

**Prevalence and factors associated with brucellosis among febrile  
patients attending Ijara District Hospital, Kenya**

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**A thesis submitted in partial fulfillment for the degree of Master of  
Science in Applied Epidemiology in the Jomo Kenyatta University  
of Agriculture and Technology.**

**2012**

**DECLARATION**

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

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## **DEDICATION**

I dedicate this research to my husband; Wanyeki, my sons; Halifax and Caleb and my Parents; Japhet and Sophia.

## **ACKNOWLEDGEMENT**

I would like to most sincerely thank my supervisors; Dr. Jared Omolo, Field Epidemiology and Laboratory Training Program, (FELTP), Dr. Joseph Oundo, FELTP Laboratory Resident Advisor and Dr. Gideon Kikvi of Jomo Kenyatta University of Agriculture and Technology, (JKUAT) for their unlimited support towards modelling me all through during my research work. My gratitude to the Centers for Diseases Control (CDC) for funding my studies, Jomo Kenyatta University of Agriculture and Technology for the opportunity to admit me into this course and Ministry of Public Health and Environmental Sanitation for hosting and thoroughly equipping me carry out this research. I thank all the officers and lecturers who spent a lot of time in training me throughout the programme and the much assistance accorded to me during research proposal development and also in carrying out this study to completion. Many thanks to the CDC laboratory director, Dr Kariuki Njenga for his full support in laboratory analysis, logistics and all the International Emerging Infections Program (IEIP) laboratory staff for their immense assistance with the PCR assays. My gratitudes to Dr Samwel Amwayi and Mr Mohamed Abade for their great inputs towards proposal development. I am indebted to Dr David Mutonga, (Division of Disease Surveillance and Response) for being such an inspiration to me. I wish to express my appreciation also to the Ministry of Livestock Development staff; Drs Peter Ithodeka, Joseph Macharia, Peter Mbatha, Salome Kairu and Romona Ndanyi for being such a strong source of inspiration and also for offloading many tasks from me so I could carry out my research on time.

I truly thank the Ijara Hospital team including District Medical Officer of Health (Dr Sonde), Medical Superintendent (Dr Abdalla A), laboratory staff; Peter Wachira, Mohammed Bare, Joseph Kariuki, Steven Mwendwa and the Clinical officer, Ahmed Mohammed who readily assisted me in many ways while collecting data at Ijara District Hospital.

Many thanks also to Christine Ouko CDC Kenya, Marion Mwangi and Gabriel Oluoch (FELTP) for their great dedication and administrative support over the whole period of this study. Last but not least, I thank my househelp Pietrena Nyambura and my brother Elias Kiambi for the overwhelming domestic support they accorded to me.

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## **LIST OF ABBREVIATIONS AND ACRONYMS**

<b>µl</b>	Microlitres
<b>C/O</b>	Clinical officer
<b>CDC</b>	Centers for disease control and prevention
<b>DMOH</b>	District medical officer of Health
<b>DNA</b>	Deoxyribonucleic acid
<b>ERC</b>	Ethical review committee
<b>FAO</b>	Food and Agricultural Organization
<b>FELTP</b>	Field Epidemiology and Laboratory Management Training Program
<b>IEIP</b>	International Emerging Infections Program
<b>ITROMID</b>	Institute of Tropical Medicine and Infectious Diseases
<b>JKUAT</b>	Jomo Kenyatta University of Agriculture and Technology
<b>KEMRI</b>	Kenya Medical Research Institute
<b>Mls</b>	Millilitres
<b>OIE</b>	World health organization for animals
<b>PCR</b>	Polymerase chain reaction
<b>PVN</b>	Predictive value negative
<b>PVP</b>	Predictive value positive

<b>RBPT</b>	Rose Bengal plate test
<b>Rpm</b>	Revolutions per minute
<b>SAT</b>	Serum Tube agglutination test
<b>SOP</b>	Standard operating procedure
<b>SSA</b>	Sub-Saharan Africa
<b>SSC</b>	Scientific steering committee
<b>STAT</b>	Standard Tube agglutination test
<b>WHO</b>	World health organization

## ABSTRACT

Brucellosis is a re-emerging zoonotic disease that causes more than half a million infections to humans every year. The disease is common in most developing countries although its prevalence often remains unreported due to low suspicion index by health workers and insufficient capacity to correctly diagnose the disease in humans. Rapid serologic kits are commonly used in human brucellosis diagnosis resulting to doubtful data due to false positives and negatives and thus little influence on policies in brucellosis control efforts.

In Sub-Saharan Africa, approximately 16% of livestock harbour the disease yet its treatment in animals is not recommended. Instead animals should be culled and this practice is not possible in the developing countries due to economic implications and poor compensation rates by the governments. This therefore has resulted to endemicity of the disease and continued source of infection to humans.

Although human mortality due to brucellosis is only about 2%, the disease causes severe disabling sequelae like rheumatism, infertility in males, spontaneous abortion and also results to wastage of resources through prolonged treatment, up to six weeks, and loss of income through loss of working hours. *Brucella* organisms are also considered potential biological weapon which could be cheaper to produce but more devastating than chemical weapons.

Even though animal brucellosis cases have been reported from Ijara District, prevalence of the disease in humans is unknown and the associated factors as well as the effectiveness of the Febrile Rapid Diagnostic Kit<sup>®</sup> have not been determined.

This hospital based cross sectional study was therefore carried out between December 2010 and January 2011 among 384 febrile patients aged 2 years and above with the objectives of determining the prevalence and factors associated with brucellosis and also evaluation of the Febrile Rapid Diagnostic Kit<sup>®</sup> used at the facility.

About 5 millilitres of blood from each patient was drawn and analyzed by the Febrile Rapid Diagnostic Kit<sup>®</sup> and Polymerase chain reaction (PCR). Semi structured questionnaire administered to collect data. Epi- info version 3.5.1 was used for data analysis.

Seroprevalence of brucellosis was 31.8% and the true prevalence was 15.4% by PCR. Obtaining milk from the market (p value <0.00001, odds ratio 7.3, 95% confidence interval 2.5-21.1) and drinking of unboiled milk (p value <0.0001, odds ratio 8.5, 95% confidence interval, 4.2-17.3) were significantly associated with brucellosis. The sensitivity and specificity of the Febrile Rapid Diagnostic Kit<sup>®</sup> was 37 % and 69% respectively with a Predictive value positive of 18% and Predictive value negative of 86%. The level of agreement was 0.03.

The findings obtained from this study indicate that brucellosis is prevalent in about 1/6 of febrile patients attending Ijara District Hospital. Unprocessed milk from the market and consumption of unboiled milk were associated with brucellosis. Febrile Diagnostic rapid kit<sup>®</sup> underestimates positivity but overestimates prevalence of brucellosis in the febrile patients



Therefore, patients with brucellosis should be treated to prevent the devastating effect of the disease and the accompanying sequelae, public health education programs should explain modes of transmission (milk should be boiled before consumption) and Febrile Rapid Diagnostic kit<sup>®</sup> used at the facility should be replaced with better rapid diagnostic tests or PCR.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 BACKGROUND INFORMATION

Brucellosis is an infectious debilitating, zoonotic disease widely spread in the countries of Europe, North and East Africa, the Middle East, South and Central Asia, Central and South America (Robert *et al.*,2010) and is a major cause of morbidity to both humans and animals in these countries. Brucellosis has been, or is close to being eradicated from a number of developed countries although it is more of a problem in countries with poorly standardized animal and public health programs (Gul *et al.*, 2007). It is also considered a potential biological weapon (Jovanka *et al.*, 2010).

The main domestic animals that are affected include cattle, sheep, goats, pigs and dogs with the principal manifestations of reproductive failure; abortion or birth of unthrifty offspring in females, orchitis and epididymitis in males (Stacy, 1986, Young, 1995). Six major brucella species are known to cause disease in humans; *Brucella abortus*, *B. Melitensis*, *B. Suis*, *B. Canis*, *B. ovis* and *B. Neotomae* (Glynn *et al.*,2008) all of which circulate in animals.

Although brucellosis is a common cause of morbidity in humans in developing countries, it is often unrecognized and more frequently goes unreported (Corbel, 2006, Kunda *et al.*, 2002) due to low suspicion index by the clinicians and weak laboratory capacity to confirm diagnosis.

Human infections occurs when they ingest animal products such as unpasteurized dairy products or semi-cooked meat from infected animals.

It is also a common problem to farmers and animal health workers who come into contact with infected materials like abortuses, fetuses, placenta and postparturent discharges from infected animals (Gerald *et al.*, 2009, Marjorie *et al.*, 2008, Hasanjani *et al.*, 2004, Kunda *et al.*, 2009).

In humans, brucellosis occurs in all age groups (Mantur *et al.*, 2007) and is characterized by influenza like clinical disease with undulating fever, sweats, malaise, weakness, anorexia, headache, myalgia and back pain (Corbel, 1997, Maichomo *et al.*, 2000). The infection could sometimes persist and results in various complications as described by Georgios *et al.*, 2003, Isaias *et al.*, 2008, Amalia 2001, Yousuf *et al.*, 2001, Nicholas *et al.*, 2001, Abhay *et al.*, 2007, Wang *et al.*, 1999, Cem *et al.*, 2009 and Dalal *et al.*, 2009. Clinical diagnosis is therefore very difficult in the absence of laboratory confirmation due to its similarity to other common tropical diseases like malaria, Q fever, typhoid and tuberculosis among others (Maichomo *et al.*, 2000, Muriuki *et al.*, 1997).

## **1.2 Statement of the problem**

Over 16% of cattle in Sub-Saharan Africa are infected with brucellosis yet more than a third of Africa's population depend solely on livestock and livestock products for their livelihoods (Mangen *et al.*, 2002). Treatment of brucellosis in animals is not recommended, instead animals should be culled (OIE, 2009), and this however has not been possible in the developing countries due to economic consequences hence maintaining carrier or chronic animals. Consequently, this has led to endemicity of human brucellosis in Africa (Corbel, 2006), since livestock are the main source of infection to humans (Nicoletti, 1992, Tzaneva *et al.*, 2009).

Although mortality due to brucellosis is minimal, 2-5%, (Wafa *et al.*, 2009, Wang *et al.*, 1999) the disease could result to permanent and disabling sequelae like sterility, spontaneous abortions, spondylitis, arthritis, neurobrucellosis among others (Yousuf *et al.*, 2001, Nicholas *et al.*, 2001, Abhay *et al.*, 2007, Wang *et al.*, 1999) if not adequately treated. Consequently, there will be considerable medical expenses in addition to loss of income due to loss of working hours (Isaias *et al.*, 2008), yet With prompt diagnosis and treatment the losses could be minimized (Wafa *et al.*, 2009, Corbel, 2006).

In Kenya, animal brucellosis has been reported every year particularly from the arid and semi-arid pastoral areas of the country (Kenya DVS Annual reports, 1999-2010). However, there is scarcity of data on human infection although prevalence ranging between 12 % and 21% among the pastoral communities in Kenya between the years 1997 to 2010 (Muriuki *et al.*, 1997, Maichomo *et al.*, 1998, Maichomo *et al.*, 2000, Richards *et al.*, 2010) have been reported.

### **1.3 Justification of the study**

Ijara community live within the tenets of strong cultural beliefs of consumption of raw dairy products in order to retain nutritive values yet the glaring of zoonoses like brucellosis and its predisposing factors have not been established. Although brucellosis is of great public health and economic concern, laboratory capacity is also very weak mainly relying on rapid agglutination tests for diagnosis. These tests are generally inconclusive, giving a lot of false positives or negatives thus they are insufficient in providing satisfactory evidence to attract any policies that would direct and reinforce control strategies both in human and livestock.

Exploration of this health problem could give evidence based data that would guide in interventions since brucellosis requires multidisciplinary control approach. Its also important that humans are correctly diagnosed and adequately treated to avoid relapses and chronicity of the disease that would result to permanent and disabling sequelae.

This study therefore, investigated the prevalence of brucellosis among febrile patients attending Ijara District Hospital and identified risk factors of infection as well as evaluated the diagnostic rapid kit used for brucellosis testing at the facility.

#### **1.4 Research Questions**

1. What is the prevalence of brucellosis among febrile patients attending Ijara District Hospital?
2. What factors are associated with brucellosis among febrile patients attending Ijara District Hospital?
3. What is the sensitivity and the specificity of the Febrile Diagnostics Rapid Kit<sup>®</sup>?

#### **1.5 Null Hypothesis**

There is no *Brucella* infection among febrile patients attending Ijara District Hospital and there are no factors associated with brucellosis among these patients.

#### **1.6 Objectives of the study**

##### **1.6.1 General objective**

To determine the prevalence and factors associated with brucellosis among febrile patients attending Ijara District Hospital and evaluate the performance of Febrile Rapid Diagnostic Kit<sup>®</sup>.

### **1.6.2 Specific objectives**

1. To determine the prevalence of brucellosis among febrile patients attending Ijara District Hospital
2. To determine the factors associated with brucellosis infection among patients with fever attending Ijara District Hospital
3. To evaluate the performance of the brucellosis Febrile Rapid Diagnostics kit<sup>®</sup> used at the hospital

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Definition and brief history of Brucellosis

Brucellosis is an infectious debilitating, zoonotic disease caused by gram-negative intracellular nonmotile coccobacillus, and one of the oldest diseases of man causing more than 500,000 new each year (Corbel, 2006). It was first recognized as a disease affecting humans on the Island of Malta in the early 20<sup>th</sup> century. Captain David Bruce was the first scientist to isolate and identify *Brucella melitensis* then called *Micrococcus melitensis* in the year 1887 (Wyatt *et al.*, 2005, Rahman *et al.*, 2006) although the history of brucellosis ranges far back before that time. There were many descriptions of disease which could possibly have been describing brucellosis including abortion epidemics in animals and fever in humans. Martson in 1859 (Wyatt *et al.*, 2005) also described an illness which differed from typhoid that had affected the Crimean war sailors aboard ships that was a debilitating chronic illness which was getting complicated with rheumatism and for this reason many Royal Navy seamen were grounded every year.

#### 2.2 Aetiology of Brucellosis

Six major *Brucella* species have been classically characterized according to the major reservoir: *Brucella abortus* (cattle), *B. Melitensis* (Sheep and goats), *B. Suis* (pigs), *B. Canis* (dogs), *B. ovis* (Sheep and goats) and *B. Neotomae* (fish); though they are not host-specific, and may transmit to other animal species under appropriate conditions (Glynn, 2008).

All these *Brucella* species cause disease in humans with *B. abortus* being the most frequently occurring (Wafa *et al.*, 2009), and *B. melitensis* being the most important clinically in humans due to its severity (Corbel, 1997).

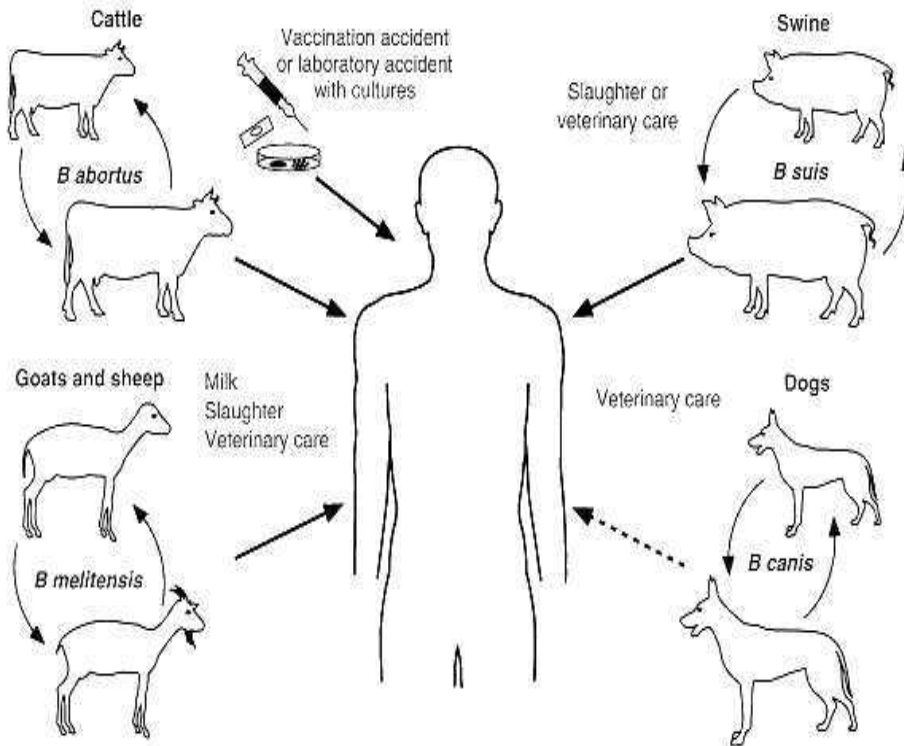
### **2.3 Transmission of brucellosis to humans**

Nearly every case of human brucellosis has an animal origin (Nicoletti, 1992, Tzaneva *et al.*, 2007). Large quantities of the bacteria are excreted with the foetus, placenta and the uterine fluid,

mainly at the time of calving. After an abortion or parturition, the organism continues to be excreted mainly via milk of infected cows serving as continued source of infection to humans (Mangen *et al.*, 2002). Human to human transmission and congenital infection have also been documented (Oded *et al.*, 2007, Frank *et al.*, 1993). Exposure through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted foetuses or placentas are also possible routes of transmission of the disease (Gerald *et al.*, 2009).



Several transmission pathways of brucellosis to humans have been described; Figure 2.1.



**Figure 2.1: Pathways involved in transmission of brucellosis to humans**

(Source: Robinson, 2003)

Intake of contaminated dairy products is the prime mode of transmission and the major risk factor for acquiring brucellosis in urban areas (Marjorie *et al.*, 2008).

Occupational airborne infection in laboratories and abattoirs has also been documented. Accidental inoculation of live vaccines such as *B. abortus* Strain 19 and *B. melitensis* can also occur, resulting in human infections (Marjorie *et al.*, 2008).

## **2.4 Transmission of brucellosis in animals**

Brucellosis is essentially a herd disease and spread between herds usually occurs by the introduction of asymptomatic chronically-infected animals (Perry *et al.*, 2002, Mangen *et al.*, 2002, Nicoletti, 1992). Initial infection in the reservoir species is often followed by abortion and subsequent delayed or permanent infertility. Infection is usually chronic in animals, and treatment is rarely undertaken (Mangen *et al.*, 2002) making brucellosis endemic in countries that lack standardized control measures (Gul *et al.*, 2007). Infected animals shed the organisms in uterine discharges following abortion and subsequent parturition, and also in the colostrum and milk (Mangen *et al.*, 2002). It is spread within the herd primarily by ingestion of contaminated material although venereal infections can also occur, but this is mainly seen with *B. suis* infections (McDermott *et al.*, 2002, Mangen *et al.*, 2002). Congenital (in utero) or perinatal infections may also occur, with the ensuing development of latent infections. High levels of bacteria are found in the birth fluids of an infected animal.

## **2.5 Epidemiology of human brucellosis**

Diagnosis of brucellosis is often difficult to establish, largely through similarity with clinical presentations of other infections prevalent in sub-Saharan Africa and weakness in the laboratory capacity to confirm the disease (Mutanda *et al.*, 1998, Maichomo *et al.*, 1998).

Over the years, brucellosis has been controlled adequately in most developed countries mainly due to various sanitary socioeconomic, and political reasons, together with the evolution of international travel (Pappas *et al.*, 2009).

Though the true incidence of human brucellosis is unknown globally, (Corbel, 1997), several areas traditionally considered to be endemic like France, Israel, and most of Latin America have achieved control of the disease. The new foci of brucellosis have emerged in other countries like in Central Asia and Syria and the disease is still present in European countries and in the United States of America (Pappas *et al.*, 2009). The infection is increasing in other countries like those in the Mediterranean region, Middle East, Central Asia, Western Asia, parts of Africa and Latin America. In Brazil, a prevalence of 4.1% was recorded in 2008 (Ramos *et al.*, 2008), 34.2% in Iraq (Aminzadeh *et al.*, 2010) and 4.8% in Turkey (Zafer *et al.*, 2005).

Endemicity of animal brucellosis in Africa and particularly in Sub-Saharan Africa which has approximately 16% prevalence continues to serve as constant source of infection to humans (Corbel, 2006). A brucellosis prevalence of 13.3% in Uganda (Mutanda, 1998) and 6.2% in Tanzania (Kunda *et al.*, 2007) have been recorded.

Kenya is equally incapacitated by limited data and knowledge of brucellosis so that many cases go unrecognized and unreported. However, human brucellosis is more common where extensive cattle production systems predominate with almost a prevalence of 14% to 21% being documented (Muriuki *et al.*, 1997, Richards *et al.*, 2010).

## 2.6 Clinical manifestation of brucellosis in humans

Brucellosis is a multisystemic infection that vary considerably and may last three days to six months and occasionally for longer than a year (Hugh, 2000). Patients may present with an acute systemic, febrile insidious chronic infection or a localized inflammatory process (Sisirak *et al.*,2009, Madkour *et al.*, 2005). There are no pathognomonic signs of brucellosis and patients present with non-specific signs as fever, malaise, sweats, fatigue, anorexia, muscle or joint aches (Wafa *et al.*, 2009, Sisirak *et al.*, 2008, Hugh, 2000). Infection may be severe and may be followed by chronic intermittent relapses (Hugh, 2000).

Other forms of presentations of the disease include respiratory system involvement (Georgios *et al.*,2003), ocular complications (Isaias *et al.*, 2008), epididymoorchitis in males (Amalia, 2001, Stamatiou *et al.*,2009) and spontaneous abortion (Yousuf *et al.*,2001).

In-utero infection of the fetus (Ziba *et al.*, 2005), visceral abscesses (Nicholas *et al.*, 2001), cardiovascular complications with implantable cardioverter defibrillator replacement (Abhay *et al.*,2007, Wang *et al.*, 1999) and osteoarticular and gastrointestinal system complications (Ali *et al.*, 2003) have been known to occur.

Brucellosis usually does not cause leukocytosis, and patients may be neutropenic. *B. melitensis* tends to cause more severe, systemic illness than the other *Brucella* species and *B. Suis* is more likely to cause localized suppurative disease (Wafa *et al.*, 2009). Mortality due to brucellosis in humans is less than 5% (Wafa *et al.*, 2009) and 80% of this mortality is due to endocarditis (Wang *et al.*, 1999).

## **2.7 Clinical manifestations of brucellosis in animals**

Brucellosis is a herd disease and abortion is the principle clinical manifestation in an infected herd with abortions occurring at about 5-7 months of pregnancy, (Nicoletti, 1992, Corbel, 2006). Full-term calves may be born but die soon after birth. In fully susceptible herds, abortion rates may vary from 30% to 80% (Corbel, 2006). Retained placenta and secondary metritis are common in infected females and may lead to permanent sterility. Subsequent gestations are normal, after a period of temporary sterility and only 5% of infected females have residual sterility. Most cows will shed the bacteria in the milk and this is the main source of infection to humans (Nicoletti, 1992). In bulls acute or chronic infections of the reproductive tract; orchitis, epididymitis and seminal vesiculitis may occur and this contributes to continued infection to the females especially if the bulls are used for breeding or producing semen for artificial insemination. Hygromas, particularly of the carpal joints, occur in some animals in chronically affected herds (FAO, 2006).

## **2.8 Diagnosis of brucellosis in humans**

Clinical diagnosis of brucellosis is often difficult to establish, largely through similarity with clinical presentations of other infections prevalent in sub-Saharan Africa such as malaria, tuberculosis, typhoid and joint diseases among others (Mutanda, 1998, Maichomo *et al.*, 1998).

Apparently, also the patients attend the health facilities for testing when the symptoms persist rather than due to the severity of the symptoms ( Kunda *et al.*, 2007).

However, since Brucellosis is not readily recognized by medical practitioners, it goes unreported (Corbel, 2006) and sometimes leading to very expensive outcomes, either directly or indirectly (Bax *et al.*, 2007).

### **2.8.1. Criteria for diagnosis of brucellosis:**

#### **2.8.1.1 History of the patient**

Nearly every case of human brucellosis has an animal origin (Nicoletti, 1992); therefore a thorough history eliciting details of appropriate exposures such as attending to or living with animals, possibility of exposure to contaminated animal products, and environmental exposures like improper disposal of arbotuses is a very important tool towards diagnosis of brucellosis (Daniel *et al.*, 2008). Key risk factors include ingestion of unprocessed contaminated food, exposure to infected animal and their products, (Cooke *et al.*, 2004), inhalation of infected aerosols, or splashes from infected material onto conjunctivae, travel to endemic area, occupation involving animals and/or animal products (farmers, animal handlers, abattoir workers, veterinarians) and laboratory workers (Robinson, 2003).

#### **2.8.1.2 Clinical presentation of the patient**

This can only be suggestive of the disease as the signs and symptoms are not pathognomonic of the disease (Sisirak *et al.*, 2009, Hugh, 2000). They include; fever or chills which occur in 53% to 100% of infections, and if left untreated can show an undulating pattern, constitutional symptoms such as sweating, lethargy, and weight loss are a feature of infection in up to 97% of patients, gastrointestinal complaints in 80% of the patients (Corbel, 2006).

### 2.8.1. 3 Diagnosis by serological tests

Most of the hospital laboratories in rural sub-Saharan Africa have limited capacity for the diagnosis of brucellosis. Brucellosis is commonly tested after failure to respond to malaria, typhoid or tuberculosis treatments (Muriuki *et al.*, 1997). Rosebengal plate test (RBPT) is the commonest rapid laboratory tool used for diagnosis of brucellosis in the local clinics while superior serological tests like Serum agglutination tests (SAT) are only available in the higher medical testing facilities (Maichomo *et al.*, 2000, Muriuki *et al.*, 1997). Immunoglobulin (IgM and IgG) ELISAs have the lowest sensitivity and specificity ranging between 60% and 84%, respectively (Gomez *et al.*, 2008).

In the sub-acute or chronic phase of brucellosis, the agglutination tests may be particularly difficult to interpret or may be negative and other tests need to be done to confirm the results. This is because the serum agglutination test depends very much on the presence of IgM that could be low or absent in chronic and sub-acute states. This also explains why the SAT is negative during the incubation period and following abortion (Mittal *et al.*, 1983).

In a comparative study done to compare RBPT relative to ELISA and Standard tube agglutination test , the sensitivity of RBPT was found to be 42.42% (Ghodasara *et al.*, 2010). When compared with Serum agglutination Test, the sensitivity and specificity of RBPT were 96% (Zafer *et al.*, 2005). Other studies have shown the sensitivity to be as low as 33% and specificity as high as 100% according (Rajkhowa *et al.*, 2005).

Although competitive Enzyme Immunoassay is the most commonly used test for diagnosis of Human Brucellosis (Lucero *et al.*, 1999), Standard tube agglutination tests (STAT), micro plate agglutination test, indirect hemagglutination technique and Growth agglutination test are also available at higher health facility levels. Growth agglutination test is more sensitive over the rest (Mittal *et al.*, 1983).

#### **2.8.1.4 Polymerase Chain Reaction (PCR)**

Diagnosis of brucellosis by PCR is relatively simple and accurate. Sensitivity and specificity of PCR provides a valuable and quick tool for diagnosis of brucellosis (Conchi *et al.*, 1994) and danger to staff exposure is minimal such that, requirement for level three laboratory for containment is not mandatory and therefore cost is also reasonable (Wei, 2006). Real time PCR using the IS711-based assay has been shown to be the most sensitive, specific, efficient, and reproducible method to detect *Brucella spp* (Bounaadja *et al.*, 2009, Wei *et al.*, 2010). False negatives in PCR assays are rare and mainly occur due to amplification of the present polymerase inhibitors like hemoglobin, urine, heparin, phenol, and sodium dodecyl sulfate (Navarro,1999) hence accurate sampling techniques that minimizes contamination are critical.

#### **2.8.1.5 Culture and Isolation**

A definitive diagnosis of brucella infection is based on culture and isolation of the organism from different samples. Blood culture in Castaneda medium gives 80 to 92% accuracy (Gotuzzo *et al.*, 1986, Yagupsky, 1999).



However, culture has a disadvantage of long turn-around time of more than 2 months (Daniel *et al.*, 2008 and it is important that samples are not disposed before twenty one days since they can take even more than a month to grow (Joaquin *et al.*, 1997) .

## **2.9 Treatment of human brucellosis**

The essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time (Corbel, 2006). Generally, the treatment recommended by the WHO for acute brucellosis in adults is rifampicin 600mg to 900 mg and doxycycline 200mg daily for a minimum of six weeks ( WHO, 1986). In Children younger than 8 years, rifampin and trimethoprim-sulfamethoxazole (TMP-SMX) for 6 weeks is the therapy of choice (Wafa, 2011).

Treatment of uncomplicated brucellosis in persons above eight years of age with intake of 100mg of doxycycline twice a day for six weeks combined with 1gm streptomycin daily for two to three weeks is also adequate (Corbel, 2006).

It has also been suggested that the combination of doxycycline and an aminoglycoside in addition of rifampicin may be a better option (Navarro *et al.*, 2001, Keren *et al.*,2008, Skalsky *et al.*,2008). In complicated brucellosis, 100mg of Doxycycline twice daily for six weeks plus rifampicin 600 to 900 mg daily for six weeks could be adequate (Corbel, 2006).

## **2.10 Prevention and control of brucellosis**

No effective human brucellosis vaccine exists as trials have shown less protection against brucellosis and adverse allergic reactions to those vaccinated (Theodore *et al.*, 1999, Corbel, 1997, Hadjichristodoulou *et al.*,1994).

Prevention of brucellosis in humans still depends on the eradication or control of the disease in animal hosts, the exercise of hygienic precautions to limit exposure to infection through occupational activities, and the effective heating of dairy products and other potentially contaminated foods ( Gul *et al.*, 2007).

*Brucella* species persist for several days in milk (even when it turns sour) and is known to flourish in soft fresh small ruminant cheese. It may also persist for weeks in ice cream and months in butter. Therefore, these products always require to be made from pasteurized milk (Memish *et al.*, 2004). Various groups at risk of contracting brucellosis like veterinarians, abattoir workers, farmers, and dairy workers need to work with protective gear as well as practice hygienic precautions.

### **2.11 Economic impact of brucellosis**

Due to its effects on multiple animal species and humans, the impact of brucellosis is considered great in Sub-Saharan Africa (Perry *et al.*, 2002). However, valuing these economic impacts across species is complicated due to lack of adequate data on brucellosis both in animal and livestock. Prices can be estimated for direct losses due to morbidity and mortality and indirect losses due to treatment costs. Control programs can then be evaluated based on the potential benefits derived by avoiding these direct and indirect losses (Dijkhuizen *et al.*, 1997). However, for human morbidity and mortality losses, a different measure, disability-adjusted life years (DALY) are applied (Murray *et al.*, 1996). This avoids the complication of financially valuing human morbidity and mortality.

In assessing the impact of control programs on human brucellosis, cost-effectiveness analysis is used, ranking programs based on their costs per DALY averted.

For example in Kampala, informally marketed milk in urban Kampala was contaminated with *B.abortus* at purchase and the annual incidence rate was estimated to be 5.8 per 10,000 people. The researchers estimated that risk of acquiring brucellosis in the study area could be reduced by 47.4% if milk was boiled at a central place before selling (Makita *et al.*, 2010).

For bovine brucellosis, the major direct losses are on reproduction (abortion and impaired fertility) and thus also milk production (McDermott *et al.*, 1987). The disease also causes carpal hygromas in livestock. These levels of direct losses are relatively modest compared to more devastating epidemic diseases associated with high mortality.

Indirect losses, particularly those that require brucellosis-free status to access regional or international livestock markets, have not been estimated in sub-Saharan Africa but could be a considerable constraint to future trade. The impact of brucellosis and other zoonoses affecting livestock production are considerably magnified by their consequences in humans. On a global scale, brucellosis does not rank among the top diseases based on DALY losses.

## **2.12 Factors associated with human brucellosis**

Nearly every case of human brucellosis has an animal origin (Nicoletti 1992) and endemicity of the disease in animals poses a continuous risk for human infection (McDermott *et al.*, 2002). Human-to-human transmission, spread from mother to infant in utero (Yousuf *et al.*, 2001) and transmission to the infant through milk while suckling from an infected mother has been recorded ( Hossein *et al.*, 2008).

Congenital Infection has also been known to occur (Oded *et al.*, 2007, Frank *et al.*, 1993). Both males and females in all age groups are affected equally in particular when dairy is the most common source of infection (Mantur *et al.*, 2007).

However, the disease may be more common in males in areas where it is an occupational hazard of farmers and shepherds, butchers or veterinarians (Mantur *et al.*, 2007, Young *et al.*, 2000). In children brucellosis is very common especially in areas where *B. melitensis* is the main aetiological agent (Mantur, *et al.*, 2007, Ciftci *et al.*, 2003, Mangen *et al.*, 2002. Additionally other risk factors include, contact with aborting animals and abortuses, slaughtering/butchering infected animals, consumption of unpasteurized dairy products and having a member of the family who is infected by Brucellosis (Aminzadeh *et al.*, 2010, Geoffrey *et al.*, 2002, Kenneth *et al.*, 2009, Ramos *et al.*, 2008). Working with live cultures in the laboratory and organ placement from an infected individual could result to brucellosis (Abhay *et al.*, 2007). Brucella organisms could also be released as a biological warfare (Jovanka *et al.*, 2010).

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Study site**

This was a cross sectional study carried out at Ijara District Hospital that is located in the semi-arid Garrissa County in the Eastern part of Kenya (Figure 3.1). The district borders Fafi district to the North, Lamu District to the South, Tana Delta to the South West, Tana River to the West and the Republic of Somalia to the East. Ijara District lies approximately between latitude  $1^{\circ} 7'S$   $2^{\circ} 3'S$  and longitude  $40^{\circ} 4'S$  and  $41^{\circ} 32E$  (Kenya Bureau of Statistics, 2009). The region receives an average annual rainfall of less than 40mm and over 80% of the land is used for livestock production.

There are approximately 100,000 persons in Ijara district with about 55% of these living in the urban/ semi-urban areas. The local communities settled in the district include the Awer (hunter-gatherers) and the nomadic Somali, Abdalla, whose main economic activity is livestock rearing. The main types of livestock include Boran cattle, Masaai sheep and goats. Search for pasture and water during dry seasons, involves movement of animals to the Boni forest located about 80km from Ijara and River Tana Delta.

Milk is a staple diet among the Ijara communities and even during drought periods, milk is normally mixed from different animals in common five to ten litre containers from where it is transported to the open air market of Ijara for the the urban dwellers to purchase. Drinking of unboiled milk is a common practice among these residents as it is believed boiling milk reduces its nutritive value.

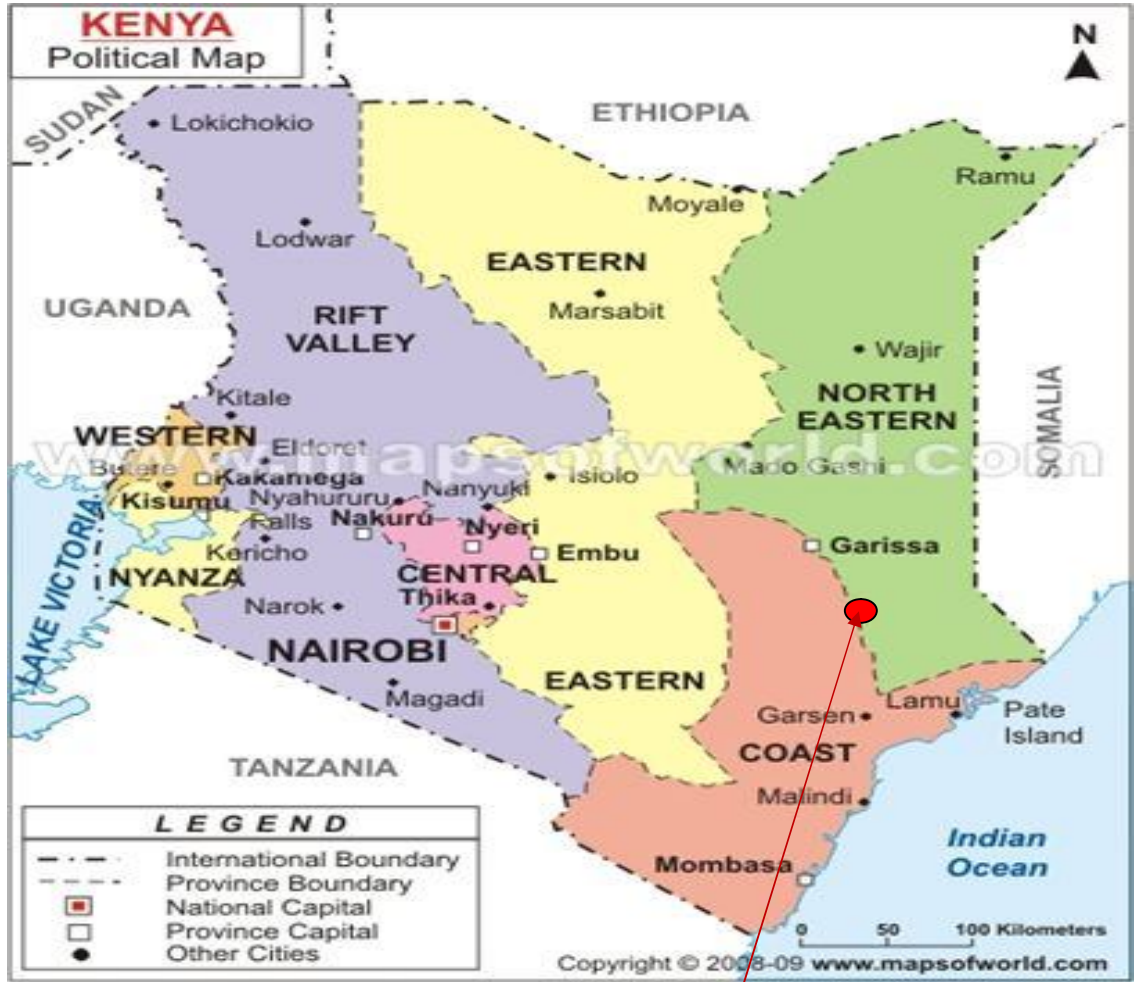


Figure 3.1 : Map of Kenya. Shaded red is the location of Ijara District

(Source:WWW.maps of world.com)

### **3.2 Sample size determination**

The Cochran formular, (Cochran, 1977) was used to determine the sample size for this study assuming a 50% prevalence so as to attain the adequate sample size to estimate the population prevalence with a good precision.

$$N = Z^2 P (1-P) /d^2$$

Where:

N = Required sample size

Z = Confidence level at 95% (standard value of 1.96)

P = Proportion (0.5)

d = Level of Precision at 5%

= 384 samples

#### **Assumptions**

-prevalence of 50 %

-Confidence interval at 95%

-precision of 0.05

-that sample is independent and randomly selected

### **3.3 Study participants**

Patients aged two years and above attending Ijara District Hospital between December 2010 and January 2011, who had reported or recorded fever during their current illness, were eligible for enrolment into the study. A total of 384 participants were systematically selected upon informed written consent for those 18 years and above (Appendix 1,2) or assent for patients aged 8 to 17 years of age (Appendix 3,4). For those below eight years, consent was sought and obtained from the parent or guardian. Sampling interval was calculated from the estimated 40 feverile patients seen daily for a period of three months (September, October and November 2010 at the hospital and every third patient who fulfilled the case definition and consented/assented was enrolled into the study.

### **3.4 Case definition**

**3.4.1 Suspected case:** Patients 2 years of age and above with recorded or reported history of fever during the current illness. Fever was defined as any patient whose temperature was recorded by the clinician to be above 37<sup>0</sup>C during the current examination at the hospital or the patient reported that they experienced episodes of fever during their current illness.

**3.4.2 Probable case:** All suspected patients whose blood tested positive on the rapid testing by the Febrile Diagnostics Kit<sup>®</sup>.

**3.4.3 Confirmed case:** All patients whose blood tested positive for brucellosis by PCR test.



### **3.5 Inclusion criteria**

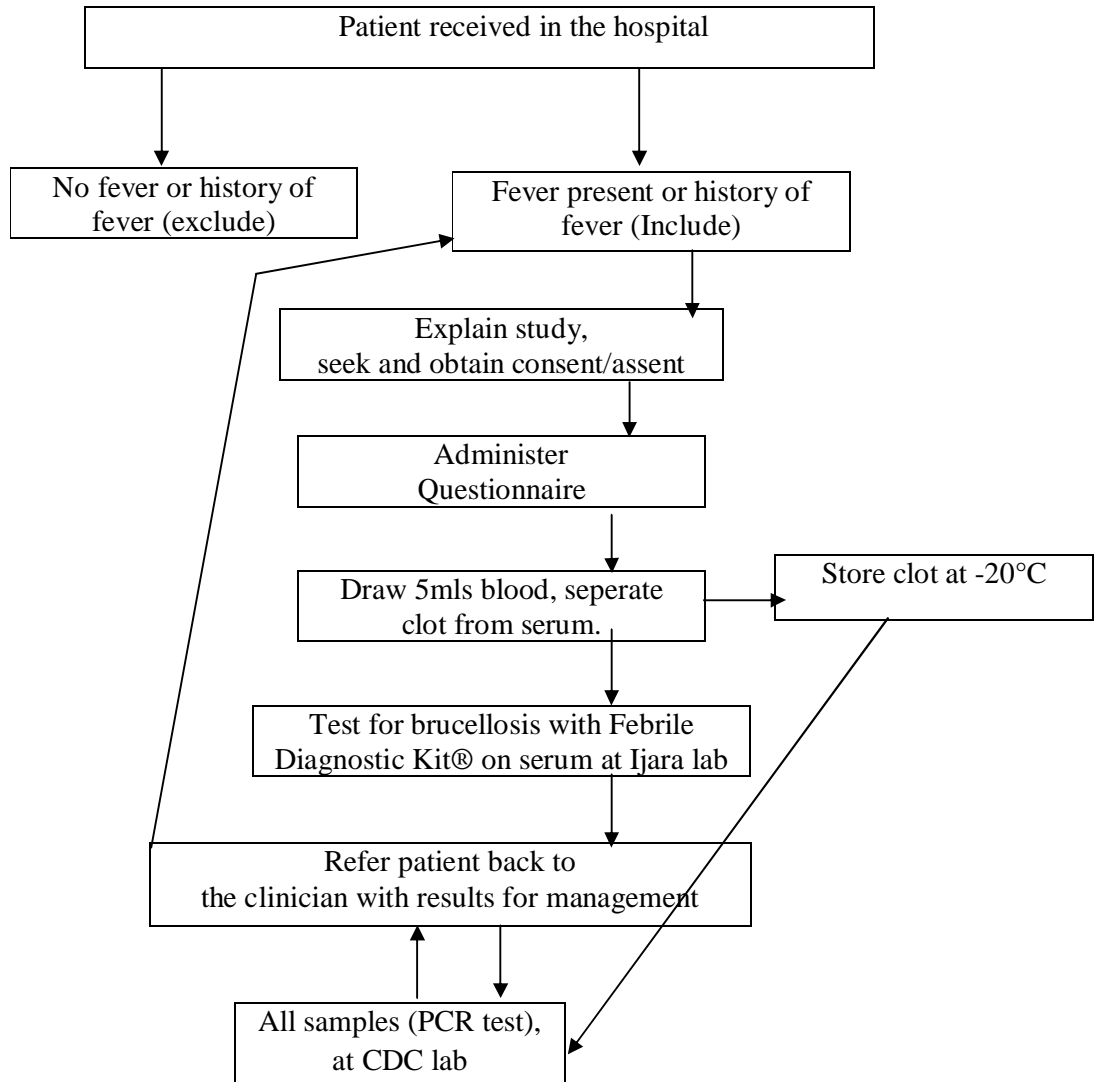
All patients meeting the case definition were asked to give a written consent and allowed to ask for any clarifications prior to enrolment. The adults were asked to give a written consent while the children, below 18 years were asked to assent upon their parents/ guardians giving consent.

### **3.6 Exclusion criteria**

Children  $\leq 2$  years and the others who did not meet the case definition, those who failed to give consent,  $\geq 18$  years, (assent children 8 to 17 years) were excluded from the study.

### **3.7 Patients enrolment criteria**

The purpose of the study was explained to the patient before withdrawal of blood and prior to administration of questionnaire (Appendix 5,6). Systematic steps were followed through the enrolment process as described in Figure 3.2 and patient management was based on the results from the Febrile Diagnostics Rapid Kit<sup>®</sup>.



**Figure 3.2: Patient enrolment criteria**

### **3.8 Data collection**

#### **3.8.1 Samples collection and laboratory procedure**

Approximately five millilitres of blood was aseptically drawn from cephalic vein by venepuncture from each patient into a vacutainer containing clot activator. Blood samples were processed by allowing to stand in a rack for about 10 minutes before centrifuging for five minutes so as to separate blood clot from serum. Serum was used to perform the serological test at Ijara District Hospital for patient management while the clots were stored at -20<sup>0</sup>C before being transported to the Centre for Disease Control and prevention (CDC) laboratory in Nairobi for PCR assays.

#### **3.8.2 Serum agglutination test by Febrile Diagnostics Rapid Kit®**

Febrile Diagnostics Rapid Kit® was used to test for brucellosis at the Ijara District hospital. This kit allows for detection of brucella antibodies against *Brucella abortus* and *Brucella melintensis* during the acute phase of the disease. The protocol was followed according to the manufacturers' instructions (Appendix 7). About 50µl (a drop) of serum was mixed with another drop of the rapid test reagent on a white tile, and gently stirred while observing for any precipitation/agglutination, which indicated a positive reaction. Lack of precipitation/agglutination was an indicator of a negative test. Positive and negative controls from the kit were used to compare the results.

#### **3.8.3 Diagnosis of brucellosis by PCR**

To obtain Deoxy-ribonucleic acid (DNA), extraction was performed from blood clots using the QIAamp DNA Mini Kit (QIAGEN Inc, Amsterdam, Netherlands) according to the manufacturer's instructions.

About 20µl of Qiagen Protease (Proteinase K) was Pipetted into the bottom of a 1.5 ml micro centrifuge tube followed by additional of 200µl of the blood clot. About 200µl of Buffer AL (lysis buffer) was then added to the to the sample before vortexing for 15 seconds and incubating at 56°C for 10 minutes. Briefly, the mixture was centrifuged in order to remove drops from the inside of the lid. Approximately 200µl ethanol (96-100 %) was added to the sample and mixed again by pulse-vortexing for 15 seconds followed by brief centrifugation.

The mixture was then added to the QIAmp Mini spin column (in a 2ml collection tube) without wetting the rim and then centrifuged at 8000 rpm for one minute. The QIAmp Mini spin column was then placed in a clean 2ml collection tube followed by discarding the tube containing the filtrate. Carefully, the QIAmp Mini spin column was opened and about 500µl of buffer AW1 was added followed by centrifugation at 8000 rpm for one minute. The QIAmp Mini spin column was then placed in a clean 2 ml collection tube and the tube containing the filtrate discarded.

Approximately 500µl of buffer AW2 was then added carefully and the mixture centrifuged at 14,000 rpm for 3 minutes. The QIAmp Mini spin column was then placed in a clean 2ml collection tube and the tube containing the filtrate discarded. The QIAmp Mini spin column was then placed in a new 2ml collection tube and centrifuged at 14000 rpm for one minute so as to eliminate the chance of possible Buffer AW2 carryover. The QIAmp mini spin column was placed in a clean 1.5 micro centrifuge tube and discarded the collection tube containing the filtrate.

Carefully, 50µl buffer AE (Elution buffer) was added to the QIAmp Mini spin column and incubated at room temperature (15-25°C) for 5 minutes, and centrifuged at 8000 rpm for one minute. The DNA obtained was then stored at – 80°C awaiting analysis.

Real Time Polymerase Chain Reaction (Real Time-PCR) assays were performed using the AgPath-ID One-Step RT-PCR Kit (ABI, Foster City, California) and gene specific primers for Brucella as per CDC protocol. The assays were performed on the ABI 7500 Fast Real-Time PCR instrument (ABI, Foster City, California). Each reaction mix included 50µM of gene specific forward and reverse primers, 10 µM of the gene specific probe, 5µl of the DNA in a final reaction of 25 µl.

The primer and probe sequences that were used in the assay are IS711 (F) GCTTGAAGCTTGCGGACAGT, IS711 (R) GGCCTACCGCTGCGAAT and IS711 (P) AAGCCAACACCCGGCCATTATGGT. The IS711-based real-time PCR assay has been shown to be specific, highly sensitive, efficient and reproducible method for the rapid and safe detection of the genus Brucella (Bounaadja *et al.*, 2009, Wei *et al.*, 2010).

#### **3.8.4 Data collection by questionnaires and rapid kit evaluation**

Interviewer administered semi-structured pretested questionnaire were also used to collect data on demographics and possible factors associated with brucellosis. Demographic information that was recorded included name of patient, age, gender, residence, occupation, religion, and highest level of formal learning.

Data on possible risk factors for contracting brucellosis like drinking unboiled milk, handling abortuses from livestock, herding animals, assisting animals during abortion, removing after-birth or being involved in livestock slaughter was also collected.

### **3.9 Data management and statistical analysis**

Data were entered in Epi-info version 3.5.1 (CDC, Atlanta, USA) for analysis. Data cleaning and validation by correcting errors that might have occurred during data entry like duplicates, checking omissions and coding was performed. To ensure confidentiality, computer access was restricted by password protection. Data coding was done using the Statistical Package for Social Sciences ( IBM SPSS Statistics 19.0 - August 2010) while graphs were made with Excel 2007 (Microsoft Corporation, Redmond, Washington, USA ).

Brucella prevalence among sub-groups was calculated. Descriptive analysis in terms of time, place and person was done to determine frequencies and proportions. Risk factors for brucella infection were assessed by uncorrected Chi -square tests, with Fisher's exact test, applied when any expected cell counts were <5. Odds ratio was used as the measure of association where brucellosis outcome was used as the independent variable.

Variables with a P-value < 0.01 in bivariate analysis were considered significant but even those with a P value of 0.1 or less were entered into multivariate logistic regression through backward stepwise elimination method to obtain the final model of factors that were independently associated ( $p < 0.05$ ) with brucellosis.

### **3.10 Evaluation of Febrile Diagnostic Rapid Kit®**

Results from Febrile Diagnostics Rapid Kit® were compared with those of PCR in order to obtain the required parameters for evaluation, calculated using the formulas:

Sensitivity = True positive/ True positive + False negative (x 100)

Specificity = True negative/ True negative + False positive (x 100)

Predictive value positive= True positive/True positive+ False positive

Predictive value Negative= True negative/True negative+ false negative

Kappa statistics was used to assess the level of agreement between Febrile Diagnostic Rapid Kit® and PCR using the formula described by Anthony *et al.*, 2005. Ratings for agreement were interpreted according to the guidelines provided (Appendix 9).

### **3.11 Ethical considerations**

Approval to carry out the study was sought and obtained from Kenya Medical Research Institute (KEMRI) Scientific Steering Committee(SSC number 1887) and National Ethical Review Committee (ERC) (Appendix 10).The study was also approved by the Board of Postgraduate Studies of Jomo Kenyatta University of Agriculture and Technology.

## **CHAPTER FOUR**

### **4.0 RESULTS**

#### **4.1 Demographic characteristics of study participants**

A total of 384 febrile patients participated in the study with brucellosis seroprevalence of 31.8% (n=122) and the true prevalence was 15.4% (n=59). More women participated in the study with a frequency of about 61% of whom 65% were housewives and the rest were in formal set up as teachers, community health care workers, nurses, army among others. Majority of the participants were Muslims (92%) and about 93% of the participants were residents of the urban centre of Ijara District. More than half of those who took part in the study (70%) had informal occupation mainly casual jobs like fetching water, looking after livestock, offloading trucks for the relief foods and general messenger duties within the town. Approximately 46% of the patients had attained formal education either at primary level, secondary or tertiary. Majority (29%) had acquired primary education, 8% tertiary and 11% had been trained on religious protocol in the Muslim Madrasa schools. Nearly 34% had never acquired any form of education.

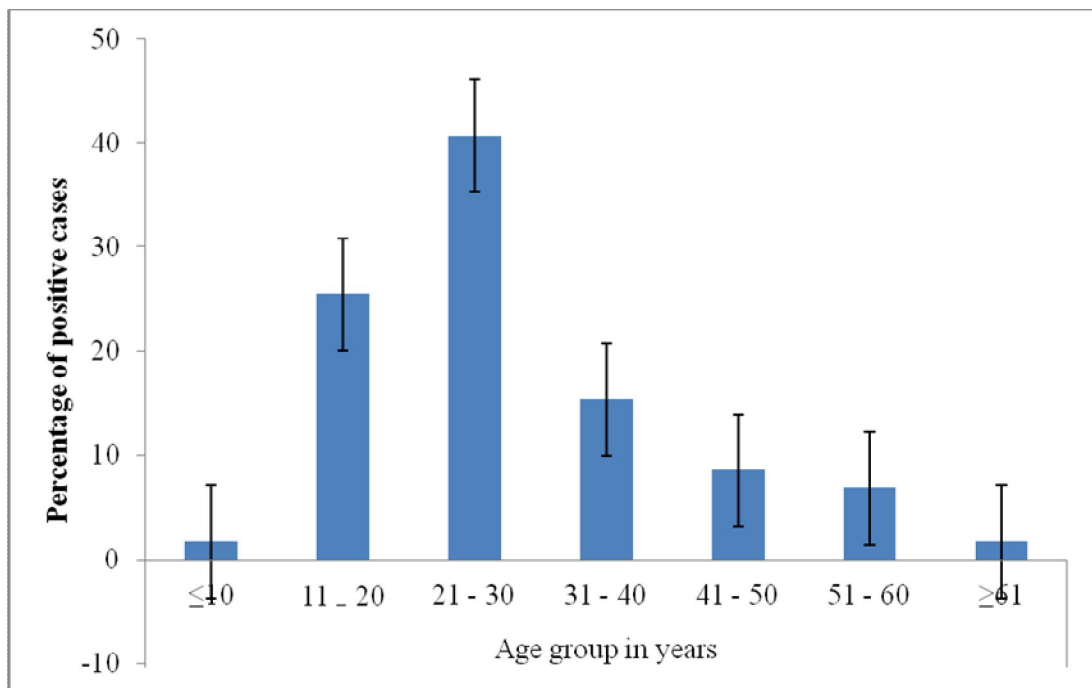
#### **4.2 Other characteristics of the study participants**

Half of the respondents had taken some medication prior to attending the hospital; of these 50% had obtained drugs from ordinary shops without prescriptions while the rest obtained drugs either from hospitals, neighbour, relative, friends or herbalists. The medications taken included paracetamol (83%), antimalarials (60%), antibiotics (31%) and herbal medicines (2%). Malaria was diagnosed in 88 (23%) of the study participants, including 19 (32%) of those positive for brucella.



### 4.3 Distribution of brucellosis cases by age groups.

Although age group was not statistically significant as a risk factor for brucellosis (P value >0.01) there were more cases among the participants aged 11- 20 and 21-30 years and least affected were those in the age groups below 11 years and over 61 years (Figure 4.1). The age range was 6 to 82 years and a mean of 29years.



**Figure 4.1 Distribution of cases by age groups (N=59)**

#### 4.2 General clinical signs and symptoms of the study participants

Headache, muscle aches, malaise and chills and fatigue were the most common clinical signs and experienced by about 50% among the participants (Figure 4.2). Brucellosis patients tended to have more chills and fatigue, though these were marginally statistically significant ( $P$  value  $> 0.01$ ). Other clinical signs and symptoms were similar among patients with and without brucellosis ( $P$  value  $> 0.01$ ).

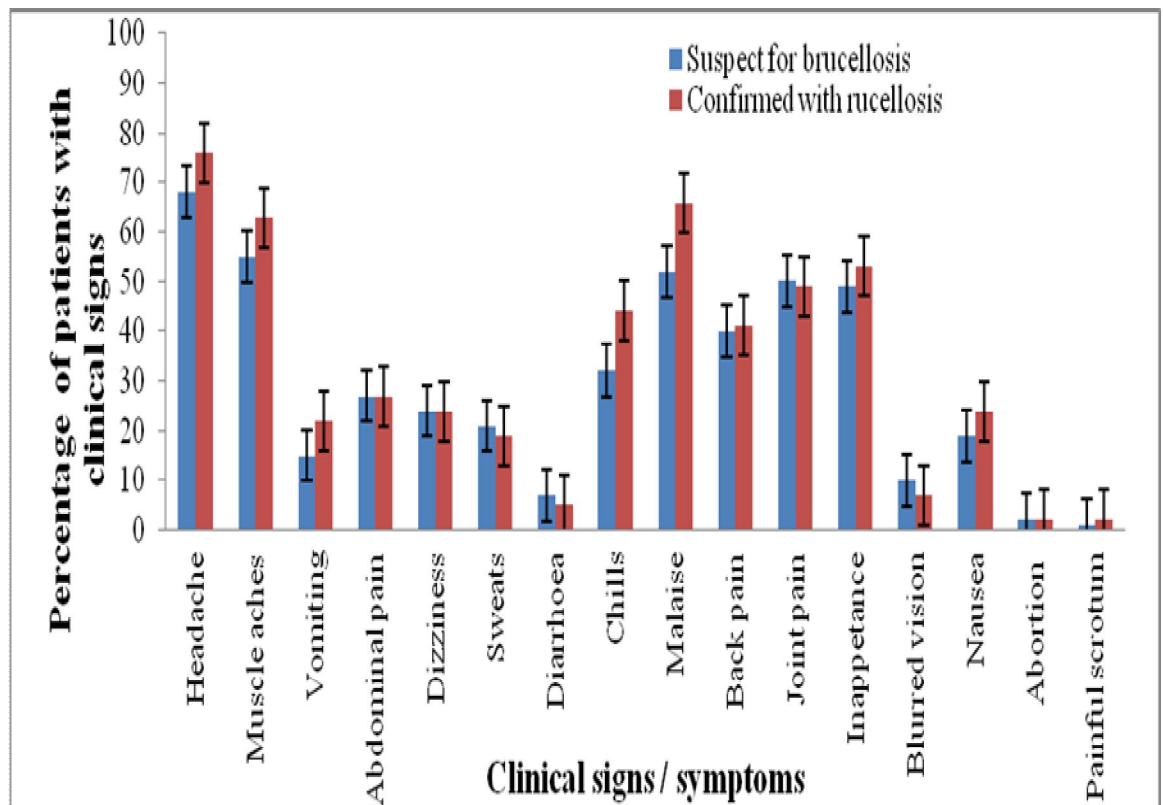


Figure 4.2: General clinical signs among the study participants

#### **4.4 Bivariate analysis**

Demographic and behavioral factors that were examined were not associated with brucellosis (P value >0.05) as measured by PCR. These included: being of any gender and age, whether one had formal or informal education, if one was a muslim or a christian or whether the respondents resided within the urban centre or came from rural areas. Among the risk factors assessed like: keeping animals at home, involvement in herding animals, handling animals that aborted recently, involvement in milking or animal slaughter and having an animal with retained placenta were all statistically insignificant as independent factors associated with brucellosis.

Two factors whose P value was  $\leq 0.01$  drunk unboiled milk (confidence interval 3.1-17.9) and obtaining unprocessed milk from the open air market (confidence interval 2.5-20.2) were significant at bivariate analysis as risk factors for brucellosis.

Participants who had handled sick animal recently had a P value of <0.1 and confidence interval ranging 0.09-0.9 and since this was marginally significant, it was incorporated in the final test model. These three factors were therefore eligible for backward stepwise elimination method at multivariate analysis level (Appendix 11).

#### **4.5 Multivariate analysis**

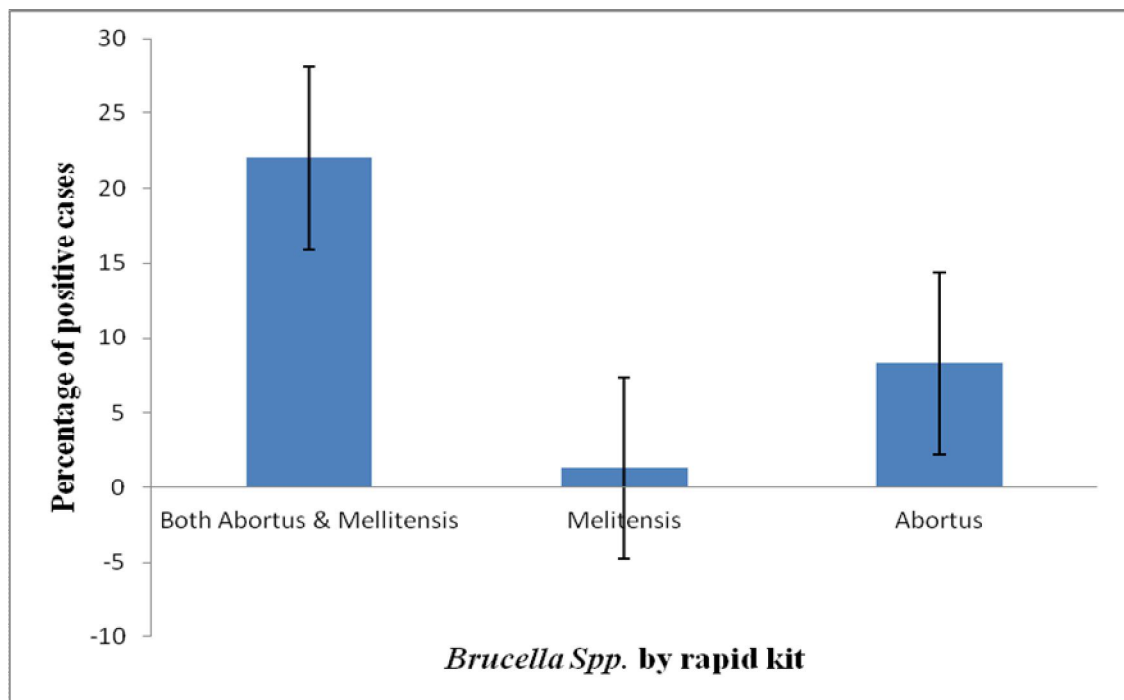
Drinking unboiled milk (OR 8.5, CI P value <0.00001) and obtaining unprocessed milk from the market (OR 7.3, P value <0.00001) were identified the factors that were independently associated with brucellosis Table 4.1

**Table 4.1: Multivariate analysis (Unconditional logistic regression\_ “Final best model”**

<b>Variable</b>	<b>OR</b>	<b>(95% CI)</b>	<b>P. value</b>
Drink unboiled milk	8.5	4.2021-17.2694	<0.00001
Source of milk (market)	7.3	2.5110-21.1051	<0.0001

#### 4.6 Evaluation of the Febrile Diagnostics Rapid Kit®

The kit used for brucellosis screening at the health facility detected a seroprevalence of 31.8% (N=122/384). Mixed infection from both *B. melitensis* and *B. abortus* was more common among patients (22%) than single infections (Figure 4.3)



**Figure 4.3: Brucella species by the rapid kit analysis**

When compared with PCR results, the sensitivity of the Febrile Diagnostics Rapid Kit<sup>®</sup> was 37% and specificity of 69%. There rapid kit diagnosed 37 false negatives and 100 patients who were false positive (Table 4.2).

**Table 4.2: Comparison of rapid kit and PCR analysis**

<b>Febrile Diagnostic kit<sup>®</sup></b>	<b>Polymerase chain reaction (PCR)</b>		<b>Total</b>
	<b>Positive</b>	<b>Negative</b>	
Positive	22	100	122
Negative	37	225	262
Total	59	325	384
	Sensitivity=37.3%	Specificity=69.2%	
	Predictive value positive =18%	Predictive value negative =86%	
Concordance = 0.03			

## CHAPTER FIVE

### 5.0 DISCUSSION

This hospital based research highlights the prevalence of brucellosis in febrile patients from a pastoral community in an urban set up of Garissa County. The prevalence discussed in this study is comparable to that described among febrile patients attending Garissa Provincial Hospital (Richards *et al.*, 2010) which is about 400km from Ijara town. Since most of the participants in both studies are mainly of Somali ethnicity, its likely that the practice of drinking unboiled milk is common and acceptable to them. Although this study did not evaluate the presence of brucellosis in livestock, its presumed that livestock could be harbouring the disease in great numbers since transmission to humans is almost always from animals (Corbel, 2006, Nicoletti, 1992). Prevalence obtained in this study is much lower than 21% established in Narok, Kenya (Muriuki *et al.*, 1997). This could be due to the fact that Muriuki *et al* evaluated for brucellosis at a wider scope investigating in more than 60 health facilities. He also worked in an area where people were directly in contact with livestock. Therefore might have been able to incorporate as many patients with diverse exposures and therefore getting such a high prevalence. In the current study, although the communities are culturally pastoralists, minimal animal contact was experienced since majority lived within the urban area while a few took the animals far away for several months in search for water and pasture.

In Kampala, Uganda, a brucellosis prevalence of 13% (Mutanda *et al.*, 1998) among febrile patients attending health facility was recorded.

This prevalence was somewhat similar to that of this current study while in Tanzania, Kunda estimated a lower prevalence of about 6.2% (Kunda *et al.*, 2007).

Although there are differences in figures obtained from these studies in neighbouring countries, it is evident that brucellosis is present in and most likely animals are also infected since about 16% of cattle in Sub-Saharan Africa are infected (Corbel, 2006, Mangen *et al.*, 2002).

Other countries in Africa have also reported brucellosis like Eritrea, Mali and Namibia with brucellosis prevalences ranging between 2 to 10%, though other studies have reported even higher rates like 35% in Tunisia (Pappas *et al.*, 2009). This could be explained probably by the high incidence and unstandardized control protocols of the disease in livestock as well as inability to appropriately manage brucellosis in humans (Corbel, 2006, Nicoletti 1992) which is contrary to practices in developed countries.

Though brucellosis has continued to be a challenge across Sub-Saharan Africa, it has also been reported in other parts of the world with prevalences varying from 4.8% in Turkey (Zafer *et al.*, 2005), 4.1% in Brazil (Ramos *et al.*, 2008) and to as high as 34.2% in Iraq (Aminzadeh *et al.*, 2010). The study in Iraq mainly looked for the disease among febrile patients who were principally abattoir workers and herdsmen and therefore this may not be much different if such a study was conducted among the Ijara herders and other animal handlers.

In this current study brucellosis infection was present across all the genders and agegroups, which corresponds with study by Mantur *et al.*, 2007.

Since milk is a staple diet among the study population, it is likely that all members of the family are exposed after consuming the same milk that is available. However other studies (Young, 2000, Mangen *et al.*, 2002) have indicated that brucellosis to be more prevalent in the males than females especially when occupational exposure is attributable.

While education is presumed to enlighten people on the way of life, level of education was not significant in this study as a protective factor for brucellosis.

This was similar to what was observed by Zafer *et al.*, 2005 who reported that level of education did not always translate to good hygienic standards. In some set-ups, like Ijara, everyone is taught to observe the culture despite how far one could be educated. Therefore, even among those who had attended school at one level or another, was exposed to drinking unboiled milk.

Clinical presentation of the illness among the study participants was similar although fatigue, chills and headache were the most clinical symptoms experienced by brucella positive cases. Indiscriminate clinical signs is a common phenomenon that has been reported from other studies (Hugh, 2000, Wafa *et al.*, 2009, Sisirak *et al.*, 2008, Maichomo *et al.*, 2000 and Mutanda, 1998). It is therefore very difficult to diagnose brucellosis clinically and requires confirmation by various laboratory tests. This becomes a big challenge to patients when the health facilities are not able to confirm diagnosis. On the other hand, it is also anticipated that most patients premedicate themselves before attending the hospital facility and therefore alleviating the severity of the clinical presentation.



In this study, Prior medication was observed in more than half of the patients similar to the study done in Tanzania by Kunda *et al.*, 2007. General shops readily sell antimalarial drugs, antibiotics and paracetamols and therefore patients find it easier and cheaper to purchase and try selfmedication. The repercussions following self medication is delayed or misdiagnosis and delayed or incorrect treatment which in turn may lead to chronic form of the disease that may not be detected by the common agglutination tests (Kunda *et al.*, 2007).

Though bacterial resistance has not been noticed by the common treatment regimes for brucellosis, (Wafa *et al.*, 2009), self medication may mask the disease and lead to more complicated forms of brucellosis like complicated respiratory disease (Georgios *et al.*,2003), osteoarticular brucellosis (Madkour *et al.*, 2005) or congenital complications in the infants in pregnancy (Ziba 2005 , Yousuf *et al.*, 2001).

Among the many risk factors that were considered in this study, obtaining unprocessed milk from the market and consuming it raw were independently associated with brucellosis a finding that compares to other studies (Geoffrey *et al.*, 2002, Kenneth *et al.*, 2009, Mutanda *et al.*, 1998). Consumption of unboiled milk could be attributed to their cultural practices in the attempt to preserve nutrients by drinking raw milk. Although this study did not establish the source of brucella organisms, it was clear that milk was the source of infection to humans. At Ijara, milk is pooled into larger five to twenty litre containers and transported from the grazing holds to the open air market, available for all residents to purchase. Milk contamination could therefore have occurred at the market level during mixing of infected and noninfected milk while selling or buying.

A study to establish the prevalence of brucella antibodies in selected markets of Kenya realized a burden of about 3% infection in marketed milk ( Kang'ethe *et al.*,2000). These findings corresponds with those of a study done in Kampala that showed market level contamination of about 12% of the milk with brucella organisms and its direct cause of disease among those who drunk it (Makita *et al.*, 2010). This also agrees with findings by Marjorie *et al.*,2008 who found contaminated milk from the market as source of infection among study participants who were urban dwellers. Even though the burden of the disease was not established in livestock, this study could indirectly be an indication of the real burden of brucellosis in livestock since about 16% of livestock in Sub-Saharan Africa are known to be infected (Corbel, 2006).

Handling aborting animals/products and slaughtering/butchering of infected animals were not statistically significant in this study although this is a known risk factor for brucellosis.A study by Kenneth *et al.*, 2009 indicated a high brucellosis prevalence among the study participants who had hanlded animals or their products in one way or the other. In this study, patients attending Ijara District Hospital were mainly urban dwellers who may not have had opportunities to directly interact with the animals as they are usually grazed away from homesteads for long periods. It is also unlikely that animal handlers would attend Ijara Hospital. Instead, they would attend nearby hospitals to where they graze and therefore if this study was done in a more widespread geographical area, to incorporate animal handlers, may be the brucellosis prevalence would even be higher and also handling animals would be an associated factor for brucellosis.

Febrile Diagnostics Rapid Kit<sup>®</sup> indicates a high seroprevalence of brucellosis with *B.abortus* more commonly occurring than *B. mellitensis* similar to findings by Wafa *et al.*, 2009. Previous studies have also shown that simple and rapid tests particularly in endemic areas are very important tools to correctly manage the disease due to its diversity in clinical signs and multispecies reservoirs (Hussein *et al.*, 2005). Although Febrile Diagnostics Rapid Kit<sup>®</sup> is rapid and simple to perform, it has very low sensitivity and specificity therefore making it overestimate seroprevalence but underestimate positivity of brucellosis. In brucella endemic areas like Ijara, it is expected that a large proportion of the population may have persistent *Brucella*-specific antibodies. The primary immunodeterminant and virulence factor for *Brucella* species is the cell wall surface lipopolysaccharide, which is antigenically similar to the lipopolysaccharide of other gram-negative rods.

This property of the bacteria, therefore increases the chances of cross reactions with antibodies due to common tropical illnesses like *Yersinia enterocolitica* and certain *Salmonella* species (Corbel, 1997) and consequently false positive test results with *Brucella* antibody tests. Consequently, results obtained by the serologic tests may be difficult to interpret and requires confirmation with *Brucella*-specific agglutination testing (CDC, 1997).

When a test is unable to correctly diagnose a disease enormous resources are wasted through incorrect treatment, loss of income through prolonged illness and consequently loss of working time. On the other hand, too much trauma is inflicted to the patients if they have to go through the full regime for brucellosis treatment, (minimum of three weeks).

Conversely, when the true positive cases are not diagnosed and treated due to low specificity of the screening kit, leads to further suffering of the patients, more expenses in incorrect treatment and progression of the disease to chronic forms or more complicated scenarios like neuro-brucellosis, respiratory system involvement, osteoarthritis, in-utero infection of the infants and complicated cardiovascular involvement and consequently mortalities.

This investigation highlights the need to confirm screening serologic test results by using established reference testing methods before committing a patient to prolonged antimicrobial therapy for brucellosis. Otherwise, overdiagnosis and overtreatment and suffering of the false negatives will continue until more specific tests are developed, based on the detection of *Brucella* antigens or on the isolation of the organism. Improvement of brucellosis diagnosis will go along way in alleviating suffering to both affected and infected since antibiotic resistance have not been reported with brucellosis.

## **5.1 CONCLUSIONS**

1. Brucellosis is prevalent in one of every six febrile patients attending Ijara District Hospital
2. Unprocessed milk from the market and consumption of unboiled milk were associated with brucellosis
3. Febrile Diagnostic Rapid Kit® underestimates positivity but overestimates prevalence of brucellosis in the febrile patients

## **5.2 RECOMMENDATIONS**

1. Patients with brucellosis should be treated to prevent further suffering and the devastating effect of the disease and the accompanying sequelae
2. Public health education programs should explain modes of transmission (milk should be boiled before consumption)
3. The Febrile Rapid Diagnostic Kit® used at the facility should be withdrawn and replaced with better rapid diagnostic tests or PCR

## **5.3 STUDY LIMITATIONS**

This study was limited by the fact that it studied fever patients in a single hospital and therefore the results may not be generalizable to general population. The study also did not test brucellosis in animals to identify source of milk contamination by Brucella organisms.

#### **5.4 PUBLIC HEALTH ACTIONS**

Findings of this study were disseminated via various meetings in order to assist in making critical decisions. It contributed to the decision to incorporate brucellosis surveillance in Integrated Disease Surveillance and Response (IDSR) technical guidelines within the Ministry of Public Health and Sanitation (MoPHS). Together with other animal data, this report also assisted the Department of Veterinary Services (DVS) to decide on recognizing brucellosis as a notifiable disease and thus development of the National strategy for control of brucellosis in livestock. Several multi-sectoral proposals for funding and collaboration have also been drawn mainly to map brucellosis in the country so as to have coordinated control and also build laboratory capacity for brucellosis in both human and animal laboratories. The study findings have also been shared in various scientific conferences and developed into a manuscript submitted to African Journal for Laboratory Medicine and it is hoped to steer up more research.

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## **APPENDICES**

### **Appendix 1: Consent form for above 18 (English)**

#### **Part A:**

##### **Title of study**

Prevalence and factors associated with brucellosis among patients attending Masalani district hospital

##### **Introduction**

Brucellosis is a zoonotic disease that is of public health importance. It is transmitted from animals to human being when people get exposed to infected livestock and their products.

##### **Purpose of the study**

We are requesting for your participation / permission for your child to participate in this study whose main objective is to determine the prevalence and factors associated with brucellosis among patients attending Masalani district hospital.

##### **Expectations of the study**

We wish to test patients who have a fever so as to determine whether they could be suffering from brucellosis. If you agree to take part in the study we will withdraw about a teaspoonful of blood from a vein in your arm which we shall test for brucellosis. We shall ask you some questions for about 10 minutes. Your brucellosis result will be provided to your doctor as soon as possible so that you can be provided with treatment if you need it.

**Risks involved in this research**

Except for minor pain, bruising and bleeding that may be a part of taking blood; there are minimal risks from being in this study.

**Research benefits**

The direct benefit from this study is that you shall be provided with rapid results of your brucellosis status without paying for it, and later we shall confirm the diagnosis without charging you or taking more blood. Indirectly, information gathered from this study will help the ministry of public health and sanitation realize the importance of brucellosis in this country (and specifically in your community) and why there's need to invest in diagnosis, treatment and control. The factors associated with brucellosis will assist the ministry of public health and sanitation to come up with health awareness messages.

This study will also help the clinicians and the community as a whole to think of brucellosis and other infections other than the ordinary malaria, typhoid and other common fevers. The results from this study will also be shared with Ministry of livestock so that they can possibly control the disease in animals since that's the source of infection to humans.

**Confidentiality**

The information collected from you will be strictly private and confidential and intended for research purpose only. Only researchers involved in the study will be allowed to work with your blood and see your information. Your name will not be used in any report of this study, publications or presentations.

The reason I am asking your name is for forwarding your results to your hospital (Masalani district hospital), whether negative or positive for brucellosis.

### **Participation information**

Participation is voluntary and it is your decision and free will to participate or not to participate in this study. If at any time you wish to withdraw from participating in this study, you can do so freely without any consequences against you.

### **Contacts and questions**

The researcher conducting this is Stella Kiambi. You may ask any questions you have now, or if you have any questions later, you are encouraged to contact her through mobile telephone number: 0724283920, P.O Box 43781-00100 Nairobi or email [gaichugi@yahoo.com](mailto:gaichugi@yahoo.com).

If you have concerns regarding your rights as a person in the study, please contact:

The Chairman,

KEMRI National Ethical Review Committee

P.O Box 54840 00200 Nairobi, Kenya

Tel: +254 20 2722541,2713349,0722205901,0733400003

Email: [info@kemri.org](mailto:info@kemri.org)

**Agreement**

Please ask any questions or clarification before you sign this form to enrol in the study.

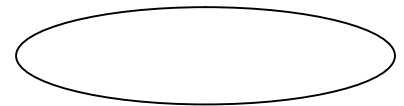
I, Mr/Miss/Mrs.....  
have been explained this study. I have had a chance to ask all questions and I have been answered adequately. I therefore give consent to Stella Kiambi to include me in the proposed study.

The risks and benefits have been explained to me. I understand that I can withdraw from the study at any time if I so wish without any consequences. I Consent voluntarily to participate in this study. I will receive a copy of this form if I require.

**I agree to join the study**

Left thumb print (For those who cannot sign)

.....



Signature

Date.....

Witness..... Date.....



## **Appendix 2 : Fomu ya makubaliano na mgonjwa (miaka 18 na kuendelea juu)**

### **Sehemu ya A**

#### **Kichwa cha Utafiti**

Kiwango na njia zinozohuzika na ugonjwa wa maziwa kwa wagonjwa wenye joto mwilini wanaohudumiwa katika hospitali ya wilaya ya Masalani.

#### **Mwanzo**

Ugonjwa wa maziwa huambukizwa kati ya wanyama na binadamu na una umuhimu sana kwa afya ya umaa. Huenezwa kutoka kwa wanyama hadi kwa binadamu punde mtu anaposhirikiana na mnyama au mazao ya mnyama aliye na viinii vya huu ugonjwa na hii ndiyo chanzo cha maambukizi.

#### **Lengo la utafiti**

Tunakuomba ujumuike nasi ama umruhuzu mwanao kujiunga nasi katika utafiti huu ambao kiini chake ni kuweza kuelewa sera na mienendo inayohuzika na ugojwa wa maziwa kati ya wagonjwa wanahudhuria hospitali ya wilaya ya Masalani.

#### **Matarajio ya utafiti**

Twatarajia kuwapima wagonjwa waliyo na joto mwilini ili tuthipitishie kama wanauguwa ugojwa wa maziwa. Iwapo utakubali kushiriki katika utafiti huu tutaweza kutoa kiasi kidogo cha damu ya yako kutoka kwa mshipa wa mkono na tutapima ugojwa wa maziwa yake. Nitakuulizwa maswali chache kwa mda usiyo sidi dakika kumi.

### **Mahangaiko ya utafiti**

Mbali na uchungu kiasi pahali shindano itandungwa ndio tutoe ndamu kidogo hatutarajii shida zingine kuhusiana na huu utafiti.

### **Manufaa ya utafiti**

Manufaa ya kwanza ni kuwa utaweza kupimwa na kupata matokeo ya haraka bila malipo ye yote, kisha hiyo damu itapimwa na kipimo iliyo juu zaidi kuhakikisha ile ripoti umepata, bila kulipa au kutolewa damu ingine. Tena, habari zitakazo sanywa kutokana na utafiti huu zitasaidia wizara ya afya ya uma na usafi kuelewa umuhimu wa ugonjwa huu wa maziwa katika nchi hii na kwa nini wanahitaji kueleza zaidi katika uchunguzi, Matibabu na kuzuiya ugonjwa huu. Ripoti itakayotokana na utafiti huu itapelekwa kwa Wizara ya wanyama ndipo waweze kuzuilia huu ugonjwa kwa mifugo kwani binadamu huambukizwa kutoka kwa wanyama.

### **Kuweka siri**

Habari zozote tutapata kutoka kwako zitahifadhiwa kwa siri na zitakuwa za utafiti pekee yake. Hasitaenezwa kwa mtu mwingine. Watafiti wanaohuzika na kazi hii ndio wataruhusiwa kuchunguza damu yako na habari ulizotupatia. Jina lako halitatumika katika kuchapisha ripoti hii. Sababu ya kutaka jina lako ni wakati wa kurudisha majibu yako iwapo uko na huu ugonjwa wa maziwa au hakuna.

### **Habari za kushiriki**

Kushiriki ni kwa hiyari yako. Ni uamsi wako kushiriki au kutoshiriki katika utafiti huu. Na iwapo unataka kujiondoa kwa wakati wowote ule una uhuru wakufanya hivyo na hakuna yeyote atakushiniza wala kukuuliza kwa nini umejiondoa.

### **Mawasiliano na maswali**

Mtafiti anayeandaa utafiti huu ni Stella Kiambi. Unaweza uliza swali lolote lile ambalo linakukera kwa wakati huu ama baadaye, na unaombwa kuwasiliana naye kupitia nambari ya rununu 0724 283 920, SLP 43781 -00100 Nairobi au barua pepe [gaichugi@yahoo.com](mailto:gaichugi@yahoo.com).

Na iwapo una mchakacho wowote kuhusiana na haki zako za kibinadamu katika utafiti huu, twakuomba uwasiliane na;

Mwenyekiti,

Kamati ya maelezo ya KEMRI,

Sanduku La Posta 54840 00200,

Nairobi.

Simu: +254 20 2722541, 2713349, 0722205901, 0733400003

Barua pepe; [infor@kemri.org](mailto:infor@kemri.org)

**Makubaliano**

Tafadhali uliza maswali yoyote na ufafanisi ungependa kabla ya kukubali kushiriki katika utafiti huu.

Mimi, Bwana /Bi..... Nimeelezwa kuhuzu utafiti huu na nimepata nafasi ya kuuliza maswali yote na nimebiwa viilivyo. Sasa naomba Stella Kiambi anishirishe katika utafiti huu.

Miteko na manufaa ya kazi hii imefafanuliwa vyema kwangu. Ninaelewa kuwa naweza kujiondowa wakati wowote ule bila shinikizo yoyote. Ninajitolea kushiriki kwa hiyari yangu katika utafiti huu. Nitapokea copi ya cheti hii Iwapo nitahitaji.

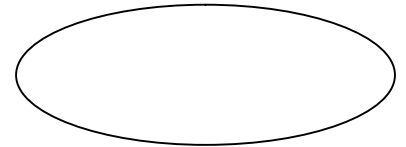
Ninakubali kujiunga katika utafiti huu

Kidole cha gumba cha kushoto

(Kwa wale ambao hawana sahihi)

.....

Sahihi



Tarehe.....

Shahidi..... Tarehe.....

### **Appendix 3 : Consent form for patients below 18 years (English)**

#### **What is the purpose of this study?**

We have sought permission from your parents/ guardian to talk to you about the study we are carrying out. Your parent /guardian has allowed us to talk to you. We are asking you if you could participate in our study on brucellosis. Brucellosis is a disease that can be acquired from animals like cow, goats, and sheep if one drinks unboiled milk or gets into contact with fluids from infected animals like when they abort or give birth.

#### **Expectations of the study**

We will take a small amount of blood from your upper arm. We will also ask your parents some questions concerning you. If you test positive for brucellosis we will guide your parents on how to get you to be treated. If your parents agree we will also keep some blood for future use.

#### **Risks in the study**

You may feel a slight pain when we stick the needle in your arm to take blood. The needle we use is clean and the amount of blood we take will not harm you. You may bleed a little bit but we will put on a bandage to prevent excessive bleeding, but this should stop almost immediately.

#### **Benefits from the study**

The results of your test will guide in understanding the presence of the disease and therefore help the doctors to treat you and give a hint about others coming with a similar problem.

The direct benefit from this study is that you shall be provided with rapid results of your brucellosis status without paying for it, and later we shall confirm the diagnosis without charging you or taking more blood. The factors associated with brucellosis will assist the ministry of public health and sanitation to come up with health awareness messages.

This study will also help the clinicians and the community as a whole to think of brucellosis and other infections other than the ordinary malaria, typhoid and other common fevers. The results from this study will also be shared with Ministry of livestock so that they can possibly control the disease in animals since that's the source of infection to humans.

### **Questions about the study**

If you have any questions we can answer them now and in case you need to ask more questions later I have given your parent/ proxy our contacts.

### **Confidentiality**

The information collected from you will be strictly private and confidential and intended for research purpose only. Only researchers involved in the study will be allowed to work with your blood and see your information. Your name will not be used in any report of this study, publications or presentations. The reason I am asking your name is for forwarding your results to your hospital (Masalani district hospital), whether negative or positive for brucellosis.

**Freedom to participate**

We do not want you in the study by force. You are free not to participate if you don't feel like.

Would like to participate in this study? Please sign this form in presence of your gurdian or your gurdian can sign it on your behalf.

**Yes**

**No**

Sign .....Date.....

#### **Appendix 4: Fomu ya makubaliano watoto miaka 17 na chini**

##### **Ni nini kiini cha utafiti huu?**

Tumepewa ruhusa na wazazi wako tuweze kukufafanulia kuhusu utafiti huu tunaofanya. Wazazi wako wameturuhusu kuongea nawe. Tunakuuliza kama utaweza kushiriki katika utafiti huu ama la. Ugonjwa wa maziwa huambukizwa kutoka kwa wanyama kama Ng'ombe, Mbuzi, na Kondoo kufatia kunywa maziwa ambayo haijachemshwa ipasavyo kutoka kwa wanyama walio na viini vya ugonjwa huu. Na pia kushikashika maji yatokayao kwa mwili wa mnyama aliyeafya mimba ama kuzaa.

##### **Matarajio ya utafiti**

Tutachukuwa kiasi kidogo cha damu kutoka kwa mshipa wa mkono na pia tutakuuliza maswali kuhusiana na wewe. Kama baada ya kupimwa utapatikana kuwa na viini vya ugonjwa huu tutawaeleza wazazi wako kupitia kwa daktari jinzi ya kukusaidia upate matibabu. Na iwapo wazazi wako watakubali pia tutahifadhi damu hii kwa matumizi ya baadaye

##### **Mapampano ya utafiti**

Utahisi chungu kidogo wakati damu inatolewa kutoka kwa mshipa wako. Sindano inayotumiwa ni safi na haina madhara yeyote kwako. Kiasi cha damu tutakayotoa haina madhara kwako.

##### **Manufaa ya utafiti huu**

Manufaa ya kwanza ni kuwa utaweza kupimwa na kupata matokeo ya haraka bila malipo ye yote, kisha hiyo damu itapimwa na kipimo iliyo juu zaidi kuhakikisha ile ripoti umepata, bila kulipa au kutolewa damu ingine.



Tena, habari zitakazo sanywa kutokana na utafiti huu zitasaidia wizara ya afya ya uma na usafi kuelewa umuhimu wa ugonjwa huu wa maziwa katika nchi hii na kwa nini wanahitaji kuelewa zaidi katika uchunguzi, Matibabu na kuzuiya ugonjwa huu. Ripoti itakayotokana na utafiti huu itapelekwa kwa Wizara ya wanyama ndipo waweze kuzuilia huu ugonywa kwa mifugo kwani binadamu huambukizwa kutoka kwa wanyama.

**Maswali kuhusiana na utafiti huu**

Kama uko na maswali yoyote tunaweza kujibu kwa sasa na iwapo unataka kuuliza zaidi baadaye nimewapatia wazazi wako mawasiliano yangu.

**Uhuru wa kushiriki**

Hatukulasimishi kushiriki bali tunakuomba ushiriki kwa hiyari yako na uko na uhuru wa kukataa kushiriki katika huu utafiti.

Ungependa kushiriki katika zoezi hili? Tafadhali tia sahihi kweny fomu hili ama msimamizi wako anaweza kutia sahihi kwa niaba yako.

Ndio  La

Sahihi.....Tarehe.....

Questionnaire No.

**Appendix 5: Questionnaire in English**

Patient ID Number .....

Name of interviewer.....

Date of interview.....

**Socio-demographic data**

Name of the patient..... Age of patient.....

Gender..... Ethnicity.....

Residence/ City/ village..... Religion.....

District..... Province.....

Telephone number..... Cell.....

1. Occupation

- a) Self-employed                      b) Formal employment
- c) Un-employed Student              d) Others (Specify).....

2. Level of highest education attained

- a) Primary school                      b) Secondary
- c) Tertially                              d) Did not attend school
- d) Other school.....

3. Marital status

- a) Married                              b) Separated
- c) Divorced                              d) Widowed

**Disease information**

1) Axillary temperature of the patient .....

2) Malaria screening:

method.....Result:.....

3) How many days have you been with fever? .....

4) Have you had any of these signs/ symptoms with the present illness?

- Muscle aches                      Abdominal pain                      dizziness
- Sweats Chills                      Malaise                      Back pain
- Fatigue                      Loss of appetite                      Headache
- Nausea                      Vomiting                      Joint pain
- Diarrhea                      Convulsions                      Blurred vision
- Spontaneous abortion (women) Swelling and pain scrotum and testicles (men)

Have you taken medicine for this disease Y/ N

If yes, Antibiotics .....

Antimalaria.....

Paracetamols.....

**Questions on possible risk factors**

1. Do you keep these animals in your home?

Cattle.....Sheep.....Goats.....dogs.....Camel.....other

(specify).....

2. Do you herd animals? Y/N

If yes what animals?

Cattle.....Sheep.....Goats.....Camel.....other

(specify).....

3. Has your animals been sick recently? Y/N

If yes, what animals?

Cattle.....Sheep.....Goats.....dogs.....Camel.....other

(specify).....

4. Have you been involved in slaughtering an animal recently? Y/N

If yes what animal?

Cattle.....Sheep.....Goats.....Camel.....other

(specify).....

5. Have you eaten meat from any animals that died or were killed because they were sick? Y/N. If yes, what animal?

Cattle.....Sheep.....Goats.....Camel.....other

(specify).....

6. Have any of your animals had abortions recently? Y/N

If yes, what animals?

Cattle.....Sheep.....Goats.....dogs.....Camel.....other

(specify).....

7. If yes, how did you dispose the abortus?

a) With bare unprotected hands.....

b) With protected hands .....

c) Other (specify).....

8. Where did you dispose the abortus?

a) In dip pit latrine                      b) In a compost pit

c) In the bush                              d) Fed to the dogs

e) Other (specify).....

9. Has any of your animals had retained after birth recently after calving?

If yes what animal?

Cattle.....Sheep.....Goats.....dogs.....Camel.....other

(specify).....

10. How was the retained after birth removed?

a) Called upon animal health worker to remove

b) Removed it myself with protected hands

c) Removed it myself with unprotected hands

d) Neighbor removed with protected hands

e) Neighbor removed with unprotected hands

f) Others (specify).....

11. What is the source of your milk?

- a) Buy from market
- b) Buy from the shop as powder or packet milk
- c) Get directly from my animals

12. Do you milk animals? Y/N

If yes what animals?

Cattle.....Sheep.....Goats.....Camel.....other

(specify).....

13. Have you drunk milk from any sick animals recently? Y / N

If yes, from what animal?

Cattle.....Sheep.....Goats.....Camel.....other

(specify).....

14. Do you boil milk before you drink? Y/N

15. If yes, how often do you boil?

- a) every time before consuming
- b) Sometimes
- c) Others (specify).....

16. If no, why?

- a) Lack of time
- b) To retain nutrient contents
- c) No need to boil
- d) Others (specify).....

Nambari ya fomu

## Appendix 6: Fomu ya mahojiano

### Kichwa cha utafiti:

Kiwango na njia zinozohuzika na ugonjwa wa maziwa kwa wagonjwa wenye joto mwilini wanaohudumiwa katika hospitali ya wilaya ya Masalani.

Kitambulisho cha mgonjwa.....	Makaazi/ Jiji/ Kijiji.....
Tarehe ya mahojiano.....	Dini.....
Jina la mgonjwa.....	Wilaya.....
Umri wa mgonjwa(miaka).....	Kabila.....
Tarehe na mwaka wa kuzaliwa.....	Mkoa.....
Jinsia.....	Nambari ya simu.....
Ukoo .....	

#### 1. Shughuli:

- |                                 |                             |
|---------------------------------|-----------------------------|
| a) Kazi ya binafsi (eleza)..... | b) Ajira rasmi (eleza)..... |
| c) Mkulima                      | d) Mwanafunzi               |
| e) Nyinginezo (eleza).....      |                             |

#### 2. Kiwango cha elimu

- |  |                   |
|--|-------------------|
| a) Shule ya msingi                       | b) Shule ya upili |
| c) Chuo                                  | d) Hujaenda shule |
| e) Inginginezo kama Madrasa (eleza)..... |                   |

3. Hali ya Ndoa

- a) umeoa/umeolewa
- b) Sijaolewa/Sijaoa
- c) Tumetengana
- d) Mjane

**Habari kuhusu ugonjwa**

1. Hali ya joto ya mgonjwa .....
2. Uchunguzi wa malaria: .....
3. Umekuwa na dalili za ugonjwa kwa muda wa siku ngapi?.....
4. Ni dalili zipi unazohisi?

- Maumivu ya misuli
- Maumivu ya tumbo
- Kizunguzungu
- Jasho chembamba
- Hisia ya unyonge
- Maumivu ya mgongo
- Uchofu
- Kukosa hamu ya kula
- Maumivu ya kichwa
- Kuchafuka roho
- Kutapika
- Maumivu ya viungo
- Kuharisha
- Kutoona vizuri
- mimba kutoka bila kusujudia (wanawake)
- uvimbe/ uchungu kwa sehemu nyeti (wanaume)

5) Umetumia tiba yeyote kwa huu ugonjwa Ndio/La

(Kama Ndio eleza) .....

- a) Dawa za malaria.....
- b) Dawa za kua viini.....
- c) Dawa za kumaliza uchungu.....



6) Ulizipata hizo dawa wapi?

- a) Hospitali
- b) Duka
- c) Jirani/Rafiki/jamaa
- d) Dakitari wa Kienyeji
- e) Nyingine
- (eleza).....

**Maswali kuhusu maambukizi**

7) Unafuga Wanyama Ndio/La?

8) Ni Wanyama wangapi umefuga kwako?

- a) Ng'ombe.....
- b) Kondoo.....
- c) Mbuzi.....
- d) Mbwa.....
- e) Ngamia.....
- f) Wengine (eleza).....

9) Maziwa ya kutumia unayatoa wapi?

- a) Sokoni
- b) Dukani (pakiti/unga)
- c) Kutoka kwa wanyama wangu

10) Unawachunga wanyama malishoni? Ndio/La

kama Ndio wanyama aina gani?

- a) Ng'ombe.....
- b) Kondoo.....
- c) Mbuzi.....
- d) Mbwa.....
- e) Ngamia.....
- f) Wengine (eleza).....

11) Kuna mnyama yeyote amekuwa mgonjwa hivi karibuni?

kama ndio ni mnyama yupi? Ndiyo/La

- a) Ng'ombe.....
- b) Kondoo.....
- c) Mbuzi.....
- d) Mbwa.....
- e) Ngamia.....
- f) Wengine (eleza).....

12) Umechinja manyama yeyote hivi karibuni? Ndio/La

Kama Ndio mnyama yupi

- a) Ng'ombe.....
- b) Kondoo.....
- c) Mbuzi.....
- d) Ngamia.....
- e) Wengine (eleza).....

13) Misoga ya wanyama wagonjwa hupelekwa wapi?

- a) Kuuzwa kwa mikahawa
- b) Inauziwa majirani
- c) Inarushwa/ inasikwa/inachomwa/inarushwa kwa msitu
- d) Inakawanywa kiholela

14) Umekula nyama yeyote itokanayo na mynma aliyechinjwa sababu ya ugojwa? Ndiyo/La

Kama ndio ni mnyama yupi?

- a) Ng'ombe.....
- b) Kondoo.....
- c) Mbuzi.....
- d) Ngamia.....
- e) Wengine (eleza).....

15) Kuna Mnyama yeyote ametoa mimba hivi karibuni? Ndio/La

Kama ndio ni mnyama yupi?

- a) Ng'ombe.....
- b) Kondoo.....
- c) Mbuzi.....
- d) Mbwa.....
- e) Ngamia.....
- f) Wengine (eleza).....

16) Umemusaidia mnyama yeyote anapotoa mimba, anajifungua ama kutoa mabaki ya kujifungua? Ndiyo/La

17) Kama Ndio eleza jinsi unavyofanya kazi hii

- a) Mikono kafu bila kinga.....
- b) Unatumia kinga kwa mikono
- c) nyingine (eleza).....

18) Hupeleka wapi vijuzi/ mabaki ya kutoka mimba?

- a) Unatupa bila kufaa kinga kwa mikono.....
- b) Unatupa kama umefaa kinga kwa mikono.....
- c) unawapa mbwa kama chakula
- d) Nyingine (eleza).....

19) Kuna mnyama yeyote ameshindwa kutoa mabaki ya uzazi baada ya kuzaa?

Ndio/La

Kama ndio ni mnyama yupi?

- a) Ng'ombe.....
- b) Kondoo.....
- c) Mbuzi.....
- d) Ngamia.....
- e) Wengine (eleza).....

20) Vipi mabaki haya yalitolewa?

- a) Ilitolewa na dakitari wa mifugo
- b) Mimi mwenyewe nilitoa
- c) Nilisaidiwa na jirani/rafiki kutoa
- d) Mbinu Nyinginezo (eleza).....

21) Unakamua wanyama wako? Ndiyo/La

Kama ndio ni mnyama yupi?

- a) Ng'ombe.....
- b) Kondoo.....
- c) Mbuzi.....
- d) Ngamia.....
- e) Wengine (eleza).....

22) Umekunya maziwa kutoka kwa mnyama mgonjwa hivi karibuni? Ndiyo/La

Kama ndio ni mnyama yupi?

- a) Ng'ombe.....
- b) Kondoo.....
- c) Mbuzi.....
- d) Ngamia.....
- e) Wengine (eleza).....

23) Unachemsha maziwa kabla ya kutumia? Ndio/La

Kama ndiyo huchemsha vipi?

- a) Kila wakati kabla ya kutumia
- b) Wakati mchache
- c) Hutumia bila kuchemsha
- d) Nyingine (eleza).....

24) Kama hauchemshi maziwa kabla ya kutumia ni kwa nini?

- a) Ukosefu wa mda
- b) Kuihifadhi maadini
- c) Hakuna haja ya kuchemsha
- d) Ingingine (eleza).....

**Asante sana kwa kushiriki katika utafiti huu.**

## Annex 7: Protocol for rapid kit analysis

**FEBRILE SERODIAGNOSTICS**  
**AGGLUTINATION FOR SLIDE AND TUBE TESTS**

INDIVIDUAL FEBRILE SUSPENSIONS		
STORE AT 2-8°C		
FOR IN-VITRO DIAGNOSTIC USE ONLY		

**Principle:**  
Salmonella Febrile Antigens are standardised suspensions of stained bacteria prepared for the rapid detection and semi-quantitation of serum antibodies developed during the acute stage of the disease. The antigens agglutinate in the presence of the homologous antibodies in the sample tested.

**Presentation:**

Contents	Code	Quantity
Salmonella Typhi O	FEBSTO05	5ml
Salmonella Paratyphi AH	FEBSAH05	5ml
Salmonella Paratyphi BH	FEBSBH05	5ml
Salmonella Paratyphi CH	FEBSCH05	5ml
Salmonella Typhi O	FEBSTO05	5ml
Salmonella Paratyphi AO	FEBSAO05	5ml
Salmonella Paratyphi BO	FEBSBO05	5ml
Salmonella Paratyphi CO	FEBSCO05	5ml
Brucella Abortus	FEBBAB05	5ml
Brucella Melitensis	FEBBME05	5ml
Proteus OXK	FEPCK05	5ml
Proteus OX2	FEPCK205	5ml
Proteus OX19	FEPCK905	5ml
Polyvalent Positive Ctrl	FEBPC001	
Polyvalent Negative Ctrl	FEBNC001	

**Composition:**  
Salmonella Febrile Antigens      Blue stained Antigens specific to somatic 'O' antigens.  
Red stained Antigens specific to somatic flagellar 'H' antigens

Positive Control      Human Serum  
Negative Control      Sodium Azide 0.95g/L.  
Animal Serum  
Sodium Azide 0.95g/L.

**Storage:**  
Store components at 2-8°C.

**Samples:**

- Serum stable for 7 days at 2-8°C.
- Samples should be free from contamination, haemolysis and Lipemia.

**Additional Equipment:**  
Glass Slides and a Mechanical Rotator set at 100 r.p.m.

**Qualitative Test Procedure:**

1. Bring the reagents and samples to room temperature.
2. Place 50µl or one drop of the sample and 1 drop of each control into separate circles on the card.
3. Resuspend the antigen gently.
4. Add one drop of the latex reagent to each circle next to the sample which is to be tested.
5. Mix with the disposable pipette / stirrer and spread over the entire area enclosed by the ring. Use a new stirrer for each sample.
6. Rotate the cards at 100 r.p.m. for 2 minutes.

**Semi-Quantitative Procedure:**

1. Using a semi-automatic pipette, dispense the following quantities of undiluted patient serum to 5 test circles:

Circle 1	80µl
Circle 2	40µl
Circle 3	20µl
Circle 4	10µl
Circle 5	5µl

2. Add 1 drop of Febrile Antigen Suspension to each circle.
3. Mix well using a pipette / stirrer.
4. Rotate the slide by hand or on a mechanical rotator at 100 r.p.m. for 2 minutes.
5. Agglutination in any of the circles is indicative of the following results:

April 2000 Manufactured in U.K.

### Appendix 8: Kappa test for evaluation of concordance of the tests

$P_e = [(n_1/n) \times (m_1/n)] + [(n_o/n) \times (m_o/n)]$ , Where;

$P_e$  = Expected agreement (how much agreement would be expected to be present by chance alone)

$P_o$  = Observed agreement (how much agreement is actually present)

$n_1$  = No. of times Febrile Diagnostic Rapid Kit<sup>®</sup> indicate positive results

$n_o$  = No. of times Febrile Diagnostic Rapid Kit<sup>®</sup> indicate negative results

$m_1$  = No. of times PCR (Gold standard test) results are positive

$m_o$  = No. of times PCR (Gold standard test) results are negative

$n$  = Total number of samples

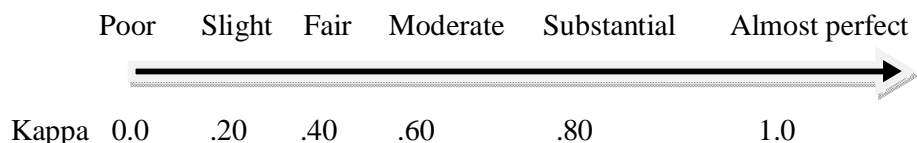
To calculate for the level of agreement (K) between the two tests:

Kappa,  $K = (p_o - p_e) / (1 - p_e)$ , Where;

$P_o$  = observed agreement

$P_e$  = expected agreement

#### Interpretation of Kappa



Kappa Agreement

< 0 Less than chance agreement

0.01–0.20 Slight agreement

0.21– 0.40 Fair agreement

0.41–0.60 Moderate agreement

0.61–0.80 Substantial agreement

0.81–0.99 Almost perfect agreement

## Appendix 9: Ethical clearance letter



# KENYA MEDICAL RESEARCH INSTITUTE

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E-mail: director@kemri.org info@kemri.org Website: www.kemri.org

KEMRI/RES/7/3/1

January 10, 2011,

**TO: STELLA KIAMBI (PRINCIPAL INVESTIGATOR)  
FELTP, KENYA**

**THRO': DR. SAMUEL KARIUKI,  
THE DIRECTOR, CMR,  
NAIROBI**

*Forwarded 12/1/2010*

**RE: SSC PROTOCOL NO. 1887 (RE-SUBMISSION): PREVALENCE AND  
FACTORS ASSOCIATED WITH BRUCELLOSIS AMONG FEBRILE  
PATIENTS ATTENDING MASALANI DISTRICT HOSPITAL, KENYA.**

Make reference to your letter dated December 8, 2010 received on December 15, 2010. Thank you for your response to the issues raised by the Committee. This is to inform you that the issues raised during the 184<sup>th</sup> meeting of the KEMRI/ERC meeting held on 23<sup>rd</sup> November 2010, have been adequately addressed.

Due consideration has been given to ethical issues and the study is hereby granted approval for implementation effective this **10<sup>th</sup> day of January 2011**, for a period of twelve (12) months.

Please note that authorization to conduct this study will automatically expire on **9<sup>th</sup> January 2012**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **15<sup>th</sup> September 2011**.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the ERC prior to initiation. You may embark on the study.

Yours sincerely,

*RSTKithinji*

**Caroline Kithinji,  
FOR: SECRETARY,  
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE**

## Appedix 10 :Multivariate analysis

### *Step 1 Unconditional logistic regression*

Factor	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
Age most active	0.5953	0.2507	1.4136	-0.5187	0.4413	-1.1755	0.2398
Keeping animals	0.8410	0.4283	1.6516	-0.1731	0.3443	-0.5028	0.6151
Drink unboiled milk	<u>9.7657</u>	<u>4.6498</u>	<u>20.5102</u>	2.2789	0.3786	6.0192	<u>0.0000</u>
Handled sic animals recently	0.3886	0.1057	1.4291	-0.9451	0.6644	-1.4226	0.1549
Source of milk (Market)	<u>5.3236</u>	<u>1.7582</u>	<u>16.1191</u>	1.6721	0.5652	2.9583	<u>0.0031</u>
CONSTANT	*	*	*	1.8784	0.7567	2.4825	<u>0.0130</u>

Keeping animals was eliminated in stage 2 since it had the highest p value; see table below:

### *Step 2 Unconditional Logistic Regression*

Factor	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
Age most active	0.6722	0.2946	1.5336	-0.3973	0.4209	-0.9439	0.3452
Drink unboiled milk	<u>8.5951</u>	<u>4.2262</u>	<u>17.4802</u>	2.1512	0.3622	5.9394	<u>0.0000</u>
Handled sic animals recently	0.3690	0.1029	1.3235	-0.9968	0.6516	-1.5298	0.1261
Source of milk (Market)	<u>5.9216</u>	<u>2.0090</u>	<u>17.4538</u>	1.7786	0.5515	3.2250	<u>0.0013</u>
CONSTANT	*	*	*	1.7309	0.7335	2.3598	<u>0.0183</u>

Age most active was eliminated in step 3 due to higher p value than the others; see table below:



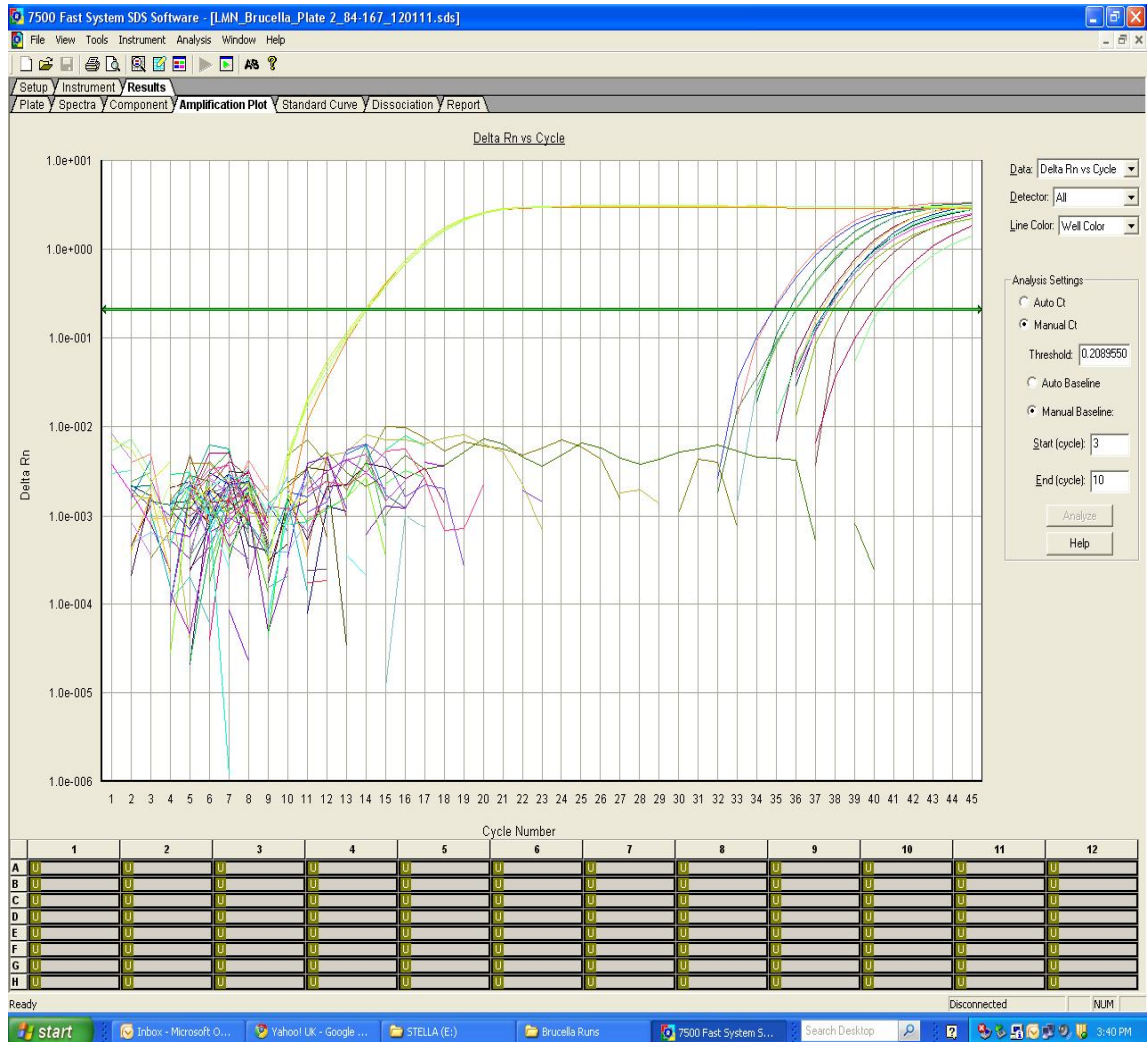
*Step 3 Unconditional Logistic Regression*

<b>Factor</b>	<b>Odds Ratio</b>	<b>95%</b>	<b>C.I.</b>	<b>Coefficient</b>	<b>S. E.</b>	<b>Z-Statistic</b>	<b>P-Value</b>
<b>Drink unboiled milk</b>	<u>8.6363</u>	<u>4.2505</u>	<u>17.5473</u>	2.1560	0.3617	5.9606	<u>0.0000</u>
<b>Handled sic animals recently</b>	0.3692	0.1033	1.3203	-0.9963	0.6501	-1.5325	0.1254
<b>Source of milk (Market)</b>	<u>6.1684</u>	<u>2.1015</u>	<u>18.1057</u>	1.8194	0.5494	3.3118	<u>0.0009</u>
<b>CONSTANT</b>	*	*	*	1.4013	0.6398	2.1904	<u>0.0285</u>

Handling sick animals recently was further eliminated due to higher p value; see table below

<b>Factor</b>	<b>Odds Ratio</b>	<b>95%</b>	<b>C.I.</b>	<b>Coefficient</b>	<b>S. E.</b>	<b>Z-Statistic</b>	<b>P-Value</b>
<b>Drink unboiled milk</b>	<u>8.5187</u>	<u>4.2021</u>	<u>17.2694</u>	2.1423	0.3606	5.9416	<u>0.0000</u>
<b>Source of milk (Market)</b>	<u>7.2797</u>	<u>2.5110</u>	<u>21.1051</u>	1.9851	0.5431	3.6552	<u>0.0003</u>
<b>CONSTANT</b>	*	*	*	0.4805	0.1884	2.5498	<u>0.0108</u>

## Appendix 11: A sample PCR run



## Appendix 12: Results for the participants PCR runs

*Plate Type: Absolute Quantification*

User: Lnderitu

Operator: Stella Kiambi

Last Modified: January 2011

Tuesday 18 13:21:22

Instrument Type: Applied Biosystems 7500 Fast Real-Time PCR System

Comments:

SDS v1.4

Thermal Cycler Profile

Stage Repetitions Temperature Ramp Rate

9600 Emulation Mode

PCR Volume: 0 µL

Sample Name	Detector	Ct	Sample Name	Detector	Ct
MAS/F/207	Brucella	29.42	MAS/M/115	Brucella	37.25
MAS/M/164	Brucella	31.54	MAS/M/112	Brucella	37.28
MAS/M/165	Brucella	32.11	MAS/M/375	Brucella	37.34
MAS/F/1	Brucella	32.29	MAS/M/315	Brucella	37.36
MAS/F/158	Brucella	34.48	MAS/M/092	Brucella	37.82
MAS/F/169	Brucella	34.74	MAS/M/172	Brucella	37.86
MAS/F/148	Brucella	34.81	MAS/F/072	Brucella	38.19
MAS/M/107	Brucella	35.1	MAS/F/061	Brucella	38.2
MAS/F/080	Brucella	35.48	MAS/F/345	Brucella	38.5
MAS/F/087	Brucella	35.51	MAS/M/111	Brucella	37.5
MAS/F/131	Brucella	35.55	MAS/M/162	Brucella	37.53
MAS/F/090	Brucella	35.6	MAS/F/096	Brucella	38.56
MAS/F/174	Brucella	35.99	MAS/F/120	Brucella	37.42
MAS/F/109	Brucella	36.01	MAS/F/071	Brucella	38.58
MAS/F/124	Brucella	36.02	MAS/M/170	Brucella	38.59
MAS/F/215	Brucella	36.02	MAS/F/130	Brucella	38.62
MAS/F/078	Brucella	36.45	MAS/M/135	Brucella	38.76
MAS/F/275	Brucella	36.53	MAS/M/189	Brucella	38.81
MAS/F/088	Brucella	36.58	MAS/F/074	Brucella	38.98
MAS/F/059	Brucella	36.68	MAS/F/136	Brucella	39.09
MAS/F/082	Brucella	37.07	MAS/M/077	Brucella	39.18
MAS/F/194	Brucella	37.12	MAS/M/144	Brucella	39.26
MAS/M/133	Brucella	37.15	MAS/F/083	Brucella	39.36
MAS/M/084	Brucella	37.23	MAS/M/123	Brucella	39.95

<b>Sample Name</b>	<b>Detector</b>	<b>Ct</b>	<b>Sample Name</b>	<b>Detector</b>	<b>Ct</b>
MAS/M/182	Brucella	40.08	MAS/F/141	Brucella	NEG
MAS/F/139	Brucella	40.22	MAS/F/142	Brucella	NEG
MAS/F/041	Brucella	41.95	MAS/F/143	Brucella	NEG
MAS/F/002	Brucella	36.28	MAS/F/149	Brucella	NEG
MAS/F/003	Brucella	35.84	MAS/F/151	Brucella	NEG
MAS/F/004	Brucella	38.59	MAS/F/153	Brucella	NEG
MAS/F/048	Brucella	NEG	MAS/F/154	Brucella	NEG
MAS/F/051	Brucella	NEG	MAS/F/155	Brucella	NEG
MAS/F/053	Brucella	NEG	MAS/F/156	Brucella	NEG
MAS/F/053	Brucella	NEG	MAS/F/157	Brucella	NEG
MAS/F/054	Brucella	NEG	MAS/F/159	Brucella	NEG
MAS/F/059	Brucella	NEG	MAS/F/168	Brucella	NEG
MAS/F/061	Brucella	NEG	MAS/F/171	Brucella	NEG
MAS/F/062	Brucella	NEG	MAS/F/173	Brucella	NEG
MAS/F/066	Brucella	NEG	MAS/F/176	Brucella	NEG
MAS/F/067	Brucella	NEG	MAS/F/177	Brucella	NEG
MAS/F/069	Brucella	NEG	MAS/F/178	Brucella	NEG
MAS/F/070	Brucella	NEG	MAS/F/180	Brucella	NEG
MAS/F/073	Brucella	NEG	MAS/F/181	Brucella	NEG
MAS/F/075	Brucella	NEG	MAS/F/183	Brucella	NEG
MAS/F/086	Brucella	NEG	MAS/F/184	Brucella	NEG
MAS/F/089	Brucella	NEG	MAS/F/185	Brucella	NEG
MAS/F/091	Brucella	NEG	MAS/F/186	Brucella	NEG
MAS/F/095	Brucella	NEG	MAS/F/187	Brucella	NEG
MAS/F/098	Brucella	NEG	MAS/F/188	Brucella	NEG
MAS/F/099	Brucella	NEG	MAS/F/190	Brucella	NEG
MAS/F/100	Brucella	NEG	MAS/F/193	Brucella	NEG
MAS/F/101	Brucella	NEG	MAS/F/195	Brucella	NEG
MAS/F/106	Brucella	NEG	MAS/F/196	Brucella	NEG
MAS/F/117	Brucella	NEG	MAS/F/197	Brucella	NEG
MAS/F/118	Brucella	NEG	MAS/F/198	Brucella	NEG
MAS/F/119	Brucella	NEG	MAS/F/199	Brucella	NEG
MAS/F/121	Brucella	NEG	MAS/F/200	Brucella	NEG
MAS/F/122	Brucella	NEG	MAS/F/201	Brucella	NEG
MAS/F/125	Brucella	NEG	MAS/F/204	Brucella	NEG
MAS/F/126	Brucella	NEG	MAS/F/210	Brucella	NEG
MAS/F/127	Brucella	NEG	MAS/F/212	Brucella	NEG
MAS/F/129	Brucella	NEG	MAS/F/213	Brucella	NEG
MAS/F/132	Brucella	NEG	MAS/F/216	Brucella	NEG
MAS/F/138	Brucella	NEG	MAS/F/217	Brucella	NEG
MAS/F/140	Brucella	NEG	MAS/F/218	Brucella	NEG

<b>Sample Name</b>	<b>Detector</b>	<b>Ct</b>	<b>Sample Name</b>	<b>Detector</b>	<b>Ct</b>
MAS/F/220	Brucella	NEG	MAS/F/291	Brucella	NEG
MAS/F/223	Brucella	NEG	MAS/F/293	Brucella	NEG
MAS/F/225	Brucella	NEG	MAS/F/296	Brucella	NEG
MAS/F/227	Brucella	NEG	MAS/F/298	Brucella	NEG
MAS/F/235	Brucella	NEG	MAS/F/299	Brucella	NEG
MAS/F/236	Brucella	NEG	MAS/F/300	Brucella	NEG
MAS/F/237	Brucella	NEG	MAS/F/301	Brucella	NEG
MAS/F/238	Brucella	NEG	MAS/F/304	Brucella	NEG
MAS/F/239	Brucella	NEG	MAS/F/305	Brucella	NEG
MAS/F/242	Brucella	NEG	MAS/F/306	Brucella	NEG
MAS/F/243	Brucella	NEG	MAS/F/307	Brucella	NEG
MAS/F/244	Brucella	NEG	MAS/F/308	Brucella	NEG
MAS/F/246	Brucella	NEG	MAS/F/311	Brucella	NEG
MAS/F/247	Brucella	NEG	MAS/F/314	Brucella	NEG
MAS/F/252	Brucella	NEG	MAS/F/317	Brucella	NEG
MAS/F/253	Brucella	NEG	MAS/F/318	Brucella	NEG
MAS/F/255	Brucella	NEG	MAS/F/319	Brucella	NEG
MAS/F/256	Brucella	NEG	MAS/F/320	Brucella	NEG
MAS/F/258	Brucella	NEG	MAS/F/321	Brucella	NEG
MAS/F/259	Brucella	NEG	MAS/F/322	Brucella	NEG
MAS/F/260	Brucella	NEG	MAS/F/323	Brucella	NEG
MAS/F/261	Brucella	NEG	MAS/F/324	Brucella	NEG
MAS/F/262	Brucella	NEG	MAS/F/325	Brucella	NEG
MAS/F/263	Brucella	NEG	MAS/F/327	Brucella	NEG
MAS/F/267	Brucella	NEG	MAS/F/328	Brucella	NEG
MAS/F/269	Brucella	NEG	MAS/F/332	Brucella	NEG
MAS/F/270	Brucella	NEG	MAS/F/334	Brucella	NEG
MAS/F/271	Brucella	NEG	MAS/F/335	Brucella	NEG
MAS/F/272	Brucella	NEG	MAS/F/337	Brucella	NEG
MAS/F/273	Brucella	NEG	MAS/F/338	Brucella	NEG
MAS/F/276	Brucella	NEG	MAS/F/339	Brucella	NEG
MAS/F/278	Brucella	NEG	MAS/F/342	Brucella	NEG
MAS/F/279	Brucella	NEG	MAS/F/343	Brucella	NEG
MAS/F/280	Brucella	NEG	MAS/F/344	Brucella	NEG
MAS/F/282	Brucella	NEG	MAS/F/346	Brucella	NEG
MAS/F/283	Brucella	NEG	MAS/F/347	Brucella	NEG
MAS/F/284	Brucella	NEG	MAS/F/348	Brucella	NEG
MAS/F/285	Brucella	NEG	MAS/F/350	Brucella	NEG
MAS/F/286	Brucella	NEG	MAS/F/351	Brucella	NEG
MAS/F/287	Brucella	NEG	MAS/F/353	Brucella	NEG
MAS/F/289	Brucella	NEG	MAS/F/355	Brucella	NEG

<b>Sample Name</b>	<b>Detector</b>	<b>Ct</b>	<b>Sample Name</b>	<b>Detector</b>	<b>Ct</b>
MAS/F/356	Brucella	NEG	MAS/M/081	Brucella	NEG
MAS/F/357	Brucella	NEG	MAS/M/085	Brucella	NEG
MAS/F/358	Brucella	NEG	MAS/M/094	Brucella	NEG
MAS/F/359	Brucella	NEG	MAS/M/097	Brucella	NEG
MAS/F/361	Brucella	NEG	MAS/M/102	Brucella	NEG
MAS/F/366	Brucella	NEG	MAS/M/103	Brucella	NEG
MAS/F/368	Brucella	NEG	MAS/M/105	Brucella	NEG
MAS/F/369	Brucella	NEG	MAS/M/108	Brucella	NEG
MAS/F/371	Brucella	NEG	MAS/M/110	Brucella	NEG
MAS/F/373	Brucella	NEG	MAS/M/113	Brucella	NEG
MAS/F/374	Brucella	NEG	MAS/M/114	Brucella	NEG
MAS/F/376	Brucella	NEG	MAS/M/116	Brucella	NEG
MAS/F/378	Brucella	NEG	MAS/M/128	Brucella	NEG
MAS/F/379	Brucella	NEG	MAS/M/129	Brucella	NEG
MAS/F/380	Brucella	NEG	MAS/M/134	Brucella	NEG
MAS/F/381	Brucella	NEG	MAS/M/137	Brucella	NEG
MAS/F/382	Brucella	NEG	MAS/M/145	Brucella	NEG
MAS/F/383	Brucella	NEG	MAS/M/146	Brucella	NEG
MAS/F/384	Brucella	NEG	MAS/M/150	Brucella	NEG
MAS/F/6	Brucella	NEG	MAS/M/152	Brucella	NEG
MAS/F028	Brucella	NEG	MAS/M/160	Brucella	NEG
MAS/M/019	Brucella	NEG	MAS/M/161	Brucella	NEG
MAS/M/025	Brucella	NEG	MAS/M/163	Brucella	NEG
MAS/M/029	Brucella	NEG	MAS/M/167	Brucella	NEG
MAS/M/030	Brucella	NEG	MAS/M/175	Brucella	NEG
MAS/M/034	Brucella	NEG	MAS/M/179	Brucella	NEG
MAS/M/036	Brucella	NEG	MAS/M/191	Brucella	NEG
MAS/M/039	Brucella	NEG	MAS/M/192	Brucella	NEG
MAS/M/040	Brucella	NEG	MAS/M/202	Brucella	NEG
MAS/M/042	Brucella	NEG	MAS/M/203	Brucella	NEG
MAS/M/044	Brucella	NEG	MAS/M/205	Brucella	NEG
MAS/M/049	Brucella	NEG	MAS/M/206	Brucella	NEG
MAS/M/050	Brucella	NEG	MAS/M/208	Brucella	NEG
MAS/M/052	Brucella	NEG	MAS/M/209	Brucella	NEG
MAS/M/055	Brucella	NEG	MAS/M/211	Brucella	NEG
MAS/M/056	Brucella	NEG	MAS/M/214	Brucella	NEG
MAS/M/060	Brucella	NEG	MAS/M/219	Brucella	NEG
MAS/M/063	Brucella	NEG	MAS/M/221	Brucella	NEG
MAS/M/064	Brucella	NEG	MAS/M/222	Brucella	NEG
MAS/M/065	Brucella	NEG	MAS/M/224	Brucella	NEG
MAS/M/076	Brucella	NEG	MAS/M/228	Brucella	NEG

<b>Sample Name</b>	<b>Detector</b>	<b>Ct</b>	<b>Sample Name</b>	<b>Detector</b>	<b>Ct</b>
MAS/M/230	Brucella	NEG	MAS/M/316	Brucella	NEG
MAS/M/231	Brucella	NEG	MAS/M/326	Brucella	NEG
MAS/M/232	Brucella	NEG	MAS/M/329	Brucella	NEG
MAS/M/233	Brucella	NEG	MAS/M/330	Brucella	NEG
MAS/M/240	Brucella	NEG	MAS/M/331	Brucella	NEG
MAS/M/241	Brucella	NEG	MAS/M/333	Brucella	NEG
MAS/M/248	Brucella	NEG	MAS/M/336	Brucella	NEG
MAS/M/249	Brucella	NEG	MAS/M/340	Brucella	NEG
MAS/M/250	Brucella	NEG	MAS/M/349	Brucella	NEG
MAS/M/254	Brucella	NEG	MAS/M/352	Brucella	NEG
MAS/M/257	Brucella	NEG	MAS/M/354	Brucella	NEG
MAS/M/264	Brucella	NEG	MAS/M/360	Brucella	NEG
MAS/M/265	Brucella	NEG	MAS/M/362	Brucella	NEG
MAS/M/266	Brucella	NEG	MAS/M/363	Brucella	NEG
MAS/M/268	Brucella	NEG	MAS/M/364	Brucella	NEG
MAS/M/274	Brucella	NEG	MAS/M/365	Brucella	NEG
MAS/M/277	Brucella	NEG	MAS/M/367	Brucella	NEG
MAS/M/281	Brucella	NEG	MAS/M/370	Brucella	NEG
MAS/M/288	Brucella	NEG	MAS/M/372	Brucella	NEG
MAS/M/290	Brucella	NEG	MAS/M/377	Brucella	NEG
MAS/M/292	Brucella	NEG	MAS/M/385	Brucella	NEG
MAS/M/295	Brucella	NEG	MAS/M/386	Brucella	NEG
MAS/M/302	Brucella	NEG	MAS/M/387	Brucella	NEG
MAS/M/303	Brucella	NEG	MAS/M/388	Brucella	NEG
MAS/M/309	Brucella	NEG	MAS/M/389	Brucella	NEG
MAS/M/310	Brucella	NEG	MAS/M/390	Brucella	NEG
MAS/M/312	Brucella	NEG	MAS/M/6	Brucella	NEG
MAS/M/313	Brucella	NEG			

Positive controls		
Sample Name	Detector	Ct
POS Ctrl	Brucella	13.68
POS Ctrl	Brucella	13.7
POS Ctrl	Brucella	13.74
POS Ctrl	Brucella	13.76
POS Ctrl	Brucella	13.87
POS Ctrl	Brucella	14.01
POS Ctrl	Brucella	14.01
POS Ctrl	Brucella	14.04
POS Ctrl	Brucella	14.05
POS Ctrl	Brucella	14.11
POS Ctrl	Brucella	14.21
POS Ctrl	Brucella	14.24
POS Ctrl	Brucella	14.26
POS Ctrl	Brucella	14.28
POS Ctrl	Brucella	14.49
POS Ctrl	Brucella	14.52
POS Ctrl	Brucella	13.82
Pos ctrl	RNP	30.75
Pos ctrl	Brucella	22.57

Negative controls		
Name	Detector	CT
NTCs	Brucella	NEG
NTCs	Brucella	NEG
NTCs	Brucella	NEG
NTCs	Brucella	NEG
NTCs	Brucella	NEG
NTCs	Brucella	NEG
NTCs	Brucella	NEG
NTCs	Brucella	NEG
NTCs	Brucella	NEG
NTCs	Brucella	NEG
NTCs	Brucella	NEG
NTCs	Brucella	NEG
Water	Brucella	NEG
Water	Brucella	NEG
Water	Brucella	NEG
Water	Brucella	NEG
Water	Brucella	NEG
Water	Brucella	NEG
Water	Brucella	NEG
Water	Brucella	NEG
Water	Brucella	NEG
Water	Brucella	NEG
Water	Brucella	NEG