SERO-EPIDEMIIOLOGICAL STUDY OF BRUCELLOSIS IN CAMELS
(CAMELUS DROMEDARIUS) IN BORENA LOWLAND PASTORAL AREAS,
SOUTHERN ETHIOPIA

BY
BEKELE MEGERSA BATI

JUNE 2004
DEBRE ZEIT, ETHIOPIA
A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial fulfillment of Degree of Master of Science in Tropical Veterinary Epidemiology

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Academic Advisor:

Dr. Bayleyegn Molla  ____________________
DECLARATION

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

Name __________________________

Signature _______________________

Date of Submission _______________

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

Dr. Bayleyegn Molla (Associate Professor)

___________________________
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ABBREVIATIONS AND ACRONYMS

µg microgram
µl micro liter
µm micrometer
BgvV Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärimedizin
BZDPED Borena Zone Department of Planning and Economic Development
CFT Complement Fixation Test
Chi2 Chi square
CI Confidence Interval
CO2 Carbon dioxide
CVL Central Veterinary Laboratory (Brucellosis Reference Laboratory)
ELISA Enzyme Linked Immunosorbent Assay
FAO Food and Agriculture Organization of the United Nation
FHD Full Hemolytic Dose
GTZ Deutsche Gesellschaft für Technische Zusammenarbeit
H2O2 Hydrogen peroxide
Ig Immunoglobulins
ILRI International Livestock Research Institute
kg Kilogram
km2 Square kilometer
l liter
LR Logistic regression
LPS Lipopolysaccharide
m a s l Meter above sea level
m Meter
mg Milligram
MHD Minimum hemolytic dose
mm Millimeter
MOA Ministry of Agriculture
ABBREVIATIONS AND ACRONYMS

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>MRT</td>
<td>Milk Ring Test</td>
</tr>
<tr>
<td>MZN</td>
<td>Modified Zeihl Neelsen Stain</td>
</tr>
<tr>
<td>NAHRC</td>
<td>National Animal Health Research Center</td>
</tr>
<tr>
<td>OIE</td>
<td>Office International des Epizooties</td>
</tr>
<tr>
<td>OR</td>
<td>Odd Ratio</td>
</tr>
<tr>
<td>PH</td>
<td>Measure of acidity and alkalinity</td>
</tr>
<tr>
<td>RBPT</td>
<td>Rose Bengal Palate Test</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>spp</td>
<td>Species</td>
</tr>
<tr>
<td>SORDU</td>
<td>South rangeland development unit</td>
</tr>
<tr>
<td>SRBC</td>
<td>Sheep Red Blood Cells</td>
</tr>
<tr>
<td>V</td>
<td>Volume</td>
</tr>
<tr>
<td>VCM</td>
<td>Veronal buffer diluent with calcium and magnesium</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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ABSTRACT

A cross-sectional study was conducted from August 2003 to January 2004, to determine the prevalence of Brucella species in camels and to identify risk factors for brucellosis infection in camels (Camelus dromedarius) in two districts of Borena lowland. A total of 3218 camels in 250 herds were included in the study from Liben (2232) and Yabello (986 animals) districts. Of these 78.6% (2528 out of 3218) and 21.4% (690 out of 3218) were female and male camels, respectively. The herd size ranged from 3 to 42 animals with mean herd size of 13.6 ± 7.8. A total of 3218 blood samples were collected. All serum samples were initially screened by RBPT. All RBPT positive reactors were further tested by CFT for confirmation. CFT confirmed 58 seropositive cases out of 72 RBPT reactors.

The study showed the distribution of Brucella species antibodies in 1.8% (95% CI = 1.4 – 2.3) of the tested samples. Forty herds were found seropositive among the 250 herds included in the study (16%, 95% CI = 11.6 – 21.1). Seroprevalence rate in those seropositive herds (within herd prevalence) varied from 2.7% (1 out of 37) to 45.5% (5 out of 11) with average prevalence of 9.5%. Slightly higher seroprevalence was recorded in Yabello (2.0%, 95% CI = 1.2 – 3.1) than Liben district (1.7%, 95% CI = 1.2 – 2.3), though not statistically differing from each other (p > 0.05). Female camels had higher prevalence (2.06%, 95% CI = 1.5 – 2.7) than male animals (0.9%, 95% CI = 0.3 – 1.9).

The effect of sex was observed to be significant for seroprevalence (p < 0.05) with the risk of infection 2.3 (95% CI = 1.1 – 5.3) times higher in females than male camels. Similarly, there was significant increase in seropositivity with respect to increasing herd size (p < 0.05) with chances of disease occurrence 1.4 times higher in herd of 11 – 20 camels and 2.4 times higher in herd above 20 animals compared to small sized herds (< 11 animals). Immature animals (2 – 4 years) had statistically lower reactors than adult camels (p < 0.05), the odds of infection being 2.2 (95% CI = 1.1 – 4.6) times lower in immature camels. Conversely, parity and herding experience did not affect the status of seroprevalence among the respective categories (p > 0.05). The multivariate analysis of presumed risk factors revealed herd size as the major risk factors associated with seropositivity (p < 0.05). Advance in age and herd size were also found to have putative effect on seroprevalence (p < 0.05).

Abortion, stillbirth and birth to weak calf affected 20%, 8.3% and 18.3% of the investigated herds. Percent of abortion, stillbirth and birth to weak calf were found to be 8%, 3.6% and
7.4%, respectively per females per annum. Live birth, abortion and stillbirth percent were not significantly different among positive and negative breeding females. This together with low seroprevalence recorded in this study may not suggest abortion and stillbirth as clinical manifestation for brucellosis in the study area.

From the questionnaire data it was identified that 95% and 81.3% of herdsmen keep at least one and more than one ruminant species along side camels, respectively. A household owned 83.3% of the herds while 16.7% belonged to extended families. Accordingly, infection rate ranging from 1.7 to 1.9% were observed in those camel herds kept with more than one ruminant while no or less reactor was found in limited numbers of camel herds kept alone or with one ruminant species.

In spite of knowledge gap about brucellosis herders traditionally do isolation of calving and aborted dams from the rest of the herd during the day (98.3% and 46.7%) and night (98.3% and 33.3%), respectively for variable durations. To the contrary, camel pastoralists could be exposed to infection in several ways including raw milk consumption and close contact with animals without any protection.

The results of the present study provide the status of seropositivity to *Brucella* species in camel in the Borena lowland pastoral areas and the risk factors that contribute to seropositivity in dromedaries. Habitual consumption of raw milk and close contact with infected animals signify possible zoonotic importance of brucellosis in the study area.

**Keywords:** Dromedary camels, Brucellosis, Serological tests, CFT, RBPT, Epidemiology, Risk factors, Liben, Yabello, Borena lowland, Southern Ethiopia
1. INTRODUCTION

Camels (*Camelus dromedarius*) 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. 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 agropastoral 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 (Figure 1). In these areas, the livelihood of the pastoral communities is certainly ensured by dromedaries (Teka, 1991; Wossene, 1991).

The Borena pastoralists, who traditionally are based on cattle husbandry for milk production and wealth storage, have recently developed considerable interest in camel production. Ecological changes, social conditions (religion, marriage linkage, conflict) and extensive seasonal migration have been the main deriving forces behind the increased camel production in the Borena plateau (Biffa and Chaka, 2003). Although the exact figure on population is not available, data from Borena Zone Department of Planning and Economic Development (BZDPED) indicates about 467,119 camels are found in the area of which 70% is being produced in Liben district alone (BZDPED, 1998).

In spite of its vital importance particularly to the marginalized communities in the dry zones of tropics and subtropics, studies about camel 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 carrier of or susceptible 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 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 area (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). The disease is known to cause abortion and birth of non-viable offspring in female, and orchitis and epididymitis in male animals and infertility in both cases (Radostits et al., 1994; Agab, 1997; Straten et al., 1997). In production system where livestock diversification is practiced, the disease circulates in sheep, goats and cattle, and further spreads to dromedaries (Andreani et al., 1982; Radwan et al., 1992).

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 1.2% 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.

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).
1993; Agab et al., 1994; Abou-Eisha, 2000) has certainly demonstrated the danger of camel milk to public health. However, available studies on camel brucellosis are so scanty and do not provide detail epidemiological information of the disease in Ethiopia. Therefore, the present study was undertaken to determine the seroprevalence status of the brucellosis in camels and to identify risk factors associated with the disease occurrence in the study areas.

2. LITERATURE 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” 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). The Borena rangeland of southern Ethiopia is the third important camel production region of the country, the first and second being Somale and Afar regions (Figure 1). According to Hukka (1998) the Borena pastoralists probably started camel production in early 1560 in the Gedda period of Abbay Orro. It has been also speculated that the Muslim Gebra communities were said to the instrumental in introducing camels to the Borena plateau (Coppock, 1994). Thereafter, social and changes, extensive seasonal migration and change in rangeland ecosystem (decreasing pasture for grazers and increasing browse vegetation species) are major factors for the expansion of dromedaries into the area (Biffa and Chaka, 2002).

Table 1: Camel population in some selected countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Number (, 000)</th>
<th>Density (No per km²)</th>
<th>Proportion to total national ruminants (%)</th>
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<tbody>
<tr>
<td>Djibouti</td>
<td>60</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>Egypt</td>
<td>170</td>
<td>0.16</td>
<td>5.8</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>1030 - 1040</td>
<td>0.83</td>
<td>3.4</td>
</tr>
<tr>
<td>India</td>
<td>1100</td>
<td>0.33</td>
<td>0.4</td>
</tr>
<tr>
<td>Kenya</td>
<td>620 - 780</td>
<td>1.08</td>
<td>5.3</td>
</tr>
<tr>
<td>Niger</td>
<td>415</td>
<td>0.32</td>
<td>8.3</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>165</td>
<td>0.00</td>
<td>14.9</td>
</tr>
<tr>
<td>Somalia</td>
<td>5800 - 63500</td>
<td>8.93</td>
<td>46.6</td>
</tr>
<tr>
<td>Sudan</td>
<td>2800 - 3100</td>
<td>0.99</td>
<td>11.1</td>
</tr>
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</table>

Source: Adopted from Wilson et al. (1990) and Schwartz and Dioli (1992)

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. 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 form 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 it’s 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 Productions

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 in the Borena areas. 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 area (Richard, 1979; Demeke, 1998). Besides the prevailing diseases, camel keeping is relatively a recent event in 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 (Biffa and Chaka, 2002). 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

**2.4.1.1 Economic Importance**

Brucellosis is characterized by abortion, non-viable offspring birth in female, and orchitis and epididymitis in male animals (Radostits *et al.*, 1994; Seifert, 1996). 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.*, 1994). 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.

2.4.1.2 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., 1994; 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 herdsmen 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).

2.4.2 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. Reported infection rates from camel rearing areas of Ethiopia ranges from 4.2 to 5.5. Table 2 summarizes the prevalence rates of camel brucellosis in Ethiopia and different neighboring countries.

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).

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

<table>
<thead>
<tr>
<th>Country</th>
<th>Authors</th>
<th>Year</th>
<th>No of camels tested</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eritrea</td>
<td>Omar et al.</td>
<td>2000</td>
<td>98</td>
<td>3.1</td>
</tr>
<tr>
<td>Azwai et al.</td>
<td></td>
<td>2001</td>
<td></td>
<td>3.5</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>Domenech</td>
<td>1977</td>
<td>977</td>
<td>4.4</td>
</tr>
<tr>
<td>Richard</td>
<td></td>
<td>1980</td>
<td>762</td>
<td>5.5</td>
</tr>
<tr>
<td>Teshome et al.</td>
<td></td>
<td>2003</td>
<td>1442</td>
<td>4.2</td>
</tr>
<tr>
<td>Kenya</td>
<td>Kagunya and Waiyaki</td>
<td>1978</td>
<td>174</td>
<td>4.6 – 10.3</td>
</tr>
<tr>
<td>Waghela et al.</td>
<td></td>
<td>1978</td>
<td>172</td>
<td>14.0</td>
</tr>
<tr>
<td>Wilson et al.</td>
<td></td>
<td>1982</td>
<td></td>
<td>6.0 – 38.0</td>
</tr>
<tr>
<td>Somalia</td>
<td>Ahmed and Ibrahim</td>
<td>1980</td>
<td>802</td>
<td>8.0 – 11.0</td>
</tr>
<tr>
<td>Andreani et al.</td>
<td></td>
<td>1982</td>
<td>250</td>
<td>10.4</td>
</tr>
<tr>
<td>Baumann et al.</td>
<td></td>
<td>1990</td>
<td></td>
<td>3.1</td>
</tr>
<tr>
<td>Baumann and Zessin</td>
<td></td>
<td>1992</td>
<td>1039</td>
<td>0.3 – 1.9</td>
</tr>
<tr>
<td>Bornstein et al.</td>
<td></td>
<td>1984</td>
<td></td>
<td>8.5 – 11.5</td>
</tr>
<tr>
<td>Elmi</td>
<td></td>
<td>1982</td>
<td>514</td>
<td>12.6</td>
</tr>
<tr>
<td>Sudan</td>
<td>Abbas et al.</td>
<td>1987</td>
<td>238</td>
<td>3.0</td>
</tr>
<tr>
<td>Abudamir et al.</td>
<td></td>
<td>1984</td>
<td>740</td>
<td>4.9</td>
</tr>
<tr>
<td>Agab et al.</td>
<td></td>
<td>1993</td>
<td>453</td>
<td>24.0</td>
</tr>
<tr>
<td>Majid et al.</td>
<td></td>
<td>1999</td>
<td>805</td>
<td>13.9 – 43.9</td>
</tr>
<tr>
<td>Osman and Adlam</td>
<td></td>
<td>1987</td>
<td>137</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Source: Adopted from Wernery and Kaaden, (2002) with modification
2.4.3 Epidemiology of Camel Brucellosis

2.4.3.1 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. neotomae* (Corbel and Morgan, 1984; Radostits *et al.*, 1994). *Brucella* are small, short rod, coccobacilli or short rod (measuring 0.5 x 0.7 to 0.6 x 1.5µm) 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.*, 2002). Most strains are aerobic (some are micro-aerophilic) but many of them are carboxyphilic (capnophilic) and best grow in CO₂ enriched atmosphere. Growth is unlikely on an ordinary media. They are catalase and oxidase positive, produce H₂S, hydrolyse urea and reduce nitrate with some exceptions. The organisms neither produce indole, acetyl methyl carbinol nor utilize citrate (Corbel, 1990; Quinn *et al.*, 2002).

*Brucella* are generally susceptible to heat, direct sunlight, acidic conditions and common disinfectant (Radostits *et al.*, 1994). However, in favorable 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 (*Camelus dromedarius*) in different countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Authors</th>
<th>Organs or specimens</th>
<th>Species isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iran</td>
<td>Zowghi and Ebadi (1988)</td>
<td>Lymph node</td>
<td><em>B. melitensis</em>, biovar 1</td>
</tr>
<tr>
<td>Kuwait</td>
<td>Zowghi and Ebadi (1988)</td>
<td>Lymph node</td>
<td><em>B. melitensis</em>, biovar 3</td>
</tr>
<tr>
<td></td>
<td>Al-Khalaf and El-Khaladi (1989)</td>
<td>Lymph node, Fetal stomach</td>
<td></td>
</tr>
<tr>
<td>Libya</td>
<td>Gameel et al. (1993)</td>
<td>Milk, aborted fetus, vaginal swab</td>
<td><em>B. melitensis</em>, biovar 1</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>Radwan et al. (1992)</td>
<td>Milk</td>
<td><em>B. melitensis</em>, biovar 1,2</td>
</tr>
<tr>
<td></td>
<td>Radwan et al. (1995)</td>
<td>Milk</td>
<td>“<em>B. melitensis</em>”, biovar 1, 2, 3</td>
</tr>
<tr>
<td></td>
<td>Ramadan et al. (1998)</td>
<td>Milk, Carpal hygroma</td>
<td><em>B. melitensis</em></td>
</tr>
<tr>
<td>Senegal</td>
<td>Verger et al. (1979)</td>
<td>Milk</td>
<td><em>B. abortus</em>, biovar 1, 3</td>
</tr>
<tr>
<td>Sudan</td>
<td>Agab et al. (1994)</td>
<td>L. node testes, vaginal swab</td>
<td><em>B. abortus</em>, biovar 3</td>
</tr>
</tbody>
</table>

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

2.4.3.2 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*., 1994). 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).

2.4.3.3 Host Factors

Infection may occur in animals of all age groups, but persists commonly in sexually mature animals (Radostits *et al.*, 1994). 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 *et al.*, 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.*, 1994).

The extent to which infection rate varies due to breed difference is not well known. Wernery 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.

2.4.3.4 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).
2.4.4 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 (Radostist 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 H₂O₂ 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 (Werney 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).

2.4.5 Immune Responses

2.4.4.1 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., 1994). IgM antibodies, which appear initially after infection and low levels of IgG, will cause complement-mediated lysis of Brucella. Secretary 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 hands, the immunogeneity 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).
2.4.4.2 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 intermediate. Both CD$_4$ and CD$_8$ 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 dismudase and catalase (Frenchick *et al*., 1985; Harmon *et al*., 1988; Quinn *et al*., 2002). Tatum *et al.* (1992) suggested that anti-oxidant Cu-Zn superoxide dimudase plays a role in the survival of *Brucella* species in phagocytic cells.

2.4.6 Diagnostic Methods

2.4.6.1 Bacteriological Methods

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). Commonly used basal media include: serum dextrose (Agab *et al*., 1994), serum tryptose agar, glycerol dextrose agar, trypticase, and 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, 2000).

Milk samples, vaginal swabs, semen and aborted fetus are useful for recovering the organisms antemortem. Samples collected at necropsy include multiple lymph nodes, spleen, udder,
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). Cultures then, incubated at 37°C with 5-10% CO\(_2\) enrichment for three days and above (Alton et al., 1975; Gameel et al., 1993; Agab et al., 1994). Characteristics 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-Zeihl 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. Florescent antibody test and polymerase chain reaction methods have been described for Brucella species identification (WHO, 1997; Walker, 1999; Quinn et al., 2002).

2.4.6.2 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 (Ajoig and Adamu, 1998; Quinn et al., 2002). Accordingly, RBPT is considered as satisfactory screening test (Nicoletti, 1992; OIE, 2000). 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., 1983; 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, 1996). The test distinguishes reaction caused by other factors like vaccines and other bacteria infections. Escherichia coli O:157, Yersinia enterocolitica O:9, Vibrio colerae, Psuedomonas malophilia 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 aerugenosa (Corbel, 1990; Cloeckaert 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 minimize the risk of cross-serological reactions between Brucella and these groups of bacteria (Vizcaino et al., 1991; OIE, 2000).
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 detecting *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., 1983). It can also be used both for screening and confirmatory tests (FAO, 1996). 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, 2000).

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).

2.4.7.1 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).
2.4.7.2 Management practices 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, the following points can be attempted in reducing infection rates (Hunter, 1994; Radostits et al., 1994):

- Public awareness is of vital importance in successful control and prevention of brucellosis
- Isolation of infected animals and female at parturition
- Proper disposal of aborted fetus, placental tissue and uterine discharge and
- Disinfecting of contaminated areas.
3. MATERIAL AND METHODS

3.1 Study Area

The study was conducted in two districts namely, Liben and Yabello, which are located in north eastern and northern part of Borena lowlands of southern Oromia region, respectively (Figure 1). The towns of Liben and Yabello are found at 600 km and 565 southeastern of Addis Ababa. Generally, the Borena area represents a vast lowland area of southern Ethiopia covering an area of about 95,000 km$^2$. The area is bordering with Kenya to the south, Somale region to the east, Guiji zone to the north and southern people, nation and nationalities region to the west. The Borena plateau gently slopes from high mountain massifs in the north (1650 m. a.s.l) to the south bordering Kenya (1000 m.a.s.l) with slight variation due to central mountain ranges, and scattered volcanic cones and craters (Coppock, 1994).

The climate is generally semi-arid with annual average rainfall ranging from 300 mm in the south to over 700 mm in the north. The rain pattern is bimodal type with the main rainy season locally “ganna” (65%) extending from March to May and small rainy season “haggaya” from mid September to mid November. Annual mean daily temperature varies from 19°C to 24°C with moderate seasonal variation. The other two seasons are the cool dry season “adoleessa” extending from June to August and the warmer dry season “bonna” December to February. Seasons affect herding strategies due to its effect on forage and water resource availability (Coppock, 1994). Consequently, herd splitting is practiced to cope up with shortage of resources (Demeke, 1998; Desta, 2000).

The vegetation is dominated by savannah type containing mixture of perennial and woody plants. The savannah community varies from open grassland to bush encroached areas. There is shift in composition in response to heavy grazing, browsing, burning and drought. Grazing shift the community to more trees where as browsing and burning favors the grass. Several plant species in the area are recognized as valuable livestock forage. Acacia are dominating bushes species in the area (Coppock, 1994; Desta, 2000).
Figure 1: Map of study areas and other camel rearing regions of Ethiopia
Surface water is a serious problem in the area. Traditional deep wells “ellas”, ponds, perennial springs, permanent rivers (Dawa and Genale), and seasonal sources (streams, ephemeral ponds and shallow wells) are water sources for both human and livestock. Deep wells and large ponds (machine excavated) are used in dry seasons while seasonal streams, ephemeral ponds and shallow wells are used in wet seasons (Helland, 1982).

Animal husbandry is characterized by extensive pastoral production system and seasonal mobility. Cattle are the dominating animal species followed by goats, camels and sheep. Camel and cattle herd splitting into mobile “forra” and home-based “warra” is practiced as strategy to mitigate forage and water shortage (Desta, 2000). Camel herd movement may be moving the whole herd to water point and to relatively high altitude where green forage is available or partial herd away from home base (Demeke, 1998). Table 4 shows the distribution of camels and other livestock in Borena lowland

3.2. Study population

There are about 328,080 and 11,036 camels being kept in Liben and Yabello districts respectively (Table 4). Approximately, a total of 339,116 camel populations exist in the area and this was considered as study population.

Table 4: Number and distribution of livestock in the six Borena lowland districts

<table>
<thead>
<tr>
<th>Districts</th>
<th>Liben</th>
<th>Arero</th>
<th>Yabello</th>
<th>Dire</th>
<th>Moyale</th>
<th>Teltele</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camel population</td>
<td>328080</td>
<td>22606</td>
<td>11036</td>
<td>44697</td>
<td>59760</td>
<td>940</td>
<td>467119</td>
</tr>
<tr>
<td>*Other livestock population</td>
<td>713175</td>
<td>229227</td>
<td>294432</td>
<td>631680</td>
<td>198570</td>
<td>281180</td>
<td>2348264</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1041255</td>
<td>251833</td>
<td>305468</td>
<td>676377</td>
<td>258330</td>
<td>282120</td>
<td>2815383</td>
</tr>
</tbody>
</table>

* Other livestock population includes cattle, goats, sheep and equines.
Source: BZDPED (1998)
3.3 Sampling method

The two districts were selected purposively due to easier accessibility and camel population. Sampling is then conducted by randomizing the pastoral association. At the present there are 38 and 30 pastoral associations (PA) in Liben and Yabello (though the number is non-stable), respectively. PA is the lowest administrative unit within a district that was considered during the survey. Camel herds in 16 PA’s from Liben and 6 from Yabello were sampled during the study based camel population of the two districts. Finally herds were sampled by cluster sampling (about 5% of camel herds per PA). All animals in a herd that were two years of age and above were sampled. 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, 1995):

$$ g = 1.96^2 \left( \frac{n \times V_c + p (1-p)}{d^2} \right) $$

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 alternatives 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. There are two reports; Domenech (1977) and Teshome et al. (2003) reported seroprevalence rates of 1.18% (2 out of 170) and 5.2% (39 out of 743), respectively from the study areas. The average expected prevalence rate being 4.5% (41 out of 913 animals) for the area. Subsequently, the formula used for this option to calculate sample size (n) is as follows:

$$ n = 1.96^2 \times p \times (1-p) $$

$$ d^2 $$

Let $d = 0.01$, 1.96 (CI = 95%), average expected prevalence, $p = 0.045$, $1 - p = 1 - 0.045 = 0.955$, thus the desired sample size for $p = 0.045$ will be $n = 1650$.

In cluster sampling, sample size will relatively be large (Thrusfield, 1995) at the same time the prevalence rate is so low in the area. Therefore, it was save to inflate the sample size twice
as far as practically possible. Hence, \( n = 1650 \times 2 = 3300 \). Demeke (1998) reported the average herd size to be 14 animals, \( 3300/14 = 236 \) herds (clusters). Therefore, it was planned to sample a total of 3300 camels camel population 70% (2310 camels or 165 herds) from Liben and the rest 30% (990 camels or 71 herds) from Yabello. Subsequently, serum samples were collected from 3218 camels (250 herds), 2232 and 986 animals from Liben and Yabello districts, respectively.

### 3.4 Study methodology

#### 3.4.1 Questionnaire survey

Sixty randomly selected camel owners from the two districts were interviewed by using structured questions. Two questionnaire formats; one for serum sampled individual animal history and the other structured questionnaire format for herders were developed and used in this study. By doing so, risk factors that have possible association with the brucellosis occurrence were investigated and used to support serological results.

#### 3.4.2 Serological survey

Blood samples were collected from jugular vein of each camel of selected herds using plain vacutainer tubes. The blood samples were allowed to clot at room temperature. Then, serum was separated from clotted blood by decanting to other tubes. Separated sera were stored at \(-20^\circ\text{C}\) until laboratory test was performed by both RBPT and CFT.

#### 3.4.3 Rose Bengal Plate Test (RBPT)

All sera samples collected were initially screened by RBPT using RBPT antigen (Institut Pourquier 325, rue de la galèra 34097 Montpellier cedex 5, France). Sera samples were kept in refrigerator at \(4^\circ\text{C}\) before testing. Sera and antigen were left at room temperature for half an hour before the test to maintain to room temperature.
The test procedure recommended by Alton et al. (1975) was followed:

- 30 µl of RBPT antigen was added to each circle on the plate,
- 30 µl of test serum was placed alongside the antigen,
- The antigen and test serum were mixed thoroughly by wooden applicator,
- The plate was shacked for 4 minutes
- After four minutes, the degree of agglutination reactions were read and recorded as + + + (coarse clumping and clearing), + + (clumping and some clearing), + (visible fine agglutination), + (weak fine agglutinations using magnifying glass) and in case of positive reactions, and 0 (no agglutinations) in negative reactions.

3.4.4 Complement Fixation Test (CFT)

Positive sera with RBPT were further tested with CFT for confirmation using Standard Brucella abortus antigen (CVL, New Haw, Weybridge, Surrey KT15 3NB, UK). The CFT test proper and reagent preparation procedures were following the procedures outlined by Alton et al. (1975) and OIE (2000). The reading was as complete fixation (no hemolysis) with water clear supernatant was recorded as + + + +, nearly complete fixation (75% clearing) as + + +, partial hemolysis (50%) + + and some fixation (25% clearing) as +. Complete lack of fixation (complete hemolysis) was recorded as 0. For positive reactions final titration was recorded.

Materials used in the laboratory for CFT:

- Micro titer plates (U-shaped), multi channel and single channel micropipettes, pipette tips, plate sealer tape, test tubes, universal bottles for preparation of solutions, stirrers (magnetic), measuring cylinders weighing balance thermometers, pH indicator, Incubator, water bath, refrigerator, deep freezer and centrifuge (NAHRC laboratory, Sebeta)
- Standard Brucella abortus antigen (CVL, New Haw, Weybridge, Surrey KT15 3NB, UK) with 1: 10 working strength), complement (Biome`rieux, France 1: 20 strength), hemolysin or amboceptor (Institut Pourquier, France 1: 700 working dilution), positive and negative control sera (BgVV, Berlin, Germany)
- Sheep RBC (from local breed male sheep), veronal buffer and Alsever`s solutions prepared in the laboratory (NAHRC laboratory, Sebeta).
I. Preparation of veronal buffer with calcium and magnesium (VCM):

- Sodium chloride                      42.5 g
- Barbituric acid                         2.85 g
- Sodium diethyl barbiturate      1.85 g
- Magnesium sulphate               1.018 g
- Calcium chloride                     0.047 g
- Distilled water                        1000 ml,

This solution was diluted by four volumes of 0.04% gelatin solutions before use.

II. Alsever’s solution preparations

- Glucose                              18.66 g
- Sodium chloride                 4.18 g
- Sodium citrate                    8.00 g
- Citric acid                           0.55 g
- Distilled water                    1000 ml.

The solution may be autoclaved or passed through a seitz filter so that SRBC preserved aseptically in a refrigerator.

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

- 7.5 ml of sheep red blood cells (SRBC) was drawn into 12.5 ml Alsever’s solution.
- The SRBC was centrifuged at 2500 rpm for 5 minutes.
- The supernatant was discarded and replaced by veronal buffer in calcium and magnesium solutions (VCM).
- The sheep red blood cells were resuspended in diluents completely.
- This procedure was repeated 4 times.
- Before discarding the supernatant after the last washing, the volume of the packed cell was measured by placing an identical tube next to the blood containing tube filled up to the level of blood by a measured amount of water until the meniscus of SRBC was reached.
- SRBC was diluted in VCM to 2% suspension and stored at +4°C. SRBC for CFT should be at least two days old.
IV. Amboceptor (Hemolysin) titration:
1. 1:500 to 1:8000 serial dilutions of Amboceptor were prepared.
   - 5 test tubes were prepared and 1 ml of VCM was added to tubes 2 to 5
   - 10 µl amboceptor was mixed with 4990 µl VCM in the first tube and 1 ml transferred up to fifth tube
2. 1: 750 amboceptor was prepared and serially diluted up to 1: 12000.
   - 5 test tubes were prepared and 1 ml of VCM was added to tubes 2 to 5
   - 10 µl amboceptor was mixed with 7490 µl VCM in the first tube and 1 ml transferred up to fifth tube

<table>
<thead>
<tr>
<th></th>
<th>1: 500</th>
<th>1: 1000</th>
<th>1: 2000</th>
<th>1: 4000</th>
<th>1: 8000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>1: 500</td>
<td>1: 1000</td>
<td>1: 2000</td>
<td>1: 4000</td>
<td>1: 8000</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>1: 750</td>
<td>1: 1500</td>
<td>1: 3000</td>
<td>1: 6000</td>
<td>1: 12000</td>
</tr>
</tbody>
</table>
3. 0.5 ml from each of these tubes were transferred to a second set of tubes starting with the 1: 12000 dilution (working from lower to higher concentrations allows using one pipette)
4. 1 ml of VCM added to each of the tubes
5. 0.5 ml of 2% sheep red blood cells added, shaken well.
6. The tubes were placed on the bench for 10 minutes.
7. 1 ml of complement at a dilution of 1: 20 added.
8. The tubes were incubated for 30 minutes in water bath at 37°C
9. SRBC sensitized by mixing equal volume of 2% erythrocytes with diluted amboceptor and allow standing for 30 minutes at room temperature or with agitation.
10. The last tube showing complete hemolysis, minimum hemolytic dose (MHD) was read and recorded

V. Fresh complement preparations:
- Four male guinea pigs bled and serum separated from the clot and pooled to produce complement
- Stock solution A:
  - Boric acid (H<sub>3</sub>BO<sub>3</sub>) 0.93 g
  - Borax (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O) 2.29 g
  - Sorbitol (C<sub>6</sub>H<sub>14</sub>O<sub>6</sub>·0.5H<sub>2</sub>O) 11.74 g
  - Saturated sodium chloride solutions 100 ml
- Stock solution B:
  - Borax 0.57 g
  - Sodium azide 0.81 g
  - Saturated sodium chloride solutions 100 ml
8 parts of guinea pigs serum was mixed with 1 part of solution B followed by 1 part of solution A.
The complement was stored frozen at –20°C or below. Before use each 1 ml preserved complement was added to 7 ml of distilled water to restore tonicity.

VI. Evaluation of complement:
1. Freeze dried complement was reconstituted with diluent according to its instructions
2. A 1:100 complement was prepared.
3. Complement was added into the 9 wells increasing by 5 µl every time, starting with 10 µl.
4. Diluent was added into the 9 wells in decreasing amount by 5 µl, starting with 40 µl.
5. 25 µl of antigen added into the wells.
6. The plate was placed in water bath at 37°C for 1 hour.
7. 25 µl hemolytic system (2% sheep red blood cells premixed with equal volume of Amboceptor) added in all wells.
8. The plate was shaked and put again in the water bath of 37°C for another 30 minutes
9. The test was read by recording the minimum hemolytic dose of complement (MHD), which is represented by the first well showing complete hemolysis. The next well contains the full hemolytic dose (FHD).
   The complement dilution = 2FHD/dilution of complement, i.e. 2FHD/100.

VI. Titration of antigen:
Micro titer plate I:
1. 25 µl of VCM was added to every well of U-plate.
2. 25 µl prediluted antigen was added to wells of row A, B, C …G.
3. By serial doubling (two fold) dilution 25 µl of antigen was transferred from row A to B, and again from row B to C, etc. until row G by multi-channel pipette. 25 µl mixture was discarded from row G (row H will only contain the diluent)

Micro titer plate II:
1. 50 µl of VCM was to be added to all wells.
2. 50 µl of prediluted positive control serum was added to all wells of column 1.
3. 50 µl was serially transferred by two fold dilution, from column 1 to 2 and again from column 2, 3 etc. until column 11. 50 µl was discarded from column 11.
Mix plate I and II:
1. 25 µl was transferred from plate II to Plate I.
2. 25 µl of complement at working dilution was to added to all wells of plate I.
3. Plate I was incubated at 37°C for 30 minutes (covered with second empty plate or sealed).
4. 25 µl of 2% sheep red blood cells, amboceptor premixed, equal volume, i.e. 25 µl of sheep red blood cells and 25 µl working dilution of amboceptor was added to all wells.
5. The plates were covered with sealing tape, shaked well and kept in water bath at 37°C for 30 minutes.
6. The last well with 50% sedimentation was read and recorded. The highest dilution of antigen with 50% sedimentation is the right antigen concentration.

The test proper, multiple sera technique:
1. The sera were prediluted to 1:2.5 and incubated at 58°C in a water bath for 30 minutes in order to inactivate the native complement.
2. 25 µl of diluted test sera was placed in wells of first and second rows of U-bottom plate, and 25 µl of veronal buffer was added to all wells except those of the first row.
3. Serial doubling dilutions were then made by transferring 25 µl volume of serum from the second row onwards continuing for at least four dilutions.
4. 25 µl of antigen diluted to working dilution excluding those of the anticomplementary controls, which received 25µl VCM instead.
5. 25 µl of complement in working dilution was added to all wells except control wells.
6. Control wells containing: serum control has serum + complement + diluent and antigen control has antigen + complement + diluent. Complement control has complement + diluent and hemolytic system has diluent set up to contain 75 µl total volume in each case before hemolytic system was added.
7. The plates were incubated at 37°C for 30 minutes with agitations (warm fixation).
8. 25 µl of volume sensitized 2% SRBC suspension was added to each wells. The plates were sealed and reincubated at 37°C for 30 minutes with agitations.
9. The results were read after the plates were centrifuged at 2500 rpm for 4 minutes to deposit unlysed red blood cells.

Interpretation: Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1: 5 (Dohoo et al., 1986) or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive (Alton et al., 1975; OIE, 2000).
3.4 Data Analysis

Data on serum sampled individual animals and questionnaire were stored in Microsoft Excel spread sheet (Microsoft Corp. 1985 – 2000) as database. The seroprevalence for animal level was calculated on the basis of RBPT and CFT positivity, dividing the number of Brucella reactors by total number of tested animals. Similarly, herd level prevalence was computed as the number of herds with at least one positive animal divided by the total number of herds tested. The within herd seroprevalence was also calculated by dividing the number of reactors in a herd by total number of animals tested in that herd.

To fulfill the assumptions of independent variables, multi co-linearity of variables were checked initially using Intercooled Stata 7.0 (Stata Corp. 1984-2001, College Station, Texas 77845, USA). Those factors not having strong linear relationship (r < 0.4) were used in the analysis. This enables to exclude confounding effects among the factors. Intercooled Stata 7.0 was used to generate categories of risk factors on which statistical analysis was performed. Univariate logistic regression analysis was employed to determine the associations of risk factors with occurrence of camel brucellosis. 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 animals exposed to a variable and the odds of the disease occurring among animals not so exposed (Thrusfield, 1995). Association of risk factors among different parity groups was analyzed using Chi–square for independence.

All risk factors having p < 0.2 on univariate analysis were subjected to multivariate analysis using logistic regression to determine the major risk factors. A stepwise approach (forward selection and backward elimination) was constructed to analyze those factors having putative effects on disease occurrence, based on a p-value <0.20 as the significance threshold for entry or removal. The linear relationship between infection rates and continuous variables were also calculated.

Questionnaire data were analyzed by descriptive statistics using Microsoft Excel and Intercooled Stata 7.0. The results were compared with the serological findings.
4. RESULTS

4.1 Serological Results

A total of 250 camel herds (3218 animals): 172 herds (2232 animals) from Liben and 78 herds (986 animals) from Yabello were sampled. RBPT identified 72 positive reactors out of 3218 serum samples (2.2%). Further confirmation by CFT showed 58 positive cases out of 72 (80.6%). RBPT agglutination intensities and CFT titers showed strong positive linear relationship (Figure 2) with correlation coefficient (r) of 0.73. All RBPT agglutinations above 1+ were found positive by CFT except for two samples at 2+ degrees of agglutinations. Most of CFT titers (74%) were distributed between 1/20 and 1/320. 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.

![Graph showing the relationship between RBPT degree of agglutination and CFT titer]

Figure 2: Linear relationship between RBPT degree of agglutinations and CFT titers on 72 RBPT reactors that further tested by CFT

The seroprevalence of the two districts with respect to age and sex is shown in Table 5. Slightly higher animal prevalence was recorded in Yabello (2.0%) than in Liben (1.7%). The two districts showed similarity of seropositivity (p > 0.05). An overall animal level seroprevalence rates of 1.80% was recorded. In both districts young animals had lower reactor
rates (ranging from 0.8% to 1.4%) than adults (ranging from 2.1% to 2.2%). Similarly, higher reactor rates were observed in females compared to male camels in respective districts. The lowest and highest infection rates of 0.4% and 2.5% were recorded for young males in Liben and adult females in Yabello, respectively.

Table 5: *Brucella* species seroprevalence in the two districts stratified by sex and age

<table>
<thead>
<tr>
<th></th>
<th>Liben district</th>
<th>Adult camels (&gt; 4 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young camels (2 - 4 years)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Total sample</td>
<td>Positives</td>
</tr>
<tr>
<td>Male</td>
<td>280</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>436</td>
<td>5</td>
</tr>
<tr>
<td>Sub total</td>
<td>716</td>
<td>6</td>
</tr>
</tbody>
</table>

|                  | Yabello district |                      |
| Male             | 168            | 1         | 0.6| 68           | 1         | 1.5| 0.9       |
| Female           | 190            | 4         | 2.1| 560          | 14        | 2.5| 2.4       |
| Sub total        | 358            | 5         | 1.4| 628          | 15        | 2.2| 2.0       |
| Total            | 1074           | 11        | 1.0| 2144         | 47        | 2.2| 1.8       |

4.2.1 Risk factors of camel *Brucella* species seroprevalence

Significantly higher infection rate was recorded in females 2.1% (52 of 2528) than males 0.9% (6 of 690) (p < 0.05) with the likelihood of brucellosis occurrence 2.3 times higher in females than male animals (Table 6). Adult dromedaries (> 4 years) had higher *Brucella* antibody distribution than young animals (p < 0.05), the odds of seroprevalence being 2.2 times higher in adult camels. Herd size was classified into three categories (small 3 -10, medium 11 – 20 and large > 20 animals) as judged with respect to enclosure space and local herding condition context. Correspondingly, significantly increasing positivity was recorded with respect to increasing herd sizes (p < 0.05) with risk of seroconversion increasing from small size to medium size, and from small to large by 40%, and 140%, respectively.
Table 6: Risk factors for the occurrence of seropositivity to *Brucella* spp. at animal level

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Category</th>
<th>Total sample</th>
<th>% Positives</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total Positives</td>
<td>(95% CI)</td>
<td>P - value</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>690</td>
<td>6</td>
<td>0.9 (0.3 - 1.9)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2528</td>
<td>52</td>
<td>2.1 (1.5 - 2.7)</td>
</tr>
<tr>
<td>Age</td>
<td>2 - 4</td>
<td>1074</td>
<td>11</td>
<td>1.0 (0.5 - 1.8)</td>
</tr>
<tr>
<td></td>
<td>&gt; 4</td>
<td>2144</td>
<td>47</td>
<td>2.2 (1.6 - 2.9)</td>
</tr>
<tr>
<td>Herd size</td>
<td>3 - 10</td>
<td>777</td>
<td>9</td>
<td>1.2 (0.5 - 2.2)</td>
</tr>
<tr>
<td></td>
<td>11 - 20</td>
<td>1523</td>
<td>24</td>
<td>1.6 (1.0 - 2.3)</td>
</tr>
<tr>
<td></td>
<td>&gt; 20</td>
<td>918</td>
<td>25</td>
<td>2.7 (1.8 - 4.0)</td>
</tr>
<tr>
<td>Parity</td>
<td>No parturition</td>
<td>198</td>
<td>4</td>
<td>2.0 (0.6 - 5.5)</td>
</tr>
<tr>
<td></td>
<td>Single parity</td>
<td>371</td>
<td>7</td>
<td>1.9 (0.86 - 3.8)</td>
</tr>
<tr>
<td></td>
<td>More than one</td>
<td>1333</td>
<td>32</td>
<td>2.4 (1.6 - 3.8)</td>
</tr>
<tr>
<td>Herding experience</td>
<td>Started recently</td>
<td>1432</td>
<td>28</td>
<td>2.0 (1.3 - 2.8)</td>
</tr>
<tr>
<td></td>
<td>Life time</td>
<td>1786</td>
<td>30</td>
<td>1.7(1.1 - 2.4)</td>
</tr>
</tbody>
</table>

** Significant at 95% level of significance

As indicated in Figures 3 and 4, a strong positive linear relationship between increasing age and herd size, and seroconversion (r = 0.98 and 0.97) was observed. On the other hand, seroconversion was not significantly affected by different parities among breeding females (p > 0.05). Higher reactor rate was recorded in multi parous animals compared to other group of camels. However, those with single parity had slightly lower reactors than without parturition, where the reverse was expected. Herding experience by herdsmen of lifetime experience and those started recently did not have any effect on the status of *Brucella* antibodies in their respective animals. Those with lifetime experience were presumed to have better hygienic and management conditions.

Figures 3 and 4 illustrate a strong positive linear relationship between increasing herd size and age groups, and positivity, with the correlation coefficient (r) of 0.97 and 0.98, respectively.
Figure 3: Linear relationship between increasing herd size and animal level seroprevalence

Figure 4: Linear relationship between increasing age and seroprevalence

Multivariate analysis of risk factors determined herd size as the major risk factor for the occurrence of seropositivity to *Brucella* spp in camels. Table 7 shows herd size was more significantly associated with occurrence of seroprevalence in camels (p < 0.05) compared to other factors like sex and age.
Table 7: Multivariate logistic regression analysis of risk factors

<table>
<thead>
<tr>
<th>CFT</th>
<th>Odds Ratio</th>
<th>Std. Error</th>
<th>z</th>
<th>P&gt;z</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1.9</td>
<td>0.9</td>
<td>1.4</td>
<td>0.17</td>
<td>0.1- 4.6</td>
</tr>
<tr>
<td>Age</td>
<td>1.4</td>
<td>0.2</td>
<td>1.7</td>
<td>0.09</td>
<td>1 - 1.7</td>
</tr>
<tr>
<td>Herd size</td>
<td>1.5</td>
<td>0.3</td>
<td>2.1</td>
<td>0.03*</td>
<td>1.03 - 2.2</td>
</tr>
</tbody>
</table>

LR chi2 (3) = 12.79    Prob > chi2 = 0.005

*Herd size was statistically found to be the risk factor for camel brucellosis occurrence (P< 0.05)

Advance in age and herd size were significantly associated with infection rate (p < 0.05) when the putative effects of different factors subjected to step wise backward reduction method. Table 8 shows that increasing age and herd size having significantly joint effect on seropositivity in dromedaries when other factors removed (p < 0.05).

Table 8: Putative effects of advance in age and herd size on seroprevalence

<table>
<thead>
<tr>
<th>CFT</th>
<th>Odds Ratio</th>
<th>Std. Error</th>
<th>z</th>
<th>P&gt;z</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.4</td>
<td>0.2</td>
<td>2.4</td>
<td>0.02</td>
<td>1.1 - 1.9</td>
</tr>
<tr>
<td>Herd size</td>
<td>1.5</td>
<td>0.3</td>
<td>2.2</td>
<td>0.03</td>
<td>1.0 - 2.2</td>
</tr>
</tbody>
</table>

LR chi2 (2) = 10.66    Prob > chi2 = 0.005

4.1.2 Seroprevalence and risk factors at herd level

The overall herd level and within herd seroprevalence rates for the study area were 16% and 9.5%, respectively. The herd level and maximum within herd prevalence recorded in this study were 14.5% (95% CI = 9.6 – 20.7) and 45.5% (5 out of a herd of 11 animals) in Liben and 19.2% (95% CI = 11.2 – 29.7) 12.5% in the other district. There was a highly significant increase in herd level seroprevalence with respect to herd size (P <0.01).

The risk for the occurrence of positivity being 2.8 time higher in medium and 4.9 times higher in large herds than small sized herds. Seroprevalence was not different between herds owned by those who had lifetime experience and recently started (P > 0.05) similar to animal level
Table 9: Risk factors associated with seroprevalence occurrence at herd level

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>Number of herds</th>
<th>Infected herds</th>
<th>% (95% CI)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 – 10</td>
<td></td>
<td>113</td>
<td>9</td>
<td>8.0 (3.7 – 14.6)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>11 – 20</td>
<td></td>
<td>93</td>
<td>18</td>
<td>19.4 (11.9 – 28.9)</td>
<td>2.8 (1.2 - 6.4)</td>
<td></td>
</tr>
<tr>
<td>&gt; 20</td>
<td></td>
<td>44</td>
<td>13</td>
<td>29.6 (16.8 – 45.2)</td>
<td>0.00*</td>
<td>4.9 (1.9 - 12.2)</td>
</tr>
<tr>
<td>Herding</td>
<td>Life time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experience</td>
<td>Recently</td>
<td>151</td>
<td>20</td>
<td>13.8 (8.3 – 19.7)</td>
<td>0.2</td>
<td>1.7 (0.8 - 3.3)</td>
</tr>
<tr>
<td></td>
<td>Yabello</td>
<td>78</td>
<td>15</td>
<td>19.2 (11.2 – 29.7)</td>
<td>1.4</td>
<td>1.4 (0.7 - 2.8)</td>
</tr>
<tr>
<td>Districts</td>
<td>Liben</td>
<td>172</td>
<td>25</td>
<td>14.5 (9.6 – 20.7)</td>
<td>0.4</td>
<td>1.4 (0.7 - 2.8)</td>
</tr>
</tbody>
</table>

*Herd size is highly significant (p < 0.01) at herd level

Table 10: Multivariate logistic regression analysis of risk factors at herd level

<table>
<thead>
<tr>
<th>CFT</th>
<th>Odds Ratio</th>
<th>Std. Error</th>
<th>z</th>
<th>P&gt;z</th>
<th>[95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herding experience</td>
<td>0.53</td>
<td>0.24</td>
<td>-1.38</td>
<td>0.17</td>
<td>0.2 - 1.3</td>
</tr>
<tr>
<td>Herd size</td>
<td>1.12</td>
<td>0.03</td>
<td>4.17</td>
<td>0.00</td>
<td>1.06 - 1.2</td>
</tr>
</tbody>
</table>

LR chi2 (2) = 23.55 Prob > chi2 = 0.000

As illustrated in Figure 5, the distributions of reactor animals per infected herds showed 50% of reactor animals, more than one reactor per herds, were clustered in 11 out of 40 herds (28%) indicating clustering of the disease in some herds.
Figure 5: The frequency distributions of positive reactors in 40 seropositive herds

Figure 6 shows the occurrence of live birth, abortion and stillbirths percent in seropositive and negative breeding females (n = 1704), that had aborted given birth or stillbirth as an outcome of pregnancy during their production life in the past.

Figure 6: Effects of brucellosis on reproductive parameters in breeding females in Liben and Yabello based on breeding females history profile (outcome of pregnancy)
An apparent difference was observed in reproductive parameters between seropositive and negative breeding females. However, this difference was not significant as the 95% binomial confidence intervals of the two groups overlapped for respective variables. Live birth percent was slightly higher for seronegative females while abortion and stillbirths were less in positive group. The values for stillbirth are too small to compare the groups. Perhaps some herdsmen consider stillbirth as abortion and might have missed it. Figure 7 shows that seroprevalence were increased with increasing abortion frequencies in 573 aborted females and reached peak (5%) in animals that aborted three times and declined thereafter.

![Graph showing seroprevalence with respect to abortion frequencies](image)

Figure 7: Status of seroprevalence with respect to abortion frequencies in breeding females with abortion history

### 4.2 Description of Demographic Production Characteristics

Pastoral communities of Borena, Guji, Arsi and Somale clans dwelling in Liben, and Borena, Gebra and Guji in Yabello district produce camels. The Borena and Guji have started camel production recently and have less experience (3 to 30 years) than Somales and Gebra (lifetime experience). High milk production is primary purpose of camel production in the area followed by transport, cash income by sale and meat production (slaughter). In addition to high milk production, drought mitigation and to some extent as alternative means against bush encroachment are decisive forces behind to start camel keeping among the Borena pastoralists.
Herds either belong to a household (83.3%) or extended family (16.3%). As a result herding can be separate (83.3%), with family herds (13.3%) or with village herds (3.3%). Consequently, majority of the herders have their own breeding bull (85%) whereas some of them use village bulls (15%). Labor division among family varies with a type of activities in that most of the herding activities done by youngsters while adult men do watering and delivery assistances. Women and men also are engaged in milking activities. The recorded mean herd size was $13.6 \pm 7.8$ with herd size ranging from 3 to 42 animals. Those owners with less than three animals do not keep alone and mix with family or village herds until the number increases. The herd composition was mainly consisting of breeding females (50%) followed by calf, young females, and young male (Figure 8). In addition to camels, majority of the pastoralists keep cattle and small ruminant (81.7%), some of them (13.3%) keep either cattle or small ruminants along side with camels whereas only few groups possessed camels only (Table 11). The status of seropositivity among herds kept with other ruminant is similar. Those kept only camels (3 out of 60) and with cattle only (3 out of 60) had no reactor whereas herds raised with more than one ruminant had seroprevalence ranging from 1.7 to 1.9%. The number of tested animals in those groups with no reactor is too small to make comparison and justify ruminants as risk factor.

![Figure 8: Herd composition based on 60 investigated herds in Liben and Yabello districts of Borena area](image-url)
Table 11: Ruminants kept alongside investigated camel herds by herdsmen (n= 60) in Liben and Yabello districts of Borena lowland and seroprevalence status of respective herds.

<table>
<thead>
<tr>
<th>Compositions of ruminant species</th>
<th>Number of camel herds</th>
<th>% out of total</th>
<th>Number of camels tested</th>
<th>Seroprevalence status: Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only camels</td>
<td>3</td>
<td>5</td>
<td>37</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cattle and camels only</td>
<td>3</td>
<td>5</td>
<td>84</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cattle and small ruminant and camels</td>
<td>49</td>
<td>81.7</td>
<td>697</td>
<td>13 (1.9)</td>
</tr>
<tr>
<td>Cattle and camel or small ruminant</td>
<td>52</td>
<td>86.7</td>
<td>781</td>
<td>13 (1.7)</td>
</tr>
<tr>
<td>Small ruminant and camels or cattle</td>
<td>54</td>
<td>90</td>
<td>793</td>
<td>14 (1.8)</td>
</tr>
<tr>
<td>Small ruminant and camels only</td>
<td>5</td>
<td>8.3</td>
<td>96</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
<td>914</td>
<td>14 (1.5)</td>
</tr>
</tbody>
</table>

Common water points that herders usually use in the area include traditional wells (60.8%), ponds (machine excavated ponds) (26.6%) and river with mean watering interval of 6.0 ± 1.8 days based on accessibility though mobility does not restrict herds to a specific water source. Traditional wells are more numerous and distributed in the area than other sources. Water from traditional wells (salty in nature) is also more preferable for dromedaries due to their high salt requirement. In the current study regardless mobility it was observed herds that often use ponds and traditional wells water points had higher prevalence (23.8%) than those using traditional wells alone (13.6%), and river and wells together (9.5%). Animals can have direct access to pond during watering unlike that of traditional wells, which is lifted from the wells and added to trough for the animals to drink. Subsequently, the chance of being contamination by discharges is higher in the former case. Moreover, a larger number of different animals come together at pond than at traditional wells.

Dromedaries are produced under a number of constraints of which diseases are very crucial. Trypanosomiasis, respiratory diseases, neck paralysis syndrome, tick paralysis, contagious skin necrosis, abscess, wasting disease of calves, black quarter, camel pox and internal parasites are priority diseases that were identified by the owners. Poor veterinary services together with lack of awareness by herders to use the existing opportunities have worsened the problem.
Only 24.7% of them have so far used veterinary services and the rest large proportions (75.4%) do health care to their animals either by self-treatment with drugs (42.3%) or using traditional healers (33.1%). 41.7% have never used veterinary service due to being far away from it or considering the service as not helpful for camels.

4.2.1 Indicators for the clinical manifestation of brucellosis in herds

Percent of abortion, stillbirth and birth to weak calf were found to be 8%, 3.6% and 7.4%, respectively per breeding females per annum. Abortion and stillbirth affected 20% and 8.3% of the investigated herds. Table 12 shows reported manifestations that may indicate brucellosis in 60 investigated herds in Liben and Yabello districts; those herds had a total of 598 breeding females with total live birth of 131 camel calves.

Table 12: Brucellosis indicators in 60 investigated herds in Liben and Yabello districts

<table>
<thead>
<tr>
<th>Manifestations</th>
<th>Affected herd per annum</th>
<th>Affected animals per annum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observations (%)</td>
<td>Observations</td>
</tr>
<tr>
<td>Abortion</td>
<td>12 20</td>
<td>12 8</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>5 8.3</td>
<td>5 3.6</td>
</tr>
<tr>
<td>Birth to weak calf</td>
<td>11 18.3</td>
<td>11 7.4</td>
</tr>
<tr>
<td>Total live birth*</td>
<td>52 86.7</td>
<td>131 88.5</td>
</tr>
<tr>
<td>Sub total</td>
<td>56 93.3</td>
<td>148 100</td>
</tr>
<tr>
<td>Cycling females</td>
<td>5 8.3</td>
<td>17 2.8</td>
</tr>
<tr>
<td>Retained placenta</td>
<td>11 18.3</td>
<td>11 7.4</td>
</tr>
</tbody>
</table>

*Birth to weak calves included in total live birth,

The knowledge on brucellosis is almost nil among the pastoralists of the area. Some of the respondents indicated that a disease that causes abortion is unknown to them and does not have specific local name. Figure 9 shows suggested causes of abortion according to herders. Abortion is multifactorial and the major causes being trypanosomiasis (21.6%), self-kicking due to fly biting (18.7%) and high temperature (16.9%). This event was reported to be high during the wet seasons.
D+: a disease that causes abortion but not known by specific vernacular name

Figure 9: Suggested causes of abortion according to herders in Liben and Yabello districts

4.2.2 Factors influencing brucellosis infection in animals and human beings

Some measures that traditionally taken by camel owners of the area possibly may reduce the spread of brucellosis from infected to other animals. These include isolation of calved and aborted females from the herd during the day (98.3% and 46.7%) and night (98.3% and 33.3%), respectively for variable durations. Those with smaller herds may not practice it. Measures also taken to frequently aborting and cycling females by selling (56.3% and 85.8%) and attempting treatment for sometimes (18.8% and 11.1%), respectively. Few of them do dispose aborted fetus (6.8%) and fetal membrane (3.3%) away from reach of camels. Others either take no action (54%) or skinning (39.7%) aborted fetus for fostering and milking.

On the other hand, several activities and existing habits might have role in the transmission of the disease from animal to human. Raw fresh milk is habitually consumed by the majority of the inhabitants (93.3%) whereas only few groups consume sour milk and milk boiled with tea. It does not necessarily mean that those that indicated often consume boiled milk with tea and sour milk never consume raw, it was reported due to inadequacy and fresh camel milk is not appreciated by those who adapted to the taste of cattle milk (Table 13).
To the contrary meat is consumed cooked by almost all except for chopped raw liver and hump (63.3%) that is liked by some groups of pastoralists. Besides consumption of camel products, owners are usually in close contact with their animals. They do delivery assistance, clean thin membrane from newborn, assist suckling and carry the newborn from field to home without any self-protection.

Table 13: Milk and meat consumption habits (n = 60) in Liben and Yabello districts

<table>
<thead>
<tr>
<th>Milk consumption habits (usually)</th>
<th>Meat consumption habits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response</td>
<td>Fresh</td>
</tr>
<tr>
<td>Yes</td>
<td>56</td>
</tr>
<tr>
<td>No</td>
<td>4</td>
</tr>
<tr>
<td>% (Yes)</td>
<td>93.3</td>
</tr>
</tbody>
</table>

** Raw others include raw hump and liver
5. DISCUSSION

Pastoral communities of Borena lowland produce camels primarily for milk production followed by transport, cash income by sale and meat production. Demeke (1998) also reported similar reasons for camel keeping while Coppock (1994) stated, as transportation is the main purpose in Borena except for Gebra and Somale ethnic groups who keep camels mainly for milk production. Borena and Gujis are basically cattle herders and recently started camel husbandry as asset diversification for uncertainties, drought mitigation and coping up with changing rangeland ecology (Biffa and Chaka, 2003). As a result they had less herding experience ranging from 3 to 30 years, resulting in less indigenous knowledge in camel husbandry.

Herd size. The average herd size recorded in this study is equivalent to that of Demeke (1998) but less than the reported values of 22.7 and 35.2 from Shinile and Jijiga in eastern Ethiopia, respectively (Getahun and Kassa, 2000). Larger proportion of females in herds of the area indicates a strong desire to maximize the herd size by herders. High degree of ruminant diversification was also observed in the area. Such strategy is common to other areas and has economic and ecological advantages (Ayan, 1984; 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).

A large number of livestock herds are congregated at water points facilitating the spread of disease. Traditional wells, ponds and few rivers are major permanent water sources in the area (Helland, 1982). Unlike traditional wells (water lifted by people and added to trough), animals have direct access to pond water and contaminate by discharges. Correspondingly, a higher infection rate was recorded in herds often using traditional wells and ponds together. However, the mobile nature of camel herds may not restrict them to a specific category of the water resources, making conclusion difficult.

Brucellosis remains widespread in domesticated and wild animal population, and present a great economic and public health problems in African countries (Chukwu, 1985; Chukwu, 1986). According to Chukwu (1985), the high prevalence of the disease in Africa is probably due to the fact that many African countries have not started control or eradication schemes.
The disease in camels is either caused by *B. melitensis* or *B. abortus* (Wernery and Kaaden, 2002). It seems that *B. melitensis* is the most frequent isolates of camels in Middle East (Table 3) whereas both species were reported in Africa (Abbas and Agab, 2002).

*Brucella* antibodies were detected in 68.8% (11 out of 16) pastoral association (PA) in Liben and all 6 sampled PAs in Yabello districts, showing the widespread occurrence of camel brucellosis even in lower administrative units of the study area. Seroprevalence ranging from 0 to 66.7% and 0 to 5.7% at herd and animal level were recorded from these PA’s, respectively (Annex 8.1). Slightly higher seroprevalence was recorded in Yabello (2.0%, 95% CI = 1.2 – 3.1) than the other district (1.7%, 95% CI = 1.2 – 2.3), making an over all seroprevalence of 1.8% (95% CI = 1.4 – 2.3) for the area. The seroprevalence of *Brucella* spp in the two districts is not statistically different from each other probably due to similarities of those identified risk factors.

The seroprevalence finding of the present study is similar to the previous reports from different countries (Mustafa and Awad El-Karim, 1971; Okoh, 1979; Abu-Damir *et al*., 1984; Abbas *et al*., 1987; Baumann and Zessin, 1992; Abou-Eisha, 2000; Omar *et al*., 2000; Azwi *et al*., 2001; Teshome *et al*., 2003). However, it is lower than some studies in Ethiopia (Domenech, 1977; Richard, 1979), Kenya (Waghela *et al*., 1971), Nigeria (Ajogi and Adamu, 1998), Sudan (Ginawi, 1997; Majid *et al*., 1999), Somalia (Andreani *et al*., 1982), Kuwait (Al-Khalaf and El-Khaladi, 1989) and Saudi Arabia (Radwan *et al*., 1992).

The seroprevalence of brucellosis in camels is low in extensively kept pastoralists camels. Thus, prevalence ranging between 2 and 5% were reported from most countries where camels are produced by pastoralists (Abbas and Agab, 2002; Wernery and Kaaden, 2002). 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). In such production system, large herds together with overcrowding in restricted area provide more chances of contact between animals leading to increased likelihood of infection.

Even under pastoral conditions, individual herds could have an appreciably higher brucellosis than the regional risk. Abbas and Agab (2002) indicated that a seroprevalence of camel brucellosis in certain camel herds in Sudan was ranging from 26.5 to 30%. Majid *et al*. (1999) also reported a seroprevalence rate ranged from 14% to 43.9% from the same country. Likewise, within herd prevalence ranged from 2.7% (1 of 37) to 45.5% (5 of 11) with average
of 9.5% recorded in the present study. As indicated by Figure 5, 50% of infected animals were found in 11 out of 40 infected herds, having more than one reactor per herds, implying clustering of the seropositivity in certain herds.

Teshome et al. (2003) recorded comparable results, 1.2% in small animal number (2 out of 170) from Borena to the current finding, which is lower than the finding of the study in eastern Ethiopia. Low prevalence rate of the present finding is probably due to the existence of relatively higher number of small sized herds (113 of 250 herds) as camel production is started recently compared to larger herd size kept in the eastern Ethiopia (Getahun and Kassa, 2000). The existing tradition of isolating calved and aborted females from other animals in the herd perhaps might reduce the infection rate. Selling of frequently aborting and cycling females might reduce the risk of infection source to other animals. Long calving intervals in combination with late age at first calving in dromedaries kept under extensive production system (Wilson, 1998) limit transmission within and subsequently among herds particularly in areas with low prevalence (Abbas and Agab, 2002). Since, brucellosis is considered as a disease of herd importance, considerably high herd level seropositivity (16%, 95% CI = 11.7 – 21.1) was recorded in this study regardless of low animal prevalence.

Several factors may affect the result of serological findings. Higher seroprevalence of camel brucellosis might be recorded using multiple serological tests in parallel (Waghela et al., 1978; Al-Khalaf and El-Khaladi, 1989) or using tests that had poor specificity (Andreani et al., 1982). Majid et al. (1999) reported higher seroprevalence rate (ranging from 14 to 43.9%) using RBPT alone (highly sensitive test). Reported lower prevalence rates by some authors also could be the result of tests with low diagnostic sensitivity (Baumann and Zessin, 1992) or as a consequence of serial multiple tests (Abbas and Agab, 2002). 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 immune suppressive effects of trypanosomiasis, often prevalent where camels are raised, were reported in vaccinated cattle and goats implying possible impact on serological findings (Chukwu, 1985). Ouma and colleagues (1997) have observed classical pass way hemolytic complement activity was negatively correlated with parasitaemia, indicating immunosupression by trypanosomiasis. Sample selection bias also might affect serological findings. Ajogi and Adamu (1998) recorded seroprevalence as high as 27.8% from
camels slaughtered at three camel rearing regions of northern Nigeria. Animals kept under extensive pastoral management are selected for slaughtering if the production performances decline substantially.

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., 2002). Accordingly, RBPT is considered as satisfactory screening test (Nicoletti, 1992; OIE, 2000; Quinn et al., 2002). The highest specificity of CFT deserved it to be used as confirmatory test in serial testing (OIE, 2000). 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 (Dohoo et al., 1986; Teshome et al., 2003). Improvement of test diagnostic specificity is particularly useful in control programs when test and slaughter policy is adopted

In camels there is yet no standards set for the diagnostic test protocol and diagnostic titer for brucellosis. OIE (2000) recommends the test procedure outlined for the diagnosis of bovine brucellosis to be applied for camels. It is also not well defined to what extent biochemical and physiological peculiarities of camelids contribute to the test result variability. 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). Likewise, 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 effect is not clear.

In the present study complement fixation at 1/10 and above dilutions were considered positive for CFT. As a result seropositivity was confirmed in 58 out of 72 RBPT positive reactors (80.6%) by CFT up on serial testing. RBPT degree of agglutinations showed a positive correlation (Figure 2) with CFT titers and all agglutination above 2+ except two confirmed positive. Most of the CFT titer (74.1%) found to be between 1/20 and 1/320 dilution rates. This may suggest that the obtained result is possibly due to brucellosis. The highest titer recorded was lower than what has been reported by Teshome et al. (2003) in which 30% had a dilution rate of 1/640. The difference could be attributed to the test procedure as warm fixation was used in this study. However, cold fixation was used in the later. Cold fixation was found to yield seropositivity at higher dilutions than warm fixation, though it is time consuming (Alton et al., 1975).
The study revealed that seroprevalence was significantly higher in female than male dromedaries \((p < 0.05)\) with the likelihood of seroconversion 2.3 times higher in females than male animals (Table 6). This result is inconsistent with the reports from Sudan (Yagoub et al., 1990; Agab et al., 1997) and Nigeria (Ajogi and Adamu, 1998). Relatively higher susceptibility of females could be due to the fact that females have more physiological stresses (Walker, 1999). On the contrary, other authors reported equal distribution of \textit{Brucella} antibodies between both sexes (Waghela \textit{et al.}, 1978; Abbas \textit{et al.}, 1987; Abu Damir \textit{et al.}, 1984). Okoh (1979) reported that male animals had higher infection rate but the sample size of females was too small to make the comparison.

Infection may occur in animals of all age groups, but persists commonly in sexually mature animals (Radostits \textit{et al.}, 1994). Younger animals tend to be more resistant to infection and frequently clear infections although few latent infections may occur (Radostits \textit{et al.}, 1994; Walker, 1999). The presence of growth factors such as erythritol and hormones favor infection in mature animals (Quinn \textit{et al.}, 2002; Walker, 1999). Camels produced under extensive production system reach maturity at about 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, animals above 4 years considered mature (adult) for this study. Statistically significant difference was observed between young and mature animals \((p < 0.05)\), the probability of disease occurrence being 2.2 times in mature camels than young animals. As the age increased so did the seropositivity and strong positive correlation was observed with increasing age and reactor rates (Figure 4), the correlation coefficient \((r) = 0.98\). This result was in agreement with the findings of others (Majid \textit{et al.}, 1999; Abou-Eisha, 2000; Teshome \textit{et al.}, 2003). In opposition to this, Radwan \textit{et al.} (1992) observed similarity of seroprevalence among various age groups of adult camels (but young animals were not compared). The current study also illustrated that herd size was significantly associated with brucellosis in camels \((p < 0.05)\) with odds of disease occurrence increasing by 40\% and 140\% when small herd is compared with medium and large herds. Consequently, herd size was statistically identified to be the major risk factor for brucellosis to occur in relation to other factors \((p = 0.03)\). 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 \textit{et al.}, 1993; Agab \textit{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).

*Brucella* antibody distribution was similar in camels of those herdsmen who started camel husbandry recently and who kept them for generations. This could be explained by smaller herd size kept by inexperienced herders reduce the infection rate in spite of presumed poor management. Parity had also no effect on positivity in breeding camels. This might be either due to equal susceptibility of breeding females or possibly as a result of negative impact of brucellosis on fertility; infected animals may had lower number of parity (Wernery and Kaaden, 2002). Radwan et al. (1992) also reported similarity of seroprevalence among various age groups of adult female dromedaries.

Mixed herding and frequent contact with small ruminants and cattle are contributing factors to infection rate. Herders in Borena and elsewhere invariably keep small ruminants and cattle alongside with camels. Herds raised with more than one ruminants had seroprevalence ranging 1.7 –1.9%. Small number of animals tested in camel herds kept with one ruminant or alone may not enable to make comparison and justify more than one ruminants as risk factor in this study. There is high chance of brucellosis transmission from these ruminants to dromedaries as they live in free range in promiscuity 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 (Ismaily et al., 1988; 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 (Table 3) perhaps magnify the role of small ruminants in the transmission of brucellosis to camels.

The seroprevalence status of brucellosis in Borena cattle was reported to be 11.5% (Zewude, 1989) from Dida tiyura ranch (established by local cattle in Yabello), and 6.2% and 3.5% (Bekele et al., 2000) from the ranch and pastoral cattle, respectively. This indicates the potential role of cattle, possibly sheep and goats in the transmission of the disease to camels though there is no information on small ruminant brucellosis status in the area.

In this study percent of abortion was slightly higher in brucellosis positive breeding females than non-infected group but not significantly (Figure 6). Seroprevalence was also increased with abortion frequencies with peak (5%) reached in animals that aborted three times and then
declined. Due to low seroprevalence recorded in this study, it may not be possible to suggest that those clinical manifestations were due to brucellosis. Percent of abortion, stillbirth and birth to weak calf were found to be 8%, 3.6% and 7.4%, respectively per annum. 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, culling and comparatively low milk production (Chukwu, 1987; Wernery and Kaaden, 2002).

The disease can also 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 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)
The current study has shown the distribution of *Brucella* antibodies in 1.8% of the tested dromedaries in Liben and Yabello districts of Borena lowlands. The herds level and within herd seroprevalence were 16% and 9.5%. Despite the fact that low overall seroprevalence of brucellosis was recorded in this study, the herd level and within herd infection rates are considerably high the later ranging 2.7% - 45.5%. This implies that animals and family members of those infected herds are above all at risk. Univariate analysis of sex, age groups and herd size categories has shown significant association between seroprevalence and these factors. The multivariate analysis of presumed risk factors indicated herd size as a major risk factor associated with camel brucellosis. Increase in age and herd size was found to have been jointly associated with brucellosis. Lack of awareness about brucellosis together with existing habit of raw milk consumption and close contact with animals can serve as means of infection in human beings. Results of the present study provide the status of camel brucellosis in the Borena lowland pastoral areas and the risk factors that contribute to the occurrence of the disease in dromedaries as well as possible zoonotic implications in human beings. Based on the results of this study the followings are recommended:

- The low prevalence rate of camel brucellosis observed in the study area may suggest the implementation of a test-and-slaughter policy. However, this remains unfeasible for the time being due to the free movement of herds in the pastoral areas and unaffordable compensation to the owners. Therefore improving management practices can assist in reducing the spread and maintenance of the infection in camel herds.

- Camel pastoralists are often marginalized from public services, facilities and information. Thus, awareness (public health education) on modern animal husbandry, disease prevention and risk of zoonotic diseases is quite necessary.

- Further research work that intended to the isolation of causative agent and identification of species and biotypes in Ethiopia is important.

- Dromedaries thrive, produce and sustain life under number of constraints. Hence, research work that features these animals and maximizing its performance is recommended.
7. REFERENCES


8. ANNEXES

8.1: Summary of *Brucella* antibodies distributions in pastoral associations (PA) of the two districts

<table>
<thead>
<tr>
<th>Districts</th>
<th>PA</th>
<th>Number of herds</th>
<th>Number of animals</th>
<th>RBPT &amp; CFT+ Herd</th>
<th>RBPT &amp; CFT+ Animal</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liben</td>
<td>Adadi</td>
<td>3</td>
<td>61</td>
<td>2</td>
<td>2</td>
<td>66.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td>Balanbal</td>
<td>6</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Bitata</td>
<td>5</td>
<td>77</td>
<td>2</td>
<td>2</td>
<td>40.00</td>
</tr>
<tr>
<td></td>
<td>Boba</td>
<td>6</td>
<td>119</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Bulbul</td>
<td>10</td>
<td>77</td>
<td>1</td>
<td>1</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>Dedeko</td>
<td>6</td>
<td>59</td>
<td>1</td>
<td>1</td>
<td>16.67</td>
</tr>
<tr>
<td></td>
<td>Dhakakala</td>
<td>6</td>
<td>85</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Genale</td>
<td>6</td>
<td>85</td>
<td>1</td>
<td>1</td>
<td>16.67</td>
</tr>
<tr>
<td></td>
<td>Gofiambo</td>
<td>23</td>
<td>233</td>
<td>2</td>
<td>2</td>
<td>8.70</td>
</tr>
<tr>
<td></td>
<td>Kararo</td>
<td>8</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Karsamale</td>
<td>5</td>
<td>64</td>
<td>1</td>
<td>1</td>
<td>20.00</td>
</tr>
<tr>
<td></td>
<td>Nuraumba</td>
<td>20</td>
<td>239</td>
<td>1</td>
<td>1</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>Marsa</td>
<td>4</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Madha</td>
<td>36</td>
<td>391</td>
<td>3</td>
<td>3</td>
<td>7.32</td>
</tr>
<tr>
<td></td>
<td>Hadhesa</td>
<td>19</td>
<td>333</td>
<td>8</td>
<td>15</td>
<td>42.11</td>
</tr>
<tr>
<td></td>
<td>Walenso</td>
<td>7</td>
<td>159</td>
<td>3</td>
<td>9</td>
<td>42.86</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td>172</td>
<td>2232</td>
<td>25</td>
<td>38</td>
<td>14.53</td>
</tr>
<tr>
<td>Yabello</td>
<td>Dadim</td>
<td>12</td>
<td>205</td>
<td>5</td>
<td>6</td>
<td>41.67</td>
</tr>
<tr>
<td></td>
<td>Dharitu</td>
<td>13</td>
<td>120</td>
<td>2</td>
<td>3</td>
<td>15.38</td>
</tr>
<tr>
<td></td>
<td>Dida hara</td>
<td>21</td>
<td>289</td>
<td>4</td>
<td>4</td>
<td>19.05</td>
</tr>
<tr>
<td></td>
<td>Didayabello</td>
<td>10</td>
<td>95</td>
<td>1</td>
<td>1</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>Haro bake</td>
<td>7</td>
<td>112</td>
<td>1</td>
<td>3</td>
<td>14.29</td>
</tr>
<tr>
<td></td>
<td>Surupa</td>
<td>15</td>
<td>165</td>
<td>2</td>
<td>3</td>
<td>13.33</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td>78</td>
<td>986</td>
<td>15</td>
<td>20</td>
<td>19.23</td>
</tr>
<tr>
<td>Grand total</td>
<td></td>
<td>250</td>
<td>3218</td>
<td>40</td>
<td>58</td>
<td>16.00</td>
</tr>
</tbody>
</table>

58
### 8.2: Questionnaire formats for serum sampled individual camels

**Formats 1: Serum sample collection format for individual camels**

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

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Sex</th>
<th>Age</th>
<th>Herd size</th>
<th>Owner experience (year)</th>
<th>Breeding female history in the herd</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>calving abortion stillbirth</td>
<td></td>
</tr>
</tbody>
</table>
I. General information
Region ..................................  Zone ............... District ..............................................
PA ............................................. Village.
Name of respondent .............................................. Age .............. Sex .....................
Herd size: ........................................... Owner experience (years)  ..............................................
Family size ..............................................

II. Comparative importance of camels and its products
1. Types of livestock kept and purpose

<table>
<thead>
<tr>
<th>Species</th>
<th>Purpose</th>
<th>More liked</th>
<th>Relative importance</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 ...............  Un weaned females ..............
5. What is 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

<table>
<thead>
<tr>
<th>Descriptions</th>
<th>Fresh</th>
<th>Boil</th>
<th>Sour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usually consumed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rarely consumed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delicacy / more liked</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.2 what is the shelf life of camel milk (days)? ……………………………

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, predator)

<table>
<thead>
<tr>
<th>Animal types</th>
<th>Animals entered the herd</th>
<th>Animals left the herd</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This year</td>
<td>Last year</td>
<td>This year</td>
</tr>
<tr>
<td>Breeding female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young (F)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young (M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult (M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf (M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf (F)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

11. Activities and labor divisions

<table>
<thead>
<tr>
<th>Activities</th>
<th>Youngsters</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Herding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watering</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery assistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating assistance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12. Water points in different seasons

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Water sources</th>
<th>Frequencies (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>River</td>
<td>Ponds</td>
</tr>
<tr>
<td>Dry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

14.1 List and prioritize ten top camel diseases

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

<table>
<thead>
<tr>
<th>Events in the camel herd</th>
<th>Since one years</th>
<th>Since three years</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes or no</td>
<td>Number</td>
<td>Yes or no</td>
</tr>
<tr>
<td>Abortion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Still birth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth to weak calf</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycling female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bull with swollen tests and joints</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retained placenta</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

15.1 What do you think that cause abortion in camels?

15.2 What do you do with camels that frequently abort?

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

15.3 How do you manage aborted fetus/ fetal membrane?

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

15.4 What do you do with female that doesn’t conceive?

a. Sell,  b. slaughter,  c. keeping  d. other
16. What is the source of bull?
   a. From own herd          b. village bull          c. others……………

17. How do you herd Camels?
   a. Separately,            b. with village herd,  c. with cattle,         d. with small ruminants

17.1 How is night resting?
   a. Separate,            b. share with cattle,    c. Share with small ruminants

17.2. 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
18. Comparative age of male and female (years)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at first mating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive life</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Life span</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.8.1 Age at first calving (Years)……………… Gestation length (Month)…………………
   Caving interval (Month)………………… Lactation length (Month)…………………

19. Seasonal variations of reproductive traits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dry season</th>
<th>Wet season</th>
<th>Both seasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calving (yes/no)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating (yes/no)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milking frequencies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk off take per day (liters)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixing breeding time (yes/no)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
9. CURRICULUM VITAE

1. Personal data
   Name: Bekele Megersa Bati
   Place of birth: Ada’a Berga, West Shewa (central Ethiopia)
   Date of birth: October 18, 1970
   Sex: Male
   Occupation: Civil servant
   Nationality: Ethiopian
   Address: Tel: 01 33 87 80(home) or 09 63 48 15(friend)
            e-mail: bekelebati@yahoo.com

2. Academic background
   Primary education: 1978 – 1986, Ula Gora and Enhini, Ethiopia
   University education:
   Undergraduate study: 1991 – 1996, Addis Ababa University, Faculty of Vet. Medicine
                     Degree: Doctor of Veterinary Medicine (DVM)
   Postgraduate study: 2002 – 2004, Addis Ababa University, Faculty of Vet. Medicine
                     Degree: M Sc in Tropical Veterinary Epidemiology
   Trainings:
   : 1999, Veterinary Epidemiology Course for 5 weeks in Germany
   : 1999, Participatory Rural Appraisal and Training of Trainers (TOT)

3. Research background
   : DVM thesis on small ruminant Seroprevalence of *Pastuerella hemolytica* serotypes in 1996
   : MSc thesis on Seroepidemiological study of brucellosis in camel in Borena lowland pastoral area in 2004

4. Work experience
   : District veterinarian in Borena zone from Oct 1996 to Sep, 1997
   : Deputy head, Borena zone department of agriculture, up to Aug 2002

5. Computer skills
   : Microsoft Word, MS Excel, Access and Statistical softwares

6. Language
   : Oromiffa, Amaric and English languages

7. References
   : Dr Bayleyegn Molla (Associate Professor, AAU, FVM D. Zeit)
   : Dr. Laike Mariam Yigezu (NAHRC, Sebeta).