FACTORS ASSOCIATED WITH BRUCELLOSIS AMONG PATIENTS ATTENDING SELECTED HOSPITALS IN KAJIADO COUNTY, KENYA

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Factors associated with brucellosis among patients attending selected hospitals in Kajiado County, Kenya

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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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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

I dedicate this thesis to Imani.

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ABBREVIATIONS AND ACRONYMNS

CDC	United States - Centres for Disease Control and Prevention
CFSPH	Centre for Food security and Public Health
CI	Confidence Interval
DHIS	District Health Information System
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immuno-Sorbent Assay
FAO	Food and Agriculture Organization
ICSP	International Committee on Systematics of Prokaryotes
ILRI	International Livestock Research Institute
KBS	Kenya Bureau of Statistics
KDDT	Kajiado District Development Trust
MOALF	Ministry of Agriculture, Livestock and Fisheries
MOH	Ministry of Health
OIE	World Organization for Animal Health
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
RDT	Rapid Diagnostic Test
SLPS	Smooth Lipopolysaccharide
WHO	World Health Organization
ZDU	Zoonotic Disease Unit

ABSTRACT

Brucellosis is a bacterial zoonotic disease with serious public health implications and high socio-economic burden among afflicted populations. Brucellosis is a priority zoonotic infection in Kenya but there is limited information on the true burden of the disease due to weak surveillance. Livestock rearing and trade is a key source of livelihood for majority of residents of Kajiado County. The close linkage between pastoralists and livestock, characterized by high dependence on livestock products increase the risk of spillover of infection between humans and animals. Identifying and understanding the modifiable risk factors for brucellosis in necessary to inform public health interventions. There are few studies in Kenya which are address brucellosis risk factors through incident cases. The objective of the study was to describe socio-demographic and clinical characteristics of brucellosis cases in Kajiado, determine risk factors for infection among patients attending select health facilities in Kajiado County and to evaluate the performance of a brucellosis rapid diagnostic kit (*Brucella* agglutination test) through comparative testing with Enzyme-Linked Immunosorbent Assay test (ELISA). The study area was Mashuru sub-county in Kajiado and the study design was an unmatched, hospital-based case-control study. Patients with fever and two or more clinical features descriptive of brucellosis as per the World Health Organization case definition and a positive ELISA test were classified as cases. Data on sociodemographic, clinical and occupational characteristics were collected using a structured questionnaire and analyzed using Epi info version 7 software. Descriptive data was analyzed using means and proportions while risk factor analysis was done using bivariate and multivariate analysis. Unconditional logistic regression was used to study the association between exposure variables and brucellosis. Forty-three cases and 86 controls were recruited for the study. The mean age for the cases was 49 years (SD=20) while that of the controls was 38 years (SD = 18.8). Majority of both cases (62.7%) and controls (58.1%) were female. The most reported symptoms for cases were headache (83.7%) back pains (62.8%) and joint pains (60.6%). Most controls also reported similar clinical presentation and there was no significant difference in reported symptoms between *brucellosis* cases and controls. Regular consumption of un-boiled milk and assisting animals in delivery were significantly associated with brucellosis by adjusted odds ratio (aOR) 7.7 (95% CI 1.5–40.1) and aOR 3.7 (95% CI 1.3–13.5), respectively. The sensitivity and specificity of the Brucellosis Rapid Diagnostic Kit (RDT) in use in the study health facilities was 20 % and 90 % respectively with a Predictive positive value of 33% and Predictive negative value negative of 84%. The lack of a significant difference in clinical presentation between cases and controls conforms to other reports that identify brucellosis as one of the most misdiagnosed diseases because it causes a systemic infection that presents in a myriad of non-specific signs. This means that clinical symptoms alone cannot be adequately used for presumptive diagnosis and clinicians should consider epidemiologic linkages and laboratory results in management of suspect brucellosis cases. The risk factor analysis shows that animal handlers; primarily farmers and animal health workers and people who consume unpasteurized milk are at the greatest risk. Public health education on brucellosis transmission and prevention, specifically use of protective personal equipment when assisting animals in delivery and boiling of milk should be offered to

farmers and the general public. The results from the comparative testing are indicative of the challenge in diagnosis of brucellosis using point of care tests with low validity values. The low sensitivity values mean a big proportion of cases are being misdiagnosed. More specifically, a significant number of cases are being missed in this high burden region due to low-test sensitivity. As such there is need to validate and replace low quality *Brucella* test kits with better diagnostic tools.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Human brucellosis is a neglected disease of poverty often found in highly agrarian, livestock dependent societies (Godfroid *et al.,* 2010). Brucellosis is an infectious debilitating, zoonotic disease widely spread in the countries of North and East Africa, the Middle East, South and Central Asia, Central and South America (M. J. Ducrotoy *et al.,* 2014). Despite being one of the oldest diseases known to man, brucellosis remains one of the world's most widespread bacterial zoonotic diseases and is now classified as an emerging and re-emerging threat to public health (Seleem *et al.,* 2010). In livestock, brucellosis is a herd problem transmitted primarily by ingestion of contaminated material and through venereal infection. Congenital or perinatal infections also occur, with ensuing development of latent infections in off-springs. Spread between livestock herds usually occurs by the introduction of asymptomatic chronically infected animals into a naive herd of animals where the infected animals shed the organisms in uterine discharges during abortion and subsequent parturitions (Cárdenas *et al*., 2019).

In humans, *Brucella abortus*, biovars 1-6, 9; *Brucella melitensis*, biovars 1-3 ; *Brucella suis*, biovars 1,3 and 4 are the most common causes of brucellosis infection although *B .melitensis* is considered as the most pathogenic (Doganay & Aygen, 2003). Transmission of infection to humans occurs through direct contact with blood, body tissues and fluids such as vaginal discharges, aborted fetuses or placental discharges. 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 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 (Pandit & Pandit, 2013). The occurrence of human brucellosis is assumed to be related to the prevalence in animals and practices that expose humans to infected animals or their products, poor hygiene coupled with close contact with infected animals and consumption habits are the main contributory factors to the spread of the disease in humans (Cárdenas *et al.*, 2019)

Brucellosis is a febrile disease capable of masquerading as a myriad of entities, both infectious and non-infectious. The disease has a tendency towards chronicity and persistence, becoming a granulomatous disease capable of affecting any organ system. The timely and accurate diagnosis of human brucellosis continues to challenge clinicians because of its non-specific clinical features, slow growth rate in blood cultures, and the complexity of its sero-diagnosis *(Franco et al.,* 2007). Establishing the factors associated with brucellosis among the most at-risk populations in Kenya and understanding the livestock-human link in disease transmission is the first step in designing effective control programs that address the problem in Kenya.

1.2 Statement of the problem

Brucellosis remains one of the most common zoonotic diseases worldwide and one of the top priority zoonotic diseases in Kenya (Munyua *et al.,* 2016). Despite a low mortality index, brucellosis is a disease of public health importance due to productivity losses attributed to the chronic nature of the disease and high costs of management of sequelae (Njeru, Wareth, *et al.*, 2016). Clinical management of brucellosis is of particular concern because of high initial treatment failure and relapse rates (Njeru *et al*., 2016). Besides the health complications in humans, brucellosis infection in animals causes heavy economic losses in animal production resulting from clinical disease, abortion, neonatal losses, reduced fertility, decreased milk production, slaughtering of the infected animals, cost of veterinary care, and replacement animals. In addition, the disease is an impediment to free animal movement (Tasiame *et al.,* 2016), and it is also a

limiting factor for international trade of animals and their products (Radostits *et al*., 2007).

In Kenya, the actual national prevalence and incidence remain unknown. A review of existing literature however, indicates that brucellosis, both in livestock and humans is an endemic problem (Njeru, Wareth, *et al*., 2016). In a study to find the serological responses to HIV sero-positive patients, (Paul *et al.,* 1995) found that 35 out of 100 patients; 65 of whom were HIV positive but whose routine microbiological cultures were negative for *Brucella*, were positive for *Brucella* specific antibodies. The study found eight study participants had *Brucella*-specific IgM and IgG, six had IgM only and 21 had IgG only, suggesting relatively high levels of exposure to *Brucella* in the study cohort. In conclusion the investigator stated that brucellosis is probably under-diagnosed in Kenya and *Brucella* serology may be helpful in the diagnosis of patients with non-specific symptoms in Kenya, regardless of HIV status. Between December 2011 and January 2012, (Kiambi *et al.,* 2020) found that one out of six febrile patients attending Ijara district hospital were positive for brucellosis antibodies, translating to a prevalence of 17%. A study found 14% of the human population in Kajiado was positive for *Brucella* antibodies, with 10% of the respondents reporting they had been treated for brucellosis before and 75% reporting they knew somebody who has been affected by the disease (Osoro *et al*., 2015). Another study found a *Brucella* antibody prevalence rate of 12.3% in milk supplies sampled at 219 consumer households in urban Kenya proving that large urban households are at risk of developing the disease through consumption of unpasteurized milk (Kang'ethe *et al.,* 2000). These are some of the published studies that prove that human brucellosis is indeed a disease of public health importance in Kenya.

1.3 Justification of the study

Livestock rearing and trade is a key source of livelihood for a majority resident of Kajiado County, a region predominantly inhabited by Maasai pastoralists. The close

linkage between pastoralists and livestock, characterized by high dependence on livestock products increase the risk of spillover of infection between humans and animals. Despite this link, weak surveillance characterized by underreporting and poor-quality diagnostic tools in primary health centers that serve the region results in poor understanding of the true burden of disease *(J Njeru, Henning, et al.,* 2016). This consequently results to a false perception that the impact of brucellosis is low on the communities and limited resource are allocated for control and prevention. Although previous studies have shown high levels of human exposure to *Brucella* (Osoro *et al.,* 2015), limited studies have been conducted to describe drivers of infection among clinical patients (J Njeru, Wareth, *et al.,* 2016). Improved understanding of the risk factors of brucellosis in Kajiado will provide evidence to guide interventions and in turn improve livestock production and minimize socio-economic losses associated with *Brucella* infection.

1.4 Research questions

1. What is the clinical presentation and socio-demographic factors of brucellosis cases in Mashuru Sub-County of Kajiado County?
2. What are the risk factors associated with brucellosis in patients attending select hospitals in Mashuru Sub-County, Kajiado County?
3. What is the performance (sensitivity and the specificity) of the Brucella RDT in use in Kajiado County?

1.5 Null Hypothesis

There are no *brucellosis* among patients attending select health facilities in Kajiado County and there are no factors associated with brucellosis cases in Mashuru, Kajiado County.

1.6 Objectives

1.6.1 General Objective

To determine the socio-demographic factors, clinical presentation and factors associated with brucellosis among patients attending selected hospitals and to evaluate the performance of the Brucella rapid diagnostic kit in use in select health facilities in Mashuru sub-county, Kajiado County.

1.6.2 Specific objectives

1. To determine socio-demographic factors and clinical presentation of brucellosis cases in select hospitals in Kajiado County
2. To determine risk factors associated with brucellosis patients attending selected hospitals in Kajiado County
3. To evaluate the performance of the brucellosis rapid diagnostic kit used in Kajiado

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of brucellosis

Most developing countries including Kenya face significant challenges in having an effective national surveillance program (Pappas *et al.,* 2006). Difficulties in case ascertainment arise because infection is chronic in both humans and animals, variable incubation periods and difficulties in laboratory confirmations (Dean, Crump, Greter, Hattendorf, *et al*., 2012). This results to gross under-reporting of the true burden of the burden. There is evidence however, that the epidemiology of human brucellosis, the commonest zoonotic infection worldwide, has drastically changed over the past two decades due to implementation of phytosanitary measures and improved socioeconomic status in most developing and developed countries. As such, several areas traditionally considered to be endemic, such as most of Latin America and Mediterranean Europe have achieved control of the disease (Lounes *et al.,* 2014).

Developing nations have the highest burden of brucellosis resulting in significant economic losses and reduced quality of life in afflicted populations (M. Ducrotoy *et al.,* 2017). Regions most heavily burdened by the disease include countries in parts of Central Asia, Middle East, Latin America and Sub-Saharan Africa (M. Ducrotoy *et al.,* 2017). Brucellosis is known to be endemic in Sub-Saharan African countries where conditions for its incidence and transmission exist, however the true of picture of disease distribution in Sub-Saharan countries including Kenya is unknown. According to data from OIE for 2019, Kenya is one of the Sub-Saharan countries that reported cases of human brucellosis (OIE, 2009) but brucellosis data pertaining to both animals and humans in Kenya is limited. Available data is primarily passive, clincial surveillance

data by county level veterinary and human health departments. A review of human health surveillance data reported through the intergrated disease surveillance system shows atleast 100,000 cases are reported annually in Kenya. This gives an annual incidence of 202/100,000. These figures, although an estimation of the disease situation due to issues that pertain to data quality and specificity of the surveillance system are an indication of the high incidence in the country.

2.2 Etiology of brucellosis

Ten Brucella species are currently documented but only seven of them that affect terrestrial animals: B. neotomae, B. ovis, B. abortus, B. melitensis, B. canis, B. suis, and B. microti (Scholz et al., 2010). Two species, B. melitensis and B. abortus are the primary cause of majority of human infection (Franco et al., 2007). B. melitensis is the species is considered the most pathogenic and virulent of all zoonotic species. The organism is primary associated with infection in goats and sheep, but other species can be affected (Seleem et al., 2010).

B. abortus infection in human is often sub-clinical and where disease does occur, it is usually less severe than caused by *B. melitensis* or *B. suis*. Cattle are by far the most common source of *B. abortus* but wildlife of the Bovidae family are also affected. *B. canis* is a widespread infection of dogs in many countries, it is infrequently associated with human disease. Reported cases have usually been mild and not as severe as those transmitted from food animals (Godfroid *et al.,* 2013).

2.3 Morphology of Brucella species

Brucella species are gram-negative, on-spore-forming and non-capsulated cocco-bacilli or short rods 0.6 to 1.5 μm long by 0.5 to 0.7 μm in width, arranged singly and less frequently in pairs. Although they are described as non-motile, they carry all the genes except the chemotactic system, necessary to assemble a functional flagellum (Fretin *et al.,* 2005). *Brucella* are not truly acid-fast but resist discoloration by weak acids, thus

stain red by the Ziehl-Nielsen method, which is sometimes used for the microscopic diagnosis of brucellosis from smears of solid or liquid specimens. The morphology of *Brucella* species is fairly constant except in old cultures, where pleomorphic forms may be evident (Godfroid *et al.,* 2010; OIE, 2009). On suitable solid media *Brucella* colonies are visible after 2 days incubation. After 4 days incubation, *Brucella* colonies are round, 1-2 mm in diameter, with smooth (S) margins, translucent and a pale honey color when plates are viewed in the daylight through a transparent medium. When viewed from above, colonies appear convex and pearly white (Fretin *et al.,* 2005).

2.4 Taxonomy of Brucella species

The genus *Brucella* was classified as a monospecific genus, with *B. melitensis* as the sole species and the other species should be considered as biovars (Xavier *et al*., 2009;Mittal *et al*., 2018). Conversely, several molecular genotyping methods have been developed and applied to characterize *Brucella* species, indicating that significant DNA polymorphisms occur between species, which favor the current multi-species classification of *Brucella* (Halling *et al*., 2005). Importantly, comparison of genome sequences of *B. suis* and *B. melitensis* demonstrated that exist clusters of genes that are unique in both species (designated genetic islands). It is reasonable to hypothesize that these unique genes may contribute to the differences in host specificity between *Brucella* species (Foster *et al.,* 2012). Furthermore, recent studies based on comparative whole genome analysis of several *Brucella* species indicate that there is limited divergence with a large number of pseudo genes. Interestingly, these genomic analyses do not clearly explain the host preferences of *Brucella* species (Wattam *et al.,* 2009). One of these studies indicates that at the *B. ovis* is the basal lineage to the rest of the *Brucella* species and that apparently most *Brucella* species diverged from their common *B. ovis* ancestor in the past 86,000 to 296,000 years (Olsen & Palmer, 2014).

The International Committee on Systematics of Prokaryotes (ICSP), Subcommittee on the taxonomy of *Brucella* recommended a taxonomic classification that includes different species within the genus, either classical or new, which are still considered as individual species. Therefore, the genus currently group ten species, namely *B. melitensis, B. abortus, B. suis, B. neotomae, B. ovis, B. canis, B. ceti, B. pinnipedialis, B. microti and B. innopinata* (Ficht, 2010).

2.5 Brucella infection in humans

Human brucellosis is distributed globally but with varying incidence in different parts of the world depending on implementation of control measures in livestock (Franc *et al*, 2018). The incubation period of brucellosis normally is 1–4 weeks, but it can be several months before cases show signs of infection. *B. melitensis* is associated with acute, severe infection whereas the infections with other species are usually sub-acute and prolonged (B G Mantur *et al*., 2007). *B. melitensis* is the most virulent *Brucella* for humans and usually causes a debilitating infection (Fugier *et al*., 2007). The WHO laboratory biosafety manual classifies *Brucella* (and particularly *B. melitensis*) in risk group III (OIE, 2009.).

Humans acquire brucellosis mainly through ingestion of contaminated milk and unpasteurized dairy products. Contact of mucosa and skin abrasions with fluids and tissues from aborted fetuses of infected animals are also important sources of *Brucella* transmission (Fugier *et al.,* 2007) Furthermore, people may be infected by inhalation of contaminated dust or aerosols. Thus, *Brucella* is one of the most common laboratory acquired pathogens worldwide and is included in the potential biological weapon list (Maza *et al.*, 2020).

The disease is primarily an occupational risk among animal health workers, laboratory technicians and professionals who work with animals and animal products. The primary source of infection is through direct or indirect contact through the skin or mucous

membranes or ingestion of contaminated products, especially fresh dairy products. Unpasteurized dairy products are the main source of infection for people who do not have direct contact with animals. However much of the milk which is consumed is rendered safe by pasteurization or boiling. The handling of raw wool has been identified as a potential source of infection of workers involved. *B. melitensis* is also easily acquired by laboratory infection (EFSA Panel on Animal Health and Welfare *et al.*, 2017 ; Lu *et al.*, 2020).

Human infections with *B. melitensis* have different clinical presentation that can become chronic and debilitating. Most patients present with constitutional symptoms, such as fever, malaise and chills. The severe form of the disease can be accompanied with osteo-articular signs (spondylitis, arthritis and osteomyelitis) or genitourinary tract changes (orchitis, epididymitis, glomerulonephritis and kidney abscesses (Buzgan *et al.*, 2010). Human brucellosis is also known for complications and involvement of internal organs and its symptoms can be diverse depending on the site of infection and include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis (Zheng *et al.*, 2018). Spontaneous abortions, mostly in the first and second trimesters of pregnancy, are seen in pregnant women infected with *Brucella* (Ali *et al*., 2016). Although a rare complication, *Brucella* endocarditis (<2% of cases) is most commonly associated with *B. melitensis* infection and is the most severe complication. It accounts for at least 80% of deaths due to brucellosis (Akhvlediani *et al.*, 2010). Lack of appropriate therapy during the acute phase may result in localization of *Brucella* in various tissues and organs and lead to sub-acute or chronic disease that is very hard to treat (Jiang *et al.*, 2019). Nervous, genitourinary, hepatosplenic and cardiovascular complications been observed. Brucellosis is termed *chronic* when it includes one or more of the signs described above and persists or recurs over a period of six months or more. *Brucella* dermatitis traditionally known as “allergy" to *Brucella* has also been associated with *B.melitensis* (Dean, Crump, Greter, Hattendorf, *et al*., 2012)

Symptoms and signs of brucellosis, usually referred to as fever of unknown origin can be confused with other diseases including enteric fever, malaria, rheumatic fever, tuberculosis, cholecystitis, thrombophlebitis, fungal infection, autoimmune disease and tumors (Basappa G Mantur *et al.,* 2006). Live animal vaccines *B. melitensis* Rev. 1 and *B. abortus* strain 19 are known to cause disease in humans. The course of the disease with vaccine strains is usually shorter and more benign (Dorneles *et al.,* 2015; Goodwin & Pascual, 2016). Direct person-to-person spread of brucellosis is extremely rare. Mothers who are breast-feeding may transmit the infection to their infants and sexual transmission has also been reported (Haghdoost *et al.,* 2015; Tuon *et al.,* 2017)

2.6 *Brucella* infection in livestock

The major route of infection is through the mucous membranes of mouth and upper respiratory tract or conjunctiva during feeding on contaminated pastures (Corbel *et al.,* 2006.). Sexual transmission is also common in herds that use bulls for breeding. After gaining entrance to the body, the organisms encounter the cellular defenses of the host, but generally succeed in arriving via the lymph channels at the nearest lymph node. The fate of invading bacteria is mainly determined by the cellular defenses of the host, chiefly macrophages and T lymphocytes, though specific antibody undoubtedly plays a part (Aparicio, 2013). The outcome depends on the ruminant species infected, age, immune status of the host, pregnancy status, and the virulence and number of the invading *Brucella*. When the bacteria prevail over the body defenses, a bacteraemia is generally established. This bacteraemia is detectable after 10 to 20 days and persists from 30 days to more than two months (Mittal *et al.,* 2018). If the animal is pregnant, bacteraemia often leads to the invasion of the uterus, surrounding lymph nodes and other organs like the udder and the spleen. During this first stage of infection, the major clinical sign is abortion but other signs due to a localization of *Brucella* may be observed such as orchitis, epididymitis, hygroma, arthritis, metritis, subclinical mastitis, occasionally retained placenta, some animals however, become asymptomatic carriers (Zinsstag *et al.,* 2005). Abortion generally does not occur if the female becomes infected

at the third trimester of pregnancy (Poester *et al.*, 2013; Xavier *et al.*, 2009). The second stage is characterized by either elimination of *Brucella* or, more frequently, by a persistent infection of mammary glands and supramammary and genital lymph nodes (Xavier *et al.*, 2009).

Animals generally abort once during the second trimester, but re-invasion of the uterus occurs in subsequent pregnancies with shedding in fluids and membranes. The pregnancy can also continue to full-term. The percentage of infected females lambing/kidding in a flock may reach 40% (Radostits *et al.*, 2007). Females that are born into an infected environment and subsequently infected generally abort less frequently as compared to those born in uninfected environment but later get infected. This explains the high level of abortions in newly infected flocks and their relatively low frequency in flocks where infection is enzootic (Radostits *et al.*, 2007) Greatly reduced milk yield follows abortion, and infection of the udder following a normal birth also leads to a considerable reduction in yield. In spite of this, clinical signs of mastitis are seldom detectable in naturally infected goats (Godfroid *et al.*, 2005, 2010)

2.7 Diagnosis of brucellosis in humans

2.7.1 Clinical manifestation of brucellosis in humans-clinical diagnosis

Human brucellosis has a wide spectrum of clinical manifestations, earning it a place alongside syphilis and tuberculosis as the diseases with most varying clinical presentation (Franco *et al.*, 2007). The clinical features of brucellosis depend on duration of infection and body systems affected. *Brucella* has been reported to compromise the central and peripheral nervous system, and the gastrointestinal, hepatobiliary, genitourinary, musculoskeletal, cardiovascular and integumentary systems (de Figueiredo *et al.*, 2015) .

Infection presents with constitutional symptoms that may have with acute onset. The main symptoms are intermittent fever (hence the name undulant fever), chills and

sweating, fatigue, anorexia, malaise, headaches, joint pains and generalized aching. Abscess formation is a rare complication. *Brucella* endocarditis and neurobrucellosis cause most deaths (Corbel *et al.,* 2006) Studies have shown that intermittent fever is the most common clinical presentation, followed by osteoarticular complications and non-specific symptoms. On physical examination, the most common findings are hepatomegaly and splenomegaly, which occur in about one-third of patients. Lymphadenopathy is seen in about 10% of patients. Osteoarticular manifestations (sacroilitis, spondylitis, peripheral arthritis, and osteomyelitis) account for over half of the focal complications. Neurological findings are not as uncommon as they are often portrayed; A study from Turkey reported that in a series of 305 patients with brucellosis, 20 (6.6%) patients presented with neurological involvement (Guler *et al.,* 2014). Neurological findings can be diverse and could include peripheral neuropathies, chorea, meningo-encephalitis, transient ischaemic attacks, psychiatric manifestations, and cranial nerve compromise. Pulmonary manifestations, including pleural pneumonias, can be found in up to 16% of complicated cases of brucellosis. Leucocytosis is observed in about 9% of patients and if found, focal complications should be excluded Leucopenia (11% of patients) and thrombocytopenia (10% of patients) are seen in similar frequencies (Franco *et al.,* 2007). Anemia is seen more frequently, affecting 26% of patients. Common disease findings, however, vary between different areas and populations. Endocarditis—with the aortic valve being the most affected structure and multiple valve involvement being common within this subset of patients—is the most serious complication, accounting for most of the 5% total mortality rate of human brucellosis (Dean, Crump, Greter, Hattendorf, *et al*., 2012; Franco *et al*., 2007). The most common osteoarticular finding in children is monoarticular arthritis (Adetunji *et al.,* 2019).

2.7.2 Culture and isolation of *Brucella*

Like in other bacterial infections, blood culture is the gold standard in the diagnosis of brucellosis (Corbel *et al.,* 2006). The biphasic Ruiz-Castaneda system is the traditional

method for the isolation of *Brucella* species from clinical samples, it has now largely been replaced by automated culture systems—such as the lysis centrifugation method with increased sensitivity and reduced culture times. The sensitivity of blood culture varies depending on multiple factors the main ones being the stage of infection and antibiotics use history. For instance, in acute cases, the sensitivities of the Ruiz-Castaneda method and lysis centrifugation have been reported as high as 80% and 90%, respectively, but as low as 30% and 70%, respectively, in chronic cases. Besides blood culture, brucellosis can be cultured from bone marrow samples, pus, tissue samples and skeletal joint fluids (Franco *et al.,* 2007; Khan & Zahoor, 2018; Smirnova *et al.,* 2013).

2.7.3 Sero-diagnosis of brucellosis

In the absence of culture facilities, the diagnosis of brucellosis relies on serological testing with a variety of agglutination tests such as the Rose Bengal test, the serum agglutination test, and the antiglobulin or Coombs' test (Corbel *et al.,* 2006). In general, the Rose Bengal test is used as a screening test, and positive results are confirmed by the serum agglutination test. Agglutination tests are based on the reaction of antibodies against smooth lipopolysaccharide. However, epidemiology of the disease should be considered when translating results. In endemic areas, high background values could occur that may affect the diagnostic value of the test due to persistence of antibodies. Also, the *Brucella* smooth lipopolysaccharide antigen tends to show cross reactivity with other Gram-negative bacteria such as *Escherichia coli* O:157, and *Francisella tularensis*. The sensitivity of the Rose Bengal test is very high and the specificity of the assay is also fairly high, however differences in the quality of the reagent used may affect quality of test results (Díaz *et al.,* 2011)

Enzyme Linked Immunosorbent Assay (ELISA) has become popular as a well standardized assay for brucellosis. The sensitivity of ELISAs prepared in the laboratory may be high, especially when the detection of specific IgM antibodies is complemented with the detection of specific IgG antibodies. The specificity of ELISA, is reported to be

lower than that of the agglutination tests (Rahman *et al.,* 2013). Since ELISA for *Brucella* is based on the detection of antibodies against smooth lipopolysaccharide, the cut-off value may need adjustment to optimize specificity when used in endemic areas, and this may influence sensitivity. Test performance of commercial ELISAs, as described in their package inserts, is based on comparison with commercial ELISAs of other brands, and not culture. Cut-off values should be established based on local epidemiological conditions (Franco *et al.,* 2007; Memish *et al.,* 2002; Zheng *et al.,* 2018)

2.7.4 Other methods for diagnosis of *Brucella* organisms

Rapid tests such as the fluorescent polarization immunoassay (FPA) for brucellosis and the immunochromatographic *Brucella* IgM/IgG lateral flow assay which is a simplified version of ELISA are in use for diagnosis of brucellosis (Praud *et al.,* 2016). The FPA test is done by incubation of a serum sample with *Brucella* O-polysaccharide antigen linked to a flouorescent probe. The sensitivity of this test at the selected cut-off value is 96% for culture-confirmed brucellosis, and the specificity was determined to be 98% for samples from healthy blood donors (Franco *et al.,* 2007). The LFA uses a drop of blood obtained by finger prick, does not require specific training, is easy to interpret, and can be used at the bedside. The components are stabilized and do not require refrigeration for transportation or storage. The sensitivity and specificity of LFA are high (more than 95%), and the test can be used at all stages of disease (Franco *et al.,* 2007).

Polymerase Chain Reaction (PCR) is considered a good tool in the diagnosis of brucellosis because of improved sensitivity compared with culture (Bricker, 2002). Several genus-specific PCR systems using primer pairs that target 16S RNA sequences and the genes of different outer membrane proteins have been developed. Each of these PCR systems produces a discrete DNA product, whose length is identical for and specific to all *Brucella* species (Godfroid *et al.,* 2010). Adequate comparisons of the different PCR systems and primers are still lacking, and results may presumably depend

on the nature of the clinical specimen, the sample preparation procedure, and the duration and stage of illness. PCR could be particularly useful in patients with specific complications such as neurobrucellosis, or other localized infections, since serological testing often fails in such patients. However, because these PCR systems are complex, time consuming, and have a high risk of contamination, they are less suitable for routine diagnostic purposes; real-time PCR systems have been developed that are faster and less prone to contamination and are thus more clinically useful (Seligsohn *et al.*, 2020) . Comparative analysis of the various real-time PCRs is needed to assess their diagnostic value (Franco *et al.,* 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Kajiado County, a semi-arid, livestock keeping county of Kenya, bordered by Tanzania to the southwest and in part by Taita-Taveta, Machakos, Kiambu and Narok counties (figure 3.1) Kajiado county has a population of 1,117,840 and an area of 21,292.7km$^{2.}$ The county is divided into five administrative regions: Loitokitok, Mashuru, Kajiado Central, Kajiado North and Isinya (*2019 Kenya Population and Housing* Census - *Kenya National Bureau of Statistics*, 2019).

Mashuru sub-county, was purposively selected to be the study area because of the predominant livestock keeping population in the sub-county and the limited number of studies done in that area. Mashuru is primarily a rural semi-arid area with an approximate population of 167,000 people (KNBS, 2009). Four out of 17 sub-locations within Mashuru; Arroi, Nkama, Mashuruu and Sultan Hamud were randomly selected (using simple random sampling tool in Microsoft excel) as the study areas. The four sub-locations have an estimated human population of 15,036, population density of 20.4 /km^2 and 3210 households (KNBS 2009). One health facility in each of the four sub-locations was selected as a study site based on patient load and catchment area: Mashuru health facility (Mashuuru), Ilmukutani dispensary (Arroi), Father Adriano health (Sultan Hamud) facility and Nkama dispensary (Nkama).]

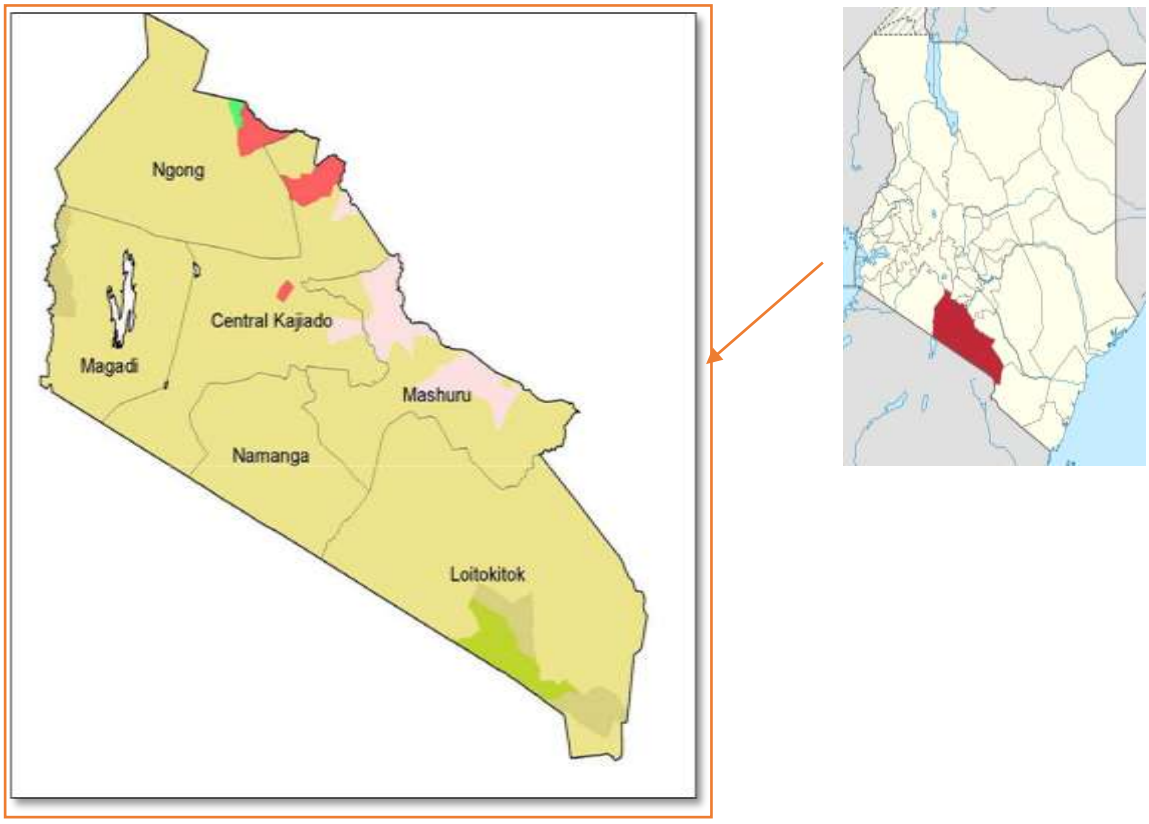

Figure 3.1: Map of Kajiado County in relation to the country and Mashuru sub-county in relation to Kajiado county (Source: https://reliefweb.int/report/kenya/kajiado-county-2016-long-rains-food- security-assessment-report-august-2018)

3.2 Study design

The study was an unmatched hospital-based case-control study conducted from January-December 2015.

An unmatched case control study design was selected to allow the investigator to analyze for age and sex (two of the most common matching variables) and to avoid the risk of overmatching which would bias the study (Marsh *et al.,* 2002). Test negative controls was used as a precaution to minimize selection bias (Lipsitch *et al.*, 2010).

3.3 Study population

The study population comprised of residents of the four sub-locations who had resided in the study area for the past one year. This criterion was developed to make the study representative of residents of Kajiado County.

3.4 Inclusion criteria for cases and controls

The case definition was adopted from the WHO (Corbel, 2006).

Case: A case was defined as per the WHO guide on brucellosis in humans and animals. The case definition was an illness occurring in a consenting patient or patient with assent above five years who had been a permanent resident of one of the study sub-locations for the past one year and was diagnosed with brucellosis on the basis of at-least two of the following symptoms; intermittent or irregular fever (>37.5°C), sweating, fatigue, anorexia, weight loss, headache, arthralgia and generalized aching and ELISA immunoglobulin M or G test which was used the confirmatory test. Children below two years

were not included in the study because of the lack of pediatric services in the study health facilities.

***Control*:** A control was defined as a consenting patient above two years with no recent (one year) history of brucellosis or fever of undetermined origin who was a permanent resident of one of the four study sub-locations and who visits study facilities with unknown illness or fever of unknown origin but does not meet the laboratory criteria put down for the cases during the study period (test negative controls). To minimize the chances of misdiagnosed cases being selected as controls, patients with recent history of brucellosis or fever of unknown origin were excluded as controls.

3.5 Exclusion criteria for cases and controls

Patients who did not fulfill the inclusion criteria and those who refuse to consent to the study.

3.6 Sample size determination

Sample size was calculated using the open-Epi version 2 open source calculator (https://www.openepi.com/Menu/OE_Menu.htm). In the calculator, Fleiss formulae for unmatched case control studies as shown in Figure 3.2 (Charan & Biswas, 2013) was applied to determine the appropriate sample size for a power of 0.8 and significance level of 0.5 to detect an odds ratio (OR) of ≥ 3 for exposure factors present in 20% of controls (Shehada & Abu Halaweh, 2013).

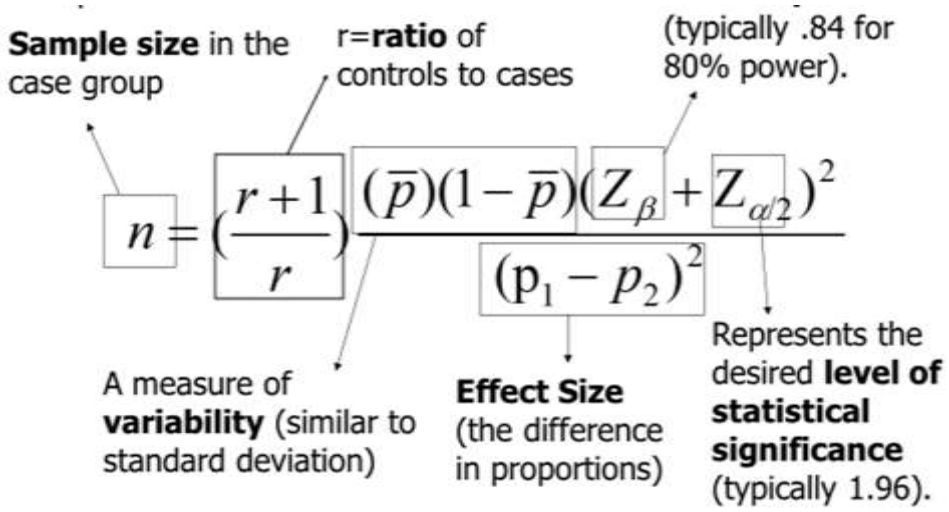

Figure 3.2: Sample size formula showing variables used in the sample size calculation

Control to case ratio was 2:1 to improve study power. The minimum sample size for cases was 47 (1/3) and 94 for controls (2/3) giving a sample size of 141.

Table 3.1: Sample size calculation for unmatched case-control study assumptions

Two-sided confidence level (1-α)	**95%**
Power (Zβ)	80%
Ratio of controls to cases (r)	2
Hypothetical proportion of controls with exposure (P2)	20
Hypothetical proportion of cases with exposure (P1)	42.86
Least extreme odds ratio to be detected	3

3.7 Sampling design- patient enrollment criteria

3.7.1 Enrolment of cases

The sampling frame was all patients attending the study facilities within the study period. Usual hospital procedures for clinical triage of patients were used. Patients who were eligible for enrollment were approached, the study purpose explained, and their consent sought (Appendix i, ii). The set case definition was strictly used to ensure the cases represent a homogenous entity. Any patient who fit the clinical description of a case was tested by laboratory personnel on duty using RBT which was the screening test. Those who were positive had their serum samples taken and tested for confirmatory testing using ELISA at KEMRI-CDC laboratory in Nairobi (case status on the questionnaire was then be filled based on the ELISA result). A questionnaire was then administered to all persons eligible for entry into the study. The patient testing criteria is shown on Figure 3.3.

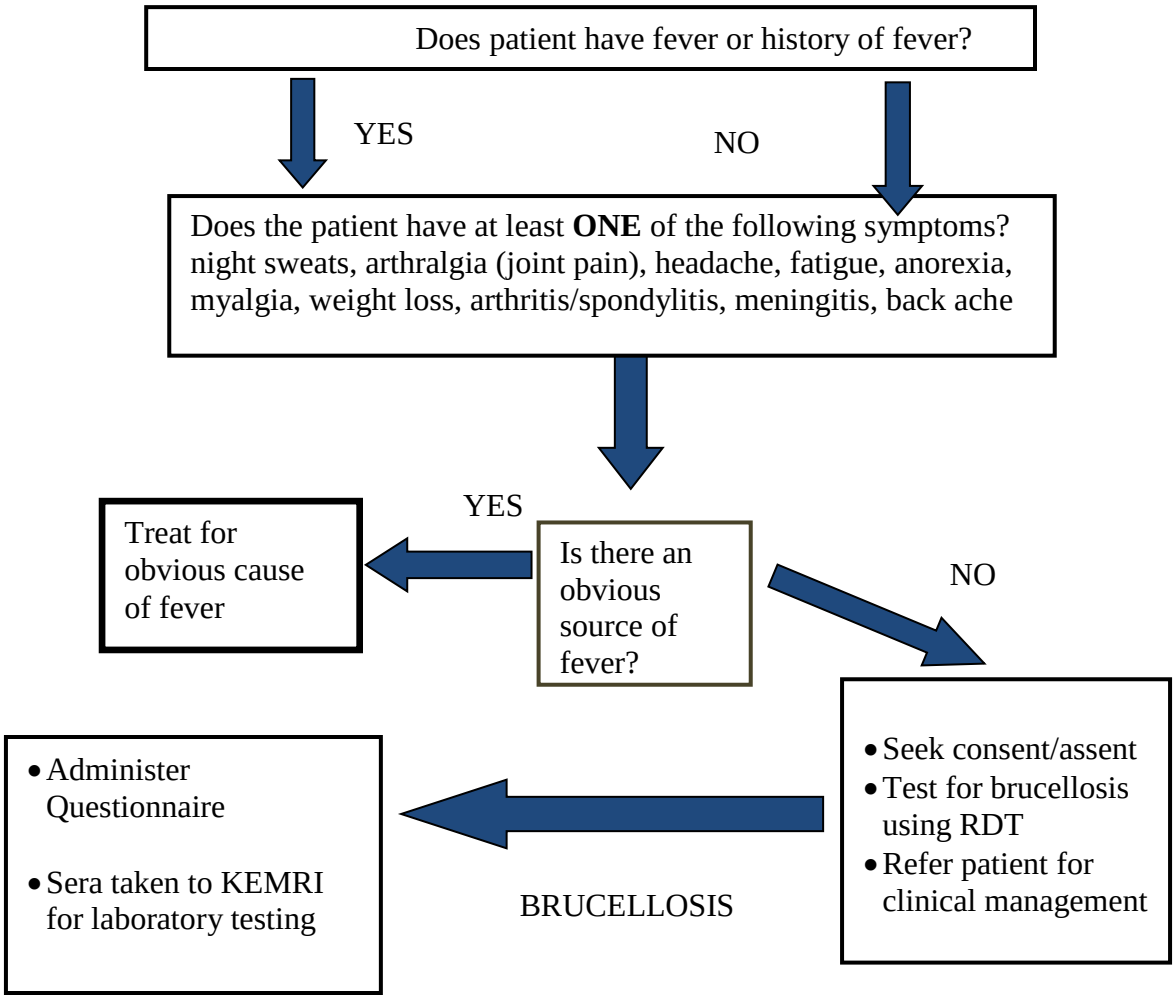

Figure 3.3: Patient enrollment criteria

3.7.2 Enrolment of controls

All patients who visited the selected facilities and satisfied the inclusion criteria for controls were eligible for selection. It was not possible to enumerate the total number of potentials controls for the study period and this made it impossible to randomly or systematically sample controls. Two controls were selected for each case during analysis

based on the following criteria; the controls for each case were the next two consecutive patients who visited the health facility and fit the criteria for inclusion as controls. A questionnaire was administered to all controls.

3.8 Data collection tool

The physician on duty at the facility examined all patients and those who met the case definition clinical and laboratory criteria were interviewed after informed consent was given. A structured questionnaire (appendix ii) was used to capture demographic information, clinical information and risk factor information for both cases and controls.

3.9 Procedure for sample transport

Serum was collected from the vacutainers using a disposable plastic Pasteur pipette, dispensed to an Eppendorf tube and stored at 4^{o}C to 8^{o}C and transported to the laboratory after every 5 days. During collection a bar coding labeling system was utilized. The labels were placed onto the consent form and the sample vial. A barcode label was immediately placed on the consent forms and sample vials as soon as clinical examination was finished and samples were prepared. The identity of the label would be checked twice to ensure that it is identical to the forms and sample vials. Eppendorf tubes were then be transported to the KEMRI-CDC laboratory in Nairobi between 0^{o}c and 4^{o}c in chilled cool boxes and stored in a freezer at -20°C until used for serological testing.

3.10 Laboratory diagnosis

3.10.1 Diagnosis by ELISA

Five milliliters of blood was collected from study participants, processed to serum and aliquoted into sterile bar coded cryovials and transported in liquid nitrogen dry shippers to Kenya Medical Research Institute (KEMRI) laboratory for testing. Specimens were tested for the presence of *Brucella*-specific IgM and IgG antibodies using indirect ELISA as described using standard manufacturer operating procedures. Briefly for ELISA testing, 50µl of diluted patient serum was added to micro titer plate pre-coated with antigen (Kirkgaard and Perry Laboratories, Maryland, USA). This was followed by incubation at 37ºC for one hour and then washing. Fifty (50) µl of *Brucella* antigens were added and the plates were then read using a micro plate Elisa reader. Any sample with optical density (O.D) ≥0.6 was considered positive.

3.10.2 Rapid Diagnostic test Kit

A *Brucella* rapid test kit was used for clinical diagnosis of brucellosis in the study health facilities. The kit was an antibody test that detected antibodies to *B. abortus* and *melitensis*. The test procedure was as follows (based on manufacturer testing protocol). Two drops of serum was mixed with an equal volume of the test reagent on a white tile or enamel plate to produce a zone approximately 2 cm in diameter. The mixture was agitated gently for four minutes at ambient temperature, and then observed for agglutination. Any visible reaction was considered to be positive indication while lack of agglutination indicated a negative test.

3.10.3 Evaluation of the performance of *Brucella* RDT

To evaluate the performance of the *Brucella* rapid test kit in use in the select health facilities in Kajiado County, patient results from the RDT were compared with ELISA. The evaluation was calculated the following formulas from variables (Table 3.2.)

Table 3.2: Tabulation for comparison of ELISA and *Brucella* RDT results

	ELISA		
Brucella RDT	POSITIVE	NEGATIVE	TOTAL
POSITIVE	A (True Positive)	B (False Positive)	
NEGATIVE	C (False Negative)	D (True Negative)	
TOTAL			

Sensitivity = A/(A+C) X 100

Specificity = D/(D+B) X 100

Predictive positive value = A/(A+B) × 100

Negative predictive value = D/(D+C) × 100

3.11 Ethical considerations

This study was reviewed and approved by the Kenyatta National Hospital Ethical Review committee number KNH-ERC/A/22 (appendix ii). Cases and controls were enrolled after verbal and written consent and no personal identifiers were recorded on the questionnaire.

3.12 Data management

Data was entered into Epi info software, cleaned, validated and coded. The data was checked for any wrong or double entries and corrected. Back up was created in case of damage and or loss of original data and it will be password protected.

3.13 Data analysis

Data was analyzed using Epi info version 7. Data from questionnaires on risk factors was entered, cleaned and analyzed using EPI Info 7 (CDC, Atlanta, GA, USA) and Ms. Excel 2007 (Microsoft, Seattle, WA, USA). Univariate analysis was performed where proportions were calculated for categorical variables and means and medians for

continuous variables. Bivariate regression analysis was carried out to evaluate the association between potential risk factors and cases Odds ratios and 95% confidence intervals were used to determine significance.

Multivariate analysis was done by subjecting the significant factors from bivariate analysis to unconditional logistic regression and final model of independent factors associated with brucellosis A forward stepwise simple logistic regression analysis was done including the significant variables (factors at bivariate analysis with p-value ≤ 0.5 were considered statistically significant) to control for confounding and to get a final logistic regression model. Only those factors that remained statistically significant in the final model were presented.

CHAPTER FOUR

RESULTS

4.1. Enrollment of participants

During the period of the study, a total of 236 participants were recruited and interviewed in the study health facilities. From these group, 129 participants (43 cases and 86 controls) were enrolled for the case-control study based on the inclusion and exclusion criteria, sample quality validation and after data cleaning to eliminate non-response.

4.2. Socio-demographic characteristics of cases and controls

The mean age for the cases was 48.7 years (SD =20, range =10-85) years while that of the controls was 37.6 (SD = 18.8, range =8-72). Among cases, 70% (n=30) were aged between 20-59 years. The dominant gender for both cases (62.7%) and controls (58.1%) was female. Majority of both cases and controls were non-skilled laborers with "housewife" being the most reported occupation between both groups. There was no significant difference in socio-demographic characteristics (sex, religion, occupation, marital status and education) between cases and controls besides age (Table 4.1). Majority of participants (88%) were not in formal employment (defined as salaried skilled workers). Majority of controls had completed secondary education (84%) compared to cases at 12%. The level of completed education was however not statistically significant between cases and controls.

Table 4.1: Socio-demographic characteristics of participants

Characteristic	Cases		Controls		P-value
	No.	%	No.	%	
Sex					
Male	16	37%	36	42%	0.704
Female	27	63%	50	58%	0.679
Mean age in years (SD)[range]	48.7(20.4) [10-85]		37.6(18.8) [2-95]		0.002
Employment status					
Employed full time on farm	1	2%	2	2%	0.545
Salaried off farm non-skilled	12	28%	19	22%	
Salaried off farm skilled	5	12%	16	19%	
Student	2	5%	12	14%	
Housewife	21	49%	32	37%	
Minor	2	4%	5	5%	
Highest educational level completed	38	88%	14	16%	0.12
Primary or below	5	12%	72	84%	
Secondary and above					

There were more cases for in the older categories of above 51 years than controls. Most controls were between 21-30 years as shown in Figure 4.1.

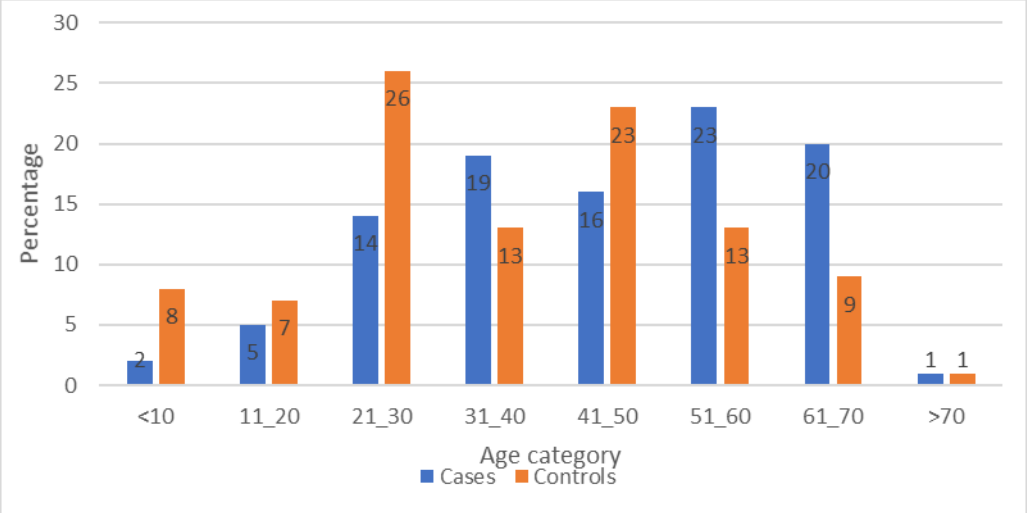

Figure 4.1: Age distribution of cases and controls

4.3. Clinical information of cases and controls

The most reported symptoms for cases were headache (83.7%) back pains (62.8%) and joint pains (60.6%). This was like the symptoms reported by the controls; headache (82.6%), back pains (47.7%) and joint pains (60.9%) as shown in Table 4.2. Most of the cases (60%) of the cases presented at-least seven days after the onset of the first symptom while 37% presented between 11-60 days after onset of symptoms. The mean number of days between onset of symptoms and visit to hospital was 12 days (SD 13.3).

Table 4.2: Clinical presentation of cases and controls

Mean no. of days since illness onset (SD)[range]	**14.3(21.1) [2-120]**		**10.7(12.3) [2-60]**		**0.307**
Patient has fever	13	30%	33	38%	0.363
Headache	36	84%	71	83%	0.869
Joint pains	26	61%	60	70%	0.291
Back pains	27	63%	41	48%	0.105
Had similar illness in the last 12 months	22	51%	27	31%	0.019

The difference in symptoms between cases and controls was not statistically significant (Table 4.2). However, more cases reported that they had a similar illness in the last one year compared to controls. This difference was statistically significant ($P \leq 0.05$) as shown in Table 4.2.

4.4. Risk factors practices for brucellosis among participants

Most of the cases 40 (95.3%) and all the controls 86 (100%) consumed cow milk more frequently as opposed to consuming milk from sheep, camels or goats. On livestock ownership, 31 (72.1%) of the cases had livestock in their households prior to the current illness compared to 56 (65.1%) of the controls. More cases (53.5%) reported to have handled animal hides and skins in the past 3 months compared to controls (34.9%) while seven out of the 43 cases (16%) reported to drink fresh blood compared to six out of the 86 controls (7%) as shown in table 4.4

4.5. Bivariate analysis

On bivariate analysis consuming un-boiled cow milk, drinking fresh blood, occasionally slaughtering animals (cattle, wild animals), assisting goats in giving birth, handling animal hides were significantly associated with increased risk of brucellosis ($P \leq 0.1$). Of these factors, handling skins and hides, assisting goats with delivery, and consuming un-boiled goat milk were significantly associated with disease (at $P \leq 0.05$). Having cattle in the household was found to be protective as shown in the Table 4.3.

Table 4.3: Bivariate analysis of risk factors for human brucellosis

Variables	Controls (N=86)	Cases (N=43)	Crude OR (95%CI)	P-Value		
	n	%	n	%		
Consume fresh goat milk						
More Than 3 Times A Week	14	16%	14	33%	2.4(1.0-6.0)	0.114
Less Than 3 Times A Week	21	24%	8	19%	0.9(0.4-2.4)	
No	51	60%	21	49%	1.0	
Consume cow milk						
Boiled	82	98%	35	84%	7.7(1.5-40.1)	0.016
Un-boiled	4	2%	8	16%		
Consume fresh cow milk						
More Than 3 Times A Week	1	1%	1	2	2.1(0.1-34.1)	
Less Than 3 Times A Week	4	5%	3	7	1.6(0.3-7.3)	0.756
No	81	94	39	91	1.0	
Drink fresh blood						
Yes	6	7	7	16	2.6(0.8-8.3)	0.098
No	80	93	36	84		
Had Cattle in the household						
Yes	55	64	26	61	0.1(0.0-0.9)	0.035
No	31	36	17	39		
Slaughter cattle at home						
Occasionally	54	70	32	84	2.3(0.8-6.2)	0.102
Never	31	30	6	16		
Herding Sheep						
Several times a week	16	22	14	38	2.0(0.5-7.8)	0.196
Occasionally	49	66	19	51	0.9(0.2-3.2)	
Never	9	12	4	11		
Assisting sheep in kidding						

Several Times A Week	1	1.4	1	3	4.0(0.2-72.2)	
Occasionally	45	61	30	79	2.7(1.0-6.9)	0.116
Never	28	38	7	18	1.0	
Home slaughter of goats						
Several Times A Week	1	1	1	3	4.8(0.3-90.3)	0.115
Occasionally	53	68	33	85	3.0(1.0-8.6)	
Never	24	31	5	12	1.0	
Assisting goats in delivery						
Occasionally	48	61.6	31	83.8	3.7(1.3-10.7)	0.043
Never	29	37.2	5	13.5		
Slaughtering/butchering wild animals						
Yes	1	1	3	7		
No	82	99	40	93	6.4(0.6-63.2)	0.073
Cleaning animal barns						
At-least 3 times a week	57	47	5	26		
Occasionally	19	53	14	74	0.4(0.1-1.3)	0.132
Handle Animal Hides						
Yes	30	35	23	54		
No	56	65	20	46	2.1(1.2-4.5)	0.043

4.6. Multivariate analysis

On multivariate logistic regression analysis consuming un-boiled cow milk (OR 7.7, 95 % CI 1.5–40.1) and assisting animals in delivery (OR 3.7, 95% CI 1.1-13.5) remained significantly associated with brucellosis as shown in Table 4.4 This means that people who drink un-boiled cow milk and assist animals in delivery are up-to 7 and 3 times (for the latter) likely to get brucellosis.

Table 4.4 Multivariate logistic regression of factors associated with brucellosis

Variable	Adjusted OR(95%CI)	P-value
Slaughter animals	6.2(1.1-34.7)	0.350
Handling animal hides	1.3(0.5-3.6)	0.563
Own cattle	0.6(0.2-1.6)	0.327
Drinks fresh blood	3.1(0.8-11.2)	0.088
Assisting livestock in delivery	3.7(1.3-13.5)	0.031
Drinking un-boiled cow milk	7.7(1.5-40.1)	0.036

4.7. Evaluation of the performance of *Brucella* RDT

A total of 190 patients were tested using a *Brucella* IgG rapid test routinely used at the health facilities to inform management of brucellosis. Sera samples from these patients was tested using ELISA. The sensitivity and specificity of the kit was 20% and 90% respectively. The positive and negative predictive value was 33% and 84% respectively. The results are presented in Table 4.5

Table 4.5: Comparison of *Brucella* rapid diagnostic kit and ELISA

	ELISA		
Brucella RDT	POSITIVE	NEGATIVE	TOTAL
POSITIVE	A (True Positive)	B (False Positive)	
	7	14	21
NEGATIVE	C (False Negative)	D (True Negative)	
	27	141	168
	34	156	190

Sensitivity = A/(A+C) X 100
(7/34) 100 = 20%
Specificity = D/(D+B) X 100
(141/156) 100 = 90%
Predictive positive value = A/(A+B) × 100

(7/21) 100 = 33%
Negative predictive value = D/(D+C) × 100
(141/168) 100 = 84%

CHAPTER FIVE

DISCUSSION

5.1 Discussion

A case-control study was conducted to determine risk factors for human brucellosis in a predominantly pastoralist Maasai community of South Western Kenya. Brucellosis is a disease of public health concern because of high initial treatment failure and relapse rates *(Christopher et al.,* 2010). The non-specific presentation of brucellosis is a major challenge in diagnosis of the disease, especially in resource limited settings with inadequate diagnostic capacity (Franco *et al.*, 2007). The disease presents in a myriad of ways depending on period of infection and body system affected and infecting species (Franco *et al.,* 2007). The study established that there was no significant difference in clinical presentation between cases and controls. This is an indication that clinical presentation alone cannot be used to diagnose brucellosis, a finding reported in literature from other endemic regions (Dean, Crump, Greter, Hattendorf, *et al*., 2012; Dean, Crump, Greter, Schelling, *et al*., 2012; Franco *et al*., 2007; J Njeru, Henning, *et al.*, 2016). Headache, joint pains and fever were the commonly reported symptoms, however due to their non-specific nature, majority of controls also reported similar symptoms. Other brucellosis studies in clinical setups have reported patients with similar clinical presentation (Akhvlediani *et al.,* 2010; M. Ducrotoy *et al.,* 2017; Kiambi *et al.,* 2020). Study participants were asked if they had a similar illness in the last one year before the hospital visit. More cases had significantly more reports of a similar illness than controls. This can be hypothesized to be due to the challenges in treatment of the disease and the chronic and undulant nature of the infection (Franco *et al.,* 2007; Mirnejad *et al.,* 2017).

Our study found a significant difference in the mean age of cases and controls. This result is consistent with findings from a study in Mongolia that reported people older

than 45 years had a higher risk of being infected than lower age groups and one in Kenya that reported an association between age and infection (Osoro *et al*., 2015). This can be explained by continued exposure due to endemicity of the disease in our study area. Some studies show education and occupation are significant risk factors contrary to our data that shows there was no significant difference on the two variables between cases and controls. A possible explanation is the study area is a rural, predominantly Maasai agro-pastoral community where most households practice a traditional livestock rearing lifestyle. This means the cases and controls had a very high chance of having similar occupation and education levels.

This study found three statistically significant risk factors in the bivariate analysis; consumption of raw cow milk, assisting goats in delivery and handling of raw animal hides. However, only two factors remained statistically significant after adjustment for confounding in the logistic regression analysis; assisting livestock in delivery 3.7(1.3-13.5) and drinking un-boiled cow milk 7.7(1.5-40.1). The association between assisting animals with delivery and increased risk of infection has been reported in other studies in similar settings in East Africa. A study in Tanzania (John *et al.,* 2010), in Kenya (Njuguna *et al.,* 2017; Osoro *et al.,* 2015) and in Ethiopia (Edao *et al.,* 2020) all reported similar findings. Brucellosis is a reproductive system infection that has a predilection for fetal fluids (causing placentitis and resultant abortion) and testes (causing epididymitis and orchitis) in sexually active animals (Neta *et al.,* 2010). Assisting animals in delivery; either miscarriages, abortions or full-term pregnancies, increases the chance of exposing animal handlers to contaminated reproductive fluids, this risk being even higher in regions with high prevalence of livestock brucellosis like our study area (Osoro *et al.,* 2015). Although this study does not make specific inference to assisting aborting animals, it is common practice for animal handlers to assist animals in delivering products of abortion, premature births and retained placentas, all of which are classical signs of a brucellosis infected herd (Corbel *et al.,* 2006). This could explain why assisting livestock in delivery was a significant risk factor in this study. Consumption of

un-boiled cow milk was found to be a significant risk factor in this study, a common finding in other studies (Kang'ethe *et al.,* 2000; J Njeru, Wareth, *et al.,* 2016; Osoro *et al.,* 2015). Although majority of livestock keepers know consumption of raw milk is one of the main routes of infection as reported by studies among Uganda pastoralists (Kansiime *et al.,* 2014), markets in Europe (Jansen *et al.,* 2019) and Kenya (Namanda *et al.,* 2009; Obonyo & Gufu, 2015) consumption of raw milk is a common practice. The findings from this study are consistent with reports from other studies that although a wide range of factors are associated with infection, they all relate to contact with livestock, either through consumption of unpasteurized animal products or through daily animal husbandry practices in the absence of sanitary measures. Although opinion differs between authors on whether direct contact with livestock (assisting in delivery, milking and feeding) or indirect contact with livestock (consumption of animal products) is a stronger risk factor, our study findings suggest greater association with the latter and disease transmission. This finding agrees with other studies carried out within the East Africa region (Kiambi *et al*., 2020; J Njeru, Henning, *et al*., 2016; Osoro *et al*., 2015; Tumwine *et al*., 2015), although a study in Jordan found contact with livestock through milking was a slightly stronger risk factor than consumption of unpasteurized milk (Shehada & Abu Halaweh, 2013). Several studies have shown consumption of unpasteurized milk is a common practice in Kenya. A study in the cosmopolitan Kenyan town of Eldoret reported up to 77% of households consume informally sold unpasteurized milk (Namanda *et al.,* 2009). The practice is even more common among pastoralists who hold a traditional belief boiling milk has an effect on the nutritional value (Njeru, Henning, *et al.,* 2016).

Diagnostic tools are very important in clinical management of brucellosis due to the non-specific nature of clinical presentation of the disease (Al Dahouk & Nöckler, 2011). Culture is the diagnostic gold standard but is often impractical due to the long lag time before results are obtained, the low sensitivity of the process due to the fastidious nature of *Brucella* and biorisk management requirements for this diagnostic method (Sagi *et*

al., 2017). In most resource poor settings like the study area, this leaves rapid diagnostic serological kits as the main diagnostic method. The results from the comparative testing in this study are indicative of the challenges in *Brucella* diagnosis using low quality point of care tests, a finding reported by other studies in Kenya (de Glanville *et al.*, 2017; Kiambi *et al.*, 2020; Njeru *et al*., 2016). The low sensitivity of the test kit used in Kajiado means there is a high number of false negatives or missed cases. This could explain the finding that more cases in our study reported having a similar illness in the past year, the hypothesis being this could be a result of earlier misdiagnosis and mistreatment. Incorrect diagnosis usually results to underreporting, a consequence of which is continued neglect of the disease in high burden regions and continued prevalence of infection (Franc *et al.,* 2018). Misdiagnosis also results in economic losses due to both direct economic losses and losses attributed to progression of the disease to more debilitating severe forms like cardiovascular disease (Olsen & Palmer, 2014). The complex problem of brucellosis misdiagnosis particularly in resource poor, endemic rural areas where appropriate and optimal assays and equipment are not available has been reported and calls for action to establish validated *Brucella* tests in endemic areas (Njeru *et al*., 2016).

There were some limitations to the study. Case-control studies are prone to selection bias, however specific measures were taken to minimize the same by use of test negative controls who visited the health facility on the same day as cases. The other limitation was the study was not able to achieve the total number of cases in the sample size. However, the difference was minimal, and the study was able to achieve the desired level of power and significance.

5.2 Conclusions

1. Brucellosis symptoms are non-specific, and cases could present as a myriad of other illnesses. In high burden, endemic areas clinicians should investigate

epidemiologic linkages and utilize laboratory investigation as part of clinical management

2. The findings of this study showed a significant association between infection and consumption of unpasteurized milk and assisting animals with delivery. These findings show that animal handlers; primarily farmers and animal health workers and people who consume unpasteurized milk; a common practice in Kenya, are at the greatest risk.
3. RDT has low sensitivity values thus underestimates positivity, meaning a significant proportion of positive cases (up to 80%) are being missed.

5.3 Recommendations

There is need for:

1. Training of clinicians on brucellosis diagnosis, primarily on investigating epidemiologic linkages and interpreting results from rapid diagnostic kits in endemic areas.
2. Public health education packages on brucellosis transmission and prevention, specifically need for biorisk management through use of protective personal equipment when assisting animals in delivery and boiling of milk should be offered to farmers and the general public respectively.
3. Validation of all brucellosis test kits in all health facilities in Kajiado to reduce the number of false positives and negatives and consequently enhance early detection.

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4.

APPENDICES

Appendix i: Informed Consent

Human adult consent form for the brucellosis incidence study (English):

Purpose:

Brucellosis is the most common of all the bacterial zoonotic infection resulting in significant agricultural economic losses and human sufferings. In Kenya, the prevalence of brucellosis in animals is unknown. However, it is clear that to control the cases of human brucellosis one must start by controlling the incidence of animal brucellosis due to the fact that human to human transfer is not known to occur. Researchers from the United States and Kenya's Ministry of Health and Ministry of Livestock Development would like to stop this disease by doing a study to find factors that contribute to infections in humans as well as animals.

Why You Have Been Chosen: We are asking you to join the study because you show signs of illness characterized by acute or insidious (slow) onset, with continued, intermittent (on and off) or irregular fever of variable duration (length, amount of time), profuse (lots of) sweating particularly at night, fatigue (tiredness), anorexia (not wanting to eat, loss of appetite), weight loss, headache, arthralgia (joint pain), generalized aching, and possibly local infection of various organs and/or your animals that had shown signs of illness and were tested were found to be sick with this disease. . We are doing this study in this health facility and or in the homes because Mashuru and Loitoktok divisions had the highest number of illness in animals and people in a study we did before. We may come again and take sample more than once. Your permission will be sought each time we come.

Procedure: If you or your child chooses to be in this study we will draw 4 mls of blood (a teaspoon) from the vein in your or his/her arm. This blood sample will be tested for

germs of the brucellosis bacteria, or other disease-causing germs at the CDC/KEMRI laboratory in Kisumu. We ask to draw a second sample of blood of the same amount after 4-6 weeks later. Tests may show us that you or your child may have been sick with brucellosis before or is sick with it now. A small number of blood samples will be sent to CDC in Atlanta, Georgia U.S.A. Researchers at CDC will do the test again to see if they get the same test results. The remaining amount of the sample will be stored in the freezer for possible testing for other germs in future. No human genetic testing will be done on the sample. We will also ask you and your child questions for 30-45 minutes. Neither of you have to answer the questions if you do not want to.

Confidentiality

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

Risks. Except for minor pain, bruising and bleeding that may be a part of taking blood sample, there are minimal risks from being in this study. In rare cases, an infection can result from drawing blood. If such infection occurs, the project will assume costs of treatment of the infection. In addition, it is possible that other people will find out that you participated in this study. However, we are taking all precautions to avoid such occurrences.

Benefits. You will not receive any benefit from this study. However, the study will pay for treatment of diseases detected by the specific blood cultures used by the study. In addition, information obtained from this study may help the Ministry of Health decide when and where brucellosis disease may occur. The result will be provided to your doctor/ or to the health facility as soon as possible so that you can be provided with treatment.

Voluntary Participation. You are free to join the study or not to join. You may leave the study at any time, for any or no reason. If you decide not to join or to drop out, you will not lose any health care services you are entitled to at the Hospital. You will not get any direct benefit or payment for being in this study, but you will help us know more about this disease.

Contact Persons: If you have question regarding this study or suffer any injuries, please contact Dr. Mathew Muturi (Phone: 0723982878). If you have concerns regarding your rights in being in the study, please contact the Ethics Review Committee, Kenya Medical Research Institute (KEMRI), P.O. Box 20778-00202, GPO, Nairobi. Telephone 0202722541 or 0722205901 or 0733400003.A signed copy of this consent form will be given to you for your personal records.

Consent

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

If you agree to participate in the study, please sign here _____

_____

Do you agree to provide a second blood sample? Yes No (circle one)

Date: _____

Name of Patient _____

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

Date: _____/_____/_____

Human adult consent form for the brucellosis incidence study (Swahili):

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

Tathmini ya Mzigo wa Ugonjwa wa Brucellosis katika Kenya

Lengo / Maazimio:

Ugonjwa wa Brucellosis ni kati ya zile maambukizi ya bakteria yanayo sababisha hasara kubwa za kiuchumi wa kilimo na mateso kwa binadamu. Katika Kenya, kiwango cha maambukizi ya brucellosis katika wanyama pori za ndani na si inajulikana. Hata hivyo, ni wazi kuwa ili kudhibiti matukio ya brucellosis kwa binadamu ni lazima kuanze kwa kudhibiti matukio ya brucellosis kwa wanyama kutokana na ukweli kwamba kuhamia kwa ugonjwa kutoka kwa binadamu hadi mwengine haujaonekana ukitokea. Watafiti kutoka Marekani na kwa Wizara ya Afya nchini Kenya na pia kwa Wizara ya Ustawi wa Mifugo wangependa kudhibiti ugonjwa huu kwa kufanya utafiti kupata sababu zinazochangia maambukizi kwa binadamu pamoja na wanyama.

Sababu ya wewe kuchaguliwa. Unaulizwa kushiriki kwa sababu una ugua ugonjwa unao anza au wa polepole, na homa unaoendelea, ama unaokuja kwa vipindi au kwa muda wa kawaida, kutoa jasho hasa nyakati za usiku, uchovu, kuto kula, kupoteza uzani, kuumwa na kichwa, magonjwa ya magoti, na maambukizi ya viungo mbalimbali kwa ujumla huweza kutokea ama kwa sababu mifugo wako wameonyasha dalili zinazoabatana na ugonjwa wa brucellosis. Pia tutachukuwa sampuli kutoka kwa watu ambao wanamiliki wanyama ambao wako na ugonjwa wa brucellosis. Utafiti huu unafanyika kwa zahanati na pia kwa boma kwa sababu wilaya za Mashuru na Loitoktok zilipatikana na kiwango

kikubwa zaidi za mifugo na binadamu wa kiwa na ugojwa huu kwa utafiti tulio ufanya hapo awali.

Tunaweza kurudi ili tuchukue sampuli nyingine. Tutahitaji idhini yako kila wakati tutakapo rejea.

Utaratibu: Ukiwa wewe au mtoto wako ata amua kushiriki katika utafiti huu, tuta toa mililita 4 ya damu (kijiko cha chai) kutoka kwa mshipa wa damu wa mkono. Sampuli hii ya damu itapimwa ili kubaini wadudu wa bacteria wanaosambaza ugonjwa wa brucellosis, au vijidudu wanaosambaza magonjwa mengine katika maabara ya CDC / KEMRI jijini Kisumu. Tutanauliza kuridia kutoa damu mara ya pili baada ya wiki 4-6. Uchunguzi unaweza kutuonyesha kwamba wewe au mtoto wako ameweza kuwa na ugonjwa wa brucellosis hapo mbeleni au ni mgonjwa sasa.

Idadi ndogo ya sampuli za damu yasiyokuwa zaidi ya mililita 500 kwa kila mmoja yatapelekwa CDC Atlanta, Georgia, Marekani. Watafiti wa CDC watafanya uchunguzi tena ili kuona kama watapata matokeo sawiya na yetu. kiasi iliyobaki ya sampuli itahifadhiwa katika barafu kwa ajili ya kupima baadayeili kubaini uwezekano wa kuwa kwa vijidudu wengine. Hakuna upimaji wa kimaumbile ya binadamu yatakayo timizwa kwenye sampuli. Maswali yetu kwako na kwa mtoto wako yatachukuwa muda wa dakika 30-45. Hamja lazimishwa kujibu maswali haya.

Siri:Ni wale watafiti wanaoshiriki kwenye utafiti huu peke yao watakao ruhusiwa kufanya kazi na damu yako na kuona taarifa yako. Jina lako na kitu chochote ambacho kinaweza kukutambua itatolewa na kuwekwa mbali na matokeo na majibu ya maswali yako kabla ya kuonekana katika taarifa.

Tahadhari. Ila kwa sababu ya maumivu madogo, kusuguliwa na kutokwa kwa damu ambayo inaweza kuwa sehemu ya kuchukua damu, kuna hatari ndogo kutokana na kuwa katika utafiti huu. Katika matukio machache, maambukizi inaweza kusababishwa

kutokana na kutolewa damu. Maambukizi yakitokea, mradi utadhani gharama za matibabu ya maambukizi. Aidha, inawezekana kwamba watu wengine watajua ya kwamba ulishiriki katika utafiti huu. Kila njia itatumiwa kuzuia madhara yoyote ile.

Manufaa. Hautapata faida yoyote wa moja kwa moja kutoka kwa utafiti huu. Hata hivyo, utafiti utalipia gharama kwa ajili ya matibabu ya magonjwa yanayosababishwa na baadhi ya vijidudu watao tumiwa katika utafiti. Aidha, taarifa zilizopatikana katika utafiti huu inaweza kusaidia Wizara ya Afya na kuamua pahali na wakati ambapo ugonjwa wa brucellosis unaweza kutokea. Aidha, matokeo yatatolewa kwa daktari wako ama kwa zahanati unahudhuria haraka iwezekanavyo ili uweze kupatiwa matibabu.

Kushiriki kwa hiari. Uko huru kushiriki au kutoshiriki kwenye utafiti huu. Unaweza kujiondoa kwenye utafiti wakati wowote, ama kwa sababu yoyote. Ukiamua kujiunga au kuacha, huwezi kupoteza haki yako ya huduma yoyote za afya katika Hospitali. Hauta pata faida yoyote ya moja kwa moja au malipo kwa ajili ya kuwa katika utafiti huu, lakini mtatusaidia kujua zaidi juu ya ugonjwa huu.

Mawasiliano. Endapo una swali kuhusu utafiti huu au kuteseka majeraha yoyote, tafadhali wasiliana na Dkt Mathew Muturi on kwenye nambari ya simu 0723982878. Kama una hoja kuhusu haki yako kama mshiriki katika utafiti, tafadhali wasiliana na Kamati ya Maadili , Taasisi ya Utafiti ya Kimatibabu Kenya (KEMRI), SLP Box 54840-00202, GPO, Nairobi. Nambari ya simu 0202722541 ama 0722205901 au 0733400003).

Unaweza kuchukua nakala ya fomu hii iliyo wekwa kidole .

Idhini.

Nime elezewa kiini cha utafiti huu . Nimepata nafasi ya kuuliza maswali. Nimefahamishwa kwamba ni chaguo langu kushiriki katika utafiti huu na ninaweza kuamua kujiondoa wakati wowote bila adhabu yoyote.

Kama utakubali kushiriki katika utafiti tafadhali weka sahihi hapa……

Utakubali kupeana damu mara ya pili? Ndio Hapana
(chagua moja)

Tarehe……

Sahihi/ alama ya Mkono wa mgonjwa ……

Sahihi ya shahidi (endapo mgonjwa hawezi kuweka mkono)……

Tarehe……..……..

Mhoji……..…….

Idhini

Nimekubali kuruhusu tarakibu ya damu yangu kuhifadhiwa KEMRI kwa uwezekano wa kupima baadaye kubainisha chanzo cha homa yangu. Huu upimaji hautahushu ukaguzi wa maumbile ya binadamu.

Tarehe…….……..

Sahihi/alama ya Mkono wa mgonjwa……

Sahihi ya shahidi (endapo mgonjwa hawezi kutia mkono)……..

Tarehe:…….. ……..

Mhoji:…….

……..

Human adult consent form for the brucellosis incidence study (Maa):

Tathmini ya Mzigo wa Ugonjwa wa Brucellosis katika Kenya

Enkipirta: biotisho na emoiyan nayau oloirerio.kore ana moiyan naa keipang'u taatwa shoo nikiramat o ltung'ana. Keing'wa abaki ana moiyan swam nebaki ltung'ana. Kepuku ana moiyan te ng'ojitin kumok enkop na nyolo oltung'ana lo alang' 50000 kemwai te kila laari. Iyiolo oloirerio lo yau ana moiyan kejing atwa ltung'ana teneibung' swaam,tenenya nkirri ashaa teneok kule naati oloirerio . iyiolo te Kenya kotumi ana moiyan taatwa swaam oltung'ana kake mikiyolo nkikena oltung'ana o swaam na ata ana moiyan. ikiyolo abaki ajo iyiolo ltung'ana pooki loata ana moiyan na ketum taatwa swaam naibung'a apa te dukuya. Kesipa ajo tinikimbooyo ana moiyan taatwa oltung'ana keishaa piking'asu aibooyo taatwa swaam.

Kore lelo layaa loing'waa retore e Kenya ye afya (MOH o Retore nturoto, ramatisho, sinkir o Kenya medical research institute o nkae naji US centre o moyaritin o mbooto. Keyeu neyelou nkikena o ltung'ana o swaam naata ana moiyan o nekuna ana moiyan nkishui e nche o ntumot.

Yiunot ino makion njing'ata: indim atijing'a asha iyany ijing' enkisoma. Endim atung'ai ta ngata pooki tangarake yieunot nigelu. Meitoki nikingilikuan te ngoitei pooki tinigeleu aing'waa nkisoma. Tinigelu aing'wa menturaa mbioto ino nikinchooki te sipitali

Mitum o yaa paida ashaa laata teatwa ena kisoma. Lakini iret yoo mateyol nkumok kuusu ana moiyan, niret yoo mateyei nkoitei nikimbooya ana moiyan taatwa itung'ana o swaam.

Anyo pikitegeluaki: I kimpar yie pijing atwa nakisoma amu itodolwa mpukunot yana moiyan na kore swam inono ketodolwa mpukunot yana moiyan na keipimaki netumiek aju kemwai naa keata ana moiyan.

Kiasita ana kisoma taatwa ana bioto o taatwa nkang'ite amu kore mashuru o luitoktok keata nkikena sapuk yana moiyan taatwa ltung'ana o swaam teatwa nkisoma nikitaasa tedukaya.

Nkoitei o sipatisho: Tanaa yie asha nkerai ino nagelu ana kisoma,kimpar yie piincharu lkini sarge 4ml loing'wa atwa ng'ony e nkaina. Nikimpar atoki yie piintai kikae kiti sarge baada ye wiki 4-6. Kore sarge lino ashaa le nkerai ino keipim teatwaa oloirerio le Biotisho te EDC/KEMRI laboratory. Kore ana kipimoto keitodolu nkikena o sarge nairewayieki CDC te Atlanta, Georgia, usa, kore layaak le CDC keas nkimata atoki peing'ura tanaa ketum nkimata narisio. Iyiolo lekunoto iolo sarge kepiki atwa Freezer petumi aipima to ngolong'naaponu meatae nkoito o sesen naipimi. Naa ikimpar yie nkuti kiparat na ya ldakikani 30-445. Lakini meishaa pilimu nimiyolo.

Iyiolo limoto nilimu meitodoli kikae tung'ani meitodoluni abaki nkarna ino te ltung'ani lemara layaani lena kisoma. Keisudori nkarna ino nemepiki abaiki atwa na ripoti yana kisoma. Kore limot nilimu kepiki te nebo o noo ikule tung'ana lena kisoma oishaa peas siai teilo sarge neing'uraa ikikwai aibula kenang'ari na limo take nintaya Eing'urari.

Mion – tanamara ke nkini mion, ndung'to, o ikini bukoroto o sarge naidim ayeu. Keikini oleng'meto teatwa ana kisomo. Kore to nkatitin keidim moyaritin neponu te nkitainoto o sarge lakini teneasai nana keya inia retore laata pooki.

Sidanisho enyorrata: pikilaki o toki itii atwa na kisomo lakini kore lkilikwai lincho yoo koret yoo meteyolo ana moiyan neret atoki yoo meteyei nkoitei nikimboonyo ana moiyan taatwa swaam o ltung'ana.

Ltung'ana lioshoki: tanaa iyata nkipara ashaa pooki tuki yana moiyan tapasali tooshoki Dr Mathew Muturi te 0723982878. Tanaa iyata pooki toki ye haki no iti atwa nakisoma

Tapasali tooshoki, **Ethics Review Committee (ERC) Kenya Medical Research institute (KEMRI). P.O. BOX 54840 00202 G.P.O Nairobi Telephone. 0202722541 ashaa 0722205901 ashaa 073340003.**

Ikinchori nkardasi naijo na teyeunoto ino makoon piiya.

Loyeu

Katilikaki ana kisoma. Natumo nkata naiparu nkiparat. Katilikaki ajo nepesheu ana kisoma paajing' ashaa majing' kaiding atupuku tangata pooki nayeu.

Tanaa itoyayorayie ajing' nkisoma. Tipika ikumojinoo.

Ne……

Nkarna ino……..

Lchaidi tanaa masain makoon…….

Ntarikini …….

Loing'ur……..
ntarikini…….

Kanyaraa aishoyo sarge la:metipiki atwa KEMRI te ng'urarata etaisere peyelouni nyo nayau ana moiyan. Iyolo ana ikipimata mejung'ore nkiri o sesen o ltungani omwai.

Ntarikini…….. saini
…….

Lchaidi ……. Ntarikini……..

Assent form for children aged 7-17 years old for giving specimens for the Brucellosis study

The investigator will read this consent to the child at the time of enrollment.

Introduction

We are asking you to give samples for a study about what germs cause illnesses in people in this area. We want to find out how big a problem these germs are and how to treat them. The compound head for your house has already agreed to be part of this study. Today we are just asking you if you will give some samples to find out what germs may be causing problems to both humans and animals.

The samples we want to get are these:

Drawing blood from the arm for a blood test for germs causing Brucellosis (a teaspoonful).

Benefit from being in this study:
If you agree to have samples taken today, some of the tests being done might help the medical staff in treating you better.

Risks from being in this study:
Drawing blood can cause brief pain. Rarely, it might cause bleeding and bruising. Serious injury due to drawing blood is very rare.

To give samples today is your free choice. If you do not want to, that decision will not harm you in any way. If you do not want to, nobody will be mad at you. If you agree to give samples, but then change your mind, you can stop at any time.

We have already asked your parents about this and they said it was okay to ask you if you wanted to do this. If you have any further questions about this study, please ask your parents or me.

Will you be a part of this study and give samples? □ Yes □ No

_____

Name of child (Print) _____

_____ Date_____Child Signature (Signature or mark of consent) _____

To be signed by witness:

The above statement has been read to the child and the child agrees to participate in the research project.

Name of witness (Print) _____

Date_____Witness Signature (Signature or mark of consent) _____

Assent form for children aged 7-17 years old for giving specimens for the Brucellosis study-Swahili

Tuna kuuliza utowe sampuli kwa ajili ya utafiti kuhusu wadudu wanaosababisha magonjwa kwa watu katika eneo hili. Tunataka kujua jinsi ya ukubwa wa tatizo hili na pia hali ya kulithibiti. Mwenye boma yenu tayari amekubali kushiriki kwa utafiti huu. Leo tuta kuuliza tu kama utaweza kutoa sampuli ili ijulikane ni vijidudu ipi zinazoweza kusababisha matatizo kwa binadamu na wanyama.

Sampuli tunazotaka kupata ni haya:

Kutoa damu kutoka mkononi kwa ajili ya vipimo vya damu kwa ajili ya wadudu wanao sababisha ugonjwa wa Brucellosis (kijiko kimoja cha chai).

Faida ya kuwa katika utafiti huu:

Ukikubali sampuli zichukuliwe leo, baadhi ya vipimo zitakazo fanywa zitasaidia wahudumu wa afya kutibu kwa njia bora .

Hatari kutokana na kushiriki katika utafiti huu:

Kutoa damu inaweza kusababisha maumivu ya muda mfupi. Pia inaweza kusababisha kutokwa na damu na jeraha kwa . Hata hivyo, majeraha yanayofuata kutolewa kwa damu ni nadra sana

Kutoa sampuli leo ni kwa hari yako mwenyewe . Kama hautaki, msimamo huo hauta kudhuru kwa njia yoyote ule. Wala hautaleta hasara kwako. Hakuna atakaye kulaumu. Kama utakubali kutoa sampuli, lakini ubadili nia baadaye, unaruhusiwa kuacha wakati wowote.

Tayari tumeuliza wazazi wako kuhusu kushiriki kwako na wakaona ni vyema tukuulize wewe kama utakubali kufanya hivyo. Kama una maswali yoyote zaidi kuhusu somo hili, tafadhali waulize wazazi wako au mimi.

Je, utajiunga na utafiti huu na kutoa sampuli? □ Ndiyo □ Hapana _____

Jina la mtoto (Nukta kubwa) _____

Tarehe_____Sahihi ya Mtoto (Sahihi au alama ya idhini) _____

Kusainiwa na shahidi:

Taarifa hili lime somwa kwa mtoto na mtoto amekubali kushiriki katika mradi wa utafiti.

Jina la shahidi (Print) _____

Tarehe_____Sahihi ya Shahidi (Sahihi au alama ya idhini)_____

Appendix ii: Research Questionnaire

Questionnaire no.....Date of interview (mm/dd/yr).....

Interviewer initials..... Health Facility name.....

Household ID..... Patient no. or hospital record number.....

A. PATIENT INFORMATION

A1. Name(*at least two names*) ……..

A2.Phone no.....

A3. Village.....

A4. Sub-location(of residence)..... A5. Home GPS coordinates.....

A6. Parent/Guardian name (if respondent under 18) ……

A7. Household ID.....

A8. Was the patient referred to the facility by community study coordinator ? ☐

1 Yes 2 No

B. PATIENT DEMOGRAPHICS

B1.Sex:

1 Male 2 Female

B 2.Age in years:____ ____

B3.Date of birth(dd/mm/yy) __ / __ /__

B4.Employment :

1 Employed full time on farm 2 Student

3 Housewife 4 Salaried off farm non-skilled

5 Salaried off farm skilled 6 Other (*specify*).....

B5.Education level ;

1 College/university 2 Secondary school

3 Primary school 4 No formal education

C. LAB INFORMATION & CASE CLASSIFICATION

C1. Test result RBT ..... cELISA.....

C2.Clinical case classification (To be filled after cELISA result)

Case

Control

C3. Test result (titre level on cELISA).....

D. CLINICAL INFORMATION

D1.Date of onset of first symptom.....

D2.What signs and symptoms did/ do you exhibit? (*DO NOT PROMPT. IF TICKED, INDICATE DURATION IN DAYS)* DURATION IN DAYS

- ☐ Hotness of body, Intermittent ……
- ☐ Hotness of body, Constant ……
- ☐ Chills ……
- ☐ Weight Loss ……
- ☐ Night Sweats ……
- ☐ Headache ……
- ☐ Malaise ……
- ☐ Lack of appetite ……
- ☐ Stiff or painful neck ……
- ☐ Joint or muscle pain ……
- ☐ Back pain ……
- ☐ Abdominal pain ……**.....**
- ☐ Other.....

D3. Have you had a spontaneous abortion (Miscarriage) (FOR FEMALES ABOVE 15 YEARS) ☐

1 Yes 2 NO

D4. Have you been treated for any febrile illness in the last 12 months? (IF NO SKIP TO E1) ☐

1 YES 2 NO 3 UNKNOWN

D5. If yes, where were you treated?

☐ Public health facility

☐ Local chemist

☐ Traditional healer

☐ private clinic

☐ mobile clinic

☐ Self medicated.

D6. If you self medicated, where did you acquire the medicine from?

☐ Shop

☐ Friend

☐ Relative

☐ Self medication with Herbs

Other (specify) _____

D7. What medicines did you take?

☐ Antibiotics

☐ Anti malarials

☐ Pain killers

☐ Herbal

☐ Don't know

Other (specify) _____

D8. For how long did you take the medication? NUMBER OF DAYS…_____

D9. How many times have you been treated for this illness in the last 12 months?

☐ Once

☐ 2-3 times

☐ More than 3 times

☐ Don't know

D10. Have the symptoms resolved? ☐

1 Yes 2 No

D11. If you went to a health facility were any samples taken? ☐ (IF NO, SKIP TO D12)

1 Yes 2 No 3 Can't remember

D12. If yes, which samples were taken?

☐ Blood ☐ Urine ☐ Stool ☐ Sputum ☐ Don't remember

D13. Were you informed of the diagnosis during that visit? ☐

1 Yes 2 No 3Can't remember

D14. If yes, what were you told was the diagnosis? ……

E. RISK FACTOR INFORMATION- FOOD CONSUMPTION AND PREPARATION

E1. Do you use milk? ☐ *IF NO SKIP TO G12*

E2. Do you drink cattle milk? ☐

1Yes, everyday 2 Yes, but not every day 3 No

E3. Do you drink goat milk? ☐

1 Yes, every day 2 Yes, not every day 3 No

E4. How do you consume the milk? DO NOT PROMPT. TICK ALL THAT APPLY

☐ Tea ☐ Add to vegetables ☐ Drinking ☐ Other (specify)

E5. Where do you get the milk? TICK ALL THAT APPLY

Commercial (packaged) ☐

From own animals ☐

Neighbor's animals ☐

Unprocessed milk from market ☐

E6. How often do you consume the following? USE THE FOLLOWING CATEGORIES

1-DAILY

2- WEEKLY

3-MONTHLY

4-OCCASIONALLY

5-NEVER

E7. Animal type	E8. Unboiled milk	E9. Boiled milk	E10. Fermented milk; made from		E11.Packaged milk
			E9. Boiled milk	E10.Unboiled milk	
Cow					
Goat					
Sheep					
Camel					

E12.Do you consume uncooked or undercooked meat? ☐

1 Yes 2 No

E13. If yes, how often?

Daily ☐ Weekly ☐ Monthly ☐

Occasionally ☐

Other, specify_____

E14. Do you consume uncooked blood? ☐

1 Yes 2 No

E15. If yes, how often?

☐ Daily ☐ Weekly ☐ Monthly ☐

Occasionally ☐

Other, specify_____

E16. Do you consume dairy products like yoghurt, cheese? ☐

1Yes 2 No

E17. If yes how often?

☐ Daily ☐ weekly ☐ monthly ☐

Occasionally ☐

Other, specify_____

E.17 Where do you obtain the fermented milk from?

☐

Commercial (packaged from factory)

☐ Prepare at home using milk from own animals

E18. If you prepare from own milk at home, do you use?

☐ Use unboiled milk

☐ Use boiled milk

☐ Obtain from neighbor or locally produced (not prepackaged)

E19. Has any member of your family been diagnosed with brucellosis in the past? ☐

1 YES 2 NO

E20. If yes, specify family member.....

E21. How long ago were there diagnosed with brucellosis? (specify if in days/weeks/months).....

F. RISK FACTOR INFORMATION- ANIMAL CONTACT

F1.Do you milk, feed, water, slaughter or herd animals? ☐ *(NO SKIP TO F3)*

1 YES 2 NO

F2. If yes, which animals? DO NOT PROMPT. TICK ALL THAT APPLY

☐ Cattle ☐ Sheep ☐ Goats ☐ Camels ☐ Other _____

F3. Do you handle (feed, graze, milk) cattle on a regular basis? ☐

1 YES 2No 3 Don't know

IF NO OR DON'T KNOW, GO TO QUESTION F12

F4. How often do you handle **cattle** for;

F5. Feeding/watering?

◯ Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never

F6. Milking?

◯ Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never

F7.Slaughter/handle meat?

◯ Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never

F8. Movement/herding of animals?

◯ Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never

F9. Assistance with births?

◯ Frequently ◯ Sometimes ◯ Rarely ◯ Never

F10.Removal of retained placentas?

◯ Frequently ◯ Sometimes ◯ Rarely ◯ Never

F11. Contact with aborted fetus?

Frequently ◯ Sometimes ◯ Rarely ◯ Never ◯

F12. Do you handle sheep on a regular basis? ☐ *(IF NO OR UNKNOWN, GO TO QUESTION F21*)

1Yes 2 No 3 Unknown

F13. How often do you handle sheep for:

F14. Feeding/watering?

Daily ◯ Several times a wee◯ Several times ◯month ◯Rarely Never ◯

F15.Slaughter/ handle meat?

Daily ◯ Several times a wee◯ Several times a ◯onth ◯Rarely Never ◯

F16. Movement / herding of animals?

Daily ◯ Several times a wee◯ Several times a m◯th ◯arely Never ◯

F17. Shearing?

Daily ◯ Several times a wee◯ Several times a m◯th ◯arely Never ◯

F18. Assistance with births?

Frequently ◯ Sometimes ◯ Rarely ◯ Never ◯

F19. Removal of retained placentas?

Frequently ◯ Sometimes ◯ Rarely ◯ Never ◯

F20. Contact with aborted fetus?

Frequently ◯ Sometimes ◯ Rarely ◯ Never ◯

F21. Do you handle goats on a regular basis?

1 Yes 2 No 3 Unknown

IF NO OR UNKNOWN, GO TO QUESTION F30

F22. How often do you handle **goats** for:

F23. Feeding/watering?

Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never ◯

F24. Milking?

Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never ◯

F25.Slaughter/ handle meat?

Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never ◯

F26. Movement of animals?

Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never ◯

F27. Assistance with births?

Frequently ◯ Sometimes ◯ Rarely ◯ Never ◯

F28. Removal of retained placentas?

Frequently ◯ Sometimes ◯ Rarely ◯ Never ◯

F29. Contact with aborted fetus?

Frequently ◯ Sometimes ◯ Rarely ◯ Never ◯

F30. Do you handle camels on a regular basis? IF NO OR UNKNOWN, GO TO QUESTION F39.

◯ Yes ◯ No ◯ Unknown

F31. How often do you handle **camels** for:

F32. Feeding/watering?

Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never ◯

F33. Milking?

Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never ◯

F34. Slaughter/ handle meat?

Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never ◯

F35. Movement/ herding of animals?

Daily ◯ Several times a week ◯ Several times a month ◯ Rarely ◯ Never ◯

F36. Assistance with births?

Frequently ◯ Sometimes ◯ Rarely ◯ Never ◯

F37. Removal of retained placentas?

Frequently ◯ Sometimes ◯ Rarely ◯ Never ◯

F38. Contact with aborted fetus?

Frequently ◯ Sometimes ◯ Rarely ◯ Never ◯

F39. Do you practice hunting? ☐

1 Yes 2 No

F40. If yes, what animals?

Antelope ☐

Bush back ☐

Wild beasts ☐

Buffaloes ☐

☐ Other (Specify) _____

F41. Are you involved in slaughter and butchering wild animals? ☐

1 Yes 2 No

F42. Do you clean/sweep animal barns/boma?

1Yes 2 No

F43. If yes, how often

☐ Daily ☐ At least 4 days in a week ☐ Once a week Occasionally ☐

F44. Do you handle/use animal manure or fresh animal waste (dung)? ☐

1 Yes 2 No

F45. Do you handle/work with animal hides and skins? ☐

1 Yes 2 No

F46. If yes, what form of hides?

Raw hides ☐

Appendix iii: Ethical Approval

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: [illegible]
[illegible]

KNH/UON-ERC
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KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: [illegible]
Fax: [illegible]
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/22 [illegible]

6th February 2014

KENYATTA NATIONAL HOSPITAL APPROVED 06 FEB 2014 KNH/UON-ERC

Kung'u Mathew Muturi
TM 312-0659/12
JKUAT

Dear Mathew

RESEARCH PROPOSAL: FACTORS ASSOCIATED WITH BRUCELLOSIS AMONG PATIENTS ATTENDING SELECTED HOSPITALS IN KAJIADO COUNTY, KENYA 2013 (P803/11/2013)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and approved your above proposal. The approval periods are 6th February 2014 to 5th February 2015.

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. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. Prof. A.N. Guantai, Chairperson, KNH/UoN-ERC
The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
Assistant Director, Health Information, KNH
Supervisors: Prof. Zipporah Ng'ang'a, Dr. Hamadi Njenga

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Appendix iv: Publication

Risk factors for human brucellosis among a pastoralist community in South-West

Kenya, 2015

Mathew Muturi , Austine Bitek , Athman Mwatondo , Eric Osoro , Doris Marwanga , Zeinab Gura , Phillip Ngere , Zipporah Nganga , S. M. Thumbi and Kariuki Njenga

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Abstract

Introduction: Brucellosis is one of the top five priority zoonosis in Kenya because of the socio-economic burden of the disease, especially among traditional, livestock keeping communities. We conducted a 1 year, hospital based, unmatched case–control study to determine risk factors for brucellosis among Maasai pastoralists of Kajiado County in 2016.

Methods: We conducted a 1 year, hospital based, unmatched case–control study to determine risk factors for brucellosis among Maasai pastoralists of Kajiado County in 2016. A case was defined by a clinical criteria; fever or history of fever and two clinical signs suggestive of brucellosis and a positive competitive enzyme-linked immunosorbent assay test (c-ELISA). A control was defined as patients visiting the study facility with negative c-ELISA. Unconditional logistic regression was used to study association between exposure variables and brucellosis using odds ratios (OR) and 95% confidence intervals (CI).

Results and conclusion: Forty-three cases and 86 controls were recruited from a population of 4792 individuals in 801 households. The mean age for the cases was 48.7 years while that of the controls was 37.6 years. The dominant gender for both cases

(62.7%) and controls (58.1%) groups was female. Regular consumption of un-boiled raw milk and assisting animals in delivery were significantly associated with brucellosis by OR 7.7 (95% CI 1.5–40.1) and OR 3.7 (95% CI 1.3–13.5), respectively.

Keywords: Brucellosis, Risk factors, Kenya