STUDIES ON HUMAN BRUCELLOSIS IN THE MIKUMI SELOUS ECOSYSTEM, MOROGORO, TANZANIA

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ABSTRACT

Febrile zoonotic diseases from animals such as brucellosis are common and share many clinical manifestations with other fever causing diseases like malaria. The febrile zoonotic diseases are rarely routinely diagnosed or misdiagnosed in patients presenting with feverish conditions in many of the health facilities. Brucellosis in Morogoro region may be exacerbated by a number of factors including the presence of human-livestock-wildlife interface in the Mikumi Selous Ecosystem. The influx of large livestock herds owned by pastoral enhances possibility of maintenance and persistence of brucellosis. The aims of this study were to determine the seroprevalence of brucellosis in human, to assess knowledge on brucellosis and to identify risk factors associated with transmission of *Brucella* species to human in the study area. A multistage sampling was applied in a case control cross sectional study design to select patients with fever and patients with no fever (non fever patients) in 10 selected health facilities in the catchment of Mikumi Selous Ecosystem. Brucellosis screening was carried out by Rose Bengal Plate Test (RBPT) in serum and thereafter the patients were followed at their homes for interview by using structured questionnaires. A total of 1509 fever group and 298 non fever groups were enrolled. A highly significant ($p = 0.0001$) infection rate was found in fever group 23.9% ($n = 1509$) than individuals in non-fever group 3.7%. Comparison of prevalence of brucellosis district wise showed that Mvomero district had higher prevalence (36.1%) than other districts. Brucellosis was found to be misdiagnosed as malaria, typhoid fever and venereal disease and the general community had poor knowledge about the diseases. Rural dwellers in Morogoro region mostly (49.3%) practiced self-medication whenever felt sick and some (30.1%) were using traditional healers to get health services. Contact with cattle manure, milking, contact with placenta during assisted parturition and home slaughter were the main risk factors for transmission of brucellosis by direct contact. However, drinking

of raw unpasteurized milk, undercooked or raw meat and seeping raw blood were the foodstuffs that constituted a major threat of brucellosis to the community in the study area. Findings of this study show that brucellosis is a problem and the prevalence is high necessitating prompt control measures. Control of brucellosis in animal populations, public health education and creation of awareness on dangers posed by handling animal placenta during abortion and consumption of improperly cooked foods of animal origin will be necessary measures for prevention of the disease in human.

DECLARATION

I, LAWRENCIA WANKYO JAMES, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own work and has neither been nor being concurrently submitted for a higher degree award in any other institution.

_____ _____

Lawrencia Wankyo James Date

(Msc. candidate)

The declaration is confirmed by

_____ _____

Prof. Rudovick R. Kazwala Date

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DEDICATION

This work is dedicated to lovely Marcelina, all stakeholders in this study and my young brothers and sisters Marwa Mathew James, Arkadius Wambura William, Augustine Manyinyi James, Pauline Nyamtura James, Joan Mgombwe James, Leah Nyageti and lovely Rosemary James.

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LIST OF ABBREVIATIONS AND SYMBOLS

µl	-	Micro liter
CFU	-	Colony Forming Unit
DFID	-	Department for International Department
ELISA	-	Enzyme Linked Immunosorbent Assay
FAO	-	Food and Agriculture Organization
ICONZ	-	Integrated Control of Neglected Zoonoses
IgG	-	Immunoglobin G
IgM	-	Immunoglobin M
LPS	-	Lipopolysaccharied
MAT	-	Micro plate Agglutination Test
NGO	-	Non-Government Organization
OIE	-	World Organization for Animal Health
OR	-	Odds ratio
RB 51	-	Rose Bengal 51
RBPT	-	Rose Bengal Plate Test
S19	-	Strain 19
SAT	-	Standard Tube Agglutination Test
TMP (SMZ)	-	Trimethoprism-Sulphamethoxale
UTI	-	Urine Transmitted Infection
VDRL	-	Venereal Diseases Research Laboratory
VLA	-	Veterinary Laboratory Agency
WHO	-	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Although many researches and initiatives have been carried out by various national, regional and international public health agencies to reduce the burden of infectious diseases, still emerging and re-emerging infectious diseases pose great threats and challenges to public health worldwide (Bourgarel *et al.,* 2011; Olano and Walker, 2011). Of these diseases, 60% that affect human have zoonotic background (Molyneux *et al.*, 2011) simply because human life is dependent on interactions with other creatures like livestock. More than 600 million people globally depend on livestock keeping for their livelihood in which 70% of the population lives in the most marginalized areas (Sherman, 2010).

Infectious diseases disproportionately affect poor and marginalised populations which are subjected to a cycle of ill-health (Maudlin *et al.,* 2007; Molyneux *et al.,* 2011). These include zoonoses, which are intensely entrenched in African rural agricultural systems and associated with poverty, poor farming practices and various forms of neglect such as denial of their human rights (Maudlin *et al.*, 2007). These diseases do not affect only the health of individuals but also their livelihoods by reducing productivity or even causing morbidities and mortalities of their livestock (Cleaveland *et al.*, 2001).

Despite of neglected diseases being known to affect 500 million people in sub-Saharan Africa (Gwida *et al.,* 2010); there is still dearth of information about prevalence of various diseases. These stems from the fact that many of them are neglected diseases and do not feature as priority diseases in local, regional or international agencies (WHO, 2006; Jaffary

et al., 2009). The prevalence of neglected diseases is important as it will help various agencies to accord them the needed attention.

One of such neglected diseases is brucellosis, caused by gram negative bacteria of genus *Brucella*. Brucellosis is wide spread in many developing countries and poorly diagnosed in both human and animals due to poor health facilities, diagnostic facilities and limited awareness of the disease among medical practioners (Kunda *et al.*, 2010). Its diagnosis is complicated by the fact that it shares symptoms with malaria, a common cause of fever and a leading cause of morbidity and mortality in Sub-Saharan Africa, especially in children under 5 years (Pappas *et al.*, 2006). Sharing of clinical features with malaria and other febrile conditions can likely lead to misdiagnosis and mismanagement of cases and hence perpetuating human vulnerabilities (Memish *et al.*, 2004; Bosilkovski *et al.*, 2009; Jergefa *et al.*, 2009).

The burden of brucellosis is mainly on the poor individuals as they are often forced to live in close contact with their animals and so are more likely to become infected (Kunda *et al.*, 2007). The worldwide prevalence of brucellosis in human has been reported to be more than 500,000 new cases annually (Pappas *et al.*, 2006). The disease result to prolong health problems which may cause permanent disabilities and is an important cause of travel-associated morbidity (Zinsstag *et al.*, 2007). The global epidemiology of the disease has significantly evolved over the past decade (Pappas *et al.*, 2006).

OIE (2010), reports that Tanzania is among the countries which has existence of animal and human cases of brucellosis. Prevalence in some of the pastoral and agro pastoral communities in northern Tanzania ranges from 0.7% to 13% in regions such as Manyara and Arusha (Minja, 2002; Shirima, 2005). Once the pastoralists are infected, they are less

likely to have access to and receive proper treatment because they live in remote rural areas and may not be able to afford the time and money for repeated visits to a health centre (Maudlin *et al.*, 2007). The burden of looking after a seriously ill family member may push the household further into poverty and illness or death of a breadwinner (WHO, 2006).

Public health significance of brucellosis in human is a severely debilitating disease that requires prolonged treatment with a combination of antibiotics leaving health problem and disabling results (Zinsstag *et al.*, 2007). It also results in considerable medical expenses in addition to loss of income due to reduced working hours (Zinsstag *et al.*, 2007). In livestock, brucellosis results in reduced productivity, abortions, retained placenta, metritis, infertility and weak offspring and is a major hindrance for trade and export (Kunda *et al.*, 2010).

1.2 Problem Statement and Justification

In developing countries malaria has attracted people's attention than other fever causing diseases and huge amount of resources have been invested on disease. In Tanzania and other developing countries most of health facilities misdiagnose diseases with febrile symptoms as malaria while other differential diagnoses are not considered. Human brucellosis is hardly and routinely diagnosed in African hospitals despite suggestions that the magnitudes of infections are greater than appreciated (Njoku, 1995; Rajish *et al.*, 2003).

Most health facilities in Tanzania do not test for brucellosis (Kunda *et al.*, 2005). Sparse and scanty information available from Medical department shows the disease prevalence tend to be variable (Kunda *et al.*, 2005). The paucity of data may be due to other diseases

like typhoid fever, malaria, and urinary tract infection which have similar clinical signs and are endemic and hence often diagnosed (Bax *et al.*, 2007).

Kunda *et al.* (2010), account on the awareness among the medical personnel can result into difficult in recognition of the brucellosis in human. Although some studies have been carried out in relation to diagnosis of brucellosis in Tanzania, there are still other parts where have not been carried out. This calls for enhanced detection and diagnosis capacities at the level of rural health facilities with the aim of allowing for prompt detection of brucellosis in human.

Studies by Shirima *et al.* (2003); Kunda *et al.* (2007) and Mellau (2009) report that the relationship between human-livestock-wildlife facilitates the maintenance of brucellosis. The irregular fever complains in health facilities which do not respond to anti malaria and anti typhoid drugs implicate other suffering such as brucellosis. Eating habits of animal products and limited knowledge of brucellosis are the driven aims of gathering of epidemiological information of human brucellosis in Mikumi-Selous ecosystem health facilities.

The aim of this study therefore, was to establish the prevalence of brucellosis in patients with febrile illness who reported in health facilities in Mikumi-Selous Ecosystem. This study is expected to add prevalence information of human brucellosis and the design strategic intervention for prevention and control in human-livestock-wildlife interface.

1.3 Objectives

1.3.1 Overall objective

To determine the seroprevalence of human brucellosis in human-livestock –wildlife interface in Morogoro region, Tanzania.

1.3.2 Specific objectives

i. To establish the seroprevalence of human brucellosis in patients with febrile and non febrile illness history in selected health facilities of Mikumi Selous Ecosystem, Morogoro region.

ii. To establish knowledge of brucellosis in fever cases and non fever cases of brucellosis in Mikumi Selous Ecosystem

iii. To establish risk factors associated with transmission of human brucellosis in brucella fever and non fever cases in Mikumi Selous Ecosystem.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 An Overview of Brucellosis

Brucellosis was firstly documented in Malta in 1859 (David and Arthur, 1998) and hence named Malta fever. However, others have named the disease as undulant fever, Rock of Gibraltar fever, and epizootic abortion, slinking of claves, infectious abortion and Bang's disease (Sathyanarayan *et al.,* 2011). The disease has very old history, since when organisms resembling *Brucella* was detected in carbonized cheese during the Roman era (Martson, 1861; Bruce, 1887).

2.2 Aetiology

Brucellosis is caused by gram-negative bacilli, of the genus *Brucella* (*Brucella abortus, B. suis, B. melitensis*, *B. canis, B.neomatoe* and *B.ovis*). The bacteria are facultative intracellular parasites that cause chronic disease, which usually persists for life. *Brucella* species are related to soil organisms and have prolonged survival in both hot and cold environments, particularly in moist conditions. Under favorable conditions they may survive for up to two years in the environment, thus constituting a risk to both animals and humans (HPA, 2009).

Pastures and animal houses on farms can remain contaminated for months. However, *Brucellae* are very sensitive to direct sunlight, moderately sensitive to acid and can be destroyed by pasteurization or cooking. They are also sensitive to common disinfectants used at the appropriate concentration and temperature (HPA, 2009). Under normal conditions, *Brucellae* spp. can survive in organic materials such as manure, abortion fluids and milk for up to six months. It may survive up to 8 months in an aborted fetus in the

shade (Aiello and Moses, 2010). Survival rate of *brucellae* in dairy products is related to a variety of factors such as type and age of product, moisture content, temperature, changes in pH, humidity level biological action of other bacteria present and conditions of storage. In raw milk *brucellae* at 25-37^{0}C can survive for 24 hours, at 8^{0}C can survive for 48 hours while at -40^{0}C can survive for 2.5 years (Alton *et al.,* 1975). Survival is longer when the temperature is low, particularly when it is below freezing. It should be noted that the bacteria are particularly susceptible to heat and desiccation and direct sunlight will rapidly destroy exposed organisms. All standard disinfectants destroy *Brucella* spp.

2.3 Species Affected

Brucellosis at acute or sub-acute affects many species but mostly those that produce food such as cattle, goats, sheep, pigs and wild animals. In early phase the disease may not be recognized while mature phase the disease tend to localize in reproductive system and produces placentitis followed by abortion in pregnant female animals during the last trimester. Clinical signs may not be seen unless further diagnosis is done (Sathyanarayan *et al.,* 2011).

2.4 Distribution of Brucellosis in Human

The epidemiology of brucellosis is complex and important factors that contribute to the commonest zoonotic disease worldwide. According to Pappas *et al.* (2006) the global epidemiology of human brucellosis has significantly evolved over the past decade. It is estimated worldwide that the real number of infected people is 26 times higher than the reported 500,000 new cases annually (Bosilkovoski *et al.,* 2009). The prevalence and occurrence has been varying in different areas, for example among the African countries Algeria is the leading country with brucellosis in human worldwide. The disease is also prevalent in Sub-Saharan countries and East Africa (Pappas, 2006). Other endemic areas

are Asia and South America (Memish *et al.*, 2004, Jergefa *et al.*, 2009) while European countries such as Austria, Belgium, Denmark, Finland, Germany, Luxembourg, Netherland, Norway, Switzerland, Sweden and United Kingdom have been declared to be free states of human brucellosis (Pappas *et al.*, 2006).

2.5 Sources of Infection

B. arbortus is the most common infection in cattle. Infection is introduced into clean herds through the purchase of infected animals. Lack of hygienic measures in animal husbandry, food handling, food habits and consumption of undercooked or properly cooked animal products accounts for sources of infection (Regassa *et al.*, 2009).

2.6 Risk Factors for Brucellosis Infection in Humans

Brucellosis in human is transmitted by poor hygiene, close contact with infected animals contact, and consumption of unpasteurized dairy products and undercooked meat products. For example, consumption of traditional delicacies such as infected raw liver can cause human infection. Acquiring infection through direct contact is possible to occupational groups such as veterinarians, famers, butcher men, milkers, laboratory workers and inseminators. The routes of infection are through contamination of broken skin, inhalation of aerosols containing organism and contamination of the conjunctiva or other membranes (Regassa *et al.*, 2009).

Person-to-person spread is rare, possible modes of transmission include blood transfusion, bone marrow/organ transplant, sexual, transplacental and breast feeding have been documented (Palanduz *et al.*, 2000). The increase in business and leisure travel to brucellosis-endemic countries has led to importation of the disease into non-endemic areas (OIE, 2010).

Limited knowledge on brucellosis, attitude and practices of pastoralists are risk factors which can also lead to transmission of diseases. According to Kunda *et al.* (2007) limited knowledge about the different manifestations of brucellosis may delay diagnosis and treatment resulting in further spread of the disease. Studies in Tanzania by Kunda *et al.* (2005) shows that even the health worker personnel's have low knowledge on zoonotic diseases. Unawareness of medical personnel's especially the physician can cause the diagnosis of brucellosis to be problematic (Smits and Kadri, 2005).

2.7 Status of the Disease in Humans in Tanzania

Brucellosis in Tanzania is not prioritized due to limited data on the burden of the disease, poor recognition of the disease among health personnel's, policies and resources for implementation are poor (WHO, 2006). OIE (2010) reveals that Tanzania is among the countries where brucellosis is endemic in human and animals. Presence of brucellosis in animals is an indication of the disease in human. Brucellosis in animals, has been reported in the Eastern zone with herd prevalence ranging from 12-14.1% (Shirima, *et al.*, 2004; Temba, 2012), 12.2% in Kilimanjaro (Swai *et al.*, 2005) and 15.2% in Southern zone (Otaru, 1985). A study by Karimuribo *et al.* (2007) found a sero-prevalence ranging from 0.6 % to 3.6% in Iringa and Tanga. In central Tanzania a study by Mdegela *et al.* (2004) reported a prevalence of 3.9% and 4.8% in diary and Tanzanian short horned Zebu.

The prevalence of brucellosis in human in Arusha region was reported to be 7.7% (Kunda, 2007). In another study by Mellau *et al.* (2009) reported the increase of brucellosis from 35.6% in 2004 to 58.1% in 2005 in livestock-wildlife interface in Serengeti ecosystem. Similarly, Swai (2008) reported a prevalence of brucellosis in humans of up to 5.52% in Tanga region. No study has been conducted in Morogoro regarding prevalence of brucellosis in human.

2.8 Public health implications of the disease

Brucellosis is the public health threat in animals and human. Economic importance in animal is mostly due to abortions which results into loss of calves for replacement of stock. The infection can result in retained placenta which may lead to endometritis, infertility, reduced milk production and high costs incurred in treating sick animals (Dinka *et al.*, 2009). In human, brucellosis is a problem in developing countries especially in rural areas where diagnostic facilities are lacking. Misdiagnosis and mistreatment of infected patients result in long illness and reduced ability to participate in economic activities.

2.9 Pathogenesis

Brucellae enters the body via the ingestion, conjunctival mucosa, respiratory tract, or skin and localization within regional lymph nodes. Virulent *Brucellae* have the ability to survive in both polymorphonuclear and mononuclear phagocytes and also can depress chemotaxis and phagocytosis by polymorphonuclear leucocytes (HPA, 2009).

Brucellae multiply in the lymph nodes as parasites and then enter the blood and produce the bacteraemia followed by the acute febrile phase of the disease after phagocytosis. From the blood, the organisms are distributed throughout the reticuloendothelial system and become present in large numbers in the liver and spleen. They also localize in many other sites such as joints, heart, kidneys, the central nervous system and genital tract (HPA, 2009). The response to infection may relate to the specific *Brucella* species, and the nature of the lesions produced depends upon the type of infecting organism; *B. abortus* has been related to the presence of granulomas in patient's livers (HPA, 2009).

2.10 Clinical Manifestation

The most common clinical features of brucellosis in humans include: (fever, fatigue, headache, sweating, loss of appetite, muscular pain, lumber pain and weight loss) (Habib *et al.,* 2003; Bosilkovski *et al.,* 2007 and Minas, 2007). The infection also causes focal lesions in bones, joints, genitourinary tract and other organs. Complications may include arthritis, sacroiliitis, spondylitis and disorders of the central nervous system. *Brucella* can cause abortions in women mostly in the first and second trimesters of pregnancy while in males can exhibit epididymo-orchitis (Wattam *et al*., 2009).

The onset may be sudden, over a few days, or gradual, over weeks to months with multiple and non-specific features which contribute to difficulties in the diagnosis of brucellosis in areas where diseases with similar clinical features such as malaria, tuberculosis, typhoid and joint diseases co-exist (Gul and Khan, 2007).

2.11 Diagnosis

The clinical illness of brucellosis is often nonspecific when considered in the individual patient. Therefore evaluation of patients often includes a number of tests dictated by the differential diagnosis (Deepak *et al.,* 2003). When a patient is suspected of having brucellosis, blood specimen, bone marrow and tissues aspirates can be taken for culture (Gotuzzo *et al*., 1986). Laboratory testing is a prerequisite to a proper diagnosis of human brucellosis. Recently, diagnosis is based on clinical observation, complemented by serology, culture and molecular techniques (Deepak *et al.,* 2003). Brucellosis diagnostic tests are subdivided into three groups namely demonstration of *Brucella* organisms, detection of immunoglobulin and allergic reaction dependent (Alton *et al.,* 1975).

2.11.1 Clinical Signs

Diagnosis is based on prolonged at least a week by presence of clinical signs like repeated fever, history of working with animals, food and eating habits, abortions, arthritis and epididymo-orchitis may be suggestive of a disease (David and Arthur, 1998). However, clinical signs may be misleading due to unusual cases with a typical lesions necessitating confirmation of suspected cases by laboratory tests (Corbel, 2006).

2.11.2 Culture

Culture is considered to be the "gold standard" and the definitive test for diagnosis of brucellosis (Alton *et al.,* 1975). However, the method is not common in routine diagnosis of the disease (Bax *et al.,* 2007). It involves taking appropriate samples like blood and bone marrow and culture on either basal media (e.g. Brucella medium base) or selective media (e.g. Farrell's medium) and subsequently followed by identification of the *Brucella* by macro and micro-morphology. Biochemical tests also identify *Brucella* phenotypically and enhance biotyping. Though phenotypic typing provides large numbers of isolates, their general applicability is limited by difficulty of obtaining standard antisera and phage reagents, and lack of standardization of protocols between laboratories (Akhvlediani *et al.*, 2010).

2.11.3 Serological test

A number of serological tests are commonly used in diagnosis of brucellosis in humans and animals (Corbel, 2006). Rose Bengal plate test (RBPT) is a common diagnostic test for screening of brucellosis in animal and human samples (WHO, 2006). It is very sensitive, but sometimes gives a positive result because of S19 vaccination or of false-positive serological reactions. Therefore positive reactions need to be further confirmed using other tests. Other serological tests include complement fixation test (CFT), enzyme linked

immunosorbent assays (ELISAs), fluorescence polarisation assay (FPA), brucellin skin test, serum agglutination test, milk i-ELISA and milk ring test (WHO, 2006; HPA, 2009).

The mainstay of diagnosis is the tube or standard agglutination tests (SAT), or its variant the micro agglutination test (MAT). Whilst the tests should be performed in any suitable laboratory samples and sent for confirmation to the *Brucella* Reference Unit (BRU) or the Veterinary Laboratory Agency (VLA) as appropriate (Akbarmehr and Ghiyamirad, 2011).

Conventional testing uses antigens of *B. melitensis* and *B. abortus* but serological responses do not completely distinguish between the two species. These tests cross react with *B. suis* but not with *B. canis* infection. ELISA and micro ELISA techniques are preferred in some laboratories and have comparable results and problems (Akhvlediani *et al*., 2010). Patients living in enzootic areas or exposed in their occupation have background serological positivity, affecting the diagnostic specificity of individual titers. Separation of IgG and IgM responses is only partially successful at distinguishing acute and chronic infections (Sathyanarayan *et al.,* 2011).

Biological false positive reactions occur after vaccination of Gram negative infections including *Francisella tularensis* and some *Salmonella* spp. In a non-endemic area, biological false positive serological reactions are expected to be more common than true positives. Over-reaction to such reports can provoke inappropriate bioterrorist alerts (Greenblatt *et al.,* 1999). In normal circumstances, 90% of patients with acute infection have positive serological results at clinical presentation. Most of the 10% that are negative seroconvert after a further 14 days, and the majority of the 90% that are positive at presentation will have further increase in titres after 14 days. The pattern of prolonged

serological positivity (months to years) varies both after treatment and without treatment, and is a matter for expert interpretation (Bosilkovski *et al.*, 2009).

2.11.4 Molecular methods

PCR-based techniques are potentially useful for the diagnosis of brucellosis particularly in chronic cases; however these tests are not yet in routine clinical use (Nimri, 2003). The advents of molecular biology techniques in recent years are reported to be accurate and precise for diagnosis, genotypic identification and typing methods for *Brucella*. Several molecular methods are available and are used in detection of *Brucella* DNA in samples from humans and animals (Akbarmehr and Ghiyamirad, 2011). The methods include PCR, RT- PCR, restriction fragment length polymorphism (RFLP), Southern blot and Pulse-field gel electrophoresis.

2.12 Treatment

To prevent disease progression and the development of complications, treatment should start as early as possible and also in patients show signs of spontaneous improvement. In all cases it is important that the patient finishes the full course of medication because the risk of incomplete recovery and relapse is otherwise increased considerably (Smits and Kadri, 2005). Combinations of antibiotics are recommended like100 0mg doxycycline twice a day for 6 weeks plus 1g streptomycin daily for 2 to 3 weeks. Instead of streptomycin, rifampicin may be given in combination with doxycycline (200 mg/day orally for 6 weeks) at a dose of 600-900 mg for 6 weeks (Doganay and Aygen, 2003; Deepak *et al.*, 2003).

2.13 Control of Brucellosis

Prevention, control and eradication of brucellosis are a major challenge for public health programmes. Although controlled or eradicated in a number of developed countries, re-introduction of brucellosis remains a constant threat, while in others, especially in the developing world, this disease continues to exert its devastating impact perpetuating poverty (Smits *et al.,* 2004). A lot of initiatives or strategies should be done from household to the government levels under multi-disciplinary approach to form a good base for control of the brucellosis.

Control of brucellosis in human depends much on control of the disease in animals. Currently no vaccine has been established for human brucellosis. Systematic vaccination of animals is recommended in the absence of an adequate surveillance system and where the prevalence is greater than 5% (Holveck *et al*., 2007). S19 and RB 51 vaccine increases individual resistance to systemic infection, and in infected animals decreases the probability of placental infection, abortion and massive shedding of infectious organisms.

These combined facts interact at the herd level, by improving herd immunity, to confer good overall protection, provided that individual animals are properly vaccinated. All female animals kept for reproduction should be vaccinated (Kitaly, 1984). A combined approach of systematic vaccination and test and slaughter is suggested for situations where 1-5% of animals are infected and test and slaughter alone in cases where the prevalence is less than 1%. Other control measures include control of animal movement and testing and isolation of infected animals.

In the absence of the infrastructure and technologies for the commercialization of safe milk, education is the most effective tool for prevention of brucellosis transmission to

humans (Abbas and Aldeween, 2009). Treatment (adequate boiling, fermentation) of milk at the household level is the consumer's opportunity to reduce or eliminate the risk of infection from brucellosis and other organisms that may have contaminated the milk in the processing system and distribution.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Area description

This study was conducted in Morogoro, a third largest region in Tanzania that lies between latitude 5^{o} 58" and 10^{o} 0"to the South of the Equator and longitude 35^{o} 25" and 35^{o} 30"to the East Greenwich with an area of 72, 939 square kilometers of the total Tanzania mainland (MRSEP, 2006). The region has six districts that include Morogoro Municipal, Morogoro Rural, Kilosa, Mvomero, Ulanga, and Kilombero districts at the vicinity of Mikumi National Park and Selous Game Reserve ecosystems as illustrated on (Fig.1). Morogoro is boarded by seven regions which are Dar es Salaam, Tanga, Pwani, Dodoma, Iringa, Mtwara and Ruvuma.

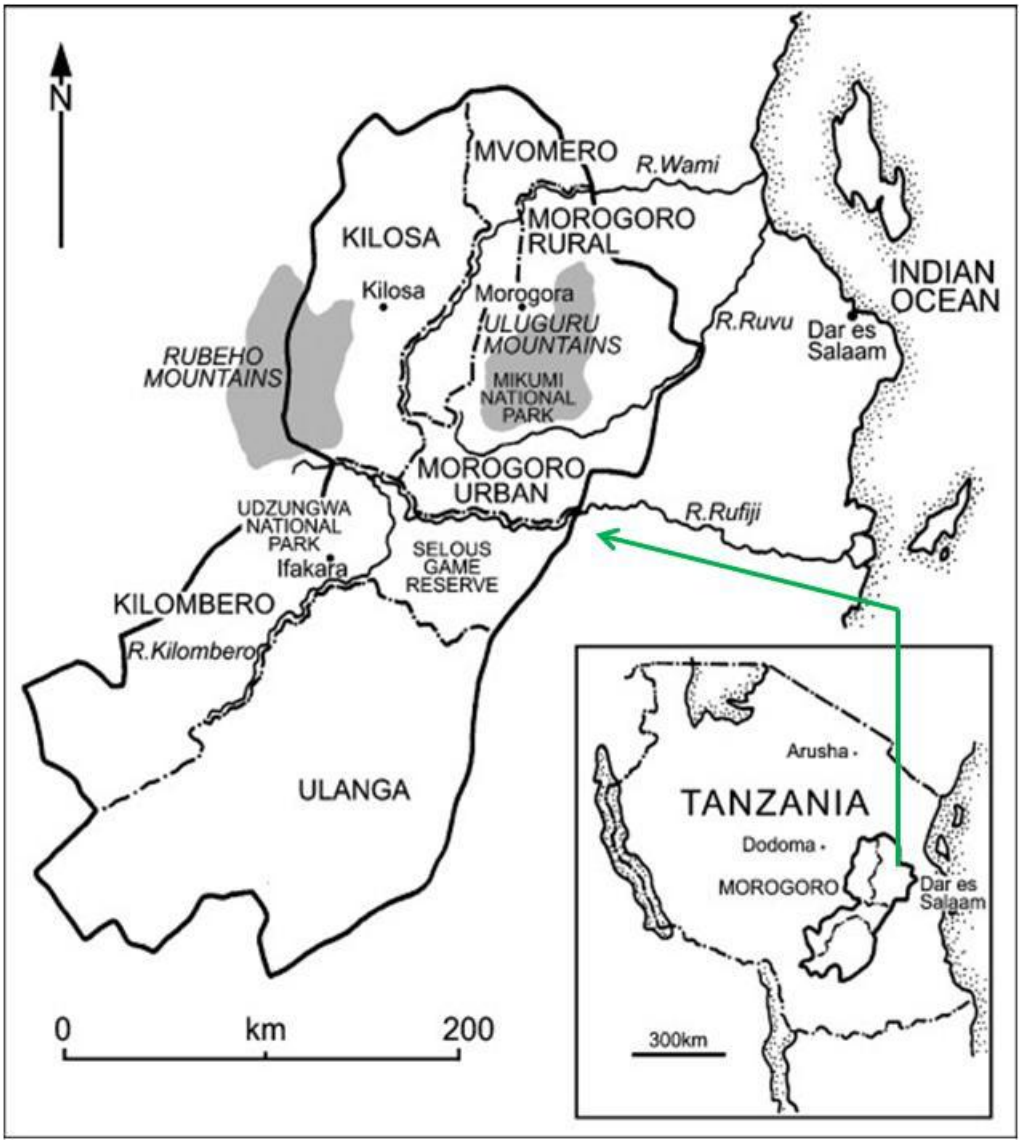

Figure 1. Map showing Morogoro region with its six districts. (Insert is the map of Tanzania which shows the location of Morogoro region).

Morogoro was selected as the study area based on presence of high interactions among human-animal-wildlife interface (i.e. presence of Mikumi National Park and Selous Game Reserve ecosystem) which could play a role in maintenance of the disease. The region has crop farming, pastoral and agro-pastoral activities and is an intermediate place for transporting animals to and from different parts in Tanzania.

Numerous agricultural, pastoral and agro-pastoral tribes live in Morogoro due to fertile land, water and climatic conditions which favor there activities. The numerically dominant tribes are the Waluguru, Wasagara, Wakaguru, Wandamba Wapogoro, Sukuma, Masaai, Barbaig and Wagogo. Approximately 90% of the people in the Morogoro are employed in small-scale agriculture, with the majority of these being subsistence farmers (MRSEP, 2006). Traditionally agro-pastoralist tribes have settled on the outskirts of villages and practice agro-pastoral lifestyle by, both farming and raising relatively large herds of livestock.

The region receives an annual average rainfall of 600-1200 mm (MRSEP, 2006). The mean annual temperatures vary with altitude from the valley bottom to the mountain top. The average annual temperature varies between 18^0C on the mountains to 30^0C in river valleys. In most parts of the region, the average temperatures are almost uniform at 25^0C. In general the hot season runs from September to November.

3.2 Study Design and Sampling Techniques

Multistage sampling was applied in a case control cross sectional study design followed by follow up of patients at their homes whereby data collection was done once at a single point (Martin, 1987). Ten health facilities were selected purposively as according to Bennett *et al*, (1991) formulae and based on ability to do serological tests as not all health facilities in the study area can diagnose serological test. The health facilities included Morogoro Regional hospital and Shalom Medical centre both are in Morogoro Municipality, Bwagala hospital in Mvomero district, St. Francis hospital in Kilombero district, Mtimbira health centre, Lugala hospital and Ulanga district hospital in Ulanga district, St. Kizito hospital, Ulaya health centre, and Kilosa district hospital in Kilosa district.

Patients attending at health facilities with complaints of fever and willing to participate in the study were enrolled in the study. According to Bennet *et al.* (1991) a total of 180 patients from each hospital were to be tested for brucellosis thus makes a total of 1800 patients. Fortunately all 1800 who were willing to participate in the study agreed to donate blood samples for brucellosis analysis.

The study aimed at getting equal number of fever and non fever respondents but due to the nature of people's habit of attending at health facilities it was hard to get equal numbers of respondents. Usually people visit hospitals when they are sick that's why few of them came with non fever complaint. Animal brucellosis prevalence data were sourced from other parallel sub project working with animals in same study areas under the main ICONZ project (Temba, 2012) and used for correlation with human prevalence in this study.

3.3 Determination of Sample Size

The approach was based on cluster sampling according to Bennett *et al*, (1991).

$$c = \frac{p(1-p)D}{(s.e._x)^2 b} \qquad \text{……...…….. (1)}$$

Whereby

$$D = 1 + (b-1)\rho$$

P = prevalence (50%) to maximize sample size

ρ (roh)= Interclass correlation coefficient (0.2) suggested for zoonotic diseases

b =Number of patients (180) per hospital

c = the number of hospital (clusters=10)

D =the design effect that accounts for the variations between clusters (18)

Se= standard error (0.05).

Cluster sampling was established (equation 1) and the design effect calculated so that the final sample size (b*c) was obtained. A total of 1800 patients were to be tested and participate in the study. However, during the interview more patients were willing to participate and requested to be included in the study. In total 1807 of respondents were included in the study.

3.4 Data Collection

3.4.1 Laboratory based data

The Standard Operating Procedure (SOP) for brucellosis was provided to laboratory technicians for precise results and uniformity in all 10 hospitals. Human blood samples were collected from febrile and non fever patients in 10 health facilities in Morogoro region. Collection of blood samples was done by qualified medical personnel after seeking consent of the patients. For children who were under five, their guardians were given the consent form to sign if they agreed to participate in the study and were responsible to give history of the children. Disposable sterile syringes and needles were used for aseptically blood sampling from the cephalic veins of the patients into properly labeled sterile plain vacutainer tubes. The blood samples were centrifuged at 1,500 g for 10 minutes to obtain sera which were tested for brucellosis by using Rose Bengal Plate Test (RBPT).

3.4.1.1 Rose Bengal Plate Test

Rose Bengal Plate Test (RBPT) was done according to Alton *et al.* (1975). Briefly, a drop of the test serum (30 µl) was taken using a clean micro-pipette and placed onto test plate beside an equal (30 µl) drop of RBPT antigen. These then were mixed well using a sterile applicator stick. The mixture was then rocked manually for 4 minutes before examination. The presence of distinct pink granules (agglutination) was recorded as positive case while samples which had no granules were recorded as negative cases. RBPT was used in this

study because of its sensitivity and specificity and it is cheap and simple to perform as suggested by Omer *et al.* (2002).

3.4.2 Sociological data

Both quantitative and qualitative data collection methods were used to obtain primary data. The main instrument for quantitative data was a structured questionnaire containing both closed and open-ended questions (Appendix I). Preliminary study survey was conducted in order to test the clarity, sequence of the questions and the discussion guides proposed as well as estimated time for each questionnaire. The revised version of the questionnaire that was used in the study was translated into 'Kiswahili', the national language understood by majority of Tanzanians. The researcher administered the questionnaires to respondents. Socio-demographic characteristics data such as sex, age category, fever status, marital status, education level, religion, occupational status and knowledge on brucellosis and risk factors for transmission of the disease were collected from the respondents. A total of 185 fever and 184 non fever patients were interviewed from 1807 cases. However, it was difficult to reach some of the patient due some problems such as remoteness of the place with no passable roads. Public engagement was done to the study area by using different tools such as focus group discussions at village level, power point presentation to explain the research aims and reasons for its conduction.

3.5 Ethical Consideration

Confidentiality of laboratory information was observed and maintained and the screened sera samples were used to detect brucellosis antibodies only. Ethical clearance was obtained from National Institute of Medical research (NIMR/HQ/R.8a/Vol.ix/1119) (Appendix II) before data collection since the research involved human subjects. After

processing of sample, results were given to the medical personnel at respective health facility for further follow up of the patients

3.6 Data Analysis

Data were entered and coded in excel then were transformed to Epi Info7 statistical package for analysis (Epi Info, 2012). These allowed computer entries of responses given in open questions or options and combinations in closed questions. Prevalence of brucellosis in animals was used to show associations with human brucellosis prevalence.

3.6.1 Analysis of sero prevalence brucellosis in human

Descriptive statistics was used to analyze frequencies, means, percentages, confidence intervals while χ^2 test was used to show the association between variables in contingency table in quantitative data. Graphs and charts were also drawn in excel showing correlation between disease and the risk factors.

3.6.2 Analysis of knowledge of brucellosis in human

Descriptive statistics was used to analyze frequencies, means and percentages of variables associated with knowledge of brucellosis between two fever and non fever group.

3.6.3 Analysis of risk factors for transmission in human

Logistic regression (univariate) and multivariate analysis were carried out to show the association of the disease and the risk factors between fever and non-fever groups. Chi square was used for comparison; odds ratio and relative risk were also calculated to show likelihood and magnitude of respondents exposed to risk factors and the brucellosis between fever and non-fever groups.

CHAPTER FOUR

4.0 RESULTS

4.1 Sero prevalence of Human Brucellosis in Morogoro Region

The overall sero-prevalence of brucellosis in human was 20.5% (95% CI: 18.7-22.5, n=1807). A highly significant (P = 0.0001) infection rate was found in fever group 23.9% (n = 1509) than in individuals in non-fever group 3.7%. Comparison of prevalence of brucellosis district wise showed that Mvomero district had higher prevalence (36.1%) than other districts. The difference of prevalence between districts was statistically significant (P = 0.0001, OR = 1.42). The sero-prevalence of human brucellosis with regard to sex, age category, district of residence and either an individual was in fever or non-fever groups are presented in Table 1.

Sex was found not to be a significant factor of brucellosis infection, (P = 0.23) despite females having a slightly higher proportion of infection 21.6% (n=1015) compared to males 19.1% (n=792). Assessment of age showed that there was no significant difference between proportion of different age categories which were brucellosis positive (P = 0.34) although the children of 0-4 years had a relatively high prevalence (28.8%, n = 52).

Table 1. Prevalence of brucellosis in humans in Morogoro

Variable	Category	Number tested (n)	Prevalence (%)	OR	CI 95%	P value
Sex	Female	1015	21.6	1.2	19.1-24.3	0.23
	Male	792	19.1		16.5-22.2	
Age category (years)	0-4	52 (F=35, M=17)	28.8		17.1-43.1	0.34
	5-14	245 (F=142, M=103)	20.8		15.9-26.4	
	15-24	283 (F=163, M=120)	23.0		18.2-28.3	
	25-34	515 (F=284, M=231)	16.9		16.3-23.4	
	35-44	289 (F=161, M=128)	18.7		14.4-23.7	
	45-54	234 (F=121, M=113)	20.1		15.2-25.8	
	55-64	96 (F=56, M=40)	17.7		10.7-26.8	
	65>	93(F=53, M=40)	22.6		14.6-32.4	
Groups	Fever	1509	23.9	8.2	21.7-78.3	0.0001
	Non fever	298	3.7		1.9-6.5	
Districts	Mvomero	249	36.1	1.4	30.2-42.5	0.0001
	Morogoro municipal	330	27.3		22.6-32.5	
	Kilosa	446	26.2		22.3-30.6	
	Morogoro	69	21.7		12.7-33.3	
	Kilombero	219	14.6		10.2-20.0	
	Ulanga	494	5.5		37.0-8.0	

F= Female, M= Male,

4.1.1 Correlation between human livestock ratio and human prevalence of brucellosis

There was a positive correlation between human livestock ratio and brucellosis prevalence in human (Fig 2) whereby R^2=0.6059.

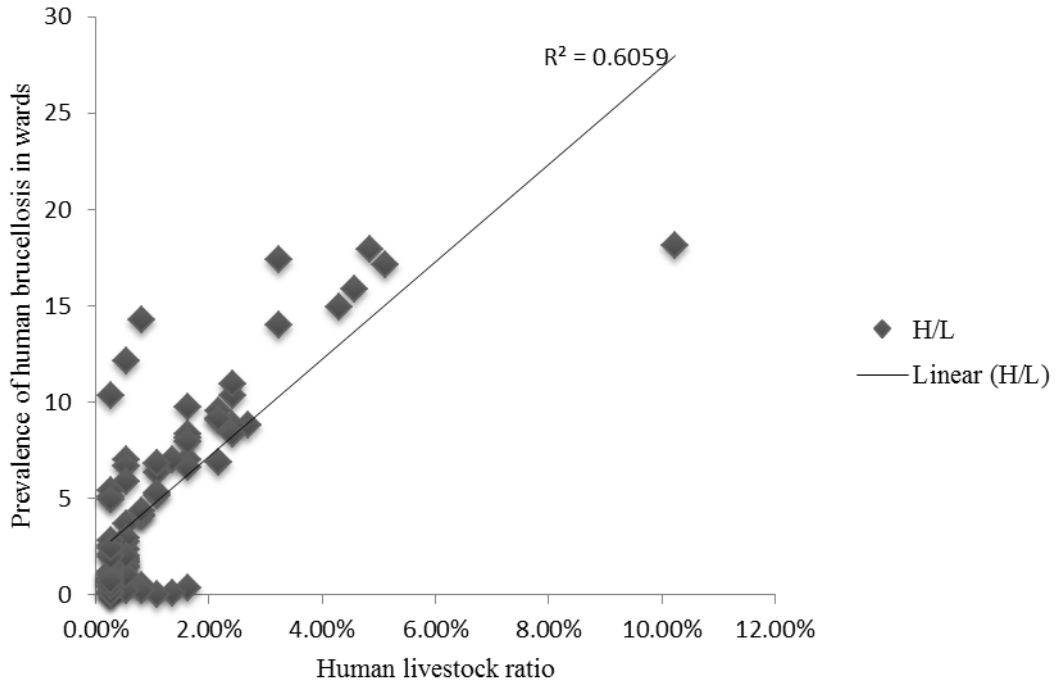

Figure 2. Correlation between prevalence of human brucellosis and human livestock ratio

4.1.2 Common clinical manifestations among the brucellosis patients

Several clinical manifestations were recorded in brucellosis positive patients. The commonest included fever, muscular pains, headache, neuralgia, joint pains, night sweat and back pain (Table 2).

Table 2. Common clinical symptoms among the brucellosis positive patients

Clinical signs	Confirmed brucellosis +ve cases (%) N=371
Fever	360 (97.0)
Muscular pains	240 (64.7)
Headache	204 (55.0)
Neuralgia	160 (47.6)
Joints pain	162 (43.6)
Night sweat	157 (42.3)
Back pain	152 (41.0)
Gastritis	146 (39.4)
Sleeping disturbance	146 (39.4)
Loss of weight	143 (38.5)
General weakness	132 (35.6)
Others symptoms	125 (33.7)

4.1.3 Association of human brucellosis with other confirmed disease conditions

Table 3 shows confirmed diseases that share similar symptoms with brucellosis in which a significantly high number (32.7%; $P < =0.05$) of patients were misdiagnosed as malaria, typhoid and venereal disease cases. However laboratory confirmation for malaria typhoid and venereal diseases were being routinely diagnosed.

Table 3. Distribution of other confirmed diseases cases

Confirmed diseases	Confirmed brucellosis +ve cases (%) N=371
Malaria	120 (32.3)
Typhoid	125 (31.8)
Rapid plasma regain/vdrl	118 (33.7)

4.1.4 Spatial distribution of visited tested cases

Fig.3 illustrates the distribution of positives cases in Morogoro region. More cases were concentrated in Morogoro municipal, Mvomero and Kilosa than Ulanga, Morogoro and Kilombero districts.

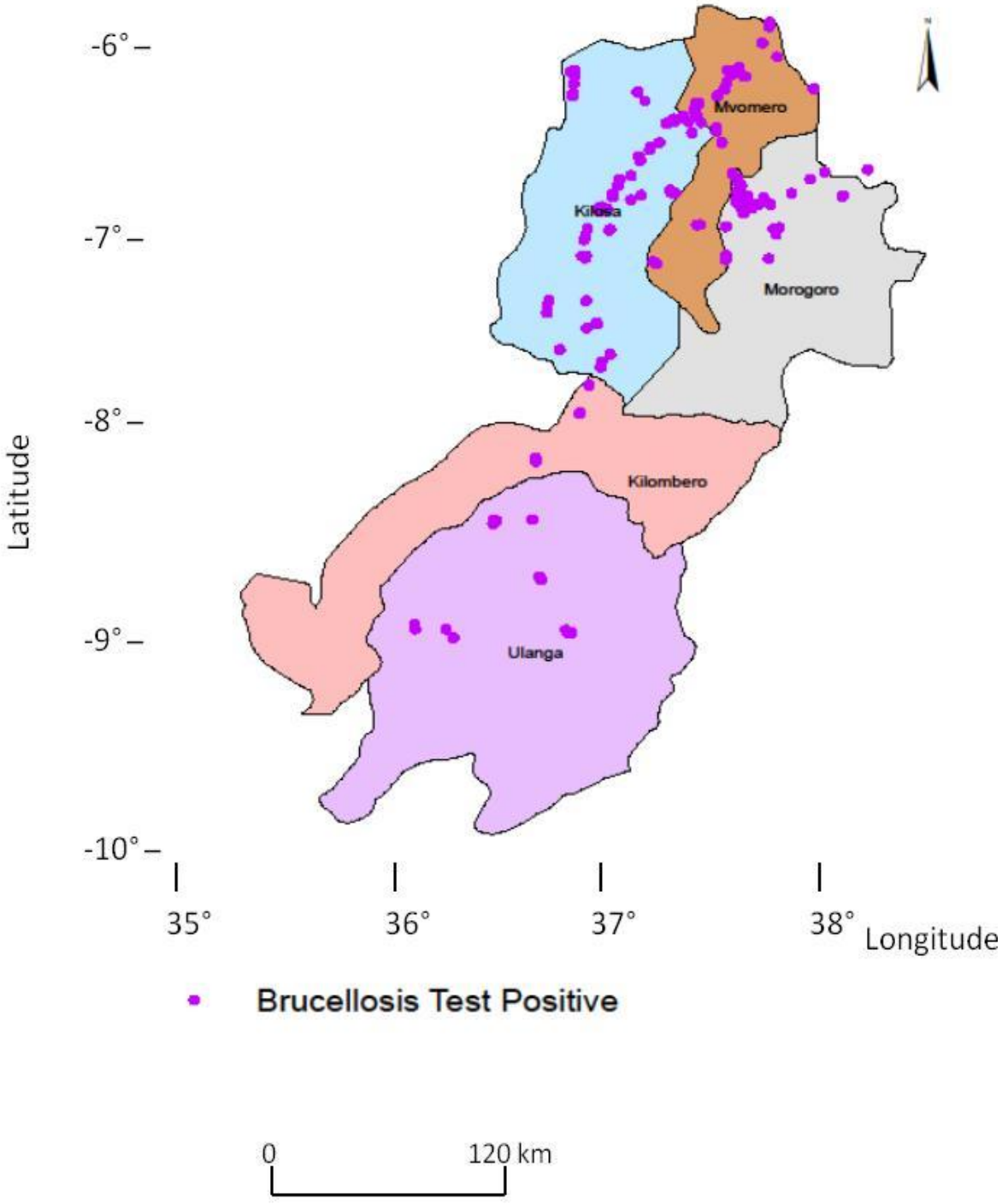

Figure 3. Spatial distribution of visited cases of brucellosis

4.2 Knowledge of Brucellosis in Humans

In this part two of the study, a total of 369 respondents who had been screened for brucellosis were recruited for interview on knowledge and risk factors for the brucella

infection in humans. Of the 369 respondents recruited in the study, 184 belonged to the non-fever group while 185 were in fever group (Table 4).

4.2.1 Socio-demographic characteristics of the respondents

Socio demographic characteristics of the recruited respondents are shown in Table 4. The results indicated that the occupations of the respondents in the fever and non-fever groups differed significantly (P=0.0001). Similarly, there was a statistical significance between education levels of the respondents in the fever and non-fever groups (P=0.0016) (Table 4). The respondent's age category, ethnicity and religion showed insignificant difference in both fever and non-fever groups.

Table 4. Socio demographic profile of study population

Variable		Fever group (%) n=185	95% CI	Non fever group (%) n=184	95% CI	p-value
Sex	Female	102 (55.1)	47.7-62.4	97 (52.7)	45.3-60.1	0.6413
	Male	83 (44.9)	37.6-52.3	87 (47.3)	39.9-54.8	
Age	0-4	10 (5.4)	2.6-9.7	6 (3.3)	1.2-6.9	0.601
category	5-14	21 (11.4)	7.2-16.8	23 (12.5)	8.1-18.2	
	15-24	30 (16.2)	11.2-22.3	29 (15.8)	10.8-21.8	
	25-34	55 (29.7)	23.3-36.9	47 (25.5)	19.4-32.5	
	35-44	26 (14.1)	9.4-2.9	38 (20.7)	15.1-27.2	
	45-54	24 (13.0)	8.5-18.7	21 (11.4)	7.2-16.9	
	55-64	8 (4.3)	1.9-8.3	11(6.0)	3.1-10.4	
	65>	11 (6.0)	3.1-10.4	9 (5.0)	3.1-10.4	
Ethnicity	Others	72 (39.0)	31.9-46.4	101 (54.9)	47.4-62.2	0.1487
	Masai	43 (23.2)	17.4-30.0	31 (16.9)	11.7-23.1	
	Mang'ati	25 (13.5)	8.9-19.3	9 (4.9)	2.3-9.1	
	Sukuma	18 (9.7)	5.9-14.3	16 (8.7)	5.1-13.7	
	Pare	17 (9.2)	5.4-14.3	14 (7.6)	4.2-12.4	
	Luguru	10 (5.4)	2.6-9.7	13 (4.9)	3.8-11.8	
Occupation	Pastoralist	83 (47.6)	40.2-55.1	41 (22.3)	16.9-28.9	0.0001
	Agro-pastoralist	44 (23.8)	17.8-30.6	28 (15.2)	10.4-21.2	
	Peasants	15 (8.1)	4.6-13.1	28 (15.2)	10.4-21.2	
	Abattoir workers/ Butchermen	12 (6.5)	3.4-11.1	1 (0.5)	0.01-2.9	
	Employees	8 (4.3)	1.9-8.3	36 (19.5)	14.1-26.04	
	Students	7 (3.8)	1.5-7.6	16 (8.7)	5.1-13.7	
	Housewife	7 (3.8)	1.5-7.6	11 (5.6)	3.02-10.4	
	Others	4 (2.2)	0.6-5.4	23 (12.5)	8.1-18.2	
Education level	Informal	89 (48.1)	40.7-55.7	57 (31.0)	24.4-38.2	0.0016
	Primary	51 (27.6)	21.3-34.6	66 (35.9)	28.9-43.3	
	Secondary	26 (14.1)	9.4-19.9	31 (16.9)	11.7-23.1	
	University	10 (5.4)	2.6-9.7	11 (6.0)	3.02-10.4	
	Collage	7 (3.8)	1.5-7.6	6 (3.3)	1.2-6.9	
	Advanced level	7 (0.5)	0.01-2.9	6 (3.3)	1.2-6.9	
	Koran	1 (0.5)	0.01-2.9	7 (3.8)	154-177.7	
Religion	Christian	111 (60)	52.6-67.1	94 (51.1)	43.6-58.5	0.2068
	Muslim	41 (22.2)	16.4-28.8	57 (31)	24.4-38.2	
	Others	31 (16.8)	11.6-22.9	29 (15.8)	10.8-21.8	
	None	2 (1.1)	0.1-3.9	4 (2.2)	0.6-5.5	

4.2.2 Respondents knowledge's on brucellosis

During the survey, it was found that typhoid fever and malaria were two major diseases reported by most of the respondents (Table 5). Majority of the respondents (49.3%) practiced self-medication whenever felt sick. However, a relatively large number (30.1%) were using traditional healers to get health services. Small proportions (12.2%) of respondents had heard of brucellosis but were not fully knowledgeable on the disease.

Table 5. Distribution of knowledge of respondents on brucellosis

Variable	Category	Number of respondents (%)
Disease problems	Typhoid fever	129 (34.9)
	Malaria and typhoid fever	127 (34.4)
	Malaria	95 (25.7)
	Others	18 (4.9)
Health services	Self medication	182 (49.3)
	Traditional healers	111 (30.1)
	Health centers	46 (12.5)
	Spiritual leaders	19 (5.1)
	None	1 (0.3)
Knowledge on brucellosis		
Heard of brucellosis?		
	Yes	45 (12.2)
Source of information	From doctor	22 (5.9)
	Friend/Relative	17 (4.6)
	School	2 (0.5)
Causative agent	Yes	6 (1.6)
Symptoms	Yes	6 (1.6)
Risks for transmission	Yes	12 (3.3)
Diagnosis	Yes	3 (0.8)
Control	Yes	9 (2.4)

4.3 Risk Factors for Transmission of Brucellosis in Humans

4.3.1 Risk factors for transmission of brucellosis by direct contact

Contact with cattle manure, milking, placenta and home slaughter were considered as risk factors for transmission of brucellosis by direct contact. The results from the 369 respondents from both fever and non-fever groups are summarized in Table 6. Up to 38% (n=140) respondents in both fever and non-fever groups had the history of coming into

direct contact with cattle manure during activities such as cleaning of animal house, fertilizing crops with manure, milking, handling animals and other activities related with livestock keeping. Out of the 140 who had come into contact with manure, 62% (n=87) cases were positive of brucellosis. When hand milking as risk factor for transmission of brucellosis by direct contact was assessed, it was found that up to 72.1% (n= 266) of respondents had the history of hand milking of livestock. Up to 64.2% (n=237) of the respondents had been come in direct contact with placenta especially during assisted delivery. In addition, a total of 174 respondents (47.2%) had ever practiced home slaughter and come into direct contact with fresh blood, meat, hides/skin and other by products from slaughtered animals. When prevalence of brucellosis in fever and non-fever groups were compared taking into account the fore mentioned risk factors (manure, milking, contact with placenta and home slaughter), the prevalence was significantly higher (P= 0.0001) in the fever group (97.3%, n=359) than the non-fever group (2.5%, n=9) (Table 6).

Table 6. Distribution of risk factors for transmission of brucellosis by direct contact

Variables		Fever group (n=185)		Non fever group (n=184)		P-value	Odds ratio
		Brucellosis +ve (%)	Brucellosis -ve (%)	Brucellosis +ve (%)	Brucellosis -ve (%)		
Contact with cattle manure	Yes	84 (46.7)	0 (0.0)	3 (60.0)	53 (29.6)	0.0003	2.19
	No	96 (53.3)	5 (100.0)	2 (40.0)	126 (70.4)		
Milking	Yes	117 (65.0)	1(20.0)	1 (20.0)	45 (25.1)	0.0001	5.28
	No	63 (35.0)	4 (80.0)	4 (80.0)	134 (74.9)		
Contact with placenta	Yes	102 (56.7)	2 (40.0)	4 (80.0)	69 (38.6)	0.0001	2.39
	No	78 (43.3)	3 (60.0)	1 (20.0)	110 (61.5)		
Home slaughtering of livestock	Yes	100 (55.6)	2 (40.0)	4 (80.0)	68 (38.0)	0.0005	2.09
	No	80 (44.4)	3 (60.0)	1 (20.0)	111 (62.0)		

4.3.2 Risk factors for transmission by consumption

Foods of animal origin (milk, meat and blood) were considered to be the risk factors for transmission of brucellosis by ingestion as shown in Table 8. The results showed that all the food materials were found to be potential risk factors for brucellosis infection. Comparing the prevalence of brucellosis in fever and non-fever groups categorizing based on different foods of animal origin, the prevalence was significantly higher (P= 0.0001) in the fever group than the non-fever group (Table.7). The prevalence of brucellosis of up to 56.1% and 60.2% was recorded in individuals drinking raw milk and seeping raw blood respectively making the two foodstuffs to constitute a major threat to the pastoralist communities.

Table 7. Distribution of brucellosis risk factors by consumption of food of animal origin

Variable	Response	Fever group (n=185)		Non fever group (n= 184)		p-value	Odds ratio
		Brucellosis +ve (%)	Brucellosis -ve (%)	Brucellosis +ve (%)	Brucellosis -ve (%)		
Milk consumption	Yes	167 (92.8)	5 (100.0)	5(100.0)	125 (69.8)	0.0001	5.04
	No	13 (7.2)	0 (0.0)	0 (0.0)	54 (30.2)		
Frequency of milk consumption	Daily	133 (73.9)	4 (80.0)	4 (80.0)	100 (55.9)	0.6580	1.12
	Rarely	47 (26.1)	1 (20.0)	1 (20.0)	79 (44.1)		
Species:							
Cow's milk	Yes	140 (77.8)	4 (80.0)	4 (80.0)	20 (11.2)	0.0001	2.82
	No	40 (22.2)	1 (20.0)	1 (20.0)	159 (88.8)		
Goat's milk	Yes	29 (16.1)	4 (80.0)	4 (80.0)	20 (11.2)	0.0147	2.24
	No	151 (83.9)	1 (20.0)	1 (20.0)	159 (88.8)		
Meat consumption	Yes	161 (89.4)	5 (100.0)	5 (100.0)	139 (77.7)	0.0032	2.42
	No	19 (10.6)	0 (0.0)	0 (0.0)	40 (22.4)		
Frequency of meat consumption	Daily	141 (78.3)	5 (100.0)	2 (40.0)	112 (62.6)	0.0039	1.94
	Rarely	39 (21.7)	0 (0.0)	3 (60.0)	67 (37.4)		
Species:							
Cattle meat	Yes	158 (87.9)	5 (100.0)	5 (100.0)	139 (77.7)	0.012	2.058
	No	22 (12.2)	0 (0.0)	0 (0.0)	40 (22.4)		
Goat meat	Yes	161 (89.4)	5(100.0)	5 (100.0)	139 (77.7)	0.0032	2.42
	No	19 (10.6)	0 (0.0)	0 (0.0)	40 (22.4)		
Wildlife meat	Yes	120 (66.7)	5 (100.0)	4 (80.0)	96 (53.6)	0.0172	1.75
	No	60(33.3)	0 (0.0)	1 (20.0)	83 (46.4)		
Consumption of other meat	Yes	159 (88.3)	5 (100.0)	5 (100.0)	138 (77.1)	0.0006	2.23
	No	21 (11.7)	0 (0.0)	0 (0.0)	41 (22.9)		
Blood consumption	Yes	85 (47.5)	2 (40.0)	1 (20.0)	55 (30.6)	0.0018	0.50
	No	94 (52.5)	3 (60.0)	4 (80.0)	125 (69.4)		
Seeping of raw blood	Yes	49 (27.4)	5 (100.0)	1 (20.0)	28 (15.6)	0.0051	0.47
	No	130 (72.6)	0 (0.0)	4 (80.0)	152 (84.5)		

4.1.3.3 Multivariate analysis of risk factors for transmission

Multivariate analysis was further done to all risk factors that showed to be significant in univariate analysis and the results are summarized in Table 8. Milking, milk consumption, cow's milk, frequency of meat consumption, goat's meat, other meat (such as pork) and seeping of raw blood were found to be statistically significant.

Table 8. Multivariate analysis of risk factors for transmission of brucellosis

Variable	Odds Ratio	C.I.	Coefficient	S. E.	Df	Z-Statistic	P-Value
Contact with cattle manure	1.1367	1.1367	0.1281	0.2731	1	0.4693	0.6389
Milking	4.5029	4.5029	1.5047	0.2445	1	6.1555	0.0001
Contact with placenta	1.2367	1.2367	0.2124	0.2660	1	0.7985	0.4246
Home slaughtering of livestock	1.5101	1.5101	0.4122	0.2565	1	1.6068	0.1081
Consumption of milk fresh	4.5645	4.5645	1.5183	0.3958	1	3.8357	0.0001
Species:							
Cow's milk	1.8717	1.8717	0.6269	0.2926	1	2.1427	0.0321
Goat's milk	1.7429	1.7429	0.5556	0.3413	1	1.6277	0.1036
Meat consumption	1.3844	1.3844	0.3253	0.3734	1	0.8711	0.3837
Frequency of meat consumption	1.8150	1.8150	0.5961	0.2945	1	2.0241	0.0430
Species: Goat meat	2.7746	2.7746	1.0205	0.3089	1	3.3038	0.0010
Consumption meat other	2.0855	2.0855	0.7350	0.3036	1	2.4214	0.0155
Seeping of raw blood	0.5549	0.5549	-0.5890	0.2661	1	-2.2131	0.0269

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of Human Brucellosis in Morogoro Region

The study has demonstrated that the overall sero-prevalence of brucellosis in human was 20.5% suggesting that the disease is a problem in Morogoro. Comparable results of human brucellosis (1-19.1%) in other regions in Tanzania were reported by Minja, (2002); Shirima (2005); Swai, (2008) and Kunda, (2008). A recent study in animals in Tanzania reported the sero-prevalence of brucellosis to be 14.3% in cattle, 0.5% in goats, 0.6% in sheep and 13.6% in African Buffalo (*Syncerus caffer*) (Temba, 2012). Other studies on sero-prevalence of brucellosis in cattle in different parts of Tanzania reported presence of the disease ranging from 1 - 30% (Kitaly, 1984; Otaru, 1985; Swai, 1997, Minja, 2002; Karimuribo *et al.*, 2007). Elsewhere, brucellosis has also been reported in humans with sero-prevalence of 18 - 24% in Uganda (Ndyabahinduka and Chu, 1984), 3.8% in Chad (Schelling *et al*., 2003), 0.04 - 35% in Saudi Arabia (Memish, 2001), India 25.5% (Kumar, 1997) and 37.7% in Algeria (Habib *et al.,* 2003).

From these results, it signifies that brucellosis is endemic in many countries worldwide assuming its zoonotic nature. However, brucellosis prevalence of 20.5% in humans in Tanzania which was observed during this study is at the higher side. This may be caused by poor prioritization of brucellosis as a disease in both human and animals at national level. Also poor records keeping in hospitals, low recognition among public health practitioners and policy makers, lack of resources for implementation of the policies and sharing of clinical symptoms with other fever diseases results to misdiagnosis hence underreporting the magnitude of brucellosis (Kunda *et al.,*2005; Kunda *et al.,* 2010). Misdiagnosis of brucellosis may be due to lack of awareness on brucellosis by medical

staff, limited diagnostic facilities, lack of experience with laboratory testing (Kunda *et al.*, 2007). Unless deliberate control measures are instituted, the diseases will keep on increasing especially in pastoral and agro pastoral communities living under poor and marginalised rural environment with limited health facilities.

During screening of patients at different health facilities, a highly significant infection rate was found in fever group (23.9%) than in individuals with no fever (3.7%) suggesting that brucellosis could have contributed to fever syndrome. It is known that brucellosis is among the fever causing conditions which are normally misdiagnosed with other common fever causing agents in the tropics. During the current study, a significantly high number (32.7%; $P < =0.05$) of fever patients who were confirmed to be brucellosis positive had been misdiagnosed as malaria, typhoid fever and venereal disease. The current finding is in line with other studies elsewhere where brucellosis was misdiagnosed as typhoid fever, malaria and venerial disease (Mantur *et al.*, 2006; Franco *et al.*, 2007; Mustaafa and Hassan, 2010).

Misdiagnosis of brucellosis to other fever causing diseases was further supported by the findings that most of the community reported typhoid fever and malaria as the two major diseases affecting majority of the people in Morogoro. The other common signs which were associated with brucellosis positive patients included muscular pains, headache, neuralgia, joint pains, night sweats and back pain. This concurs with signs in brucella positive patients which have been reported by different authors in different studies (Mantur *et al*., 2006; Diju, 2009; Akhvlediani *et al.*, 2010; Mustafa and Hassan, 2010; El-Metwally *et al*., 2011).

On the distribution, most of the brucellosis cases were recorded in Mvomero, Morogoro municipality and Kilosa districts. The high number of cases in Mvomero district may be

due to high number of pastoralists and agro-pastoralist households having large herds of indignous cattle which rarely receive veterinary services. Concurrently, Mvomero district had higher prevalence (14.9%) of brucellosis in livestock compared to other districts in Morogoro region (Temba, 2012). This may have predisposed the local people to brucellosis infection. Furthermore, because of its fertile and vast availability of land, Mvomero district is highly populated and this may increase the interatctions between human and animals. This further predisposes more the community to brucellosis and other disease conditions.

Furthermore, the current study found that both male and female were equally predisposed to brucellosis infection although female had a relatively higher infection rate than males. The results in this study concur with the study by Swai *et al.* (2009) on occupational risk factors in Tanga, Tanzania. However, there have been mixed results on rates of infection between male and females (Minas *et al.*, 2007). Differences in prevalence rates between the sexes may be attributed to different behavioural attitudes towards livestock handling and preparation of food of animal origin. Under pastoral and most agro-pastoral setup, females do most of the work associated with harvesting of livestock products (such as. milking), cleaning of livestock houses, house repair using cattle dung and handling of the newly borne calves, which may predispose them to infection. However, in African settings, most women attend health facilities and hence possibilities for diagnosing different diseases which may be the case also of brucellosis.

On the other hand, the current study found that all the age groups were found to be equally predisposed to brucellosis infection. Surprisingly, children of 0-4 years had a relatively high infection rate (28.8%) compared to other age groups. Contrary to a study by Mustafa and Hassan, (2010) who did not detect brucellosis among 0-5 year's patients. Factors that

are likely to contribute to high infection rates in children in the study area are poor hygiene and sanitation, closeness to animals, malnutrition and low immunity because of first exposures. However, early exposure to consumption of raw unpasteurized milk could be the factor. Most of the supplementary and post weaning food for children in pastoralist and agro-pastoralist is milk. While other parents start feeding their children with milk even from day one. In line to these facts, it was noticed during the questionnaire survey that most respondents used raw unpasteurized milk as among the staple food. In addition, another age category which had high prevalence was of 15-24 years (prevalence of 23%). This age group is a peer group whereby they have habits of consuming junky and most popular foods such as chips with grilled meat (*mishikaki*), ice creams, uncontrolled unpasteurized milk and milk products. Such kind of eating habit may further predispose them to brucellosis infection.

The study signifies that, there was a positive correlation between human livestock ratio and human prevalence as illustrated in Fig. 2. Brucellosis in human tend to increase with the increase of human livestock ratio which can be attributed by other factors such as poor animal husbandry practices, consumption of infected animal products, lack of initiatives to control the disease in animals and ignorance of people to the disease.

5.2 Knowledge of brucellosis in human

The study found that most of education level attained among respondents affects knowledge on causative agent, symptoms, mode of transmission, diagnosis and control of brucellosis. Also they were not aware of brucellosis as among the diseases affecting human being since they were mixing it with other feverish diseases such as typhoid and malaria. Worse still, most people were doing self-medication whenever felt sick and others were using traditional healers. This signifies that brucellosis problem is likely to keep on

increasing affecting a large community members and the currently reported prevalence may be underreported and treated with wrong drugs. A recent study by Bouley *et al*., (2012) showed that the diseases are responsible for high proportion of febrile illness in humans in Northern Tanzania and account for more hospital admission of fever but misdiagnosed as malaria (Cleaveland *et al*, 2001). The results from this study concurs with other studies (Shirima *et al.,* 2003) and is In contrast to Holt *et al.* (2011) who found that people were most aware of the disease. Swai *et al.* (2010) revealed that most livestock keepers are knowledgeable of diseases such as rabies, anthrax, or tuberculosis as zoonoses, but not brucellosis, as a zoonotic disease.

5.3 Risk Factors Associated with Transmission of Brucellosis in Human

Transmission by direct contact is the way one can acquire brucellosis directly by contacting the animal or animal products or waste products. The study found that contact with cattle manure, milking, contact with placenta and home slaughter were considered as major risk factors for transmission of brucellosis by direct contact. This occurs due to activities associated with livestock husbandry such as cleaning of animal house, fertilizing crops with manure, milking, handling of animals, assisted parturition and animal slaughter at home. The finding of this study is in agreement with other studies by Mfinanga *et al*. (2003); Regassa *et al.,* (2009); Swai *et al.* (2010); Mustafa and Hassan, (2010) which reported that livestock keepers who had in direct contact with livestock excreta, livestock products like contaminated milk, meat and hides/skin are the potential sources of brucellosis infection. It is documented that *Brucella* bacteria from infected animals are secreted in placenta, fetal fluids, aborted fetuses, other uterine discharges, milk, feces, vaginal mucus, urine, semen and other body fluids which all serve as sources of infections to humans (Blood *et al*., 2007). Direct contact with blood and meat from infected animals are also potentially dangerous. It has been reported also that *Brucella* spp. can be recovered

from cattle manure that has remained in cool environment for more than 2 months (Aillo and Moses, 2010). Therefore contact with cattle manure can be potentially dangerous as it is with secretion from the reproductive organs.

Furthermore, eating habits may expose an individual to brucellosis infection if the consumed foodstuffs (milk, meat and blood) come from infected livestock. In the current study, drinking of raw unpasteurized milk, undercooked or raw meat and seeping raw blood were the foodstuffs that constituted a major threat of brucellosis to the community in Morogoro region. These findings were in agreement with the previously reported findings in Tanzania by Swai *et al.* (2009) and Kunda *et al.* (2010) that food preferences and eating behaviour play major roles in brucellosis infection especially in pastoral and agropastoral communities. Other studies in Africa report that risk factors of transmission of brucellosis are the same but tend to vary widely depending on customs and taboos of referred community (Dogany and Aygen, 2003; Regassa *et al.*, 2009; El-Metwally *et al.*, 2011).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions and Recommendations

Based on the present results on studies of human brucellosis in Mikumi–Selous ecosystem, the following are conclusion and recommendation to be made:

I. Observed prevalence of brucellosis in human was high indicating its public health importance. This calls for research to isolate and identify the existence of other *Brucella* species in areas where the disease has been reported in animals. It should be considered in the differential diagnosis of fever like diseases such as typhoid and malaria in all hospitals.

II. The spatial distribution of brucellosis cases showed that almost all districts in Morogoro region were affected with high number of cases in Mvomero, Morogoro municipality and Kilosa.

III. Knowledge on brucellosis among the respondents was low. This put the pastoral and agro-pastoral communities at high risk of being infected with brucellosis.

IV. There is a need to carry out education campaigns to raise awareness of brucellosis and risk factors for transmission of the disease from animal and animal products to human.

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APPENDICES

Appendix 1: Questionnaire

QUESTIONNAIRE FOR FEVER AND NON FEVER CASES OF BRUCELLOSIS AND

ITS SOCIO ECONOMIC IMPACTS IN PASTORALIST ZONES

Draft1: Questionnaire number|__|__|
Good morning/afternoon,
My name is Lawrencia, W. James, from the Sokoine University of Agriculture in Morogoro In collaboration with the university and the research programme called 'Integrated Control of Neglected Zoonoses', we are carrying out the study at the interface between Mikumi and Selous ecosystems to assess the epidemiology of brucellosis and its socio-economic impacts in pastoral communities. This will form the basis for future control of brucellosis in the Mikumi-Selous vicinity as well as encourage advocacy, policy-making and prioritization of the disease in health interventions at a national level. All information will be treated as confidentially and therefore we request you to be free to provide any useful information. You can ask also any questions you like to. We will ask you questions about the household's activities for income generation and about the health in the household and your personnel health care seeking and behaviour

Interviewer instructions:
Fill in numbers |__|__| make sure you put a 0 if the answer is no so we know the question has been *answered.*
Where there are choices tick the answer given in the box given |__| and fill in the 'other' where *Relevant* Write answers on lines _____

1. Interview identification
101. Household id |__|__|
102. Time interview started (24hr clock: hh-mm) |__|__| - |__|__|
103. Date of interview Interview date (mm-dd-yy)
|__|__| -|__|__| - |__|__|
104. District name _____
105. Village name _____
106. Name of person being interviewed
_____
107. Sex |__|
108. Age
1. 1-4 |__| 3. 15-19 |__|
2. 5-14 |__| 4. 20-24 |__|
5. 25-29 |__| 6. 30-34 |__|
7. 35-39 |__| 8. 40-44 |__|
9. 45-49 |__| 10.50- above adult |__|
109. What is your ethnicity? _____

110. What is your religion among the following?

1. Muslim |__|
2. Christian (Catholic) |__|
3. Christian (Protestant) |__|
4. Animist |__|
5. Others |__|

111. What is the highest level of schooling completed in the following?

1. None |__|
2. Primary|__|
3. Ordinary secondary|__|
4. Advance secondary|__|
5. University|__|
6. Collage|__|
7. Koran|__|

112. What is your main occupation?

1. Peasants |__| 2 Abattoir workers/Butchermen.|__|

3. Pastoralist |__| 4.Agro Pastoralist|__|
5. Employees |__| 6.Student|__|
7.Housewife |__| 0.None|__|
8.Other_____

2. Health Care Seeking

201. Where do **you** seek cure for your illness if you are sick? More than one answers possible

Spontaneous response	**Probed response**
1. Self medication |__|	**2.** Self medication |__|
3. Traditional health |__|	**4.** Traditional health |__|
5. Spiritual leader |__|	**6.** Spiritual leader |__|
7. Health centre |__|	**8.** Health centre |__|

202. Why from the answer you choose?

1. ----------
2. ----------
3.----------

203. Since when did you feel that you are ill? |__||__| |__||__| |__||__||__||__|

204. What have you been told is the name of your illness in the hospital?

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205. What are the signs and symptoms of your illness you had?

Spontaneous answer	Probed answer
a. Fever 1. Yes |__| 0. No |__|	b. Fever 1. Yes |__| 0. No |__|
c. Headache 1. Yes |__| 0. No |__|	d. Headache 1. Yes |__| 0. No |__|
e.Inappetite and/or loss of weight	f.Inappetite and/or loss of weight
e. Joint and bone paint1. Yes |__| 0. No |__|	f. Joint and bone paint 1. Yes |__| 0. No |__|
g. Back pain1. Yes |__| 0. No |__| if yes, which part?	h. Back pain 1. Yes |__| 0. No |__|
i.Gastritis	j. Gastritis
k. Muscle pain 1. Yes |__| 0. No |__|	l. Muscle pain1. Yes |__| 0. No |__|
m. Weakness 1. Yes |__| 0. No |__|	o.Weakness 1. Yes |__| 0. No |__|
p. Night sweat 1. Yes |__| 0. No |__|	q. Night sweat 1. Yes |__| 0. No |__|
o. Sleep disturbance 1. Yes |__| 0. No |__|	p. Sleep disturbance 1. Yes |__| 0. No
r Neuralgia 1. Yes |__| 0. No |__|	s. Neuralgia 1. Yes |__| 0. No |__|
t.Others|__|	u.Others|__|

206. What are the main human health problems in your household? Provide list and rank.

1. _____
2. _____
3. _____
4. _____

207. Do you know a disease called Brucellosis

- **1.** Yes
- **0.** No
- **1.** Not sure

208. If yes, what is it?

_____

209. If yes, what are the symptoms?

_____

210. If yes, what are the causes – how do you catch it?

1._____
2._____
3._____

211. How is the disease being diagnosed?

1._____
2._____
3._____

212. What is the treatment of the disease?

1._____
2._____
3._____

213. If you have heard of brucellosis earlier, from where? 1. Radio |__| 2.Televesion |__| 3. From friend/ family member |__| 4. From Doctor |__|

3. Risky behavior with regard to brucellosis

301. What do you think were your illness comes from?

_____

302. What it is caused by?_____

303. Do you consume fresh milk?

1. Yes |__| 0. No |__|

If yes, how often of from which livestock species?

	1. Daily	2. Weekly	3. Monthly
1. Cattle	|__|	|__|	|__|
3. Sheep	|__|	|__|	|__|
4. Goat	|__|	|__|	|__|

304. Do you boil milk?

1. Yes|__| 2. No|__|

305. Does your milk form a foam?

1.Yes |__| 2. No |__|

306. Do you consume products made by raw milk?

1. Yes |__| 0. No |__|

If yes, how often of from what kind of?

Name of products		Daily	Weekly	Monthly	Yearly
1. Fresh milk		|__|	|__|	|__|	|__|
2. Sour milk		|__|	|__|	|__|	|__|
3. Cream		|__|	|__|	|__|	|__|
3.Other products		|__|	|__|	|__|	|__|
		|__|	|__|	|__|	|__|
		|__|	|__|	|__|	|__|
		|__|	|__|	|__|	|__|

307. What meat do you prefer? 1. Bloody |__| 0. Dried one |__|

From which livestock species?

	Daily	Weekly	Monthly	Yearly
	|__|	|__|	|__|	|__|
1. Cattle	|__|	|__|	|__|	|__|
2. Goat	|__|	|__|	|__|	|__|
	|__|	|__|	|__|	|__|

306. What method do you prefer when preparing meat ?

Method of cooking		Daily	Weekly	Monthly	Yearly
1. Roasting		|__|	|__|	|__|	|__|
2. Boiling		|__|	|__|	|__|	|__|
3. Stewing		|__|	|__|	|__|	|__|
3.Other method		|__|	|__|	|__|	|__|
		|__|	|__|	|__|	|__|
		|__|	|__|	|__|	|__|
		|__|	|__|	|__|	|__|

307. Do you wash your knife after cutting meat?
1.Yes |__| 2.No|__|
308. Do you wash your hands with soap after you have cut meat?
1. Yes |__| 2.No |__|
309. Do you wash you keep animal at home?
1. Yes|__| 2.No|__|

310.Do you slaughter animals at home?
1. Yes|__| 2 No|__|.

If yes, how often

Type of animal	Daily	Weekly	Monthly	Yearly
1.	|__|	|__|	|__|	|__|
2.	|__|	|__|	|__|	|__|
3.	|__|	|__|	|__|	|__|
	|__|	|__|	|__|	|__|

311.Did you consume blood ?
1. Yes |__| 0. No |__|
If Yes,
1.Cooked |__| 2.Not cooked|__|
How Often and from which animals

Type of animal	Daily	Weekly	Monthly	Yearly
1.	|__|	|__|	|__|	|__|
2.	|__|	|__|	|__|	|__|
3.	|__|	|__|	|__|	|__|
	|__|	|__|	|__|	|__|

312. Do you clean animal structures
1.Yes |__| 2.No|__|
If Yes from what animals and how often?

Type of animal	Daily	Weekly	Monthly	Yearly
1.	|__|	|__|	|__|	|__|
2.	|__|	|__|	|__|	|__|
3.	|__|	|__|	|__|	|__|
	|__|	|__|	|__|	|__|

313. Do you help animal during parturition
1.Yes |__| 2.No|__|
If Yes from which animals and how often?

Type of animal	Daily	Weekly	Monthly	Yearly
1.	|__|	|__|	|__|	|__|
2.	|__|	|__|	|__|	|__|
3.	|__|	|__|	|__|	|__|
	|__|	|__|	|__|	|__|

314. Did you **contact directly aborted** foetus and retained placenta during the past year?
1. Yes |__| 0. No |__|

If yes, which species?

Type of animal
1.
2.
3.

315. How did you **remove aborted foetus and retained placenta** by one of the following methods?

Type of animal	Method used
1.	|__|
2.	|__|
3.	|__|
	|__|

316. **Do you clean or wash your hands after use?** 1. Yes |__| 0. No |__|
If yes, how? .....

317.Where did you throw the placenta?----------
318. Do you keep weak **newborns in your house**? 1. Yes |__| 0. No |__|

If yes, when? 1. Dry season |__| 2. Wet season |__| 3. Other (Write)……

319.Do you wash your hands with soap after contact with animals and milking?
1. Yes |__| 0. No |__|

320. Do your animals share pasture with other wildlife?
1. Yes |__|
0. No |__|

315. Where do you fetch your water for drinking?
1. At the tap |__|
2. Dams |__|
3. River |__|
4. Borehole |__|

316. Do you share the same water source with animals?

1. Yes |__|
2. No |__|
3. None |__|

317. Where do you get your animal products?

1. You buy |__| 2. From your own livestock |__|

Thank you very much again.

Appendix 2: Ethical clearance certificate

THE UNITED REPUBLIC OF
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15th April 2011

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CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: The epidemiology of brucellosis and its socio-economic impact in pastoralist communities in eastern Tanzania, (Wankyo L J *et al)*, has been granted ethics clearance to be conducted in Tanzania.
The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Approval is for one year: 15th April 2011 to 14th April 2012.

Name: Dr Mwelecele N Malecela

Signature

CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Dr Deo M Mtasiwa

Signature

CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL WELFARE

CC: **RMO**
DMO