SERO-PREVALENCE OF HUMAN AND LIVESTOCK BRUCELLOSIS AND ASSOCIATED RISK FACTORS IN MARSABIT COUNTY, KENYA

A thesis submitted in partial fulfillment of requirements for the Masters of Science in Veterinary Epidemiology and Economics degree of University of Nairobi (MVEE)

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DECLARATION

This thesis is my original work and has not been submitted before for any degree or examination in any other university.

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I dedicate this thesis to my wife Loise Karungari, my son Bill Kahariri and my daughter Lisa Wamunyu for persevering my long absence from home during the work.

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ACRONYMS

KAPs	Knowledge Attitude and Practices
B. abortus	*Brucella abortus,*
B. Melitensis	*Brucella Melitensis*
B. suis	*Brucella suis*
B. Canis	*Brucella Canis*
B. ovis	*Brucella ovis*
B. Neotomae	*Brucella Neotomae*
CAT	Card Agglutination Test
RBPT	Rose Bengal Plate Agglutination Tests
CFT	Complement fixation test
ELISA	Enzyme-Linked Immunosorbent Assays
IgM	Immunoglobulin M
IgG	Immunoglobulin G
SAT	Serum agglutination Test
CO_2	Carbon dioxide
PCR	Polymerase Chain Reaction
WHO	World Health Organization
TMP-SMX	Trimethoprim-sulfamethoxazole ()
ST	Streptomycin,
OTC	Oxytetracycline
LA-OTC	Oxytetracycline long-acting,
IMI	Intra-mammary Infusion
HH	Households
GPS	Global Positioning System
KEMRI	Kenya Medical Research institute
ERC	Ethical Review Committee
ACUC	Animal Care and Use Committee.
NaCl	Sodium Chloride
BSL-3	Biosesty level 3
AIC	Arkaike Information Criterion
CDC	Centre for Disease Control
PDA	Personal Data Assistant

ABSTRACT

Brucellosis is among the world's widest spread zoonotic diseases and recognized as a public health concern in both developed and developing countries. It is a bacterial zoonotic infection resulting in significant health and economic losses in Kenya. Human infection of brucellosis occurs only from contact with infected animals or animal products. There is limited information on the public health implication of brucella particularly in the pastoral areas of Kenya. Thus, the objectives of the current study were 1) To estimate the sero-prevalence of brucellosis in human and animals; 2) To determine risk factors associated with human sero-positivity; and 3) To study the knowledge attitude and practices (KAP) of the local community in relation to brucellosis transmission and control.

A cross-sectional survey was conducted within Marsabit County which represents a pastoral ecosystem. The study was conducted in a two-stage cluster sample whereby sub-locations and households were randomly selected. All persons living in the selected household were listed and three randomly selected. Sampling of livestock was conducted at the herd level where the maximum number of animals sampled per herd per species was fifteen animals randomly selected. Blood samples from the selected animal species (cattle, sheep, goats, camels) were tested for *Brucella* antibodies using an ELISA test.

A total of 227 households were selected. Blood samples were aseptically drawn from the selected human and animals. Thereafter, the samples were tested for *Brucella* immunoglobulin G (IgG) antibodies. Questionnaires were administered via personal interviews to the head of the study household. The human *Brucella* sero-prevalence was estimated at 44% and the animal sero-prevalence was; 16.09% in goats, 11.89% in sheep, 11.24% in cattle, and 11.14% in camels. The household and herd sero-prevalence was 73.13% and 68%, respectively. In univariate analysis, individual level factors that were associated with testing positive to Brucella antibodies included; male gender (OR=1.5, p=0.0049), age category (Youth (OR=2.2,

p=0.0022), Mid age (OR=2.4, p=0.0007), Old (OR=3.4, P=0.0001)), Education level (Primary (OR=0.4, p=3.02e-6), Secondary (OR=0.3, p=0.0045), Post-secondary (OR=0.2, p=0.0196)), Primary occupation (Student (OR=0.4, p=0.0036), Skilled workers (OR=0.2, p=0.0023)), Packed milk (OR=0.3, p=0.0023), consumption of animal milk (OR=1.6, p=0.0051), consumption of market milk (OR=0.4, p=0.0002), consumption of raw blood (OR=1.4, p=0.0391), handling hides and skins (OR=1.3, p=0.0490), Milking (OR=1.7, p=0.0008), herding (OR=1.5, p=0.0145), slaughtering (OR=1.8, P=0,0002), cleaning barns (OR=1.4, p=0.0419), assisting in delivery (OR=1.5, p=0.0033). In the multivariate analysis, the significant factors were; being a male (OR=1.8, p=0.0477), herding (OR=0.5, p=0.0365), primary occupation (student (OR=0.3, p=0.0009), skilled workers (OR=0.2, p=0.0021)), consumption of milk from the market (OR=0.5, p=0.0447), consumption of packed milk (OR=0.3, p=0.0035). At the household level, factors that were significantly associated with testing positive to *Brucella* antibodies in univariate analysis included; using milk from own animals (OR=0.1, p=0.0002), feeding aborted materials to dogs (OR=0.4, p=0.0039), assisting in delivery (OR=7.0, p=0.0058), keeping sheep (OR=2.3, p=0.0075), boiling milk before use (OR=0.3, p=5.968e-05). However, only 4 factors remained significant in multivariate analysis including; using milk from own animals (OR=0.2, p=0.0024), boiling milk before use (OR=0.4, p=0.0155), assisting in delivery (OR=5.4, p=0.0312), keeping sheep (OR=2.3, p=0.0151).

Although majority (85.5%) of the respondents said they knew about brucellosis, only a few could identify the disease by clinical signs in both man and animals. The vast majority (88.5%) engaged in practices that were likely to enhance *Brucella* transmission and thus spread. These practices included: assisting animals during birth, without protective clothing; consumption of raw milk; and feeding aborted fetuses to the dogs or throwing them in the environment leading to contamination. In conclusion, brucellosis is endemic in Marsabit County affecting both man and livestock. There is scarce knowledge of the disease in the study area. Thus, there is a need

for control and preventive strategies to be implemented in Marsabit. Such measures would include livestock vaccinations, education and public campaigns on how to control the infection.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Brucellosis is among the world's widest spread zoonotic diseases and recognized as a public health concern in both developed and developing countries. Its causative agents are in the genus *Brucella* (Gwida *et al.*, 2010). Brucellosis still remains one of the commonest public health and livestock production problems, especially in Kenya's pastoral communities. Global, morbidity of brucellosis is poorly understood. The disease is found in almost all continents and at least 500,000 people are infected annually (Karakas, 2013). In Africa, countries like Uganda and Eritrea have reported 5-48 new cases per million of the human population (Pappas *et al.*, 2006). *Brucella melitensis* is the most frequent cause of brucellosis in human worldwide (Pappas *et al*., 2006). In East Africa alone 21,104,976 cases of livestock brucellosis are reported annually (McDermott *et al.*, 2013). Human infection occurs only from exposure to infected animals or contaminated animal products.

Brucella suis is known to result in venereal infections in pigs (Díaz Aparicio, 2013). Brucellos is referred to as a herd or flock problem. The disease is spread within the herd mainly by ingestion or consumption of material contaminated with *Brucella* organisms. *In utero* (congenital) or perinatal infection may also occur, with the subsequent development of a latent infection. Spread of the disease among herds normally occurs by the introduction of chronically-infected animals that are not showing signs into a clean herd or flock. The organism mainly affects the sexually mature animals and its predilection sites are placentas, fetal fluids and testes in males (Zinsstag *et al*., 2005). The mode of transmission of the disease in animals is by contact (direct or indirect) with contaminated materials. Domestic and wild animals are

susceptible to *Brucella* infection and may function as carriers for other domestic animals (Radostits, 2000).

Mode of transmission of *Brucella* to humans is through the skin openings/cuts, direct or indirect contact with tissues, urine, vaginal discharges, blood, placentas and aborted fetuses (Al-Majali *et al*., 2009). Foodborne infection may occur following consumption of un-boiled milk among other dairy products, but consumption of uncooked meat from infected animals rarely transmits the infection (Maldonado *et al.*, 2014). Aerosol infection in abattoirs and laboratories is also possible (Ohishi *et al*., 2004). Human infections resulting from unintentional inoculation of live vaccines has also been reported. Congenital and venereal infections in humans are also possible (Al-Majali *et al*., 2009)

The first infection by *Brucella* in the reservoir host is usually preceded by abortions and subsequently other reproductive complications like infertility. In animals the infection is normally chronic if no treatment regime is undertaken. The *Brucella* organisms are shed in the environment by the infected animals through milk, colostrum, uterine discharges soon after abortion and subsequent parturition (Ofukwu *et al*., 2007).

The human *Brucella* infections have a variable incubation period which ranges from several days to several months. The major clinical signs and symptoms are mainly continued, intermittent or irregular fever of variable duration, profuse sweating, headaches, lethargy, depression, chills, and loss of weight (Lucero *et al*., 2010). The disease can take a different course depending on whether the patient has been adequately treated or not (Billard *et al*., 2005).

Laboratory diagnosis of clinical brucellosis in humans and animals is initially done serologically or by use of other immunological tests. Confirmatory diagnosis is done by bacteriological isolation and identification of the agent (Muendo *et al.*, 2012).

Designing an effective surveillance programme for brucellosis is hampered by the clinical picture of the disease. The disease is mainly chronic in animals and humans, clinical manifestations and incubation periods vary, and confirmatory diagnosis are rarely done. The association and link of human infection to the animal reservoirs is poorly understood. In areas where brucellosis is of highest importance, the populations of animal may be poorly identified, inaccessible for long periods and not enumerated (Robinson-Dunn, 2002). Earlier attempts to relate the variation in *Brucella* sero-prevalence to ecological system and risk factors have been done qualitatively. The estimated prevalence of the disease in Malindi, Kilifi County and Maralal, Samburu County in Kenya were 25% and 27%, respectively (Kadohira *et al*., 1997).

1.2 Justification for the study

Brucellosis continues to be a major animal and public health problem in many countries of the world especially where livestock are a major source of livelihood. This is despite the fact that the disease has been, or is close to being, eradicated from a number of developed countries.

In Marsabit County in Kenya, livestock contributes immensely to the livelihood of the residents of whom are predominantly pastoralists keeping mainly sheep, goats, cattle and camels. The high dependence on livestock makes people vulnerable to zoonotic diseases. There are many reasons why brucellosis may still remain endemic in Marsabit County, Kenya including and not limited to: the expansive livestock herds and flocks; uncontrolled livestock movements; inadequate veterinary support services; and vaccines and husbandry practices that increase the risk of infection. Human brucellosis cases occur due to consumption of un-boiled milk and other dairy products and close contact with infected animals. There is inadequate knowledge about the status of the disease and the associated risk factors in Marsabit County. In some cases, it's misdiagnosed or under reported, thus the aim of this study was to estimate the sero-prevalence of the disease in Marsabit County and assess the risk factors associated with human and livestock infections.

1.3 General objective

To estimate the sero-prevalence and assess the risk factors associated with human, and livestock Brucellosis in Marsabit County, Kenya.

1.4 Specific objectives.

1. To estimate the sero-prevalence of human and livestock brucellosis in Marsabit County, Kenya.
2. To identify the risk factors associated with human sero-positivity to brucellosis in Marsabit County.
3. To assess livestock owners' Knowledge, Attitude and Practices (KAPs) regarding brucellosis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition, Etiology and History of Brucellosis

Brucellosis is a zoonotic disease that is infectious and debilitating. It is caused by a gram-negative intracellular non-motile coccobacillus (Bricker *et al*., 2000), and one of the oldest diseases of man causing more than 500,000 new cases annually. Six main *Brucella* species have been classified and characterized according to the major reservoir: *Brucella abortus (*cattle*), B. suis* (pigs), *B. Neotomae* (fish), *B. Melitensis (*sheep and goats*), B. Canis* (dogs) and *B. ovis* (sheep and goats) (Whatmore *et al*., 2007). Although they are host-specific, they can transmit infection to other species of animal under favorable conditions (Kim *et al*., 2011)

Brucella suis and *Brucella abortus* infections may sometimes occur in small ruminants, but clinical manifestation seems to be rare. Infection caused by *B. ovis* arise in sheep in sub-Saharan Africa leading to epididymitis, orchitis, and infertility in rams (McDermott and Arimi, 2002). All these *Brucella* species cause disease in humans with *Brucella abortus* being the most frequently occurring (Bouaziz *et al*., 2010) while *Brucella melitensis* is the most important clinically in humans due to its severity (Adone *et al*., 2011).

In livestock, brucellosis is a major impediment to local and international trade for both livestock and livestock products. It results in losses arising from reduced productivity, abortions and weak offspring. The disease affects almost all domestic animal species. *B. melitensis* is the most virulent for humans and other hosts such as in cattle, sheep and goats. *B. melitensis* infections are especially problematic as the attenuated *B. abortus* cattle vaccine fails to protect from disease that it causes.

Although brucellosis has been controlled in many developed countries, it still remains endemic in several parts of the world, including the Middle East, Latin America, parts of Africa, Spain

and western Asia (Memish and Balkhy, 2004). The disease poses occupational hazard among herders, slaughterhouse workers, veterinarians, milk-industry professionals, and laboratory personnel (Ali *et al.*, 2013).

2.2 Epidemiology of Brucellosis

Previous studies in Kenya have reported a brucellosis prevalence range of between 5% - 45% in livestock as well as over 20% in humans in selected regions (Osoro *et al*., 2015). Risk factors for human brucellosis observed in one agro-ecological system cannot easily be extrapolated to another agro-ecological system where husbandry practices are different (Ogola *et al*., 2014). In humans, the disease manifestations include undulant fever, debility and generalized aching, whose duration vary from months or years.

In Kenya there is inadequate information and knowledge of brucellosis among the communities so that many cases go unrecognized and unreported (Osoro *et al*., 2015). However, brucellosis in human is rampant where extensive livestock production systems are practiced with almost a prevalence of 14% to 21% being documented (Ogola *et al*., 2014). The disease is frequently underreported due to the nonspecific clinical manifestations, the limitations of current diagnostic tests, low utilization of health care services, and the widespread use of non-prescribed antibiotics in many endemic countries (Ulu-Kilic *et al*., 2013).

Several studies in suspected brucellosis endemic areas in East Africa have assessed the antibody positivity among family members of brucellosis cases, and have found proportions ranging from 7.7% in Arusha, Tanzania to 21.2% in Narok, Kenya (Ogola *et al*., 2014)

In Kenya, hundreds of abortions that are associated with *Brucella* in livestock are reported every year, suggesting that brucellosis is a key source of morbidity and mortality (Ogola *et al*., 2014). The extent and rate of the disease transmission within and among herds increases with the frequent

illegal animal movements and the large herd sizes found in semi-arid areas where nomadic pastoralism is practised.

Pastoralism is use of grassland grazing for the purpose of livestock production and predominantly practiced in Africa, although it is also present in parts of Asia, South America and Europe pastoralism is characterized by high mobility and low population density. This mobility supports a population based on seasonal water and pasture availability, in regions where landscapes are less productive (Racloz *et al*., 2013).

2.3 Transmission of Brucellosis to Humans

Almost all the human brucellosis cases originate from animals (Makita *et al*., 2011). Shedding of large quantities of the bacteria occurs mainly at calving through the fetus, placenta and the uterine fluid. The shedding continues after an abortion or parturition, mainly through milk of infected cows. This becomes a consistent source of infection to humans for the entire lactation period (Mangen *et al*., 2002). Transmission among human and congenital infection have also been documented (Mesner *et al*., 2007). Exposure through skin breaks, direct contact with tissues, vaginal discharges, urine, blood, aborted fetuses or placentas are also possible routes of transmission of the disease.

The U.S.A has identified *Brucella spp* as a potential biological weapon (Pappas *et al*., 2006). In a theoretical scenario, it was projected that spread of *Brucella* in aerosols under optimal environments for spread would cause 82,500 infections and 413 deaths (Kaufmann *et al*., 1997) thus making it an excellent biological weapon. Laboratory-acquired *Brucella* infections are the flawless examples of airborne infection of the disease (Kutlu *et al*., 2014).

2.4 Risk factors Associated with Brucellosis Infection

Key risk factors for human brucellosis include: ingestion of unprocessed contaminated milk; exposure to infected animal and their products (Sharma *et al*., 2008); inhalation of infected

aerosols or splashes from infected material onto conjunctivae; increased contact with animals (farmers, animal handlers, abattoir workers, veterinarians); and laboratory workers. In addition, contact with aborted fetuses and consuming dairy products obtained from vendors or neighbors are risk factors for human brucellosis. Knowledge of the transmission mechanism of brucellosis is lowers the possibility of getting infected. This emphasizes the importance of awareness creation in prevention of brucellosis. Sheep, goats and their dairy products are the major sources of *Brucella* infection (Kansiime *et al*., 2014). Subsequently, the disease is an occupational hazard for livestock producers, veterinarians and employees in the animal products value chain business (Kutlu *et al*., 2014). Other infection sources include use of raw goat cheese and un-boiled milk (Maldonado *et al*., 2014). Human to human transmission of *Brucella* is very rare (Ruben *et al*., 1991).

2.5 Diagnosis of Brucellosis

2.5.1 History and Clinical Presentation in Humans

Brucella infection in humans can be challenging to diagnose as its presentation resembles many other conditions. In the early presentation (days to weeks after exposure), non-specific signs like fever, malaise, myalgias and arthralgia predominate (Karcaaltincaba *et al*., 2010; Jackson *et al*., 2014). Some patients proceed to develop focal complications, with osteoarticular complications being relatively common and very debilitating. Other patients may have chronic, mild symptoms for many months, making brucellosis difficult to recognize as the cause of their infection (Buzgan *et al*., 2010). A certain proportion of patients relapse, usually with non-specific symptoms, even after an extensive course of initial therapy. With all of these manifestations of brucellosis, accurate diagnostic testing is imperative (Dean *et al.*, 2012).

2.5.2 Diagnosis of Brucellosis in Humans

Almost all cases of human brucellosis originate from animals (Zinsstag *et al*., 2005). Thus, a thorough history eliciting details of appropriate exposures such as attending to or living with animals, possibility of exposure to contaminated animal products, and environmental exposures like improper disposal of aborted fetuses and other materials is a very important tool towards diagnosis of brucellosis (Tena *et al*., 2007). This can only be suggestive of the disease as the signs and symptoms are not pathognomonic (Bamaiyi *et al*., 2010). The signs include: fever or chills which occur in 53% to 100% of infections, and if left untreated can show an undulating pattern (Prasad *et al*., 2013), constitutional symptoms such as sweating, lethargy, and weight loss are a feature of infection in up to 97% of patients and gastrointestinal complaints in 80% of the patients (Corbel, 1997).

2.5.3 Diagnosis by serological tests

Serology has been commonly used for a probable brucellosis diagnosis, or for flock screening. The tests cannot differentiate reactions arising from *Brucella melitensis* infection from reactions due to other microorganisms, mainly *Yersinia enterocolitica*. In small ruminants, the buffered *Brucella* antigen tests, the complement fixation test (CFT), Rose Bengal Plate Agglutination Tests (RBPT) and the Card Agglutination Test (CAT) are the most frequently used serological tests. Indirect or competitive ELISAs could also be used (Stemshorn *et al*., 1985). The ELISA is also a technique that is gaining wide usage for diagnosis of Brucellosis (Fadeel *et al*., 2006).

In case of unvaccinated sheep and goats, the brucellin allergic skin test could also be useful in testing for *B. melitensis*. This is done by introducing the allergen into the lower eyelid, (Godfroid *et al*.,2002)

During advanced phases (the sub-acute or chronic phase) of brucellosis, it may be difficult to interpret the agglutination tests. Therefore, other tests must be conducted to confirm the results. Serum agglutination test (SAT) depends on presence of IgM which is low or absent in advanced phases of the disease. This also explains why the Serum Agglutination Test (SAT) is mainly negative throughout the incubation stage and after abortion (Mittal and Tizard, 1980).

2.5.4 Staining and Microscopy of *Brucella* Organisms

Microscopic examination of smears stained with the Stamp's modification of the Ziehl-Neelsen method can be used to give presumptive diagnosis. Direct examination can be complemented with serology. *Brucella* species, are not affected by weak acids. The organisms are coccobacilli, normally arranged singly although can occur in small groups or pairs. This is not a confirmatory test because various organisms that cause abortions like *Coxiella burnetii and Chlamydophila abortus* have some resemblance with *Brucella.* Similarly, *B. ovis* which causes orchitis and epididymitis in rams, also has some resemblance with *B. melitensis.* Immuno-staining is occasionally used to recognize *Brucella* in smear (Alton *et al*., 1975).

2.5.5 Culture of *Brucella* Organisms

Culturing the *B. melitensis* from an animal can give a conclusive diagnosis (Yi *et al*., 2014). Isolation of *Brucella* species can be done on various types of selective or plain media (Hornsby *et al*., 2000). The ideal samples to isolate *B. melitensis* from live animals are vaginal and preputial swabs and milk samples. Culturing of *B. melitensis* can also be done from aborted fetuses (spleen, lung and stomach contents) or the placenta (Moshkelani *et al*., 2011). The spleen, udder, mammary lymph nodes, inguinal lymph nodes and late pregnant or early post-parturient uterus are the most dependable samples to collect at post mortem. *Brucella* organism

culture can also be obtained from the testis, semen, epididymis, and hygroma or arthritis fluids (Khamesipour *et al*., 2013).

Brucella species are aerobic in nature. Some strains need an environment with at least 5-10% carbon dioxide (CO_2) for optimum growth to occur e.g. *B. abortus* wild type (biovars 1-4). Others, like *B. abortus* S19 vaccine strain, *B. abortus* wild type (biovars 5, 6, 9), *B. suis* and *B. melitensis*, do not require CO_2 for growth (Sun *et al*., 2005). The optimal pH varies from 6.6 to 7.4. For optimum growth to occur at about pH 6.8 culture media should be adequately buffered (Bricker *et al*., 2000).

To isolate *Brucella* in blood, milk and other body fluids, a non-selective, biphasic medium, called Castaneda's medium is used (Ewalt *et al*., 1983). The medium is more appropriate because *Brucella* dissociates in broth medium, hence interfering with biotyping by conventional bacteriological techniques. Identification of *B. melitensis* species and at biovars level can be achieved by phage typing, biochemical, serological, and cultural characteristics (Radwan *et al*., 1992).

2.5.6 Polymerase Chain Reaction (PCR)

Diagnosis of brucellosis by PCR is simple and accurate. Sensitivity and specificity of PCR provides a valuable and quick tool for diagnosis (Navarro *et al*., 2004) and danger to staff exposure is minimal such that, requirement for level three laboratory for containment is not mandatory and therefore cost is also reasonable (Yu and Nielsen, 2010). Real-time PCR conducted using the IS711-based assay was shown to be the most specific, sensitive, reproducible and efficient method to detect *Brucella spp* (McDonald *et al*., 2006; Qasem *et al*., 2015). False -negatives in PCR assays are rare and mainly occur due to amplification of the present polymerase inhibitors like hemoglobin, urine, heparin, phenol, and sodium dodecyl

sulfate hence accurate sampling techniques that minimizes contamination are critical (Navarro *et al*., 2004).

2.6 Treatment of Human Brucellosis

The important aspect in the management of various forms of human brucellosis is the use of the right antibiotics for the appropriate time period (Corbel, 2006). Generally, the recommended approach for acute cases of brucellosis in grown-ups is rifampicin 600mg to 900 mg and doxycycline 200mg daily for at least for six weeks (Corbel, 2006).

In individuals younger than 8 years, rifampin and trimethoprim-sulfamethoxazole (TMP-SMX) 6 weeks' period is the therapy of choice (Teker *et al*., 2014). In persons above eight years of age, 100mg of doxycycline twice a day for six weeks combined with 1gm streptomycin daily for two to three weeks is also adequate to treat uncomplicated cases (Corbel, 2006). It has also been suggested that the combination of aminoglycoside and doxycycline in addition to rifampicin may be a better option (Skalsky *et al*., 2008). In complicated brucellosis, 100mg of Doxycycline taken two times per day for six weeks with rifampicin 600 to 900 mg every day for six consecutive weeks could be adequate (Corbel, 2006).

2.7 Treatment of Animal Brucellosis

There is no effective and reliable treatment for animal brucellosis. A number of chemical agents have been used recently for management of brucellosis in cattle but have not been totally successful. Many chemical agents, trace elements, minerals and mixtures of vitamins (A and E), general antimicrobials (phenols or dyes), have been tried unsuccessfully (Olsen and Palmer, 2014). Use of antibiotics like sulfonamides or penicillin does not stop the shedding of the organisms from the udder discharges of diseased cows, in some cases it only led to short-term solution (Basdew and Laing, 2011).

The use of single or combined broad-spectrum antibiotics like aureomycin, terramycin, streptomycin (ST) and tetracyclines has ensued in the drop in numbers of abortions in individual cows or diseased herds (Solera, 2000). However, the treatment cost, the existence of the drug residues in animal products and the poor response for udder infections treatment, in various instances, have rendered the therapeutic options inappropriate for bovine brucellosis.

Following improvement of long-acting (LA) and Oxytetracycline (OTC), the use of these products alone or in a combination with ST eliminates the symptoms of this disease and minimizing the spread of *Brucellae* organism by diseased cows (Guerra and Nicoletti, 1986). Subsequently, these therapeutic regimens have been used to prevent abortions and decrease the spread of brucellosis in infected herds. However, complete cure has not been achieved by these regimens. A different study to assess the efficacy in a number of long-term therapeutic regimens making use of a combination of LA-OTC, ST and OTC intra-mammary infusion (IMI) in eliminating *B. abortus* or *B. melitensis* from cows that are naturally infected was undertaken (Radwan *et al*., 1992). The study showed the management regime to be relatively inexpensive, practical, effective and without side-effects in terminating the symptoms of the diseases and getting rid of the pathogen from diseased cows. This treatment could perhaps be only undertaken in high cost breeding animals due to the cost involved and the residues.

2.8 Control and Prevention

2.8.1 In Animals

Effective control programs must locate and contain the infection. This is done through testing schemes. The major component of the control and eradication program are: testing and slaughter of the infection reservoir and quarantine of the rest of the animals and depopulation in cases where all animals are exposed (Radostits *et al*., 2000). Vaccinations have been used to control the spread of brucellosis in animals but it does not eliminate the risk and therefore constitutes a perpetual infection risk to consumers of raw animal products (Muendo *et al*.,

2012). *Brucella abortus* strain 19 attenuated is most widely used. However, it is not recommended for use in bulls due to its potential to cause orchitis and epididymitis (Corbel, 2006).

2.8.2 In Humans

Effective and non-reactogenic vaccines for human brucellosis are not currently available and therefore, human vaccination is not recommended. To eliminate the risk of brucellosis, pasteurization is recommended for milk and milk products before human consumption. Strict hygiene practices are important in control of infection that is transmitted through contact. (Racloz *et al*., 2013)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study sites

The study was conducted in Marsabit County. The County is largely semi-arid to arid and is located in the northern part of Kenya (Fig 3.1). Livestock keeping is the main economic activity and livestock keepers practise pastoralism. It has a total human population of 291,166 people and a population density of 4 people per Km^2(CBA, 2014).

Marsabit County is in the former Eastern Province of Kenya and covers an area of 70,961.3 Km^2 (Ngene *et al.*, 2010). Ninety-two percent (92%) of the population live below the poverty line (WorldBank (E), 2014).

Due to the proximity of the County to Lake Turkana on the western side, the average amount of humidity in the area is estimated to be sixty-five percent all year round (Kirubi *et al.*, 2000). As a result of high temperatures coupled with low rainfall, most of the plant species that survive under these conditions are shrubs which have certain physiological features such as small leaves and deep roots (Reynolds *et al.*, 1999). The soils are generally not fertile in most parts of the county with low amounts of basic plant nutrients. This explains why the communities living in the area are nomadic pastoralists.

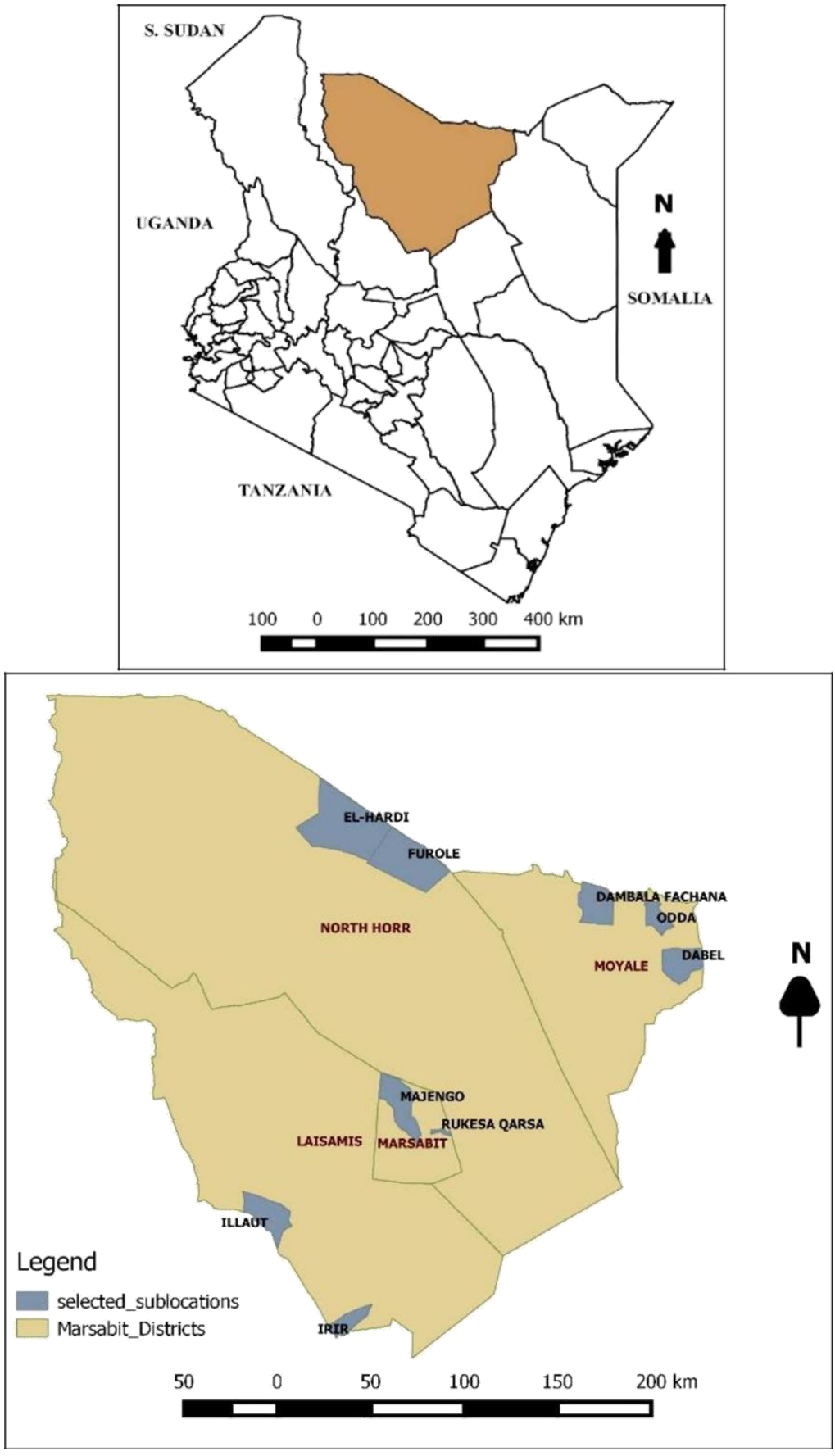

Figure 3.1 Map showing selected sub-locations in Marsabit, Kenya

3.2 Study Design and Sampling

A cross-sectional study was undertaken using a multi-stage sampling method. The study population were all persons aged 5 years old and above. The animal study population included cattle, sheep, goats and camels.

3.2.1 Selection of Sub locations and Households

Multi-stage sampling method was applied. Nine Sub-locations were randomly selected from the list of all the Sub-locations in Marsabit County as per the Kenya National Bureau of Statistics. For the purpose of this study, a household (HH) was a group of people who used a common cooking area. The HHs were then selected randomly in the selected sub-locations. In each selected Sub-location, random geographical coordinates were generated in ArcGIS corresponding to the number of HHs to be sampled. Sampling was then conducted in one household per geo-code within 5 km radius. The selected sub-locations and households are shown in Fig. 3.2.

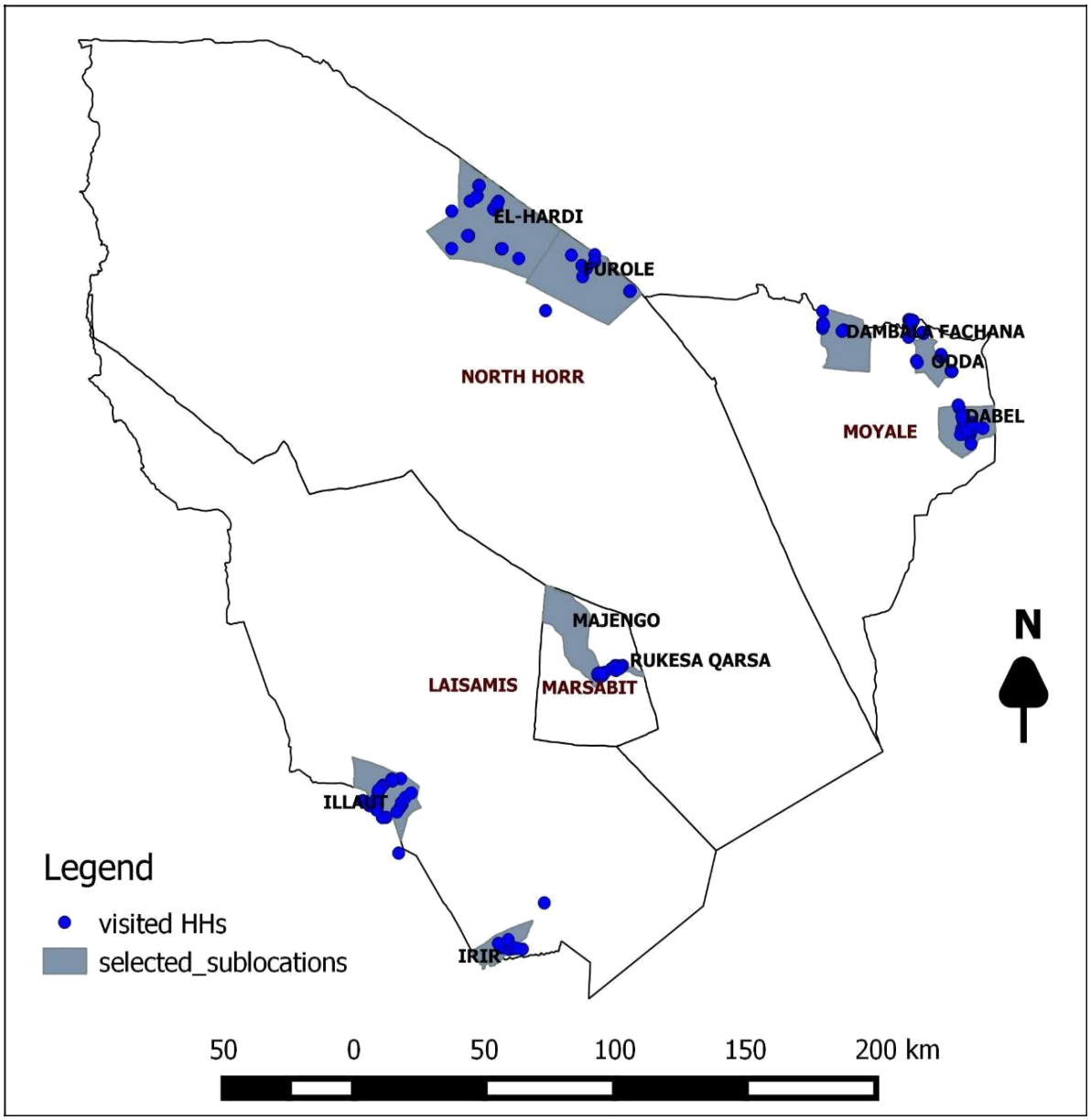

Figure 3.2 A map of showing study Sub-locations and Households in Marsabit County, Kenya

3.3 Identifying Households

A handheld Global Positioning System (GPS) receiver was used to navigate to the selected geocode. A household to be sampled was then randomly identified using 'spin the bottle' method (WHO, 2005). In this method, a pen or bottle was spun on a flat surface and the first household towards the direction where the mouth of the bottle or pen tip pointed to was selected.

3.4 Sampling of Humans and Livestock

In the selected households, a maximum of 3 persons were sampled. All persons living in the selected household including the herders were listed and three randomly selected.

The animals owned by the randomly selected households formed the herd. Random sampling of livestock was conducted at the herd level where the maximum number of animals sampled per herd per species was fifteen animals.

3.5 Sample Size determination

The minimum number of households/herds and individuals required for the study was determined using the formula in Dohoo *et al. (*2003):

$$n = \frac{Z\alpha^2\, pq}{L^2}$$

Where n = required sample size.

Zα = value of statistic that corresponds to a level of confidence of 95% (1.96)

P = A priori estimate of the prevalence (15%) of Brucellosis (Kadohira *et al.*, 1997)

q=1 −p

L= precision of the estimate set at 0.05 (5%)

With solution:

$$n = \frac{1.96^2 \times 0.15 \times 0.85}{0.05^2}$$

n = 196 human and animals per species

Adjusting for clustering (Dohoo *et al.*, 2003).

$$n' = n(1+ \rho(m-1))$$

Where n' = adjusted sample size

n = unadjusted sample size (196)

ρ = intra-household and herd correlation coefficient set at 0.04 (Correa *et al*., 2012)

M = average household size of five.

With the solution:

n'=196(1+0.04(5-1)

n'= 815 human samples (or 271 households)

n'= 815 animals per species (or 54 herds)

3.6 Blood Sample Collection

Bar coding labeling system was used to label all the cryovials and vacutainer tubes that were used in sampling both humans and animals. The bar code labels were pre-printed with the sample code to minimize writing errors and ink rubbing during shipping or storage. The sample codes were serialized such that each 5 labels bear the same code, e.g. Z08105225 for animals and H08105226 for human specimens. Care was taken to ensure that a matching label was fixed to the vacutainer tube (after blood collection), human tracking sheet (human) and/or animal tracking sheet (animals). The remaining two labels were transported and delivered to the laboratory with the sera zip-lock bag together with the sample collection form. The identity of the label was counter checked to ensure that it was identical to the forms and sample vials.

After restraining the animal, 10-15 milliliters of blood was drawn via venipuncture of the jugular vein using barcoded plain vacutainer tube. The vacutainer tubes were left to stand in a shade at ambient temperature (30°C) for approximately 15 minutes, then transported to the field laboratory where serum was separated on the same day by centrifuging at 12,000 revolutions per minutes to separate the cells and serum. A pasteur pipette was used to aliquot two milliliters of the separated serum into Barcoded 5 milliliters cryovials and kept at -80^0C for transportation to the diagnostic laboratory in Kabete. In humans 5 to 7 ml whole blood was drawn by a qualified technician via venipuncture of the median cubital vein using barcoded plain EDTA vacutainer tubes. The sample was allowed a minimum of 15 minutes for clot formation. Human sera were shipped to the Kenya Medical Research Institute

(KEMRI)/Centers for Disease Control and Prevention (CDC) laboratory in Kisumu while the animal sera were transported to the Central Veterinary Laboratory in Kabete for serology work.

3.7 Data Collection

Data collection was done using a standardized questionnaire (Appendix 1) in Personal Digital Assistant (PDA) and geographical coordinates of the sampled households recorded using GPS receivers. The structured questionnaire was administered via personal interviews to the household head. The data collected were on potential risk factors associated with brucellosis, household history of brucellosis, education level, socio-economic status, herd management and demographic characteristics. The questionnaire also assessed the knowledge, attitude and practices of the community towards brucellosis.

3.8 Laboratory Procedures

Diagnosis of *Brucella* spp. was based on serological tests. Tests on human samples were done using the IBL-America IgG ELISA kits while Svanova Biotech AB (Uppsala, Sweden) ELISA kits were used in testing animal samples. The SVANOVIR® *Brucella*-Ab I-ELISA test kit was used on bovine sera and the SVANOVIR® *Brucella*-Ab C-ELISA test kit on camel, Caprine and ovine sera. The 96-well plates pre-coated with either inactivated *Brucella* antigens or antibody.

3.8.1 Preparation of Human Serum Samples and Reagents

All reagents were allowed to settle at 18- 26^0C and mixed by gentle swirling or vortexing before use. The PBS- Tween Solution 20X concentrate 1:20 was diluted in distilled water. Then 500 mL per plate was prepared by addition of 25mL PBS- Tween solution to 475mL distilled water and thoroughly mixed to ensure dissolution of any precipitated salts. All other reagents provided in the kit (microtest strip, conjugate, substrate and stop solution) were ready to use as per the manufactures instructions. Freeze-dried mouse monoclonal antibody was reconstituted with 6mL

Sample Dilution Buffer, carefully added into the bottle and gently mixed. The laboratory equipment's required were also set for delivering and aspiration of wash solution, microplate cover (lid, aluminum foil, adhesive) vortex mixture and Microplate mixture, disinfectant (Dettol or 70% ethanol), paper towels for tapping to remove wash buffer) in readiness to start ELISA assay.

3.8.2 Procedure for Animal Sera

Five µl of diluted PBS Buffer was added into every well of smooth lipopolysaccharide (S-LPS) coated wells on micro titer plates which would be used for samples, control and conjugate controls. Then 45µl of positive, weak and negative serum control was then added into each of the appropriate wells, respectively. Sample Dilution Buffer (5 µl) was added into two wells designated as Conjugate Control after which 5µLof the test sample was added to each of the appropriate wells. Then 50µL of mouse monoclonal antibody solution was added into all the wells used for control and test samples. The pre-diluted samples, positive and negative controls was dispensed into the suitable wells of the microtiter plate after mixing thoroughly by pipetting up and down severally. The content of the wells were mixed by shaking gently using a microtiter plate shaker, so that the antigens or antibodies passively attaches to the solid phase after incubation.

The microtiter plate was covered using the plastic adhesive seals and incubated in a humid chamber at 37°C for 30 minutes to bind the antigen to the antibodies if present in the coated plate. All the liquid content was emptied from the micro-wells. Each well was washed with approximately 300µl of wash solution thrice by overflowing and emptying of the wells using buffered solution to detached bound (reacted) from unbound reagents, without drying the plate between washes and before adding the next reagent. Finally, the residual wash fluids from each plate were firmly tapped into absorbent material to remove the wash solution as much as possible. To each well 100µl of goat anti-mouse horseradish peroxidase (HRP) conjugated IgG

was added and the plate incubated in a humid chamber 37°C for 30 minutes. The liquid content from the micro-wells was emptied completely, washed with 300µl of wash solution thrice by simply overflowing and emptying of the wells using PBS-Tween buffer to separate bound from unbound reagents. Subsequently, the residual wash solution was tapped firmly from each plate into absorbent material and 100µl substrate dispensed into each well, the o-Phenylenediamine (OPD) substrate was incubated at 18-26^{0}C for 15 min (RT±1min) to satisfactorily produce a blue color reaction. Then 50µl of stop solution was added to each well.

The stop solution halts the reaction between enzyme and substrate resulting to darkbrown colour development. The results were measured using a microplate-photometer (plate reader machine) at a wavelength of 450nm within 15min after addition of Stop Solution to avoid fluctuation of Optical Density (OD) values. All samples were run in duplicates.

3.8.3 Interpretation of Results

The mean OD values were calculated for each of the control and test samples. The percent inhibition (PI) values for the control and test samples were calculated by use of the following formula:

$$\mathbf{PI=\frac{100-OD_{\text{samples or control}}}{OD_{\text{conjugate control (Cc)}}}\times 100}$$

To ensure validity, the PI value of the controls had to be within the ranges below:

OD Cc	= 0.75- 2.0%
PI Positive control	= 80-100%
PI Weak positive control	= 30-70%

The status of test sample was determined as follows;

PI	Status
<30%	Negative
≥ 30%	Positive

3.8.4 Procedure for Testing Human Samples

Dilution of human sera at 1:101 with the kit sample diluent was done then added to microtiter plates pre-coated with *Brucella* (*Brucella abortus*, strain W99; lysate of a NaCl extract) antigen. All sera and controls were run in duplicates. This was incubated at room temperature for 1hr after which the plates were washed, conjugate then added and incubated for 30min. Following a wash cycle, substrate was added and incubated for 20min. The conjugate-substrate reaction was terminated by the addition of a stop solution. Optical densities (ODs) for the samples were read at 450 nm.

Calculations

Calculation of Percent Positivity Values (PP)

All optical density values for the test samples and the Negative Controls (NC) were linked to the OD value of the positive control as follows:

$$PP = \frac{OD_{\text{sample or Negative control}}}{OD_{\text{positive control}}} \times 100$$

To guarantee validity, the duplicate optical density values of the positive control must not differ beyond 25% from mean of the two duplicates. The limits of the control values are as shown

OD	Positive Control	> 1.0
PP	Negative Control	< 10

The test sample results were interpreted as follows:

Sample material	**PP**		**Interpretation**
Serum sample	<	10	Negative
	≥	10	Positive

In case of doubt, the sample was re-tested.

3.9 Biosafety Measures

The following biosafety measures were adhered to:

1. Diagnostic specimens were handled in BSL-2 conditions. Work was performed in accordance with BSL-2 conditions, under a licensed BSL-2 safety cabinet. The blood tubes/tissue/organism did not leave the hood unless contained in a vessel. The laboratory personnel wore BSL-3 attire, including laboratory coats, bonnets, boots, double gloves, and N95 masks.
2. All *Brucella* positive specimens for culture were forwarded to the KEMRI/CDC BSL 3 laboratory. No aliquots were stored in BSL-2 laboratories.

3.10 Data Handling and Analysis

Data were cleaned in excel before being imported to R statistical software version 3.0.2 for analysis. Descriptive statistics were generated using the same software. The Knowledge, Attitude and Practices (KAP) analysis was presented as proportions, frequencies and means. The brucellosis sero-prevalence was determined by dividing the total number of positive tests

by the total number of samples tested. Univariate and multivariate logistic models were used to assess the association between human brucellosis infection and the risk factors. The response variable used was the serological test result. The apparent sero-prevalence was determined at household, herd and individual level. Any household where at least one person was seropositive was defined as a seropositive household. A seropositive herd was any herd with at least one animal seropositive.

The independent variables for risk factors of brucellosis in univariate analysis comprised the socio-economic and demographic variables that included level of education, age, primary occupation and gender. The animal-related human factors that were analyzed included: animal contact, livestock ownership, working with hides/skins, symptoms experienced within the last year, milk and meat consumption habits and contact with manure and other byproducts. Animal herd characteristic factors analyzed included age, breed, grazing and breeding systems.

Univariate analysis was done for each potential explanatory risk factor. Thereafter, multivariable models were developed by backwards elimination procedure. Starting with all potentially significant variables, explanatory variables were sequentially dropped if their effect on the model was not significant ($p>0.2$). Multivariate analysis was conducted at individual level. The model included all the significant variables (p-value $\leq$ 0.2) from the univariate analysis. The model with the lowest Arkaike Information Criterion (AIC) was considered as the most parsimonious. The goodness-of-fit of the final model was then tested using residual deviance chi-square.

3.11 Ethical Considerations

Ethical approval and clearance was sought and obtained from KEMRI Ethical Review Committee (ERC) and Animal Care and Use Committee (ACUC) of University of Nairobi.

Other approvals were obtained from the Ministry of Agriculture Livestock and Fisheries and the Ministry of Health. In each of the selected households, consent was sought from the household head/ or any eligible adult to allow for sampling of animals and access to household members to obtain individual consent. Consent (Appendix 2) was obtained from all participants in the study after being informed about the study. For minors, assent was obtained from the minor as well as informed consent from the guardian or parent. No personal identifiers were collected. All smart phones used for data collection was password protected and kept in a locked area with restricted access. All records in the smart phones were downloaded into a password secured Microsoft Access database every evening.

CHAPTER FOUR

4.0 RESULTS

4.1 Household Demographics and Characteristics

A total of 227 households were visited and included in the study. The average household size was five people and 89% of household members consumed milk from their animals. A high proportion (92%) of household members consumed unpasteurized milk. A vast majority (87%) of households disposed aborted fetuses and placenta from animals inappropriately by feeding to dogs and leaving it on the pastures. Almost all the surveyed households (88.5%) practised nomadic pastoralism and the rest, particularly those from the arable part of Marsabit, were Agro-pastoralists.

A total of 755 individuals from the 227 out of the projected 271 households participated in the study giving a response rate was 84%. Slightly more than a half (50.1%) of the respondents were males and the rest were females (Table 4.1). The average age was 35years. Sixty-eight percent of the respondents had no formal education while only 5.28% of the respondents had formal education beyond the primary school level. The primary occupation of 50.3% of the respondents was livestock farming (Table 4.1).

Table 4.1 Demographic characteristics of human respondents in sampled households, Marsabit County, 2013

Variable	Level	Number	Proportion (%)
Sex			
	Female	378	49.9
	Male	377	50.1
Education Level			
	No education	511	67.7
	Primary	190	25.2
	Secondary	33	4.4
	Post-Secondary	16	2.0
	Other	5	0.7
Occupation			
	Works Farm/Farmer	380	50.3
	Salaried off farm skilled	42	5.56
	House wife	56	7.4
	Salaried skilled workers	88	11.7
	Student	172	22.8
	Others	17	2.3

4.2 Sero-prevalence of Brucellosis

4.2.1 Brucellosis Sero-prevalence in Humans

Three hundred and thirty-two people out of 755 tested positive for *Brucella* antibody equivalent to a sero-prevalence of 44%. There were variations in the brucellosis sero-prevalence according to sub-locations ranging from 60% in Furole Sub-location to 25% in Gurumesa. (Table 4.2).

Table 4.2 Distribution of sero-prevalence of human brucellosis by sub-location in Marsabit County, 2013

Sub location	No. tested	Number positive	Proportion (%)Positive
Dabel	114	64	56.1
Dambala Fachana	30	17	56.7
El Hadi	66	38	57.6
Furole	40	24	60
Gurumesa	97	24	24.7
Illaut	72	41	56.9
Irir	31	15	48.4
Majengo	118	40	33.9
Odda	81	30	37.0
Rukesa Qarsa	106	39	36.8
	755	332	44.0

Seventy-three percent (73%; 166/227) of the sampled households had at least one member testing positive for *Brucella* antibodies for a household prevalence of 73% and 14% of the seropositive households had all three individuals sampled testing positive.

4.2.2 Brucellosis Sero-prevalence in Animals

A total of 5444 animal serum samples were tested of which 734 (13.6%) were positive for *Brucella* antibodies. There were no statistical differences in the sero- prevalence of the four animal species tested (Table 4.3). Out of the 277 herds tested 189 had at least one animal testing positive for a herd prevalence of 68.2%.

Table 4.3 Sero-prevalence of Brucella antibodies in animal species sampled in Marsabit County, 2013

Species	No. Tested	No. positive	Proportion (%) Positive
Bovine	712	80	11.2
Ovine	1472	175	11.9
Caprine	2380	383	16.1
Camel	880	98	11.1
Total	**5444**	**736**	

4.3 Risk Factors Associated with Human Sero-positivity to Brucellosis.

4.3.1 Individual - Level factors

4.3.1.1 Univariate Analysis

Univariate analysis was done for each potential explanatory risk factor in sampled humans against individual predisposing factors as shown in Appendix 3. Gender, age category, education level, primary occupation, consuming packed milk, consuming milk from own animals, consuming milk from the market, consuming undercooked or uncooked blood, handling hides, milking, herding, slaughtering, cleaning barns and assisting in delivery were significantly ($p< 0.05$) associated human sero-positivity to brucellosis. The old people were more likely (OR=3.3571) to test positive to brucellosis compared to adolescents, youth and middle aged people. People who consumed milk from their own animals or raw blood also had higher risk (OR=1.613, 1.4331, respectively) of testing positive. Herders who were in close contact with livestock (those who milked, slaughtered animals, cleaned barns, assisted delivery and handled fresh hides) were at higher risk (OR=1.7, 1.8, 1.4, 1.5, 1.3, respectively) of exposure to the Brucella pathogens. On the other hand, any form of formal education and skilled farm labour reduced chances of exposure to Brucella pathogen. Consumption of packed milk as opposed to consuming unprocessed milk also reduced exposure (Appendix 3).

Students were less likely (OR=0.3984) to test positive to *Brucella* compared to people in other occupations. Similarly, people who had formal education were less likely to test positive to *Brucella* relative to those with no education. People who drunk packed milk and those who bought their milk from the market were less likely to be infected with brucellosis.

4.3.1.2 Multivariate analysis

In multivariate analysis, only five factors were associated with brucellosis seropositivity including sex, herders, occupation, whether milk for consumption is pasteurized or not (Table 4.4) This was an indication that the univariate results were confounded by either some measured or unmeasured variables. Males were at a higher risk of testing positive to *Brucella* (OR=1.8) relative to females. Surprisingly, herders were 0.5 time less likely to seroconvert relative to non-herders (Table 4.4). Students and off farm skilled workers were also less likely to sero convert relative to housewives (OR 0.3, 0.2, respectively) Similarly, those who drunk milk bought from the market and those who bought pasteurized milk (in packets) were at lower risk of testing positive to *Brucella* antibodies (Table 4.4).

Table 4.4 Multivariate analysis of individual level factors and their association with brucellosis sero-positivity in Humans in Marsabit County, 2013

Variable	Estimate	Odds Ratio	Confidence intervals (95%)		P value
			Lower	Upper	
Gender (ref=female)					
Male	0.5953	1.8136	1.0109	3.2932	0.0477
Herding (ref=no)					
Yes	-0.6412	0.5267	0.2857	0.9542	0.0365
Occupation (ref=housewife)					
Student	-1.1864	0.3053	0.1507	0.6134	0.0009
off farm skilled	-1.6044	0.2010	0.0692	0.5435	0.0021
Market milk (ref=no)					
Yes	-0.6227	0.5365	0.2880	0.9753	0.0447
Packed milk (ref=no)					
Yes	-1.3670	0.2549	0.0925	0.5988	0.0035

4.3.2 Household level factors

4.3.2.1 Univariate analysis

In the univariate analysis of household level factors, assistance of the livestock by household members during delivery and keeping of sheep together with other livestock, were positively associated with sero-positivity to *Brucella*. Households where assistance during delivery was practised were 7 times more likely to have at least one-member positive to *Brucella* antibodies relative to those where assistance during delivery was not given (OR=7.04; Table 4.5) Similarly, household with sheep were 2.3 times more likely to have a household member testing positive to *Brucella*. Three household factors were negatively associated with testing positive to *Brucella* antibodies including those who drunk milk from their own animals (OR=0.13), those who did not handle and fed aborted fetuses to dogs (OR=0.41), and those who boiled milk before drinking (OR=0.26; Table 4.5)

Table 4.5 Univariate analysis of factors associated with sero-positivity to Brucella at household level in Marsabit County, 2013

Variable	Estimate	Odds Ratio	Confidence intervals (95%)		P value
			Lower	Upper	
Use milk-own animals					
(ref=No)					
Yes	-1.9772	0.1385	0.04648706	0.3700407	0.000145
Feeding aborted materials to dogs					
(ref=no)					
Yes	-0.8834	0.4134	0.2256842	0.7519644	0.00391
HH member assist delivery					
(ref=no)					
Yes	1.9521	7.0432	1.8873	33.5636	0.0058
Keeping sheep					
(ref=no)					
Yes	0.8145	2.2580	1.2479	4.1333	0.00749
Boil milk before use					
(ref=no)					
Yes	-1.3652	0.2553	0.1306	0.4953	5.968e-05

4.3.2.2 Multivariate analysis

Of the five household factors that were significant in univariate analysis, four remained significant in the final multivariate logistic model (Table 4.6) The factor “feeding fetuses to the dog” lost its significance indicating its association with *Brucella* positivity was confounded by another factor most likely the factor “assisting in delivery” because its OR changed dramatically from 7 (Table 4.6) in univariate analysis to 5.4 (Table 4.6) in the multivariate analysis. This was not surprising because both factors involved handling of aborted fetuses and both of the practices leads to the exposure to the *Brucella* organisms. The ORs of the others factors maintained the same direction of associations and their ORs did not change much from the univariate analysis to multivariate analysis (Table 4.5, 4.6) indicating confounding was not serious.

Table 4.6 Multivariate analysis of risk associated with sero-positivity of Brucella at household level in Marsabit, 2013

Variable	Estimate	Odds Ratio	Confidence intervals (95%)		P value
			Lower	Upper	
Taking milk from own animals (ref=no)					
Yes	-1.7407	0.1754	0.0538	0.5258	0.0024
Boiling milk before use (ref=no)					
Yes	-0.9062	0.4040	0.1939	0.8462	0.0155
Assisting in delivery (ref=no)					
Yes	1.6939	5.4407	1.2168	29.1572	0.0312
Keeping sheep (ref=no)					
Yes	0.8495	2.3385	1.1856	4.6997	0.0151

4.4 Community Knowledge Attitude and Practices on Animal Brucellosis

Majority (85.5%; 237/277) of the respondents had heard about brucellosis but only 25.11% (70/277) identified some animal species affected by the disease like cattle, sheep and goats, camel and antelopes. Only 4% (11/277) of the respondents identified the contact between wild and domestic animals as important transmission route for *brucellosis*. Less than half (41.4%; 115/277) of the respondents did not know of any method through which Brucellosis could be prevented in animals while 5.7% (16/277) identified that the disease could be prevented through vaccination and 15.9% (44/277) thought drug treatment would prevent the disease. About a third (31% (86/277)) identified key symptoms of the disease; 16.3% (45/277) identified abortions in livestock and 15% (42/277) identified joint pains in human while the rest of the respondents could not identify any symptoms of the disease.

Out of the 277 households, only 1% reportedly disposed-off aborted materials either through burying or throwing them into pit latrines. Majority (63%; 175/277) disposed the aborted materials by feeding them to dogs and the rest (36%; 100/277) left the materials out in the pasture.

4.5 Community Knowledge Attitude and Practices on Human Brucellosis

The vast majority (75.3%; 209/277) of the community members were aware of the fact that *Brucellosis* is a zoonotic disease. However, only a small proportion (1.8%; 5/277) could identify the mode through which the disease is transmitted to humans; contact with aborted animal fetuses; drinking and/or eating raw dairy products (1.3%; 4/277) and consumption of uncooked or undercooked meat from an infected animal (3.5%; 10/277). About a third (32%; 89/277) of the household respondents had no information on the transmission mechanism while 2.2% (6/277) reported that milking infected animals could transmit the disease to humans. The only clinical signs identified in sick persons by the respondents were headaches and fever (21%; 58/277) while 79% (219/277) did not know of any clinical signs associated with the

disease. About 66% (182/227) of the respondents were familiar with the disease since either a relative or friend was at one time diagnosed with *brucellosis*.

Knowledge on the prevention of human brucellosis among the community was scanty as 81.06% (225/227) of the respondents did not know of any method of prevention. However, 18.94% (53/227) mentioned a few ways of prevention including; boiling milk before consumption and drug treatment. A small proportion (16.74%; 46/277) of the respondents mixed milk from different animals. When animals give birth, 92.51% (256/277) were assisted by either the father or other male members of the family. It was noted that 55.51% (154/277) of the community had received general information on brucellosis mainly from community meetings (baraza) and friends.

CHAPTER FIVE

5.0 DISCUSSION

The human seroprevalence of brucellosis was estimated at 44% in Marasabit County. This was in sharp contrast to the seroprevalence of 5.7% estimated in Kiambu County (Ogola *et al*., 2014) but was in close agreement with the seroprevalence of 32% estimated in Kajiado County (Nakeel *et al*.,2016) which like Marsabit, nomadic pastoralism is practiced. In Kiambu County the livestock production system is zero-grazing implying that there is minimal contact of animals between herds and thus the risk of infection with *Brucella* organisms is reduced (McDermott and Arimi, 2002). Similarly, the animal seroprevalence of brucellosis was the same in Marsabit (13.6%) and Kajiado (12.9%). The human household seroprevalence of brucellosis was 73% while the herd seroprevalence was 68%. These estimates were in contrast with those made in the mixed agro-pastoral Kajiado County and the agro-based Kiambu County of 27% and 18% respectively, (Osoro *et al*., 2015). In addition, the results of the Marsabit study are in accordance with the results of Kadohira *et al*. (1997) who estimated bovine seroprevalence at 2% and 15% in high potential areas of Kenya and semi-arid and pastoral areas of Kenya, respectively.

Several factors increase the risk of brucellosis in pastoral areas including: migration of stocks in search of water and pastures; high concentration of stocks at the watering points; and limited access to education by the pastoralists. Similar observations have also been made elsewhere in Africa including Ethiopia and Uganda (Jergefa *et al*., 2009). Therefore, for disease-management strategies to be effective, it is important to take into account the strong cultural and economic dependence of the pastoral communities on livestock. Strategies in sustainable pasture management would play a pivotal role in management of the livestock migrations in search for the pasture. The control of brucellosis should also include maintenance of the ecosystem

services of the pastoral areas through initiatives like, limiting livestock densities, land reforms and integrated social and economic development (Racloz *et al.,* 2013).

At the human individual level risk factors associated with *brucella* sero-positivity included old age, gender (male), regular consumption or taking un-boiled milk, handling hides, exposure and contact with livestock (herding, feeding, milking), and consuming milk. Other factors like boiling milk before consumption were protective against *Brucella* infection. The findings agree with those of similar studies which majorly attributed transmission of human brucellosis to direct contact with animals and also some animal products or indirectly through ingestion of their products (Kozukeev *et al*., 2006; John *et al.,* 2010).

The findings of the current study are also consistent with a study by Osoro *et al*. (2015) who identified increasing age by decade, being male, regularly ingesting raw milk, exposure to goats (herding, milking, and feeding), and handling animal hides as the risk factors associated with human sero-positivity to *Brucella* at individual level.

Male household heads are likely to propagate some cultural practices, which promote Brucella transmission, for example, not boiling milk before drinking. Males in the hoouseholds were mostly involved in assisting delivery among other activities identified as risk factors for human brucellosis. This may explain why being a male was a risk factor for human brucellosis in the pastoral community.

At the household level, of the five household factors that were significant in univariate analysis, four remained significant in the final multivariate logistic model The factor “feeding fetuses to the dog” lost its significance indicating its association with *Brucella* positivity was confounded by another factor most likely the factor “assisting in delivery” because its OR changed dramatically from 7 in univariate analysis to 5.4 in the multivariate analysis. This was not surprising because both factors involved handling of aborted fetuses and both of the practices

leads to the exposure to the *Brucella* organisms. The others factors maintained the same direction of associations indicating confounding was not serious.

Household members who consumed milk from own animals and kept sheep were at a higher risk of infection. Boiling milk before use was protective factor against exposure to *Brucella* organism. These findings were consistent with the study by John *et al*. (2010) who identified that brucellosis transmission to humans was associated with a wide range of risk factors, all of which related to transmission through direct contact with animals or their products or indirectly through consumption of their products. Raw milk from an infected animal may contain the *Brucella* pathogens which if ingested by humans, transmits the infection. However, boiling or high temperature pasteurization will kill *Brucella* in milk (Racloz *et al.*, 2013). Therefore, to control the disease in human population, there is need to sensitize members of the community on the need to boil milk or heat treat all dairy products for human consumption.

The surveyed farmers had limited knowledge on brucellosis particularly on identification of sick animals and prevention methods such as proper disposal of reproductive materials to minimize spread of the disease to the animals at risk. The most predominant methods of disposing aborted fetuses, placentas and remains from still births were feeding to the dogs and leaving them out in pasture. Other disposal methods like burning and burying the materials were not utilized by the members of the community. The inappropriate methods of disposal could lead to increased spread of the infection in among the susceptible populations. The practice of leaving the aborted fetuses, placentas and still borns on the pastures exposes dogs and perhaps wild animals to brucella infection. While assisting in the deliveries, only 5.73% of them used gloves, a practice that suggested great contacts between human and the animals' reproductive materials and fluids. This increases the risk of brucellosis spread among the populations though contact.

The community of Marsabit County is largely (88.5%) pastoralist and engage in practices that enhance human contact with animals and animal products and thus the transmission of the disease. Consumption of raw milk was very predominant (82.82%) in Marsabit. It is a practice that greatly contributed to the high animal to human brucellosis transmission. This is consistent with other studies that associated transmission of *Brucella* from animals to human with consumption of raw milk (Osoro *et al*., 2015). Majority of the pastoralist who showed limited knowledge on major aspects of the brucellosis exposure to human were mainly from the most remote areas of the county where there was also limited access to education.

Brucellosis was present in the study population both in human and animals. All the factors that increase the risk of infection were identified and this creates a need for awareness creation in order to control the disease among the pastoral community of Marsabit County and indeed in other pastoral communities in Kenya.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENTATIONS

6.1 Conclusions

The following conclusions were drawn from the study;

- The sero-prevalence of brucellosis was estimated at 44% in human and at 11.2% in cattle, 11.9% in sheep, 16.1% in goats and 11.1% in camels. The herd sero-prevalence was 68.2% and household sero-prevalence was 73%.
- In univariate analysis, individual level factors that were associated with testing positive to Brucella antibodies included; gender (OR=1.5, P=0.0049), age category (Youth (OR=2.2, P=0.0022), mid age (OR=2.4, P=0.0007), old (OR=3.4, P=0.0001)), education level (primary (OR=0.4, P=3.02e-6), secondary (OR=0.3, P=0.0045), post-secondary (OR=0.2, P=0.0196)), primary occupation (Student (OR=0.4, P=0.0036), skilled workers (OR=0.2, P=0.0023)), packed milk (OR=0.3, P=0.0023), consumption of animal milk (OR=1.6, P=0.0051), consumption of market milk (OR=0.4, P=0.0002), consumption of raw blood (OR=1.4, P=0.0391), handling hide (OR=1.3, P=0.0490), Milking (OR=1.7, P=0.0008), herding (OR=1.5, P=0.0145), slaughtering (OR=1.8, P=0,0002), cleaning barns (OR=1.4, P=0.0419), assisting in delivery (OR=1.5, P=0.0033). However only 5 factors remained significant in multivariate analysis including male gender (OR=1.8, P=0.0477), herding (OR=0.5, P=0.0365), primary occupation (student (OR=0.3, P=0.0009), skilled workers (OR=0.2, P=0.0021)), consumption of milk from the market (OR=0.5, P=0.0447), consumption of packed milk (OR=0.3, P=0.0035).
- At the household level, factors that were significantly associated with testing positive to *Brucella* antibodies in univariate analysis included using milk from own animals (OR=0.1, P=0.0002), feeding aborted materials to dogs (OR=0.4, P=0.0039), assisting in delivery

(OR=7.0, P=0.0058), keeping sheep (OR=2.3, P=0.0075), boiling milk before use (OR=0.3, P=5.968e-05). However, only 4 factors remained significant in multivariate analysis including using milk from own animals (OR=0.2, P=0.0024), boiling milk before use (OR=0.4, P=0.0155), assisting in delivery (OR=5.4, P=0.0312), keeping sheep (OR=2.3, P=0.0151).

- The vast majority (85.5%) of the respondents knew brucellosis in both man and animals. However, only a few (31%) could identify the disease by clinical signs in both humans and animals.
- The pastoral community of Marsabit engaged in practices that were likely to enhance *Brucella* transmission in the population including: consumption of raw milk (92%), assisting animals in delivery without protection (92.5%), feeding of afterbirths to dogs (63%), and throwing them in the environment (36%) leading to contamination.

6.2 Recommendations

- In order to deal with the high (44%, 13.6%) sero-prevalence of brucellosis in human and animals respectively, there is need to put in place control measures in both animals and human within Marsabit County to minimize the disease burden. These control measures would include, animal vaccinations and public education on prevention measures such as necessity to always drink boiled milk, use of protection while assisting animals to deliver and either burning or burying aborted fetuses to avoid environmental contamination.
- Since nomadic pastoralism poses a challenge to the implementation of disease control programs, the stock migration routes should be identified, regularly updated and mapped while intensifying disease surveillance in the areas in order to have disease control programs customized to take care of all the dynamics.

- There is a great need for community sensitization campaigns on public health implications of some practices like consumption of raw milk among others that pose a great risk to the human health.

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APPENDICES

Appendix 1: Questionnaire on Knowledge attitude and practices

1. Have you ever heard of brucellosis before today? Yes ☐ No ☐ Unsure ☐
2. If yes, do you know which animals are affected by brucellosis?
3. Which animals are affected by brucellosis? Bovine, caprine, ovine, porcine, canine, camels, Don't Know other (specify)
4. How is the disease spread among animals?

   ☐ Food,

   ☐ Water,

   ☐ Wild animals,

   ☐ Sexual,

   ☐ Other (specify),

   ☐ Don't know

5. How is brucellosis prevented in animals? ☐ Vaccination ☐ medications, ☐ other(specify), ☐ don't know
6. How can you tell if your animal has brucellosis?? Abortions, swollen joints, retained placenta, reduced milk production, swollen testes, others (don't know), infertility,
7. How do you normally dispose aborted fetuses?

   Eaten by dogs,

   Buried/thrown in pit latrine,

   Left out in the pasture,

Eaten by humans,

Others (specify)

8. Does brucellosis affect humans? Y/N/Don't Know. ☐

   ☐ If yes, how do humans get brucellosis?

   ☐ Contact with aborted animal fetus;

   ☐ Drinking/eating raw dairy products

   ☐ Close contact with infected animal,

   ☐ Slaughtering animals

   ☐ Drinking animal blood,

   ☐ Eating uncooked meat from an infected animal

   ☐ Don't know

   ☐ Other (specify)

9. How can you tell that somebody has brucellosis? (tick one)

Hotness of body, chills, fatigue, lack of appetite, joint pains, headache. Others, don't know, abortions

10. Have you ever known anyone with brucellosis?

1. □ Yes □ No □ Unsure

   If yes, who?

   Family member □ Relative □ Friend □ Co-worker □ other _____

11. Have you heard of ways to prevent brucellosis?

□Yes □No □Unsure

12. If yes, how is brucellosis prevented in humans?

☐ Vaccination,

☐ Medications,

☐ Other (specify),

☐ Don't know

13. Where do you get milk for domestic use?

☐ Raw milk from own animals

☐ Raw milk from shop/farmer

☐ Pasteurized milk from shop

☐ Both raw and pasteurized

14. Do you boil milk before drinking? ☐ Always ☐ Sometimes ☐ No

15. Do you prepare fermented milk from raw milk at home? ☐ Always ☐ Sometimes ☐ No

16. How is milk from your household consumed? (Tick one) Neighborhood Sales: Local Market; Feed young Animals Only; Home Consumption; Local Hotels; Cooperatives, others

17. Do you mix milk from different animals? ☐ Yes ☐ No

18. In case of domestic consumption, how do you treat the milk? ☐ No Preservation; ☐ Boiling; Chilling; ☐ Other Specify)

19. Which milking methods/gadgets do you use? (tick one) Hand Milking; Machine Milking; Other (specify).

20. At what age are young children started on other milk other than human milk?

Cow milk _____months

Goat milk _____months

Camel milk _____ months

21. Do you assist animals during birth deliveries? Y/N ☐

22. If so, do you use gloves at the time of assistance? Yes/ No ☐

23. Have you ever received information on brucellosis from anyone? (Y/N) ☐

24. If yes, from whom?

- ☐ Animal health workers
- ☐ Human health workers
- ☐ Posters
- ☐ Electronic media
- ☐ Print media
- ☐ Friends
- ☐ Religious leader
- ☐ Community meetings (baraza)
- ☐ Other

25. Where do you typically get health information?

☐ Doctor or nurse

☐ Health clinic

☐ News

☐ Community member

Older adult in household Friends/Neighbors Other (specify) ☐

26. Where would you like to receive health information?

☐ Health Clinic

☐ TV

☐ Radio

☐ Community member

☐ Family member

☐ Religious leader

☐ Other (specify)

Appendix 2: Consent form

Voluntary Participation. You are free to join the study or not to join. You may leave the study at any time, for any reason. If you decide not to join or to drop out, you will not lose any health care services you are entitled to at the Hospital. You will not get any direct benefit or payment for being in this study, but you will help us know more about this disease.

Why You Have Been Chosen: We are testing persons from households selected randomly within Kajiado districts, (Rift Valley province), Kiambu (Central province), and Marsabit (Eastern province). These districts are chosen because they have reported a high number of animal brucellosis cases through the MoLD.

Procedure and Confidentiality: If you or your child chooses to be in this study we will draw **4 ml** of blood (a teaspoon) from the vein in your, his or her arm. This blood sample will be tested for germs of the brucellosis bacteria, or other disease causing germs at the National Public health laboratories in Nairobi and/or the CDC/KEMRI lab in Kisumu. Tests may show us that you or your child may have been sick with brucellosis before or is sick with it now. A small number of blood samples not exceeding 500 vials per species will be sent to CDC in Atlanta, Georgia U.S.A. Researchers at CDC will do the test again to see if they get the same test results. The remaining amount of the sample will be stored in the freezer for possible testing for other germs in future. No human genetic testing will be done on the sample**.** We will also ask you and your child questions for10 minutes. Neither of you have to answer the questions if you do not want to.

Only researchers involved in the study will be allowed to work with your blood and see your information. Your name and anything that can identify you will be taken off the test results and the questions you were asked before it is looked at and reported.

Risks. Except for minor pain, bruising and bleeding that may be a part of taking blood, there are minimal risks from being in this study. In rare cases, an infection can result from drawing blood. If such infection occurs, the project will assume costs of treatment of the infection. In addition, it is

possible that other people will find out that you participated in this study. SSC 2193 Version 4_11102012 Page 86

Benefits. You will not receive any benefit from this study. In addition, information obtained from this study may help the Ministry of Health decide when and where brucellosis disease may occur. In addition, the result will be provided to your doctor as soon as possible so that you can be provided with treatment.

Contact Persons: If you have concerns regarding, injuries please contact Dr Kahariri Samuel on 0720227118. If you have concerns regarding your rights in being in the study, please contact the Ethics Review Committee, Kenya Medical Research Institute (KEMRI), P.O. Box 54840-00202, GPO, Nairobi. Telephone 0202722541 or 0722205901 or 0733400003.

You will receive a copy of this signed consent form to take away with you

Consent

This study has been explained to me. I have had a chance to ask questions. I have been informed that it is my free choice to be in this study and if I join the study, I can drop out at any time without any penalty.

If you agree to participate in the study, please sign/thumb print here

Name of participant

Witness signature (if participant cannot sign his/her name) _____

Date: _____/_____/_____

Consentor _____Date: _____/_____/_____

I agree to allow my blood sample to be stored at KEMRI for possible future testing to determine the cause of my fever. This testing will not include genetic testing of the patient.

Date: _____ Signature of participant _____

Witness signature (if participant cannot sign his/her name) _____

Date: _____/_____/_____

Consenter _____Date: _____/_____/_____

Appendix 3: Univariate analysis at individual level factors in Marsabit County, Kenya in 2013.

Variable	Estimate	Odds Ratio	Confidence intervals (95%)		P-value
			Lower	Upper	
Gender (ref=female)					
Male	0.4148	1.5141	1.1348	2.0233	0.0049
Age category (ref=child)					
Adolescent	-0.3691	0.6914	0.3885	1.2163	0.2037
youth	0.8056	2.2380	1.3463	3.7943	0.0022
mid-age	0.8710	2.3892	1.4575	3.9997	0.0007
old	1.2111	3.3571	1.8281	6.2789	0.0001
Education level (ref=no education)					
Primary	-0.8407	0.4314	0.3016	0.6114	3.02e-6
Secondary	-1.1825	0.3065	0.1273	0.6640	0.0045
Post-secondary	-1.5094	0.2210	0.0502	0.6956	0.0196
Other	-1.42935	0.2395	0.0122	1.6323	0.2025
Primary occupation (ref=housewife)					
Student	-0.9204	0.3984	0.2135	0.7402	0.0036
Farmer	0.1476	1.1591	0.6596	2.0369	0.6062
Salaried off farm non skilled	-0.2744	0.7600	0.3866	1.4894	0.4238
Salaried off farm skilled	-1.4470	0.2353	0.0881	0.5770	0.0023
Packed milk (ref=no)					
Yes	-1.3861	0.2500	0.0928	0.5696	0.0023
Own animals milk (ref=no)					
Yes	0.4781	1.6130	1.1578	2.2605	0.0051
Market milk (ref=no)					
Yes	-0.8980	0.4074	0.2498	0.6458	0.0002
Cooked meat (ref=no)					
Yes	0.6520	1.9195	0.3163	2.4636	0.4765
Raw blood (ref=no)					
Yes	0.3598	1.4331	1.0181	2.0188	0.0391
Handle raw meat (ref=no)					
Yes	-0.0023	0.9977	0.7479	1.3307	0.9876
Hunting (ref=no)					
Yes	-0.1643	0.8485	0.1113	5.1489	0.8576
Hides (ref=no)					
Yes	0.2913	1.3382	1.0015	1.7892	0.0490
Milking (ref=no)					
Yes	0.5409	1.7175	1.2527	2.3662	0.0008
Herding (ref=no)					
Yes	0.3775	1.4587	1.0790	1.9776	0.0145
Slaughtering (ref=no)					
Yes	0.5923	1.8082	1.3276	2.4674	0.0002
Cleaning barns (ref=no)					
Yes	0.3212	1.3788	1.0133	1.8822	0.0419

Variable	Estimate	Odds Ratio	Confidence intervals (95%)		P-value
Assisting in delivery (ref=no)					
Yes	0.4342	1.5437	1.1563	2.0644	0.0033
District (ref=marsabit)					
Marsabit central	-0.6758	0.5088	0.2249	1.0688	0.0862
Marsabit south	0.6090	1.8385	1.1245	3.0217	0.0156
Marsabit north	0.7743	2.1690	1.1337	4.2226	0.0204
Moyale	0.0372	1.0379	0.7165	1.5068	0.8444
North horr	0.5928	1.8090	1.0062	3.2754	0.0483
Sololo	0.6460	1.9079	0.8487	4.3745	0.1191