CROSS-SECTIONAL STUDY OF BRUCELLOSIS AND MASTITIS IN CAMELS (*Camelus dromedarius*) AND CAMEL MILK MARKETS NETWORK IN BABILLE WOREDA, EAST HARARGAE ZONE, ETHIOPIA

HAFEZ MOHAMMED SEID

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Joint Masters in Transboundary Animal Disease Management

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NOVEMBER, 2011

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ACKNOWLEDGMENTS

I would like to thank my academic advisors Dr. Maximilian Baumann, Dr. Mekonnen Hailemariam and Dr. Gizat Almaw for their support, professional advice, guidance and correction of the research work and provision of valuable reference materials.

My thanks also goes to Dr. Hartmut Lentz from Friedrich Loeffler Institute, Germany for evaluating and forwarding comments regarding the NA part of the study.

My special thanks goes to Dr. Mohammed Osman from Babille Agricultural Bureau, for his unreserved cooperation and tireless field assistance and his outstanding familiarity with the local languages facilitated the cooperation of herd owners and the taking of questionnaire survey. The kind cooperation of herd owners and local leaders of various PAs deserve special acknowledgement.

The laboratory technical support provided by Ato Belachew Bura of the National Animal Health Diagnostic and Investigation Center (NAHDIC) and W/o Webet Welde Medhin of National Veterinary Institute (NVI) is highly appreciated.

I would like to thank the European Union, the African, Caribbean and Pacific Group of States (ACP), the ACP-EU Cooperation Programme in Higher Education (EDULINK) and the Programme Management Unit (PMU) for supporting this Masters Programme and the Rural Capacity Building Project (RCBP) of Ministry of Agriculture, Ethiopia, for the generous financial support extended throughout the study. Ato Moges Hiluf, the training specialist of RCBP, deserves special thanks for his keen support.

I am greatly indebted to Moa Bekema, Elishadai Bekema, Sileshi Ambaw, Ali Kasahun, Anteneh Ashenafi, Mustefa Ademnur, Dr. Fitsum Alemayehu and Dereje

Tesfu for their assistance in many ways. My family members, Haji Mohammed Birhan seid, W/o Zeineb Atta and Amina Mohammed, deserve special thanks for their encouragement and support. Many thanks to my wife Hanan Abubaker for her patience support and love.

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ABBREVIATIONS AND ACRONYMS

AP	Apparent Prevalence
Ato	Mr. in *Amharic* language
BWAB	Babille Woreda Agricultural Bureau
cELISA	Competitive ELISA
CFT	Complement Fixation Test
CI	Confidence Interval
Cm	Centimetre
CMT	California Mastitis Test
CSA	Central Statistics Agency
ECRA	Ethiopian Customs and Revenue Authority
ELISA	Enzyme-Linked Immunosorbent Assay
Euc Norm	Euclidean Norm
FAO	Food and Agriculture Organization
FL	Front Left quarter
FR	Front Right quarter
G	gram
HRS	Harari Regional State
I-ELISA	Indirect ELISA
km	Kilometre
km^2	Square Kilometre
MARD	Ministry of Agriculture and Rural Development
Mi	Miles
ml	Millilitre
mm	Millimetre
mRBPT	Modified RBPT
N	Number (of)
NA	Network Analysis
NW	Network
Obs	Observations
OIE	World Organization for Animal Health

OR	Odds Ratio
ORS	Oromiya Regional State
PAs	Peasant Associations
PSU	Primary Sampling Units
RBPT	Rose-Bengal Plate Test
RL	Rear Left quarter
RR	Rear Right quarter
Se	Sensitivity
Sp	Specificity
Spp.	Species
SRS	Somali Regional State
SSQ	Sum of Squares
Std Dev.	Standard Deviation
TB	Tuberculosis
TP	True Prevalence
UAE	United Arab Emirates
UK	United Kingdom
UN-EUE	United Nations Emergency Unit for Ethiopia
Vc	Between-cluster Variance
W/o	Mrs. in *Amharic* language
Ml	Microliter

ABSTRACT

A cross-sectional study was conducted with the objectives of determining the prevalence of camel brucellosis and clinical and sub-clinical mastitis and associated risk factors, and making a preliminary assessment of the camel milk markets network in Babille Woreda. Field data gathering and observation was carried out from August to October, 2011. Two- stage cluster sampling was employed to select 378 camels in 12 peasant associations of Babille Woreda. Serum samples were taken from these camels and 8 serum samples (2.1%) were lost during handling. The 370 sera were screened for brucellosis by RBPT and 26 serum samples (7.0%) were found positive and further tested by CFT where 9 sera (2.4%) were reacting positive. Hence, the overall seroprevalence was 2.4% (n=370). Seroprevalence of *Brucella* infection in male and female camels were 2.9% (7/241) and 1.6% (2/129), respectively, with no statistically significant difference. The seroprevalence of brucellosis was relatively higher (3.8%, n=79) in young (below 4 years) than in adult camels (above 4 years) (2.0%, n=291), however, not statistically significant. No statistical association of parity and origin of camels nor abortion with the occurrence of *Brucella* infection could be determined. All camels with an abortion history were reacting seronegative. Questionnaire survey results of 40 herders revealed an average herd size of 12.4 camels and camels were the only livestock in 8 of the herds (20%) and were reared together with other livestock in 80% of the herds. Contact with other herds was common in 90% of the herds. Abortion seemed rare in Babille Woreda: only 6 of the respondents (15%) reported abortion in the past. Fever, trypanosomosis infection, ingestion of toxic plants, drought and bulls were claimed to be the causes of abortion. For clinical and sub-clinical mastitis prevalence determination again two-stage sampling was undertaken and 544 quarters were examined from 136 lactating camels reared in 34 herds and 9 quarters were blind (1.7%). The remaining 535 quarter milk samples (98.4%) were screened for sub-clinical mastitis using CMT. Clinical examination of the udder and inspection of milk were employed to determine the presence of clinical mastitis; however, clinical mastitis was not observed at all. The prevalence of sub-clinical mastitis was 14.4% at quarter level. Sub-clinical mastitis cases were prevalent in 31.6% of the camels studied. A significant association ($p-< 0.05$) was found between sub-clinical mastitis and age groups. Sub-clinical mastitis and udder lesions were also found to be significantly associated ($p-< 0.05$). The presence of sub-clinical mastitis was not significantly

associated with udder conformational disorders, tick infested udders and lactation stage classes. Out of a total number of 34 herds, 25 herds (73.5%) presented at least one camel with one quarter milk sample positive for CMT. Associations of practices of cauterization for treatment of udder problems, use of anti-suckling equipment and unhygienic practices with herd level sub-clinical mastitis positivity were not found. The questionnaire survey results showed that clinical mastitis seemed to be rare in the study area; only 4 owners (11.8%) confirmed the presence of mastitis in their herds. Twenty two respondents (64.7%) attributed the cause of mastitis in camels to tick infestation. Cleaning of the udder before milking was seldom done, only 3 owners (8.8%) cleaned the udder. However, 31 owners (91.2%) washed their hands before milking. Through direct observation and interviews information was gathered to analyze the camel milk markets network in Babille Woreda and neighboring towns The nodes included all 22 PAs within Babille Woreda and 5 small and big towns to which the milk was sold; arcs represented milk trade relationship among PAs and of PAs and towns. A network with 27 nodes and 33 edges (relationships) was identified and analyzed. The network consisted of 3 components: one large component and two other components with 24, 2 and 1 nodes, respectively. The overall density of the milk market network was 0.047, indicating a very loosely connected network with an average degree of 1.2. Babille, Harer, and Jijiga scored high in-degree values indicating their role as terminal markets. Aw-Sherif having the highest degree centrality score had a very important and influential position in the network. There was substantial centralization in this whole network as the network centralization for out-degree and in-degree were 15.1% and 19.1%, respectively. The network closeness centrality measures revealed that Harer had the highest incloseness score followed by Jijiga and Dire Dawa. The majority of the PAs had lower scores of closeness centrality measures as they were peripherally located. Actor betweenness measures ranged from zero to 20 with wide variation. Aw-Sherif, Erer Ibada and Gemechu appeared to be more powerful than others by this measure followed by Tula and Erer Guda and others scoring zero. This study has shown that camel brucellosis and sub-clinical mastitis are prevalent in the area. Both diseases can cause extensive economic losses in camel production and pose a public health hazard as a result of raw camel milk consumption and traditional husbandry practices. This is warranting control and prevention programmes and public health education. Such undertakings should be based on further in-depth epidemiological investigations. Network analysis of milk markets can help in policy formulations,

evaluation of risks, estimation of potential spread of infectious agents and control as well as in designing and implementing risk-based surveillance and milk market development activities.

Keywords: Dromedary camels, brucellosis, mastitis, network analysis, milk markets, Ethiopia

1. INTRODUCTION AND OBJECTIVES

According to FAO statistics (2009) there are more than 25.3 million camels in the world, of which 21.5 million are found in Africa. Somalia with 7 million heads has the largest herd in the world followed by Sudan with 4.5 million. Ethiopia has an estimated 2.5 million camels, which are mainly distributed in arid and semi-arid areas of the country (CSA, 2007).

The dromedary camel is a multipurpose animal adapted to the harsh environments of semi-arid and arid zones, mainly kept for milk and meat production and transportation. It also constitutes a financial reserve assisting in drought-prone risk management for pastoralists and plays an important role in social prestige and wealth (Woubit *et al.*, 2001).

Camel milk is one of the most valuable food resources for pastoral people in arid and semi-arid areas. In the last years milk consumption among urban population was increasing (Younan, 2004). As a result camel milk marketing becomes a rich source of income for pastoralists (FAO, 2006). Current indications are that the commercialization of camel milk in Somalia and in the Somali region and adjacent areas of Oromiya Region of Ethiopia is expanding and increasingly represents a vital asset to ensure food security and promote the socio-economic development of a larger number of pastoral households (Farah *et al.*, 2007; ECRA, 2008). An increasing trend of raw camel milk export has been observed from Ethiopia (ERCA, 2008).

However, the zoonotic risk of camel milk should be considered as camel milk is usually consumed raw and is marketed through an informal market (Radwan *et al.*, 1992; Younan, 2004). Reports of mastitis in the camel have increased tremendously during the past decade (Younan, 2004). Cases of mastitis in camels have been reported from almost all camel keeping countries in Africa and in Asia.

Camels are not known to be primary hosts for any *Brucella* organisms, although they are susceptible to both *B. abortus* and *B. melitensis* (Musa and Shigidi, 2001). The presence of brucellosis in camels pose an important health threat to consumers of

marketed fresh camel milk (Younan, 2004); camel brucellosis was also reported from Ethiopia (Bekele, 2010; Teshome *et al.*, 2003; Teshome and Molla, 2002), Somaliland (Yassir *et al.*, 2009) and Central Somalia (Baumann and Zessin, 1992).

In the near future there will be an increasing demand for clean milk from consumers and by regulatory food safety agencies (Younan, 2004). Thus, detailed knowledge of such diseases is a prerequisite to design control measures and safeguard the health of the public. This study is to determine the prevalence of camel brucellosis and mastitis and to identify the risk factors associated in selected areas where camel milk production for sale to urban centers is flourishing.

Understanding the structure and function of networks, in both business-to-business and consumer markets is essential to marketing (Webster and Morrison, 2004). Furthermore, network studies would help in policy formulations, evaluation of risks, estimation of potential spread of infectious agents and its control as well as in designing and implementing risk-based surveillance activities (Kao *et al.*, 2006; Baptista *et al.*, 2008; Aznar *et al.*, 2011). Analyses of the network structures are expected to provide an opportunity to identify targets for interventions such as control procedures and surveillance (Aznar *et al.*, 2011).

Therefore, the **objectives** of the study are:

- to determine the prevalence of brucellosis and of clinical and sub-clinical mastitis in camels and to assess some of the risk factors that affect the occurrence of these diseases in Babille Woreda and

- to identify camel milk flow, to characterize the pattern of camel milk markets and to analyze network structures and connections found between milk markets in PAs and towns.

2. LITERATURE REVIEW

2.1. Domestication of Camels in the Horn of Africa

The genus *Camelus* has two species; the dromedary or Arabian camel (one-humped) and the Bactrian camel (two-humped) (Al-Eknah, 2000). The one-humped camel was domesticated about 3000 B.C. in Southern Arabia, mainly for its meat and milk (Valérie, 2007). Camels originated in North America when the land masses were still joined and the animals migrated to other parts of the world, finally disappearing from their original area (Leuner, 1963 in Valérie, 2007).

Some of the camels migrated to the deserts and semi-deserts of Northern Africa and the Middle East. Remains of camels have been found in old Palestine, dating to 1800 B.C. Further migration of camels in Africa was prevented by their susceptibility to tsetse-borne trypanosomosis (Field, 1979 in Valérie, 2007). In Ethiopia, the camel seems more likely to have come across the Red Sea from Arabia. Camels have been attested in Northern Ethiopia from about 1000 B.C onward (Henze, 2000).

2.2. Camel population in Africa

According to FAO (2009) statistics there are a little more than 25.3 million camels in the world, of which 21.5 million are found in Africa. Approximately, more than 14.8 million dromedaries, representing 58.6% of the world's camel population, are found in the arid areas of North East Africa, i.e. Somalia, Sudan, Ethiopia and Kenya (Table 1).

Table 1: Estimated camel populations in Africa (FAOSTAT Database 2009)

Country	Camel population	Country	Camel population
Algeria	295,000	Libya	50,000
Chad	1,391,045	Mauritania	1,495,000
Djibouti	70,000	Morocco	50,000
Egypt	110,000	Somalia	7,000,000
Ethiopia	2,400,000	Sudan	4,521,000
Kenya	947,000	Tunisia	235,000

2.3. Camel breeds and distribution in the Horn of Africa

Camel breeds are not as differentiated and classified as breeds in other livestock. Systematic selection for productive traits has never been done in camels, except for racing animals (Kappler, 1998 in Valérie, 2007). In most camel rearing societies, breed classifications are based on names of the ethnic group, clan as well as on the geographical localities (Farah, 2004). Wardeh (2004) suggested a new classification system on the basis of production performance as meat, dairy, dual purpose and race animals.

Attempts to classify Ethiopian camels have not been satisfactory so far. Wilson (1984) classified and described the major camel breeds in the country as the Afar, Borena, Anfi and Somali/Ogaden breeds.

Somali camels cross the whole length of the frontier with Somalia and Kenya in the South-East and Borena camels extend into Northern Kenya in the South-Central area (MARD, 2007b). The Somali camels are of three different types: Horr, Sifdarh and Gellab (MARD, 2007a).

Hoor camels are the most numerous and are adapted to the more arid regions of Somalia. They are characterized by their small size, short legs and white color. They

mature between 3-5 years of age, but practically breed at 5-6 years and are dairy camels (Wardeh, 2004).

Sifdar camels are found in South-Central Somalia. They are tall, light, and grey to reddish in color and reach maturity at 5-6 years, but breed at 6-7 years (Wardeh, 2004).

The Gellab type is known for its multipurpose use and is said to be more resistant to drought and feed shortage. It has a short gestation period and provides tastier milk at shorter intervals (3.61/day) (MARD, 2007a).

2.4. Importance of the camel in the Horn of Africa

The dromedary camel is a multipurpose animal adapted to the harsh environments of semi-arid and arid zones, mainly kept for milk and meat production and transportation. It also constitutes a financial reserve assisting in drought-prone risk management for pastoralists and plays an important role in social prestige and wealth (Woubit *et al.*, 2001). Because of increasing desertification and recurrence of drought and famine in Sub-Saharan Africa, particularly in East Africa, the camel plays a very significant role as a source of milk, meat and draft power (Woubit *et al.*, 2001).

Camel milk is one of the most valuable food resources for nomads in arid regions and can contribute to a better income for pastoralists, especially as in the last years milk consumption among the urban population is increasing (Farah, 2004). The fact that milk is mainly consumed in its raw state, the high ambient temperature and the lack of refrigeration facilities in many arid areas are the main reasons for hygienic problems (Radwan *et al.*, 1992). The annual camel milk production in Ethiopia is estimated to be at 75,000 tons (Felleke, 2003). According to FAO (2009) Ethiopia produced about 194,000 tons annually and Somalia produces the largest overall share, amounting to 870,000 tons a year.

Camel meat, however, is an important by-product mainly as a source of income. Sale of live camels, usually males and unproductive females for slaughter, is very common in

East Africa and there are now increasing numbers of camel butcheries in many urban centers. There is also a growing export trade of slaughter camels to the Arabian Peninsula (Farah, 2004). Camel meat is regarded as high-quality food with medicinal value and as a least cost source of meat. Camel meat is preferred to that of any other livestock by Somalis: it is also available, especially during the dry season when beef is in short supply (Kurtu, 2004). In Ethiopia, the total camel meat production in 2005 was estimated at 4,560 tons (MARD, 2007b). FAO (2009) estimated a relatively higher meat production in Ethiopia which is about 12,000 tons in 2009 showing an increasing trend.

2.5. Infectious diseases of food safety importance

2.5.1. Camel brucellosis

Brucellosis is a zoonotic disease occurring in humans and various species of domesticated and wild animals. Initial infection in the reservoir species is often followed by abortion and subsequent delayed or permanent infertility. Infected animals shed the organisms in uterine discharges following abortion and subsequent parturition and also in milk (including the colostrum) (FAO, 2003). The disease represents a public health hazard, also poses a barrier to trade of animals and animal products and is, thus, an impediment to free animal movement (WHO, 2006).

Brucella abortus antibodies in infected camels were reported from a number of countries (Table 2 and 3).

Table 2: Seroprevalences of camel brucellosis and types of *Brucella* antigen used for diagnosis in different camel rearing countries

Country	**Seroprevalence**	***Brucella* antigen used**	**Author(s) and year**
Oman	8%	not described	Ismaily, *et al.*, 1988
Saudi Arabia	8%	not described	Radwan, *et al.*, 1995
Sudan	7.8%	*B. abortus*	Musa and Shigidi, 2001

	37.5%	not described	Omer *et al.*, 2010b
Egypt	10.7%	not described	Barsowm *et al.*, 1995
Jordan	14%	not described	Dawood, 2008
United Arab Emirates	0.1%	*B. abortus*	Moustafa *et al.*, 1998
Iran*	1.93%	*B. abortus*	Khadjeh *et al.*, 1999

*Incidence rate

Camel brucellosis seroprevalence rates were also reported from Ethiopia, Somaliland and Central Somalia (Table 3.).

Table 3: Seroprevalences of camel brucellosis in Eastern Africa.

Country	**Seroprevalence**	**Type of *Brucella* antigen used**	**Author(s) and year**
Ethiopia	2%	*B. abortus*	Bekele, 2010
	2.8%	*B. abortus*	Teshome *et al.*, 2003
	5.9%	*B. abortus*	Teshome and Molla, 2002
Kenya	14%	not described	Waghela *et al.*, 1978
Somaliland	3.1%	*B. abortus*	Yassir *et al.*, 2009
Central Somalia	0.3%	*B. abortus* *B. melitensis*	Baumann and Zessin, 1992

Etiology of camel brucellosis

The three species of *Brucella* of major concern are: *Brucella abortus* (biovars 1–6), affecting primarily cattle, other bovidae and cervidae; *Brucella suis* (biovars 1–5), affecting primarily swine; and *Brucella melitensis* (biovars 1–3), affecting primarily sheep and goats. All the above *Brucella* species are not host-specific, and may be transmitted to other animal species under appropriate conditions (FAO, 2003).

Brucella melitensis biotype 3 was isolated from four aborted camel fetuses in Jordan (Al-Majali *et al.*, 2008) and in Saudi Arabia (Omar *et al.*, 2010a). In Libya and Iran, *Brucella melitensis* biovar 1 was identified from camels (Gameel *et al.*, 1993; Khadjeh

et al., 1999). Brucellosis in camels in Saudi Arabia (Radwan *et al.*, 1992; Radwan *et al.*, 1995) and Oman (Ismaily *et al.*, 1988) was linked to *Brucella melitensis.* In Sudan, *Brucella abortus* biovar 6 was isolated from camels (Omer *et al.*, 2010b).

Risk factors for camel brucellosis

Camels are not known to be primary hosts for any of the *Brucella* organisms, but they are susceptible to both *B. abortus* and *B. melitensis* (Musa and Shigidi, 2001). As camels and goats are often herded together it is assumed that camels become infected from goats and, eventually, *Brucella* organisms are excreted in the milk of camels with subsequent human infections (Ismaily *et al.*, 1988).

On both herd level and individual camel level, larger herd size and contact with small ruminants were identified as risk factors for camel brucellosis (Al-Majali *et al.*, 2008). At the herd level, use of disinfectants was identified as a protective factor (Al-Majali *et al.*, 2008). Locality, herd size, rearing with other ruminants and contact with other camels were considered as risk factors in Somaliland (Yassir *et al.*, 2009). Keeping camels with more than two species was reported to be a risk factor in Ethiopia (Megersa, 2010). The lack of adequate sheep and goat *Brucella* control programmes was reported to increase the prevalence of *Brucella* in camels in mixed herds (Al-Majali *et al.*, 2008).

Diagnosis of camel brucellosis

Brucellosis is a sub-acute or chronic disease affecting many species of animals. In cattle, sheep, goats, other ruminants and pigs the initial phase following infection is often not apparent. In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy, and epididymitis and orchitis in the male. Clinical signs are not pathognomonic and diagnosis is dependent upon demonstration of the presence of *Brucella* species either by isolation of the bacteria or detection of their antigens or genetic material, or by demonstration of specific antibodies or cell-mediated immune responses (WHO, 2006).

Presumptive evidence of *Brucella* is based upon demonstrating the *Brucella* organism morphology by modified acid-fast staining of abortion material, aborted fetuses, vaginal/ uterine discharges, udder secretions or selected tissues, such as lymph nodes and/or male and female reproductive organs. *Brucella* species are to be isolated in culture using plain or selective media. Species and biovars are identified by phage lysis, and by cultural, biochemical and serological criteria. Polymerase chain reaction methods provide additional means of detection (OIE, 2009).

Serological tests such as the buffered *Brucella* antigen tests, i.e. Rose-Bengal Plate Test (RBPT) and Buffered Plate Agglutination Test, the Complement Fixation Test (CFT), the Enzyme-linked Immunosorbent Assay (ELISA) or the Fluorescence Polarization Assay (FPA), are suitable tests for screening herds and individual animal. The indirect ELISA or milk ring test performed on bulk milk samples are effective for screening and monitoring dairy cattle for brucellosis, but the milk ring test is not suitable for camel milk. Another immunological test is the brucellin skin test, which can be used as a screening or as a confirmatory herd test when positive serological reactors occur in the absence of obvious risk factors in unvaccinated herds (OIE, 2009).

Teshome *et al.* (2003) and Megersa *et al.* (2010) reported camel brucellosis from Ethiopia by using RBPT and CFT. SAT, RBPT and CFT were used in a cross-sectional study of brucellosis in Sudan (Musa and Shigidi, 2001). In Central Somalia, SAT and CFT were used to study camel brucellosis (Baumann and Zessin, 1992) and the seroprevalence of brucellosis was determined by RBPT and I-ELISA in Somaliland (Yasser *et al.*, 2009). A recent study in Sudan by Omer *et al.* (2010b) employed RBPT, modified RBPT (mRBPT), SAT, and competitive ELISA (cELISA) in a serological test evaluation.

2.5.2. Camel mastitis

Mastitis is defined as inflammation of the mammary gland and is associated with chemical, physical and most commonly bacteriological changes in the milk and pathological changes in the mammary tissue. Mastitis causes suffering for the animal, reduces milk production and poses public health risk. Mastitis also influences the

technological properties of milk. Reports of mastitis in the camel have increased tremendously during the past decade (Younan, 2004). Camel mastitis has been reported from a number of camel rearing countries (Table 4).

Table 4: Camel mastitis reports from various camel rearing countries

Country	**Authors**
Ethiopia	Abera *et al.*, 2010; Abdurahman, 2006; Abdel Gadir *et al.*, 2006; Mohammed *et al.*, 2005; Bekelle and Molla, 2001; Woubit *et al.*, 2001; Almaw and Molla, 2000
Iraq	Al-Ani and Al-Shareefi, 1998.
Israel	Guliye *et al.*, 2002
Jordan	Hawari and Hassawi, 2008
Pakistan	Fazlani *et al.*, 2009
Saudi Arabia	Agab, 2006; Barbour *et al.*, 1985
Somalia	Abdurahman *et al.*, 1991
Sudan	Obied *et al.*, 1996

Etiology of camel mastitis

Bacteria which can cause mastitis are present in air, water and other surfaces in the environment including human and animal skin. *Streptococcus agalactiae*, other *Streptococcus* species, *Staphylococcus aureus*, coagulase-negative *staphylococci*, and *Escherichia coli* are incriminated as the major bacterial causes of mastitis in the camel. There is evidence that *Streptococcus agalactiae* and *Staphylococcus aureus* are the two most important mastitis pathogens in camels (Younan, 2004). *Staphylococcus, Streptococcus, E. coli* and *Bacillus* species were found to be the major isolates in Eastern Ethiopia (Abera *et al.*, 2010) (Table 5).

Table 5: Pathogens isolated in cases of camel mastitis in different countries

Country	Author(s)	Mastitis Pathogens
Ethiopia	Abera *et al.*, 2010	*Staphylococcus, Streptococcus, E. coli* and *Bacillus*
	Abdurahman, 2006	*Staphylococcus aureus, Streptococcus agalactiae* and coagulase negative *staphylococci*
	AbdelGadir *et al.*, 2006	*Staphylococcus aureus, Staphylococcus hyicus, E.coli, Streptococcus uberis, Streptococcus epidermidis* and *Bacillus cereus*
	Woubit *et al.*, 2001	*Staphylococcus, Streptococcus, Micrococcus, Corynebacterium , Bacillus, Actinomyces pyogenes, E. coli* and *Pasteurella haemolytica.*
	Bekelle and Molla, 2001	*Staphylococcus aureus,* coagulase-negative *staphylococci, Streptococcus agalactiae, S. dysgalactiae,* and other species of *streptococci, Pasteurella haemolytica* and *E. coli*
Sudan	Suheir *et al.*, 2005	*Staphylococcus aureus, Streptococcus spp, Corynebacterium, Enterobacteria , Micrococcus spp. *Mycoplasma arginini, *Acholephasma laidlawii*
Kenya	Younan, 2002	*Streptococcus agalactiae*
Pakistan	Fazlani *et al.*, 2009	*Bacillus cereus, Corynebacterium pyogenes, Escherichia coli, Micrococcus luteus, Pasteurella haemolytica, Pasteurella multocida, Pseudomonas aeruginosa* and *Staphylococcus*
Jordan	Hawari and Hassawi, 2008	*Micrococcus spp, Staphylococcus aureus, Streptococcus spp* and *Corynebacterium spp.*
Saudi Arabia	Barbour *et al.*, 1985	*Micrococcus* species., *Staphylococcus aureus, Streptococcus* species and *Corynebacterium*

*Fungi and yeasts

Risk factors for camel mastitis

Mastitis is considered to be a multifactorial disease, closely related to the production system and the environment the animals are kept. Mastitis risk factors or disease determinants can be classified into three groups: host, pathogen and environmental (including management) determinants (Younan, 2004).

Abera *et al.* (2010) reported that tick infestations, udder lesions, and increased age and parity of the animals are predisposing factors for camel mastitis. Stage of lactation and parity were also reported as risk factors for the occurrence of mastitis in camel from Sudan (Suhir *et al.*, 2005). Heavy tick infestation, use of anti-suckling devices and thorny bushes in pastoral areas that might be responsible for udder deformities and abnormalities could be possible risk factors for camel mastitis. Trauma of the udder was also reported to be positively correlated with camel mastitis (Bekele and Molla, 2001). Younan (2004) considered that unhygienic and traumatic application of intramammary treatment could very likely do more harm than good.

Types of camel mastitis

Mastitis in camels can be clinical (peracute, acute, and chronic) or sub-clinical. As in other dairy animals, clinical mastitis in camel is self-evident and can be detected without special tests by careful examination of the udder and the milk (Abera *et al.*, 2010). There are changes in color, consistency, floccules etc. of the secreted milk and/or the udder (red, swollen) and other generalized signs exhibited by the animal (fever, anorexia, deteriorating body condition) (Woubit *et al.*, 2001; Younan, 2004).

Mastitis with visible signs of inflammation includes acute and edematous swelling of the udder and usually formation of pus in the mammary exudates resulting in a visible alteration of the milk was reported from Jordan (Hawari and Hassawi, 2008). Clinical forms of mastitis were reported from Ethiopia by Abdel Gadir (2001), Woubit *et al.* (2001), Abdurahman (2006), Abera *et al.* (2010) and Eyassu and Bekele (2010). It was also reported from other countries such as Pakistan (Ahmed *et al.*, 2011), Saudi Arabia (Barbour *et al.*, 1985; Ibrahim *et al.*, 2011) and Jordan (Hawari and Hassawi, 2008).

Detecting sub-clinical mastitis is, however, difficult and depends on various test procedures aiming at identifying the etiology by detecting indicators of inflammation in the milk. These include microbial examination, Somatic Cell Count (SCC), California Mastitis Test (CMT), Adenosine Triphosphate (ATP), N-acetyl-D-glucosaminidase (NAGase) and Serum Albumin determination (Abdurahman, 1995; Guliye *et al.*, 2002).

Sub-clinical mastitis in camels was reported from Ethiopia (Abdurahman, 2006; Abdel Gadir *et al.*, 2006; Abera *et al.*, 2010), Sudan (Suheir *et al.*, 2005) and Jordan (Hawari and Hassawi, 2008). Abera *et al.* (2010) reported a prevalence of 29.0% at animal level. At quarter level prevalences of 28.8% (Woubit *et al.*, 2001) and 17.9% (Abera *et al.*, 2010) were recorded in Ethiopia. Abdel Gadir *et al.* (2006) reported quarter level prevalence of 46.2%, 45.4% and 37.1% of sub-clinical camel mastitis at three localities in Ethiopia.

Diagnosis of camel mastitis

Clinical mastitis is self-evident and can be detected without special tests (Younan, 2004). On the other hand, diagnosis of sub-clinical mastitis requires diagnostic tools and tests as described above. Mastitis indicator paper that detects changes in pH of milk and bacteriological isolation was used to determine sub-clinical mastitis in Ethiopia by Abera *et al.* (2010) and associations of CMT and SCC scores and bacteriological isolation were assessed by Woubit *et al.* (2001) and Abdurahman (2006).

A study of sub-clinical mastitis using CMT and bacteriological examination was carried out to assess the appropriateness of CMT as diagnostic tool (Woubit *et al.*, 2001; Suheir *et al.*, 2005). Woubit *et al.* (2001) reported an agreement of 100% for CMT scores 3+ and 2+ and bacteriological results but only 35%, 71% and 85% agreements for CMT scores of 0, trace and 1+, respectively. However, Abdurahman (2006) reported that 82.7 % of bacteriologically negative samples had CMT score 1 and 80% of bacteria positive milk samples had CMT score 2 or more. In another study, 60.9% of CMT positive cases were associated with major pathogens and 27.8% with

minor pathogens; however, 11.28 % of CMT positive cases were from non-infected quarter milk samples (Abdel Gadir, 2001).

2.6. Network analysis (NA)

Network theory is a very general term that refers to the study of relationships. It can refer to relationships among people, chemicals, atoms, animals, organizations, companies, or countries (Gretzel, 2001). Network analysis (NA) tries to determine how structural properties affect behaviour. Thus, NA concentrates on studying how the patterns of ties in a network provide significant opportunities and constraints because it affects the access of people and institutions to such resources as information, wealth and power as relational ties are channels for transfer of resources, material or nonmaterial (Wellman, 1993).

Network theories have been applied to a wide range of marketing issues, including, communication, trade relationships, agriculture, industry, and information flow (Webster and Morrison, 2004; Mueller *et al.*, 2007).

Studies have been published applying social network theories to the study of animal movement patterns in various countries, such as Portugal (Baptista *et al.*, 2008), Argentina (Aznar *et al.*, 2011), Great Britain (Kao *et al.*, 2006) and Germany where they use an advanced animal movement database to monitor livestock movements (Lentz, pers. commu). The authors recommended that such studies would help in policy formulations, evaluation of risks, estimation of potential spread of infectious agents and control as well as in designing and implementing risk-based surveillance activities.

2.6.1. Network elements

A network, also called a graph in the mathematical literature, is made up of points, usually called nodes, actors or vertices, and lines connecting them, usually called edges

or relational ties (Webster and Morrison, 2004; Mueller *et al.*, 2007). Relational ties link network members (Figure 1). These linkages may differ in direction (symmetric or asymmetric), valence (positive, neutral or negative), strength (weak, moderate or strong), and content (resource sharing) (Webster and Morrison, 2004).

2.6.2. Network data

Relational data matrix

A standard network dataset includes a set of informants or actors (the rows of the matrix) and their responses to a set of questions about their attributes in regards to specific issues (the columns of the matrix). The result is an *actor-by-attribute matrix*. A relational dataset in network analysis also includes a set of informants (the rows of the matrix), but with their responses to a set of questions about their relationships to specific actors to get an *actor-by actor matrix* (Webster and Morrison, 2004).

The most common form of a matrix in social NA is a very simple square matrix with as many rows and columns as there are actors in our data set. The "elements" or scores in the cells of the matrix record information about the ties between each pair of actors. The simplest and most common matrix is binary. That is, if a tie is present, a "1" is entered in a cell and if there is no tie, a "0" is entered (Hanneman and Riddle, 2005). For relationships in which a *valued* information has been obtained (strength, duration, intimacy) a real number is entered (Webster and Morrison, 2004).

Data collection for network analysis

Defining which nodes to include in a NA is the challenge at the beginning. The boundaries of the populations studied by NA are of two main types, either imposed or created by the actors themselves or naturally occurring clusters. So, in a sense, social network studies often draw the boundaries around a population that is known, a priori,

to be a network. This implies there is an assumption of the existence of network in the study population. Alternatively, a network analyst may take a more "demographic" or "ecological" approach to defining population boundaries. Researchers collecting network data must first decide what kinds of networks and what kinds of relations they will study (Hanneman and Riddle, 2005; Marin and Wellman, 2009).

Data collection also depends on the focus of the research. When the goal is to map a complete network consisting of the relational ties among members of a single bounded community, it is referred to as a *socio-centric* study. Relevant relational data are obtained for each actor in the network. *Ego-centric* or *personal networks* are centered on a focal actor (ego) and ego's relationships to a set of others (ego's alters), plus the relationships among these alters (Baptista *et al.*, 2008). After identifying network members, information on the inter-relationships among network members is required to investigate structural issues. There are four broad categories of relations: similarities, social relations, interactions, and flows (Marin and Wellman, 2009).

All of the standard data collection techniques of surveys, interviews, observations, experiments, and documentary analysis can be used to obtain network data (Webster and Morrison, 2004). In surveys and interviews, a list of network members should be prepared and members are asked to indicate actors they share ties. When making a list is not practical, respondents are asked to make a list by recalling the people with whom they share the relevant relations. Network data can also be collected through observation from archives and historical materials (Marin and Wellman, 2009) and from electronic databases (Baptista *et al.*, 2008; Aznar *et al.*, 2011).

2.6.3. Network data analysis

Graphs

Among the major advantages of NA is its use of the graph theory. Graphs allow for a visual representation of the structural and relational positions of network members. NA

uses (primarily) one kind of graphic display that consists of points (or nodes) to represent actors and lines (or edges) to represent ties or relations. When a relation is mutual, the ties between actors are shown with arrows headed at both ends. A relation that is headed by a single arrow indicates a tie directed from one actor to another (Webster and Morrison, 2004; Hanneman and Riddle, 2005). Figure 1 illustrates simple directed binary graph of four nodes.

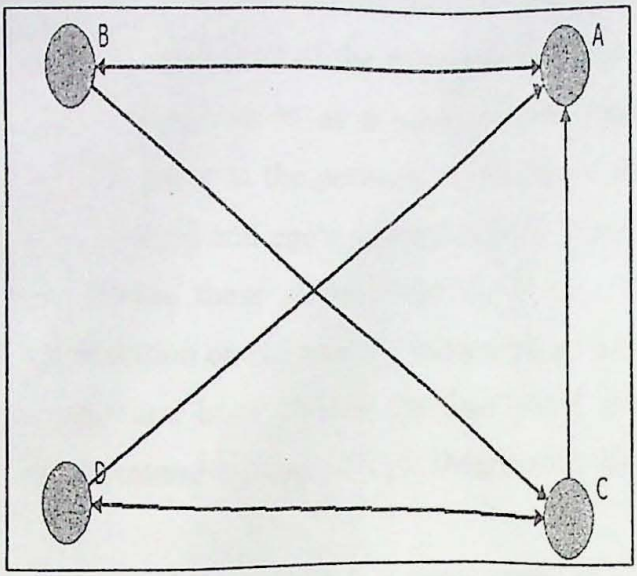


Figure 1: Nodes and directed relationships of a simple network graph

In this figure, A, B, C and D represent actors or nodes and the arrowed lines indicate the directed relationships that exist between nodes.

Quantitative measurements

There are more than 50 network measurements (Webster and Morrison, 2004). The following measures are relevant to this study.

Basic demographics, cohesion and clustering

Network size: The size of a network is often very important. Size is critical for the structure of social relations because of the limited resources and capacities that each

actor has for building and maintaining ties. Usually the size of a network is indexed simply by counting the number of nodes (Hanneman and Riddle, 2005).

Density: The density of a network (the number of ties present in a network divided by the number of possible ties) is a concept relating to the network as a whole. The value ranges between 0 and 1, with higher values indicating denser networks, and lower values indicating sparse networks (Webster and Morrison, 2004).

Actor degree: this is the number of connections an actor has. The distribution of how connected individual actors are can tell a good bit about the social structure (Hanneman and Riddle, 2005).

Components: components are sets of nodes that are tied directly or indirectly to one another but are not tied directly to nodes in other components (Webster and Morrison, 2004).

Position or Role

Degree centrality: It can be calculated for both ties that are incoming, the total number of ties received, and outgoing, total number of ties reported (Webster and Morrison, 2004). The node that has the most direct connections is the most active node in the network and can also be called a "hub". Identifying hubs may also reveal the critical nodes of the network, i.e., the most important points for disease spread and control (Baptista *et al.*, 2008).

Betweenness centrality: With binary data, *betweenness centrality* views an actor as being in a favored position to the extent that the actor falls on the geodesic paths between other pairs of actors in the network. It is a measure of a node's centrality in a network equal to the number of shortest paths from all vertices to all others that pass through that node (Hanneman and Riddle, 2005). It measures of the extent that a network member's position falls on the geodesic paths between other members of a network (Marin and Wellman, 2009). A node with high betweenness has great influence over what flows within the network, because it connects important constituents (Baptista *et al.*, 2008).

Closeness centrality: It reveals the nodes that have the shortest paths to all others, indicating how quick a node can access other nodes in the network (Baptista *et al.*, 2008). Unlike *degree centrality*, it considers both an actor's direct and indirect ties. An actor with a high closeness score is able to get in contact quickly with many others. Also, actors with high closeness scores can be efficient at communicating information or their opinions throughout the network, and may be less dependent on others for the passing on of information (Marin and Wellman, 2009).

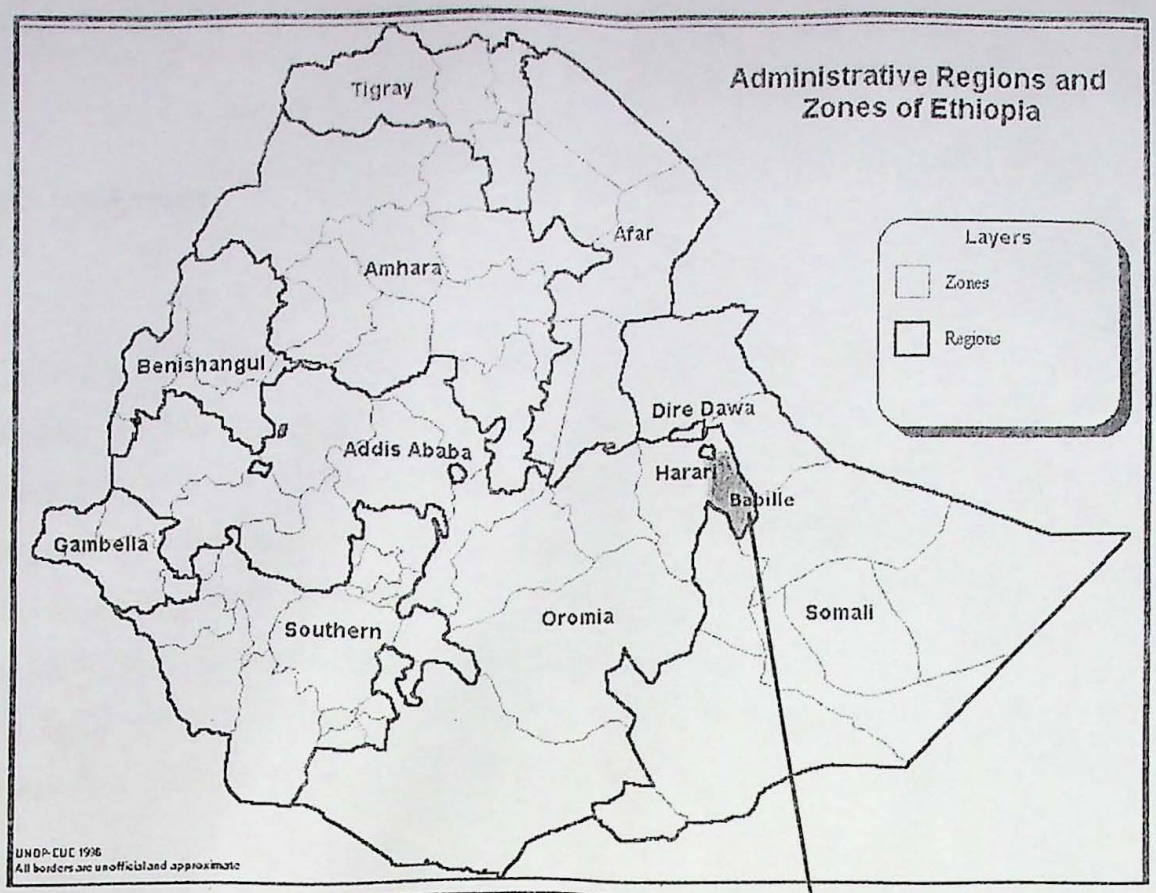
3. MATERIALS AND METHODS

3.1. Study area

This study was conducted in Babille Woreda of the Oromiya Regional State of Ethiopia. Babille is found in Eastern Ethiopia in the Eastern Harargae Zone, at 553 km South-East of Addis Ababa, and has a latitude range of 8° 09' and 9° 23' North and longitude of 42°15' and 42° 53' East. The total land area of the Woreda is 3,500km^2. The altitude varies between 950 and 2000 meters above sea level (asl) and the terrain consists of mainly plains, 90% of the surface, and the rest are isolate hills, with very few forest areas (woodland savannah, bushes and shrub grassland). The temperature ranges from 16 to 26°c with an average annual rainfall of 671mm (BWAB, 2011).

According to CSA (2007) the total population of the Babille Woreda is estimated to be 93,674. Out of this, 81.1% live in rural set-ups while the remaining 18.9% live in Babille town, the Woreda seat. The Woreda is divided into 21 peasant associations (PA) and one urban area (Babille town).

Babille town is located at the intersection of Oromiya (ORS), Somali (SRS) and Hareri (HRS) Regional States, along the surfaced road connecting both cities. The population is engaged in crop production and livestock husbandry. In the Babille Valley agro-pastoralists reside as well as pastoralists who keep their camels in the valley all year round. Milk from camels of the surrounding areas is collected and marketed to Harer, Jijiga, Dire Dawa, and Awedi and sometimes to Addis Ababa and to Hargessa. Babille is said to offer a potential for camel milk traded to Eastern and Central Ethiopian markets (CSA, 2005).



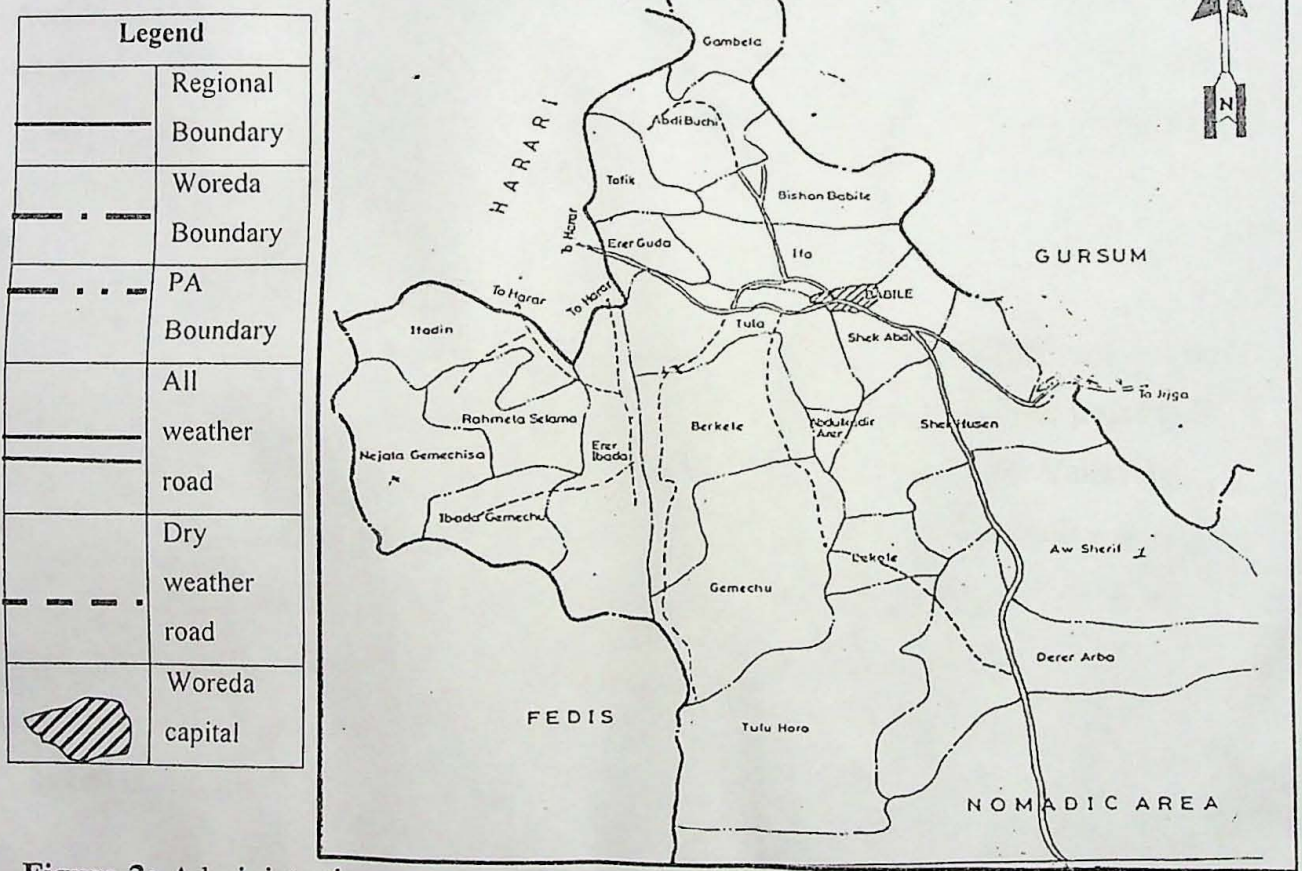


Figure 2: Administrative regions and zones of Ethiopia, depicting Babille Woreda in East Hararge Zone (UN-EUE, 2011) and PAs in Babille Woreda (BWAB, 2011).

3.2. Study design

A cross-sectional survey approach was employed to determine the prevalence of brucellosis and clinical and sub-clinical mastitis in camels in the study area. The same cross-sectional study method was also used to assess the camel milk markets network. All the samples were taken from August to October, 2011.

3.2.1. Camel brucellosis

Study population and sampling method

The study animals for the brucellosis were traditionally managed camels of different herds in purposively selected 12 accessible peasant associations (PAs).

To determine the seroprevalence of brucellosis, a two-stage cluster sampling method was applied as the sample size is fixed (Thrusfield, 2007). The primary sampling units (PSUs) were selected herds in the purposively selected 12 peasant associations, and the second stage sampling units (SSUs) were randomly selected camels within these selected herds. A herd was defined as a collection of livestock that was owned by an individual or a group and kept together sharing the same rangeland and water resources.

Sample size determination

The required sample size was determined at 95% confidence level using the following formula (Thrusfield, 2007).

Equation 1: $$n = \frac{1.96^2\ Pexp\ (1-Pexp)}{d^2}$$

Where n is required sample size, $Pexp$ expected prevalence, and d desired absolute precision.

Determination of the expected prevalence depended on previous reports of camel brucellosis from Ethiopia. Prevalences of 2.2% (Megersa *et al.* 2010), 4.2% (Teshome

et al. 2003) and 5.9% (Teshome and Molla, 2002) were reported from various parts of Ethiopia. Based on these an average prevalence of 4.1% was obtained as expected prevalence (*Pexp*). With desired absolute precision (*d*) of 2%, the required sample size was 378 camels.

Then, the number of clusters to be sampled when the total sample size is fixed was calculated using the formula below (Thrusfield, 2007). Note that, in this study the term cluster referred to herds.

Equation:2: $$g = \frac{1.96^2 TVc}{d^2 T - 1.96^2 Pexp\,(1-Pexp)}$$

Where g are number of clusters to be sampled, *Pexp* expected prevalence, *T* total number of animals to be sampled, *Vc* between-cluster variance, and *d* desired absolute precision.

This formula required determination of the between-cluster variance. However, this estimation again requires previous cluster sample data which were not available for this study. Alternatively, the between-cluster variance component can be guessed in such that the standard deviation (i.e., the average difference expected between individual cluster prevalence and the overall mean cluster prevalence) is guessed and then squared to give the between-cluster variance component (Thrusfield, 2007). As brucellosis occurs usually at low prevalence rates (below 5%) in nomadic or extensively kept camels (Abbas and Agab, 2002) a very low, 0.1%, deviation was estimated.

Thus, at a fixed sample size of 378 animals the number of herds to be sampled, assuming the between-cluster variance, *Vc*, of $(0.001)^2$, with required absolute precision of 2%, would be 10 herds.

3.2.2. Camel mastitis

Study population and sampling method

Lactating camels from 5 purposively selected PAs were sampled for clinical and sub-clinical mastitis prevalence determination. A similar two-stage stratified cluster sampling method was applied as described for the brucellosis study.

Sample size determination

The number of quarters from lactating camels to be sampled was calculated based on an expected prevalence (*Pexp*) of 19.6%, being the average prevalence calculated from various prevalence rates that have been reported from Ethiopia by different researchers: 17.9% by Abera *et al.* (2010), 28.8% by Woubit *et al.* (2001) and 12.1% by Bekele and Molla (2001) at 95% confidence level and a desired absolute precision of 5% using the formula (Equation 1) stated in Thrusfield (2007). The required sample size was 242 quarter milk samples. In order to increase precision the calculated sample size was increased to a little above 2 fold to 544 quarter milk samples (Thrusfield, 2007). This could allow prevalence estimation at individual camel level. Thus, milk samples were collected from 136 lactating camels based on accessibility and owners willingness.

As no information was available from cluster sample data of previous reports calculation of the between-cluster variance (*Vc*) was not possible. However, with the anticipated prevalence of 19.6% the average difference between this and the individual-cluster prevalence was guessed to be 0.12. Hence, the between-cluster variance (*Vc*) was estimated at 0.0144 and the number of clusters to be sampled was 33. In order to make prevalence estimation at individual level 34 clusters (herds) were selected and from each herd 16 quarters were sampled which were equivalent to 4 camels per herd.

3.2.3. Camel milk markets network

There is an assumption that there exists a network of camel milk markets in Babille Woreda, which is exclusively informal. The informal market involved direct delivery of fresh camel and cow milk by producers to consumer in the immediate neighborhood of PAs and sale to collectors or traders or consumers in nearby PAs, and small and big towns. In the informal market, milk is traded from producers to consumers directly but also passes through two or more market agents.

The flow of milk from each PA to each other PA, and big towns within Babille Woreda and outside Babille Woreda was observed to assess the milk markets network structure. This is expected to shed light on the inter-PA and PAs and towns interactions as well.

Nodes: The population in this study was all informal camel milk markets in PAs within Babille Woreda. The sampling frame included all areas that produce camel milk and the areas where the camel milk flows to either for consumption or reselling or for sale at terminal markets. The nodes were all PAs within Babille Woreda and small and big towns to which the milk was sold. The nodes were on the milk value chain line starting from producers to consumers.

Relationships: Milk produced in one PA was traded to another PA and/or to small or big towns. The PAs were mainly milk suppliers for the towns. The relationship studied was the simple presence or absence of a milk trade relationship among PAs and between PAs and towns.

3.3. Data collection

3.3.1. Camel brucellosis

Blood sample collection and laboratory tests: Blood samples of about 10 ml were aseptically collected using plain tubes from camels older than 6 months of age through jugular venipuncture. Sera were separated within 12 hours of collection and transported in an ice box to be stored at −20°C. The sera were then transported to the National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia, for testing with RBPT and positive sera samples were further tested by CFT at the National Veterinary Institute, Debre Zeit, Ethiopia. A total number of 378 sera was collected and 370 sera were tested as 8 were lost during handling. Information on potential risk factors was gathered during blood sampling by means of a questionnaire (Appendix 1).

Rose-Bengal Plate Agglutination Test (RBPT): All sera (370) were screened for antibodies against *Brucella abortus* by RBPT according to the procedures described by OIE (2009) (Appendix 4). The test system used was manufactured by Lillidale Diagnostics (Dorset, UK) and a bovine positive serum (*B. abortus* PM 248) which was manufactured by Central Veterinary Laboratory, UK, was used for validation of the test.

Complement Fixation Test (CFT): All RBPT positive serum samples were further tested using CFT as described in OIE (2009) (Appendix 5). Camels with both positive RBPT and CFT results were considered as *Brucella* seropositive camels.

Questionnaire survey: Data for each camel sampled included: location, owner, sex, age, pathological conditions, history of abortions, breeding records, status of the camel at the time of sampling (suckling, weaned, adult, pregnant or repeat breeder), whether reared individually, with other species or in a herd, and herd information such as herd size, management system, history of abortion at herd level, management of abortion, restocking and access to veterinary services were gathered (Appendix 1). Gathering this information on all individual examined camels was difficult as some hired camel keepers did not have the required information or the information provided was unreliable as a result 40 herd owners in 6 PAs were selected and interviewed.

3.3.2. Camel mastitis

Milk sample collection: For the mastitis investigations the suckling calf was used to induce milk let-dawn. The teats were disinfected with cotton wool moistened with 70% ethyl alcohol. The first streaks of milk were discarded and about 10ml of milk was collected in a sterile universal bottle. Camels were milked early in the morning and then allowed to browse in nearby rangelands that made animal side CMT test for each camel difficult. As a result samples were kept in an ice-box and transported to the lab immediately and placed at -20^0 C before the application of CMT. CMT tests were done in the afternoon of the day of collection. Information on age, parity, and previous history of udder problem was collected during sampling (Appendix 2).

Clinical examination of the udder: In a clinical examination, udder abnormalities such as swelling, presence of lesions, ticks or blind teats or anatomical malformations were investigated. The size of the rear and forequarters, indurations and fibrosis were also examined by deep digital palpation. The milk was examined macroscopically for its consistency, color and other visible abnormalities (Appendix 2). An acute clinical mastitis was diagnosed when pathologic udder changes such as swelling, pain, redness and heat, and changes in milk color, presence of pus, inclusion of red blood cells, flakes

and clots were present. Pathological changes of the udder such as hardening of the udder, blockage of the teats, atrophy or fibrosis and abscess formation were considered as indicators of cases of chronic mastitis.

California Mastitis Test (CMT): CMT was carried out according to the methods outlined by the test manufacturer, PARK Scientific Limited, UK. About 3ml of CMT reagent and 3ml of camel milk were mixed and the reaction was graded and interpreted as Negative, Weak/Positive, Positive and Very Positive, hence, forming four categorical classes (Appendix 6).

Questionnaire survey: All 34 camel herd owners from whose herd's milk samples were collected were interviewed to assess their knowledge about causes of mastitis, its transmission, treatment, control measures applied and management practices (Appendix 3). Tick infestations and use of anti-suckling devices were recorded.

3.3.3. Camel milk markets network

A list of all PAs within Babille woreda and neighboring towns and cities was made. The methods applied include direct observation and interviews.

Direct observation: Milk markets in the PAs of Babille Woreda were visited to observe and assess milk catchment areas, mode of transport of products, role of producers and traders, and the supply routes.

Interviews: Milk producers, traders, transporters, and animal health experts were interviewed to generate facts regarding the camel milk trade routes and trade relationships among nodes.

3.4. Data management and statistical analysis

A Microsoft Excel (Windows 2007) spreadsheet was used for data entry. Data analysis was carried out using IBM SPSS statistics version-19 software. Descriptive statistics were obtained for all variables.

The *Brucella* seroprevalence was planned to be estimated by adjusting the apparent prevalence to the sensitivities and specificities of the two serological tests, CFT and RBPT, (in series) using the following formula:

$$\text{TP} = \frac{AP - (1 - sp1) - (1 - sp2)}{se1se2 - (1 - sp1) - (1 - sp2)}$$

Where TP is the true prevalence, AP is the apparent prevalence, $sp1$ and $sp2$ are RBPT and CFT tests specificities, respectively, and $se1$ and $se2$ are RBPT and CFT sensitivities, respectively (Dawood, 2008). However, the manufacturers of the tests did not indicate the specificities and sensitivities of the tests which made calculations of the TP difficult. As a result the AP was calculated. Prevalence is expressed as the number of diseased animals in relation to the number of animals in the population at risk of developing the disease (Thrusfield, 2007). The seroprevalence at the animal level was calculated on the basis of both RBPT and CFT seropositivity using the formula below.

$$\text{Brucellosis seroprevalence} = \frac{\text{No. camels tested positive in RBPT and CMT}}{\text{Total no. camel serologically tested}}$$

Similarly, sub-clinical mastitis prevalence rates at quarter, animal and herd levels were determined by the following formulae.

$$\text{Quarter prevalence} = \frac{\text{No. quarter milk samples positive by CMT}}{\text{Total no. quarter milk samples tested by CMT}}$$

$$\text{Animal level prevalence} = \frac{\text{No. camels with CMT positive quarter(s)}}{\text{Total no. camels examined}}$$

$$\text{Herd prevalence} = \frac{\text{No. herds with at least one camel with positive CMT}}{\text{Total no. herds examined}}$$

Assessment of the associations of different risk factors with brucellosis seropositivity and with the occurrence of sub-clinical mastitis was made using Chi-square (x^2) test. Odds ratio (OR) was used to quantify the risk factor association with the disease occurrence, and calculating the 95% confidence intervals. OR is the ratio of the odds of disease in exposed individuals to the odds in those unexposed (Thrusfield, 2007).

A logistic regression model was employed to obtain odds ratios between different risk factors and the occurrence of sub-clinical mastitis at the 95% confidence level.

Binary adjacency matrix data were stored in a UCINET dataset. UCINET 6 for Windows, a social network analysis software, was used to generate quantitative measurements, such as density, degrees, components, degree centrality, betweenness centrality and closeness centrality (Borgatti *et al.* 2002) (Appendix 7 and 8). NetDraw 2.111 software was used for visual representation of the graphs (Borgatti, 2002). The free trial version of the software was downloaded at www.analytictech.com/ucinet/.

4.1. Camel Brucellosis

4.1.1. Field investigation

Table 6 illustrates some husbandry practices and the herd structure of camels in 6 PAs that were used for brucellosis investigation. The average camel herd size was 12.4 over all PAs. Camels were the only livestock in 8 herds (20%). Almost half (47.5%) and 13 (32.5%) of the respondents disclosed that camels were usually reared together with cattle and with small ruminants, respectively. Eleven herd owners (27.5%) reared camels separate from other livestock. However, contact with other herds was commonly practiced by 90% of the herd owners.

Table 6: Summary of the questionnaire survey responses by camel herders in six PAs in Babille woreda, Ethiopia, 2011

	Awu-Sherif	Sheik Abdi	Bishane Babille	Babille	Iffa	Tula	Total
	n (%)						
No of Respondents	6	7	7	6	7	7	40
Herds (Camels only)							
Small (1-10)	4 (66.7)	6 (85.7)	5(71.4)	5(83.3)	6 (85.7)	2(28.6)	28 (70)
Medium (11-20)	2(33.3)	1(14.3)	1(14.3)	0.0	1(14.3)	1(14.3)	6 (15)
Large (above 21)	0.0	0.0	1(14.3)	1(16.7)	0.0	4(57.1)	6 (15)
Herd (All Livestock)							
Small (1-10)	1(16.7)	1(14.3)	1(14.3)	3(50)	4 (57.1)	1(14.3)	11(27.5)
Medium (11-20)	4 (66.7)	4(57.1)	3(42.9)	1(16.7)	1(14.3)	0.0	13(32.5)
Large (above 21)	1(16.7)	2(28.6)	3(42.9)	2(33.3)	2(28.6)	6(85.7)	16(40)
Average herd size (camels)	7.8	8.3	10.4	10.5	4.9	31.3	12.2
Herd composition							
A. Camels only	3(50)	2(28.6)	1(14.3)	0.0	0.0	2(28.6)	8 (20)
B. Camels &cattle	1(16.7)	1(14.3)	4 (10)	4 (66.7)	6(85.7)	3(42.9)	19(47.5)
C. Camels &small ruminants	2(33.3)	4(57.1)	2(33.3)	2(33.3)	1(14.3)	2(28.6)	13(32.5)
Contact with other herds							
A. Present	4 (66.7)	7(100)	6(85.7)	5(83.3)	7(100)	7(100)	36 (90)
B. Absent	2(33.3)	0.0	1(14.3)	1(16.7)	0.0	0.0	4 (10)

Management system							
A. Separate	3(50)	2(28.6)	1(14.3)	3(50)	0.0	2(28.6)	11(27.5)
B. With other animal	3(50)	5(71.4)	6(85.7)	3(50)	7(100)	5(71.4)	29(72.5)
Presence of Abortion							
A. Absent	4 (66.7)	7(100)	6(85.7)	5(83.3)	6(85.7)	6(85.7)	34 (85)
B. Present	2(33.3)	0.0	1(14.3)	1(16.7)	1(14.3)	1(14.3)	6(15)
Cause of Abortion							
A. Fever, Toxic plants, Tryps.	2(33.3)	0.0	0.0	0.0	0.0	0.0	2(5)
B. Toxic plants, Drought	0.0	0.0	0.0	1(16.7)	0.0	1(14.3)	2(5)
C. Toxic plants, Drought, Bull	0.0	0.0	0.0	0.0	0.0	1(14.3)	1(2.5)
D. Fever, Toxic plants, Bull	0.0	0.0	1(14.3)	0.0	1(14.3)	1(14.3)	3(7.5)
Treatment of Abortion							
A. No treatment	1(16.7)	0.0	0.0	1(16.7)	1(14.3)	1(14.3)	4(10)
B. Vet. Services	1(16.7)	0.0	1(14.3)	0.0	0.0	0.0	2(5)
Transmission							
A. Bull	0.0	0.0	1(14.3)	0.0	1(14.3)	1(14.3)	3(7.5)
B. None	2(33.3)	0.0	0.0	1(16.7)	0.0	0.0	3(7.5)
Disposal of Aborted fetus							
A. Discard	2(33.3)		1(14.3)	1(16.7)	1(14.3)	1(14.3)	6(15)
B. Burial	0.0	0.0	0.0	0.0	0.0	0.0	0.0

n: number of observations **(%)**: percentage

The age of the camel included into the study ranged from 1 to 16 years; those below 4 years were considered as young, and those 4 years and above as adults. Age categorization was based on the reproductive maturity of camels for breeding purposes. Out of the total, the proportion of young and adults were 21.4% (n=79) and 78.6% (n=291), respectively.

Abortion seemed rare in Babille Woreda. Only 6 of the respondents (15%) reported the presence of abortions in the past. Fever, trypanosomosis infection, ingestion of toxic plants, drought and bulls were claimed to be the causes of abortion and only 2 of the owners (5%) sought veterinary treatment for aborted female camels. Transmission was attributed to bulls and aborted fetuses and fetal membranes were simply discarded.

Camels were mainly kept for milk production in 47.5% of the herds and 20% of herd owners kept camel exclusively for trade purposes (Figure 3). Camels are bought by traders at Babille market, fattened for some months and are re-sold at the same market for better prices. Babille livestock market is one of the biggest markets in Eastern Ethiopia. All the herd owners disclosed that restocking and selling of all livestock species were taking place at this market.

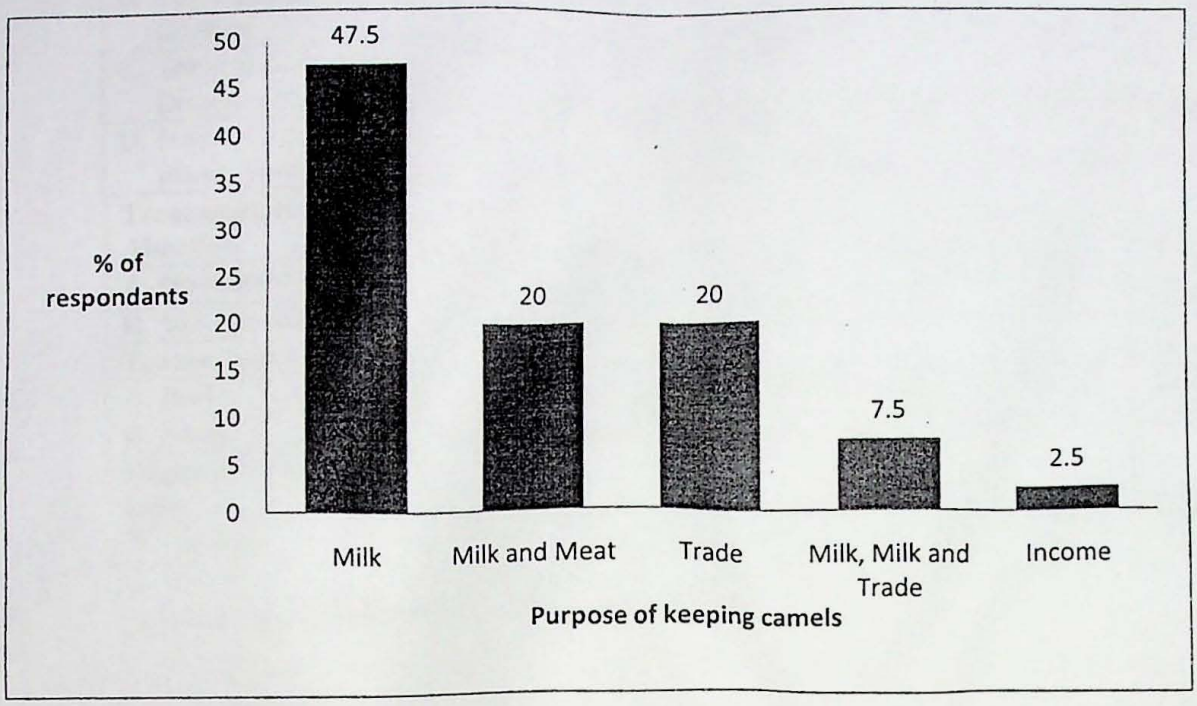


Figure 3: The main purposes of keeping camels in Babille Woreda as reported by herders in 2011

4.1.2. Laboratory analysis of blood samples

Out of 378 serum samples collected, 8 sera (2.1%) were lost during handling. The sera were drawn from 241 male (65.1%) and 129 female (34.9%) camels.

The 370 serum samples were screened for brucellosis with RBPT and 26 sera (7.0%) tested positive. The positive serum samples were further tested using CFT and 9 sera (2.4%) were positive reactors (Table 7). As there were no prescriptions regarding the

sensitivities and specificities of both tests from the manufacturers, the true prevalence (TP) could not be computed based on the apparent prevalence (AP) obtained from test results. Hence, the overall seroprevalence was 2.4%. Seroprevalence rates of *Brucella* infection in males and females were 2.9% and 1.6%, respectively, with no statistically significant difference (x^2 =0.649, p-value= 0.42).

Table 7: Laboratory analysis results of camel brucellosis in various PAs of Babille Woreda, Ethiopia, 2011

Location	Camels examined			Males			Females		
	Total No	Posit.	%	Total No	Posit.	%	Total No	Posit.	%
Awu-Sherif	31	0	0	18	0	0	13	0	0
Sheik Hussien	27	2	7.4	8	0	0	19	2	10.5
HoroBati	32	0	0	20	0	0	12	0	0
Iffa	35	0	0	23	0	0	12	0	0
Babille	31	0	0	28	0	0	3	0	0
Daketa	31	3	9.7	26	3	11.5	5	0	0
Tula	29	1	3.4	22	1	4.6	7	0	0
Sheik Abdi	30	0	0	11	0	0	19	0	0
BishaneBabille	31	0	0	21	0	0	10	0	0
Berkelle	30	0	0	19	0	0	11	0	0
ErerIbada	31	2	6.5	24	2	8.3	7	0	0
ErerGuda	32	1	3.1	21	1	4.8	11	0	0
Total	**370**	**9**	**2.4**	**241**	**7**	**2.9**	**129**	**2**	**1.6**
x^2 = 17.458, *p*-value= 0.095									

The seroprevalence of *Brucella* was relatively higher (3.8% versus 2.0%) in young (below 4 years) than in adult camel (above 4 years), however not statistically significant (x^2 = 0.789, *p*-value= 0.375). No statistical association of parity and source of camels nor with abortion and the occurrence of *Brucella* infection could be determined. All camels with an abortion history were reacting seronegative (Table 8).

Table 8: The association between the occurrence of camel brucellosis and risk factors in Babille Woreda, Ethiopia, 2011

Variable	Camels examined	Positive camels	Prevalence (%)
Age groups			
Young	79	3	3.8
Adult	291	6	2.0
Total	**370**	**9**	**2.4**
$x^2 = 0.789$, p-value= 0.375			
Sex			
Male	241	7	2.9
Female	129	2	1.6
Total	**370**	**9**	**2.4**
$x^2 = 0.649$, p-value= 0.420			
Parity			
No parturition	49	0	0
Single parity	24	0	0
More than one parity	54	2	3.7
Total	127	2	1.6
$x^2 = 2.747$, p-value= 0.253			
Outcome of last pregnancy			
Full term delivery	74	2	2.7
Aborted	6	0	0
Still birth	0	0	0
Total	**80**	**2**	**2.6**
$x^2 = 0.171$, p-value= 0.679			
Origin of camel			
From birth until now in herd	290	7	2.4
Purchase	80	2	2.5
Total	**370**	**9**	**2.4**
$x^2 = 0.002$, p-value= 0.965			

Herd level prevalence of brucellosis could not be determined as the required information could not be gathered from the mostly hired camel keepers when sampling the selected individual camels. Thus, only 40 herd owners in 6 PAs were selected and interviewed further.

4.2. Camel mastitis

4.2.1. Camel data

Thirty four herd owners in 5 PAs were included in the questionnaire survey. All the respondents were not well aware of sub-clinical mastitis. Table 9 shows the summary of milking and udder health problem and handling practices as indicated by the camel herders.

Table 9: Summary of the questionnaire survey responses by camel herders in 5 PAs of Babille Woreda, Ethiopia, 2011

	Tula	Iffa	Erer Guda	Babille	Erer Ibada	Total
			n (%)			
No of respondents	7	7	7	7	6	34
Cause of mastitis						
A. Tick	5 (71.4)	4 (57.1)	4 (57.1)	5 (71.4)	4 (66.7)	22 (64.7)
B. Flies	1 (14.3)	2(28.6)	1 (14.3)	1 (14.3)	2(5.88)	7 (20.6)
C. Both	2(28.6)	2(28.6)	2(28.6)	1 (14.3)	3 (50)	10 (29.4)
D. Trauma	1 (14.3)	1 (14.3)	2(28.6)	1 (14.3)	2(33.3)	7 (20.6)
E. Other*	1 (14.3)	0.0	0.0	1 (14.3)	0.0	2(5.9)
Types of treatment						
A. Veterinary Drugs	5 (71.4)	4 (57.1)	5 (71.4)	6 (85.7)	5 (83.3)	25 (73.5)
B. Traditional	1 (14.3)	0.0	1 (14.3)	0.0	1 (16.7)	3 (8.8)
C. Both	1 (14.3)	2(28.6)	1 (14.3)	1 (14.3)	1 (16.7)	6 (17.7)
D. Nothing	0.0	1 (14.3)	1 (14.3)	0.0	1 (16.7)	3 (8.8)
Application of cauterization						
A. Yes	1 (14.3)	2(28.6)	1 (14.3)	0.0	2(33.3)	6 (17.7)

B. No	6 (85.7)	5 (71.4)	6 (85.7)	7 (100)	4 (66.7)	28 (82.3)
Use of anti-suckling device						
A. Yes	2(28.6)	1 (14.3)	2(28.6)	0.0	1 (16.7)	6 (17.7)
B. No	5 (71.4)	6 (85.7)	5 (71.4)	7 (100)	5 (83.3)	28 (82.3)
Tick control						
A. Acaricides	7 (100)	5 (71.4)	5 (71.4)	7 (100)	5 (83.3)	29 (85.3)
B. Manual Removal	0.0	2(28.6)	1 (14.3)	0.0	1 (16.7)	4 (11.8)
C. Both	0.0	2(28.6)	1 (14.3)	0.0	0.0	3 (8.8)
E. Nothing	0.0	0.0	2(5.88)	0.0	0.0	2(5.9)

n: number of herd owners; **(%)**: percentage of owners; *superstitious causes

All respondents disclosed that feed scarcity, water shortage and diseases were the major constraints to camel keeping and, in particular, to keep lactating camels and their calves near the homesteads for milk production. Cleaning of the udder before milking was seldom done, only 3 owners (8.8%) cleaned the udder. However, 31 owners (91.2%) washed their hands before milking.

Clinical mastitis seemed to be rare in the study area; only 4 owners (11.8%) confirmed the presence of clinical mastitis in their herds. Twenty-two respondents (64.7%) attributed the cause of mastitis in camels to tick infestation. Whenever udder problem occurred in the herd, 25 owners (73.5%) took affected animals to veterinary clinics. However, 3 owners (8.8%) did not know any type of treatment for mastitis and did consider udder problem as transient. Application of cauterization for udder problem and use of anti-suckling devices were used by 6 owners (17.7%).

4.2.2. CMT test results

Quarter level prevalence of sub-clinical mastitis

Five hundred and forty-four udder quarters from 136 traditionally managed lactating camels were investigated for this study; 9 quarters (1.7%) were blind. The remaining 535 quarter milk samples (98.4%) were screened for mastitis using CMT. The results are given in Table 10.

Table 10: Distribution and percentage of quarters examined and CMT results at quarter level across PAs in Babille Woreda, Ethiopia, 2011

	Quarters				Total
	FL*	FR*	RL*	RR*	
	(%)				
Site					
Tula	20.6	20.6	20.6	19.9	20.5
Iffa	19.9	19.1	20.6	19.9	19.9
Erer Guda	19.9	19.9	20.6	20.6	20.2
Babille	19.9	20.6	20.6	20.6	20.5
Erer Ibada	17.6	16.9	17.6	17.6	17.5
Blind teats	2.2	2.9	0.0	1.5	1.7
Total - n (%)	136 (100)	136 (100)	136 (100)	136 (100)	544 (100)
CMT Result					
Total CMT tested (n)	133	132	136	134	535
Negative	**86.5**	**87.1**	**84.6**	**84.3**	**85.6**
Weak/positive	4.5	2.3	5.1	3.7	3.9
Positive	5.3	6.8	7.4	9.7	7.3
Strong positive	3.8	3.8	2.9	2.2	3.2
Total - (% positive)	**13.5**	**12.9**	**8.8**	**15.7**	**14.4**

*__FL__: front left; __FR__: front right; __RL__: rear left; __RR__: rear right

__(%)__: percentage of samples; __n__: number of samples

The overall prevalence of sub-clinical mastitis at quarter level was 14.4% (77/ 535). There was no significant association (x^2 = 14.320, p-value= 0.133) between quarter sub-clinical mastitis prevalence and localities (PAs).

Individual lactating camel level prevalence of sub-clinical mastitis

A lactating camel was considered as having sub-clinical mastitis when at least one of its quarter milk samples yielded a positive result to CMT. In this study, a total number of 136 lactating camels were examined by detailed clinical examination of the udder and sub-clinically using CMT.

A breakdown of the camels investigated for mastitis by age classes and lactation stages is given in Table 11 whereby the age classes roughly compare to parity numbers.

Table 11: Distribution of age and lactation stages of the examined camels in Babille Woreda, Ethiopia, 2011

	Frequency	Percent
Age class		
5 to 7 years	61	44.9
> 7 to 10 years	31	22.8
>10 to 13 years	23	16.9
>13 to 18 years	21	15.4
Total	**136**	**100.0**
Lactation stages		
1 - 3 months	76	55.9
> 3 to 6 months	47	34.6
> 6 to 9 months	13	9.6
Total	**136**	**100.0**

The majority of examined camels (72.1%) had ticks on the udder. Teat and/or udder lesions were observed in 8 camels (5.9%). Nine camels (6.6%) had one blind teat resulting in a blind teat prevalence of 1.7% and 6.6% at quarter and animal levels, respectively.

In this study, clinical mastitis cases were not detected. However, sub-clinical mastitis cases were prevalent in 31.6% (43/136) of the camels studied. Associations between age groups, lactation stages, parity, ticks on udder, udder lesions and teat conformation disorders with the prevalence of sub-clinical mastitis was assessed. A significant association (x^2 = 19.846, p-value= 0.00) was found between sub-clinical mastitis and age groups (Table 12).

Table 12: Prevalence of sub-clinical mastitis at individual lactating camel level in Babille Woreda, Ethiopia, 2011

	Animal level		
	No of animals examined	**Percent**	**Positive**
Clinical mastitis	136	100	0 (0.0%)
Sub-clinical mastitis	136	100	43 (31.6%)
Sub-clinical mastitis by age class			
5 to 7 years	61	44.9	9 (14.8%)
> 7 to 10 years	31	22.8	14 (45.2%)
>10 to 13 years	23	16.9	14 (60.9%)
>13 to 18 years	21	15.4	6 (28.6%)
Total	**136**	**100**	**43 (31.6%)**
$x^2 = 19.846$, *P*-value= 0.00			
Lactation stages			
1 - 3 months	76	55.9	20 (26.3%)
> 3 to 6 months	47	34.6	20 (42.6%)
> 6 to 9 months	13	9.6	3 (23.1%)
Total	**136**	**100**	**43 (31.6%)**
$x^2 = 4.026$, *P*-value= 0.134			

Parity numbers also showed significant association($x^2 = 16.497$, *p*-value= 0.006) with sub-clinical mastitis occurrence. The prevalence rate showed a declining trend in multiparous camels beyond the third parity (Figure 4).

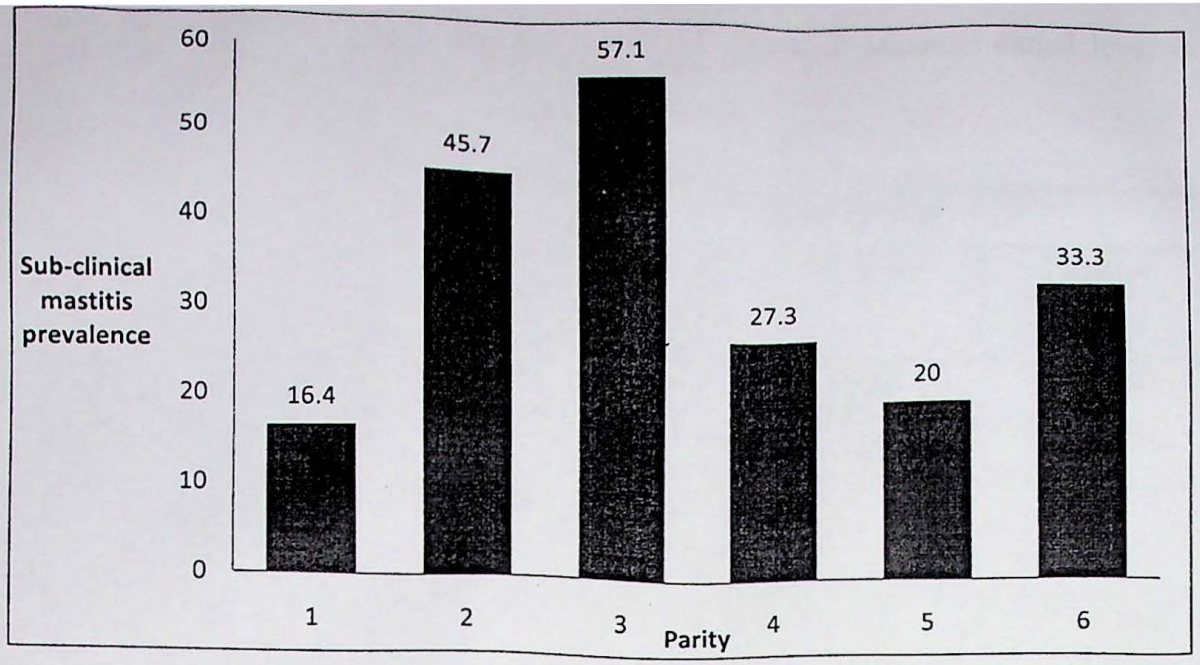


Figure 4: Parity numbers and corresponding sub-clinical mastitis prevalence rates in camels in Babille Woreda, Ethiopia, 2011

Sub-clinical mastitis and udder lesions were also found to be significantly associated (x^2= 18.384, *p*-value= 0.00). **All lactating camels with udder lesions were positive for CMT tests (Table 13).**

Table 13: Univariate analysis of some risk factors and occurrence of sub-clinical mastitis at individual lactating camel level, Babille Woreda, Ethiopia, 2011

	Animal level		
	Number of camels examined	**Percent**	**Positive**
No. camel examined	136	100	
Udder lesions			
• Absent	128	94.1	35 (27.3%)
• Present	8	5.9	8 (100%)
x^2 = 18.384 , *P*-value= 0.00			
Conformational disorders			
• Absent	127	93.4	40 (31.5%)
• Present	9	6.6	3 (33.3%)
x^2 = 0.013, *P*-value= 0.909			

Ticks on udder			
• Absent	38	27.9	9 (23.7%)
• Present	98	72.1	34 (34.7%)
x^2 = 1.535, *P*-value= 0.215			

Camels between 8 and 13 years of age had higher risks of sub-clinical infection than younger camels (5 to 7 years). The probability of sub-clinical infection in camels of 8 to 10 years is almost 5 times (OR=4.8) and in camel between 11 to 13 years almost 9 times (OR=8.99) the risk of infection of younger camels of 5 to 7 years (Table 14).

Table 14: Sub-clinical mastitis prevalence in different age groups, lower and upper confidence limits and odds ratio

Factor	Sub-clinical mastitis prevalence	OR	*p*- value	95% CI	
				Lower	Upper
Age groups					
5 to 7 years	14.8		0.00		
> 7 to 10 years	45.2	4.758	0.02	1.750	12.941
>10 to 13 years	60.9	8.988	0.00	3.003	26.901
>13 to 18 years	28.6	2.311	0.165	0.709	7.536

Herd level prevalence of sub-clinical mastitis

Within 34 herds 136 individual lactating camels were subjected for the study of sub – clinical mastitis. A herd was considered as having sub-clinical mastitis when at least one lactating camel was found positive for the CMT test. Accordingly, 25 of the herds (73.5%) were found to be affected by sub-clinical mastitis (Table 15).

Table 15: Herd level sub-clinical mastitis prevalence in selected PAs in Babille Woreda, Ethiopia, 2011

PAs	No of herds examined	Percent	Positive herds
Tula	7	20.6	6 (85.7%)
Iffa	7	20.6	6 (85.7%)
Erer Guda	7	20.6	5 (71.4%)
Babille	7	20.6	5 (71.4%)
Erer Ibada	6	17.6	3 (50%)
Total	**34**	**100**	**25 (73.5%)**

Associations of practices of cauterization for treating udder problems, use of anti-suckling devices and unhygienic practices (not washing hands and udder before milking) with herd sub-clinical mastitis positivity were not found to be significant (Figure 5).

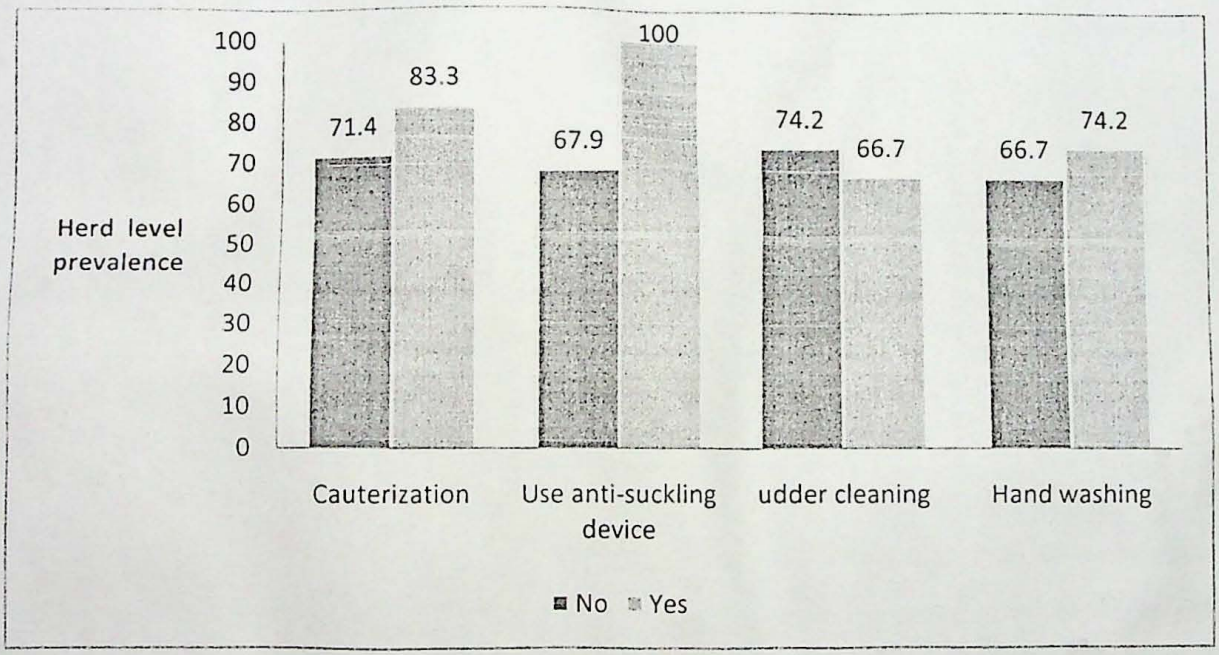


Figure 5: Traditional husbandry practices and occurrence of sub-clinical mastitis in herds in Babille Woreda, Ethiopia, 2011

4.3. Camel milk markets network

Twenty seven was the number of nodes in the milk markets network, representing the network size out of which 22 nodes represent the PAs within Babille Woreda where camel milk was produced. Five nodes (Jijiga, Dire Dawa, Harer, Awedi and Hargessa) represent relatively big towns which serve as terminal markets for camel milk. One node (Hargessa) is located in Somaliland, outside of Ethiopia. As there were no official information on frequency, volume of products and other quantitative measures, only presence or absence of trade links were recorded and used in the analysis.

4.3.1. Quantitative measurements

Overall, the analyzed network consisted of 3 components; one large component consisting of 24 nodes (88.9% of all the nodes), another component with only two nodes (Abdigurich and Gambella) and the last one was just an isolate (Najata).

The overall density (the number of ties present in a network divided by the number of possible ties) of the milk market network was 0.047, That is only 4.7% of all the possible ties were present, indicating a very loosely connected network. The number of ties in the network was 33, with an average degree of 1.2.

Babille, Harer, Jijiga and Dire Dawa had in-degrees of 6, 5, 3 and 2, respectively (Table 16). These towns received milk produced from PAs for consumption. Among the PAs, Aw-Sherif, Erer Ibada, Gemechu, in-degree scores of 4, 4 and 2, respectively were recorded.

Table 16: In-degree and out-degree scores of towns in camel milk markets network, Ethiopia, 2011

Towns	Out-degree	In-degree	Total direct connections
Babille	0.0	6.0	6.0
Harer	0.0	5.0	5.0
Jijiga	0.0	3.0	3.0
Dire Dawa	0.0	2.0	2.0
Awedai	0.0	1.0	1.0
Hargessa*	0.0	1.0	1.0

* Capital of Somaliland

All towns recorded out-degree scores of 0. From the PAs Aw-Sherif had the highest out-degree score of 5 followed by Tula and Berkele with scores of 3 (Table 17). All the remaining PAs had out-degree scores of 1, 2 and Abdigruch and Najata, an isolate, had 0 (Appendix 7).

Table 17: PAs with high in-degree and/or out-degree scores in Babille Woreda, Ethiopia, 2011

Pas	Out-degree	In-degree	Total direct connections
Aw-Sherif	5.0	4.0	9.0
Tula	3.0	1.0	4.0
Berkele	3.0	0.0	3.0
Gemechu	2.0	2.0	4.0
Rahmata Selama	2.0	0.0	2.0
Erer Ibada	2.0	4.0	6.0
Abdilkadir Erer	2.0	0.0	2.0
Erer Guda	1.0	2.0	3.0

In this study, the network connections were of directed types. Based on *degree centrality* measures, an actor that received many ties was considered to be prominent,

or to have high prestige (Hanneman and Mark, 2005). Babille, Harer, and Jijiga, with respective in-degrees of 6, 5, and 3, were of higher importance as terminal markets for milk. Many actors were connected to them. Aw-Sherif having direct connection of 9 was able to exchange with many other actors followed by Erer Ibada, Tula and Gemechu. It had a very important and influential position in the network. On average, actors had a degree of 1.2, which was very low, given that there were 26 other actors. In the current study, the network centralization for out-degree and in-degree were 15.1% and 19.1%, respectively.

The network *closeness centrality* measures revealed that Harer had the highest incloseness score followed by Jijiga and Dire Dawa (Table 18). The majority of the PAs had lower score of *closeness centrality* measures (Appendix 8).

Table 18: *Closeness centrality* measures for some PAs and towns in camel milk markets network, Babille Woreda, Ethiopia, 2011

Nodes	InCloseness	OutCloseness
Harer	8.7	3.7
Jijiga	6.5	3.7
Dire Dawa	5.0	3.7
Babille	4.8	3.7
Erer Ebada	4.7	4.0
Awedai	4.5	3.7
Hargessa	4.5	3.7
Aw-Sherif	4.4	4.6

Broad variation in actor betweenness (from zero to 20) was observed and with a wide standard variation of 3.97 relative to a mean betweenness of 1.3. Aw-Sherif (20), Erer Ibada (6.5) and Gemechu (4.5) appeared to be more powerful than others using this measure. Tula (2.5) and Erer Guda (2) had a relatively lower betweenness power in the system (Table 19). Other nodes had *betweenness centrality* measures of 0 (Appendix 9).

Table 19: *Betweenness* and *normalized betweenness centrality* measures of PAs in Babille Woreda, Ethiopia, 2011

Nodes	Betweenness	nBetweenness
Aw-Sherif	20.0	3.1
Erer Ibada	6.5	1.0
Gemechu	4.5	0.7
Tula	2.5	0.4

4.3.2. Visual representation: graphs

Network analysis (NA) uses a graphic display that consists of points to represent actors and lines representing relations. In the current study, the nodes were PAs, and towns and the lines represented traded camel milk flow. The graphs generated were binary and directed, and the relationships being represented were simplex. As directed graph, arrow heads were used for connecting nodes, indicating the direction of milk flow. Below are three graphs that depict the milk markets network based on various attributes represented by different colors and potential spread of brucellosis through milk trade (Fig. 6, 7 and 8).

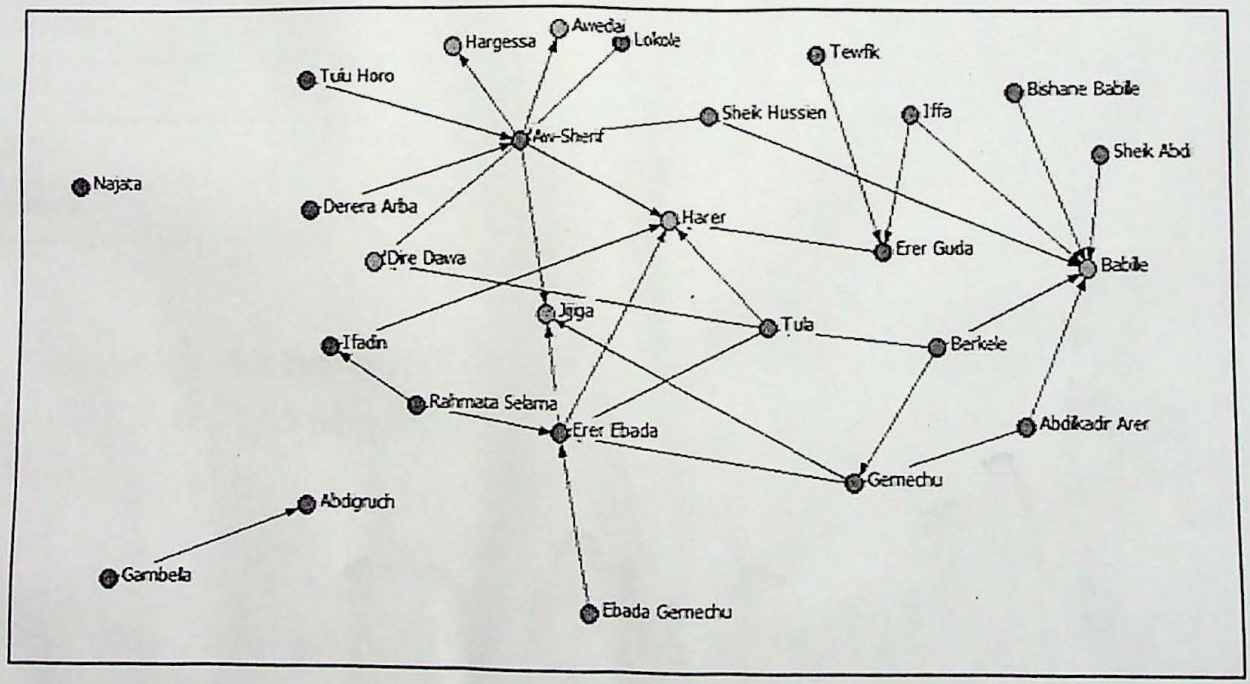


Figure 6: Camel milk markets network, illustrating the three components; red circles represent PAs and light green circles towns.

Figure 6 shows the three components of the milk markets network. All towns (100%) and 86.4% of the PAs were inter-connected in the large component. One PA (Gambella) supplied milk to only another PA (Abdigruch). No milk trade relationship was observed for Najata as it was an isolate.

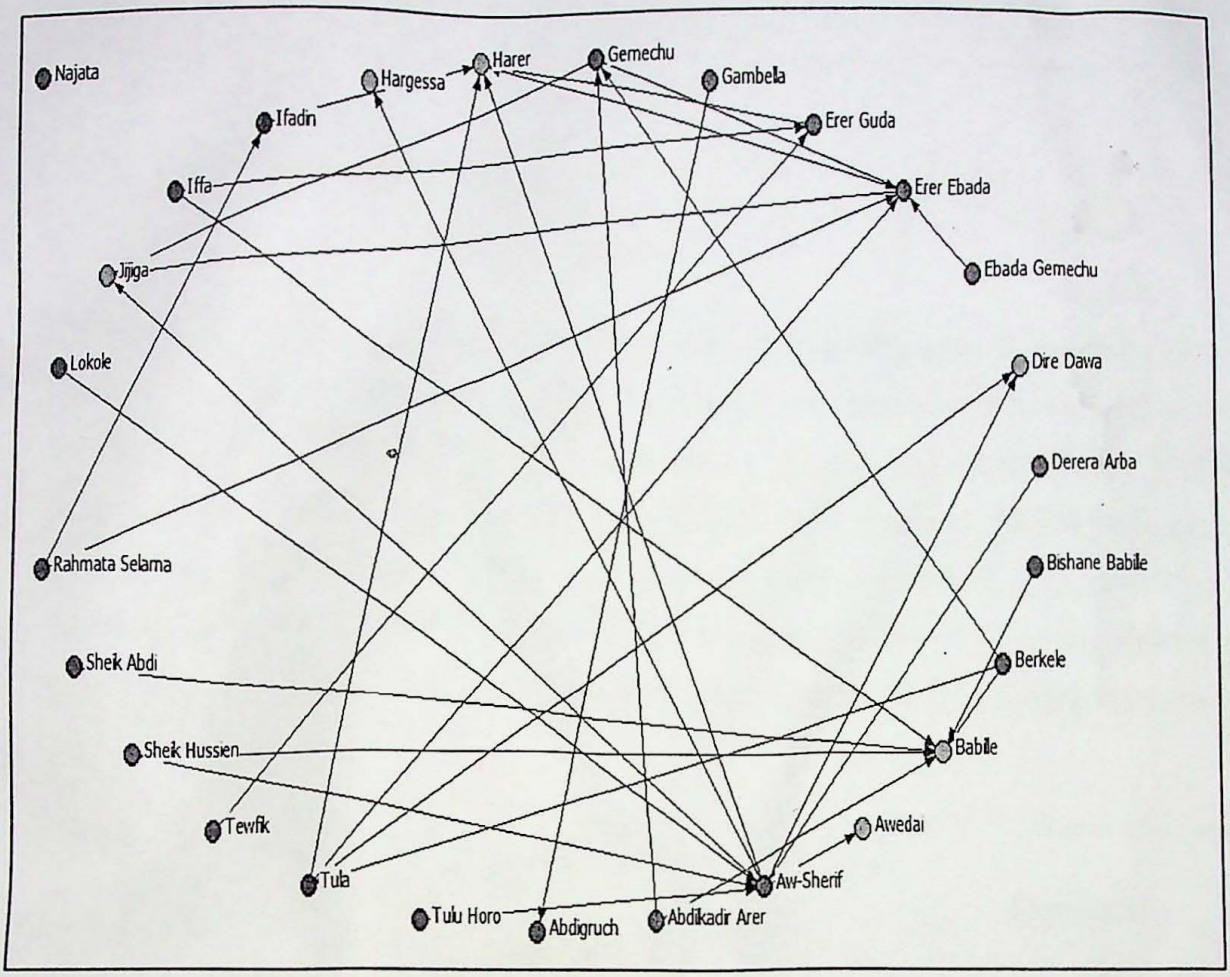


Figure 7: Circular layout of the camel milk markets network - red circles represent PAs and light green circles represent towns

Figure 7 is another way of visualizing the overall network. The nodes are arranged in a circular pattern which can help to assess individual and neighborhood connections, as well as the density of the network.

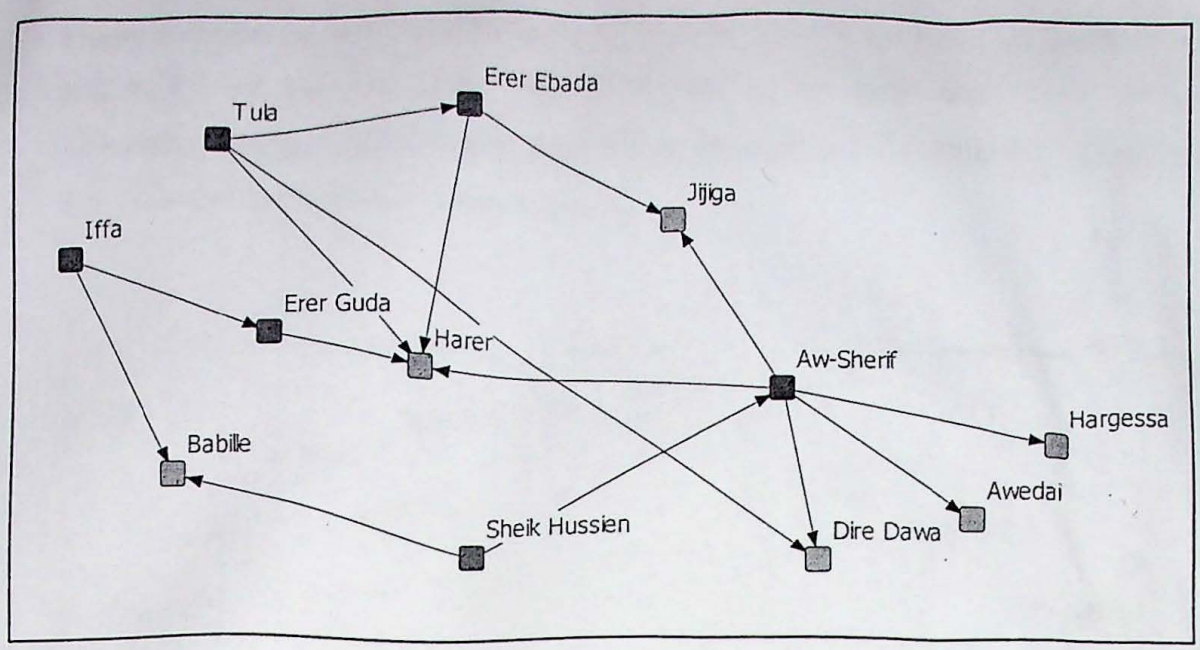


Figure 8: Potential spread of Brucella organisms via milk; seroreactors were found in red nodes and green nodes are towns.

In the above figure the nodes represented in red were PAs where brucellosis seropositive camels were detected. The light green nodes are towns that received camel milk from PAs with seropositive camels. The blue node, Aw-sherif, is the hub for the trade relationship. As milk from brucellosis seropositive camels reaches the hub, it could be distributed to many other nodes, increasing the risk of spread.

5. DISCUSSION

5.1. Camel brucellosis

The results of the questionnaire survey revealed that camels were reared alone in 20% and with cattle and /or small ruminants in 80% of the herds in the study area. Mohammed *et al.* (2011) reported that the majority of herds (84.5%) in Dire Dawa, Eastern Ethiopia consisted of only camels but camels reared together with small ruminants (12.2%) showed higher *Brucella* prevalence. Contact with small ruminants was identified as a risk factor in Jordan (Al-Majali *et al.*, 2008) and in Ethiopia (Megersa *et al.*, 2010). As camels are not the primary hosts to *Brucella* organisms, infection rates in camels depend on the infection rates in primary animal hosts in contact with them (Dawood, 2008). Cross-species transmission was confirmed by isolation of *Brucella melitensis* (Radwan *et al.*, 1992; Gameel *et al.*, 1993; Radwan *et al.*, 1995; Khadjeh *et al.*, 1999; Al-Majali *et al.*, 2008) and *Brucella abortus* in camels (Omer *et al.*, 2010b) suggesting that camels mainly acquire infections from cattle and goats.

Babille livestock market is one of the biggest livestock markets in Eastern Ethiopia. Herders use the market for selling and restocking their livestock. Different kind of herders who are both herders and traders are emerging taking advantage of the nearby market. Almost a quarter of herders (20%) disclosed that they keep camels for trade purposes, buying and reselling after a fattening period of some months for better prices. Such trends favour the contact rate of camels with other livestock originated from other regions and zones, increasing the possibility of infection not only with brucellosis but also with other diseases. Megersa (2004) reported that herders in Borena zone usually sell aborting she-camels frequently. The fact that *Brucella* organisms were isolated from aborted fetuses (Al-Majali *et al.*, 2008; Dawood, 2008) and the strong association of brucellosis with abortion in camels (Mohammed *et al.*, 2011) shows that unregulated animal movement increases the spread of infection.

Although camels were kept separately at homesteads, free contact with other herds occurs in rangelands and watering points: in Somaliland, camels in contact with other herds showed a significantly higher level of *Brucella* infections (Yasser *et al.*, 2009). For strict brucellosis control, it would be necessary to keep camels separately which might not be practical in pastoral settings, however, control measures in other ruminants should be undertaken to protect camels. Eradication programs directed at all livestock with concurrent annual disease surveillances in UAE decreased the seroprevalence of camel brucellosis from 5.8% in 1991 to level as low as 0.1% in 1995 and 1996 (Moustafa *et al.*, 1998). Al-Majali *et al.* (2008) suggested that adequate *Brucella* control programs in small ruminants may contribute to a reduction in the prevalence of this disease in camels.

However, there is a very low level of brucellosis awareness among herd owners as only a small proportion of herders (5%) sought veterinary treatment for aborted she-camels and 15% of the herders are careless in the disposal of aborted fetuses and fetal membranes as well as in consumption of raw camel milk. Lack of knowledge about brucellosis by camel herders was reported not only from Ethiopia (Mohammed *et al.*, 2011) but also from Sudan (Omer *et al.*, 2010) and Chad (Schelling *et al.*, 2003). *Brucella* organisms were isolated from aborted fetuses (Al-Majali *et al.*, 2008), aborted fetuses and milk (Dawood, 2008) and from milk samples (Radwan *et al.*, 1995; Omer *et al.*, 2010b).

Brucellosis is known to affect herd owners and individuals in close contact with livestock. Positive antibody titers by RBPT from 12 camel herd owners (60%) and 3 abattoir workers (9%) were reported from the Sudan (Omer *et al.*, 2010b). Higher brucellosis prevalence rates of 4.0%, 8.9%, 5.4% in abattoir workers, butchers and veterinary personnel than in administrative personnel (1.1%) were reported from Saudi Arabia indicating the occupational hazard associated with brucellosis infection (Al-Sekait, 2005). Human brucellosis seroprevalence of 3.9% in Chad (Schelling *et al.*, 2003) and a morbidity in the range of 1.6% to 2.6% particularly in the rural areas in Saudi Arabia (Al-Eissa, 1999) was recorded indicating the level of threat to humans. An alarming seroprevalence of 40% in humans in Libya was recorded although the

samples taken were not representative as only volunteers were included (Ahmed *et al.*, 2010). Ingestion of raw milk and milk products from infected goats and camels and contact with aborted materials when doing unprotected obstetric tasks is the main means of acquiring a brucellosis infection by the herders. The legacy of consuming raw camel milk among consumers in urban areas indicates that the risk of human brucellosis infections in both sexes and in all ages could increase further in the future.

In the study area, herders consider febrile diseases, infections of trypanosomosis, ingestion of toxic plants and drought as leading to abortions in camels. Abortions in camels were observed following acute infection of trypanosomosis and ingestion of toxic agents (Tibary *et al.*, 2006). Herders believed that male camels used for service can transmit "abortion" to she-camels. Orchitis and epididymitis have been associated with brucellosis caused by *Brucella abortus* and *Brucella melitensis* in other species (Tibary *et al.*, 2006). A case of orchaitis was found in a seropositive camel (Ismaily *et al.*, 1988). However, Musa and Shigidi (2001) confirmed that cases of orchaitis were negative for *Brucella* antibodies and Yagoub (2005) isolated *Staphylococcus aureus*, *E.coli* and *proteus* organisms in male camels with orchitis.

The 2.4% seroprevalence of *Brucella* antibodies in camels in the study area compares well to the 2.2% seroprevalence rate reported by Megersa *et al.* (2010) in Borena and 2.8% in the Somali Region of Ethiopia as indicated by Teshome *et al.* (2003) whereas in an earlier study in Borena a relatively lower prevalence rate of 1.2% (Teshome *et al.*, 2003) and 1.6% in Dire Dawa, Eastern Ethiopia (Mohammed *et al.*, 2011) were recorded. Lower rates were also reported from other countries: 0.1% in United Arab Emirates (Moustafa *et al.*, 1998), 0.3% in Central Somalia (Baumann and Zessin, 1992) and 0.4% in Chad (Schelling *et al.*, 2003). In contrast, high prevalence rates of 5.2% in Afar, Ethiopia (Teshome *et al.*, 2003), 7.8% in Sudan (Musa and Shigidi, 2001), 15.8% in Jordan (Dawood, 2008) and 14% in Kenya (Waghela *et al.*, 1978) were indicated. Based on the figures reported above seroprevalence of camel brucellosis appears to follow two distinct patterns: low prevalences (below 5%) in nomadic or extensively kept camels and distinct high prevalences (8-15%) in camels kept intensively or semi-intensively. The fact that camels are mainly browsers, less frequently drink water, and

reach maturity late with prolonged reproduction intervals may also contribute to lower brucellosis prevalence among pastoral camels (Abbas and Agab, 2002). A very high seroprevalence of 37.5% in Sudan reported by Omer *et al.* (2010b) could not be considered as representative of the population as some of the samples were drawn both from randomly selected herds and camels destined for slaughter.

In the current study brucellosis seroprevalence and the putative risk factors age, sex, localities, and source of camel showed no significant associations. Also Mohammed *et al.* (2011) reported the absence of association between seroprevalence and these risk factors. In Sudan, no association of brucellosis with these putative risk factors was described (Omer *et al.*, 2010b).

On the other hand, higher levels of *Brucella* antibodies were detected in female (2.1%) than in male (0.9%) camels in Ethiopia (Megersa, 2004). According to Megersa (2004), Teshome *et al.* (2003) and Dawood (2008), in younger camels lower reactor rates of 1.0%, 3.6% and 35.2% were found than in adult camels, ranging from 2.2% to 64.8%. Marked associations between seroprevalence and regions were recorded in Ethiopia (Teshome *et al.*, 2003). Poor body condition was also associated with *Brucella* seropositivity in camels in Tanzania (Swai *et al.*, 2011).

5.2. Camel mastitis

The results of the questionnaire survey disclosed that all herd owners reported that feed scarcity, water shortage and diseases were the major constraints to camel keeping in general and to milk production in particular to keep lactating camels and their calves near the homestead. Sub-clinical mastitis was not well known and not implicated as a constraint. Abera *et al.* (2010) reported that sub-clinical mastitis was not appreciated at all in Eastern Ethiopia.

Only 4 owners (11.8%) confirmed the presence of mastitis in their herds in the past. Clinical mastitis seemed to be rare in the study area. However, Abdel Gadir (2001) reported the existence of udder infections in lactating camels from 95% of the respondents in Southern, North-Eastern and Eastern Ethiopia. A report by Eyassu and Bekele (2010) confirmed that camel herders in Eastern Ethiopia were well aware of clinical mastitis as a cause for significant decline in milk yield.

Cleaning of the udder before milking was done by only 3 owners (8.8%). However, almost all owners (91.2%) washed their hands before milking. Eyassu and Bekele (2010) reported that washing the udder and teats of camels prior to milking was not completely practiced in Eastern Ethiopia but the majority of camel owners (81.3%, n = 32) wash their hands before milking in Shinile areas; but only a small proportion of the respondents (30%, n = 10) in Erer Valley.

Such unhygienic practices have implications on udder health. Ahmed *et al.* (2011) reported a higher prevalence of mastitis (94.2% versus 38.6%) in she-camels with poor hygienic practice during the milking process than in camel with good hygiene. The traditional husbandry practices in the study area are characterized by application of very low hygienic standards as clean water is scarce and knowledge about the requirements of basic hygienic standards in livestock production is lacking, thus, increasing the risk of a mastitis infection. Poor application of basic hygienic standards was reported to be conducive for the occurrence of high prevalence of mastitis (Aljumaah *et al.*, 2011).

In our questionnaire survey a relatively small number of respondents (17.7%) reported the application of cauterization for udder swelling and abscesses on the skin and the use of anti-suckling devices for preventing calves from suckling whereas in Jijiga area a high proportion of respondents (80%) claimed the use of anti-suckling devices (Abera *et al.*, 2010). Abdel Gadir (2001) also reported the practice of cauterization and the use of anti-suckling devices by 65% and 60% of interviewed herd owners, respectively. In a study conducted in Eastern Ethiopia, 24.7% of herd owners practiced hot iron branding to treat udder problems (Eyassu and Bekele, 2010). Woubit *et al.* (2001) associated the use of these devices together with heavy tick infestation in Borena zone of Ethiopia as predisposing factor for bacterial udder infections, which may persist as

chronic infections. In the present study all the camels with udder lesions showed a sub-clinical mastitis. Udder lesions predispose the udder to bacterial infections (Woubit *et al.,* 2001). El-Hallawany *et al.* (2011) isolated bacteria and mixed infection of bacteria and fungi from 62.5% and 14.5% of teats with lesions. Significant association of udder and teat lesions and sub-clinical mastitis was reported by many authors (Bekele and Molla, 2001; Mohammed *et al.*, 2005; Abera *et al.,* 2010; Ahmed *et al.,* 2011).

In this study, clinical mastitis cases were not recorded. However, a number of authors (Woubit *et al.,* 2001; Abdel Gadir, 2001; Abdurahman, 2006; Eyassu and Bekele, 2010; Abera *et al.,* 2010), reported various prevalence rates of clinical mastitis in Ethiopia. Clinical mastitis was also reported from other countries such as Tanzania (Swai *et al.,* 2011), Pakistan (Ahmed *et al.,* 2011), Saudi Arabia (Barbour *et al.,* 1985; Ibrahim, *et al.,* 2011) and Jordan (Hawari and Hassawi, 2008). Due to increasing camel milk demand in the study area, she-camels are usually kept at homesteads with better housing conditions and care. This might, partly, be the reason for the absence of clinical mastitis cases in the study area. Ahmed *et al.* (2011) argued that nomadic and semi-nomadic husbandry conditions are conducive for the occurrence of higher mastitis prevalence rates.

This study employed the California Mastitis Test (CMT) to detect sub-clinical mastitis in she-camels. Several authors have validated the usefulness of CMT in detecting sub-clinical mastitis. Accuracy levels of 85.9% and 89.1% were reported for CMT taking cultural examination and somatic cell counts (SCC) as a `gold standard´, respectively (Kathiriya and Shah, 2009). Significant associations of CMT with the presence of mastitis pathogens in camel milk and SCC values were reported by many authors (Barbour *et al.,* 1985; Woubit *et al.,* 2001; Bekele and Molla, 2001; Abdurahman, 2006; Abdel Gader *et al.,* 2006; Hawari and Hassawi, 2008; Saleh and Faye, 2011). A recent study by Aljumaah *et al.* (2011) assessed the risk factors of sub-clinical mastitis and compared milk constituents from healthy and sub-clinically affected quarters using only CMT and reported associations of sub-clinical mastitis with significant decreases in protein, lactose, and solid nonfat, calcium and potassium concentrations and increase in sodium concentrations in milk being consistent with other reports.

In the present study, the overall CMT prevalence of sub-clinical mastitis at quarter levels was 14.4%. Comparable quarter level prevalence rates of 17.8% (Bekele and Molla, 2001) and 19.1% (Almaw and Molla, 2001) were reported from North-Eastern Ethiopia, based on CMT and bacteriological results. In contrast, higher prevalence of 41.6% in Dire Dawa, Afar and Borena regions of Ethiopia (Abdel Gader *et al.*, 2006), 48.1% in South-Eastern Ethiopia (Mohammed *et al.*, 2005), 28.8% in South-Western Ethiopia (Woubit *et al.*, 2001) as well as 33% in Saudi Arabian (Aljumaah *et al.*, 2011) were reported based on CMT results.

Eyassu and Bekele (2010) reported an extremely high CMT prevalence of 81.3% from Erer Valley where Abdurahman (2006) reported quarter prevalence of 37.6%, however, based on CMT and bacteriological examinations. In both studies 49.1% (Abdurahman, 2006) and 35.4% (Eyassu and Bekele, 2010) of the examined camels were infested with ticks which might explain these remarkable higher prevalences. Although ticks were present in 72.1% of the camels examined in our study, only light tick infestation burdens were observed which could be the reason for the low quarter level prevalence in the study area.

There were no significant associations between individual quarters and CMT results. Most authors did not report whether such associations existed. However, Saleh and Faye (2011) reported that highest number of CMT scores were observed in front quarters (FL and FR).

In this study, sub-clinical mastitis cases were found in 31.6% of the camels investigated. A relatively lower prevalence of 20.7% was reported by Abera *et al.* (2010) in Jijiga, Eastern Ethiopia which is adjacent to the study area based on a mastitis card test with subsequent bacteriological assessment. This could be due to the higher sensitivity of the serial tests employed. In contrast, Eyassu and Bekele (2010) reported a 67.4% prevalence of sub-clinical mastitis from Erer Valley being over twice as high as the individual animal prevalence found in the present study based on CMT.

In this study significant associations between sub-clinical mastitis and age groups and parity were found. An increasing trend of sub-clinical mastitis was observed from 5 up to 13 years and then declined above 14 years. With increasing parity numbers a decreasing prevalence trend after the third parity was observed. However, Abera *et al.* (2010) reported an increasing prevalence trend with increasing age and parity. Concurrent increase in prevalence and parity was also recorded in Saudi Arabia (Aljumaah *et al.*, 2011).

In the current study tick infested udder and presence of sub-clinical mastitis showed no significant associations. However, camels with tick infested udder showed relatively higher sub-clinical mastitis prevalences than non-infested camels. Ticks on the udder are regarded as predisposing factors for mastitis and udder lesions (Megersa, 2010). A strong relation between sub-clinical mastitis and tick infestation was reported in various studies (Woubit *et al.*, 2001; Mohammed *et al.*, 2005; Abera *et al.*, 2010; Eyassu and Bekele, 2010). Tick-burden counting and species identification should be undertaken to assess the effects of ticks on udder and as predisposing factors to mastitis.

5.3. Camel milk markets network

Camel milk is produced by individual herders and collected by herders or traders and transported to relatively populous areas, usually to towns. The trade is informal with no recordings on the volume, frequency, origin and destination of the product. In the present study the nodes were PAs and towns where camel milk is produced and marketed. However, nodes could be individual milk producers, traders and transporters to get in-depth structural characteristics of the camel milk trade in the area. Aznar *et al.* (2011) conducted an analysis of cattle movements taking the nodes as farms (producers) although movements of cattle were mediated via markets. Farms, cattle markets and cattle-shows were used as nodes in the analysis of cattle movements in Portugal (Baptista *et al.*, 2008).

The size of the network was small, only 27, as the nodes were PAs and towns. The nodes can be considered as `super nodes' (blocks) in which other types of nodes were embedded. This is the reason why there were small numbers of nodes in the camel milk market networks.

Livestock and livestock products trade in developed countries are regulated by governmental organizations that supervise the observance of laws, policies and standards. Such practices require the use of information technology at various levels. On the contrary, livestock and livestock products trade in developing countries are informal with no official recordings. NA can make use of official information for structural analysis and to follow dynamic changes in the network. Baptista *et al.* (2008) used data from central veterinary services and the National Bovine Identification and Recording System of Portugal. The livestock movement of Great Britain was studied by Kao *et al.* (2006) with data obtained from central databases and in Argentina data from a sanitary management system database were used (Aznar *et al.*, 2011).

The analyzed network consisted of 3 components with one large component consisting of 24 nodes. The large component included 88.9% of the nodes in the network. Introduction of diseases in such large components is considered to have an impact on other network actors. Brucellosis seropositivity was recorded in PAs at the periphery of the network which could easily pose a threat to humans in other PAs and towns. Baptista *et al.* (2008) and Aznar *et al.* (2011) indicated the importance of identifying larger components in cases of disease introduction to assess its potential spread.

The density of the camel milk markets network was 0.047 indicating a loosely connected network. The density of a network may give insights into the rate of information flow (Hanneman and Riddle, 2005). Access to information on milk market price fluctuations, especially in towns, may not reach producers in time. The price of milk in towns was far higher than in PAs. Producers sold their products far cheaper than the market price. The camel milk markets did not draw resourceful individuals but are managed by individuals with close relationship with producers. Webster and

Morrison (2004) note that networks with low densities can be quite rigid and only those actors with sufficient status were able to negotiate their exchange relations. On the other hand, this low density network reduces the spread of milk-borne infectious agents to other nodes.

All major towns, Babille (6), Harer (5), Jijiga (3) and Dire Dawa (2) had high in-degree scores as they received milk from PAs for consumption. This showed that towns are important terminal markets for camel milk. Based on *degree centrality* measures, Aw-Sherif showed high in-degree and out-degree scores. It acted as a major site for milk collection and distribution. It will also have epidemiological importance once a disease that can be transmitted through milk (brucellosis, tuberculosis) has been introduced into areas that are supplied by Aw-Sherif; it can then likely facilitate the spread to other parts of the network. Aznar *et al.* (2011) indicated that nodes with high *degree centrality* measures could spread diseases through animal movements. Christley *et al.* (2005) emphasized the importance of degree measures in predicting risk of infection.

Network centralization for out-degree and in-degree was 15.1% and 19.1%, respectively. This indicated the presence of a substantial concentration or centralization in this whole network. The powers of individual actors varied rather substantially and this meant that overall positional advantages were rather unequally distributed in this network. Degree centrality measures have important implications for disease surveillance and control (Christley *et al.*, 2005).

In the present network, *closeness centrality* scores showed that all the towns and two PAs (Erer Ibada and Aw-Sherif) had higher scores. *Closeness centrality* measures nodes direct and indirect ties. Hence, these nodes could get in touch with others fairly quickly. For example, increase in demand in towns could be easily reached to producer nodes. On the other hand, many of the PAs did not have quick access to other nodes as they were peripherally located.

The milk markets network showed a very low *betweenness centrality* measures, ranging between 0 to 20, indicating that few nodes had positional advantage or power. The *betweenness centrality* idea is that actors who are "between" other actors, and on whom other actors must depend to conduct exchanges (Hanneman and Riddle, 2005). In this

network many PAs depended on Aw-Sherif and other PAs with some relevant value of *betweennes centrality* scores to trade with towns. The majority of the PAs and towns were either suppliers or terminal markets that were located at the periphery of the network. In the event of identifying infectious diseases spread by milk, betweenness scores provide useful information by identifying those PAs 'controlling' the flow of milk through the network. Baptista *et al.* (2008) suggested that if a node with high *betweenness centrality* is removed from the network, spread of infection might be more reduced than if a node with a low score is removed.

6. CONCLUSIONS AND RECOMMENDATIONS

The study has shown the occurrence of *Brucella* antibodies in 2.4% of the dromedaries examined in Babille Woreda of Eastern Oromiya Zone. Despite the fact that this seroprevalence rate is low, the tradition of raw camel milk consumption increases the transmission rate to humans with individuals working in the traditional camel production and marketing being at greater risk of contracting occupationally acquired brucellosis.

Sub-clinical mastitis prevalence rates of 14.4% at quarter, 31.6% at individual animal and 73.5% at herd levels were recorded indicating the significance of sub-clinical mastitis in the study area. Camels are important milk producers in arid lands and camel milk is an essential food and means of earning income for herders. Thus, mastitis significantly reduces milk production, affecting food security and pastoral households.

In response to increasing camel milk demand in urban areas, commercial camel milk marketing has been flourishing in the study area. This study characterized network structures of camel milk markets in Babille and other towns. Characterization of the camel milk market revealed how market network structures might be associated with the potential spread of diseases via milk and consequently could help identify effective control strategies. Moreover, analysis of the network provided information on the marketing, providing insights into the opportunities and constraints faced by market players.

Based on the results of the present study the following recommendations are given

- The presence of camel brucellosis in the study area calls for the design and implementation of appropriate control and prevention measures. Such control and prevention measures should be based on epidemiological investigations of brucellosis also in other livestock species.

- The causative agent of camel brucellosis has not been identified so far in Ethiopia. Further research works with the aim of identifying the species and biotypes of *Brucella* organisms should be undertaken.

- The low level of awareness on brucellosis and sub-clinical mastitis should be addressed through public health education and training in improved husbandry practices and disease prevention.

- Camel milk markets network should be investigated further and over a longer period to better understand the dynamics of the markets.

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8. APPENDICES

Appendix 1: Sample collection format - brucellosis study

Date of sample collection _____ Sample code _____
Owner's name _____ Education:_____
Address: _____
Location:_____ Herd Code:_____

Individual Camel Record

1. Age:_____ Sex:_____ Breed(if any):_____
2. Status of Camel: (suckling, weaned, adolescent, adult, pregnant, *repeat breeder)_____
3. Source of Camel: (birth till now, purchase):_____
4. Breeding Records: Parity:_____ Calving date:_____
5. Abortion(History):_____; Status of last offspring: ___Full term; ___Aborted
6. History of Retained fetal membrane (RFM):_____

Herd Information

1. Herd size:_____
2. Herd Composition: Camel:_____ Cattle:_____ Goats:_____ Sheep:_____
3. Purpose of keeping camels:_____
4. Contact with other herds: Yes:_____ No:_____
5. Restocking: Sheep_____; Goats_____; Cattle_____: Camels_____
6. Management system: (feeding, watering, housing, etc.)_____
7. Access to veterinary services: _____
8. History of Abortion: Yes:_____ No:_____

Remark:_____

9. History of RFM:_____
10. Cause of abortion:_____
11. Treatment of abortion:_____
12. Knowledge of transmission of abortion:_____
13. Management of abortion:_____
14. Disposal of aborted fetus/fetal membrane: _____

*Repeat breeder animal is a sub-fertile animal which mated three or more times during the proper period and does not become pregnant and continually return to service in the absence of any obvious pathological disorder in the genital tract and has normal estrous cycles (El-Khadrawy *et al.*, 2011).

Appendix 2: Sample collection format- mastitis study

Date of sample collection: _____ Sample code: _____

Owner's name: _____ Address: _____

Animal identification: Age: _____ Calving date: _____ Parity: _____

Previous History of udder problem: Yes _____ No _____

Physical examinations of the udder:		
Conformation:	Even: _____	Uneven: _____
Teat lesion:	Present: _____	Absent: _____
Tick infestation:	Present: _____	Absent : _____
Blind teats	Present _____	Absent: _____
If present	FL: _____ FR: ___ RL: ____ RR: ____	

Gross milk quality:			
Watery: _____	blood tinged: _____	clots/flakes: _____	Normal: _____
CMT score (0, T, 1, 2, 3):			
FL : _____	FR : _____	RL : _____	RR : _____
Form of mastitis:			
Chronic: _____	Clinical: _____	Sub-clinical: ____	

Remarks: _____

Appendix 3 : General questionnaire - mastitis study

Date : _____ Sample Code : _____
Location : _____
Owner's name: _____ Address : _____

1. Herd size
   Number of species owned
   Sheep:_____ Goats: _____ Cattle:_____ Camels: _____ Equine: _____
2. Purpose of keeping camels:_____
3. The main constraints for camel rearing
   Feed shortage:_____ Water Scarcity:_____ Diseases: _____

Others:_____

4. Presence of mastitis: Yes: _____ No: _____
   In how many camels_____
5. Cause of camel mastitis:_____
6. Type of treatment of camel mastitis:_____
   _____
   _____
7. Application of cauterization for udder problems: Yes: _____ No: _____
8. Use of anti-suckling devices: Yes: _____ No: _____
9. Tick control
   Manual removal:_____ Use of Acaricides: _____Other: _____ No control:_____
10. Frequency of milking per day
    Once :_____ Twice: _____ Three times:_____ Four times: _____
11. Cleaning the udder before milking: Yes: _____ No: _____
12. washing the hands before milking: Yes: _____ No: _____
13. Knowledge of Transmission of mastitis: _____
    _____
    _____
    _____
    _____

Remark:_____
_____
_____
_____

Appendix 4: Rose Bengal Plate Test (RBPT) procedure (OIE, 2009)

I. Bring the serum samples and antigen to room temperature (22 ± 4°C); only sufficient antigen for the day's tests should be removed from the refrigerator.

II. Place 25–30 µl of each serum sample on a white tile, enamel or plastic plate, or in a WHO haemagglutination plate.

III. Shake the antigen bottle well, but gently, and place an equal volume of antigen near each serum spot.

IV. Immediately after the last drop of antigen has been added to the plate, mix the serum and antigen thoroughly (using a clean glass or plastic rod for each test) to produce a circular or oval zone approximately 2 cm in diameter.

V. The mixture is agitated gently for 4 minutes at ambient temperature on a rocker or three-directional agitator (if the reaction zone is oval or round, respectively).

VI. Read for agglutination immediately after the 4-minute period is completed. Any visible reaction is considered to be positive. A control serum that gives a minimum positive reaction should be tested before each day's tests are begun to verify the sensitivity of test conditions.

Appendix 5: Complement Fixation Test (CFT) procedure (OIE, 2009)

The undiluted test sera and appropriate working standards should be inactivated for 30 minutes in a water bath at 60°C ± 2°C. If previously diluted with an equal volume of veronal buffered saline these sera could be inactivated at 58°C ± 2°C for 50 minutes.

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

II. Volumes of 25 µl of antigen, diluted to working strength, are added to each well except in the first row.

III. Volumes of 25 µl of complement, diluted to the number of units required, are added to each well.

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

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

VI. The results are read after the plates have been centrifuged at 1000 ***g*** for 10 minutes at 4°C or left to stand at 4°C for 2–3 hours to allow unlysed cells to settle. The degree of haemolysis is compared with standards corresponding to 0, 25, 50, 75 and 100% lysis. The absence of anti-complementary activity is checked for each serum in the first row.

VII. *Interpretation of the results:* Sera giving a titre equivalent to 20 ICFTU/ml or more are considered to be positive.

Appendix 6: CMT score, corresponding visible reaction and expected total somatic cell count

CMT score	Visible Reaction	Total Cell count (cells/ml)
Negative	The liquidity of the mixture remains unchanged	Up to 500,000
Weak/ Positive	The mixture turns mucus.	400,000 – 1.5 million
Positive	Unmistakable mucus formation. The mixture slows down.	800,000 – 5 million
Very Positive	The mixture turns into thick mucus, almost a jelly and lumps will develop.	Above 5 million

Appendix 7: Freeman's *degree centrality* measures

	Pas	OutDegree	InDegree	NrmOutDeg	NrmInDeg
7	Aw-Sherif	5.000	4.000	19.231	15.385
11	Tula	3.000	1.000	11.538	3.846
10	Berkele	3.000	0.000	11.538	0.000
9	Gemechu	2.000	2.000	7.692	7.692
15	Rahmata Selama	2.000	0.000	7.692	0.000
19	Iffa	2.000	0.000	7.692	0.000
13	Erer Ebada	2.000	4.000	7.692	15.385
8	Abdilkadir Arer	2.000	0.000	7.692	0.000
5	Sheik Hussien	2.000	0.000	7.692	0.000
3	Tulu Horo	1.000	0.000	3.846	0.000
4	Lokole	1.000	0.000	3.846	0.000
18	Erer Guda	1.000	2.000	3.846	7.692
14	Ebada Gemechu	1.000	0.000	3.846	0.000
20	Bishane Babille	1.000	0.000	3.846	0.000
2	Derera Arba	1.000	0.000	3.846	0.000
22	Gambella	1.000	0.000	3.846	0.000
17	Ifadin	1.000	1.000	3.846	3.846
21	Tewfik	1.000	0.000	3.846	0.000
6	Sheik Abdi	1.000	0.000	3.846	0.000
1	Abdigruch	0.000	1.000	0.000	3.846
12	Babille	0.000	6.000	0.000	23.077
16	Najata	0.000	0.000	0.000	0.000
23	Jijiga	0.000	3.000	0.000	11.538
24	Dire Dawa	0.000	2.000	0.000	7.692
25	Hargessa	0.000	1.000	0.000	3.846
26	Harer	0.000	5.000	0.000	19.231
27	Awedai	0.000	1.000	0.000	3.846

Descriptive statistics

		OutDegree	InDegree	NrmOutDeg	NrmInDeg
1	Mean	1.222	1.222	4.701	4.701
2	Std Dev	1.165	1.707	4.482	6.565
3	Sum	33.000	33.000	126.923	126.923
4	Variance	1.358	2.914	20.089	43.100
5	SSQ	77.000	119.000	1139.053	1760.355
6	MCSSQ	36.667	78.667	542.406	1163.708
7	Euc Norm	8.775	10.909	33.750	41.957
8	Minimum	0.000	0.000	0.000	0.000
9	Maximum	5.000	6.000	19.231	23.077
10	N of Obs	27.000	27.000	27.000	27.000

Network Centralization (Outdegree) = 15.089%

Network Centralization (Indegree) = 19.083%

Appendix 8: ***Closeness centrality*** **measures**

Method: Geodesic paths only (Freeman Closeness)

	Pas	inFarness	outFarness	inCloseness	outCloseness
26	Harer	298.000	702.000	8.725	3.704
23	Jijiga	399.000	702.000	6.516	3.704
24	Dire Dawa	525.000	702.000	4.952	3.704
12	Babille	546.000	702.000	4.762	3.704
13	Erer Ebada	548.000	650.000	4.745	4.000
27	Awedai	576.000	702.000	4.514	3.704
25	Hargessa	576.000	702.000	4.514	3.704
7	Aw-Sherif	598.000	572.000	4.348	4.545
9	Gemechu	650.000	625.000	4.000	4.160
18	Erer Guda	650.000	676.000	4.000	3.846
11	Tula	676.000	599.000	3.846	4.341
1	Abdigruch	676.000	702.000	3.846	3.704
17	Ifadin	676.000	676.000	3.846	3.846
3	Tulu Horo	702.000	551.000	3.704	4.719
2	Derera Arba	702.000	551.000	3.704	4.719
15	Rahmata Selama	702.000	600.000	3.704	4.333
4	Lokole	702.000	551.000	3.704	4.719
6	Sheik Abdi	702.000	676.000	3.704	3.846
16	Najata	702.000	702.000	3.704	3.704
8	Abdilkadir Arer	702.000	576.000	3.704	4.514
21	Tewfik	702.000	651.000	3.704	3.994
22	Gambella	702.000	676.000	3.704	3.846
10	Berkele	702.000	524.000	3.704	4.962
5	Sheik Hussien	702.000	525.000	3.704	4.952
19	Iffa	702.000	625.000	3.704	4.160
20	Bishane Babille	702.000	676.000	3.704	3.846
14	Ebada Gemechu	702.000	626.000	3.704	4.153

Statistics

		inFarness	outFarness	inCloseness	outCloseness
1	Mean	637.852	637.852	4.239	4.116
2	Std Dev	100.610	60.986	1.073	0.417
3	Sum	17222.000	17222.000	114.466	111.131
4	Variance	10122.423	3719.237	1.152	0.174
5	SSQ	11258390.000	11085504.000	516.374	462.115
6	MCSSQ	273305.406	100419.406	31.098	4.703
7	Euc Norm	3355.353	3329.490	22.724	21.497
8	Minimum	298.000	524.000	3.704	3.704
9	Maximum	702.000	702.000	8.725	4.962
10	N of Obs	27.000	27.000	27.000	27.000

Appendix 9: Freeman *betweenness centrality* measures

	Pas	Betweenness	nBetweenness
7	Aw-Sherif	20.000	3.077
13	Erer Ebada	6.500	1.000
9	Gemechu	4.500	0.692
11	Tula	2.500	0.385
18	Erer Guda	2.000	0.308
17	Ifadin	0.500	0.077
6	Sheik Abdi	0.000	0.000
2	Derera Arba	0.000	0.000
3	Tulu Horo	0.000	0.000
10	Berkele	0.000	0.000
8	Abdilkadir Arer	0.000	0.000
12	Babille	0.000	0.000
1	Abdigruch	0.000	0.000
14	Ebada Gemechu	0.000	0.000
15	Rahmata Selama	0.000	0.000
16	Najata	0.000	0.000
4	Lokole	0.000	0.000
5	Sheik Hussien	0.000	0.000
19	Iffa	0.000	0.000
20	Bishane Babille	0.000	0.000
21	Tewfik	0.000	0.000
22	Gambella	0.000	0.000
23	Jijiga	0.000	0.000
24	Dire Dawa	0.000	0.000
25	Hargessa	0.000	0.000
26	Harer	0.000	0.000
27	Awedai	0.000	0.000

Descriptive statistics for each measure

		Betweenness	nBetweenness
1	Mean	1.333	0.205
2	Std Dev	3.967	0.610
3	Sum	36.000	5.538
4	Variance	15.741	0.373
5	SSQ	473.000	11.195
6	MCSSQ	425.000	10.059
7	Euc Norm	21.749	3.346
8	Minimum	0.000	0.000
9	Maximum	20.000	3.077
10	N of Obs	27.000	27.000

Network Centralization Index = 2.98%

9. CURRICULUM VITAE

PERSONNAL DATA

Name:	Hafez Mohammed Seid
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1991 – 1994	Tana Haik Comprehensive Secondary School
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WORK EXPERIENCES

2002 – 2003	Junior instructor, Kombolcha Technical Vocational and Educational College
2004 – 2006	Assistant Lecturer, School of Veterinary Medicine,
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2007	Lecturer, Faculty of Veterinary Medicine,
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DECLARATION SHEET

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

Name: Hafez Mohammed Seid

Signature:_____

Date of submission:_____

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

Dr. Maximilian Baumann _____

Dr. Mekonnen Hailemariam _____

Dr. Gizat Almaw _____