SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF BRUCELLOSIS AND Q-FEVER IN LIVESTOCK AND HUMANS IN KAJIADO COUNTY, KENYA

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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ACRONYMS AND ABREVIATIONS

ASAL	Arid and Semi-Arid Lands
CDCP	Centre for Disease Control and Prevention.
CELISA	Competitive Enzyme Linked Immuno Assay
CFT	Complement fixation Test
CSF	Cerebral spinal fluid
DMS	Director of Medical Services
DNA	Deoxyribose Nucleic Acid
DVS	Director of Veterinary Services
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
FAO	Food and Agricultural Organization
IdELISA	Indirect Enzyme Linked Immuno Assay
IFA	Immuno Fluorescent Assay
KNH	Kenyatta National Hospital
LCV	Large Cell Variants
LPS	Lipopolysaccharide
MOH	Medical Officer of Health
MRT	Milk Ring Test
NACOSTI	National Commission for Science, Technology and Innovation

NVL	National Veterinary Laboratories
OD	Optical Density
PCR	Polymerase chain reaction
RBPT	Rose Bengal Plate Test
SAT	Serum Agglutination test
SCV	Small Cell Variants
SCVO	Sub- County Veterinary Officer
SLPS	Smooth Lipopolysaccharide
UoN	University of Nairobi
WHO	World Health Organization

ABSTRACT

Animal and human health is inextricably linked. People depend on animals for nutrition, socio-economic development and companionship. A cross-sectional serological study was carried out to determine the sero-prevalence of two related zoonotic diseases namely brucellosis and Q-fever in cattle, sheep, goats and humans in three sub-counties of Kajiado County. Animal serum samples were collected for three months (July to September 2012) from different farms and watering points by systematically sampling a healthy adult animal in each herd. Human blood samples were collected at the health facilities serving the study areas from patients presenting with flu-like symptoms. A total of, 250 (cattle), 167 (sheep), (167) goats and 317 (humans) samples were collected. A total of 400 samples were subjected to cELISA (COMPELISA, VLA, UK) test. 382 livestock samples from the 400 cELISA test samples were subjected to Q-fever Indirect Multispecies (cattle, sheep and goats) and 90 human samples to *Coxiella burnetii* ELISA IgG test. In addition, information regarding risk factors for the two zoonoses in both livestock and humans was collected using questionnaires. Risk factors were management (grazing, watering and breeding system) introduction of new stock, level of awareness of the livestock owners regarding the zoonoses and frequency of contact with veterinary extension staff; contact with contaminated environment, consumption of raw/unprocessed/under processed livestock products, close association with livestock and awareness of the diseases for human. The harvested serum samples were tested for the presence of antibodies to the Brucella organisms using the Competitive Enzyme Linked Immunosorbent Assay (Com- ELISA) and *C burnetii* ELISA IgG.

Results indicated a low prevalence of brucellosis in humans 1.3 %but a higher prevalence of Q-fever 26%. The overall prevalence in livestock was 12.91 % and 79.3 % for brucellosis and

Q-fever respectively. The prevalence estimates in cattle, sheep and goats were 21.92%, 8.6 % and 7.3% for brucellosis respectively and 89.7% , 57.5% and 83.1 % for Q-fever respectively, indicating a high risk of transmission of the diseases to humans through contact or consumption of livestock products such as milk. A univariate analysis of risk factors showed that using a communal bull and introduction of new animals especially bulls were important risk factors. However other factors that were found to be significantly ($p < 0.05$) associated with testing positive to the disease in humans were occupation ($p<0.05$), method of processing meat ($p<0.01$), whether they process blood before taking ($p<0.001$), contact with aborted foetuses ($p<0.029$), and method of disposal of aborted foetuses and placentas ($p<0.028$), while communal grazing system($p<0.005$) and introduction of new animals was not significant ($p<0.07$). The study and the data obtained strongly suggest that the two zoonotic diseases may be enzootic in the study area in human, cattle, sheep and goats and presents a serious public health problem among the inhabitants of the county and that there is need to create awareness among all concerned on the likely high prevalence of the two diseases to avoid misdiagnosis and suffering of patients. It is recommended that the veterinary personnel in Kajiado County make an effort to investigate all cases of abortions and the possible causes retained placentas that are included in their disease surveillance reports. This calls for strengthening laboratory diagnostic capacity in the county by training more veterinary and health staff and providing diagnostic equipment and reagents. Creating awareness on the causes, modes of transmission and risk factors to the zoonoses should be undertaken as soon as possible.

CHAPTER 1

1.0: INTRODUCTION

1.1 General introduction

Brucellosis and Q-fever are important bacterial infections of livestock that cause a range of clinical conditions leading to infertility and loss of production. These diseases are also found in wildlife (Scolamacchia *et al*. (2010). In addition, they are important zoonotic infections affecting people who handle livestock or consume contaminated animal products such as milk. These diseases produce non-specific symptoms including fever, which are often misdiagnosed leading to severe chronic disease.

The economic importance and public health significance of brucellosis cannot be over emphasized. The disease is widely distributed in Africa with the highest incidence in areas where extensive livestock husbandry is practised and animal populations are high (McDermott and Arimi, 2002). It is transmitted directly or indirectly from infected animals to man with consequent debilitation and prolonged incapacitation. Six hundred and nine (609) cases based on Rose Bengal Plate Test (RBPT) of human brucellosis have been reported in Kajiado County (MOH annual reports Kajiado County Referral Hospital, 2007- 2012). Although RBPT is a screening test that is prone to giving false positives, it is a strong indicator of the magnitude of the disease in the country. Similarly eight hundred and ninety seven (897) cases of animal Brucellosis have been reported from 2003- 2011 in the whole country based on Rose Bengal Plate test (RBPT), Enzyme Linked Immunosorbent Assay (ELISA) and Complement Fixation Test (CFT) at the Central Veterinary Laboratories in Kabete (DVO Kajiado East Sub County reports of 2003-2011).

Eleven (11) cases were confirmed in Kajiado East Sub County (DVO reports 2010, 2011). However, cases of unspecified abortion and retained afterbirths were reported during that period in Kajiado County. Information regarding the prevalence of brucellosis in the county is scanty and disjointed. It was therefore necessary to carry out studies to ascertain the prevalence of the disease. Real risk prevails (Kangethe *et al*, 2000) but there are no records of any surveys conducted in the County.

Q-fever is a highly contagious zoonotic disease with multiple hosts that can serve as reservoirs of infection, but aborting domestic ruminants are typically the main source of the bacterium in humans and other animals (Rodolakis *et al.,* 2007). Early studies done in Nyanza, Coast and Central Kenya from 1955 to 1976 among domestic ruminants (Craddock and Gear, 1955;Brotherson and Cooke, 1956; Vanek and Thimm, 1976) showed the prevalence of antibodies as 7–57% in cattle and 33–34% in goats. In human population based studies done elsewhere in sub Saharan Africa showed 17-37% indicating high levels of exposure to the pathogen (Mediannikov, *et al.,*2010;)

In a more recent study done in 2007 to 2008 in cattle, sheep and goats in Western Kenya the prevalence of antibodies against *C. burnetii* was reported as 28.3% in cattle, 32% in goats, and 18.2% in sheep (Darryn *et al*., 2013) .Despite the high-profile nature of some Q-fever outbreaks, and the attention that *C. burnetii* has received as a potential bioterrorism agent, (Madariaga *et al.,* 2003), information on the prevalence of infection in sub-Saharan Africa is scanty (Mediannikov *et al.,* 2010).

In Kenya, serological evidence of Q-fever in patients with acute febrile and respiratory illness was shown in the 1950s. Other studies between 1956 and 1976 showed the prevalence of antibodies to *C. burnetii* among Kenyans to range between 10% and 20%. A more recent investigation found that four people (8%) of a group of 50 travellers to Kenya contracted Q-

fever and in another recent study investigators diagnosed acute Q-fever in 5% of febrile patients admitted to two hospitals from September 2007 to August 2008, in neighbouring northern Tanzania (Malavika *et al*., 2011). The latest study done in Western Kenya in 2009 to 2010 reported a prevalence of 30.9% in human sera that were collected between 2007-2008 (Darryn *et al*., 2013)

This study in Kajiado County provided a valuable opportunity for generating domestic animal prevalence data that could be linked to human health outcomes. To assess the current status of Q-fever among humans and infection prevalence in domestic ruminants, surveys were conducted for *C. burnetii* in cattle, goats, sheep, and tested specimens from human patients presenting to a clinic in the same area. The size and extent of these two zoonotic diseases in the Kajiado ecosystem remains largely unknown. It was therefore necessary to conduct investigations aimed at establishing their status in order to lessen the potential negative impacts they exert in the ecosystem. In this study the estimation of the prevalence of brucellosis and Q-fever was made in cattle, sheep, goats and humans in three districts that make a transect from east to west of Kajiado County. The County is inhabited mainly by the Maasai community which still practices nomadic Pastoralism to a large extent. Like other pastoralists, livestock play an important central role in their daily and ceremonial life. They depend on livestock for meat, milk and blood in addition to providing the principal currency for social and commercial transactions.

1.2 Objectives

The overall objective of this study was to estimate the sero-prevalence of brucellosis and Q-fever in animals and humans Kajiado County.

Specific objectives:

1. To estimate the sero-prevalence of brucellosis and Q-fever in livestock and humans.
2. To determine the risk factors associated with the occurrence of brucellosis and Q- fever in livestock and humans.

1.3 Justification for the study

Zoonoses have been recognized as important public health issues for centuries. Ungulates in particular are known to carry at least 315 zoonotic pathogens (Cleaveland *et al*., 2001). Many emerging and re-emerging infectious disease problems globally are zoonotic (Taylor *et al*., 2001) and there is therefore a clear need to understand these diseases in the animal populations where they may be maintained (Haydon *et al*., 2002) 2003)and in human beings who associate with animals or animal products. To achieve this, the veterinary and medical professions need to work closely in multidisciplinary teams in line with the concept of 'One Health' and once the magnitude and spread of these diseases are understood and the potential risk factors identified, targeted control/prevention measures will be made possible.

Kajiado County is one of the forty seven (47) Counties in Kenya. Most of the County lies in the arid and semi arid lands (ASAL's) and is predominantly inhabited by Maasai people who are to a large extent pastoralists. In this County, just as in other ASAL's in Kenya, delivery of veterinary services is poor. This can be attributed principally to the few veterinary personnel, difficult terrain, and lack of reliable transport. Thus disease surveillance is grossly in-adequate.

Information regarding disease outbreaks in these areas is not received in time for prompt response. The problem is not limited to the delivery of veterinary services only, but also to the provision of health care to the pastoralists; health facilities are far apart and mostly found in urban centres and therefore not readily available. Outbreaks of disease in such a complex ecosystem involving wildlife - livestock - human interaction are expected to occur at high frequency.

The clinical features and presentation of the diseases in humans overlap with many other infectious and non-infectious diseases, which present with flu-like syndromes. There was therefore a need to establish the prevalence of the diseases in man in Kajiado and create awareness. Lack of pathognomonic signs in livestock presents a challenge in the diagnosis of the two zoonoses in livestock despite the high risk of these diseases in Kajiado County. It is possible that some of the cases of abortion reported in some parts of the county are caused by one, two or more zoonotic diseases. This study therefore endeavoured to establish the status of the two diseases in livestock and also in humans.

Livestock movement is a major risk factor for these Zoonoses. The spread of the disease from one herd to another and from one area to another is almost always due to movement of an infected animal from an infected herd or area to a new one and is therefore a major cause of one or the entire zoonotic control breakdown. The prevalence is linked to the practice of animal movement to communal watering points and other areas when searching for pasture and water. Uncontrolled livestock movement is common within and outside the county.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Brucellosis

Brucellosis is an infectious disease caused by a bacterium of the genus *Brucella*. It is zoonoses presenting a worldwide problem with significant public health and economic implications. The disease affect animals, both domestic and wild, acting as reservoirs for human infection. These bacteria are primarily passed among animals and cause disease in different vertebrates. The various species affect sheep, goats, cattle, deer, elk, pigs, dogs and several other animals (Radostits *et al*., 2000). Camels are also affected (Waghela *et al*., 1978). Humans beings become infected by coming into contact with animals or animal products contaminated with these bacteria or by consuming contaminated animal products (Chomel *et al*., 1994). In animals, the disease is characterized by abortion, retained afterbirth, orchitis, epididymitis, infertility, drop in milk yield and hygromas in cattle (Blood and Radostits, 1989). In humans, the disease is characterized by undulating fever, sweating, headache, muscle pain, arthritis and neurological symptoms (Maichomo *et al.,* 2000).

2.1.1 Aetiology

Brucella, the causal organism of brucellosis is a Gram negative, facultative intracellular bacterium. The organisms are cocci, coccobacilli or short rods measuring 0.5-0.7 µm by 0.6-1.5 um, arranged singly and rarely in short chains. They are non-capsulated, non-spore forming and non-motile. There are six known species that infect land animals namely; *Brucella abortus, Brucella melitensis, Brucella suis, Brucella ovis, Brucella canis and Brucella neotomae.* All the above except *B. Neotomae* are important pathogens. *Brucella*

abortus is associated with cattle, *Brucella melitensis* with goats and sheep, *Brucella suis* with pigs, *Brucella ovis* with sheep, *Brucella canis* with dogs and *Brucella neotomae* with the desert woodland rat (*Neotoma lepida)* (Chomel *et al*., 1994).Two new species, *Brucella cetaceae* and *Brucella pinnipediaee* have been recently described from a wide variety of cetaceaen (dolphins, porpoises) and pinnipeds (seals) by (Cloeckaert *et al.*,2001). Seven biovars are recognized for *B.abortus*, three for *B.melitensis* and five for *B.suis.* However the degree of genetic relatedness, as shown by DNA hybridization studies, is consistent with the existence of a single species within the genus *Brucella*. On clear solid medium, most *Brucella* strains grow slowly and after 24 hours, colonies are 0.5-1.0 mm in diameter, raised, convex and with entire edges. Colonies are smooth, mucoid except for *B.canis* and *B.ovis* which are permanently rough (Muendo *et al.,* 2012).

2.1.2 Antigenic structure

Brucella organisms have a closely related structure which makes their differentiation in serological studies difficult. They localize and proliferate within the cytoplasm of monocytes and reticulo-endothelial cells (Jubb *et al.,* 1985) and are thus protected from host defence mechanisms. *Brucella* species are aerobic with the exception of *B.abortus*, which requires 5-10% carbon dioxide for growth. All *Brucella* strains grow well in media enriched with animal serum and glucose at an optimum temperature of 37°C (Alton *et al*., 1988). *Brucella* occurs singly, in groups or short chains and is non-motile non-capsulated and non-sporing. On solid medium they are smooth, moist, translucent and glistering colonies which may take several days to appear. It grows slowly, even on rich media to give pinpoint, translucent colonies with a smooth surface (Figure 1). In all the smooth strains, the dominant surface antigen is a lipopolysaccharide- O chain which, depending on the three dimensional structure, forms A, M

or C epitopes. These are common to all smooth species but the distribution of A and M depends on the biovar. Rough strains do not produce the lipopolysaccharide- O chain but have a common R epitope. The lipopolysaccharide has endotoxin activity and elicits antibody – mediated protection (Corbel, 1997). More complete immunity is dependent on cell – mediated, particularly cytotoxic responses elicited by ribosomal and other proteins.

Figure 1. Colony appearance of Brucella abortus

Source: CDC/Courtesy of Larry Stauffer, Oregon State Public Health Laboratory, Public Health Image Library number 1902 (Adazu *et al.*, 2005).

2.1.3 Epidemiology

There are six classical *Brucella* species, which differ from one another in their choice of animal hosts. Other differences observed include biochemical characteristics, culture appearance and the amount or number of the main antigens they posses (Stack and Macmillan, 2000). The major species are *B.abortus* which infects cattle; *B.melitensis* affecting goats and sheep; *B.suis* affecting pigs; *B.canis* which infects dogs; *B.ovis* which infects sheep and *B. neotomae* which infects desert rats. *B. microfti* has been isolated from soil and mice (Corbel, 1997; Cloeckaert *et al*., 2002). The host parasite relationship for the major species is not absolute, and both man and domestic animals are susceptible to infection by strains of all the eight species .The isolation of a distinctive *Brucella* strain tentatively named *B. maris* from marine animals in the United Kingdom, Australia and the United States extends the ecologic

range of the genus and its scope as a zoonosis (Cloeckaert *et al.,* 2001). *B. maris* has been divided into two subspecies namely *B. cetaceae* from otter/seal and *B. pinnipediae* of the whale/porpoise (Cloeckaert *et al*., 2001, 2003).

According to Cloeckaert *et al*. (2001, 2003), all the above *Brucella* species are not host-specific, and may transmit to other animal species under appropriate conditions. Initial infection in the reservoir species is often followed by abortion and subsequent delayed or permanent infertility. Infection is usually chronic in animals, and treatment is rarely undertaken. Infected animals shed the organisms in uterine discharges following abortion or parturition, and also in the colostrum and milk. Brucellosis is a herd or flock problem. It is spread within the herd primarily by ingestion of contaminated material. Venereal infections can also occur, but this is mainly seen with *B. suis* infections. Congenital (*in utero*) or perinatal infections may also occur, with the ensuing development of latent infections. Spread between herds usually occurs by the introduction of asymptomatic chronically-infected animals (CDC, 2000a; 2000b).

Human infections are characterized by a variable incubation period (from several days up to several months), and clinical signs and symptoms of continued, intermittent or irregular fever of variable duration, with headaches, weakness, profuse sweating, chills, depression and weight loss. Localized suppurative infections may also occur. The course of the disease can be variable, especially in persons either not or inadequately treated. Diagnosis of clinical brucellosis in humans and animals is initially made by use of appropriate serological or other immunological tests, and confirmed by bacteriological isolation and identification of the agent (CDC, 2000a; 2000b).

Transmission of infection to humans occurs through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted foetuses or placentas. Food-

borne infection occurs following ingestion of raw milk and other dairy products, but rarely from eating raw meat from infected animals. Occupational airborne infection in laboratories and abattoirs has also been documented. Accidental inoculation of live vaccines (such as *B. abortus* Strain 19 and *B. melitensis* Rev.1) can also occur, resulting in human infections. There are also case reports of venereal and congenital infection in humans (Cloeckaert *et al*., (2001).

Infection with *Brucella species* continues to pose a human health risk globally despite strides in eradicating the disease from domestic animals (Mantur *et al.*, 2007). Although many countries have eradicated *B.abortus* from cattle, in some areas *B.melitensis* and *B suis* have emerged as causes of this infection in cattle, leading to human infections (CDC, 2000a; 2000b). Currently *B.melitensis* remains the principal cause of human brucellosis worldwide. The recent isolation of distinct strains of *Brucella* from marine mammals as well as humans is an indicator of an emerging zoonotic disease.

Brucellosis in endemic and non endemic regions remains a puzzle due to misleading non specific manifestations and increasing unusual presentations. Fewer than 10% of human cases of brucellosis may be clinically recognized and treated or reported. If routine serological surveillance is not practised, *Brucellae* are very invasive, capable of penetrating the mucous membranes of the nose, throat, conjunctiva, urogenital tract, and epithelium of the teat canal, parenchyma of the mammary glands or the testis and normal and abraded skin (Muriuki *et al*., 1997, Machiomo *et al.,* 1998). Human infection with B*rucellae* depends upon contact with infected animals or their products or materials contaminated with animal discharge.

The disease occurs worldwide, except in those countries where bovine brucellosis (*B.abortus*) has been eradicated. This is usually defined as the absence of any reported cases for at least

five years. These countries include Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden and the United Kingdom (2002 OIE Reports., 2002). The Mediterranean countries of Europe, northern and eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America are especially affected. While *B.melitensis* has never been detected in some countries, there are no reliable reports that it has ever been eradicated from small ruminants.

The sources of infection for humans and the species of *Brucella* species found in humans vary according to geographical region. It is usually either an occupational or a food-borne infection. Both sporadic cases and epidemics occur in humans, but often the disease or infection is either unrecognized, or, if diagnosed, not reported to the public health authorities. Brucellosis is a worldwide disease affecting man, domestic animals and wildlife (Maichomo *et al*., 2000). *Brucella* species infections have been documented worldwide in a variety of terrestrial wildlife species and marine mammals. *B. abortus* and *B. suis* have been isolated from bison, elk, feral pigs, wild boar, hares, foxes, African buffalo, eland, waterbuck and may serve as carriers for other domestic animals and humans (Godfroid, 2002).

Brucellosis is considered the commonest zoonotic infection in the world (Ramirez *et al*., 2006). It is widely reported in Africa in all the livestock species and man (McDermott and Arimi, 2002) and is considered to be endemic (Ramirez *et al*., 2000). It has been reported in Chad in humans, camels and cattle, sheep and goats (Schelling *et al.*, 2003), , Nigeria (Ocholi *et al*., 1996), Eritrea (Omer ,2000), Zambia (Muma *et al.,* 2006 and 2008), Ethiopia (Mekonnen *et al*., 2011), Sudan (McDermott *et al*., 1987), Cameroon (Shey-Njila *et al*.,2005), Tanzania (Weinhaupl *et al*.,2000; Kunda, 2004) among other countries in Africa.

In Kenya, the disease was first reported in 1914. Thereafter, several reports of the disease were given in both livestock and man. A serological survey showing evidence of porcine

brucellosis in Kenya was carried out by (Waghela and Gathuma, 1975). Another survey was carried out in North Eastern province which showed evidence of the disease in camels (Waghela *et al*., 1978). The disease has since been reported in many parts of the country including Narok in humans (Muriuki *et al*., 1997, Machiomo *et al.,* 1998) Samburu, Kiambu and Kilifi in cattle (Kadohira *et al.*, 1997), The prevalence of the disease in both man and animals varies considerably depending on the livestock production system. It is higher in pastoral production system where large numbers of livestock are kept and share close communal grazing fields and watering points. In addition, the animals are in close contact with people (Kadohira *et al*., 1997; McDermott and Arimi, 2002). In contrast, the disease has low prevalence in the intensive livestock production systems such as in zero- grazing due to low cattle to cattle contact. Seropositivity to brucellosis has been shown to increase with age of animals .Sexually mature animals are very susceptible to brucellosis Females have been shown to have increased chance of testing Brucella positive (Muma *et al*., 2006)

Brucella infections are widely distributed in domesticated animals especially in the developing world. Cattle infections are commonly caused by *B.abortus* (Corbel, 1997). In cases where cattle come in contact with infected pigs or goats, *Brucella suis* and *B.melitensis* infections may take place (Corbel, 1997; Godfroid, 2002). However the two strains usually cause less severe disease in cattle. Infection is most commonly through ingestion, contact with foetal and placental contents while *B.abortus* can also be transmitted through coitus .Young cows are less susceptible compared to mature or older animals which tend to be sexually active since brucellosis is considered to be more of a sexually transmitted disease among animals (Parker, 2007). Unborn calves are usually aborted at about seven months and in case of birth, they are weak and die shortly afterwards (Corbel, 1997). In terms of milk production,

a severe drop is experienced as a result of infection in the herd (Bandara and Mahipale, 2002). There are large swellings in the joints of limbs called hygromas in infected cows (Anon., 2002).

Brucellosis mainly affects sexual organs with serious results of endometritis and epididymitis (Bandara and Mahipale, 2002). Bulls may exhibit sterility and orchitis. The infected herd may also exhibit disabilities such as discospondylitis, bursitis or arthritis (McDermott *et al*., 1994; Traboulsi *et al*., 2007). Pigs are affected most commonly by *B. suis* (Godfroid, 2002; Pappas *et al*., 2005). However, pigs may also be affected by *B. abortus* in cases where they come in contact with infected cattle. Sexual contact and ingestion may be the modes of transmission (Godfroid, 2002).

Abortion and other reproductive disorders may occur in sows. In boars, orchitis occurs and less commonly arthritis, spondylitis or abscesses in various organs may occur (Pappas *et al*., 2005). In sheep and goats, *B.melitensis* is the classical species affecting females of both animal species. In cases where infected cattle or pigs come in contact with small ruminants, infections of *B.abortus* and *B.suis* can occur *B.melitensis* infections are acquired primarily by ingestion (Alton, 1990). Abortion and mastitis usually occur in infected goats (Corbel, 1997).

Dog brucellosis is most commonly caused by *B.canis* (Pappas *et al*., 2005). However infections by *B.abortus, B.suis* and *B.melitensis* may occur occasionally when dogs eat placentas from infected farm animals. The disease is most commonly transmitted sexually and bitches abort at 40 to 60 days of gestation. In stud dogs, epididymitis, orchitis and scrotal dermatitis, which sometimes progresses to complete scrotal necrosis, may occur.

2.1.4 Sources of infection

2.1.4.1 In humans

The primary sources of the bacterium to humans are the foetal membranes and fluids, vaginal discharges which are expelled by infected females when they abort or calve, at which time Brucella organisms are present in the placenta lochia (Muriuki *et al*., 1997; Machiomo *et al.,* 1998). Other routes of less importance include inhalation via mucus membranes of the respiratory tract or through conjunctiva, and contact with contaminated material through intact and broken skin.

2.1.4.2 In livestock

Cows occasionally may be infected through coitus or when artificial insemination is done using infected semen. Calves may acquire infection in utero or they may become infected after ingesting infected colostrum or milk. Although some will rid themselves of the infection within few months, others may remain infected for life and thus spread the disease at their subsequent parturitions (Anon, 1986). *B.abortus* has special affinity for the pregnant uterus because the placenta contains a high concentration of erythritol a 4- carbon sugar- alcohol molecule which favours the multiplication of organisms. Brucella organisms metabolize this sugar preferably than other sugars and its presence in the placenta of ungulates explains the tropism of this pathogen for the reproductive organs and its capacity to induce abortions .The mammary route also allows for escape of Brucella organisms into the environment. The infected animals develop Brucella induced mastitis and shed the organisms either continuously or intermittently throughout the lactation period and sometimes continue discharging the organisms in subsequent lactations. Cattle vaccinated before infection show a

lower degree of brucella excretion in milk than those not vaccinated (Radostits *et al.,* 2000).The other mode of environment contamination is through infected carcasses. Urine and faeces of some infected animals are less important sources of the bacterium. The fluid in hygromas caused by Brucella infection may have large numbers of organisms but since they are restricted to the lesion, they do not seem to play an important role in the spread of the disease (Anon, 1986).

2.1.5 Transmission to humans

There is a direct relationship between the level of brucellosis in animal and the human infection, which has been shown to be influenced by methods of husbandry, standards of hygiene and food customs in any community. Consumption of unpasteurized raw milk and dairy products is a common method of transmission .Raw semi-cooked or pickled meat is a source of human infections (OIE, 2009). Pasteurization of milk and heat treatment of meats have reduced brucellosis to an occupational hazard in developed countries; however this is not the case in Kenya. Milk from domestic animals and water buffaloes is the most common source of infection, with *B.melitensis* being more easily transmissible by this method than *B.abortus* (CDC, 2000a; 2000b). Dairy products like cheese, cream, butter, and yoghurt prepared from unpasteurised milk are a good source of infection (CDC, 2010), with the cream fraction being more heavily laden with the organisms , thus requiring more heating to kill them. *Brucellae* can survive pickling, smoking and freezing meats. Occasionally contamination may result from vegetables and water coming in contact with infected discharge, secretion, or animal excreta.

Inhalation of infected dried materials of animal origin in houses, laboratories, abattoirs and farm premises used for housing animals may lead to infection through respiratory route and

conjunctiva (FAO/WHO, 2010). Infection can follow inhalation of contaminated aerosols or dusts. Airborne transmission of brucellosis has been studied in the context of using *Brucella* as a weapon. In fact, *B. suis* was the first agent contemplated by the U.S Army as a potential biological weapon and is still considered in that category (FAO/WHO, 2010).

B.melitensis has been isolated from vaginal mucosa, urine and milk of infected women and semen of infected men (Corbel, 2002). This does not constitute any evidence to implicate venereal transmission, though it can occur in some circumstances. There are isolated cases reported of transmission through blood transfusion (Doganay *et al.*, 2001), bone marrow transplantation and to nursing babies through mother's milk (Celebi *et al*., 2007). Transplacental transmission is another potential mode but seems unlikely as human lacks erythritol. Blood sucking arthropods have been shown to harbour the organisms which multiply and persist in both ticks and insects and still remain virulent to man. Mosquitoes and flies can be infected experimentally, but it is doubtful whether transmission occurs naturally through insect bites (Radostits *et al.,* 2000).

The relative importance of mode of transmission and pathway of penetration of the aetiological agent varies with the epidemiological area, the animal reservoirs, and the occupational groups exposed to the risk. Brucellosis is an occupational disease in shepherds, abattoir workers, veterinarians, dairy industry professionals and personnel in microbiologic laboratories. One important epidemiological step in containing brucellosis in the community is the screening of household members of infected persons (Pappas *et al*, 2005). There is limited evidence of person to person transmission of Brucella species and might only occur via the urine or blood transfusion. Human disease prevalence in any given area closely parallels animal prevalence, although the likelihood of disease in humans is further greatly

influenced by the degree of contact with animals or their excreta, and especially by ingestion of unpasteurized milk or inadequately cooked meat from infected animals (Mantur, 2007).

2.1.6 Transmission in animals

Animals are exposed to Brucella by licking or muzzling of newborns on external genitalia of infected animals, ingestion of food and water contaminated with secretions, excretions, or tissues especially when aborted animals shed the organisms on vegetation and water sources. Contact infection through skin and mucous membranes may occur from heavily contaminated bedding, while aerosols and droplets generated by tail switching and during parturition facilitate infection through airways and conjunctiva (FAO/WHO, 2004). Intra-mammary exposure through the teat canal can occur during hand milking due to cross contamination. Infected females may transmit to the conceptus in-utero or through milk post natal (Radostits *et al*., 2000). Although the organisms localize in the male and female genital tracts, venereal transmission is thought to be insignificant probably because the number of *brucellae* in semen is much lower than that required for infection per-vaginum. However, females can easily be infected during artificial insemination with semen from infected male animals when it is introduced directly into the uterus (Muendo et al., 2012). *B.ovis* infection in sheep is thought to be transmitted venereally from a ram with epididymitis shedding large numbers of organisms in the semen. Licking of infected rams penis and homosexuality is also a common route of infection (OIE manual, 2008). Embryo transfer from infected donors may be achieved without transfer of infection and super- ovulation is unlikely to reactivate the release of *Brucellae* into the uterus when embryos are normally collected. Transmission by ticks and biting insects has been demonstrated experimentally, but their role in natural transmission has not been documented (Radostits *et al.,* 2000). The organism is taken into the alimentary tract

of the housefly but is rapidly eliminated and there is no evidence for a role in natural transmission. Other possible modes of transmission are: air currents, waterways, contaminated equipment and scavengers (FAO/WHO, 2004).

2.1.7 Pathogenesis and clinical disease

2.1.7.1 In Humans

Brucellosis is a systemic disease that can involve almost any organ system. Infection through the skin causes a more severe and rapid reaction than when the organism is ingested or inhaled (Radostits *et al*, 2000). Upon penetrating the epithelial barriers, the organisms are ingested by neutrophils and tissue macrophages, which in turn, transport them to local lymph nodes. Bacteraemia develops within 1-3 weeks of exposure if the host immune system cannot contain the infection. Organisms then localize in the organs of the reticuloendothelial system, primarily the liver, spleen and bone marrow, where the formation of granulomas ensues. Large granulomas serve as a source for persistent bacteraemia. The primary virulence factor for *Brucella* species is the cell wall lipopolysaccharide (LPS). Both smooth (*B.melitensis, B.abortus, B.suis*) and rough forms (*B.Canis and B.Ovis*) exist with strains displaying rough LPS that have much less virulence in humans. After opsonisation and ingestion by phagocytic cells, organisms can be maintained extracellularly within phagosomes. Susceptibility to intracellular killing differs between species, *B.abortus* is readily killed and *B.melitensis* rarely affected. Serum lysis occurs, even in the absence of agglutinating antibodies, with *B.abortus* being much more susceptible to lysis than *B.melitensis.* Infection in human may remain latent or subclinical or it may give rise to symptoms of varying intensity and duration. Brucellosis can present as an acute pyrexia illness, which may persist for months or develop into focal infection that can involve almost any organ system (Olsen, 2004).

The characteristic intermittent waves of elevated temperature are usually seen in long standing untreated cases .The incubation period is generally 1-2 months, after which the onset of illness may be acute or insidious. Symptoms of brucellosis are protean in nature, and none are specific enough to make the diagnosis. Thereafter, symptoms may include: an intermittent, "undulating" fever, headaches, chills, depression, profound weakness, arthralgia, myalgia, weight loss, and orchitis/epididymitis in men and spontaneous abortion in pregnant women. Brucellosis lasts for days to months, and can be quite debilitating, although the case fatality rate is very low. Chronic sequelae may include sacrolitis, hepatic disease, endocarditis, colitis and meningitis.

2.1.7.2 In animals

In animals the incubation period takes about 30 to 60 days. When infection occurs in pregnant animals, the initial lesion is in the wall of the uterus and later it spreads to other parts of the organ. There is an association between the production of erythritol and the rate of proliferation of *Brucella* organisms. This leads to severe ulcerative endometritis of the intercotyledonary spaces affecting the allantoic chorion, foetal fluids, placental cotyledons and destruction of villi (Radostits *et al*., 1994). Following bacteraemia, there is localisation in the cow's gravid uterus resulting in placentitis, which enhances production of prostaglandins curtailing the corpus luteum, and then abortion occurs (Woods and Jan, 2005). In cases where the animal is not pregnant, there is localisation in the udder resulting in interstitial mastitis and involvement of the mammary glands that may cause the organisms to be excreted in milk for months or even years where the animal becomes a carrier (Mdegela *et al*., 2005; Akay *et al*., 2007).

Brucella infection is dependent on the exposure dose, virulence of the organism and natural resistance of the animal to the organism. Resistance to infection is based on the hosts' ability to prevent the establishment of a mucosal infection by the destruction of the invading

organism. Following infection of the host, the organisms are subjected to non-specific host defence mechanisms in which they are engulfed by leucocytes. In the leucocytes they multiply within the cytoplasm leading to the eventual rupture and death of the leukocyte releasing the *Brucellae i*nto the host system. The organisms are then ingested by monocytes where they are transported to the local lymph nodes where an immune response is mounted, which may lead to formation of granuloma (Jubb *et al.,* 1985). This leads to bacteraemia and generalized infection from 14 days to several months. Localization of the organisms may occur in organs, especially those of reticulo-endothelial system: liver, spleen, bone marrow and lymph nodes, as well as mammary glands, testis and uterus where the organisms may persist for years.

In bulls *Brucellae* may become localized in the testis and other genital organs (epididymis, seminal vesicle and ampullae). Orchitis and epididymitis occur occasionally. One or both scrotal sacs may be affected with acute painful swelling to twice the normal size. The seminal vesicles may be affected and their enlargement can be detected on rectal palpation. Bulls are most resistant but may get infection at younger ages or may acquire infection by ingestion. Two clinical pictures are described; one which involves the testis and the epididymis and is characterized by orchitis whereas the other involves seminal vesicular glands and the ampoulae and there may be focal areas of adhesion between tunica vaginalis and the testicle. Sperm granulomas may form with chronic fibrosis of intestinal tissue. (Mdegela *et al*., 2005).

The predominant sign in females is abortion or premature or full term dead or weak calves and retained placentas. In the adult non-pregnant cow, localization occurs in the udder and the uterus if it becomes gravid, is infected from periodic bacteraemia phases originating in the udder. Infected udders are clinically normal but are a good source of infection for calves or humans drinking the milk. (Radostits *et al*., 1994).

B.abortus can often be isolated from lesions of non-suppurative synovitis in cattle (Radostits *et al*., *2000)*. Hygromatous swelling especially on the knees should be viewed with suspicion. There are reports of progressive and erosive non- suppurative arthritis of the stifle joints occurring in young cattle, from brucellosis free herds that had been vaccinated with strain-19 vaccine. *B.melitensis* and *B. suis* may also infect cattle when they share pasture or facilities with infected pigs, goats or sheep. Such infection in cattle caused by heterogonous species of *Brucellae* may be more transient than that caused by *B.abortus*. However, such cross infections are a serious public health threat, since these *Brucellae* which are highly pathogenic to man, can pass into cow's milk. Infections by *B.melitensis* have a disease course similar to that caused by *B.abortus*. In cattle, *B.abortus* are usually the cause of brucellosis but *B.melitensis* has also been implicated in causing abortion in cattle where they are kept in close association with infected sheep or goats. Occasionally, *B.suis* may infect the mammary glands of cattle but has not been reported to cause abortion in this species.

Brucellosis in goats and sheep is mainly caused by *B.melitensis* (Shirima *et al.,* 2003). The disease in these animals is similar epidemiologically to bovine brucellosis. Infection by *B. suis* and *B.abortus* have occasionally been found. This, however, is rare. The main symptom is abortion, which occurs most frequently in the third or fourth month of pregnancy. Hygromas, arthritis, spondylitis and orchitis are some of the signs seen. In contrast to brucellosis in females of other domestic species, mastitis is a common symptom in goats and may be the first noticeable sign in a flock. Clotting in milk and small nodules in the mammary gland may be observed. Small ruminant brucellosis is mostly caused by *B.melitensis* and constitutes a public health problem (Omer *et al.*, 2002). This infection is found in areas of mixed goat and

sheep flocks. Sheep are more resistant to infection than goats and fewer sheep than goats are found to be infected (Radostits *et al.,* 2000).

B.ovis is also an important cause of orchitis and epididymitis in sheep but it is not recognized as a cause of natural infection in goats. Persistent infection is a common feature of the disease with frequent shedding of the bacterium in reproductive and mammary secretions. Brucellosis has been reported in small ruminants from different parts of the world. Prevalence rates of 3.8 % in goats and 1.4 % in sheep in Eritrea (Omer *et al*., 2000); 4 % in goats and 1 % in sheep in eastern Sudan (El – *Ansary et al*., 2001). *B.canis* causes epididymitis and orchitis in male dog and metritis in bitches and it is a rare infection in humans. Brucellosis in pigs is caused by *B.suis* and characterized by an initial bacteraemia followed by the production of chronic lesions in the bones and reproductive organs of both sexes (Radostits *et al.*, 2000).

2.1.8 Diagnosis

2.1.8.1 In Humans

Signs and symptoms of brucellosis are not specific. Pointers to the diagnosis are a history of occupational exposure or recent travel to endemic areas with consumption of milk products (Corbel, 2002). The disease is confirmed in man by isolating the organisms from blood, cerebrospinal fluid (CSF), peritoneal fluid, semen, vaginal swabs and tissues from the Spleen, liver and lymph nodes, which are specimens of choice (Anon., 2000) through culturing. Diagnosis of brucellosis usually depends on serological tests, the results of which tend to vary with the stage of the infection.

2.1.8.2 In animals

The best approach in the diagnosis of brucellosis is a combination of epidemiology, serology, clinical and bacteriological evidence (OIE, 2010). But the absence of pathognomonic signs

may lead to unreliable clinical diagnosis. The presumptive diagnosis based on clinical history of abortion, retained placenta in females and lesions in the seminal vesicles and testis in the male must be sustained with demonstration of the organism and or specific antibodies in the body fluid for making confirmatory diagnosis of brucella infection.

2.1.8.3 Laboratory diagnosis

Diagnosis requires prompt detection and identification of coccobacillus for appropriate patient management as the organism is associated with a potentially severe outcome. Isolation of the organism by culture or guinea pig inoculation is done from citrated blood. However, isolation from blood is not always possible and serological tests play a major role in the routine diagnosis.

Brucellosis is confirmed by isolating the organism from blood or other tissue samples and by serological tests. In animals, culture is attempted from abortion material, placenta, milk, semen or from samples of lymphoid tissue, mammary gland, uterus or testis collected during the post-mortem. Laboratory diagnosis relies on serological tests presently used which include; Rose Bengal plate tests (RBPT), Complement fixation test (CFT), Enzyme Linked Immunosorbent Assay (ELISA) test, Milk Ring Test (MRT), Milk ELISA and most recently Polymerase Chain Reaction (PCR)

The routine serological tests offer good results for the diagnosis of brucellosis upon use of adequate cut- off point's .Serological tests applied in human beings are modifications of those used in animals. The simulation of the immune system by Brucella antigens shows a transient character. Immunoglobulin G (IgG) and /or IgM/ IgA antibodies are secreted by peripheral blood mononuclear cells. They disappear 5 to 20 months after onset of clinical signs and 20 to 27 days after vaccination. Detection of these immunoglobulins could improve the diagnosis of

brucellosis. Serological tests presently used include; Rose Bengal plate test (RBPT), Complement fixation test (CFT), Enzyme Linked Immunosorbent Assay (ELISA) test and most recently Polymerase Chain Reaction (PCR) (OIE manual 2010).

The Rose Bengal Plate Test (RBT) is a screening test with high sensitivity (90%) but low specificity (75%). As such it does not discriminate between S19 vaccinations and natural infections (Nielsen *et al*., 1995; 1996).The Complement Fixation Test (CFT) is used as a confirmatory test for *Brucella* species with high specificity (100%) but lower sensitivity (89.9%) (Nielsen *et al*.,1996). However its major limitations are the cumbersome procedures of inactivation of nonspecific complements. Other limitations are the time consuming technical procedures and possible existence of residual anti complementary sera.

The Enzyme-linked immunosorbent assay (ELISA) in general detects antibodies that could have been missed by the RBT, SAT or CFT (Van Aert *et al*., 1984) while Indirect ELISA with its high specificity (98.9%) takes care of the limitations experienced in SAT which are cumbersome. Competitive ELISA (C-ELISA) is the best tool in that it has fewer stages compared to the other three diagnostic tests mentioned above. It is quite adaptive to reagents/automations and can discriminate vaccinated from infected cattle at the level of at least 85% specificity (Dohoo *et al*., 1986; Uzal *et al*., 1995). The principle for *Brucella* antibody C–ELISA is that serum samples are subjected to *B. abortus* smooth lipopolysaccharide (SLPS) coated wells on microtiter plates together with a mouse monoclonal antibody (mAb) specific for an epitope on the O–polysaccharide portion of the SLPS antigen. The microplates are washed after incubation and goat anti–mouse IgG antibody with horseradish peroxide is added which binds to any mAbs bound to the SLPS on the micro plate. If anti–*Brucella* natural antibodies are present in the test serum (positive) they would

compete with the mAb for the epitope sites and thus inhibits it from binding to the O–polysaccharide portion of SLPS. In case the anti–*Brucella* natural antibodies were absent in the test serum (negative), the mAb would bind to the O– polysaccharide epitope of SLPS antigen. When the conjugate is added it binds specifically to this mAb. Unbound materials are removed by rinsing before the addition of substrate solution. Colour development is due to the conversion of substrate by the conjugate. Sera from strain 19 vaccinated cattle do not compete with mAbs because of their specificity and lower affinity. The optical density is measured by a micro plate photometer at 450 nm based on the amount of light transmitted after passing through the solution. This is directly proportional to the concentration of bound antibodies in the solution.

Milk Ring Test (MRT) is used to detect antibodies in milk. The test depends on two reactions: (i) fat globulins in milk are aggregated by milk antibodies (fat globule agglutinins) and (ii) stained brucella antigens are added to the milk and will be agglutinated by the Brucella antibody in the fat globule and then rise to form a coloured cream layer at the top (Anon, 1986). This is a sensitive screening test used on bulk milk samples either to detect infected animals on a herd basis or to monitor clean herds. MRT is a simple and effective method, but can only be used with cow's milk. A drop of haematoxylin-stained antigen is mixed with a small volume of milk in a glass or plastic tube. If specific antibody is present in the milk it will bind to the antigen and rise with the cream to form a blue ring at the top of the column of milk. The test is reasonably sensitive but may fail to detect a small number of infected animals within a large herd. Non-specific reactions are common with this test, especially in brucellosis free areas. Milk ELISA may be used to test bulk milk and is extremely sensitive and specific, enabling the detection of single infected animals in large herds in most circumstances.

Molecular based techniques have replaced phenotypic characterization which classifies *Brucella* into biotypes according to the biological and physiological characteristics. These techniques comprise Polymerase Chain Reaction (PCR) using primers from 16S rRNA sequence of *Brucella abortus* and Restriction Fragment Length Polymorphism (RFLP) (Bricker, 2002a; 2002b). Primers are universal and standardized so that they can be applied across all the molecular tests where *Brucella* is suspected.

2.1.9 Treatment

2.1.9.1 In animals

Treatment is not normally done because of the intracellular sequestration of organisms in the lymph nodes, mammary glands and reproductive organs (Radostis *et al*., 2000).

2.1.9.2 In humans

Brucella infections in human respond to a combination of streptomycin or Gentamicin and tetracycline or Rifampicin and Doxycycline (OIE manual 2010). Tetracycline alone is often adequate in mild cases. Treatment should be continued for at least six weeks. The current recommendation is the combination of oral Doxycycline with Rifampicin for six weeks as a minimum. In severe disease, a third drug, Co-trimoxazole, can be added to the treatment. Some cases have to be treated for very long periods, for example one year. Some chronic cases require intermittent, repeated treatments for life in order to relieve symptoms, since complete cure cannot be achieved.

2.1.10 Control, eradication and prevention

2.1.10.1 In animals

Programmes for control must locate the infection, contain it and eliminate infected animals by employing a testing scheme. The major components of a control and eradication program are as follows: test and slaughter of reservoir of infection, quarantine of the remaining cattle, and depopulation in situations where all animals are presumed exposed (Radostits *et al.*, 2000).

Vaccination has been applied to control the spread of animal brucellosis but it does not eliminate infection and therefore constitutes a perpetual infection risk to consumers of raw animal products (Muendo et al., 2012). Live, attenuated or inactivated vaccines have been used. *B.abortus* strain 19 attenuated is the most widely used. It is capable of inducing abortion in pregnant cows but unable to spread from one animal to another. It is not recommended for use in bulls because it can cause orchitis and epididymitis.

The live attenuated smooth strain *B. melitensis* Rev 1 is used to protect sheep and goats from *B. melitensis* infection. Vaccination of pigs is not widely practiced but the attenuated B. suis strain 2 has been used in China (Corbel, 2002).

2.1.10.2 In humans

Human vaccination is not recommended because effective and non- reactogenic vaccines are not currently available. Pasteurization eliminates the risk of brucellosis from consumption of infected milk or milk products. However, there remains the possibility of infection due to contact with infected animals or their tissues. Veterinary surgeons, farmers and laboratory workers are particularly at risk. Strict hygiene practices are important in control through contact.

2.2 Q- fever

Q-fever is a disease caused by infection with the obligate intracellular bacterial pathogen, *Coxiella burnetii*. It is a zoonotic infection, typically transmitted from animal hosts to humans through inhalation of contaminated aerosols or ingestion of infected animal products such as milk or cheese. Ticks have also been implicated as vectors, and over 40 species of ticks have been found naturally infected with the agent (Maurin and Raoult, 1999).*Coxiella burnetii* has a wide host range, including wild and domestic mammals, birds, reptiles, and arthropods. Domestic ruminants primarily goats, cattle, and sheep represent the most frequent source of human infection (Maurin, and Raoult,1999; Darryn, *et al*., 2013) although transmission from dogs and cats is also documented (Darryn, *et al*., 2013). The uterus and mammary glands of female animals are sites of chronic *C. burnetii* infection, and infected females may shed large amounts of bacteria into the environment during parturition or spontaneous abortion. Once shed, the organisms may remain infective in the environment for several months (Maurin and Raoult, 1999; Darryn, *et al*., 2013).

Infection in humans, usually by inhalation, may be asymptomatic (up to 60% of infected individuals) or may manifest clinically after an incubation period ranging between 1 and 3 weeks. Clinical signs of acute Q-fever include fever of 2– 14 days' duration, atypical pneumonia, and/or hepatitis. Although the disease is typically self-limiting, severe debilitating illness requiring hospitalization can occur in a small proportion (2–5%) of acutely infected cases. Chronic disease may develop following infection, particularly in patients with predisposing conditions such as pre-existing cardiac valvulopathy, pregnancy, or immunosuppression. Common manifestations of chronic disease include endocarditis and vascular infection. *Coxiella burnetii* displays antigenic (phase) variation associated with loss of virulence and mutational variation in the lipopolysaccharide (Maurin and Raoult,1999;

Raoult.*et al*.,2005; Darryn, *et al*.,2013). High levels of antibodies to phase I antigens are detected during chronic Q-fever, whereas antibodies to phase II antigens are produced in acute disease. *Coxiella burnetii* is found worldwide, with the exception of New Zealand (Darryn, *et al*., 2013).

A recent large outbreak in Netherlands involved at least 3,523 human cases from 2007 through 2009, and was characterized by a high rate of hospitalization, with 20% of notified cases admitted to hospital in 2008–2009 (Van Der Hoek *et al*., 2010). Pneumonia was the predominant clinical presentation,(Roest *et al*, 2011; Darryn, *et al*., 2013). The Netherlands outbreak has been linked to the increase in the country's dairy goat population, which more than doubled in size between 2000 and 2009, and highlights the public health risks of Q-fever epidemics posed by domestic ruminants. Despite the high-profile nature of some Q-fever outbreaks, and the attention that *C. burnetii* has received as a potential bioterrorism agent, (Madariaga *et al*., 2003), information on the prevalence of infection in sub-Saharan Africa is scanty (Tissot-Dupont *et al.,*1995; Mediannikov et *al.,*2010).

In Kenya, serological evidence of Q-fever in patients with acute febrile and respiratory illness was shown in the 1950s (Craddock and Gear, 1955; Brotherson and Cooke 1956, Vanek and Thimm 1976) and recent times in western Kenya (Darryn, *et al*.,2013). Other studies showed the prevalence of antibodies to *C. burnetii* among Kenyans to range between 10% and 20% (Darryn, *et al*.,2013). A more recent investigation found that four people (8%) of a group of 50 travellers to Kenya contracted Q-fever, (Potasman *et al*., 2000; Darryn, *et al*., 2013) and in another recent study investigators diagnosed acute Q-fever in 5% of febrile patients admitted to two hospitals from September 2007 to August 2008, in neighbouring northern Tanzania (Prabhu *et al*., 2011; Darryn, *et al*., 2013). Among human and domestic ruminants in Kenya, the prevalence of antibodies was reported as 7–57% in cattle and 33–34% in goats (Darryn, *et*

al., 2013). Antibodies against *C.burnetii* were detected in 30.9% (N = 246) of archived patient sera and in 28.3% (N =463) of cattle, 32.0% (N = 378) of goats, and 18.2% (N = 159) of sheep surveyed in western Kenya in 2007-2008 (Darryn, *et al*., 2013).

2.2.1 Occurrence and distribution

Q-fever is widely distributed throughout the world with the exception of New Zealand. The disease affects mostly humans, cattle, sheep and goats (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005; EFSA, 2010). Antibodies to *Coxiella burnetti* have been detected in 5% of human sera in urban Tanzania and in 37% in Zimbabwe. Sero-prevalence studies completed in Tanzania have documented prevalence estimate ranging from 7% - 17% in agricultural animals suggesting their likely role as reservoirs for human infection via environmental contaminations. A recent study in Western Kenya done in 2007 to 2008 showed an overall prevalence 27.4% in human and livestock species sampled (Darryn, *et al*., 2013).

2.2.2 Aetiology

The aetiological agent, *Coxiella burnetii*, is a Gram-negative obligate intracellular bacterium, adapted to thrive within the phagolysosome of the phagocyte. It has been historically classified in the *Rickettsiaceae* family; however, phylogenetic investigations, based mainly on 16s rRNA sequence analysis, have shown that the *Coxiella* genus is distant from the *Rickettsia* genus in the *alpha* subdivision of *Proteobacteria* (Drancourt and Raoult, 2005). *Coxiella burnetii* has therefore been placed in the *Coxiellaceae* family in the order *Legionellales* of the *gamma* subdivision of *Proteobacteria*. The complete genome sequencing of *C. burnetii* has been achieved and confirms its systematic position. Unlike rickettsiae, *C. burnetii* produces a small, dense, highly resistant spore-like form. This ability has been attributed to the existence

of *C. burnetii* developmental cycle variants described from *invitro* studies as large-cell variants (LCV), small-cell variants (SCV), and small dense cells (SDC) measuring 0.2 μm wide and between 0.5 and 2 μm long or 0.4 to 0.7 μm diameter (Coleman *et al.,* 2004). The SDC and SCV represent the small morphological variants of the bacteria likely to survive extracellularly as infectious particles, a trait that is important for persistence in the environment and transmission (ECDC, 2010; EFSA, 2010; Kersh *et al.,* 2010).

Another essential characteristic is that *C. burnetii* has two antigenic forms: the pathogenic phase I, isolated from infected animals or humans, and the attenuated phase II, obtained by repeated *in-ovo* or *in-vitro* passages. An LPS (lipopolysaccharide) change occurs during serial passages: phase I cells, with full-length LPS O-chains, change to intermediate phases with decreasing LPS O-chain lengths and then to phase II, with truncated LPS. Thus, the long phase I LPS contains the phase II part. The latter has been described as a major immunogenic determinant. Currently available commercial tests allow the detection of at least the anti-*C. burnetii* phase II antibodies, which appear to be present whatever the infection stage or form. In contrast, vaccination is effective with a phase I vaccine but not with a phase II vaccine (Arricau-Bouvery *et al.,* 2005; EFSA, 2010). In general, the genomes of *C. burnetii* isolates from a wide range of biologically and geographically diverse sources are highly conserved, but notable polymorphism occurs such as rearrangements of syntenic blocks (Beare *et al.*, 2009). This genomic plasticity might contribute to different phenotypes and is of great interest for genotyping methods.

2.2.3 Transmission and clinical signs

2.2.3.1 Transmission and Clinical signs in animals

In animals, vertical transmission and sexual transmission could occur but their importance is not known. Finally, arthropods, principally ticks, may be involved in Q-fever transmission. The risk of transmission seems to be linked to wildlife animals. It could be associated with tick bites as well as with contaminated dust from dried excrement.

In cows, ewes and goats, Q-fever has been associated mostly with late abortion and reproductive disorders such as premature birth, dead or weak offspring (Arricau-Bouvery and Rodolakis, 2005). Moreover, *C. burnetii* might be associated with metritis and infertility in cattle. Given the lack of specificity of these latter signs, it is not recommended to retain them for clinical diagnosis of Q-fever (EFSA, 2010).

Domestic ruminants are mainly subclinical carriers but can shed bacteria in various secretions and excreta. In the environment, *C. burnetii* can survive for variable periods and can spread. The levels of bacterial contamination in the environment have been tackled using quantitative PCR (Polymerase Chain Reaction) for detection of *C. burnetii* DNA, but a rapid test assessing viability is required (EFSA, 2010; Kersh, 2010). For now, lack of knowledge on shedding patterns among ruminants has made the determination of Q-fever status difficult. Concomitant shedding into the milk, faeces and vaginal mucus may be rare (Rousset *et al.*, 2009a ; Guatteo *et al.*, 2007). The vaginal shedding at the day of kidding may be the most frequent (Arricau-Bouvery *et al.*, 2005). Within herds or flocks experiencing abortion problems caused by *C. burnetii*, most of animals may be shedding massive numbers of bacteria whether they have aborted or not. The global quantities are thus clearly higher than within sub clinically infected herds/flocks. At parturitions following an abortion storm, higher bacterial discharges were

measured among the primiparous compared with the other females (Guatteo *et al.*, 2008; Rousset *et al.*, 2009b). Moreover, the shedding may persist several months, describing either an intermittent or a continuous kinetic pattern. Animals with continuous shedding patterns might be heavy shedders. These latter animals seem to mostly exhibit a highly sero-positive serological profile (Guatteo *et al.*, 2007). Arthropods, principally ticks, may be involved in Q-fever transmission (Sidi-Boumedine *et al.*, 2010).

2.2.4 Transmission and clinical signs in humans

2.2.4.1 Transmission

Humans generally acquire infection through air-borne transmission from animal reservoirs, especially domestic ruminants and from contaminated raw milk. In domestic ruminants, infection is principally transmitted by inhalation of desiccated aerosol particles, and through contact with infected animals, their reproductive tissues or other animal products, like wool (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005; ECDC, 2010).

Ingestion has been often suggested, particularly through the consumption of dairy products derived from contaminated raw milk, but no good evidence has shown a significant transmission to humans by food. Indeed, there are epidemiological indications of sero-conversion but no association with clinical *Q-fever* in humans. Q-fever seems also very rarely transmissible from person to person, although exposure during childbirth, through sexual transmission or blood transfusion is possible.

2.2.4.2 Clinical signs

In humans, the infection can manifest as an acute, chronic or subclinical form (Maurin and Raoult, 1999; ECDC, 2010). Diagnosis and the treatment is often delayed because of the various and nonspecific clinical expressions. The acute forms commonly include a self-limiting febrile episode, pneumonia or granulomatous hepatitis. The main clinical manifestation of chronic Q-fever is endocarditis in patients with valvulopathies, vascular infections, hepatitis or chronic fatigue syndrome. The acute form resolves quite quickly after appropriate antibiotic therapy, but the chronic form requires prolonged antibiotic therapy (for 2 years or more), coupled with serological monitoring (ECDC, 2010).

In the absence of any appropriate antibiotic treatment, complications of the chronic form may be severe to fatal. Moreover, *C. burnetii* infection of pregnant women can provoke placentitis and leads to premature birth, growth restriction, spontaneous abortion or foetal death. Overall, the chronic disease is more likely to develop in immunocompromised individuals. The infection is endemic in many areas leading to sporadic cases or explosive epidemics. Its incidence is probably greater than reported (Roest *et al*., 2011).

Awareness for Q-fever is increased during human outbreaks, which are generally temporary and rarely comprise more than 300 acute Q-fever cases. However, the largest community outbreaks of Q-fever ever reported emerged involving at least 3,523 human cases from 2007 through 2009, in the Netherlands and was characterized by a high rate of hospitalization, with 20% of notified cases admitted to hospital in 2008–2009 (Van Der Hoek *et al*., 2010; Roest *et al*., 2011). In the subsequent years, peak incidence from February to September increased and the geographical area expanded progressively. The country reported 982 and 2305 confirmed

cases in 2008 and 2009, respectively. However, the factors leading to outbreaks are not fully understood (ECDC, 2010; EFSA, 2010).Domestic ruminants are considered the main reservoirs for *C. burnetii*, but cats, dogs, rabbits, birds have also been reported to be implicated in human disease/infection (ECDC, 2010; EFSA, 2010).There is clear epidemiological and experimental evidence that the infection is principally transmitted by inhalation of desiccated aerosol particles, and through contact with infected animals, their reproductive tissues or other animal products, like wool (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005; ECDC, 2010).

2.2.5 Diagnosis in animals and humans

Diagnosis of Q-fever in ruminants, including differentiating it from other abortive diseases, has traditionally been made on the basis of microscopy on clinical samples, coupled with positive serological results (EFSA, 2010). At present, direct detection and quantification by PCR and serological Enzyme-Linked Immunosorbent Assay (ELISA) should be considered as the methods of choice for clinical diagnosis (Sidi-Boumedine *et al.*, 2010). Proposals have been recently elaborated for the development of harmonised monitoring and reporting schemes for Q-fever, so as to enable comparisons over time and between countries (EFSA, 2010; Sidi-Boumedine *et al*., 2010). However, no gold standard technique is available and efforts are encouraged both for the validation of the methods and for development of reference reagents for quality control, proficiency and harmonization purposes (Principles of validation of diagnostic assays for infectious diseases). The Q-fever diagnostic tests are also required for epidemiological surveys of ‘at risk’ and suspected flocks in limited areas (following recent outbreaks in humans or animals), or for exchanges between herds or flocks.

Identification of the agent *Coxiella burnetii* can be demonstrated in various ways, depending on the type of sample and the purpose of investigations (Samuel and Hendrix, 2009; Sidi-Boumedine et al., 2010).

2.2.5.1 Serological tests

Among the various techniques that can be employed, the three most often used are: the indirect immuno-fluorescence assay (IFA), the ELISA and the complement fixation test (CFT). Three older serological tests are no longer used in routine diagnosis: the micro agglutination technique, the capillary agglutination test and the indirect haemolysis test. Overall, ELISAs are preferred for practical reasons. Currently, no IFA is commercially available for ruminants. Numerous reports showed a weak sensitivity of CFT compared with other methods (EFSA, 2010; Kittelberger *et al.,* 2009; Rousset *et al.,* 2007; 2009a). Serological tools allowing specific antibodies detection in sera from different animal species (not only ruminants) should be developed. The presence of specific Ig_G anti-*C.burnetii* antibodies provides evidence of a recent infection as well as a past exposure. Serological assays are suitable for screening herds or flocks, but the interpretation at the individual animal level is not possible. Indeed, a significant proportion of animals shedding *C. burnetii* bacteria and even some Q-fever aborted animals are found to be seronegative (Arricau-Bouvery *et al.*2005; Guatteo *et al.*,2007; Rousset *et al.*, 2007, 2009a). Serological cut-off values used to diagnose Q-fever are given by the suppliers. Both serological responses and bacterial evidence are often necessary for establishing the presence of the infection.

(i) Indirect Immuno-fluorescence Assay (IFA)

In human medicine, the IFA adapted as a micro-immuno fluorescence technique is the current method for the serodiagnosis of Q-fever .The procedure can be adapted to perform an immune

peroxidase assay. Briefly, both phase I and phase II *C. burnetii* antigens are used; phase II antigen is obtained by growing *C. burnetii* Nine Mile reference strain in cell culture, while phase I antigen is obtained from the spleens of laboratory animals inoculated with phase II *C. burnetii* in cell cultures. A few phase I cells may still be present in the phase II population and can be selected and propagated within animals. Antigen is diluted, dropped on to the wells of a glass microscope slide, allowed to dry, and fixed with acetone. The two forms of the infection, acute and chronic, have different serological profiles: during acute Q-fever, IgG antibodies are elevated against phase II only whereas during chronic Q-fever, high levels of IgG antibodies to both phase I and II of the bacteria are observed (Tissot-Dupont *et al.,* 1994). In addition, antigen-spot slide wells may be purchased from a supplier providing the phase II form, or the phase I and II forms of *C. burnetii*. These can be adapted by replacing the human conjugate by a conjugate adapted to the animal species. Twofold dilutions of the serum under test are placed on immunofluorescence slides with wells previously coated with one or two antigens. If specific antibodies are present, they are fixed by the antigen on the slide. The complex is then detected by examination with a fluorescence microscope following the addition of the fluorescent conjugate recognising the species-specific immunoglobulins.

(ii) Complement fixation test (CFT)

This cold fixation micro method of the type developed by Kolmer (FAO, 2006) is performed with 96-well U-bottomed microtitre plates. The test detects complement-fixing antibodies present in the serum. The CFT is specific but less sensitive than the ELISA or IFA (Kittelberger *et al.,*2009; Rousset *et al.,*2007; 2009a). The CFT is still used by laboratories in many countries. This method often uses antigen in phase II prepared from a mixture of two strains (Nine Mile and Henzerling) or antigen in phase I and II mixture prepared from Nine

Mile strain3. The reaction is done in two stages. Antigen and complement-fixing antibodies are first mixed, and sheep erythrocytes, sensitised by the anti-sheep erythrocyte serum, are added. Fixation of the complement by the antigen/antibody complex during the first step does not permit lysis of erythrocytes; in contrast, if there are no complement-fixing antibodies, the complement induces the lysis of the sensitised erythrocytes. Then the haemolysis rate is inversely proportional to the level of specific antibodies present in the sample serum.

(iii) Enzyme-linked immunosorbent assay (ELISA)

This technique has a high sensitivity and a good specificity (Rousset *et al.,* 2007; Kittelberger *et al.,*2009a). It is easy to perform in laboratories that have the necessary equipment (a spectrophotometer) and reagents. The ELISA is preferred to IFA and CFT, particularly for veterinary diagnosis, because it is convenient for large-scale screening and, as it is a reliable technique for demonstrating *C.burnetii* antibody in various animal species. Ready-to-use kits are commercially available and can detect anti-phase II antibodies or both anti-phase I and II antibodies. Wells of the microplate are coated with *C.burnetii* whole-cell inactivated antigen. Diluted serum samples are added to the wells and react to antigens bound to the solid support. Unbound material is removed by washing after a suitable incubation period. Conjugate (horseradish-peroxidase-labelled anti-ruminant IgG) reacts with specific antibodies bound to the antigen. Un-reacted conjugate is removed by washing after a suitable incubation period. Enzyme substrate is added. The rate of conversion of substrate is proportional to the amount of bound antibodies. The reaction is terminated after a suitable time and the amount of colour development is measured spectrophotometrically.

2.2.6 Treatment

Treatment in animals has not been attempted**.** In humans Fluoroquinolones, Co-trimoxazole, and Doxycycline are active against *C. burnetii* in vitro, and Ceftriaxone has been shown to have a bacteriostatic effect and could be effective in the phagolysosome of *C. burnetii*–infected cells (Maurin and Raoult, 1999). However, the treatment of choice for Q fever is Doxycycline.

Q-fever occurs in either acute form or a severe chronic form following an early infection that may go unnoticed (Maurin and Raoult, 1999; ECDC,2010).The acute form resolves quite quickly after appropriate antibiotic therapy, but the chronic form requires prolonged antibiotic therapy (for 2 years or more), coupled with serological monitoring. It causes endocarditis involving aortic valves presenting a serious condition.

2.2.7 Control

In humans, the overall impact of *C. burnetii* infection on public health is limited but there is a need for a better surveillance system. In human epidemic situations, active surveillance of acute Q-fever is the best strategy for avoiding chronic cases.

In animals, Q-fever can be controlled by preventive vaccination, manure management, changes to farm characteristics, for example separate housing for classes of animals, wool shearing management, a segregated kidding area, removal of risk material, visitor ban, control of other animal reservoirs and tick control, (Drancourt and Raoult, 2005; Kersh *et al.,* 2010).

Based on the reviewed information Q- fever is a disease that can occur in pastoral areas like Kajiado where the inhabitants live close and handle the animals on their daily operations without protective clothing. The disease can be transmitted by inhalation of contaminated particles and the community are not aware. The disease is transmitted through consumption of animal products posing a grave danger to this community. Testing of this disease is not done in all health facilities despite its manifestations of flu- like symptoms associated with other diseases.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was undertaken in three divisions of Kajiado County namely Kajiado East sub county (Mashuru), Kajiado Central sub county (Central) and Kajiado west sub county(Magadi) between July 2012 and October 2012. Kajiado is one of the 47 counties in Kenya, located at the South-Western part of the country. It borders the Republic of Tanzania to the West, Taita-Taveta County to the southeast, Machakos and Makueni Counties to the east, Nairobi County to the northeast, Kiambu County to the north and Narok County to the North-West. It lies between longitudes 36^{o} 5´and 37^{o} 5´ east and latitudes 1^{o} 0´ and 3^{o} 0´ south and covers an area approximately 21,902.9 km^2 (Kajiado County development plan 2007-2009). The land varies in altitude from about 500 meters around Lake Magadi to about 2,500 meters in the Ngong Hills area. Topographically, the County is divided into four different areas namely; Rift Valley, Kapiti Plains, Central Broken Ground and the Amboseli Plains.

The County has a bimodal rainfall pattern. Short rains fall between October and December while the long rains fall between March and May. Annual rainfall is strongly influenced by altitude. Loitokitok, which has a high elevation, has the highest average rainfall of 1,250mm while Magadi and Lake Amboseli with the lowest elevations have the lowest annual average rainfall of about 500mm. Temperatures in the County also vary with both altitude and season.

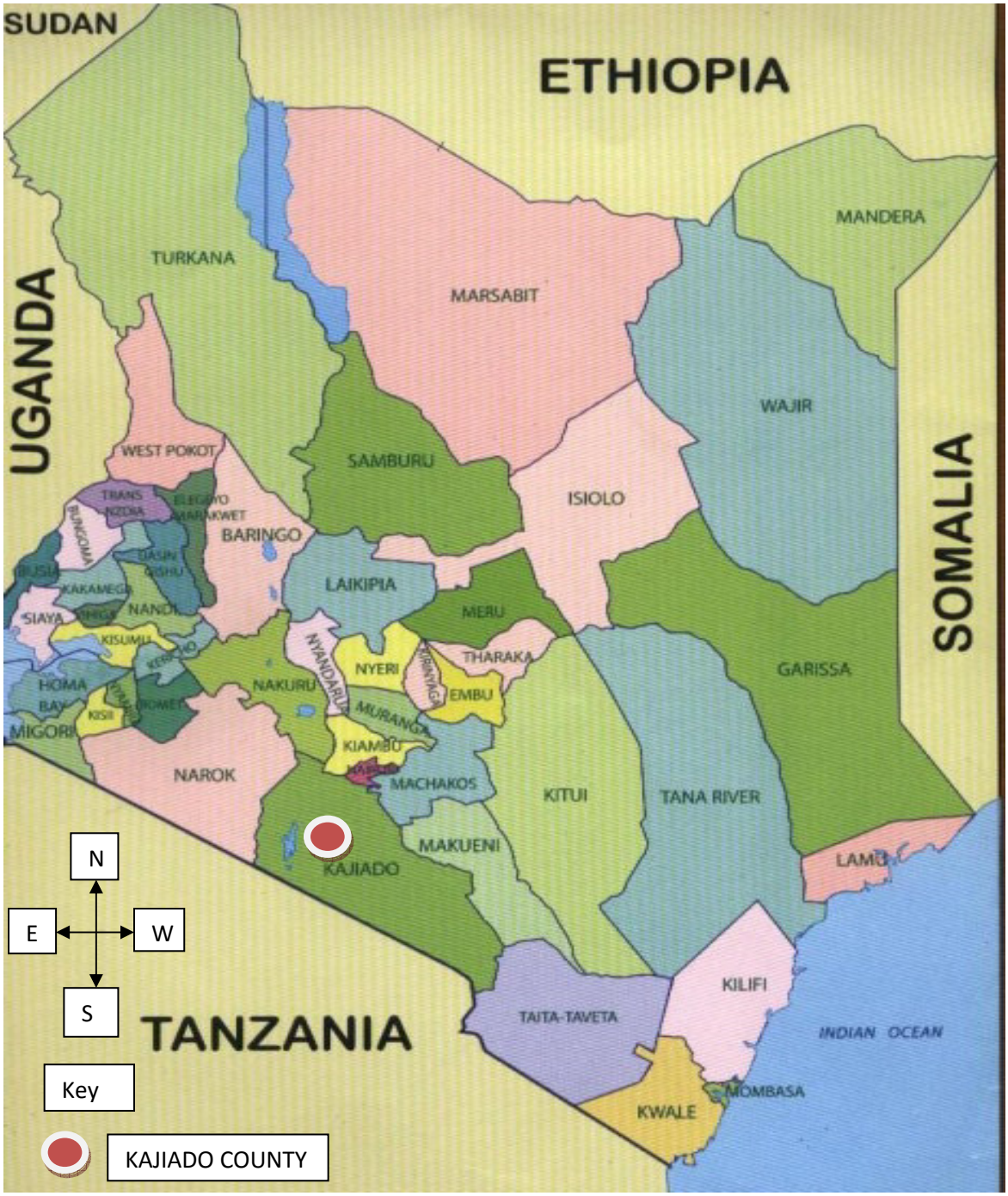


Figure 2 Map of Kenya showing Kajiado County (Source, Kenya Maps 2010)

The highest temperatures of about 34^{o}C are recorded around Lake Magadi while the lowest minimum of 10^{o}C is experienced at Loitokitok on the eastern slopes of Mt. Kilimanjaro. The coolest period is between July and August while the hottest months are from November to April throughout the County. The vegetation is predominantly wooded grassland. The county has few seasonal rivers thus the water sources for the majority of the people and their livestock are water pans and boreholes.

The study area was a transect of the county from east to west covering Kajiado East (Mashuru) Kajiado Central and Kajiado West (Magadi) Sub Counties.

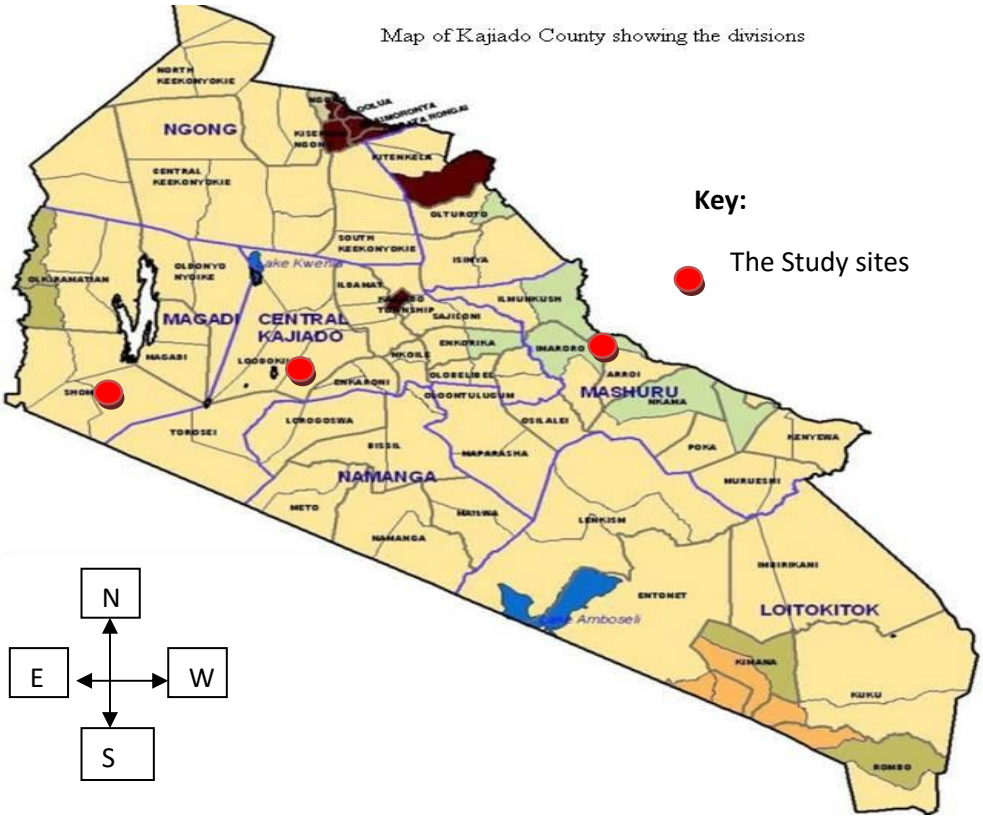


Figure 3. Kajiado County map showing the areas of study (Source, Kenya Maps 2010)

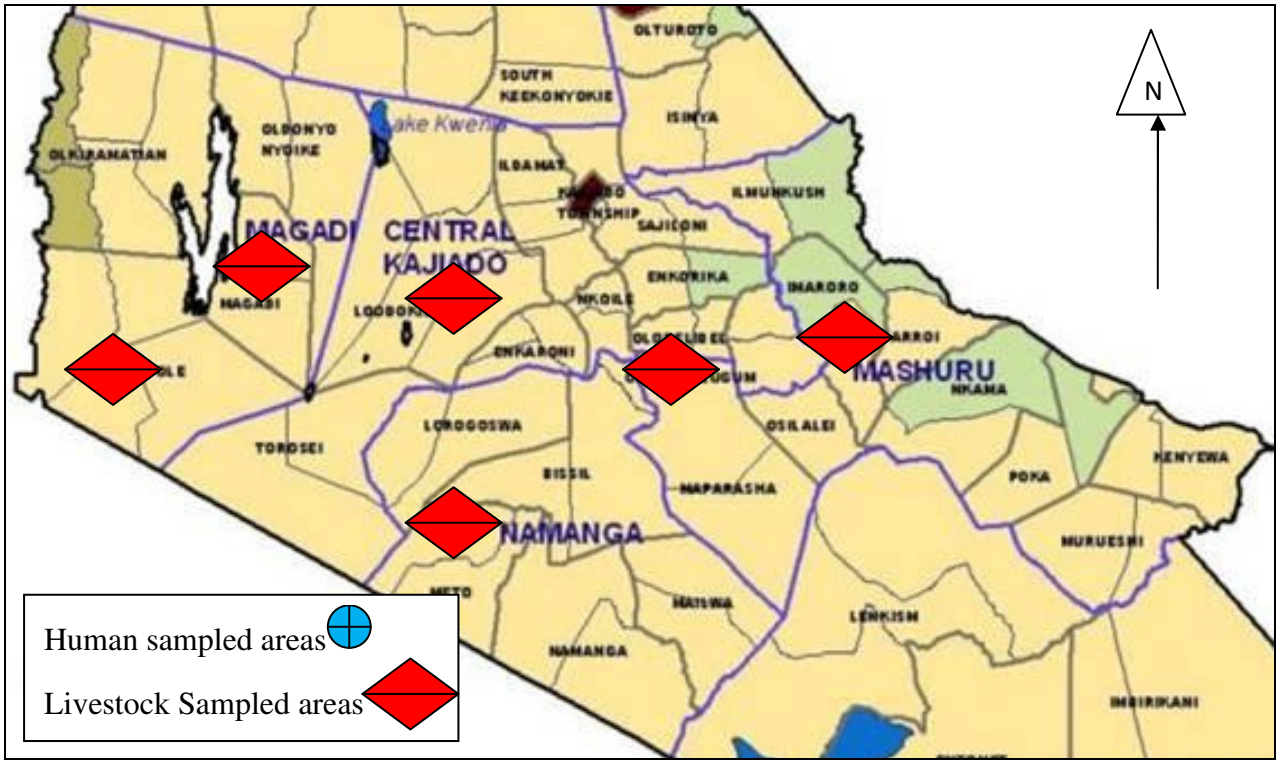


Figure 4. Kajiado County transect showing the areas sampled (Kenya Maps 2010).

Kajiado East Sub County has a human population of 35,666 and a livestock population comprising of 120,000 cattle, 152,164 sheep, 166,132 goats, 7 camels and 11,874 donkeys; Kajiado Central Sub County has a human population of 69,402 an a livestock population composed of 110,345 cattle, 99,000 sheep, 98,000 goats , 11,234 donkeys and 67 camels; Kajiado West (Magadi) has a human population of 20,111 and a livestock population composed of 25,698 cattle, 46,833 goats, 58,013 sheep and 2000 donkeys (Nyariki *et al*., 2005) distributed in three group ranches. The cattle breeds are Zebu-Sahiwal crosses tending towards pure Sahiwals, valued for their higher yield of milk and high sale value (Especially the brown colour of Sahiwal – *Rangi Ya Pesa).*

Livestock are herded individually or communally by families in communal land. Watering of animals is done daily during the rainy season using water pans and once after two days during the dry season using boreholes. Boreholes are communally owned by pastoralists in expansive

locations and serve as major sites for animal health care. The pastoralists live with their livestock and consume meat, blood and milk and use hides, skins and dung as bedding and building material.

The State Department of Livestock through the veterinary department is the major provider of animal health services by a lean staff of five. Mashuru and Kajiado have cold storage facilities but with no laboratories to support field diagnostic services.

To cater for the human health services, the area is served by Sultan Hamud hospital at the border of Mukaa and Mashuru Sub Counties in Makueni and Kajiado Counties respectively. It has a bed capacity of fifteen (15) and provides health services to residents of both Sub Counties together with very small dispensaries. Kajiado Central Sub County has about twenty (20) dispensaries owned by the Government of Kenya (GOK) or faith based groups with Kajiado County Referral hospital, with a bed capacity of 138 serving as the County referral hospital. Magadi area of Kajiado West Sub county is served by Magadi Hospital, with a bed capacity of fifty (50). It serves about 30,000 people from within and outside the Sub County. Tests for brucellosis were available in the three health facilities, but awareness of Q-fever was limited.

3.2 Study Design

Historical data of patient records in the three health facilities were compiled for the six years (2007-2012). Then a cross sectional survey was conducted to sample livestock in the three divisions of Magadi, Namanga and Mashuru to determine sero-reactors to brucellosis and Q-fever. Patients seeking medical attention in the three health facilities and presenting with flu-like symptoms in the course of the study were also sampled. Clearance for the research was sought from the National Council of Science and Technology Institute (NACOSTI), Ethical

Committee KNH/UON, Director of Medical Services (DMS), and Director of Veterinary Services (DVS).

3.3 Sample Size Determination

The sample sizes of the various livestock species and humans that were bled for sero-prevalence estimation of the two zoonoses was determined according to Dohoo *et al.*,(2010).

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

Where; n is the required sample size,

$Z\alpha = 1.96$, the normal deviate at 5% level of significance,

p A *priori* estimate of prevalence of the two diseases,

q=1-p and L is the allowable error of estimate

Cattle: Using the highest estimated prevalence of 15% for Brucellosis (Kadohira *et.al*., 1998) and setting L at 5%

The sample size required was $n = \frac{1.96^2 \times 0.15 \times 0.85}{(0.05)^2} = 196$

Sheep and goats: The estimated prevalence of 12% (Malavika Prabhu, *et al*, 2011)

$$n = \frac{1.96^2 \times 0.12 \times 0.88}{(0.05)^2} = 162$$

Humans: Using the highest estimated prevalence of 12% for Brucellosis (Maichomo 2000)

$$n = \frac{1.96^2 \times 0.12 \times 0.88}{(0.05)^2} = 162$$

3.4 Data Collection

The Sub County Veterinary Officers (SCVOs) in the three study sites were visited to discuss the sampling procedure, livestock movement, the location of the watering points and other necessary assistance. The scouts and animal handlers who helped to interview the farmers were sourced from the community. Farmers who were interviewed were systematically sampled in each farm and watering point. At the watering points, the diseases under study were introduced to the farmers in small groups as they brought their herds to water. Using semi- structured questionnaires (Appendix 1 and 11) knowledge gaps regarding the diseases were identified and information sought on the risk factors for brucellosis and Q-fever in both livestock. The information collected included the following:-

A. The two zoonoses in livestock

a) Management (grazing, breeding and watering system)

b) Introduction of new stock into the herd in the last one year.

c) Livestock owner's level of awareness or knowledge about the two zoonoses brucellosis and Q-fever.

d) Frequency of contact of the livestock owners with veterinary staff

B. The two zoonoses in Humans

a) Exposure history (how long has the patient been sick)

b) Consumption of unprocessed or under processed livestock products

c) Close association with livestock, through sharing of compound and water sources

d) Level of awareness or knowledge of the two zoonoses

For each person interviewed, the age, sex, manyattas, occupation and location were recorded in each case. Two more questionnaires were administered to the DVO (Appendix 111) and the

MOH (Appendix IV) to elicit information regarding the diseases occurrence in the District, symptoms/clinical signs, management, history of tentative and confirmed diagnosis and the trend of the diseases in the last five years for livestock and humans respectively. Laboratory records at the three health facilities, which carried out laboratory diagnosis, were taken to establish the trend of the diseases in humans over the last five years (MOH reports 2008-2012).

3.5 Sampling

Humans: The three health facilities were visited and patients presenting with flu-like symptoms recruited into the study upon consent. The hospital clinicians were in-charge of examining the patients, identifying the cases with flu-like symptoms, seeking their consent to participate in the study and taking of blood samples for testing for the presence of the two zoonoses. A total of 250 5-10ml samples were collected.

Livestock: Study herds were selected for sampling with the help of veterinary staff, based on the suspected cases of abortions and their accessibility. A total of 237 cattle, 167 goats and 167 sheep, six months old and above, were sampled using systematic random method. Blood samples (10 ml) were collected in plain vacutainers by bleeding from the jugular vein following restraint.

3.6 Serum separation

The blood samples were left to stand overnight in a cool box packed with ice to allow clotting and serum separation at Mashuru health centre, Kajiado Central Sub County veterinary office laboratory and Magadi hospital laboratory. Serum was harvested by centrifugation by decanting into plastic 2ml to 5ml vials depending on the yield. Vials were labelled appropriately and stored in freezers at the veterinary offices and hospitals.

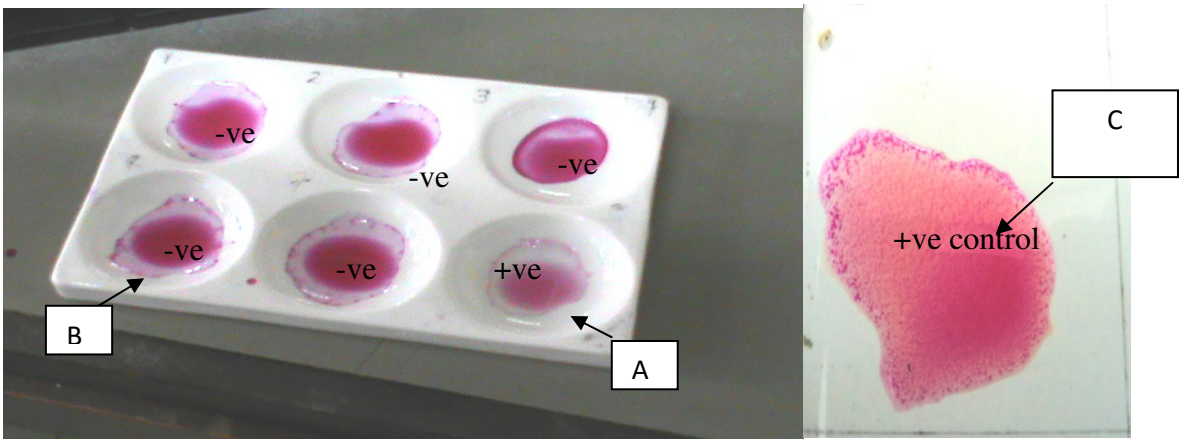
3.7 Laboratory tests

All the serum samples were aliquot into ependoffs vials to get working samples to avoid repeated thawing and refreezing of the samples. All the 579 cattle, sheep, goats and human samples were subjected to Rose Bengal Plate Test (RBPT) for brucellosis. However due to limited funds only one kit of Indirect Enzyme Linked Immunosorbent Assay (ELISA) (COMELISA, Veterinary Laboratories Agency, UK) that could test 400 samples from all the four species to detect antibodies against and Phase 1 and 11 of *Coxiella burnetti,* Competitive Enzyme Linked Immunosorbent Assay (ELISA) to detect antibodies against *Brucella.*

3.7.1 Tests for brucellosis

a) Rose Bengal Plate Test (RBPT)

Rose Bengal (RB) antigen was obtained from the National Veterinary Laboratories, Kabete. Serum samples and the test plate were first warmed to room temperature (21-22°C). Using a micropipette, 30µl of known sample were placed into the corresponding well in the plate followed by 30µl of well mixed Rose Bengal antigen. The two were then mixed thoroughly with an applicator stick and the plate then rocked on a rotator at 100rpm for four minutes. Results were read by examining macroscopically for the presence or absence of visible agglutination against a source of light immediately after removing the slide from the rotator. Agglutination denoted a positive test (+ve) while lack of it meant a negative (-ve) result (Fig. 6). Positive and negative controls were used to monitor the performance of the procedure and to compare the patterns for better interpretation.



A= Positive sample B= Negative sample C= Positive control

Figure 5. A plate of Rose Bengal Test showing positive and negative samples

b) Competitive Enzyme Linked Immunosorbent Assay (ELISA) for brucellosis

Competitive ELISA kit (COMELISA, Veterinary Laboratories Agency, UK) was used. The kit is standardized for the diagnosis of brucellosis in ruminants and human. The reagents were prepared and the tests carried out as per the instructions of the manufacturer. The optical densities (OD) were measured at 450nm in a microplate photometer (Hum reader, model 18500/1, Awareness Technology inc..Germany). Sera and controls were run in duplicates to compare the two OD readings for every sample. Positive results were denoted by lack of colour development. A positive/negative cut-off was calculated according to the manufacturer's recommendations of 60% of the mean of the optical density (OD) of the four conjugate control wells. Any test sample giving an optical density equal to or below this value was recorded as being positive. Each plate had six wells for positive control and another six wells for the negative control. In this study, cELISA was used as a confirmatory test and therefore any sera testing positive on this test was regarded as positive. The brucellosis prevalence was calculated based on this test using the formula; Prevalence in % = (Total number positive/ Total number of samples) x 100.

3.7.2 Test for Q- fever

Indirect multispecies Enzyme Linked Immunosorbent Assay (ELISA) ID Screen® Q-fever Indirect Multi-species kit (IDVET innovative diagnostics, Lillidale Diagnostics England) was used. The kit is standardized for the diagnosis of Q-fever in cattle, sheep and goats. The reagents were prepared and the tests carried out as per the instructions of the manufacturer. The optical densities (OD) were measured at 450nm in a microplate photometer (Hum reader, model 18500/1, Awareness Technology inc. Germany). Sera and controls were run in duplicates to compare the two OD readings for every sample. The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested. In the presence of antibodies (Positive), a blue solution appears which becomes yellow after addition of stop solution. In the absence of antibodies (Negative), no coloration appears. The test was validated if the mean value of the positive control optical density percentage (OD_{pc}) was greater than 0.350 and the ratio of the mean optical density (OD) values of the Positive and Negative control ($OD_{pc}/OD_{nc)}$ was greater than 3. For each sample, the sample percentage was calculated as: $s/p = OD_{sample} - OD_{nc} / OD_{pc} - OD_{nc} X 100$. The results were interpreted as shown in Table 1

Table 1. Interpretation of Q-fever ELISA test results

Serum	
Result	Status
S/P ≤ 40%	Negative
40% < S/P < 50%	Doubtful
50% < S/P ≤80%	Positive
S/P > 80%	Strongly positive

3.8 Data management and Analysis

a) Brucellosis data

All the data obtained from the field was recorded in the notebook and later entered into the computer using Microsoft Excel for serum analyzed data and SPPS v20 for questionnaire data for ease of handling. The data was analyzed and descriptive statistics of continuous variables done. All the1108 samples were tested for brucellosis using RBPT and out of the total, 400 samples were tested using cELISA.

a) Brucellosis and Q-fever

Association between the explanatory (independent) variables and the outcomes or dependent variables (prevalence of Brucellosis in cattle, sheep, goats and human; Q-fever in cattle, sheep, goats and human) were investigated by logistic regression using SPSSvs 20, IBM. The relationship between each explanatory variable and the outcome variable was investigated and any variable that was significantly associated at the $p < 0.05$ level was included in the multivariate models through forward and backward elimination, the most parsimonious models in which all explanatory variables remained significant at the $p < 0.05$ level was generated. The Z-test for independent samples (Dohoo *et al*., 2010) was used to determine whether the proportions of animals positive for *Brucella, C.burnetii,* and antibodies or a combination of the two or all the organisms differed significantly between Mashuru, Central and Magadi areas for livestock, and Sultan- Hamud, Kajiado, and Magadi hospitals for humans.

CHAPTER 4

4.0 RESULTS

4.1 Descriptive results

A total of 20 farms and watering points (from Mashuru, Kajiado central and (Magadi Sub Counties) comprising of 229 cattle 149 sheep and 140 goats were investigated for brucellosis and Q-Fever. At the same time 240 patients were investigated from three health facilities (Sultan –Hamud, Kajiado referral and Magadi hospitals). Details of the number of both livestock and human samples and Questionnaires collected are shown in Table 2.

4.1.2 Blood test results

Blood samples were obtained from 28/236 (12%) of persons eighteen years and below (≤18 years), 149/236 (63%) of humans aged 19-45 years, and 59/236(25%) of those above 45 but less than 60 years. The sex of these respondents were female 163/236 (69%) and 73/236 (31%) were male. Brucellosis serology results were available for 150 humans and 73 cattle, 69 sheep and 57 goats. A total of 90 human and 156 cattle, 80 sheep and 83 goat sera were tested for Q-fever. Complete questionnaire data was available for 236 humans and 88 livestock (see Appendix 1).

Results indicated a low overall prevalence of brucellosis in humans 1.3% (2/150) and a moderate overall prevalence in livestock of 12.9% (27/209). The prevalence estimates in cattle, sheep and goats were 21.9% (16/73), 8.6 % (6/69) and 7.3% (5/67) respectively for brucellosis. In humans the overall prevalence was found to be 1.3% with 1.8% in Kajiado district hospital, 1.5% in Sultan Hamud hospital and 0% in Magadi hospital. In Bovine, the highest prevalence was Mashuru area with 40%, followed by Magadi area with 19% but 0% in Kajiado area. The overall prevalence in the study area was found to be 21.9%. In Ovine, the

overall prevalence in the study area was 8.7%, with 14.8% in Mashuru, 5% in Kajiado and 4.5% in Magadi area. In Caprine, the overall prevalence was found to be 7.3%, with 3.3% in Mashuru, 10% in Kajiado and 11.8% in Magadi areas respectively.

The highest prevalence of Q-fever in humans was found to be 32% in Sultan Hamud hospital in Mashuru district, with 25% in Kajiado District Hospital in Kajiado district and 22% in Magadi Hospital serving residents in Magadi division. The overall prevalence of Q-fever in humans was found to be 26.7%. In Bovine the overall prevalence was found to be 89.7% with 88.5% in Mashuru, 85.2% in Kajiado and 92% in Magadi. In Ovine the overall prevalence was found to be 57.5% with 58.8% in both Mashuru and Kajiado and 55.2% in Magadi. The overall prevalence of Q-fever in Caprine was found to be 83.1%, with 79.4% in Mashuru, 86.2% in Kajiado and 85% in Magadi.

4.1.3 Household practises associated with presence of Q- fever and brucellosis.

As presented in Tables 3 and 4 the prevalence of the two diseases was higher in persons 15 years and above 82/223 (36.8%) than persons 5-14 years old and occupation of being a housewife had a high prevalence of 50/120 (41.6%) than all the other occupations because they handle livestock products. Cooking (frying) meat was more associated with sero positive 71/194 (36.6%) than roasting meat. A high number of respondents that used blood without processing 66/120 (32.4%) showed that this factor was significant in testing positive. Pastoralists that threw away foetal discharges and aborted foetuses in the bush were many at 3/7 (42.8%) as those giving it to the dogs 2/37 (5.4%).The data on livestock collected indicated that the majority of the respondents watered their livestock using borehole water (47) in dams (14) and water pans (12).

Table 2. Test results for Brucellosis and Q- fever in livestock and in human patients.

	Brucellosis		Q- fever	
Species	**Number of serum samples**	**Positive samples on cELISA(%)**	**Number of serum samples**	**Positive samples on IdELISA(%)**
Humans				
Kajiado	57	1 (1.8)	44	11 (25)
Mashuru	68	1 (1.5)	28	9 (32)
Magadi	25	0 (0)	18	4 (22)
Total	**150**	**2 (1.3)**	**90**	**24 (26)**
Cattle				
Kajiado	30	12 (40)	52	46 (88)
Mashuru	22	0 (0)	54	46 (85)
Magadi	21	4 (19)	50	48 (92)
Total	**73**	**16 (22)**	**156**	**140 (90)**
Ovine				
Kajiado	27	4 (15)	34	20 (59)
Mashuru	20	1 (5)	17	10 (59)
Magadi	22	1 (4.5)	29	16 (55)
Total	**69**	**6 (8.7)**	**80**	**46 (57.5)**
Caprine				
Kajiado	20	1 (3.3)	34	27 (79)
Mashuru	20	2 (10)	29	25 (86)
Magadi	17	2 (11.8)	20	17 (85)
Total	**57**	**5 (8.8)**	**83**	**69 (83)**

4.1.4 Factors associated with sero-prevalence of Brucellosis and Q-fever in human and livestock.

The results of logistic regression in the study area presented in Tables 3 and 4 showed that among the potential risk factors considered in human sero-prevalence in the study hospitals, occupation ($p<0.05$), method of processing meat ($p<0.01$), whether they process blood before taking ($p<0.001$), contact with aborted foetuses ($p<0.029$), and method of disposal of aborted foetuses and placenta ($p<0.028$) were significant. The age of the patients was slightly significant ($p<0.06$) while the contact with contaminated water and whether they consume milk or not were not significant.

In livestock as shown in Table 5 and 6 shows water source ($p<0.07$) is slightly significant in sheep. The results on Table 6 on the risks in livestock associated with Q- fever only gazing system in cattle particularly communal grazing was significant at ($p<0.005$). In sheep water source ($p<0.05$) and introduction of new animals ($p<0.03$) were significant.

Table 3. Factors associated with seroprevalence of brucellosis in humans (Results of backward stepwise logistic regression, significant at p <0.05)

Human brucellosis	**Group response**	**Number of respondents**	**Prevalence**	**rate%**	**95% CI**	**p-value**	**OR**
Age	5-14 years Above 15 years	14 223	2 82	(14.3) (36.8)	0.9-1.0	0.06	1.02
Occupation	Housewife Pastoralist	120 50	50 12	(41.7) (24)	0.6-0.1	0.05	0.82
Method of processing meat	Cooked Roasted	194 236	71 13	(36.6) (30.3)	0.07-0.71	0.011	0.22
Do they process blood	Yes No	5 204	1 66	(20) (32.4)	2.52-32.0	0.001	8.99
Contact with aborted foetuses	Yes No	8 229	6 78	(75) (34.1)	0.008-0.77	0.029	0.08
Method of disposal of aborted foetuses	Throw into bush Give to dogs	7 37	3 2	(42.8) (5.4)	0.9-3.8	0.028	0.11
Consume milk	Yes No	233 4	81 3	(34.7) (75)	0.82-162.5	0.07	11.6
Contact with contaminated water	Yes No	128 109	33 51	(25.8) (46.8)	0.9-3.8	0.09	1.86

Table 4. Factors associated with seroprevalence of Q-Fever in humans (Results of backward stepwise logistic regression, significant at p <0.05)

Human Q- fever	**Group Response**	**Number of Respondents**	**Prevalence**	**(Rate)%**	**95% CI**	**p-value**	**OR**
Occupation	House wife	121	50	(41.32)	.65 .99	0.04	0.80
	Student	36	12	(33.33)			
Process blood	Yes	5	1	(20)	1.59 16.3	0.006	5.10
	No	204	66	(32.25)			
How to handle aborted foetus	Throw in the bush	7	3	(42.86)	017 .79	0.028	0.11
	Give to dogs	37	2	(5.41)			
Contact with contaminated water	Yes	128	33	(25.78	.90 3.8	0.091	1.86
	No	109	51	(46.79)			
Consume milk	Yes	233	81	(34.76)	.82 162.5	0.07	11.55
	No	4	3	(75)			
Method of processing meat	Cooked	157	50	(31.85)	81 2.4	0.221	1.40
	Roasted	43	13	(30.23)			

Table 5. Factors associated with seroprevalence of brucellosis in livestock (Results of backward stepwise logistic regression, significant at p <0.05)

In Cattle	**Groups**	**Number of Respondents**	**Prevalence**	**(Rate%)**	**95 % CI**	**P value**	**Odds Ratio**
Breeding Systems	Individual bull	17	8	(47.06)	.25 5.84	0.25	1.2
	Bull from neighbour	5	3	(60)			
Introduction of new animals	Yes	6	3	(50)	.18 11.6	0.35	1.4
	No	19	10	(52.63)			
In Sheep	**Groups**	**Number of Respondents**	**Prevalence**	**(Rate%)**	**95 % CI**	**P value**	**Odds Ratio**
Water source for livestock	Water pan	12	2	(16.67)	.05 1.12	0.07	0.2
	Borehole	47	5	(10.64			
Introduction of new animals	Yes	37	5	(11.76	.05 2.2	0.268	0.3
	No	51	2	(5.77)			
In Goats	**Groups**	**Number of Respondents**	**Prevalence**	**(Rate%)**	**95 % CI**	**P value**	**Odds Ratio**
Introduction of new animals	Yes	37	18	(48.65)	.87 -6.47	0.09	2.3
	No	51	32	(662.75)			

Table 6. Factors associated with seroprevalence of Q-fever in livestock (Results of backward stepwise logistic regression, significant at p <0.05)

In Cattle	**Group**	**Number of Respondents**	**Prevalence**	**Rate%**	**95%CI**	**p-value**	**OR**
Water source	Water pan Borehole	12 47	2 5	(16.7) (10.6)	0.05-1.1	0.07	0.24
Introduction of new animals	Yes No	37 51	18 32	(48.6) (62.7)	0.87-6.4	0.09	2.37
Grazing system	Individual grazing Communal grazing	52 36	33 17	(63.5) (47.2)	0.01-1.04	0.005	0.12
In Goats	**Group**	**Number of Respondents**	**Prevalence**	**Rate%**	**95% CI**	**p-value**	**OR**
Introduction of new animals	Yes No	37 51	18 32	(48.6) (62.7)	0.87-6.4	0.09	2.37
In Sheep	**Group**	**Number of Respondents**	**Prevalence**	**Rate%**	**95% CI**	**p-value**	**OR**
Introduction of new animals	Yes No	37 51	18 32	(48.6) (62.7)	**1.2-69**	**0.03**	**9.2**
Water source	Water pan Borehole	12 47	8 24	(66.6) (50)	.27-1.01	0.05	0.5

Key:

1- One questionnaire was used for the both diseases in all patients
2- One questionnaire was used in herders for both diseases in livestock
3- Respondents were patients who had come to the hospitals and herders at their farms

CHAPTER 5

5.0 DISCUSSIONS

5.1 Overview

This study in Kajiado County provided a valuable opportunity for generating domestic animal prevalence data that could be linked with human health outcomes. To assess the current status of Q-fever among humans and infection prevalence in domestic ruminants, serological surveys were conducted for *C. burnetii* in cattle, goats and sheep, including examination of blood specimens from human patients presenting to the health clinic in the same area.

It is estimated that around 61% of the known 1415 human pathogens are zoonotic (Cloeckaert *et al*.,2001).The concept of 'one medicine' which is defined as the science of all human and animal health diseases has been around for several decades, but its uptake is still lacking in many developing countries where it could have most impact. Understanding the epidemiology of diseases such as brucellosis, and Q-fever are important veterinary issues relating to production losses and abortions. However, the zoonotic nature of these diseases means that it is also important for the medical profession to understand the extent and prevalence of these diseases in the livestock reservoir. The two diseases produce very variable non-specific symptoms in people and are generally believed to be hugely under reported largely due to confusion with malaria in developing countries where 50–80% of malaria cases may suffer fevers resulting from other causes (Maichomo *et al*., 2000).

5.2 Brucellosis and Q-fever in human

The overall sero-prevalence of brucellosis in humans was low 1.3% among the sampled patients in the three hospitals of Sultan Hamud, Kajiado referral and Magadi (Table 3).

Kajiado hospital showed a prevalence of 1.8% in Sultan Hamud 1.5% and 0% in Magadi hospital. The prevalence differed from other findings in the neighbouring counties of Narok 12% (Maichomo, 1997). The low prevalence in this study site is not consistent with other findings which show that the disease is more prevalent among nomadic pastoralists (Schelling *et al*., 2003) as Kajiado County is still being classified as nomadic pastoral area. The questionnaire data showed that despite the people being thought to be more exposed because of keeping large herds of livestock, consuming animal products, using animals by -products like skins and manure and living in close association with livestock the prevalence of brucellosis was found to be low and not high as expected. This is despite the high prevalence recorded of cases of diagnosed brucellosis on RBPT in Narok of 609 from year 2007- 2012 (Maichomo, 1997).

The prevalence of Q-fever on the other hand was found to be high overall (26.7%) despite the complete lack of awareness in the study area agreeing with 30.9% prevalence on reported in patients in western Kenya (Knobel *et al.,* 2013). Apparently Q-fever was not suspected routinely and tested for despite the common symptoms it shows with other diseases like malaria, typhoid and brucellosis. This is most likely because of lack of awareness of this disease among the medical workers and therefore the high probability of confusing it with Malaria and other diseases with similar symptomatology leading to wrong treatment and management. The highest prevalence number of cases were recorded in Sultan Hamud hospital of (32%) followed by Kajiado at (25%) and lowest at Magadi hospital (22%).In addition to sero-prevalence, this study was also carried out to determine the risk factors associated with disease occurrence in humans in this transect of the county. Assessment of risk factors showed a high level of awareness of brucellosis by the respondents

(61%) who referred to it as *"ugonjwa wa Maziwa"* transmitted through consumption of raw milk. They were also aware that it is also associated with meat. The rest (39%) were either not aware or partially aware. There was totally no awareness of Q-fever among veterinary and health staff and the respondents probably because they have not heard of the disease which clearly shows the need for public health education to create awareness. The pastoralists do not relate brucellosis to abortion, retained placenta, assisting animals during parturition, handling of aborted foetuses and throwing of foetal membranes in the bush or giving to dogs, which facilitated disease spread among animals and humans. This makes them handle abortion materials and placenta without any protection or hygienic consideration including hand washing which is not routinely practised due to shortage of water.

Among the respondents to both diseases in humans 82% (n=195) had very close contact with animals, sharing compounds, watering points and premises with neonates posing a high risk to infection and showed a prevalence of 33.7% and 30.2% of brucellosis and Q-fever respectively. On occupation 53% (n=120) of the patients interviewed were housewives in pastoral homes that showed a prevalence of 41.6 %while 20% (n=45) were pastoralist men that had a prevalence of 24%. Students of ages 5-18 years had a high prevalence of brucellosis of 34.4% and 33.3%) prevalence of Q-fever. This may be attributed to the fact that they are in close association with animals when herding, milking and handling retained placenta or aborted foetuses. Most in pastoral areas sleep within the animals at night to guard.

Laboratory tests are important in proper diagnosis of both diseases in both humans and animals. Studies have highlighted challenges encountered in remote areas in carrying out laboratory diagnosis (McDermott and Arimi, 2002). In humans the two diseases present signs

and symptoms similar to other flu-like conditions such as malaria, typhoid, streptococcal infections and rheumatic fever (Muriuki *et al*., 1997; Maichomo *et al*., 1998; 2000).

There was no significant difference in the sero-prevalence of brucellosis and Q- fever in the three hospitals among the patients sampled. The prevalence of the two diseases in humans could be much higher than was portrayed by the hospital records. This is because testing is not carried out consistently in the hospitals and most patients are tested for malaria and typhoid only and the other diseases are tested only when the patient does not respond to treatment against malaria or typhoid. Lack of reagents was also witnessed in the hospitals coupled with inadequately trained staff to carry out quick testing to take care of the high number of patients turning up especially during market days. Health facilities are located away from the people with the nearest facilities being 80kms away for most patients (MOH reports 2007-2012). The tests are also carried out at a cost making it unaffordable for many patients.

5.3 Brucellosis and Q-fever in livestock

The prevalence of brucellosis in cattle in the three districts was highest in Kajiado East (Mashuru) at 40 % followed by Kajiado West (Magadi) at 19.04%. Kajiado Central Sub County (Central) had no positive tests on cattle making the overall prevalence to stand at 21.9% across transect of the county. The overall prevalence in ovines was found to be higher in Mashuru too at 14.8% 5% and 4.5% in Magadi district. Magadi on the other hand had the highest prevalence of brucellosis in goats at 11.8% then Kajiado at 10 % and lowest in Mashuru at 3.3% . The prevalence of brucellosis was highest in cattle (21.9%) and lowest in goats at (7.25%). This could be attributed to the fact that goats are browsers and therefore feed on less contaminated pasture than cattle and sheep.

Q-fever prevalence in livestock was high in all the three species across the three Sub Counties much higher than found in recent studies by CDC in western Kenya. The overall prevalence in cattle was found to be 89.7% higher than 7-57% as reported by (Darryn, *et al.,*2013). The overall prevalence in sheep in this study was 57.5% compared to 18.2% (n=159) in the same survey and 83.1% compared to 32 % (n=378) in goats.

The likely explanation for this rather high prevalence could lie in the lack of awareness on zoonotic diseases, large numbers of animals kept, close contact of animals being herded and housed together and the sharing of watering places as opposed to better awareness, low number of animals, keeping on type of animal and individual grazing and watering of animals in western Kenya. The sharing of enclosures and grazing areas between calving animals and the others could be causing a build up of the *Coxiella* in Kajiado livestock. Majority of the livestock owners interviewed said they had encountered cases of abortion and retained placenta within livestock where the aborted foetuses are thrown to the bush or given to dogs spreading the organisms further.

The high sero-prevalence of exposure to *Coxiella burnetti* represent a major challenge both from a veterinary and a public health view point. It is likely that there is a high incidence of abortion/reproductive failure in affected herds leading to potentially high levels of exposure of livestock owners and their families which is then not being correctly diagnosed. Further studies are clearly needed to study these important zoonoses and to be able to understand the human and animal interactions and the clinical significance of these sero-prevalence in both the animal and for the human populations. Domestic ruminants are sources of human infection, through direct contact or contamination of the environment during parturition or abortion.

Although not assessed in this study, consumption of dairy products from infected ruminants is also likely to pose a risk. Although risk factor studies would be helpful to identify the principal modes of transmission to humans in this setting, the results of this study support the likely efficacy of recognized public health and hygiene measures for mitigating the risk of transmission from domestic ruminants, such as confinement of domestic animals during parturition, avoidance of contaminated pastures and contact with placental material, and boiling or pasteurization of milk before consumption. This study also suggests that investigation of the role of domestic dogs and tick-borne transmission is warranted to identify potential sources of infection and risk factors that are currently not well recognized (Haydon *et al.,* 2002)

CHAPTER 6

CONCLUSIONS AND RECOMENDATIONS

6.1 Conclusions

i. There is a high likelihood that brucellosis and Q-fever are enzootic in the study area and that they present a serious public health problem among the inhabitants of the county.

ii. Despite the prevalence of brucellosis been low the disease still poses a public health problem because of its zoonotic nature and its clinical manifestations being similar to other febrile diseases. The prevalence of Q-fever was high despite the lack of awareness and that it is not tested in any of the hospitals

iii. It should be noted that while abortions in livestock are commonly associated with the better known brucellosis, Q-fever could be contributing significantly. Investigations on brucellosis and Q-fever in livestock have virtually not been done previously in Kajiado and therefore no control measures have been put in place.

iv. Some health centres carry out limited tests for brucellosis but face shortages of test reagents and records are inconsistent and unreliable.

v. A substantial number of people lack awareness on the risk factors of the two diseases and the methods of prevention and control. The risk factors are significant in the spread of these two diseases.

6.2 Recommendations

i. The veterinary personnel in Kajiado County should make an effort to investigate all cases of abortions and retained placentas in livestock that are included in their disease surveillance reports. This calls for strengthening laboratory diagnostic capacity in the county by training more veterinary and health staff and providing diagnostic equipment and reagents.

ii. Creation of awareness among the people on the seriousness of the causes, modes of transmission, risk factors and methods of prevention of the two diseases should be undertaken as soon as possible.

iii. Both zoonoses appear to be of major public health and economic importance in humans and animals which calls for concerted efforts to control them by both veterinary and health personnel in the spirit of one health concept between the veterinary and health personnel.

iv. Effort should be made by health personnel to do a confirmatory diagnosis of all diseases presenting flu-like symptoms before treatment to avoid misdiagnosis, drug misuse and long suffering of patients.

v. Public health education on these diseases should be done and emphasised to the herders and those closely associated with livestock and/or consumers of animal products.

vi. Further studies should be carried out involving a wider population of human beings and livestock including wildlife to better understand the epidemiology scope and impact of the diseases in humans and animals

REFERENCES

Adazu, K., Lindblade, K. A., Rosen, D. H., Odhiambo, F., Ofware, P., Kwach, J., Van Eijk, A, M,, Decock, K. M., Amornkul, P., Karanja, D., Vulule, J. M., Slutsker, L.,(2005). Health and demographic surveillance in rural western Kenya: a platform for evaluating interventions to reduce morbidity and mortality from infectious diseases. *American Journal of Tropical Medical and Hygiene*, **73**: 1151–1158.

Alton, G. G., Jone, L M., Angus, R.D., and Verger J. M., (1988). Bacteriological methods, in Laboratory Techniques in Brucellosis, 2nd Ed. *World Health Organisation,* Geneva, Page 11-64.

Anon. (1986). Joint FAO/WHO. Expert Committee on Brucellosis. *World Health Organization Technical Report Series* 740, Geneva: WHO.

Anon. (2000). Manual of Standards for Diagnostic Tests and Vaccines, 3rd Edition. Paris: *Office International des Epizootics.*

Arricau-Bouvery, N., and Rodolakis, A., (2005). Is Q-fever an emerging or re-emerging zoonosis? *Veterinary Research* **36**: 327–349.

Arricau-Bouvery, N., Hauck, Y., Bejaoui, A., Frangoulidis, D., Bodier, C., Souriau, A., Meyer, H., Neubauer, H., Souriau, A., Bodier, C., Dufour, P., Rousset, E. and Rodolakis, A., (2005).Effect of vaccination with phase I and phase II *Coxiella burnetii* vaccines in pregnant goats. *Vaccine,* **23**, 4392–4402.

Bandara, A. B. and Mahipale, M. B., (2002).Incidence of brucellosis in Sri Lanka: An overview. *Veterinary Microbiology*, **90**: 197-207.

Beare, P.A., Unsworth, N., Andoh, M., Voth, D.E., Omsland, A., Gilk, S.D., Williams, K.P., Sobral, B.W., Kupko, J.J. Porcella, S.F., Samuel, J.E. and Heinzen, R.A., (2009). Comparative genomics reveal extensive transpose on mediated genomic plasticity and diversity among potential effector proteins within the genus *Coxiella*. *Infectious Immunology*, **77**, 642–656.

Blood, D. C., Radotstits, O.M., 1989. Veterinary Medicine—A Textbook of the Disease of Cattle, Sheep, Pigs,Goats and Horses. Baillière Tindall, London.

Bricker, B. J. (2002a).Diagnostic Strategies used for the Identification of *Brucella.* *Veterinary Microbiology*, **90**: 433-434.

Bricker, B. J., (2002b). PCR as a diagnostic tool for Brucellosis. *Veterinary Microbiology,* **90**: 435 – 46.

Brotherson, J. C, Cooke ER, 1956. Q fever in Kenya. *East African Medical Journal* **33**: 125–130.

Centre for Disease Control, (2011). Q-fever Outbreak Associated with Goat Farms --- Washington and Montana, 2011

Centre for Disease Control and Prevention. (2000a). Suspected brucellosis case prompts investigation of possible bioterrorism – related activity: New Hampshire and Massachusetts, 1999. MMWR: *Morbidity and Mortality Weekly Report*, **49**: 509 – 512.

Centre for Disease Control and Prevention. (2000b). Biological and Chemical terrorism: strategic plan preparedness and response. MMWR: *Morbidity and Mortality Weekly Report*, **49**: 1 – 4.

Chomel, B. B., Debess, E. E., Mangiamele, D. M., Reilly, K. F., Farver, T. B., Sun, R.. K., and Barrett, L.R., (1994).Changing trends in the epidemiology of human brucellosis in California from 1973 to 1992:A shift toward food borne transmission. *Journal of Infectious Diseases* **170**:1216–1223.

Chukwu, C C., (1987). Brucellosis in Africa. Part 11. The Prevalence. Bulletin *of Animal Health and Production in Africa.* **35***:92-98.*

Cleaveland, S., Laurenson, M. K.,Taylor, L. H., (2001) Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Proceedings of the National Academy of Sciences of the United States of America* 356: 991–999.

Cloeckaert, A., Verger, J. M., Grayen, M., Paquet, J. Y., Garin-Bastufi, B., Foster G., and Godfroid J., (2001).Classification of *Brucella spp* isolated from marine mammals by DNA polymorphism at the *Omp2* locus. *Microbes and infection*, **3**:729 – 738.

Cloeckaert, A., Vizcaino, J.Y., Nipaguet, R., Bowden, A., and Elzer P. H., (2002). Major outer membrane proteins of *Brucella* spp therefore past, present and future. *Veterinary Microbiology*, **90**: 229-247.

Clockaert, A., Grayon, M., Grepinet O., and Boume Dine K. S., (2003). Classification of *Brucella* strains isolated from marine mammals by infrequent restriction site-PCR and development of specific PCR identification tests. *Infectious Immunology,* **75**:11.

Coleman, P. G., (2002).Zoonotic diseases and their impact on the poor. *In*: Perry, B.D., McDermott, J.J., Randolph, T.F., Sones, K.R. and Thornton, P.K., (2002). *Investing in Animal Health Research to Alleviate Poverty*. International Livestock Research Institute (ILRI), Nairobi, Kenya.

Coleman, S. A., Fischer, E. R., Howe, D., Mead, D. J. and Heinzen, R.A., (2004). Temporal analysis of *Coxiella burnetii* morphological differentiation. *Journal of Bacteriology,* **186**, 7344–7352.

Corbel, M. J., (1997). Brucellosis: An overview. *Emerging Infectious Diseases* **3**: 213–221. *Coxiella burnetii* antibody in animal sera. *Journal of Clinical Microbiology*, **30**, 1595–1597.

Corbel, M. J., (2002).Brucella: A guide to infections: Pathogenesis, Immunity, Laboratory diagnosis and control. In: *Medical Microbiology*, 16th Edition. Edited by Greenwood, D., Slack, R.C.B., Peutherer, J.F. Published by Churchill Livingstone, Edinburg, **2002**. 322-325.

Coulibaly, N. D., and Yameogo, K. R., (2000). Prevalence and control of zoonotic diseases: collaboration between public health workers and veterinarians in Burkina Faso. *Acta Tropica* **76**, 53 - 57.

Craddock, A. L, Gear J, 1955. Q fever in Nakuru, Kenya. *Lancet* **269**: 1167–1169.

Cripps, P. J., (2000).Veterinary Education, Zoonoses and Public Health: A personal perspective. *Acta. Tropica***76**, 77-80.

Darryn, L. K., Maina, A., Cutler, S. S., Ogola E., Feikin D. R., Muthoni J., Allan R .L., Freiman R.. F., Clevaland S., Kariuki M. N.,(2013). *Coxiella burnetii* in Humans, Domestic ruminants and ticks in western Kenya. *American Journal of Tropical Medical and Hygiene*, 88(3), 2013, pp. 513–518 doi:10.4269/ajtmh.12-0169.Copyright © 2013 by *The American Society of Tropical Medicine and Hygiene.*

Doganay, M., Aygen, B., and Essel, D., (2001). Brucellosis due to blood transfusion. *Journal of hospital Infection* **49,** 151-152.

Dohoo, I. R., Wright, F., Ruckerbauers, G. M., Samagh, B. S., Robertson, F.J. and Forbes, L.B., (1986).Comparison of five serological tests for bovine brucellosis. *Canadian Journal of Veterinary Research*, **50**: 485 – 493.

Dohoo, I. R, Wayne, M., and Styhn, H., (2010). *Veterinary Epidemiologic Research.* Veterinary Epidemiology Research Textbooks

Director of Veterinary Services. Kenya Annual Reports; (2003- 2011)

Drancourt, M., and Raoult, D., (2005). Genus I. *Coxiella*. *In:* Bergey's Manual Of Systematic Bacteriology, **Volume 2**: East Lansing, MI, USA, 237–241.

ECDC, (European Centre for Disease Prevention and Control), (2010). Panel with Representatives from the Netherlands, France, Germany, United Kingdom, United States of America. Risk assessment on Q-fever. *ECDC Technical Report,* **40** pp.:10.2900/28860.

EFSA, (European Food Safety Authority), (2010). Panel on Animal Health and Welfare (AHAW); Scientific Opinion on Q-fever. *EFSA Journal*, **8** (5), 1595, **114** pp. doi:10.2903/j.efsa.2010.1595.

Godfroid, J., (2002). Brucellosis in wildlife. *Review of Science Technological Office of International Epizootic*s, **21**: 277-286.

Godfroid, J., Salgerman, C., and Wellemansa, V., (2002).How to substantiate eradication of bovine brucellosis when a specific serological reaction occurs in the course of brucellosis testing. *Veterinary Microbiology*, **90**:461-477.

Guatteo, R., Beaudeau, F., Berri, M., Rodolakis, A., Joly, A., (2006). Shedding routes of *Coxiella burnetii* in dairy cows: Implications for detection and control. *Veterinary Research* **37**: 827–833.

Guatteo, R., Beaudeau, F., Joly, A. and Seegers H., (2007). *Coxiella burnetii* shedding by dairy cows. *Vet. Res.*, **38 (6)** 849–860.

Guatteo, R., Seegers, H., Joly, A., and Beaudeau, F., (2008). Prevention of *Coxiella burnetii* shedding in infected dairy herds using a phase I *C. burnetii* inactivated vaccine. *Vaccine*, **26** (34), 4320–4338.

Haydon, D.T., Cleaveland, S., Taylor, L. H., Laurenson, M. K., (2002).Identifying reservoirs of infection: A conceptual and practical challenge. *Emerging Infectious Dis*eases, **8**: 1468–1473.

International Office of Epizootics (OIE), (2002).Bovine brucellosis .In: *Manual of standard diagnostic tests and vaccines*. Paris, France, OIE guidelines, pp328-345.

International Office of Epizootics (OIE), *Terrestrial Manual* 2009

International Office of Epizootics (OIE), *Terrestrial Manual* 2010

Jiwa, S. F. H., Kazwala, R. R., Tungaraza, R., Kimara, S. I., and Kalaya, W.J., (1996). Bovine brucellosis serum agglutination test prevalence and breed disposition according to prevalent management systems in the Lake Victoria zone of Tanzania. *Preventive Veterinary Medicine*, **26**,341-346.

Jubb, K.V.F., Kennedy, P.C. and Palmer, N., (1985). Pathology of Domestic Animals, Vol.3, *Academic Press*, Orlando, FL, Page 345 – 349.

Kadohira,M., McDermott, J.J., Shoukri, M.M., and Kyule, M.N., (1997).Variations in the prevalence of antibody to Brucella infection in cattle by farm, area and district in Kenya. *Epidemiology and Infection,* **118**: 35-41.

Kagumba, M., and Nandokha, E., (1978).Survey of the prevalence of bovine brucellosis in East Africa. *Bulletin of Animal Health and Production in Afr*ica, **26**: 224-229.

Kagunya, D.K.J., and Waiyaki, P.G., (1978) A serological survey of animal brucellosis in the North Eastern Province of Kenya. *Kenya Veterinarian*, **Vol. 2 No. 2** pp.35-38.

Kajiado County, (MOH annual reports, Kajiado District Hospital 2007-2012)

Kajiado District Strategic Plan, 2005-2010 for Implementation of the National Population Policy for Sustainable Development.

Kambarage, D. M., Karimuribo, E. D., Kusiluka, L. J. M., Mdegela R. H. and Kazwala R. R.,(2003) *Community Public Health Education in Tanzania: Challenges, Opportunities and the Way Forward.* FAO, Expert consultation on community based Veterinary Public Health (VPH) systems, pp 55-63.

Kang'ethe, E. K., Arimi, S. M., Omore, A. O., McDermott, J. J., Nduhiu, J. G., Macharia, J. K. and Githua A., (2000).The prevalence of antibodies to Brucella abortus in marketed milk in Kenya and its public health implications. Paper prepared for oral presentation at the 3rd all Africa conference on Animal Agriculture, 6 -9 November 2000.

Kangethe, E. K., Ekuttan, C. E., Kimani, V. N., and Kiragu, M. W., (2007). Investigation into prevalence of bovine brucellosis and the risk factors that predispose humans to infection among urban dairy and non-dairy farming households in Dagoretti Division, Nairobi, Kenya. *East African Medical Journal*, **84**: 96-100.

Kelly, P. J., (2004).Infectious Diseases of Livestock, Oxford University Press, volume **1**, chapter 43. 2nd Edition.

Kenya Population Census, (2009) .Kenya National Bureau of Statistics (KNBS)

Kersh, G.J., Wolfe, T.M., Fitzpatrick, K.A., Candee, A.J., Oliver, L.D., Patterson, N.E., SELF, J.S., Priestle, Y.R.A., Loftis, A.D., and Massung, R. F., (2010). Presence of

Coxiella burnetii DNA in the environment of the United States (2006–2008). *Applied Environmental Microbiol*ogy, **14**: 221-224

Kittelberger, R., Mars J., Wibberley G., Sting R., Henning, K., Horner, G. W., Garnett, K. M., Hannah, M. J., Jenner, A., Pigott, C. J. and O'keefe J. S., (2009). Comparison of the Q-fever complement fixation test and two commercial enzyme-linked immunosorbent assays for the detection of serum antibodies against *Coxiella burnetii* (Q-fever) in ruminants: Recommendations for use of serological tests on imported animals in New Zealand. *New Zealand Veterinary Journal*, **57** (5), 262–268.

Kunda, J., Fitzpatrick, J., Kazwala, R., French, N.P., Shirima,G., Macmillan,A., Kambarage, D., Bronsvoort, M., and Cleaveland, S.,(2007). Health-seeking behaviour of human brucellosis cases in rural Tanzania. *BMC Public Health,* **3**, 315.

Madariaga, M.G, Rezai, K., Trenholme, G. M, Weinstein, R.A.,(2003).Q-fever: A biological weapon in your backyard. *Lancet Infectious Diseases,* **3**: 709–721.

Madkour, M. M., (2001). Madkour's brucellosis. Barlin*: Berlin and Heiderberg: springerVerlay* 306.

Maichomo, M. W., McDermott, J. J., Arimi, S. M., Gathura, P. B., (1998). Assessment of the Rose-Bengal plate test for the diagnosis of human brucellosis in health facilities in Narok district, Kenya. *East African Medical Journal,* **75**, 219–222.

Maichomo, M. W., McDermott, J. J., Arimi, S. M., Gathura, P. B., Mugambi, T. J., and Muriuki, S. M., (2000).Study of brucellosis in a pastoral community and evaluation of the usefulness of clinical signs and symptoms in differentiating it from other flu-like diseases. *African Journal of Health Sci*ences, **7 (18),** 114-119.

Makau, P., (2001)**.** *Magadi Division Environmental Assessment Report*. ITDG EA.

Malavika, P., William, L. N., Aubree J. R., Gilbert, J. K. K., Fitzpatrick, A., Lindsay, D. O., Robert F. M., Anne B. M., John A. B., Jecinta .J. O., Venance P. M., Grace D. K., Wilbrod S., John, A. C.,(2011).Q-fever, spotted fever group and Typhus group Rickettsioses among hospitalized febrile patients in Northern Tanzania . *Clinical Infectious Diseases,* **53**:e8-e15.

Martin, S.W., Meek, A.H., Willebrg, P., (1987).Veterinary Epidemiology; Principles and Methods, **32-74.**

Mantur, B. G., S. K. Amarnath and R. S. Slinde. (2007). Review of clinical and laboratory features of human brucellosis. *Indian Journal of Medical Microbiology*, **25**:188-202.

Maurin, M., and Raoult D., (1999). Q-fever. *Clinical Microbiological Review*, **12**, 518–553.

Mentor, B. G., Amarnath, S.K., and Slinde, R.S., (2007). Review of clinical and laboratory features of human Brucellosis. *Indian Journal of Medical Microbiology*, **25:**188-202.

Mdegela, R. H., E. Karimuribo, L. J., Kusiluka, B., Kabula, A., Manjurano, A., Kapaga, M., and Kambarage, D. M., (2005). Mastitis in small holder dairy and pastoral cattle herds in the urban and peri-urban areas of the Dodoma municipality in Central Tanzania. Livestock Research for Rural Development Report, 17: 11.

McDermott, J. J, Arimi, S. M., (2002). Brucellosis in sub-Saharan Africa: Epidemiology, Control and Impact. *Veterinary Microbiology* **90**: 111–134.

McDermott, J. J., Deng, K. A., Jayatileka, T. N., and El Jack, M. A.,(1987). A cross sectional study; of cattle disease study in Kongor Rural Council, Southern Sudan. Brucellosis in cows: Associated Factors, Impact on production and disease control considerations. *Preventive Veterinary Medicine, **5**:125-132.*

Mediannikov,O, Fenollar, F., Socolouschi, C., Diatta, G., Bassene, H., Molez, J.F., Sokha C., Trape, J.F, Rout, D., (2010). *Coxiella burnetii* in humans and ticks in rural Senegal. *PLoS Neglected Tropical Diseases,* **4**: e654.

Ministry of Health Annual Reports Kajiado District Hospital, 2007- 2012

Muendo, E. N., Peter M. Mbatha, Joseph Macharia, Theresia H. Abdoel, Paul V. Janszen , Rob Pastoor, Henk L. Smits, (2012) . Infection of cattle in Kenya with Brucella abortus biovar 3 and Brucella melitensis biovar 1 genotypes.*Tropical Animal Health and Production January 2012, Volume **44**,* Issue 1, pp 17-20

Muma, J. B., Skjerve, E., Samui, K.L., Siamudala, V., Oloya, J., Matope, G., Munyeme, M., Mubita, C. and Omer, M.K.,(2006).Prevalence of antibodies to *Brucella spp*. in Traditional Cattle and Goats Reared in Livestock-wildlife interface Areas of Zambia. *Tropical Animal health and Production,* **38**: 138 – 206.

Muma, J. B., Samui, K. L., Lund, A., Nielsen, K., Chimana, H., and Skjerve E., (2008). Brucellosis in rural communities in Zambia and factors associated with increased anti-Brucella spp. Antibody presence. *Journal of Science and Technology*, **12**: 9-18.

Muriuki, S. M., McDermott, J. J., Arimi, S. M., Mugambi, J. T., and Wamola, I. A., (1997). Criteria for better detection of brucellosis in the Narok District of Kenya. *East Africa Medical J*ournal, **74**: 317 – 320.

Navara, E., (2003).Comparison of three different PCR, methods for detection of *Brucella species* in human blood samples. *FEMS Immunology Medical Microbiology*, **41**: 144 – 148.

Nicoletti, P. (1984).The control of brucellosis in tropical Africa and subtropical regions. *Preventive Veterinary Med*icine, **2:** 193-196.

Nyariki, D. M., Makau, B. F., Ekaya, W. N., and Gathuma,J. M., (2005). *Guidelines for Emergency Livestock Off-take Handbook*. Arid Lands Resource Management Project (ALRMP), Office of the President; Agricultural Research Foundation (AGREF), Nairobi.

Nielsen, K., and Duncan, J.R., (1990). Animal Brucellosis CRC Press, Inc., Page 203 – 206.

Nielsen, K., Gall, D., Jolley, M., Leishman, G., Balsevicius, S., Smith, P., Nicoletti, P., and Thomas, F., (1996).A homogeneous fluorescence polarization assay for detection of antibody to *Brucella abortus*. *Journal of Immunological Methods*, **195**: 161 –168.

Nielsen, K. H., Gall, L. D., Balsevicus, S., Bosse, J., Nicolette, P., and Kelly, W., (1995). Comparison of enzyme Immuno assays for the diagnosis of bovine brucellosis. *Preventive Veterinary Medicine*, **26**: 17-32.

Olsen, S. C., Theon, C. O., Cheville, N. F., (2004). *Brucella.* In: Gyles, C.E.; Prescott, J.F.; Songer, J.G.; Theon, C.O. Pathogenesis of bacterial infections in animals. Ames, L.A. Blackwell Publishing pp 309-320

Olsen, S. C., and Staff W. S., (2005). Essential role of vaccines in brucellosis control and eradication programs for livestock. *Expert Review of Vaccines*, **4**: 915-928.

Omer M., Skjerve, E., Holstad, G., Woldehiwet, Z., Macmillan, A., (2000). Prevalence of antibodies to *Brucella spp*. in cattle, sheep, goats, horses and camels in the state of Eritrea; Influence of husbandry systems. *Epidemiology and Infection* **125**: 447–453.

Omore, A. O., Muriuki, H., Kenyanjui, M., Owango, M., and Staal, S., (1999).The Kenyan Dairy Sub-Sector: A Rapid Appraisal: Research Report of the MoA/KARI/ILRI Smallholder Dairy (R&D) Project. International Livestock Research Institute. Nairobi (Kenya).51pp **170**:1216–1223.

Pappas, G., Bosilkovski, M., Akritidis, N., Mastora, M., Ketena, H., and Tsianos E., (2003). Brucellosis and the respiratory system. *Clinical Infectious Diseases*, **37**: 95-99.

Pappas, G., Arkritidis, N., Bosilkouski M., and Tsiano, E., (2005). Brucellosis, *Medical Progress*. New England Journal of Medicine, **352**: 2325-2336.

Pappas, G., Panagopoulou, P., Christou, L., and Akritidis, N., (2006). *Brucella.* as a biological weapon. *Cell Molecular Life Sciences*, **63**: 2229-2236.

Parker, R., (2007). Diseases affecting reproduction in beef cattle. College of Agriculture and Home Economics, New Mexico State University. USA.

Radostis, O M., Gay C. C., Blood, D. C., and Hinchcliff, K W., (2000). Disease caused by *Brucella species.* In: *Veterinary Medicine*: A text book of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th Edition. W. B. Saunders Co. Ltd, London, **2002.** Published by Harcourt Publishers Ltd. Pp 867-891

.

Ramirez, M., Hamdy, M. E., and Amin, M., (2002).Serologic response and time to eradication in herds with brucellosis vaccinated with strain 19 or strain RB-51. *A Archivos de medicina veterinaria**34***: 143-151.

Raoult ,D, 1996. Q fever; still a query afer all these years. *Journal Medical Microbiology*, **44**, 77-78.

Raoult, D., Marrie, T., Mege, J., (2005).Natural history and pathophysiology of Q-fever. *Lancet Infectious Diseases,* **5**: 219–226.

Ray, W. C., (1979). Brucellosis due to *B.abortus* and *B.suis*. In: CRC Handbook series in Zoonosis, Section A: *Bacterial, Ricketsial and Mycotic diseases*. Vol.1 Ed. James H. Steele pp 99-127

Rodolakis, A., Berri, M., Hechard, C., Caudron, C., Souriau, A., (2007).Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds. *Journal of Dairy Science* **90**: 5352–5360.

Roest, H. I, Tilburg, J. J, Van der Hoek, W., Vellema P., Van Zijderveld, F.G., Klaassen, C. H., Raoult, D., (2011). The Q-fever epidemic in the Netherlands: history, onset, response and reflection. *Epidemiological Infection,* **139**: 1–12.

Roushan, M. R. H., Mohraz, M., Hajiahmadi, M., Ramzani, A., and Valayati, A. A., (2006). Efficacy of Gentamycin plus Doxycycline versus Streptomycin plus Doxycycline in the treatment of brucellosis in humans. *Clinical Infectious Diseases*, **42**: 1075-80.

Rousset, E., Durand, B., Berri, M., Dufour, P., Prigent, M., Russo, P., Delcroix, T., Touratier, A., Rodolakis, A., and Aubert, M.F. (2007).Comparative diagnostic potential of three serological tests for abortive Q-fever in goat herds. *Veterinary Microbiology,* **124**, 286–297.

Rousset, E., Berri, M., Durand, B., Dufour, P., Prigent, M., Delcroix, T., Touratier, A.,and Rodolakis, A., (2009a).*Coxiella burnetii* shedding routes and antibody response after outbreaks of Q-fever-induced abortion in dairy goatherds. *Applied Environmental Microbiol*ogy, **75**, 428–433.

Rousset, E., Durand, B., Champion, J.L., Prigent, M., Dufour, P., Forfait, C., Marois, M., Gasnier, T., Duquesne, V., Thiery, R., and Aubert, M. F., (2009b).Efficiency of a phase I vaccine for the reduction of vaginal *Coxiella burnetii* shedding in a clinically affected goat herd. *CMI,* 15 (suppl 1), 1–2.

Roest, H.J., Ruuls, R., Van Rotterdam, B., Vincent, G., and Thiéry, R., (2009). A multicentre MLVA and MST typing ring trial for *C. burnetii* genotyping: An approach to

standardisation of methods.5th *Medical Veterinary Network,* Annual Scientific Conference. Madrid, Spain.

Roest, H.I, Tilburg, J.J, van der Hoek, W., Vellema. P., van Zijderveld, F.G., Klaassen C.H., Raoult, D., (2011). The Q-fever epidemic in the Netherlands: history, onset, response and reflection. *Epidemiology and Infection,* **139**: 1–12.

Samuel, J.E., and Hendrix, L.R., (2009). Laboratory maintenance of *Coxiella burnetii*. *Current Protocol Microbiolo*gy, **6 C** (suppl.15), 1–16.

Seshadri, R., Paulsen, I.T., Eisen, J. A., Read, T. D., Nelson, K. E., Nelson, W. C., Ward, N. L., Tettelin, H., Davidsent, M., Beanan, M. J., Deboy, R. T., Daugherty, S. C., Brinkac, L. M., Madupu, R., Dodson, R. J., Khouri, H. M., Lee, K. H.,Carty, H. A., Scanlan, D., Heinzen, R. A., Thompson, H. A., Samuel, J. E., Fraser, C. M., and Heidelberg, J. F., (2003).Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. *Proceedings of the National Academy of Sciences of the United States of America,* **100**, 5455–5460.

Sidi-Boumedine, K., Rousset, E., Henning, K., Ziller, M., Niemczuck, K., Roest, H.I.J., and Thiéry, R., (2010).Development of harmonized schemes for the monitoring and reporting of Q-fever in animals in the European Union. *EFSA Scientific Report on Question No EFSA-Q-2009-00511*., 48 pp.

Scolamacchia, F., Handel, I.G., Fèvre E.M., Morgan, K.L., Tanya, V.N., (2010) Serological Patterns of Brucellosis, Leptospirosis and Q-fever in *Bos indicus* Cattle in Cameroon. *PLoS ONE* 5(1): e8623. doi:10.1371

Schelling, E., Diguimbaye, C., Daoud, S., Nicolet, J., Boerlin, P., Tanner, M., Zinsstag, J., (2002). Brucellosis and Q-fever sero-prevalence of nomadic pastoralists and their

livestock in Chad. *Preventive Veterinary Medicine*, Volume **61**, Issue 4, Pages 279-293

Schelling, E., Diguimbaye, C., Daoud S., Nicolet, J., Boerlin, P., (2003) Brucellosis and q-fever sero-prevalences of nomadic pastoralists and their livestock in Chad. *Preventive Veterinary Medicine* **61**: 279–293.

Shey-Njila O, Nya, D.E., Zoli, P.A., Walravens, K., Godfroid, J., (2005) Serological survey of bovine brucellosis in Cameroon. *Revue d'Elevage et de Medicine Veterinaire des Pays Tropicaux* **58**: 139–143.

Shirima, G.M., Fitzpatrick, J., Cleaveland, S., Kambarage, D.M., Kazwala, R.R., Kunda, J., and French, N.P., (2003) Participatory Survey on Zoonoses Affecting Livestock Keeping Communities in Tanzania. *Journal of Animal and Veterinary Advances* **4**, 253–258.

Stack J.A., and Macmillan, A.P., (2000). Identification and Bio typing of *Brucella* spp. *Clinical and Vaccine Immunology*, **146**:166-167.

Swai, E.S., and Schoonman, L., (2009). Human brucellosis: sero-prevalence and risk factors related to high risk occupational groups in Tanga, Municipality, Tanzania. *Zoonoses and Public Health* **56**, 183-189.

Taylor, L.H, Latham, S.M., Woolhouse M.E.J., (2001). Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London Series B-*Biological Sciences* **356**: 983–989.

Tissot-Dupont H., Thirion X. and Raoult D. (1994). Q fever serology: Cutoff determination for microimmunofluorescence. *Clinical Diagnostic Laboratories of Immunology,* **1**,

189–196.to brucella infection in cattle by farm, area and district in Kenya. *Epidemiology and Infection* 118: 35-41.

Tissot-Dupont H, Brouqui P, Faugere B, Raoult D, 1995. Prevalence of antibodies to *Coxiella burnetii*, *Rickettsia conorii*, and *Rickettsia typhi* in 7 African countries. *Clinical Infectious Diseases* **21**: 1126–1133.

Traboulsi, R., Uttman, I., and S.S. Kani., (2007). Pre patellar *Brucella melitensis* bursitis: Case report and literature review. *Clinical Rheumatology*, **11**:1941-1942.

Uzal, F.A., Carrasco, A.E., Echaide, S., Nielsen, K., and Robles, C. A., (1995). Evaluation of an Indirect ELISA for the diagnostic of bovine brucellosis. *Journal of Veterinary Diagnosis,* **7**: 473 – 75.

Van Aert, A., Brioen, P., Dekeyser, A., Uytter halgen, R. Sijens, J., and A. Boeye. (1984). A Comparative study of ELISA and other methods for the detection of *Brucella* antibodies in bovine sera. *Veterinary Microbiology*, **10**: 13 – 21.

Van der Hoek, W., Dijkstra, F., Schimmer, B., Schneeberger., P.M, Vellema, P., Wijkmans, C., Schegget, R., Hackert, V., van Duynhoven, Y., (2010). Q-fever in the Netherlands: An update on the epidemiology and control measures. *Euro Surveill* **15**: 19520.

Vanek E, Thimm B, 1976. Q fever in Kenya. Serological investigations in man and domestic animals. *East African Medical Journal,* 53:678–684

Waghela, S., Gathuma, J. M., (1975). Serological survey of the prevalence of Brucellosis in pigs in Kenya, *Bulletin of Animal Health and .Production in Africa,* **24** (1): 251.

Waghela, S., Gathuma, J.M., Fazil, M.A., and Kagunya, D.K.J., (1978). A serological survey of Camel brucellosis in the North Eastern Province of Kenya. *Tropical Animal Health and Production* Vol. **10** No. 1 pp. 28-29. – 1978.

Weinhaupl, I., Schopf, K.C, Khaschabi, D, Kapaga, A.M, Msami, H.M., (2000). Investigations on the prevalence of bovine tuberculosis and brucellosis in dairy cattle in Dar es salaam region and in zebu cattle in Lugoba area, Tanzania. *Tropical Animal Health and Production,* **32**: 147–154**.**

WHO/FAO/OIE, (2004): Report of the WHO/FAO/OIE Joint Consultation on Emerging Zoonotic Diseases, 3–5 May 2004, Geneva, Switzerland. Available at: *http://www.who.int/ zoonoses/en (last accessed on 4/7/2008).*

WHO/FAO/OIE, (2004). *Report of the WHO/FAO/OIE Joint Consultation on Emerging Zoonotic Diseases*. 3-5 May 2004, Geneva, Switzerland. Food and Agriculture Organization of the United Nations (FAO),

WHO, (2006).The Control of Neglected Zoonotic Diseases: A Route to Poverty Alleviation. Geneva: Report of a joint WHO/DFID-AHP meeting with the participation of FAO and OIE, Geneva, 20-21 September, 2005

Woods, C., and Jan (eds), B., (2005). USAMRHD's Medical Management of Biological casualties Handbook, 6th Ed, U.S Army Medical Institute of Infectious Diseases, FortDetrick, Maryland, **53**.

APPENDICES

Appendix 1. Human response to questionnaire in the three hospitals in the study area

	Risk factor	**Category / response**	**Proportion of respondents %**	
1	Age of persons sampled	≤ 18 years	28	12%
		19- 45years	149	63. %
		>45-60 years	59	25%
2	Sex of the respondent	Female	163	69%
		Male	73	31%
3	Level of awareness of zoonoses	Completely aware	142	61%
		Partially aware	13	6%
		Not aware	81	33%
4	Occupation	Housewife	120	53%
		Business lady	14	6%
		Teacher	11	5%
		Pupil/student	24	10%
		Pastoralists	45	20%
		Hotelier	6	3%
		Farmer	4	2%
5	Contact with contaminated environment	Contact with dust	123	52%
		Contact with aborted foetus	8	4%
		Contact with animal discharges	22	9%
		Contact with urine	3	2%
		Contact with contaminated water	128	54%
6	Consumption of raw animal products	Raw milk	3	2%
		Raw meat	0	0%
		Raw blood	3	2%
		Mix raw blood and milk	31	13%
7	Contact with animals	Yes	195	82%
		Share compound	138	58%
		Share house	4	2%
		Share water	52	22%
		Not in contact	53	22%
8	Disease treatment and diagnosis	Did you seek treatment	236	100%
		How long treated + once	135	57%
		+ More times	101	43%
		Diagnosis malaria	200	85%
		Diagnosis typhoid	27	11%
		Diagnosis brucellosis	84	36%
		Diagnosis flu/cold	173	73%
		Diagnosis unknown	39	17%

Appendix 2. Number of livestock and human blood samples from three hospitals and three Sub-Counties of Kajiado.

Sub County	sampled areas	No	Percent (%)	Patient origin	No	Percent (%)
Kajiado East	Ernkau	15	19			
	Matoi	16	20	**Sultan Hamud Hospital**		
	Olerai	17	21			
	Lepolos	15	19	Non Pastoral area (Mukaa)	108	61
	Kibini	17	21	Pastoral area (Mashuru)	69	31
	Sub-Total	**80**	**100**	**Sub-Total**	**177**	**100**
Kajiado Central				**Kajiado County Referral Hospital**		
	Maili tisa	16	17			
	Nosikito	6	6			
	Mailua	20	21	Ilbissil	17	15
	Enyorrata	9	10	Kajiado	56	49
	Town area	11	11	Mashuru	5	4
	Osiligi Farm	8	8	Mile 46	5	4
	Olosutia area	6	6	Namanga	12	10
	Kwa Ndege area	4	4	Oltepes	9	8
	Tama farm	6	6	Torosei	11	10
	Ilbisil	11	12			
	SubTotal	**97**	**100**	**Sub-Total**	**115**	**100**
Kajiado West				**Magadi hospital**		
	Shompole	17	23			
	Ilkiramatian ranch	5	7			
	Magadi area	12	16	Oldonyo Nyokie	7	19
	Oldonyo Nyokie	15	22	Olkeri	10	27
	Kamukuru	12	16	Shompole	11	30
	Mitaru	12	16	Township	9	24
	SubTotal	**73**	**100**	**Sub-Total**	**37**	**100**

Appendix 3. Pastoralists Questionnaire response in the three study areas in Kajiado County

	Risk factor	**Category / response**	**Proportion of respondents**	
1	Grazing management	Individual	52/88	59%
		Communal	36	41%
2	Breeding system	1. Communal bull	17	19%
		2. Individual bull	54	61%
		3.Bull from neighbour	1	1%
3	Introduction of new animals main source - market	Yes	37	42%
		Cattle sex	40	45%
		Sheep sex	35	40%
		Goats sex	34	39%
4	Water sources for both animals and livestock	1. Water pan	4	5%
		2. Borehole	21	24%
		3. Dam	7	8%
		4. River	19	22%
		5. Borehole and water pan	32	36%
5	Watering system	1. Individual	15	17%
		2. Shared	65	74%
		3. Communal	8	9%
6	Who is consulted in case of sick animals	1. Buys drugs &treat	62	70%
		2. Calls Vet	5	6%
		3. Call AHA	16	18%
		4. Calls herbalist	3	3%
7	Disposal of aborted foetuses and placentas	1. Throw away in bush	5	6%
		2. Bury/Burn	5	6%
		3. Give to dogs	77	88%
8	Assisting the animals at parturition	Yes	61	69%
		No	27	31%
9	Presence of vet staff and call for service	1. All the time	3	3%
		2. Rarely	80	91%
		3. Not at all	5	6%
		4. Once	2	2%

Appendix 4. Test results of Brucellosis and Q fever in livestock and human patients attending hospitals

Species	Brucellosis				Q-fever		
	Questionnaire data	Serum samples	Positive cELISA	Positive samples%	Serum samples	Positive IdELISA	Positive samples %
Human							
Kajiado	115	57	1	(1.8)	44	11	(25)
Mashuru	175	68	1	(1.5)	28	9	(32)
Magadi	27	25	**0**	(0)	18	4	(22)
Total	**317**	**150**	2	**(1.3)**	**90**	**24**	**(28)**
Cattle							
Mashuru	39	30	12	(40)	52	46	(88)
Kajiado	29	22	**0**	(0)	54	46	(85)
Magadi	20	21	4	(19)	50	48	(92)
Total	**88**	**73**	16	**(22)**	**156**	**140**	**(90)**
Ovine							
Mashuru	39	27	4	(15)	34	20	(59)
Kajiado	29	20	1	(5)	17	10	(59)
Magadi	20	22	1	(4.5)	29	16	(55)
Total	**88**	**69**	6	**(8.7)**	**80**	**46**	**(57)**
Caprine							
Mashuru	39	20	1	(3.3)	34	27	(79)
Kajiado	29	20	2	(10)	29	25	(86)
Magadi	20	17	2	(12)	20	17	(85)
Total	**88**	**67**	5	**(7.3)**	**83**	**69**	**(83)**

Appendix 5. Questionnaire for humans

QUESTIONNAIRE FOR HUMANS

GENERAL INFORMATION

1. Hospital/health centre _____Date day/month/year_____
2. Name _____
3. Origin: Division_____ Location_____ Manyatta
4. Age _____ Sex _____ Occupation_____
5. Exposure history: Date _____

Condition of animals in contact with_____

Contact with contaminated environment: Dust _____ Aborted foetuses_____Contact with animal discharges _____urine _____ Contaminated water.....

CONSUMPTION OF RAW/UNPROCESSED/UNDERPROCESSED ANIMAL PRODUCTS

1. Do you consume livestock products? Yes { } No { } If yes what products

1. Milk
2. Meat
3. Blood
4. Other (specify)

2. Do you process them before consumption?

1. Milk: No _____ Yes_____ Sometimes_____
2. Meat: No _____ Yes_____ Sometimes_____
3. Blood: No _____ Yes_____ Sometimes_____
4. Do you mix blood with milk .Yes { } No { }

3. How are these livestock products processed?

1. Milk_____
2. Meat_____
3. Blood_____

4. **Do you use any other livestock products such as urine, faeces, hides/skins?**

   Yes { } No { }If yes how_____

CLOSE ASSOCIATION WITH LIVESTOCK

5. **Do you have close contact with livestock? Yes { } No [ ] If yes, how?**
   1. Sharing compound
   2. Sharing house
   3. Sharing water points

AWARENESS OF THE DISEASE

6. **Have you encountered cases of infertility, abortions and retained placenta in livestock?**

   Yes { } No { }

7. **Did human beings also get diseases with flu like symptoms at the same time?**

   Yes { } No { } If yes how many, _____

   Did they know the cause, Yes { } No { }

8. **How do you handle aborted foetuses?**
   1. Eat
   2. Throw away in the bush
   3. Bury/burn
   4. Give to dogs
   5. Do not know
9. **When were these cases of abortion seen?**
   1. Rainy season
   2. Dry season
   3. When there were floods
10. **Did you seek treatment for these ailments Yes { } No { } if Yes after how long?**
11. **What was the diagnosis?**
    1. Malaria
    2. Typhoid
    3. Brucellosis
    4. Flu/ cold
    5. Unknown

Appendix 6. Questionnaire for livestock owners

QUESTIONNAIRE FOR LIVESTOCK OWNERS

GENERAL INFORMATION

Date of interview: _____

Name of the Herder _____

Name of the Interviewer _____

Area _____ Division _____Location _____Group ranch_____

Livestock species owned and their numbers

1. Cattle .....No.....
2. Sheep.....No.....
3. Goats.....No.....
4. Donkeys.....No.....
5. Others specify).....No.....

MANAGEMENT SYSTEM

1. **Grazing system**
   1) Individual herd grazing
   2) Communal free grazing
   3) Others (specify).....
2. Breeding system
   1) Communal bull
   2) Individual bull
   3) Bull from neighbour
   4) Artificial insemination
   5) Others Specify).....
3. **Have you introduced new animals into your farm in the last one year? Yes { } No { } If yes,**

1. How many Cattle _____Sex Age _____ Source(s)_____
2. How many Sheep _____Sex Age _____ Source(s) _____
3. How many Goats _____Sex Age _____ Source(s)_____

4. How many Donkeys_____ Sex Age _____ Source(s)_____

5. How many goats _____Sex Age _____ Source(s)_____

4. Sources of water for livestock

1) Water pan
2) Borehole
3) River
4) Dam
5) Wells
6) Others (Specify).....

5. Sources of water for humans.

1) Water pan
2) Borehole
3) River
4) Dam
5) Wells
6) Others (Specify)

6. Watering system

1) Individual
2) Shared
3) Others (Specify).....

7. What do you when your livestock get sick

1) Buys drugs and treats himself
2) Consults Vet or doctor
3) Consults an AHA
4) Consult an Herbalist
5) Others (specify).....

LIVESTOCK DISEASES

8. List the livestock diseases you experience in order of importance from the most common

1) .....
2) .....
3) .....
4) .....
5) .....

9. Do you have animals that have not given a young one and it is old enough? Yes { } No{ }Cattle No _____**Sheep** No_____**Goats** No_____

10. Have you had animals having premature birth? Yes { } No { }

Cattle No _____**Sheep** No_____**Goats** No_____

11. List cases of retained placenta in your livestock

Species	**No.**	**Year**
Cattle	.....	.....
Sheep	.....	.....
Goats	.....	.....

12. How do you handle aborted foetuses?

1) Eat
2) Throw away in the bush
3) Bury
4) Burn
5) Give to dogs
6) Throw into water
7) Others (specify).....

13. How do you dispose the placenta?

1) Throw away in the bush
2) Bury
3) Burn
4) Give to dogs
5) Throw into water
6) Others (specify).....

14. Do you assist/aid the animals during birth? , Yes { } No { }

CONTACT WITH VETERINARY STAFF

1. Are there veterinary extension staffs in your area? Yes { } No { } If yes, how often do you call them to treat/ assist your animals?
    a. All the time { } rarely { } Not at all { }
2. If Not at all, how do you get the information/ assistance in case of sick animals?

    .....

Appendix 7. Questionnaire for Medical Officer of Health

QUESTIONNAIRE FOR MEDICAL OFFICER OF HEALTH (MOH)

1. **Have you encountered cases of Brucellosis, Q-fever in the patients in** the last five years? Yes { } No { }
2. **How is the general trend of these diseases over the last five years? Indicate below;**

Year	CASES			
	PASTORALISTS		URBAN DWELLERS	
	Brucellosis	Q-fever	Brucellosis	Q-fever
2007				
2008				
2009				
2010				
2011				
2012				

3. **What were the clinical signs exhibited by the patients**
    1. Headache
    2. Fever
    3. Sweating
    4. Joint and body pain
    5. General weakness
    6. Chills
    7. Others (specify)
4. **How is the diagnosis carried out?**
    1. Tentative
    2. Laboratory diagnosis
    3. Others (specify)
5. **If laboratory diagnosis , which tests**
    1. Blood for culture
    2. Urine for culture
    3. Blood staining
    4. Serological tests

    a) Rose Bengal plate Test

I. When did you start using RBPT

II. What prompted you to start

b) Serum agglutination test

c) ELISA

d) Others (specify)

6. Have you encountered any other disease exhibiting the same symptoms as the three diseases or any of the three in your hospital?

1.

2.

7. How are these other diseases managed?

1. Treatment
2. Vaccination
3. Others (specify)

8. What is the average distance in which your patients travel to the hospital?

1. 2 – 5 kms
2. 5-10 kms
3. 10-20kms
4. Beyond 20 kms

9. Do you treat your patients for free or do you charge them? Yes { } No { }

If so what is the average charge these diseases

1. Malaria----------
2. Typhoid----------
3. Brucellosis----------
4. Leptospirosis----------
5. Q-fever----------
6. Any flu/ cold symptom

10 What are you rating on the socio-economic effects of these diseases?

1. High { }
2. Medium { }
3. Low { }

Appendix 8. Questionnaire for the District Veterinary Officer

QUESTIONNAIRE FOR THE DISTRICT VETERINARY OFFICER

1. **Have you encountered cases of Brucellosis and Q-fever in the division in the last five years? Yes { } No { }**If yes indicate the number of cases below

Year	CASES			
	CATTLE		SHEEP AND GOATS	
	Brucellosis	Q-fever	Brucellosis	Q-fever
2008				
2009				
2010				
2011				
2012				

2. **What were the initial signs observed?**
   1. Infertility
   2. Storm abortions
   3. Stillbirths/ weak offspring
   4. Retained after birth
   5. Arthritis (swelling of the knee joint)
   6. Decreased milk production
   7. Others (specify).....
3. **Were these disease manifestations related to**
   1. Rainy season
   2. Dry season
   3. Flooding/Heavy rains
   4. Presence of dogs and other rodents
4. **How was the diagnosis of these diseases carried out?**
   1. Clinical (tentative)
   2. Laboratory diagnosis
   3. Others (specify).....
5. **How were the cases managed or controlled?**
   1. Treatment
   2. Vaccination
   3. Others (Specify).....
6. **Do you have adequate veterinary staff to serve the pastoralists? Yes { }No{ }**

Appendix 9. Adult Patient consent form

Title. A serological survey of Brucellosis, Q-fever in livestock and humans and their associated risk factors in Kajiado County

Adult Patient consent form

Declaration:

1. I the undersigned understand that I may refuse consent to participate in the study without any penalty or loss of benefit, and that in this case I will receive the usual investigations and treatment provided by the health facility.
2. I undersigned declare that the project purpose has been fully explained to me, that I have been given the opportunity to ask questions and that all my questions have been answered satisfactorily.
3. I undersigned consent to having my blood drawn for the intended tests only

Name.....

Identification Card No.....

Study Number.....

Signature of Patient/Guardian.....

Witness name.....Witness designation.....

Witness Signature.....Date.....

Appendix 10. Patients under 18 consent form

Title. A serological survey of Brucellosis, Q-fever in livestock and humans and their associated risk factors in Kajiado County

Patients<18 consent form

Declaration:

1. I Parent/Guardian of a patient <18 years understand that I may refuse consent to participate in the study without any penalty or loss of benefit, and that in this case I will receive the usual investigations and treatment provided by the health facility.
2. I Parent/Guardian of a patient <18 years declare that the project purpose has been fully explained to me, that I have been given the opportunity to ask questions and that all my questions have been answered satisfactorily.
3. I Parent/Guardian of a patient <18 years consent to having my blood drawn for the intended tests only

Name of Parent /Guardian.....

Identification Card No.....

Study Number.....

Signature of Patient/Guardian.....

Witness name.....Witness designation.....

Witness Signature.....Date.....

Appendix 11. Ethical clearance

KENYATTA NATIONAL HOSPITAL
APPROVED
22 JUN 2012
ETHICS & RESEARCH COMMITTEE

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/175

22 June 2012

Miriam Jepkosgei Nakeel
Dept. of Public Health, Pharmacology and Toxicology
Faculty of Veterinary Medicine
University of Nairobi

Dear Miriam

Research proposal: "A serological survey of Brucellosis, Q-Fever and Leptospirosis in Livestock and Humans and their associated risk factors in Kajiado county" (P244/04/2012\)

This is to inform you that the KNH/UoN-Ethics & Research Committee (ERC) has reviewed and **approved** your above revised research proposal. The approval periods are 22nd June 2012 to 21st June 2013.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN**

"Protect to Discover"

Yours sincerely

PROF. A.N. GUANTAI
SECRETARY, KNH/UON-ERC

c.c.

The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
The HOD, Records, KNH
Supervisors: Prof. S.M. Arimi, Prof. J.M. Njenga, Prof. J.K.Wabacha, Dr. P.M. Kitala

"Protect to Discover"

Appendix 12. Director of veterinary services clearance letter

MINISTRY OF LIVESTOCK DEVELOPMENT

Telegrams: "VETLAB", Kabete
Telephone: 020 – 631390/4/5/7, 631287 and 631291
Fax: 254-20- 631273
E-mail: vetdept@todaysonline.com
When replying, please quote:
REF: RES/GEN.VOL.XII/57
All correspondences should be addressed to:
The Director of Veterinary Services
Parcel by rail: Nairobi Station

DEPARTMENT OF VETERINARY SERVICES,
VETERINARY RESEARCH LABORATORIES,
PRIVATE BAG,
00625 KANGEMI

16th May 2012

Prof. S. M. Arimi, PhD
Department of Public Health
Pharmacology & Toxicology
University of Nairobi

RE: FIELD/LABORATORY STUDENT ATTACHMENT

Your letter of 27th February 2012 refers

This is to confirm that there is no objection to your request for authorisation to carry out scientific research on brucellosis, Q-fever and leptospirosis in livestock in Kajiado County.

You are advised to seek similar consent from the Ministry of Public Health and Sanitation regarding research in the in-contact human population.

Dr P. M. Ithondeka PhD, MBS
Director of Veterinary Services

cc PDVS Rift Valley Province

Appendix 13. Director of Medical Services clearance letter

MINISTRY OF MEDICAL SERVICES
OFFICE OF DIRECTOR OF MEDICAL SERVICES

Telegrams:"MINHEALTH". Nairobi
Telephone; Nairobi 2717077 Fax: 2715239

OFFICE OF DIRECTOR OF
MEDICAL SERVICES
AFYA HOUSE
CATHEDRAL ROAD
P.O. BOX 30016
NAIROBI

MMS/ADM/3/8/VOL.111

2nd July 2012

Miriam Jepkosgei Nakeel
Dept of Public Health, Pharmacology and Toxicology
Faculty of Veterinary Medicine
University of Nairobi

Dear Miriam

REF: AUTHORITY TO CONDUCT RESEARCH

Your request for authority to carry out research dated 27/2/2012 refers.

The title of your study is noted to be **"Survey on serological patterns of brucellosis, q-fever and leptospirosis in livestock and humans and their socio-economic impact in Kajiado County".**

Authority is hereby granted to conduct the said research and this office should be notified of the findings following its completion.

Submit a copy of the report to the office of Research and Development at Afya House.

DR. S. M. MAGADA
FOR: DIRECTOR OF MEDICAL SERVICES

Copies to: Medical superintendents
- Kajiado Hospital
- Magadi Hospital
- Sultan Hamud Border Hospital

Appendix 14. National Council of Science and Technology Authorization letter

REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telephone: 254-020-2213471, 2241349
254-020-310571, 2213123, 2219420
Fax: 254-020-318245, 318249
When replying please quote
secretary@ncst.go.ke

P.O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref: **NCST/RCD/12A/012/63**

Date: **25th June 2012**

Miriam Jepkosgei Nakeel
University of Nairobi
P.O.Box 30197-00100
Nairobi.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Survey on serological patterns of brucellosis, q-fever and leptospirosis in livestock and humans and their socio-economic impact in Kajiado County,"*** I am pleased to inform you that you have been authorized to undertake research in **Kajiado County** for a period ending **31st August, *2012.***

You are advised to report to **the District Commissioner, the District Education Officer, the District Livestock Officer and the District Medical Health Officer, Kajiado County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

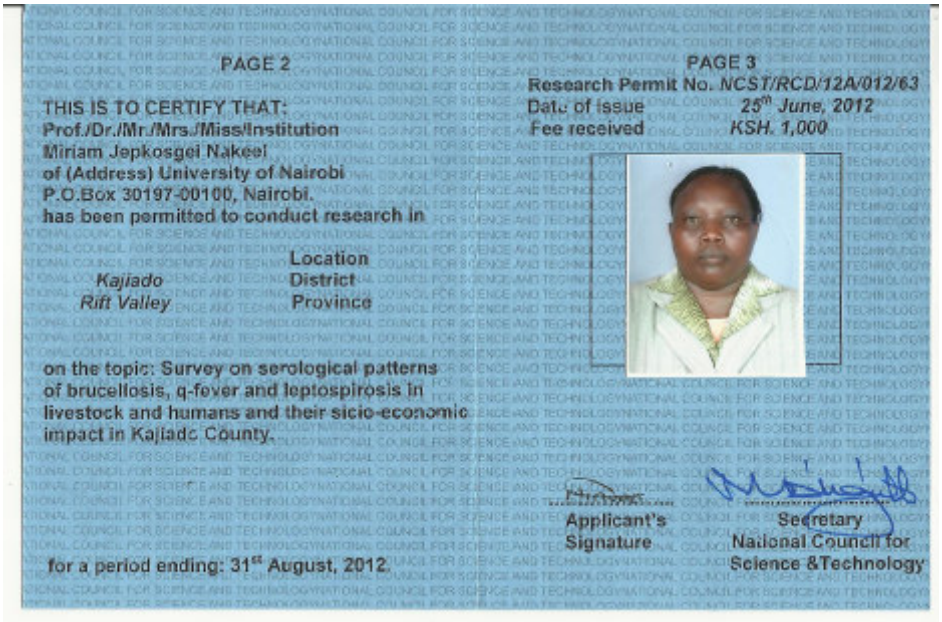
DR. M. K. RUGUTT, PhD, HSC.
DEPUTY COUNCIL SECRETARY

Copy to:

The District Commissioner
The District Education Officer
The District Medical Health Officer
The District Livestock Officer
Kajiado District.

"The National Council for Science and Technology is Committed to the Promotion of Science and Technology for National Development."

Appendix 15. National Council of Science and Technology Research Permit



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THIS IS TO CERTIFY THAT:
Prof./Dr./Mr./Mrs./Miss/Institution
Miriam Jepkosgei Nakeel
of (Address) University of Nairobi
P.O.Box 30197-00100, Nairobi.
has been permitted to conduct research in

Location
District
Province

Kajiado
Rift Valley

on the topic: Survey on serological patterns of brucellosis, q-fever and leptospirosis in livestock and humans and their sicio-economic impact in Kajiado County.

Applicant's Signature

for a period ending: 31st August, 2012

PAGE 3

Research Permit No. *NCST/RCD/12A/012/63*
Date of Issue *25th June, 2012*
Fee received *KSH. 1,000*

Secretary
National Council for Science &Technology

Appendix 16. Map of Kajiado County showing divisions