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Study on Camel and Human Brucellosis in Fentale District, East Shoa Zone, Oromia Regional State, Ethiopia

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Abstract
A cross-sectional study was conducted to determine the sero-prevalence of camel and human brucellosis and to assess the association between risk factors and seroprevalence in Fentale Districts of East Shoa Zone of Oromia Regional State from September 2009 to April 2010. Multi-stage sampling procedure was used. In the study 768 camel and 250 human sera were screened for *Brucella* antibodies using rose Bengal plate test (RBPT). RBPT positive sera were further tested using complement fixation test (CFT) as per OIE recommendation. Moreover, information on individual animal and herd-level risk factors was gathered using pre tested questionnaires. Based on CFT results, the overall individual animal sero-prevalence was 98.6% (among RBPT positives, 70/71). The herd-level sero-prevalence was 36.5% (70/192) and the within-herd sero-prevalence varied from negative animals to at least one positive reactor. The results of binary logistic regression analysis revealed that seropositivity was higher in Dhebiti followed by Kenifa, Ilaala and Saraweba; and sex ways males were more infected than females. However, no statistically significant difference was observed (p=0.872) between sex. The results also indicated that sero-prevalence and seropositivity increase with increment of age and herd size, respectively. In addition herding experience did also affect the status of seroprevalence among the respective categories ($p<0.05$); but reproductive status did not significantly affect the status of seroprevalence ($p>0.05$). History of abortion, fetal membrane retention and stillbirth was found to be significantly (p=0.000, p=0.004 and p=0.000) associated with brucellosis. Watering points /except wet season/ and culling methods of camel management and husbandry related factors were not significantly associated with seropositivity to brucellosis. Out of 250 persons (male and female) tested 15 were RBPT positives and the RBPT positives were tested using CFT accordingly all of them were seropositive, the positive reactor being herdsmen. There was a high risk of acquiring the infection during removal of retained fetal membranes (p=0.000) and in those who were both in contact with animals and drank raw milk (p=0.001). In conclusion the study showed that camel and human brucellosis was prevalent in the study area and appropriate control measures need to be introduced to alleviate the disease problem in the area.
Keywords: *Brucellosis*, CFT, Dromedary Camel, Ethiopia, Fentale, Human, Prevalence, Risk, Factors, RBPT

1. INTRODUCTION

Camels (*Camelus deromedarius*) are versatile, vital domestic animals that are best adapted to harsh environmental conditions prevailing in extreme semi arid and arid areas. They are endowed with extremely extra ordinary features that enable them to survive and perform under extreme hard conditions (Teka, 1991). They are able to produce milk from scanty and highly variable feed sources that can partly be due to their ability to feed on plants that other animals cannot feed on (Yagil, 1985; Higgins *et al.,* 1992) Camels ensure food security in pastoral communities by producing milk and meat. They are also sources of hides, which are used as bed sheets; serve as means of transportation and draught power (Yagil, 1985; Higgins *et al.,* 1992). Long lactation and ability to maintain milk production over long dry spells are important facets of camel production. In spite of all these advantages, camel production and productivity is constrained by a number of factors including infectious diseases, of which brucellosis is considered to play a major role (Kohler-Rollefson *et al.,* 2001). Brucellosis causes heavy economic losses in camels resulting from infertility, abortions, mastitis, and decreased milk production. Infertility is characterized by increased intercalving period and abortion results in loss of neonatal calves (Radositits *et al*., 2000; Wernery and Kaaden, 2002; Kulplulu and Sarimehtoglu, 2004; Al-Majali, 2005). In addition to these, brucellosis hinders international trade in live camels, their products and by products (Maria, 2006; Coelho *et al.,* 2007). Moreover brucellosis is considered to be one of the major zoonotic diseases affecting man. Brucellosis in humans impairs public health (Poester *et al.,* 2002) and hinders social and economic development. Infection is acquired mostly through consumption of raw camel milk, contact with aborted fetus or placenta, and other contaminated tissue samples (CFSPH, 2007). Brucellosis in man is characterized by undulating or fluctuating fever, breast abscess, epididymo-orchitis and spondylitis. While the acute form progress to chronic form, serious complications affecting the musculo-skeletal, cardiovascular and the centeral nervous system may develop (Morata *et al*., 2003; Coetzer and Tustin, 2004). High risk groups are veterinary personnel, butchers, camel herders and consumers of raw milk (Acha and Szyfers, 2001). Economic losses in the public health ensue from absence of work, treatment costs, physician's time and hospitalization costs (Refai, 2002). Camel brucellosis has been found to be one of the diseases associated with reproductive wastage in camel producing pastoralist areas in Ethiopia. Although studies have been carried out to determine the sero-prevalence

of camel brucellosis in different parts of Ethiopia including the Oromia Regional State by different researchers including Domenech, (1977), Richard, (1980), Teshome *et al.* (2003), Bekele *et al.* (2005) and Berhanu, (2006) with prevalences of 4.4, 5.5, 4.2, 1.8 and 2.43%, respectively, Fanatale Districts of East shoa zone where the disease is assumed to be prevailed was untouched.

Therefore, the present study was undertaken to determine the sero-prevalence of camel and human brucellosis, to identify the major potential risk factors, to assess the association between risk factors and seropositivity to camel brucellosis and to identify the public health hazard due to camel brucellosis in Fentale District, East Shoa Zone of Oromia Regional State.

2. LITERATURE REVIEW

2.1. Etiology

Brucellae are small, short rod, coccobacilli (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. Most strains are aerobic (some are micro-aerophilic) but many of them are carboxyphilic (capnophilic) and best grow in CO_2 enriched atmosphere. Growth is unlikely on an ordinary media (Quinn *et al.,* 2002). Brucellae are generally susceptible to heat, direct sun light, 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 found in varying concentration among different smooth variants (Walker, 1999). Camels can be infected by either of the main species of the genus *Brucella* (*B. abortus/* biovars 1, 2, 3 and 7 / and *B. melitensis* /biovars 1, 2, and 3/), but *B. abortus* was shown to be the main cause of brucellosis in camels, while *B. melitensis* was considered less common cause of camel brucellosis (Abbas and Agab, 2002), while Rutter and Mack (1963) considered *B. melitensis* as the agent of camel brucellosis. Both authors based their assumptions on the results of comparative serological tests. The different *Brucella* serotypes isolated from camels is shown in Table 1.

Table 12: Species of *Brucella* isolated from camels

Country	Species isolated	Organ cultured	Reference
Iran	*B. melitensis* biovar 1	Lymph nodes	Zowghi and Ebadi (1988)
Kuwait	*B. melitensis* biovar 3	Lymph nodes	Zowghi and Ebadi (1988)
Libya	*B. abortus* biovar 1	Fetal stomach contents	Gameel *et al*. (1993)
Saudi Arabia	*B. melitensis* biovar 1	Milk, aborted fetus, vaginal swab	Gameel *et al*., (1993)
	B. melitensis biovar 1, 2	Milk	Radwan *et al*.,(1992)
	B. melitensis biovar 1, 2, 3	Milk	Radwan *et al*.,(1995)
	B. melitensis	Carpal hygroma	Ramadan *et al*., (1998)
Sudan	*B. abortus* biovar 1	Lymph nodes, tests, vaginal swab	Agab *et al*., (1996)
Egypt	*B. melitensis* biovar 3	Milk	Abou-Eisha (2000)
Jordan	*B. melitensis* biovar 3	Milk, aborted fetus	Hawari (2008)
Sudan	*B. melitensis* biovar 3,	Lymph nodes	Musa *et al*.,(2008)
	B. abortus biovar 6	Lymph nodes	Musa *et al*.,(2008)

In humans, brucellosis can be caused by *B. melitensis* and *B. abortus* (Kulplulu and Sarimehmetoglu, 2004). Live vaccines for *B. abortus* and *B. melitensis*, (a less virulent strain used as an antigen for serological testing) are also pathogenic for humans. (Renukaradhya *et al.* 2002). The species of *Brucella* affecting human beings and the levels of each specific pathogenicity are summarized in Table 2.

Table 13: **Typical host specificity *of Brucella* species and their degree of pathogenicity to humans**

Brucella species	Biovar	Lipopolysaccharide	Animal Host	Virulence to Humans
B. melitensis	1-3	S	Goats, sheep	High
B. suis	1	S	Pigs, cattle	High
	2	S	Hares	Low
	2,3	S	Pigs	Low(2), High (3)
	4	S	Reindeer, caribou	Moderate
	5	S	Rodents	High
B. abortus	1-6, 9	S	Cattle	Moderate
B. canis	None	R	Dogs	Low
B. ovis	None	R	Sheep	None
B. neotomae	None	S	Rodents	None
B. maris	Unknown	Unknown	Seals, cetaceans	Possible

R=rough, S=smooth
Source=Modified from Young, (1995)

2.2. Epidemiology of camel brucellosis

2.2.1. Distribution of camels (*Camelus dromedarius*)

Camels (*Camelus dromedarius*) are mainly reared by nomadic pastoralists mostly in marginal ecozones of semi-desert lands in sub-Saharan Africa. Thus, camels have a vital role in the subsistence economy of large sectors of rural pastoral communities. Camels are kept in a wide geographical area extending from the Gobi and India in Central Asia in the east, to Mauritania in the west, in the horn of Africa including Somalia, Kenya and Ethiopia. The camel pastoralists are always moving over large areas in search of feed and water for their camels. During their continuous transhumance, camels are affected by many production limiting factors such as diseases, feed shortage, water scarcity, high calf mortality and, recently, security problems (Abbas and Omer, 2005; Ali and Majid, 2006). Environmental, social and cultural factors have great influence on the distribution and production of camels. Arid and semi arid zones of tropical and sub tropical countries of Africa and Asia are found to be convenient ecology. The greatest cultural influences in recent distribution of camels was the advent of Islam, when Arabs spread their gospel, consolidating its ranges north and east wards in Asia, and along the Mediterranean littoral. There have been many attempts to introduce camels outside the “normal” range in Brazil, Colombia, USA, Cuba, Spain Italy and France (Wilson, 1998). Generally, there has been steady increase in camel population since about 1980s. However, decrease in numbers has been observed in some countries for instance, where oil is the principal commodity and the nomadic way of life is no longer the major one (Wilson, 1998). Eastern Africa is known to be the heartland for camel production as 80% and 63% of Africa and world popular, respectively produced in the region. Subsistence camel production is practiced in dry areas of Ethiopia that cover 61% to 65% of the total land area (Abebe, 2000). The eastern part of the country is considered as the heartland for camel production, which is the home of two-third of the nations camel population (Getahun and Bruckner, 2000). The Borena range land of Southern Ethiopia is the third important camel production region of the country, the first and second being Somalia and Afar regions. Table 3 shows the camel population in some selected countries.

Table 14: **Camel population in some selected countries**

No.	Country	Number (,000)	Density (No. per km sq).	Proportion to total national ruminants
1	Djibouti	60		34
2	Saud Arabia	165	0.00	14.9
3	Niger	415	0.32	8.3
4	Egypt	170	0.16	5.8
5	Ethiopia	1030-1040	0.83	3.4
6	India	1100	0.33	0.4
7	Kenya	620-780	1.08	5.3
8	Somali	5800-63500	8.93	46.6
9	Sudan	2800-3100	0.99	11.1
10	Bahrain	1	-	-
11	Iraq	27	-	-
12	Jordan	15	-	-
13	Kuwait	8	-	-
14	Libya	190	-	-
15	Morocco	43	-	-
16	Qatar	22	-	-
17	Syria	5	-	-
18	Yemen	144	-	-

Sources (Wilson *et al*., 1990; Schwartz and Dioli, (1992); Abbas, and Agab, (2002)

2.2.2. Status of camel brucellosis in Ethiopia and other countries

Camel brucellosis is a wide spread disease in camel rearing regions of the world. These include the Middle East countries and camel producing areas of Africa. Thus, seroprevalences ranging between 2 and 5% were reported from most countries where camels are still kept by nomadic or transhumant pastoralists, extensive form of husbandry (Wernery and Kaaden, 2002). A higher seroprevalence of brucellosis (8–15%) was reported in intensively kept camels—especially in Saudi Arabia and Kuwait (Table 4), where camel dairies managing over 2000 head of camels have been established for the commercial production of camel milk (Radwan *et al.,* 1992). Even under pastoral conditions, individual herds could have an appreciably higher prevalence of brucellosis than the regional risk. Agab (1993) andBitter (1986) recorded seroprevalence of brucellosis in certain camel herds in Sudan ranging between 26.5% and 30%. Brucellosis is a widely spread disease in camel producing horn of African countries such as Ethiopia, Eritrea, Somalia and Sudan. Camel brucellosis was found to be one of the disease problems associated with reproductive wastage in camel producing pastoral areas of Ethiopia. Few field surveys have been carried out to determine the magnitude of camel brucellosis in pastoral areas. Generally the previous serological surveys showed seroprevalence rates of 4.4% (Domenech, 1977), 5.5% (Richard, 1980), 4.2% (Teshome *et al.,* 2003), 1.8% (Bekele *et al.,* 2005) and 2.43% (Berhanu, 2006). Table 4 summarizes the prevalence rate of camel brucellosis in Ethiopia and neighboring countries.

Table 15: **Prevalence of camel brucellosis in Ethiopia and some camel rearing countries**

Country	Prevalence (%)	Reference
Kuwait	14.6	Godfroid, 2002
Iraq	12.0	Refai, 2002
Iran	11.2	Refai, 2002
Saudi Arabia	8.2	Abbas and Agab, 2002
Oman	8.0	Refai, 2002
Sudan	7.2	Abbas and Agab, 2002
Egypt	5.0	Godfroid, 2002
Ethiopia	1.8	Bekele *et al.*, 2005
Ethiopia	4.4	Domenech, 1977
Ethiopia	5.5	Richard, 1980
Ethiopia	4.2	Teshome *et al*., 2003
	2.43	Berhanu, 2006
Jordan	12.1	Al-Majali *et al,* 2008
Jordan/South/	15.8	Hawari, 2008

2.2.3. Transmission and sources of infection

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 remain 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 (Abbas *et al*., 1987; Radwan *et. al.,* 1992; Abuo-Eisha, 2000). Humans usually become infected by ingestion of raw milk harboring the organisms or by the contamination of mucous membranes and abraded skin. In the laboratory and probably in abattoirs, *Brucella* can be transmitted through aerosols. Common sources of infection for people include contact with animal abortion products; ingestion of unpasteurized dairy products from cows, small ruminants or camels; ingestion of undercooked meat, bone marrow or other uncooked meat products; contact with laboratory cultures and tissue samples; and accidental injection of live *Brucella* vaccines. Human to human transmission is rare, but has been reported after blood transfusion, bone marrow transplantation or sexual intercourse. Rare congenital infections seem to result from trans-placental transmission or the ingestion of breast milk. Congenital infections might also occur, if the infant is exposed to organisms in the mother's blood, urine or feces during delivery (CFSPH, 2007).

2.2.4. Risk factors

Management

Management contributes to the transmission of the agent. Once infected, the time required to become free from brucellosis is increased by large herd size, active abortions and loose housing (Mohamed, 2002). Calving practices play a major role in the spread of brucellosis. Separate calving pens minimize exposure of infected animals. There is a positive association between population density (number of animals per land area) and disease prevalence, which is attributed to increased contact between susceptible and infected animals. Management practices directed at eliminating infected males and minimizing exposure to aborted tissue greatly reduce the incidence of the disease. Both venereal transmission and exposure to aborted fetuses and fetal membranes are crucial for maintaining infections in a herd. Introduction of infected animals can lead to rapid

spread of infection within the herd (Menachem, 2002).

Host factor

Susceptibility to infection depends on age, sex, breed and pregnancy status of the animals. Younger animals tend to be more resistant to infection and frequently clear infection, although latent infection does occur. Only 2.6% of animals infected at birth remain infective, as adults and sexually mature animals are much more susceptible to infection (Abou-Eisha, 2000), regardless of sex. Most animals infected as adult remain infected for life. After reaching sexual maturity, the state of pregnancy has a greater influence on the degree of susceptibility than age (Nicoletti, 1980). 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 mating is not important (Radostits *et al.,* 1994). Introduction of infected animals can lead to rapid spread of the disease /infection within the herd (Walker, 1999; PAHO-WHO, 2001). 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. An important aspect of the epidemiology of camel brucellosis is the role of the inter-calving interval in the transmission of infection between camels within a herd (particularly in dam-to-offspring transmission). Nomadic camels usually have a rather lengthy inter-calving interval, estimated to be between 2 and 3 years with a mean of 2.4 years (Abbas and Agab, 2002). It is generally agreed that most *Brucella* infections are contracted during calving (Higgins *et al*., 1992). Because it is also established that most brucellosis contamination occurs following an abortion or delivery by an infected female, then the long inter-calving interval might contribute to a lower incidence of brucellosis in nomadic or extensively raised camels. Kiel and Khan (1989) suggested that the epidemiology of brucellosis in camels in a country like Saudi Arabia was complicated by the consumption of raw camel milk, by importation of live animals with higher prevalence of brucellosis than in the local animals and by the uncontrolled movement of animals and humans across national borders. These factors are relevant in many countries where camels are kept.

Agent factors

Brucella is facultative intracellular bacteria, which are capable of multiplication and survival with in host phagocytes (WHO, 1997). The organisms are able to survive within host leukocytes and may utilize both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanism during the period of haematogenous spread. The inability of the leukocytes to effectively kill virulent *B. abortus* at the primary site of infection is a key factor in the dissemination to regional lymph nodes and other sites such as recticuloendothelial system and organs such as the uterus and udder (Radostits *et al*., 2000; Georgios *et al*., 2005).

Environmental and climatic factors

The survival of the organism in the environment plays a great role in the epidemiology of the disease. (Radostits *et al.,* 2000; Mc Dermott and Arimi, 2002). Atmospheric conditions and seasons of the year may have influence on the management and contact of the infected and susceptible host and hence in dry areas, water resources are sparsely distributed (Schwartz and Dioli, 1992). 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).

2.3. Pathogenesis, pathology and clinical manifestations

The initiation of *Brucella* infection depends on exposure dose, virulence of the *Brucella* species and natural resistance of the animal to the organisms (Radostits *et al*., 2000). Resistance to infection is based on the host's ability to prevent the establishment of infection by the destruction of the invading organism. Invading *Brucella* usually localize in the lymph nodes, draining the invasion site, resulting in hyperplasia of lymphoid and reticulo-endothelial tissue and the infiltration of inflammatory cells. Survival of the first line of defense by the bacteria results in local infection and the escape of *Brucella* from lymph nodes into the blood (Coetzer and Tustin, 2004). During the bacteraemic phase, which may last 2-8 weeks, bones, joints, eyes and brain can be infected, but the bacteria are most frequently isolated from supramammary lymph nodes, milk, iliac lymph nodes, spleen and uterus. In bulls, the predilection sites for infection are the reproductive organs and the associated lymph nodes. During the acute phase of infection, the semen contains large number of *Brucella* as the infection becomes more chronic, the number of *Brucella* excreted decreases and excretion may cease altogether. However, it may also continue to be execrated for years or just become intermittent (Radostits *et al*., 2000). After the *Brucella* organisms spread through the haematogenous route in females it also reaches the placenta and then to the fetus

(Lapaque *et al.,* 2005). The preferential localization to the reproductive tract of the pregnant animals is due to the presence of unknown factors in the gravid uterus. These are collectively referred to as allantoic fluid factors that would stimulate the growth of *Brucella*. Erythritol, a four-carbon alcohol, is considered to be one of these factors (Walker, 1999), which are elevated in the placenta and fetal fluid from about the fifth month of gestation (Bishop *et al.,* 1994). An initial localization within erythrophagocytic trophoblasts of the placentome, adjacent chorioallantoic membrane results in rupture of the cells and ulceration of the membrane. The damage to placental tissue together with fetal infection and fetal stress inducing maternal hormonal changes may cause abortion (Seifert, 1996). Abortion and expulsion of the fetus was thought to be the results of the placentitis caused by *Brucella*. Proliferation of *Brucella* in the uterus induces necrosis and destruction of the fetal and maternal placental membranes resulting in death and then expulsion of the fetus. The pathologic changes in the caruncules and cotyledons prevent normal separation and expulsion of the placenta. Although placentitis impairs the normal function of the placenta, *Brucella* endotoxins may also play a role in inducing abortion (Radostits *et al*., 2000). Since *Brucella* species are intracellular, among various mechanisms employed by *Brucella* organisms to survive inside the phagocytic cells include inhibiting phagolysosome fusion, blocking bactericidal action of phagocytes and suppressing the myeloperoxidase H_2O_2 halide system (Young, 2005). The enhanced virulence of the *Brucella* inside the reproductive system is supposed to be the consequence of the increased level of the sugar erythritol, a four-carbon alcohol, which is maintained in the reproductive system (Dwight and Yuan, 1999). 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 (Quinn *et al*., 1994; Walker, 1999).). In male camels, inflammation and enlargement of the epididymis, characterized by hyperplasia, degeneration of tubular epithelium, orchitis and inflammation of other accessory sex organs are common (Seifert, 1996; Wernery and Kaaden, 2002). The primary clinical manifestations of brucellosis in animals are related to the reproductive tract. Abortion that occurs in the last trimester is the most obvious manifestation. Infection may also cause stillbirth or weak calves, retained fetal membrane, lowering of fertility with poor conception rates and reduced milk yield (Nicoletti, 1984; Seifert, 1996). In humans, the onsets of clinical signs occur within 2-3 weeks of exposure to infection. Clinical signs observed include recurrent fever; chills with night sweats, fatigue, muscle joint pain, backache, depression and insomnia are common (Pal, 2007). In chronic form it may result in serious complications in which the musculo-skeletal, cardiovascular and central nervous systems are affected (Morata *et al*., 2003; Coetzer and Tustin, 2004).

2.4. Immune response

Infection with *Brucella* usually results in the induction of both humoral and cell-mediated immune responses. The magnitude and duration of these responses can be affected by many factors including virulence of the infecting strain, size of inoculum, age, sex, pregnancy, species, and immune status of the host (WHO, 1986). Although humoral immune response plays an important role in immunity to *Brucella*, it is the cell-mediated response that is most important in providing protection (WHO, 1997).

2.4.1. Humoral immune response

IgG1, IgG2, IgM, and IgA are the immunoglobulin isotypes present in serologically significant concentrations in animal serum. Similar isotypes at different relative concentrations occur in milk, although most of the IgA is present in the secretary form. Although secretary IgA in milk does play an important role in the Milk Ring Test, IgM also participates in this reaction, whereas IgG1 will produce an agglutinate at the bottom of the tube and may interfere with ring formation by other isotypes (WHO, 1986). The first immunoglobulin produced after an initial heavy infection or strain 19 immunization is IgM. This can usually be detected in the first or second week following the initial antigenic stimulus, but is soon followed by IgG antibody. IgG1 immunoglobulin is the most abundant in serum and exceeds the concentration of IgG2. The magnitude and duration of the antibody response following immunization is directly related to the age at immunization and the number of organisms administered. Following immunization with the standard dose of strain 19 during calf hood, IgG antibody concentrations usually decline to diagnostically insignificant levels over 3 - 6 months. Residual antibody, if present, is usually predominantly of the IgM class (WHO, 1986). Exposure to a relatively large dose usually produces a significant agglutinin titre within 2 to 4 weeks. With a minimum dose, the time required for development of "reactor" titres may vary from 2 to 7 months after exposure. Under natural conditions, the majority of infected animal will probably have developed a diagnostic agglutinin titre 30 to 60 days after exposure (WHO, 1977; OIE, 2000). According to WHO (1986), following exposure to virulent *Brucella abortus*, antibody may appear in 4-10 weeks or longer, depending on the size and route of entry of the inoculums and the stage of pregnancy of the animal, but even under controlled experimental conditions there is a great variation in response from animal to animal. In infected environments, animals exposed to low doses may develop transient low antibody titres, but show no clinical or bacteriological evidence of infection. A disturbing number of infected animals do not develop

antibody of the IgG class until parturition, or 1-3 weeks after parturition. These animals may have low IgM titres a few weeks earlier, but in a vaccinated population, they can not be differentiated from non-infected vaccinated animals. Antibodies of the IgA, IgM, IgG1, and IgG2 isotypes can all react in the tube agglutination test, but those of the IgM class are by far the most efficient. Antibodies of the IgG1 isotype produced in some sera, at least, have the capacity to block agglutination by other isotypes, particularly IgM. The agglutinating and precipitating activity of IgG1 antibodies is enhanced at high salt concentrations or under acid conditions and this isotype is reactive in the card and Rose Bengal tests. The reactivity of IgM in this type of test is dependent on the precise method of preparation of the antigen and the procedures used (WHO, 1986).

2.4.2. Cellular immune response

Brucella species are facultative intracellular pathogens. They are readily phagocytised by macrophages and polymorphonuclear leukocytes and in the case of virulent strains, are capable of surviving within these cells, and phagocytosis is promoted by antibody. However, since virulent *Brucella* can survive within normal macrophages for long periods, recovery from infection is likely to be dependent upon the acquisition of increased bactericidal activity by phagocytic cells. Macrophage activation occurs when T-lymphocytes of the appropriate subset are stimulated to release lymphokines (interleukins). (WHO, 1997; Coetzer and Tustin, 2004; Georgios *et al*., 2005) The release of these activating factors is dependent upon recognition of the appropriate antigen by the T-lymphocyte and is subject to regulation through the major histocompatabilty complex. Live organisms capable of establishing persistent intracellular infection and certain types of antigens, with or with out adjuvant, are the most effective inducers of cell-mediated immunity. The role of cytotoxic cells, including cytotoxic T-lymphocyts, natural killer cells (NK) and killer (K) cells, in the cell-mediated immune response to *Brucella* has not been elucidated. Further studies are needed to determine the basic processes underlying the developments of protective immunity to *Brucella* in the natural host species (WHO, 1997; Coetzer and Tustin, 2004).

2.5. Importance of camel brucellosis

2.5.1. Public health importance

Brucellosis is an important zoonotic disease that has been shown to cause human ailments for over one and half centuries. It has been known to be caused by *B. melitensis*, *B. abortus*, *B. suis* and occasionally by *B. canis* (Pal, 2007). The most pathogenic to man, who can be acquired from camels through consumption of raw milk and during assisting delivery of camels, is *B. melitensis* followed in descending order by *B. suis* and *B. abortus* (Nicoletti, 2002). *Brucella* organisms have been shown to cause wide spectrum of clinical episodes in humans ranging from the classical undulant (Malta) fever to other complications such as neurobrucellosis, breast abscess, epididymoorchitis and spondylitis (Pappas *et al.*, 2007). Various biotypes of the *Brucella* species have been isolated from human patients. Moreover, it has been evident that humans can acquire *Brucella* infection from different animal hosts. The existence of wide range of animal hosts is responsible for increased opportunity of humans to get *Brucella* infection. The diversity of the agents accounts for the increased spectrum of clinical brucellosis in humans (Nicoletti, 2002). The relative importance of each *Brucella* species or biovars in causing human brucellosis varies greatly among areas depending on the relative importance of the principal animal hosts to humans. For example, most cases of human brucellosis are caused by *B. melitensis*, particularly biovar 3 in the Middle East countries and Iran due to traditional consumption of raw camels' milk and milk products. The highest incidence of human brucellosis due to *B. melitensis* was recorded in Saudi Arabia, Iran, Palestine, Syria, Jordan and Oman (Bochiroli *et al.*, 2001). The age distribution of reported brucellosis cases from these countries indicates that children are particularly at risk. The disease has also been frequently reported in laboratory personnel dealing with diagnostic work. With the intensification of production and importation of animals (cattle, camels, sheep and goats) and establishment of big farms, the incidence of brucellosis in man was shown to rise sharply in many countries. The isolation of various *Brucella* biotypes from cattle, sheep, goats, camels and humans in endemic areas indicates the epidemiological complexity in the maintenance and spread of these biotypes. This situation could be attributed to composite livestock farming, natural grazing and unrestricted movement of camel rearing. (Bochiroli *et al.*, 2001). Table 5 shows the prevalence of human brucellosis in some countries.

Table 16: **Prevalence of human brucellosis in the Middle and Far East**

Country	Prevalence	Reference
Saudi Arabia	22.5	Refai, 2002
Iran	10.5	Godfroid, 2002
Syria	4.0	Godfroid, 2002
Jordan	3.2	Refai, 2002
Oman	2.0	Godfroid, 2002
Egypt	1.9	Refai, 2002

Brucellosis in human represents a major public health hazard, which affects social and economic development in various countries. Groups at high risk for brucellosis are animal health workers, butchers, farmers, and those who 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 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). The significance of brucellosis as a zonoosis has ever increased in recent times, as a result of expansion of international commerce in animals and animal products, increase urbanization with growing demand-supply of animals and animal products in closest proximity to people, increasing tourism (consumption local animal products), (Menachem, 2002). Brucellosis is most common in rural areas and it occurs in urban settings where animals are kept in compounds around houses and among meat packers and veterinarians (Smits *et al*., 1999). Human brucellosis is a disease with non-pathognomonic signs and characterized by acute illness with undulant fever, which may progress to a more chronic form and can produce a serious complication affecting the musculoskeletal, cardiovascular and central nervous system (Georgios *et al*., 2005).

2.5.2. Economic importance

Camels are primarily the domestic animals of pastoral communities that ensure food security. They produce milk, meat, hair and hides, and serve as a draught animal for agriculture and transport people and goods (Schwartz and Dioli, 1992). 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 is 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. 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 from scanty and highly variable feed resources. The most significant merits to perform in areas where other livestock species do not thrive and perhaps do not survive are attributed to the economic use of water in almost all metabolic functions and wide range of feed resource utilization (Yagil, 1985). In mixed species, the camel feeds on plants or part of plants that are not eaten by other livestock due to its size to browse the highest strata, thus reducing competitions and enhancing complementarities (Wilson *el al.*, 1990; Teka, 1991). Brucellosis causes heavy economic losses in camel production as a result of abortions, sterility, mastitis, decreased milk production, veterinary attendance and more importantly due to hindrance of free animal and animal product trade ((Kulplulu and Sarimehtoglu, 2004; Al-Majali, 2005). The common sequel of infertility increases the period between lactations in infected herds, and the average inter-calving period may be prolonged by several months (Radostits *et al.,* 2000). Added to the above losses is the economic impact of human brucellosis. The costs associated with medical care of *Brucella* infected humans and the duration of time the infected people are out of work account for financial losses (Refai, 2002). Brucellosis seriously impairs public health and socio-economic development. This holds true especially for livestock owners, who represent a vulnerable segment of the community in many rural populations. (Bochiroli *et al.*, 2001). Generally, heavy economic losses associated with brucellosis emanate from:

- Losses of calves due to abortion
- Reduced milk yield

- Culling of valuable animals because of reproductive problems
- Endangering animal export / trade of animal products
- Loss of man-hours and medical costs
- Government cost incurred for research and eradication programs

2.6. Diagnostic methods

Diagnosis of brucellosis is the corner stone for any control and eradication program. Especially in humans due to its heterogeneous and poorly specific clinical symptoms the diagnosis of brucellosis always requires laboratory conformation (Morata *et al*., 2003). It is made possible by direct demonstration of the causal organism using staining, immunofluorescent antibody, culture, animal inoculation and polymerase chain reaction (PCR) and indirectly by demonstration of antibodies using serological techniques (Corbel, 1997; Walker, 1999; Quinn *et al*., 2002).

2.6.1. Bacteriological method

Specimens of fetal stomach, lung, liver, placental cotyledon, vaginal discharges, are stained with Gram stain and modified Ziehl Neelsen stains. *Brucella* appears as small red-colored coccobacilli in clumps (Quinn *et al*., 2002). Blood or bone marrow samples can be taken and cultured in 5-10% blood agar. To check up bacterial and fungal contamination *Brucella* selective medias are often used. The selective Medias are nutritive media blood agar based with 5% seronegative equine or bovine serum. On primary isolation, it usually requires the addition of 5-10% carbon dioxide and takes 3-5 days incubation at 37^0C for visible colonies to appear (Quinn *et al*., 2002). The organism is catalase and oxidase positive, (*Brucella ovis* and *Brucella neotomae* are oxidase negative), reduce nitrate to nitrite (except *Brucella ovis*), *Brucella* does not cause haemolysis on blood agar, does not produce acid on agar containing glucose, and does not ferment lactose (Walker, 1999; Quinn *et al.,* 2002). Recovery of *Brucella* species from camel specimens requires rapidity in processing of samples; there is increased difficulty in isolation of the organisms with storage of samples (Agab, 1997). Some researchers have failed to isolate *Brucella* organisms from the milk of seropositive camels after milk was transported to the laboratories and processed for culture within 1–2 days (Agab, 1997) and others reported the isolation of *Brucella* spp. from some but not all of the milk samples obtained from seropositive camels (Radwan *et al*., 1995). However, the isolation of *Brucella* spp. from internal organs (particularly lymph glands, testes and vagina) is relatively easy (Agab, 1997). Guinea pigs are the most sensitive laboratory animals, two guinea pigs are inoculated intramuscular 0.5-1.0 ml of suspected tissue homogenate and sacrificed at three, six weeks post inoculation, and serum is taken along with spleen and other abnormal tissues for serology and bacteriological examination, respectively (Walker, 1999; Quinn *et al.,* 2002).

2.6.2. Molecular techniques

Molecular technologies like, Polymerase Chain Reaction (PCR) is a new approach and applied in many diagnostic works to overcome limitation and difficulties in bacterial culture and serological assays. PCR shows high sensitivity, specificity and overcame the extraneous intervention of mimicry antibodies from sources other than actual infection (Radostits *et al.,* 2000; Quinn *et al.,* 2002).

2.6.3. 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 (Ajoig and Adamu, 1998; Quinn *et al.,* 2002). Serum agglutination test (SAT) can be used to detect acute infections, as antibodies of the IgM type, usually appear first after infection and are more reactive in the SAT than antibodies of the IgG1 and IgG2 types. However, because the SAT may yield both false negative or false positive results, it effectively detects brucellosis only on a herd basis (Corbel *et al*., 1984). 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 (OIE, 2000; Nicoletti, 2002). Complement fixation test (CFT) on the other hand, is considered the most accurate one. Some researchers reported its superiority to the other mentioned tests (Mohammed *et al.,* 1981; Asfaw *et al.,* 1998). CFT detects predominately IgG antibodies as most of IgM destroyed during serum deactivation and so used as a confirmatory test (OIE, 2000). The test distinguishes reaction caused by other factors like vaccines and other bacteria infections. *Escherchia coli O:157, Yersinia entrocolitica O:9, Vibrio colerae, Psuedomonas mallophilia and Salmonella* serotypes share common chain of LPS antigen with smooth *Brucella* strains and do cross react. *Francella tularensis* also cross reacts for unknown reason. Rough *Brucella* strains also cross-reacts with *Actinobacillus equuli, Pasteurella multocida and Pseudomonas aerugenosa* (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 (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 (Bastawrows *et a*l., 2000). 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, 1983). It can also be used both for screening and confirmatory tests (WHO, 1986). 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 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). In humans, diagnosis of brucellosis is always missed, because other diseases that partially or almost totally mimic brucellosis symptoms including malaria, typhoid, paratyphoid and influenza are misdiagnosed. In humans, the definitive diagnosis is by culture or serology. Some times *Brucella* species can be isolated from blood and bone marrow early in the infection. Occasionally, they can be recovered from cerebrospinal fluid (CSF), urine or tissues. In addition, *Brucella* species can be isolated from a variety of plain or selective media such as Farrell`s medium or Thayer-Martin modified medium. Colony morphology varies with the species. Colonies of smooth species (*B. melitensis, B. abortus, B. suis and B. maris*) are round with smooth margin. When the plates are viewed in daylight, through a transparent medium, these colonies are translucent and have a pale honey color. From above, they are convex and pearly white. *Brucella ovis* and *Brucella canis* are rough forms and their colonies are round, shinny, and convex, and their rough nature can be seen by examining the colony with oblique illumination (CFSPH, 2007). Culture from the blood of a patient provides definite proof of brucellosis. *Brucella* however, is a slow growing organism and cultures are rarely positive before the fourth day of incubation. Usually cultures become positive between the first and third week, and should be kept for at least 45 days before the culture can be concluded to be negative for *Brucella* (CFSPH, 2007). The classical Rose Bengal test (RBT) is often used as a screening test. RBT is based on the agglutination of serum antibodies with a stained whole cell preparation of killed *Brucella.* For confirmation of RBT the Wright or serum agglutination test (SAT), CFT or in more sophisticated equipped laboratories enzyme linked immunosorbent assay (ELISA) may be used. ELISA is used to discriminate between the presence of specific IgM and IgG antibodies and to roughly access the stage of illness (Smits and Kadri, 2005).

2.7. Control and prevention

The control of animal brucellosis has been approached with a combination of procedures: vaccination, test-and-slaughter and hygienic measures ((Mustafa and Nicoletti, 1993). Abbas and Agab, (2002) reported that, control of camel brucellosis should be tailored to suit conditions in the particular countries where camels are raised. Most of these countries are poor and nomadic tribes raise camels. However, control of camel brucellosis can be used as a means of extending veterinary services to pastoral areas. The move is well justified and can draw funding and concern from local political circles, international agencies and non-governmental organizations. The venture can be started on an experimental scale using specified pastoralist communities in each country hence, camel-keeping countries (Table 3) can be divided into two broad categories. The first category (developing countries) contains most camel-keeping countries, where nomadic pastoralists or agro pastoralists keep camels. Prevalence of brucellosis in the camels of these countries is low (Chad, Ethiopia, Kenya, Libya, Nigeria, Somalia, Sudan and Tunisia).The second category includes countries in the former USSR, the Middle East and Iran, where camels are kept intensively and in close contact with other animals. In these countries, there is also a higher consumption of camel products by the urban communities and a higher seroprevalence of brucellosis in camels. According to the report of Abbas and Agab (2002) for countries, in category-1 they suggest that a control strategy based on whole-herd vaccination using S19 or Rev 1 vaccinal strains preceded by blood testing using the slide-agglutination test (SAT) or card test on the field. Seropositive animals should be identified by branding or special ear-mark and subjected to retesting. This marking will restrict the sale of seropositive animals. Because brucellosis prevalence is generally low in category-1 countries, the small number of *Brucella* reactors in individual camel herds should not be annoying. Camel calves should be vaccinated at 4–8 months of age, using a full adult dose of vaccine (Abbas and Agab, 2002). Radwan *et al.,* (1995) showed that camel calves vaccinated in this way were seropositive up to 8 months post-vaccination. Adult camels vaccinated by 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 (Wernery and Kaaden, 2002; Abbas and Agab, 2002) had detectable antibodies up to 3 months post-vaccination. However, in both studies, the adult camels received a reduced dose of the vaccine. Recently, there was a break through in the vaccination and control of animal brucellosis where a reduced dosage of *B. abortus* strain RB51 (SRB51) was tried successfully in adult cattle and bison with the following benefits: high immunogenecity against infection, protection against abortion, no seroconversion to the

vaccine and absence of prolonged bacterial colonization of tissues. This vaccinal strain is also not secreted into the milk (Olsen, 2000). Thus, there is need to test this new vaccine in camels because it might overcome several problems encountered with S19 and Rev 1 vaccination. In category-2 countries, the control plan should aim at an appreciable reduction of disease prevalence by a test-and-slaughter policy in the first phase, followed by a vaccination program when the prevalence is brought down to 3–5% (FAO/WHO, 1986). Countries in category-2 have wealthier economies and can afford the compensation component necessary for a successful culling policy. In both groups of countries, an extension and educational component is vital for the success of the control plan. The public should be sensitized to the problem and to the possibility of controlling the disease if both government and public sectors cooperate. Radwan *et al*., (1995) reported the successful eradication of brucellosis from a large camel herd in Saudi Arabia by vaccination of adult seronegative and young camels and antibiotherapy of seropositive or culture-positive camels. They were able to eliminate *Brucella* shedding by a combination of ox tetracycline and streptomycin treatment for 30 and 16 days, respectively (during which and for several days after the milk and meat were not marketable). This opens new possibilities for specific action to control brucellosis on herd level. Antibiotherapy is also worth trial in valuable animals (especially racing camels). Chloramphenicol (1 g per 100 kg BWT daily for 20 days) was effective in the treatment of *Brucella* infected horses and a single treatment with oxytetracycline (10 g) was recommended to eliminate brucellosis carrier cattle from infected herds (Blood *et al.,* 1983). Several authors have reported the successful treatment of brucellosis in humans by various combinations of antibiotics (McLean *et al*., 1992). Controlled experiments should be conducted to test the possibility of eliminating *Brucella* shedding and/or the carrier state in camel brucellosis through antibiotherapy; this would have a great impact on control of the disease. Besides the above mentioned control measures, improving management practices is one way of attempting to control brucellosis. The aim is to improve hygienic and reduce the chances to 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; Radostit *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

The most rational approach for preventing human brucellosis is the control and elimination of infection in animal reservoirs, as has been demonstrated in various countries in Europe and Americas. The direct human health benefits of the American bovine brucellosis eradication program during its early years may be a good example for this .In 1947, 6321 cases of human brucellosis were reported for the country (America). This number had been reduced by more than 95% to 252 in 1966 (Acha and Szyfres, 2001). Some human populations were protected by mandatory milk pasteurization. Prevention of infection in occupational groups is more difficult and should be based on health education, the use of protective clothing, wherever possible, and medical supervision (Acha and Szyfres, 2001). Ranchers, farmers, or animal managers should clean and disinfect calving areas and other places likely to become contaminated with infective material. All individual should wear sturdy (strong) rubber or plastic gloves when assisting calving or aborting animals, and scrub well with soap and water after ward. Precautions against drinking raw milk and unpasteurized milk products and by products is also important. Ultimately, the best prevention is to eliminate brucellosis from all animals in the area (USDA-APHIS, 2003). Great care should be exercised, when working with infected tissues and cultures in the laboratory. All *Brucella* cultures should be handled following bio safety level three practices because of the potential for laboratory infection. All laboratory procedures should be performed in a manner that prevents aerosol formation (Walker, 1999). Protecting refrigerator plant and slaughterhouse workers against brucellosis is particularly important because they constitute the occupational group at highest risk. Protection is achieved by separating the slaughter area from other sections and controlling air circulation. Employees should be instructed in personal hygiene and provided with disinfectants and protective clothing (Acha and Szyfres, 2001). The immunization of high-risk occupational groups is practiced in the former Soviet Union and China. In the former Soviet Union, good results have apparently been obtained with the use of a vaccine prepared from strain 19 of B. *abortus*, applied by skin scarification. Annual revaccination is carried out for those individuals not reacting to serologic tests. In china, an attenuated live vaccine made from B. *abortus* strain 104M is applied per cutaneously. These vaccines are not used in other countries because of possible side effects. Promising trials have also been conducted in France with antigenic fractions of *Brucella* (WHO, 1997; Acha and Szyfres, 2001). Routine screening testes should be introduced in health institutions, since contact between human and animal products in Ethiopia is high. Owners with infected animals should be advised to pasteurize milk and milk products derived from infected animals before consumption and carcass from clinically and serologically positive animals must be boiled, stewed, roasted before consumption. Health education and publicity campaigns should be a part of control programs (Eshetu *et al*., 2005).

3. MATERIAL AND METHODS

3.1. Study area and Population

The study was carried out in Fentale District of Eastern Shoa Zone of Oromia Regional State. Fentale District is located at about 198 kms east of Addis Ababa and lies between 8^0 54' north latitude and 36^0 23' to 39 ^{0}54' east longitude. The average annual rainfall is 486 mm. It has a mean minimum and maximum temperature of 36^OC and 42 ^OC, respectively (FARM-Africa-PLIP, 2005-2007). Fentale District is bordered on the southeast by the Arsi Zone, on the southwest by Boset District, on the northwest by the Amhara Region, and on the northeast by the Afar Region. Most parts of this District range from 900 to 1000 meters above sea level; Mount Fentale (2400 meters) is the highest point. Rivers, which flow in the area, include the Awash and the Germama; Lake Basaka is an important body of water in this District. In eleven of the eighteen peasant associations of Fentale District, the predominant agricultural practice is pastoralism. The livestock population in East Shoa Zone is about 865,106 cattle, 347,050 sheep, 549,993 goats, 13,809 horses, 8,282 mules, 194,083 donkeys, 68,331 camels and 1,015,328 poultry. The camel population of Fentale district is about 61,425, which is the study population. Camels, goats and cattle are the most common livestock; migration to the border areas of Boset district for grazing during normal years is common, but in years of low rainfall, herdsmen will migrate as far as to Negele Arsi. The vegetation is primarily acacia trees with the bushes and shrubs common to the lowland parts of Ethiopia (ESZARDO, 2008). The study area is shown in Figure 1.

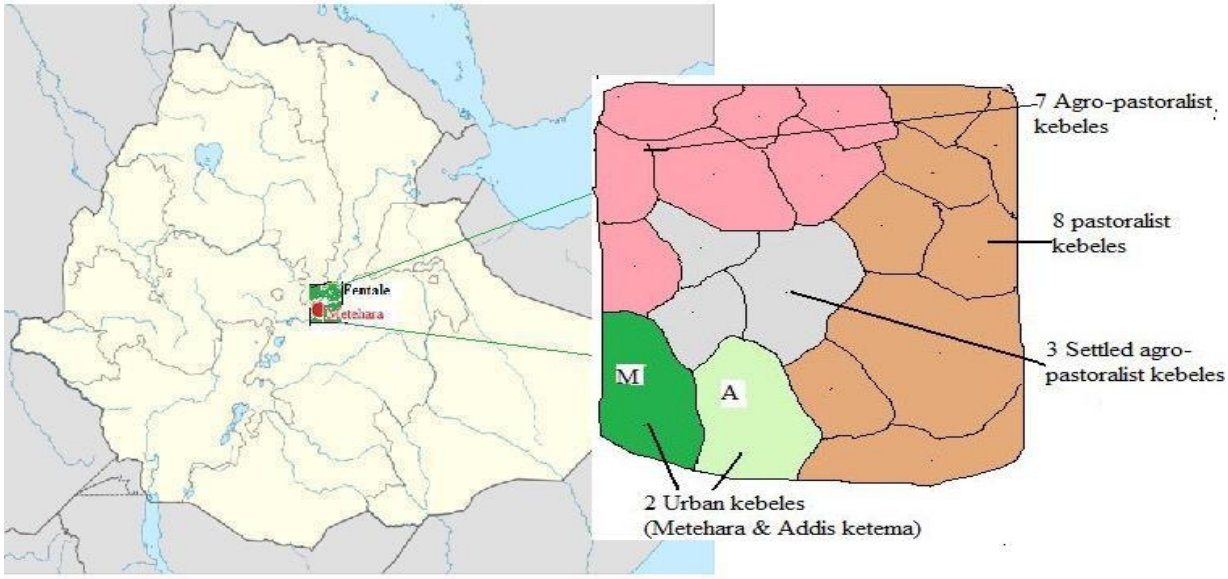

Figure 4: Map of Ethiopia and study area (Source: FARM-Africa-PLIP, 2005-2007)

Based on data from ESZARDO (2008) Fentale District has an estimated human population of 90,115, 18 peasant associations and 9,696 households out of which 24.42% are urban dwellers, which is less than the Zone average of 32.1%. With an estimated area of 1,169.85 square kilometers, Fentale has an estimated population density of 74.7 people per square kilometer, which is less than the Zone average of 181.7 (PHCE, 2009).

3.2. Study animals

The study was carried out on camels owned by pastoralists. The camels in the study area are used primarily for milk and meat production, drought mitigation and cash income generation. The true representatives of the study population of the camels were selected by simple random sampling method. Based on this, a total of 768 camel blood samples were collected. Human blood samples were collected from the occupational workers i.e. camel attendants and animal health workers in collaboration with Metehara town health center. Relevant risk factors pertaining to brucellosis like proper disposal of retained fetal membranes, drinking of raw milk and contact with infected animals were gathered during sera collection. A total of 250 human blood samples were collected purposively from those vulnerable groups.

3.3. Study design

A cross-sectional study was employed to determine the seroprevalence of brucellosis in camels and humans from September 2009 to April 2010. For the flow up of the study design, see Figure 2. The serological survey was intended to determine the human, individual animal and herd levels sero-prevalence. Densities of animal populations, herd sizes and management, as well as environmental factors thought to be important determinants

of the infection dynamics within and between herds were gathered according to Omer *et al*. (2000). A pre-tested questionnaire was used to identify risk factors for the occurrence of brucellosis in humans and camels. The details of the questionnaire surveys were given in Annex 2.

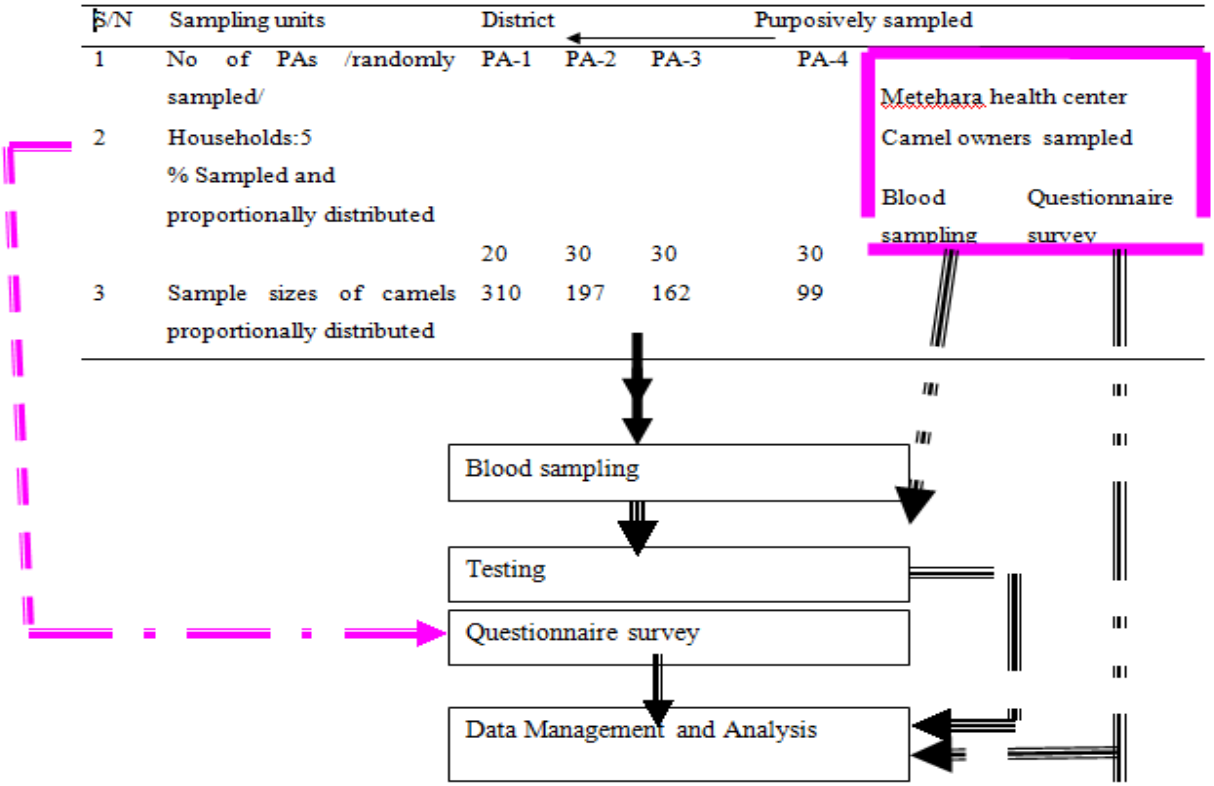

Figure 2: diagram showing sequence of study design

PA- peasant association: *PA-1* ***Dhebiti****, PA-2* ***Kenifa,*** *PA-3* ***Saraweba and*** *PA-4* ***Ilaala***

Figure 5: Diagram showing sequence of study design

3.4. Sample size determination

Sample size was determined according to Thrusfield (2005) using 95% confidence level, 5% precision. The 50% expected prevalence of brucellosis was used since there was no published prevalence of camel brucellosis in the study area.

The formula used for sample size determination was:

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

Where:

n = required sample size

P_{exp}= expected prevalence

d= desired absolute precision

Using the above formula, the minimum sample size required for the study was about 384. However, to increase the precision, the sample size was increased two-fold and 768 camels were sampled in Fantale District. For the questionnaire survey, 5% of the herders (households) were conveniently selected. Serum samples from the individuals were collected for brucellosis testing. The collection of these serum samples was carried out by the physicians in the district.

3.5. Sampling methodology

Fentale District was purposively selected for this study based on accessibility and availability of camel population. The district has eighteen peasant associations out of which 20% (4PAs) were randomly selected. Multi-stage sampling procedure was used to select study camels. The number of camels sampled from each peasant association was proportionally determined from the camel population in peasant association and the animals were sampled randomly. A total of 192 herds with an average herd size of 34 camels were sampled randomly. All camels above two years of age kept for breeding purposes were selected for this study.

3.6. Questionnaire survey

One hundred and ten randomly selected camel owners were interviewed using pre-tested structured questionnaire to determine husbandry and management risk factors which were known or thought to influence the spread and maintenance of brucellosis in camels. Relevant data on animals such as sex, age, pregnancy status and source of replacement stock, movement of camels, dry season feeding and watering points, handling of calving and abortion together with the reproductive disorders such as history of abortion, fetal membrane retention, stillbirths or births to weak young animals, conception failure, repeat breeding and other potential risk factors were recorded. Finally, the results of the questionnaire were compared with the results of serological tests. Information on potential risk factors for human brucellosis was also collected using pre-tested structured questionnaire. The study on humans was focused on risk groups (camel owners, veterinary assistants and hospital patients) and others who had contact with camels. Relevant risk factors such as removal methods of retained fetal membranes, consumption of raw milk and milk products, raw meat and meat products; clinical manifestations such as headache and back pain, insomnia, prolonged intermittent fever and chills with night sweating, and joint pain, weakness and nervous disorders pertaining to brucellosis in humans were gathered during serum collection.

Blood collection

Approximately 10 ml of blood from each camel, and 5ml of blood from each human was collected. A total of 768 blood samples from camels and 250 blood samples from humans were collected aseptically using sterile plain vacutainer tubes for serum separation. The samples were properly labeled and vacutainer tubes were left for 24 hours at room temperature for the blood samples to clot. The next day the sera were separated and transferred into sterile vials and kept at -20 ^{O}C until tested for antibodies.

3.7. Serological tests

The serological tests employed were RBPT for screening and the sera that tested positive to RBPT were further tested using complement fixation test (CFT) (OIE, 2004). Only serum samples that were positive to both RBPT and CFT were considered as positive. The RBPT and CFT procedures are described in the following sub-sections 3.7.1 and 3.7.2 respectively.

3.7.1. Rose Bengal Plate Test (RBPT)

The RBPT is an agglutination test that uses stained antigen. The latter is a suspension of *Brucella abortus* from Institut Purquier 326, Rue de la Galera 34097 MONTPELLIER CEDEX 5, France. The antigen was inactivated by heat and 0.5% phenol, adjusted to pH 3.65 and colored with Rose Bengal. The low pH of the assay prevents agglutination with IgM and agglutinates only IgG1. This reduces non-specific interactions. The procedure described by Alton *et al*. (1975) was used. The degree of agglutination was visually graded from 0 (no agglutination) to 3 (coarse clumping), with corresponding RBPT scores of 0, 1, 2, or 3. For materials required and test procedures see Annex 1. Serum of 30µl was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone of approximately equal to 2 cm in diameter. The mixture was then rocked gently for four minutes at ambient temperature and then observed for agglutination. Any visible reaction was regarded as positive otherwise negative (OIE, 2004). All collected serum samples were screened using RBPT.

3.7.2. Complement fixation test (CFT)

The complement fixation (CFT) test was undertaken at the National Veterinary Institute (NVI), Department of Immunology, Debre Zeit. Preparation of the reagents was performed according to the protocols recommended in the OIE (2004). All sera that tested positive to RBPT were further tested using the CFT for confirmation. The control sera and complement were obtained from the Federal Institute of Veterinary Medicine Berlin, Germany and the 2% RBC used were from National Veterinary Institute, Debre Zeit. As for the interpretation of test results, the positive reactions were identified by sedimentation of SRBC and absence of haemolysis. Negative reactions were those that showed haemolysis of SRBC. According to OIE (2004) sera with strong reactions of approximately 100% fixation of the complement (4+) at dilution of 1:5, sera with about 75% fixation of the complement (3+) at a dilution of 1:10, sera with about 50% fixation of the complement (2+) at a dilution of 1:20, and sera with about 25% fixation of the complement (1+) at a dilution of 1:40 were considered as positive. In this study at least with 50% fixation of complement (2+) at the lowest dilution of 1:10 and above were considered as positive for *Brucella* antibodies.

3.8. Data management and analysis

Data obtained from both serological tests and questionnaire survey were stored in Microsoft excel spreadsheet (Microsoft Corporation). These data were analyzed by descriptive statistics and binary logistic regression using SPSS version 15.0 statistical package (SPSS, 2007). Animals and humans test positives to both RBPT and CFT were defined as seropositive. Clusters (herds) having at least one seropositive camel were considered positive for brucellosis. The individual animal and human level sero-prevalence was calculated based on RBPT and CFT positive results divided by total number of animals and humans tested. Similarly, herd (cluster) level sero-

prevalence was computed as the number of clusters (herds) with at least one positive animals divided by the total number of clusters (herds) tested. Within-cluster (fherd) sero-prevalence was calculated by dividing the number of positive reactors in the cluster (herd) by the total number of animals tested in that herd as described in Thrusfield (2005). Questionnaire data that included risk factors associated with husbandry management systems like, females having infertility problems, whether sold or kept within herds (reason for sell of breeding camels), method of disposal of fetal membranes, dry and wet season watering point and those reproductive parameters thought to influence the disease were compared with that of serological results. The Chi-square test was applied to determine existence of any association between seropositivity and some risk factors in camel and association between human positivity and contacts with animals. To measure the strengths of the association binary logistic regression was applied to calculate the odds ratio. The agreement between the RBPT and CFT results was determined used Kappa statistic. The significance level used was $\alpha = 0.05$.

4. RESULTS

4.1. Serological results

A total of 768 camel sera, collected from four peasant associations of Fantale District were screened using RBPT. A total of 71 of them were positive. These were further tested with CFT for confirmation and 70 out of the 71 sera were positive. These sera that were positive to both RBPT and CFT were considered as true seropositive and were then used for the subsequent data analysis. All RBPT agglutinations 1+ and above were found positive by CFT except one sample at 2+ degrees of agglutinations from camel sera that was RBPT positive was found to be negative by CFT. Among the 192 herds investigated in this study, 71 herds had at least one positive reactor after RBPT and 70 by CFT. The results revealed an overall herd level seroprevalence of 36.5% (70/192) and within herd seroprevalence of 3.2% (70/2210). Within herd sero-prevalence varied from negative animals to at least one positive reactor.

4.1.1. Descriptive results of sero-provalence

The overall individual animal level seroprevalence of RBPT and CFT were 71(9.2%) and 70 (98.6%, among RBPT positives), respectively (Table 6). The test agreement between RBPT and CFT was 99.2%.

Table 17: The overall seroprevalence of camel brucellosis

Variables	n*	RBPT		95 % CI		CFT		95 % CI***	
		Positive	Seropre Valence	Lower	Upper	Positive	Seropre Valence	Lower	Upper
Camel brucellosis	768	71	9.2%	7.2	11.2	70	98.6%**	95.9	101.3

*=Total number of samples tested **Among RBPT positives * ** = Confidence intervals

At herd-level there were statistically significant difference in prevalence (p=0.006) among herds with the highest seroprevalencere recorded in Knifa (57.1%) and the lowest in Saraweba (18.2%). The seroprevalence at herd-level was 34.1%(14), 49.0%(25) and 31.0%(31) with herd catagories of 8-20, 21-34 and >34, respectively. There was statistically significant (p=0.000) difference among herd groups. Significant (p=000) difference was also observed in defferent groups of herding experiance (Table 7).

Table 18: Summary of the overall herd-level seroprevalence of brucellosis for potential risk factors in Fental District, East Shoa Zone, Oromia Regional State

Riskfactor	Category	n*	Seropre valence %	χ^2	P-value	95 % CI	
						Lower	Upper
PAs	Dhebiti	78	29 (37.2)			26.5	47.9
	Kenifa	49	28 (57.1)	12.429	0.006	43.2	71.0
	Saraweba	33	6 (18.2)			5.0	31.4
	Ilaala	32	7 (21.9)			7.6	36.2
Herd size	8-20	41	14 (34.1)			19.6	48.6
	21-34	51	25 (49.0)	41.003	0.000	35.3	62.7
	> 34	100	31 (31.0)			21.9	40.1
Herding experience	2-10years	30	8 (26.7)			10.9	42.5
	11-20years	92	45 (48.9)	30.307	0.000	38.7	59.1
	>20years	70	17 (24.3)			14.3	34.3

*=Total number of samples tested, $^*\chi^2$ =Chi-square

In order to assess the potential risk factors, at individual animal-level associations of seroprevalence of *Brucella* antibody with respect to sex, age, parity and reproductive status were determined based on risk factor analysis in camel brucellosis. There was no significant (p=0.872) difference in susceptibility to brucellosis between the two sex groups. The seroprevalence was 9.1 %(64) and 9.7% (6) in female and male, respectively (Table 8). There had been observed a high variation in seroprevalence of *Brucella* antibody among different age

groups. The seroprevalence in age groups was 6.1% (14), 8.5%(35) and 19% (19) respectively for 2-4, 5-10 and >10years old camels. Statistically significant (p=0.001) difference was found among different age groups. Significant (p=0.001) difference was also observed in different parity groups with different seroprevalences recorded in the study area, with the highest in camels with more than one parturation and the lowest in camels with no parturation. No significant (p=0.517) difference was observed in different categories of reproductive status where the highest and lowest seroprevalence was recorded in pregnant and lactating respectively (Table 8).

Table 19: Summary of the overall individual animal-level seroprevalence of brucellosis for potential risk factors of study area

Risk factor	Category	n*	Seropre valence %	χ^2	P-value	95 % CI	
						Lower	Upper
Sex	Female	706	64 (9.1)			7.0	11.2
	Male	62	6 (9.7)	0.026	0.872	2.3	17.1
Age	2-4years	231	14 (6.1)			3.0	9.2
	5-10years	437	37 (8.5)	14.619	0.001	5.9	11.1
	>10years	100	19 (19.0)			11.3	26.7
Parity	No parturition	217	12 (5.5)			2.5	8.5
	Single parity	180	10 (5.6)	13.662	0.001	1.1	10.1
	More than one	309	42 (13.6)			9.8	17.4
Reproductive	Non pregnant	168	16 (9.5)			5.1	13.9
status	Pregnant	329	33 (10)	1.319	0.517	6.8	13.2
	Lactating	209	15 (7.2)			3.7	10.7

*=Total number of samples tested

Statistically significant difference in seroprevalence was observed in abortions 38.7% (41) when compared with non-abortions 3.8% (23) (p=0.000) (Table 9). The seroprevalence was 34.9% (29) in female camels with fetal membrane retentions and 5.6% (35) in those with out fetal membrane retention. Statistically significant variation was observed (p=0.000) (Table 9). Significant variation was also observed (p=0.000) in camels with stillbirths and birth to weak calves, respectively.

Table 20: Seroprevalence results by females reproductive disorders of camel brucellosis

Variables	Category	n*	Seropre Valence%	χ^2	P-value	95%CI	
						Lower	Upper
Abortion	Absent	600	23 (3.8)			2.3	5.3
	Present	106	41 (38.7)	132.694	0.000	29.4	48.0
Fetal membrane	Absent	623	35 (5.6)			3.8	7.4
retention	Present	83	29 (34.9)	76.390	0.000	24.6	45.2
Stillbirth	Absent	649	30 (4.6)			3.0	6.2
	Present	57	34 (59.6)	192.466	0.000	46.9	72.3
Birth to weak	Absent	652	42 (6.4)			4.5	8.3
calf	Present	54	22 (40.7)	71.170	0.000	27.6	53.8

*n=Total number of samples tested

4.1.2. Results of binary logistic regression analysis

During the binary logistic regression analysis, one level of potential risk factor was a reference category level. Risk factors affecting seroprevalence of camel brucellosis at herd level for example PAs, herd size and herding experience were considered as potential risk factors to the seroprevalence of camel brucellosis at herd level. The differences between camel herd seroprevalence of brucellosis in each risk factor categories as well as associations are summarized in Table 10. The strength of associations among the different peasant associations were assessed and it was found that only Kenifa had significant (OR=0.168, p=0.001) association with seropositivity compared to Dhebiti and Saraweba, both of which had no significant associations with OR=0.464 (p=0.142) and OR=0.730 (p=0.617), respectively. Herd size was classified into three categories (small 8-20, medium 21-34 and large > 34 camels), to assess the association with seropositivity. The results showed that camels in small and medium herd sizes had significance associations with OR=0.124 (p=0.000) and OR=0.129 (p=0.000), respectively. At herd level, herding experience by herdsmen of those started recently OR=0.237 (p=0.005) and with medium experience OR=0.113 (p=0.000), had effect on the seropositivity to *Brucella* antibodies in their respective herds. Those with lifetime experience were presumed to have better hygienic and management conditions.

Table 21: **Associations of potential risk factors with dependent CFT *Brucella* seropositivity of camel brucellosis at herd level**

Risk factor	Category	Estimated Coefficient	S.E.	df	P-value	OR	95.0% CI for OR	
							Lower	Upper
PAs	Dhebiti	-0.768	0.523	1	0.142*	0.464	0.166	1.293
	Kenifa	-1.785	0.550	1	0.001	0.168	0.057	0.493
	Saraweba Ilaala	-0.315	0.630	1	0.617*	0.730	0.212	2.507
Herd size	8-20camel	-2.091	0.439	1	0.000	0.124	0.052	0.292
	21-34camel	-2.048	0.365	1	0.000	0.129	0.063	0.264
	>34camel							
Herding experience	2-10years	-1.441	0.514	1	0.005	0.237	0.086	0.648
	11-20years	-2.179	0.372	1	0.000	0.113	0.055	0.234
	>20years							

S.E=Standard Error of the coefficient, *=No significance differences and associations at $p<0.05$, df= Degree of freedom

The risk factors affecting seroprevalence of individual camel brucellosis, sex, age, parity and reproductive status were considered as potential risk factors affecting the seroprevalence of camel brucellosis at individual animal level. The results of associations between camel seroprevalence of brucellosis and the categories of these potential risk factors are summarized in Table 11. There was no (OR=1.075, p=0.872) associations of both male and female camels and seropositivity to camel brucellosis. Similarly, only age groups 5-10years had significant (OR=2.662, p=0.014) associations. In the assessment of reproductive potential risk factors and seropositivity to *Brucella* antibody an attempt was made to find out the associations of the seropositivity with different reproductive status. Camels were grouped into three categories as camels having no parity, single parity and multiple parities. The binary logistic regression indicated that there was significant (OR=54.244, p=0.008) associations with camels that no reported parturition. In addition, females were also categorized into three groups: non-pregnant, pregnant and lactating. Binary logistic regression analysis showed that seropositvity to *Brucella* antibodies in pregnant and non pregnant camels had no statistical significant associations, OR=0.842 (P=0.636) and OR=0.491 (p=0.120), respectively (Table 11).

Table 22: Summary results of the binary logistic regression analysis of potential risk factors with dependent CFT *Brucella* seropositivity in camel in Fentale District, East Shoa Zone, Oromia Regional State.

Risk factor	Category	Estimated Coefficient	S.E.	df	P-value	OR	95.0% CI for OR	
							Lower	Upper
Sex	Female	0.072	0.449	1	0.872	1.075	0.446	2.592
	Male							
Age	2-4years	-2.352	1.479	1	0.112	0.095	0.005	1.729
	5-10years	0.979	0.400	1	0.014*	2.662	1.216	5.829
	>10years							
Parity	No parity	3.993	1.500	1	0.008*	54.224	2.868	1025.246
	Single parity	0.815	0.443	1	0.066	2.258	0.948	5.379
	More than one							
Reproductive Status	Non pregnant	-0.711	0.458	1	0.120	0.491	0.200	1.204
	Pregnant	-0.173	0.365	1	0.636	0.842	0.412	1.720
	Lactating							

S.E=Standard Error of the coefficient, df=Degrees of freedom, *=Significance differences and associations at $p<0.05$

The associations of seropositivity of *Brucella* antibodies and reproductive disorders were done using the histories of abortions. The reports of abortions had significant associations with seropositivity (OR=5.078 and p=0.000). So, biologically the odds ratio showed that presence of abortion in camels was atleast 5 times more likely to be seropositive in contrast to non-aborted camels (Table 12). Statistical significant associations with seropositivity was also found in camels that had history of fetal membrane retention and stillbirth with OR=3.152 (p=0.004) and OR=14.480 (P=0.000), respectively. Giving birth to weak calf had no significant association OR=2.468 (p=0.052) with *Brucella* antibodies seropositivity.

Table 23: Associations between CFT *Brucella* seropositivity and history of reproductive disorders in camels in Fentale District, Eastern Shoa Zone of Oromia Regional State

							95% CI for OR	
Risk factor	**Category**	**Estimated Coefficient**	**S.E.**	**df**	**P-value**	**OR**	**Lower**	**Upper**
Abortion	Present Absent	1.625	0.364	1	0.000	5.078	2.489	10.362
Fetal membrane retention	Present Absent	1.148	0.398	1	0.004	3.152	1.445	6.875
Stillbirth	Present Absent	2.673	0.383	1	0.000	14.480	6.832	30.690
Birth to weak calf	Present Absent	0.904	0.465	1	0.052*	2.468	0.993	6.136

S.E=Standard Error of the coefficient, *= No significance difference and associations at P<0.05
df=Degrees of freedom

4.2. Questionnaire survey

High milk production is primary purpose (72.7%) of camel production in the area followed by drought mitigation (15%) and to some extent as alternative means against bush encroachment are decisive forces behind to start camel keeping among the Fentale pastoralists and cash income (4.5%) by sale and meat production (slaughter) and the rest for herd accumulation (Table 13). The majority of the herders does not have their own breeding bull but use village bull (89.1%) whereas some of them use their own bulls (10.9%). Division of labor among families varies with a type of activities in that most of the cases youngsters do herding, watering and milking activities, while adult men and women do delivery and mating assistances. According to the result of questionnaire survey, 47.3% of the members of the community in the study area use veterinary clinics for their animal health care and trypanosomiasis were the most prominent disease of the area (Table 13).

Table 24: **Frequencies and percentages for the questionnaire survey (n=110) of respondent of camel brucellosis in the study area**

Variables		n*	(%)
Importance of camel	Milk	80	72.7
	Drought mitigation	17	15
	Cash income	5	4.5
	Herd accumulation	8	7.8
Milk consumption and preservation means	Fresh	110	100
	Boil	0	0
	Other treatments	0	0
Meat consumption	Raw	65	59.1
	Cooked	3	2.7
	Both	42	38.2
Activities and labor division-herding	Young	84	76.4
	Husband	21	19.1
	Wife	5	4.5
Activities and labor division-watering	Young	91	82.7
	Husband	16	14.5
	Wife	3	2.7
Activities and labor division-milking	Young	80	72.7
	Husband	13	11.8
	Wife	17	15.5
Activities and labor division-delivery assist	Young	24	21.8
	Husband	83	75.5
	Wife	3	2.7
Activities and labor division-mating assist	Young	31	28.2
	Husband	17	15.5
	Wife	62	56.4
Health care	Traditional healer	18	16.4
	Self-drug administration	40	36.4
	Veterinary clinics	52	47.3
Diseases	Contagious skin necrosis	4	3.6
	Tick paralysis	8	7.3
	Black quarter	6	5.5
	Abortion	12	10.9
	Abscesses	7	6.4
	Ecto-parasite	10	9.1
	Anthrax	13	11.8
	Endo-parasite	17	15.5
	Pneumonia	15	13.6
	Trypanosomosis	18	16.4
Causes of abortion	Disease	30	27.3
	Mechanical trauma	5	4.5
	Both	75	68.2
Source of bull	Village bull	98	89.1
	Own bull	12	10.9

n*=Number of respondents

The results of the analysis of the questionnaire data on management risk factors and some reproductive parameters and their associations with *Brucella* seropositivity are shown in Table 14. One hundred and ten randomly selected camel owners were interviewed in the study area to correlate the associations of the management and husbandry risk factors with serological results. The status of seroprevalence among herds kept with small ruminants was 23.8%. Those kept together with camels had seroprevalence of 9.1%, while those kept separately and with cattle and had no positive reactors. The number of tested camels in those groups with no reactor was too small to make comparison and justify ruminants as risk factor. Among the management and husbandry risk factors, less *Brucella* antibody seroreactors revealed 19.6% from those who sold camels that frequently aborted. In the current study regardless of mobility, it was observed that herds which often use rivers 23.3% and lakes 12.5% during dry season and herds that often use rivers 42.3% and seasonal springs 15.5% during wet season had high seroprevalence than the other watering points (Table 14). However, except watering point in the wet season, no statistical significant variations observed ($p>0.05$) in management risk factors and

some reproductive parameters.

Table 25: **Descriptive results of management and husbandry risk factors**

Variable	Category	n*	Seropre valence %	χ^2	P-value	95 % CI of OR	
						Lower	Upper
Herding of camels	With village camel	22	2 (9.1)			-2.90	21.1
	With cattle	3	0 (0.0)	3.398	0.334		
	With small ruminants	84	20 (23.8)			14.7	32.9
	Separately	1	0 (0.0%)				
Culling method: camels that frequently abort	Sell	92	18 (19.6)			11.5	27.7
	Keep	10	4 (40.0)	3.093	0.213	9.6	70.4
	Slaughter	3	0 (0.0)				
Culling method: camels that do not conceive	Sell	94	18 (19.1)			11.2	27.0
	Keep	10	3 (30.0)	4.452	0.108	1.6	58.4
	Slaughter	1	1 (100.0)				
Management of fetal membrane	Leave on the field	97	21 (21.6)			13.4	29.8
	Give to dogs	2	0 (0.0)	1.483	0.476		
	Dispose	11	1 (9.1)			-7.9	26.1
Watering points: dry	River	86	20 (23.3)			14.4	32.2
	Pond	2	0 (0.0)				
	Artificial well	5	0 (0.0)	3.132	0.536		
	Natural well	1	0 (0.0)				
	Lakes	16	2 (12.5)			-3.70	28.7
Watering points: wet	River	26	11 (42.3)			23.3	61.3
	Pond	1	0 (0.0)				
	Artificial well	9	0 (0.0)	12.238	0.032		
	Natural well	2	0 (0.0)				
	Lakes	1	0 (0.0)				
	Seasonal springs	71	11 (15.5)			7.1	23.9

*n= Number of respondents

4.3. The seroprevalence of brucellosis in humans

The overall seroprevalence results of human brucellosis are shown in Table 15. Of the 250 human serum samples collected, 118 were from females and the rest were from males. Eleven *Brucella* seroreactors were from females and four were from males. Fifteen RBPT positive sera were further examined by CFT and all of them were seropositive to this test. All the fifteen were from camel herders.

Table 26: The overall seroprevalence of human brucellosis

variables	n*	RBPT		95 % CI		CFT		95 % CI	
		positive	Seropre valence	Lower	Upper	Positive	Seropre valence	Lower	Upper
Human brucellosis	250	15	6.0%	5.7	8.9	15	100%**		

*=Total number of samples tested, **Among RBPT positives

The results of the analysis of potential risk factors with seroprevalence to human brucellosis are summarized in Table 16. Seroprevalence was 6% in camel owners; 9.3% in females; 6.9% in age groups between 12 to 30 years old. The seroprevalence of those who drank raw milk was at least 4.0%, contacts with fetal membrane were 29%, with laceration were 16.2%, individuals who did not use gloves 6% and those who visit hospitals were 10.8%. However, statistically significant ($p<0.05$) variations were observed in sex groups, in pastoralists who consume raw milk, individuals who had made contact with fetal membranes, with lacerations and those who visited hospitals, respectively.

Table 27: Summary of human *Brucella* seroprevalence to different potential risk factors in Fental District, East Shoa Zone, Oromia Regional State.

Risk factor	Category	n*	Seropre Valence (%)	χ^2	p-value	95%of CI Lower	Upper
Occupation	Pastoralist	249	15 (6)			3.1	8.9
	Veterinarian	1	1 (0.0)	0.064	0.800		
Sex	Female	118	11 (9.3)			4.1	14.5
	Male	132	4 (3)	4.373	0.037	0.1	5.9
Age	12-30	189	13 (6.9)			3.3	10.5
	>30	61	2 (3.3)	1.059	0.303	-1.2	7.8
Raw meat consumption	Yes	163	10 (6.1)			2.4	9.8
	No	87	5 (5.7)	0.015	0.902	0.8	10.6
Raw milk consumption	Yes	241	10 (4.1)			1.6	6.6
	No	9	5 (55.6)	40.651	0.000	23.1	88.1
Contact with fetal membrane	Yes	31	9 (29)			13.0	45.0
	No	219	6 (2.7)	33.285	0.000	0.6	4.8
Laceration	Yes	68	11 (16.2)			7.4	25.0
	No	182	4 (2.2)	17.151	0.000	0.1	4.3
Use of gloves	Yes	1	0 (0.0)				
	No	249	15 (6)	0.064	0.800	3.1	8.9
Hospital visit	Yes	102	11 (10.8)			4.8	16.8
	No	148	4 (2.7)	6.993	0.008	0.1	5.3

*n=Total number of samples tested

The binary logistic regression results of the questionnaire data are shown in Table 17. Those who consumed raw milk were at least 29 times to seropositive to brucellosis (OR=29.434, p=0.001). Those individuals who had contacts with fetal membranes had OR=0.046, although it was statistically significant (p=0.000)> those with lacerations OR=0.059, however, it was statistically significant (p=0.001). All the rest of the responded questionnaire risk factor had no statistical strong associations with seropositivity to brucellosis. Similarly even though there was no significance association in those people who consumed raw meat there was biologically slight association (OR= 1.164).

Table 28: Summary results of binary logistic regression test results of potential risk factors with human brucellosis seropositivity in the study area.

Risk factor	Category	Estimated Coefficient	S.E	df	P-value	OR	95%of CI for OR Lower	Upper
Occupation	Pastoralist Vet.	-15.721	40193.0	1	1.000	0.000	0.000	.
Sex	Female Male	-0.755	0.790	1	0.339	0.470	0.100	2.211
Age	12-30 >30	-1.112	1.034	1	0.282	0.329	0.043	2.496
Raw meat consumption	yes No	0.152	0.778	1	0.845	1.164	0.253	5.345
Raw milk consumption	yes No	3.382	1.049	1	0.001*	29.434	3.767	229.97
Contact with fetal membrane	yes No	-3.074	0.786	1	0.000*	0.046	0.010	0.216
Laceration	yes No	-2.838	0.858	1	0.001*	0.059	0.011	0.314
Use of gloves	yes No	18.456	40192.97	1	1.000	1E+008	0.000	.
Hospital visit	yes No	-0.996	0.779	1	0.201	0.369	0.080	1.701

S.E=Standard Error of the coefficient, *= significance difference and associations at df=degrees of freedom and P<0.05

5. DISCUSSION

Prevalence of camel brucellosis

The overall seroprevalence found in the present study by CFT test among RBPT positives was 98.6% in Fentale District, Eastern Shoa Zone of Oromia Regional State. This proportion was considerably higher than most of those reported previously in the region and Ethiopia. Teshome *et al. (*2003) and Bekele *et al.* (2005) reported 1.2% and 1.8% of camel brucellosis respectively in Borena lowlands. Domenech (1977), Richard (1980) and Teshome *et al*. (2003) reported 4.4%, 5.5% and 4.2% of camel brucellosis in the country. This study indicated that seroprevalence of camel brucellosis at herd-level varied in different peasant associations (localities) in the same location with high prevalence in Kenifa (57.1%) and lowest in Saraweba (18.2%). These findings are higher than those reported by Bekele *et al*. (2005) who reported prevalences of 19.2 and 14.5% in Yabello and Libeen Districts, Borena Zone of Oromia Regional State. Berhanu (2006) also reported in Jijiga and Babile Districts prevalences of 12 and 5.8%, respectively, although the finding of 57.1% in Kenifa was quite high. These finding could be due to various varying husbandry and management practices, susceptibility of animals, virulence of the organisms, presence of reactor animals, and the movement of pastoralists from place to place. The movement of the animals may worsen the level herd endemicity situation of brucellosis in an area, as the spread of the disease from one herd to another and from one area to the other is usually due to the movement of an infected camel into susceptible camel herd (Radostits *et al.,* 1994). The results of the present study showed high seroprevalences in all peasant associations than those reported in other countries for example 15.8% in South Jordan (Hawari, 2008), 12.1% in Jordan (Al-Majali *et al,* 2008), 14.6% in Kuwait (Godfroid, 2002), 12% in Iraq (Refai, 2002) and 11.2% in Iran (Refai, 2002). In addition, lower seroprevalences to these study findings of camel brucellosis have been reported in some African countries for example 7.2% in Sudan (Abbas and Agab, 2002) and 5% in Egypt (Godfroid, 2002). Abbas and Agab (2002) and Wernery and Kaaden (2002) reported that seroprevalences of brucellosis of camels kept in extensive farming system, ranges from 2 to 5% in most countries. But those raised intensively they range between 8 to 15% especially in Saudi Arabia (Radwan *et al*., 1992) and Kuwait (Khalaf and Khaladi, 1989). In such production system, overcrowding is the major risk factor providing more chances of contacts between susceptible camels and cases leading to increased likelihood of transmission of brucellosis. Further, these varying seroprevalences of brucellosis in different countries may be due to differing husbandry and management conditions, the number of susceptible camel population, the rate of transmission, the virulence of the organism and other factors (Radostits *et al.,* 1994; Radwan *et al*., 1992). In addition, the absence of vaccination of herds and the existence of positive animals indicate the occurrence of natural infection (Alton *et al*., 1975). The high prevalence of *Brucella* infection in the present study could be due to close contacts of camels with other infected ruminants, which facilitates high chances of transmission of brucellosis from these ruminants to dromedaries as they live in free range in proximity in the bush and at watering points as pointed out by Radwan *et al*. (1992) in Magnolia. Specially, contact between dromedaries and small ruminants were more incriminated for the transmission of brucellosis to camels (Radwan *et al.,* 1992). Abou-Eisha, (2000) also observed higher seroprevalence in camels that were in contact with sheep and goat who reported higher frequencies of *B. melitensis* isolation from camels indicate the role of small ruminants in the transmission of brucellosis to camels. Even though no research was conducted in the study area on role of small ruminants in *Brucella* transmission, research conducted by Ashenafi *et al*. (2007) in neighboring Afar Regional State shows prevalence of brucellosis in small ruminants was 4.8% and this also indicates the importance of small ruminants in the transmission of the disease to susceptible camels in this study area. A contributing factor to the spread of the disease may be the movement of animals for grazing and watering during dry season, as aggregating the animals around watering points will increase the contact between infected and healthy animals and thereby facilitate the spread of the disease (Richard, 1979). In addition to this fact, no hygienic measures are adopted during milking and the calves are allowed to suckle both before and after milking. This means that the milker can not only carry an infection from one camel to another but also the calves may become infected, which may become an additional factor for the persistence of the infection in the area, as calves may be raised on infected milk or kept in the presence of the infection. Lack of strict control of animal movement at the border of the country and the neighboring countries may also facilitate the spread of infection.

Host potential risk factors

This study revealed that higher numbers of seropositive in females. Even though sample size of males was low in this study, seroprevalence was slightly higher in males (9.7%) than female (9.1%) dromedaries. However, the likelihood of seroconversion of only 1.1 times higher in females than male animals. Nevertheless, the apparently high seroprevalence in males agrees with the findings of Teshome *et al*. (2003) in Afar region and Berhanu, (2006) in Somali Regional State of seroprevalences of 7.2 and 2.8% in male and 4.9 and 2.3% in females, respectively. Relatively higher susceptibility of females could be because females have more physiological stresses (Walker, 1999). On the contrary, Abbas *et al*. (1987) reported equal distribution of *Brucella* antibodies between both sexes. Okoh (1979) reported that male camels had higher infection rate, but Okoh's sample size of females was too small to contrast with the findings of this study. Age categorization was made to assess an

association of the seroprevalence with the disease. Despite the increment in seropositivity with age, no significant differences were observed in camels with age 2-4 but significant difference was observed in camels with age 5-10 years. However higher seroprevalences of 19% in animals aged above 10 years followed by those in 5-10 age group had seroprevalences of 8.5% and those in 2-4 age group with 6.1% was observed. Overall in three age groups no significant differences (p=0.151) were observed. This finding is consistent with Teshome *et al*. (2003), Bekele *et al.* (2005) and Berhanu, (2006) who found similar seroprevalences of brucellosis in very old camels, while the seroprevalences in other age-groups for example young and adult camels were 2.9, 6.1; 1.0, 2.2 and 1.7; 2.6%, respectively. The increase in infection with advances in ages agrees with report of Musa and Shigidi, (2001). Walker (1999) described that younger animals tend to be more resistant to infection and frequently clear infections than sexually mature animals. The presence of growth factors such as erythritol and hormones favor infection in mature animals (Quinn *et al.,* 2002; Walker, 1999). This could be due to the fact that hormones such as erythritol might stimulate the growth and multiplication of *Brucella* organisms which tend to increase the concentration with age and sexual maturity as has been suggested earlier (Radostitis *et al*., 1994). 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 were 5 years at puberty in eastern Ethiopia. Wossene (1991) also reported the same age for puberty and first calving in Ogaden female dromedaries. In this study, camels above 5-10 years were considered adults and the probability of this was found disease 2.7 times in mature camels than young ones. The analysis on the associations and seroreactor rates among three parity stages (no parity, single parity and multiple parities) were performed. A significant association was found in camels with no parity (p=0.008) when compared to camels with one and more parities. The seroprevalence of the three parity groups of the present finding agrees with the findings of Berhanu, (2006), who reported 1.6%, 2.5% and 2.7% for heifers, single parity and more than one parities, respectively. This finding is in consistence with the report of Radostitis *et al*. 2000) who indicated that animals that which has not given birth tended to be more resistant to infection. Among the three categorized reproductive status that is not pregnant, pregnant and lactating, only high seroreactors were recorded in pregnant camels. This is in agreement with Radostitis *et al*. (2000) who reported that sexually mature and pregnant animals are more susceptible to infection with the organism than sexually immature animals of either sex. In contrast, Omer *et al.* (2002) also reported high seroprevalence in adult animals irrespective of their lactating or pregnancy status. Omer *et al.* (2002) stated that stocking densities are important potential determinants between susceptible and infected animals. This concept coincides with current study that the seroprevalence of brucellosis among three categorized herd sizes, 8-20 camel, 21-34 camel and >34 camel had significant (p=0.000) variations where higher seropositivity was recorded in the large herd size. This may be due to easy contacts between infected and susceptible camels. The significantly higher seropositivity in the large herd size categories is in concordance with several reports that large herd sizes are at risk for occurrence high prevalence of brucellosis (Teshome *et al*., 2003, Bekele *et al.*, 2005 and Berhanu, 2006). A large number of camel herds always congregate at watering points thus facilitating the spread of brucellosis. Rivers, lakes and artificial wells are major permanent water sources in this study area. Camels have direct access to water points and contaminate by discharges and hence a higher infection rate was recorded in large-sized camel herds. Nevertheless, the mobility natures of camel herds do not restrict them to a specific category of the water resources, making conclusion to specific watering pints difficult on that observation. *Brucella* seroprevalence was higher in camels of those herdsmen with medium experience than those who started camel husbandry recently. This could be explained by herdsmen who started recently might have good management and husbandry practices since they may be having smaller herd sizes. Otherwise, this finding is not consistent to that reported by Bekele *et al.*, (2005), who reported seroprevalences of 13.8% and 22.8% for herders who started recently and who kept them for generations.

History of abortion and seroprevalence of brucellosis

Brucellosis can generally cause significant economic losses through abortion, late first calving age, long calving interval time, low herd fertility, culling and comparatively low milk production (Wernery and Kaaden, 2002). In the current study, among the abortions a significant association (p=0.000) was found with seropositivity of the infection and the proportion of abortion rates were 3.8% in those herders who reported no history and 38.7% in those who reported history of abortion was recorded.

Management and husbandry risk factors and impact of the disease on some reproductive parameters

Considering the contagious nature of *Brucella* species, sharing grazing land and drinking water facilitate the transmission of the disease (Jiwa *et al*., 1996). This is in agreement to current study where one hundred and ten randomly selected camel owners in the extensive production system were interviewed between those who had camel having infertility problems/frequent abortion/ were observed. This result was common with the habit of pastoralists in the area who sell and keep their camels. This could have increased the risk of disease transmission. Walker (1999) indicated brucellosis transmission was high when infected camels were found in the herds. Moreover, pastoralists that used individual wells, as watering points had lower proportions of seroreactors

than pastoralists that used communal ones. This is compatible with Muma *et al*. (2007) who reported that animals often grazing and watering in plains along drink water standing in water up to 50 cm in depth. Since *Brucella* organisms can survive in water (at 20 ^{0}c for 2.5 months), sharing infection through water contamination was likely both within and across animal species. This is has been reported in Jijiga zone (Berhanu, 2006) who reported an association between husbandry risk factors and seroprevalence of *Brucella* antibodies. In the present study, complement fixations at 1/10 and above were considered positive for CFT. As a result, seropositivity was confirmed in 70 out of 71 RBPT positive reactors (98.6%) by CFT. All RBPT agglutinations 1+ and above were found positive by CFT except for one sample at 2+ degrees of agglutinations. Most of the CFT titers (90 %) were between 1/20 and (1/40) dilution rates. The titer recorded was higher than what has been reported by Teshome *et al.* (2003) in which 30% had a dilution rate of 1/640 but, it was similar to the work of Bekele *et al*., (2005) and Berhanu. (2006) who reported a proportion of 74.1% at a dilution rate of 1/320. Brucellosis remains widespread in domesticated and wild animal population, and presents a great economic and public health problems in African countries (Chukwu, 1985). According to Chukwu (1985), the high prevalence of the disease in Africa is probably due to the fact that many African countries have not 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 frequently isolated in camels in Middle East but also both species have been reported in Africa too (Abbas and Agab, 2002).

Human brucellosis

Proportions of seroprevalence of *Brucella* antibodies can be very high, particularly among populations in endemic areas. The prevalence of brucellosis in human is largely influenced by the prevalence of disease among domestic animals (Mohamed, 2002; Omer e*t al*., 2002). In the present study, 100% (among RBPT positives) of human sera were seropositive to *Brucella* antibodies. Among the risk groups tested were pastoralists and a single veterinarian who performed testing of *Brucella* antibodies among the pastoralists. This finding is higher than the findings of Abou-Eisha (2000) who reported 1% seroprevalence of brucellosis among nomadic people. This fact is justified by the consumption of 100% raw, unpasturaized and unboiled milk by the nomads in the study area, which is a common practice among the herdsmen. This is also true in Oman where Mohamed (2002) reported that among 375 cases of brucellosis 63% consumed raw milk and its products. Generally human brucellosis seroprevalence is high in risk groups and in others very low or zero in groups that are not at risk due to profession, lifestyles or eating habits. For instance seroprevalence of brucellosis was found in 14% of abattoir workers and zero in blood donors, and 3.8% in nomadic pastoralists in Chad (Massenet *et al.,* 1993), 6.5% in slaughterhouse workers in Dijibout (Chantal *et al*.,1996) and between 3-7% at different high risk groups in Eritrea (Omer *et al.* 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 in 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 probably put the public health in the area at risk. Abou-Eisha (2000) reported 1% (3/330) seroprevalence of brucellosis among nomadic people. The disease in human may be misdiagnosed due to other differential conditions like prevailing malaria (Abou-Eisha, 2000; El-Ansary *et al.,* 2001). In the current study, proportions of seropositivity in females and male were 9.3% and 3% respectively. However**,** these proportions were not statistically significantly different which could be attributed different sample sizes and large within sexes variations. These findings slightly differ with those reported by Cooper (1991) in Saudi Arabia of 5.4% and 6.34% in females and males respectively with but nevertheless they also note no significantly significant different. All these findings are similar to those in Mussie (2005). Brucellosis in humans is acquired from infected animals through direct contact or indirect by ingestion of animal products (Acha and Szyfers, 2001). At present study an association of *Brucella* seropositive with removal of fetal membranes was observed. This is in agreement with Al Sekait (1999) who conducted a sero-survey for brucellosis in Saudi Arabia of in those who lived in rural and urban areas. A proportion of 26.6% of brucellosis cases were found in rural people who were exposed to infected animal and 9.5% in urban people. Therefore, under the existing high need of animal products in this country there exist no justifications to ignore the role of this zoonotic disease.

6. CONCLUSIONS AND RECOMMENDATIONS

Results of the present study revealed that camel brucellosis is prevalent in Fentale District, East Shoa Zone of Oromia Reginal State. The findings of positive serological reactors do not only suggest the presence of the disease in camel populations of the area but also indicates the presence of foci of infection that could serve as sources of infection of the disease in naive camel herds. The study also showed that herd sizes and herding experience are important risk factors associated for this disease. For the human brucellosis, lack of awareness of the disease, habit of consuming raw milk and close contacts with camels were risk factors. This emphasizes the high prevalence of brucellosis in both camels and camel herders (public health) that is calling for need to implement suitable control strategies of brucellosis in the study area. Based on the above conclusions, the

following recommendations are forwarded:

- Awareness creation and continuous extension education on modern camel husbandry practices and control, prevention with eventual aim of eradicating this zoonosis among the pastoral communities.
- Proper management of aborted cases and areas possibly contaminated with aborted materials like disinfection and regular cleaning of the area and animal premises, proper disposal of aborted materials and after births, isolation of pregnant animals some weeks before and after calving should be practiced by pastorals';
- All animals having signs of brucellosis should be tested and the positive once should be removed swiftly from the healthy animals to control the transmission of the disease.
- Nationwide, strict animal movement policy should be created and implemented, not only for brucellosis but also for other related diseases.
- A close cooperation between veterinary and public health personnel is very crucial in raising public awareness with ultimate objective to control brucellosis
- Further research should be carried out with the objectives of isolating the causative agents, identification of the species and biotypes in the study area.

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