examination with our approval as university advisor.

Dr. Merga Bekana _____

Dr. Kelay Belihu _____