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

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

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## LIST OF ABBREVIATIONS AND ACRONYMS

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<tr>
<td>µl</td>
<td>Microlitres</td>
</tr>
<tr>
<td>C/O</td>
<td>Clinical officer</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for disease control and prevention</td>
</tr>
<tr>
<td>DMOH</td>
<td>District medical officer of Health</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ERC</td>
<td>Ethical review committee</td>
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<tr>
<td>FAO</td>
<td>Food and Agricultural Organization</td>
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<tr>
<td>FELTP</td>
<td>Field Epidemiology and Laboratory Management Training Program</td>
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<tr>
<td>IEIP</td>
<td>International Emerging Infections Program</td>
</tr>
<tr>
<td>ITROMID</td>
<td>Institute of Tropical Medicine and Infectious Diseases</td>
</tr>
<tr>
<td>JKUAT</td>
<td>Jomo Kenyatta University of Agriculture and Technology</td>
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<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
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<tr>
<td>Mls</td>
<td>Millilitres</td>
</tr>
<tr>
<td>OIE</td>
<td>World health organization for animals</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PVN</td>
<td>Predictive value negative</td>
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<td>PVP</td>
<td>Predictive value positive</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>RBPT</td>
<td>Rose Bengal plate test</td>
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<td>Rpm</td>
<td>Revolutions per minute</td>
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<tr>
<td>SAT</td>
<td>Serum Tube agglutination test</td>
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<tr>
<td>SOP</td>
<td>Standard operating procedure</td>
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<tr>
<td>SSA</td>
<td>Sub-Saharan Africa</td>
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<tr>
<td>SSC</td>
<td>Scientific steering committee</td>
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<tr>
<td>STAT</td>
<td>Standard Tube agglutination test</td>
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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 sequele 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® 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® used at the facility.

About 5 millilitres of blood from each patient was drawn and analyzed by the Febrile Rapid Diagnostic Kit® 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® 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® 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® 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 unpasturized 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, spodylitis, 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 loses could be minimized (Wafa et al., 2009, Corbel, 2006).

In Kenya, animal brucellosis has been reported every year particulary 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 inorder 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 alot 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®?

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®.
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® 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 20th 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 heamagglutination 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, Yagupsy, 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 continous 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 boarders 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° 7’S 2° 3’S and longitude 40° 4’S and 41° 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, Masaii 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 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.

\[ N = \frac{Z^2 P (1-P)}{d^2} \]

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%

\[ = 384 \text{ samples} \]

**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 febrile 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°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®.

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 ≤2 years and the others who did not meet the case definition, those who failed to give consent, ≥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 throughout the enrolment process as described in Figure 3.2 and patient management was based on the results from the Febrile Diagnostics Rapid Kit®.
Patient received in the hospital

No fever or history of fever (exclude)

Fever present or history of fever (Include)

Explain study, seek and obtain consent/assent

Administer Questionnaire

Draw 5mls blood, separate clot from serum.

Test for brucellosis with Febrile Diagnostic Kit® on serum at Ijara lab

Refer patient back to the clinician with results for management

Store clot at -20°C

All samples (PCR test), at CDC lab

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 \(-20^\circ\)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 melitensis* 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 inorder 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 (Bounadja 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 inorder to obtain the required parameters for evaluation, calculated using the formulars:

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 formular 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 bout 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 fo the relief foods and general messager 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 29 years.

![Figure 4.1 Distribution of cases by age groups (N=59)](image-url)
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 ≤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”

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>(95% CI)</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drink unboiled milk</td>
<td>8.5</td>
<td>4.2021-17.2694</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Source of milk (market)</td>
<td>7.3</td>
<td>2.5110-21.1051</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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](image)
When compared with PCR results, the sensitivity of the Febrile Diagnostics Rapid Kit® 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**

<table>
<thead>
<tr>
<th>Febrile Diagnostic kit®</th>
<th>Polymerase chain reaction (PCR)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>22</td>
<td>100</td>
</tr>
<tr>
<td>Negative</td>
<td>37</td>
<td>225</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>325</td>
</tr>
</tbody>
</table>

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 form 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 herdsmen 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 availed. 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 repurcussions 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 indipendently 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 occured 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 drank 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 handled 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® indicates a high seroprevalence of brucellosis with *B. abortus* more commonly occurring than *B. melitensis* 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® 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, its 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 illment 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 very 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 sequele
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 inorder 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.
REFERENCES


OIE (2009). An approach to developing coordinated and harmonised actions for the control of brucellosis: *10th Conference of the OIE Regional Commission for the Middle East Doha, Qatar, 25-29 October 2009*

http://www.oie.int/doc/ged/D7241.PDF.


Wafa Al-Nassir (Updated April 29th 2011).


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.

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
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)

Schemu 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 wanyama wana umuhimu sana kwa afya ya umaa. Huenezwa kutoka kwa wanyama hadi kwa binadamu punu 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 uafiti huu ambao kiini chake ni kuweza kueelewa 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 tuthipitishe kama wanauguwa ugojwa wa maziwa. Iwapo utakubali kushiriki katika uafiti huu tutaweza kutoa kiazi 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 utawez akipima na kupata matokeo ya haraka bila malipo ye yote, kisha hiyo damu itapima na kipimo iliyoi juu zaidi kuhakikisha ile ripoti umepata, bila kulipa au kutolewa damu ingine. Tena, habari zitakazo sanywa kutoekana na utafiti huu zitasaidia wizara ya afya ya uma na usafi kuelelewa umuhimu wa ugonjwa huu wa maziwa katika nchi hii na kwa nini wanahitaji kuekeza za idi katika uchunguzi, Matibabu na kuzuiya ugonjwa huu. Ripoti itakayotokana na utafiti huu itapima kwa Wizara ya wanyama ndipo waweze kuzuilia hu ugonjwa kwa mifugo kwani binadamu huambukizwa kutoka kwa wanyama.

Kuweka siri


Habari za kushiriki

Kushiriki ni kwa hiyari yako. Ni uamisi 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.

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

Tafadhali uliza maswali yote 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 vilivyvo. Sasa naomba Stella Kiambi anishirishe katika utafiti huu.


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 guardian or your guardian 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 kukufanulia 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 kwa daktari jinzi ya kukusaidia upate matibabu. Na iwapo wazazi wako watakubali pia tutahifadhi damu hii kwa matumizi ya baadaye.

Mapampano ya utafiti

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 iliyoi juu zaidi kuhakikisha ile ripoti umpata, 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 hihi na kwa nini wanahitaji kuekeza 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.

<table>
<thead>
<tr>
<th>Ndio</th>
<th>La</th>
</tr>
</thead>
</table>

Sahibi..........................................Tarehe..............................
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).................................
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) Inginezo 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?
   o Maumivu ya misuli  • Kuchafuka roho
   o Maumivu ya tumbo  • Kutapika
   o Kizunguzungu  • Maumivu ya viungo
   o Jasho chembamba  • Kiharisha
   o Hisia ya unyonge  • Kutoona vizuri
   o Maumivu ya mgongo  • mimba kutoka bila kusujudia
   o Uchofu  (wanawake)
   o Kukosa hamu ya kula  • uvimbe/ uchungu kwa sehemu nyeti (wanaume)
   o Maumivu ya kichwa

5) Umetumia tiba yeyote kwa huu ugonjwa Ndio/La
   (Kama Ndio eleza) …………………………….
   a) Dawa za malaria………………………………
   b) Dawa za kuua 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 manyama yipi

a) Ng’ombe…………

b) Kondoo…………

c) Mbusi………..

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 manyama yipi?

a) Ng’ombe………..

b) Kondoo…………

c) Mbusi………..

d) Ngamia…………

e) Wengine (eleza)…..

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

Kama ndio ni manyama yipi?

a) Ng’ombe………..

b) Kondoo…………

c) Mbusi………..

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? Ndiyo/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 kuchemshsa
   d) Ingine (eleza)...........

Asante sana kwa kushiriki katika utafiti huu.
Annex 7: Protocol for rapid kit analysis

**FEVERILE SERODIAGONSTICS**
**AGGLUTINATION FOR SLIDE AND TUBE TESTS**

**INDIVIDUAL FEVERILE 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 homogeneous antibodies in the sample tested.

**Presentation:**

<table>
<thead>
<tr>
<th>Contents</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Typhi II</td>
<td>FEBS105</td>
</tr>
<tr>
<td>Salmonella Paratyphi AH</td>
<td>FEBS105</td>
</tr>
<tr>
<td>Salmonella Paratyphi BH</td>
<td>FEBS105</td>
</tr>
<tr>
<td>Salmonella Paratyphi CH</td>
<td>FEBS105</td>
</tr>
<tr>
<td>Salmonella Typhi O</td>
<td>FEBS205</td>
</tr>
<tr>
<td>Salmonella Paratyphi AO</td>
<td>FEBS3005</td>
</tr>
<tr>
<td>Salmonella Paratyphi BO</td>
<td>FEBS3005</td>
</tr>
<tr>
<td>Salmonella Paratyphi CO</td>
<td>FEBS4005</td>
</tr>
<tr>
<td>Brucella Abortus</td>
<td>FEBS8005</td>
</tr>
<tr>
<td>Brucella Melitensis</td>
<td>FEBS8005</td>
</tr>
<tr>
<td>Proteus OXK</td>
<td>FEPC1005</td>
</tr>
<tr>
<td>Proteus OX2</td>
<td>FEPC1005</td>
</tr>
<tr>
<td>Proteus OX19</td>
<td>FEPC1005</td>
</tr>
<tr>
<td>Polyvalent Positive Ctrl</td>
<td>FEBP2001</td>
</tr>
<tr>
<td>Polyvalent Negative Ctrl</td>
<td>FEBN2001</td>
</tr>
</tbody>
</table>

**Composition:**
Salmonella Febrile Antigens - Blue-stained Antigens specific to some of the antigens.
Proteus Antigens - Orange-stained Antigens specific to some of the Proteus antigens.
Brucella Antigens - Mixed for 'A' and 'H' antigens.
Positive Control - Human Serum
Negative Control - Animal Serum

**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 50uL 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: 60uL
   - Circle 2: 40uL
   - Circle 3: 20uL
   - Circle 4: 10uL
   - Circle 5: 5uL
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

\[ P_e = \frac{(n_1/n) \times (m_1/n)}{n} + \frac{(n_0/n) \times (m_0/n)}{n}, \]  
Where;

\[ P_e = \text{Expected agreement (how much agreement would be expected to be present by chance alone)} \]

\[ P_o = \text{Observed agreement (how much agreement is actually present)} \]

\[ n_1 = \text{No. of times Febrile Diagnostic Rapid Kit® indicate positive results} \]

\[ n_0 = \text{No. of times Febrile Diagnostic Rapid Kit® indicate negative results} \]

\[ m_1 = \text{No. of times PCR (Gold standard test) results are positive} \]

\[ m_0 = \text{No. of times PCR (Gold standard test) results are negative} \]

\[ n = \text{Total number of samples} \]

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

Kappa, \( K = \frac{P_o - P_e}{1 - P_e} \), Where;

\[ P_o = \text{observed agreement} \]

\[ P_e = \text{expected agreement} \]

**Interpretation of Kappa**

<table>
<thead>
<tr>
<th>Kappa Agreement</th>
<th>0.0</th>
<th>.20</th>
<th>.40</th>
<th>.60</th>
<th>.80</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
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<tr>
<td>Slight</td>
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<tr>
<td>Fair</td>
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<td></td>
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<tr>
<td>Moderate</td>
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<tr>
<td>Substantial</td>
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<td></td>
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<tr>
<td>Almost perfect</td>
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</tbody>
</table>

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

KEMRI/RES/73/1

TO: STELLA KIAMBI (PRINCIPAL INVESTIGATOR)
    FELTP, KENYA
THRO: DR. SAMUEL KARIUKI,
      THE DIRECTOR, CNR,
      NAIROBI
RE: SSC PROTOCOL NO. 1887 (RE-SUBMISSION): PREVALENCE AND
    FACTORS ASSOCIATED WITH BRUCELLOSIS AMONG FEBRILE
    PATIENTS ATTENDING MASALANI DISTRICT HOSPITAL, KENYA.

January 10, 2011,

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 134th meeting of the KEMRI/ERC meeting held on 23rd 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 10th day of January 2011, for a period of twelve (12) months.

Please note that authorization to conduct this study will automatically expire on 9th 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 15th 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,

Caroline Kitinjii,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE
Appendix 10: Multivariate analysis

**Step 1 Unconditional logistic regression**

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

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

**Step 2 Unconditional Logistic Regression**

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

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

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

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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds Ratio</th>
<th>95%</th>
<th>C.I.</th>
<th>Coefficient</th>
<th>S. E.</th>
<th>Z-Statistic</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drink unboiled milk</td>
<td>8.5187</td>
<td>4.2021</td>
<td>17.2694</td>
<td>2.1423</td>
<td>0.3606</td>
<td>5.9416</td>
<td>0.0000</td>
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<tr>
<td>Source of milk (Market)</td>
<td>7.2797</td>
<td>2.5110</td>
<td>21.1051</td>
<td>1.9851</td>
<td>0.5431</td>
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<td>0.0003</td>
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<td>CONSTANT</td>
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<td>*</td>
<td>*</td>
<td>0.4805</td>
<td>0.1884</td>
<td>2.5498</td>
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</table>
Appendix 11: A sample PCR run

![Image of a PCR analysis graph]

<table>
<thead>
<tr>
<th>Cycle Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<th>6</th>
<th>7</th>
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<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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</tr>
</tbody>
</table>

[Graph showing amplification curves for different samples over 48 cycles]
Appendix 12: Results for the participants PCR runs

*Plate Type: Absolute Quantification*

User: Lnderitu  
Operator: Stella Kiambi  
Last Modified: January 18 2011  
Tuesday 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

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Detector</th>
<th>Ct</th>
<th>Sample Name</th>
<th>Detector</th>
<th>Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAS/F/207</td>
<td>Brucella</td>
<td>29.42</td>
<td>MAS/M/115</td>
<td>Brucella</td>
<td>37.25</td>
</tr>
<tr>
<td>MAS/M/164</td>
<td>Brucella</td>
<td>31.54</td>
<td>MAS/M/112</td>
<td>Brucella</td>
<td>37.28</td>
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<td>MAS/M/165</td>
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<td>Brucella</td>
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