PREVALENCE OF BOVINE BRUCELLOSIS IN SMALLHOLDER DAIRY FARMS IN MOROGORO MUNICIPALITY, TANZANIA

BERTILLA ELIAS LYIMO

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PUBLIC HEALTH AND FOOD SAFETY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

A cross sectional study was conducted to determine prevalence of bovine brucellosis in smallholder dairy farms in Morogoro Municipality. Milk and blood samples collected from 450 dairy cows in thirteen wards of Morogoro Municipality were examined for *Brucella* antibodies' using the Milk Ring Test as an initial screening test followed by Competitive Enzyme Linked Immunosorbent Assay was used as a confirmatory test. Questionnaires were also administered to 135 respondents to assess possible factors associated with transmission of brucellosis from cattle to human. Overall, 29.3% (95%CI: 25.2-33.8%) of milk samples tested positive according to MRT while 18.4% (95% CI) of the serum samples tested positive according to c-ELISA. Analysis of factors associated with occurrence of brucellosis by single table analysis showed that abortion (p=0.000) and herd size (p=0.049) were statistically significant. From this study there is evidence that brucellosis is prevalent and locally distributed in Morogoro Municipality. The study concluded by recommending, further studies, surveillance and institution of preventive and control measures like mass vaccination using S19 vaccine to be undertaken. Furthermore, public health education and formulation of by laws concerning testing of animals and animal products as well as culling of positive tested animals.

DECLARATION

I, Bertilla Elias Lyimo, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is the result of my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

Bertilla Elias Lyimo — Date

(MSc. Public Health and Food Safety Candidate)

The above declaration is confirmed by:

Prof. N. L. Kanuya — Date

(Supervisor)

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LIST OF ABBREVIATIONS AND SYMBOLS

μ	Micro-10^{-6}
χ^2	Chi square
APHCA	Animal Production and Health Commission for Asia and the Pacific
AVMA	American Veterinary Medical Association
CBPP	Contagious Bovine Pleuropneumonia
CDC	Centres for Disease Control and Prevention
c-ELISA	Competitive Enzyme Linked Immunosorbent Assay
CFIA	Canadian Food Inspection Agency
CFSPH	Centre for Food Security and Public Health
CFT	Complement Fixation Test
CI	Confidence Interval
df	Degrees of freedom
DNA	Deoxyribonucleic acid
ECF	East Coast Fever
FMD	Foot and Mouth Disease
FVM	Faculty of Veterinary Medicine
GPS	Global Positioning System
HPA	Health Protection Agency
i-ELISA	Indirect Enzyme Linked Immunosorbent Assay
Ig	Immunoglobulin
IU	International units
LSD	Lumpy skin disease

MAT	Microagglutination test
MRT	Milk Ring Test
NABC	National Agricultural Biosecurity Centres
NBS	National Bureau of Statistics
OD	Optical Density
OIE	Office International des Epizooties
OR	Odds Ratio
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
PO_4	Phosphate
RBPT	Rose Bengal Plate Test
REA	Restriction Endonuclease Analysis
R-LPS	Rough lipopolysaccharide
Rpm	Rotation per minute
RR	Relative Risk
RvPT	Rivanol Precipitation Test
S19	B. *abortus* strain 19
SAT	Serum agglutination test
SE	Standard Error
S-LPS	Smooth lipopolysaccharide
S-RB51	*Brucella abortus* rough strain 51
SUA	Sokoine University of Agriculture
URT	United Republic of Tanzania

WEO	Ward Executive Officer

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Brucellosis is one of the world's major diseases. It is amongst the 'neglected zoonoses' (WHO, 2009) largely due to lack of public awareness and yet it is one of the most important zoonotic infections, especially in pastoral and mixed crop-livestock farming systems in Africa (McDermott and Arimi, 2002). Brucellosis is endemic in most Sub-Saharan African countries including Tanzania (Faye *et al*., 2005; Karimuribo *et al.*, 2007). Though it has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand (Greening *et al.*, 1995), it remains an uncontrolled problem in regions of high endemicity such as Africa, Mediterranean, Middle East, parts of Asia and Latin America (Refai, 2002; Lopez-Merino, 1989). Several synonyms of brucellosis have been known like Malta fever, undulant fever, Rock of Gibraltar fever and Bang's disease. The disease constitutes a public health problem throughout the world, particularly in the tropics, where its control is restricted by inadequate infrastructure and limited resources (WHO, 2006). Additionally, there is a lack of information on its significance and distribution. The disease burden is more profound in the developing countries due to lack of effective public health measures, domestic animal health programs and appropriate diagnostic facilities as well as limited public awareness. The disease in humans is compounded by the resemblance of the clinical symptoms to those of other diseases such as malaria, typhoid and HIV/AIDS leading to incorrect diagnoses and under-reporting of the brucellosis (Capasso, 2002). It is possible that some cases of brucellosis are recorded

as malaria and typhoid; these difficulties have contributed to the general lack of information on the disease in Africa (El-Ansary *et al.*, 2001).

The Centres for Disease Control and Prevention (CDC, 2002) lists *Brucella* as a possible bio-terrorist agent. However, it has never been successfully used in this manner. Considering the damage done by the infection in animals in terms of decreased milk production, abortions, weak offspring, weight loss, infertility and lameness, it is one of the most serious diseases of livestock. It is also a major impediment for international trade in livestock and livestock products.

Brucellosis is caused by bacteria of genus *Brucella*. These are small, non-motile, aerobic, facultative intracellular, Gram-negative coccobacilli. The ability of *Brucella* to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity (Fishi, 2003). Almost all domestic animals can be affected with brucellosis except cats which are resistant to *Brucella* infection. The species of *Brucella* and their major hosts are *B.abortus* (cattle), *B. melitensis* (goats), *B. suis* (swine) and *B. ovis* (sheep). *B.abortus* also causes infection in horses and is commonly found in chronic bursal enlargements as a secondary invader rather than a primary pathogen (Radostits *et al.*, 2000). The disease has also been reported in camels (Abbas and Agab, 2002; Teshome *et al*., 2003) and in marine mammals such as seals, sea otters, dolphins and porpoises (Forbes *et al.*, 2000). From public health view point, brucellosis is considered to be an occupational disease that mainly affects laboratory workers, farmers, slaughter-house workers, butchers, and veterinarians.

Transmission occurs through contact with infected animals or contaminated materials like placenta, reproductive fluids and sometimes consumption of contaminated food like milk. Symptoms of brucellosis in humans can be highly variable, ranging from non–specific, flu-like symptoms (acute form) to undulant fever, arthritis, orchitis and epididymitis (Plummet *et al*., 1998). The *Brucella* bacteria may enter the body through digestive tract, or mucosal layers and intact skin. Then it may spread through blood and the lymphatic system to any other organ where it infects the tissues and causes localized infection (Lapaque *et al*., 2005). The organism is able to escape phagocytic killing through inhibiting the phagosome-lysosome fusion and reproducing inside macrophages (Young, 2005). After a variable incubation period ranging from less than one week to several months, non-specific systemic symptoms such as fever, headache, malaise, night sweats and arthralgia follows, resembling flu like disease. During the early stages of the disease, patients exhibit frequent bacteraemia that has a continuous pattern, making circulating *Brucella* easily detectable by blood culture. Once in the blood stream, the organism is seeded to multiple organs/systems, especially those rich in reticulo endothelial tissue, such as liver, spleen, skeletal and hematopoietic system (Greenfield *et al.*, 2002).

There are so many factors that can affect the prevalence of brucellosis in various species of livestock including climatic conditions, geography, species, sex, age and diagnostic tests applied. The disease has a very old history, and in Tanzania brucellosis dates back to 1927 when an outbreak of abortion was reported in Arusha region (Shirima, 2005). Since then, a number of studies have been carried out to establish the disease status in livestock. Brucellosis has been reported to occur at the prevalence of 15.2% in crossbred and indigenous cattle in Arusha region (Mahlau, 1967), 12.2% in traditional and smallholder

dairy production system in Kilimanjaro region (Swai *et al.*, 2005), 12-14% in dairy and zebu cattle in Eastern zone (Weinhaupl *et al.,* 2000) and 15.2% in crossbred and indigenous cattle in Southern zone Tanzania (Otaru, 1985). Mahlau (1967) isolated *B. melitensis* from aborting goats and *B. abortus* in aborting cows in Iringa and Arusha regions, respectively. The majority of these studies were carried out in Tanzania involving parastatal farms and indigenous traditional cattle herds, which were often used purposively. Only two studies have been carried out systematically in the smallholder dairy sub sector in Tanzania which includes studies carried out in Morogoro and Dar-es-Salaam regions (Swai, 1997) and Morogoro and Coast regions by Mdegela *et al.* (2004).

Surveys have shown that the disease occurs in cattle in various regions and zones in the country with sero-prevalence varying considerably (Shirima, 2005). These studies though carried out in different parts of the country were mainly carried out in pastoral sector which account for over 90% of total cattle population in Tanzania (Minja, 2002). This indicates that there is still paucity of information about the disease status in the smallholder dairy sub sector, where there is a close human and animal interaction.

1.2. Justification of the Study

Knowledge on the prevalence of brucellosis is of paramount importance as far as public health is concerned. According to Schelling *et al.* (2003), brucellosis is considered as one of the most widespread diseases in the world occurring in human and various species of domesticated and feral (wild) animals. Few studies have been carried out on brucellosis in the smallholder dairy sub sector in Morogoro, Dar es Salaam (Swai, 1997) and Coast Region Mdegela *et al.* (2004), but little information is known on the prevalence of bovine

brucellosis in urban areas. Furthermore, people in urban areas are most likely to be infected with brucellosis due to close proximity of animal and human houses because of limited space. Therefore this study gathered current information to contribute to understanding of the disease prevalence in urban based smallholder dairy sub sector, appropriate disease surveillance and help in informing national strategies for the control of the disease. Also findings from this study will be used to recommend some form of community intervention to minimize the health problem associated with brucellosis both human and animals. Based on the results of the study, the prevalence of brucellosis in smallholder dairy cattle in Morogoro Municipality could be clarified and would primarily help to create awareness to livestock officers, livestock keepers and to the community on the important issues of brucellosis.

1.3 Objective of the Study

This study was conducted with the general objective of establishing the status of bovine brucellosis in smallholder dairy farms in Morogoro Municipality.

1.3.1 The Specific objectives

i. Determine the occurrence and prevalence of bovine brucellosis in smallholder dairy farms in Morogoro Municipality.
ii. Assess the possible factors associated with transmission and occurrence of brucellosis infection between cattle and humans in the study area.

1.3.2 Research questions

i. What is the prevalence of bovine brucellosis in Morogoro Municipality?

ii. What are possible factors associated with transmission and occurrence of brucellosis infection between cattle to cattle and human under the prevailing smallholder production conditions?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition of Brucellosis

Brucellosis also known as undulant fever, “Mediterranean fever,” “Malta fever,” Contagious or Infectious abortion, or Bangs disease is a zoonosis and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products (WHO, 2006). Although there has been great progress in controlling the disease in many countries there still remain regions of the world where the infection persist in domestic animals and consequently transmission to the human population (WHO, 2006). Brucellosis was first diagnosed in humans by David Bruce by isolation of the causative organism from fatal cases in 1887 **(**Pier *et al*., 2004). In cattle it was first described in Denmark by Bang in 1897.

Expansion of animal industries and urbanization, and the lack of hygienic measures in animal husbandry and in food handling partly account for brucellosis remaining a public health hazard (WHO, 2006).

2.2 Aetiology of Brucellosis

Brucellae are Gram negative coccobacilli or short rods with straight or slightly convex sides and rounded ends. They do not ferment carbohydrates in conventional media (Quinn *et al*., 1999). The genus *Brucella* comprises of six species namely, *B. abortus, B. melitensis, B. suis, B. ovis, B.canis, and B. neotomae.* Seven biovars are recognised for *B.abortus,* three for *B.melitensis* and five for *B. suis.* However the degree of

genetic relatedness as shown by DNA hybridization studies is consistent with the existence of a single species within the genus *Brucella* (Briker *et al*., 2000). *B. abortus, B. melitensis, B. suis,* and *B. neotomae* generally occur in smooth form, while *B. ovis* and *B.canis* are invariable rough species (Nielsen *et al.*, 2004). A broad spectrum of smooth *Brucella* isolates have recently been described from a wide variety of cetacean and pinned marine mammals (Briker *et al*., 2000). As their overall characteristics are not consistent with those of any of the six recognised *Brucella* species it has been suggested that they comprise of more than one species, thus two new species, *Brucella pinnipedialis* for pinniped isolates and *Brucella ceti* for cetacean isolates were identified (Ross *et al.*, 1994; Godfroid *et al.*, 2005). *B. abortus, B. melitensis* and *B. suis* are morphologically and tinctorially indistinguishable, according to Berman (1981). They are small, Gram negative, non-encapsulating cocci, coccobacilli or short rods, 0.6 to 1.5 µm in length and 0.5 to 0.7 µm in width. The organism is not acid fast but does resist decolourisation by weak acids and thus stains red with stamp's modification of the Ziehl-Neelsen stain.

Most wild strains of *B. abortus* are fastidious and slow growing, and require carbon dioxide (5-10%) supplementation for primary isolation at an optimal growth temperature of 36 to 38ºC, while growth of *B. melitensis* is not dependent on an atmosphere of 5-10% carbon dioxide although they might be some exceptions (Alton and Forsth, 1998). Survival of the organism in the contaminated environment following parturition or other vaginal discharges present after an infected animal contaminate the environment is influenced by prevailing environmental conditions (Nielson and Duncan, 1990). Bacteria survival outside a host is dependent on

environmental factors including exposure to light, humidity and temperature. *Brucella* can survive for approximately 5 hours on general surface. The rate at which *Brucella* becomes non-viable on pasture is dependent on the weather conditions. In sunlight it survives for <5 days and in shade for >6 days.

2.3 Epidemiology of Brucellosis

2.3.1 Distribution and prevalence of brucellosis in livestock

Brucellosis is a widespread disease particularly among cattle and of major economic importance in most of the countries in the world. In small ruminants, the disease is more restricted to the Mediterranean region including southern Europe, West and Central Asia, South America and Africa (Nielsen and Duncan, 1990; Godfroid *et al.*, 2005), with considerable variation between herds and between areas and countries.

B. abortus has been isolated in cattle raising regions of the world except in Japan, Canada and some European countries, Australia, New Zealand and Israel where it has been eradicated (Animal Health Australia, 2005; Faye *et al.*, 2005). *B. melitensis* is particularly common in the Mediterranean, the Middle East and Central Asia around the Arabian Gulf, some countries of Central America, Africa and India (WHO, 2006). *B. ovis* has been reported from Australia, North and South America, South Africa and many countries of South and Central America, Mexico and Asia (CFSPH, 2009).

Brucellosis has also been reported in a number of African countries with different production systems and varying range of prevalence and as shown in (Table 1).

The reported prevalence of the disease in different zones and management systems in Tanzania is as shown on Table 2.

Table 1: Prevalence of brucellosis in some African countries with different animal management systems.

Country	Animal Management system	Prevalence (%)	References
Mali	Intensive rotational system	22	Mayoral,1992
Uganda	Zero grazing and extensive system	18.1	Magona *et al* 2009
Botswana	Communal and commercial management system	2.1	Brown *et al*. 1992
Sudan	Agropastorallist and courtyard system	2.27	Babiker 1997
Ethiopia	Extensive and intensive system	4.2	Berhe *et al*. 2007
Kenya	Zero, semizero and free range system	5.5-17.5	Delgado *et al.* 2001
Egypt	Controlled and seasonal rotation system	9-61.8	Campbell and Luckert (2002)
Ghana	Extensive system	6.6-9.3	Westoby *et al.* 1989
Nigeria	Extensive and intensive	7-63	Minja 2002

Table 2: Prevalence of brucellosis reported in different zones and animal management systems in Tanzania

Zone	Animal Management system	Prevalence (%)	References
Northern	Intensive and extensive system	1-30	Mtui-Malamsha, 2001;Minja, 2002;Swai *et al*. 2005
Eastern	Zero grazing and extensive system	12-14	Weinhaupl *et al*. 2000
Lake	Traditional cattle production system	4-22.5	Kagumba and Nandoka, 1978;Msanga *et al*. 1986
Central	Extensive and intensive system	2-10.6	Kitaly, 1984
Coastal	Extensive and intensive system	2-90.5	Minga and Balemba, 1990;Swai, 1997; Weinhaupl *et al*. 2000
Southern	Extensive and intensive	15.2	Otaru,1985

2.3.2 Transmission of brucellosis

2.3.2.1. Transmission of brucellosis in humans

Until the beginning of the present century, the animal origin of only a small number of human diseases had been recognized, but at the present time, more than 300 diseases are proved to be of zoonotic importance. Animal and human health is inextricably linked. Several zoonoses are almost equally harmful to man and to animals. Among others, brucellosis only rarely or slightly impairs animal health, but it may cause serious illnesses in the human body (FAO, 1959).

Brucellosis can be transmitted to humans through contact with B*rucella* organisms when exposed to infective discharge or tissues from infected animal or their products, for instance drinking raw or improperly pasteurized infective milk. Statistics show an increased incidence of human brucellosis with substantial cases per 100,000 populations during 1997 to 2002 (Kozukeev *et al.*, 2003). This is mostly in personnel who are engaged in livestock activities such as veterinarians, slaughterhouse employees, dairy farmers and workers, livestock handlers and laboratory personnel. Reported incidence of brucellosis in endemic areas varies from 0.1 to more than 200 per 100,000 populations (Lopes-Merino, 1998). Other countries such as Peru, Kuwait and parts of Saudi Arabia have a very high incidence of acute infections (Bret *et al.*, 2008). The low incidence reported in other known brucellosis endemic areas may reflect low levels of surveillance and reporting, although other factors such as methods of food preparation, heat treatment of dairy products and direct contact with animals also influence risk to the population (WHO, 2006).

The heaviest brucellosis burden lies in countries of Mediterranean basin (Portugal, Spain, Southern France, Italy, Greece, Turkey and North Africa), Arabian Peninsula, India, Mexico, South and Central America, Eastern Europe, Asia, Africa, the Caribbean, and the Middle East. This is due to increased animal production, intensive keeping of animals under poor hygienic conditions, in addition to socio-economic and behavioral factors (Abdulssalam and Fein, 1976; Al-Nassir et al., 2009). From a recent survey of the Arabic Peninsula, there was a serological evidence of exposure to *Brucella o*f almost 20 %. In Mediterranean and Middle East countries, the annual incidence of human brucellosis varies from 1 to 78 cases per 100,000. Certain communities in South European countries reported up to 77 cases per 100,000 people, and the main *Brucella* spp. in these countries was *B. melitensis* (Mousa et *al.,* 1988). Although some countries have effectively controlled brucellosis, new cases of human brucellosis have emerged from people returning from endemic countries (Feya *et al.*, 2005; WHO, 2006).

Evance and Francis (1935) reported brucellosis in humans in Tanganyika caused by *B. abortus* and *B. melitensis*. Monthly medical reports from the Lake and West regions for 1959, 1960 and 1961 and from two patients at Kihesa village in Iringa region in 1962 (Anon, 1963) also revealed presence of human brucellosis in the country. However, because clinical symptoms of brucellosis in humans are similar to malaria and typhoid (Muriuki *et al.*, 1997), it is possible that some brucellosis cases are recorded as malaria or typhoid, thus the true incidence has been estimated to be ten to twenty five times higher than reflected in existing reports (WHO, 1997).

2.3.2.2 Transmission of brucellosis in cattle

Cattle become infected by ingesting B. abortus on contaminated pasture or in feed and water. They also get infected by licking an aborted fetus, infected after birth or genital exudates from a recently aborted or recently calved infected cow.

(i). Ingestion

In cattle brucellosis is transmitted through ingestion or direct contact. Natural infection generally results from the ingestion of feed and water contaminated with reproductive discharges or tissues from aborting cow and the placenta. Ramanatha *et al.* (1992) reported that discharges from an infected bull, afterbirths, tissues and discharges from aborting cow may contaminate pastures, water sources, feed or udder and potentially lead to infection of other animals through the alimentary tract when the susceptible animals feed on the contaminated pastures, feeds and water. Animals also can contract the infection by licking tissues of the afterbirth or from abortion.

(ii). Direct contact

Brucellosis is typically transmitted when susceptible animals come into direct contact with tissues or discharges from infected animals. Full virulent *Brucellae* are highly invasive and capable of penetrating the mucosa or skin of the nose, throat, conjunctiva, urogenital tracts, teat canal and abraded skin (Davis *et al*., 1990). Infection also results when reproductive discharges come in contact with the mucous membrane of a susceptible animal.

(iii). Venereal Transmission

Generally, the bull is not credited with playing a significant role in the transmission of brucellosi*s* in cattle. A very substantial amount of research has been conducted in attempt to determine transmission rates by infected bulls to heifers or cows during breeding. Reports of these experiments ranges in time from 1926 through the 1940s, (Fitch, 1938; Manthei, *et al.*, 1950) and 1960s (Mukerji, 1960; Rankin, 1965). Although the numbers of

animals reported is relatively small in each of these studies, there is not one reported incident of brucellosis transmission through normal coitus by an infected bull. Transmission via artificial insemination on the other hand has been reported to occur with relative certainty when contaminated semen is deposited in the uterus (Rankin, 1965). Apparently, inoculation of contaminated semen directly to the uterus produces an environment in which *B. abortus* grows as compared to the vaginal environment in which the organisms die.

2.3.3 Pathogenesis of Brucella infection in cattle

After infection, *Brucella* bacteria localize in regional lymph nodes, spread via blood and reach the udder and lymph nodes surrounding the reproductive organs, where in the next gestation, infection of the placenta again occurs. Bacteria are shed in birth membranes and discharges from the female reproductive tract following normal calving or abortion, they are also shed in milk thereby endangering public health.

Brucellae organisms are capable of invading and surviving in both phagocytic and non-phagocytic cells and tends to localize in the rough endoplasmic reticulum (Zhan and Cheers, 1995). After the invasion the *Brucellae* are ingested by various local phagocytic cells and multiply in mononuclear and polymorphnuclear cells and localise temporarily in the lymph nodes of the invasion site, where they cause hyperplasia and acute inflammation. This cycle is repeated by multiplying of the *Brucellae* in the cytoplasm of the phagocytes rupturing and being ingested by new phagocytes (Smith and Fitzgeorge, 1964). From the nodes spreading occurs via the blood to other lymph nodes and the reticulo endothelial cells (Macrae and Smith, 1964). In pregnant animals, the placenta and

mammary gland are also invaded (Meador and Deyoe, 1989) and in acute cases up to 85% of the bacteria are in cotyledons, placental membranes, and allantoic fluid (Radostitis *et al.*, 2000).

In non-pregnant cows, localization occurs in the udder and uterus and in cases where the animal becomes pregnant bacteraemia phases occur from the udder. Infected udders are clinically normal but they are important as a source of infection of the uterus and also a source of infection in calves and humans by drinking the milk (Johnson, 1994).

In general the organisms escape from the lymph nodes and set up bacteraemia phase in the cytoplasm of circulating phagocytic cells. The onset of bacteraemia is variable from a few days to 2 month or up to 5 months or even more. *Brucellae* are dispersed throughout the body during the bacteraemia phase and localize in lymph nodes (Supra mammary and mammary lymph nodes), the spleen, mammary gland, uterus and in the epididymis and accessory sex glands of male (Alton, 1990).

2.3.4 Clinical manifestation

2.3.4.1 Brucellosis in livestock

Brucellosis in dairy herds result in decreased milk production, increase somatic cell count in milk, occurrence of abortions and post-partum metritis (Meador and Deyoe, 1989). The disease is characterised by late abortion associated with necro-hemorrhagic placentitis and foetal lesions, particularly fibrinous pleuritis, pericarditis and pneumonia are also common (Xavier *et al.*, 2009). Infected cows usually abort only once, and subsequent gestations

may generate calves that may be born weak or healthy. Some infected cows will not exhibit any clinical symptoms of the disease and give birth to normal calves.

Characteristic but not pathognomonic signs of brucellosis in most animal hosts are abortion or storm abortion in highly susceptible group, premature births and retained placenta (Ariza *et al.,* 1992; WHO, 2006; Matope1 *et al.,* 2010; NABC, 2011). In some parts of Africa, hygromas and abscesses are the major clinical signs in nomadic or semi-nomadic cattle herds infected with *B. abortus* biovar 3. There is lowered milk production due to premature births. Interference with fertility is usually temporary and most infected animals will abort only once and some are unaffected. Occasionally in some cases second or third abortion in the same animal may occur (Adams, 1998).

Infected cows usually abort during the second trimester to term of first pregnancy after infection. Thereafter the disease usually localizes in the lymph nodes surrounding the reproductive organs and the udder. Bacteria are shed in milk, foetal membranes and discharges from the female reproductive tract after birth or abortion. Male animal develop orchits, epididymitis, and hygromas **(**WHO, 2006; Matope1 *et al.,* 2010). The condition is associated with enlargement and pain of one or both testicles (Blood and Radostits, 1990). Infection of one testicle may not render the animal sterile, but if both testicles are infected sterility is a common feature (Minja, 2002). The severity of the disease depends upon many factors such as previous vaccination, age, sex and management such as herd size and density (WHO, 2006).

Incubation period of disease from time of exposure until a positive serological reaction or abortion varies widely depending on age of animal, stage of gestation, exposure dose and other factors (Popeuieck and Kahrs, 1981; Crawford *et al.,* 1986; Bishop *et al.*, 1994). The incubation period in cows varies according to the time at which infection occurred.

2.3.4.2 Brucellosis in humans

Brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia and prostration, and which, in the absence of specific treatment, may persist for weeks or months (Cutler *et al.,* 2005; WHO, 2006).

Typically few signs are apparent but enlargement of the liver, spleen and lymph nodes may occur as many signs referable to almost any other organ system (WHO, 2006). It affects people of all age groups and of both sexes. The incubation period is difficult to determine in humans but has been estimated at five days to three months. Most infection seems to become apparent within two weeks. Aerosolization of bacteria in biological weapons could result in shorter incubation period (OIE, 2009).

Bone and joint involvement are the most frequently complication of brucellosis occurring in up to 40% of cases. A variety of syndromes have been reported including sarcoilitis, spondylitis, peripheral arthritis, osteomyelitis, bursitis and tenosynovitis. A post infectious spongy (loarthropathy) involving multiple joints has been described and is believed to be caused by circulating immune complexes (WHO, 2006).

In humans brucellosis is a multisytematic disease with a broad spectrum of symptoms. Asymptomatic infections are common typically; brucellosis begins as an acute febrile illness with nonspecific flue like signs such as fever, headache, malaise, back pain, myalgia and generalized aches. Drenching sweats can occur particularly at night. Splenomegaly, hepatomegaly, coughing and pleuritic chest pain are sometimes seen.

Gastrointestinal signs including anorexia, nausea, vomiting, diarrhoea and constipation occur frequently in adult but less often in children. Most people with undulant form recover completely in three to twelve months. A few patients become chronically ill. Hypersensitivity reaction can mimic the symptoms of brucellosis (OIE, 2009).

2.3.4.3 Brucellosis in livestock

In cattle *B.abortus* cause abortion which usually occurs during the second half of gestation, stillbirths and weak calves. The placenta may be retained and milk yield may be decreased. After the first abortion subsequent pregnancies are generally normal. However, cows may shed the organism in milk and uterine discharges. Infertility occurs occasionally in both sexes, due to metritis or orchitis. Systemic signs do not usually occur in uncomplicated infections, and deaths are rare except in the foetus or new-born. Infections in non-pregnant female are usually asymptomatic (OIE, 2009).

2.3.5 Diagnosis of brucellosis in human and animals

Because of variable symptoms, non-distinctive clinical signs, and subclinical and atypical infections the clinical diagnosis of brucellosis in humans is particularly difficult. A wide

variety of symptoms and revealed by persons who acquirred the disease in a slaughter plant, on a farm or ranch, or from the consumption of raw milk or cheese made from raw milk, many of which did not result in an initial diagnosis of brucellosis (Young, 1983). Generally diagnostic tests fall into two categories: Those that demonstrate the presence of the organisms and those that detect an immune response to its antigens (WHO, 2006; Godfroid *et al*., 2010).

The diagnosis of brucellosis is confirmed by isolation and identification of the causative organism (Godfroid *et al*., 2010). However this approach is time-consuming, and the specific tests needed to characterize the bacteria are complicated. In order to be able to screen a large number of animals, the diagnostic tests should be "inexpensive, easy to perform, rapid, highly sensitive and fairly specific". Several serological tests have been designed to meet these requirements (Mangen *et al*., 2002).

2.3.5.1 Serological tests

The detection of specific antibody in serum or milk remains the most practical diagnosis of brucellosis (WHO, 2006). There are several common serological tests available for detecting antibody response in animals and human, thus used for screening purposes (Minga and Balemba, 1990). The tests include Serum agglutination test (SAT), Complement Fixation Test (CFT), indirect enzyme linked immunosorbent assay (i-ELISA), Competitive ELISA (c-ELISA) and Rose Bengal Plate Precipitation Test (RBPT).

(i) Serum Agglutination Test (SAT)

The word agglutination originates from the Latin word agglutinare, which means “to glue to.” This is known to occur in biology among three main examples. The first example is the clumping of the cells like bacteria or the red blood cells when in the presence of the antibody. The antibody or the other molecule then binds the multiple particles and thus joins them, helping to create a large complex. The coalescing of the small particles is thus now suspended in the solution. These larger groups or masses are normally then precipitated (Marrodan et al., 2001).

The SAT has been used extensively for brucellosis diagnosis and, although simple and cheap to perform, its lack of sensitivity and specificity mean that it should only be used in the absence of alternative techniques (Ariza *et al.*, 1992; Anderson *et al.*, 1995; Jiwa *et al.*, 1996; Swai, 1997; Mahlau and Hammond, 1962; WHO, 2006). The limitation to this test include failure to differentiate natural infections from the effects of vaccination, and failure to detect *Brucella* antibodies following abortion or early incubation, while the test can also become negative during chronic stages of the disease (Corbel, 1988; Bishop *et al*., 1994).

(ii) Complement Fixation Test (CFT)

The basic test consists of *B. abortus* whole cell antigen incubated with dilutions of heat-inactivated serum (heated to destroy indigenous complement) and a titrated source of complement, usually guinea pig serum. After a suitable time a pretitrated amount of erythrocytes coated with rabbit antibody is added. If a primary immune complex (*B.*

*abortu*s cells and test serum) formed due to the presence of certain antibody isotypes mainly IgG1, in the serum, complement was activated and therefore not available to react with the secondary immune complex of sheep erythrocytes and rabbit antibody, resulting in no or only slight lysis of the erythrocytes. Alternately, if no primary immune complex was formed, complement would cause all the sensitized sheep erythrocytes to lyse. Thus the amount of haemoglobin in solution is a measure of anti-Brucella antibody activity. The complement fixation assay has been standardized (Huber *et al.*, 1986). The sensitivity and specificity of the CFT is good, but it is a complex method to perform requiring good laboratory facilities and trained staff (WHO, 2006). It is essential to titrate each serum sample because of the occurrence of the prozone phenomenon whereby low dilutions of some sera from infected animals do not fix complement. This is due to the presence of high levels of non-complement fixing antibody isotypes competing for binding to the antigen. At higher dilutions these are diluted out and complement is fixed. Such positive samples will be missed if they are only screened at a single dilution (WHO, 2006). This test is regarded as definitive test for the serological detection of infected animals and humans (Ding, 1993; Bishop *et al.*, 1994; Batra *et al*., 1998; Omer *et al*., 2000).

2.3.5.2 Demonstration of *Brucella* organisms

(i) Molecular Diagnostic techniques

These are modern diagnostic technique based on molecular biology. The *Brucella* organism can be detected directly from specimen hence shortening time required to identify the pathogen. These techniques include Polymerase Chain Reaction (PCR), Restriction Endonuclease Analysis (REA) and Restriction Endonuclease and Hybridisation analysis which have been used for diagnosis and epidemiological studies

of the disease (Tenover, 1988; Ghassan *et al.*, 1996). Polymerase Chain Reaction assays have been designed that are specific for the *Brucella* genus (Yingst *et al.*, 2010). Speciation by PCR is possible, but it is not essential for initial diagnostics, especially for outbreak detection (Yingst *et al.*, 2010). However these techniques are too expensive to be used widely, they are more appropriate for differential diagnosis rather than for establishing prevalence.

(ii) Culture and microscopy

The only conclusive evidence of *Brucella* infection is the recovery of the bacteria from the patient. Although *Brucella* can be isolated from bone marrow, cerebrospinal fluid, wounds, pus, blood is the material most frequently used for bacteriological culture in human (WHO, 2006). In animals the preferred samples include foetal membranes, uterine discharges, milk, blood or colostrum from infected animals and stomach contents, liver and spleen of aborted foetus. Retropharyngeal or pre scapular lymph nodes may also be used but supra mammary lymph node is the most suitable specimen (Bishop *et al*., 1994; Abdel-Hafeez *et al.*, 1995). Since *Brucella* is extremely infectious for laboratory workers, this necessitates its culture to be carried out in a biohazard hood (David and Arthur, 1998).

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) or Kosters' methods. The presence of large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as

Coxiella burnetii or Chlamydia may superficially resemble *Brucella* in smears after staining (Bishop *et al*., 1994; WHO, 2006).

(iii) Rose Bengal Precipitation Test (RBPT)

The RBPT is one of a group of tests known as the buffered *Brucella* antigen tests which rely on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low pH (WHO, 2006). The RBPT is a simple spot agglutination test where drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a positive reaction (WHO, 2006). The test is capable of detecting infected animals earlier than SAT due to its ability to detect presence of IgG1, which is produced early after exposure (Nielsen *et al.*, 1996). False positive reactors are normally due to residual antibodies from vaccination history of the herd, colostrum antibodies in calves, cross-reaction with certain bacteria and laboratory errors. It is recommended that RBPT positive samples should therefore be subjected to SAT or CFT for confirmation (Arthur *et al*., 1989). It is also common to observe false positive reactions during early incubation period of the disease and immediately after abortion. Rose Bengal Precipitation Test requires minimum equipment; therefore it is an excellent test for large scale screening of sera Blood and (Radostitis, 1990). It is the most useful method if suspected weak positive are considered negative (Abduharfeil *et al*., 1998).

(iv) Enzyme Linked Immunosorbent Assay (ELISA)

The Enzyme-Linked Immunosorbent Assay (ELISA) is a technique used to detect antibodies or infectious agents in a sample. Antibodies are made in response to infection and so an antibody ELISA can indicate whether or not an animal has been in contact with a certain virus. An antigen ELISA can tell whether an animal is infected with a virus by detecting it directly (WHO, 2006). The ELISA tests offer excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form (Munir *et al*., 2008). Moreover ELISA can be used on either serum or milk samples from different species (Vanzini *et al.*, 2001). Among the ELISA methods the competitive ELISA (c-ELISA) was found to be more robust and easy to perform compared to others. The c-ELISA has several diagnostic merits and these include high sensitivity and specificity, ability to differentiate vaccinated animals from naturally infected ones, or those infected with cross-reacting organisms and its use in areas where disease prevalence is low (Nielsen *et al.*, 1996). Indirect ELISA is used to test antibodies High sensitivity: More than one labelled antibody is bound per antigen molecule Flexible: Different primary detection antibodies can be used with a single labelled secondary antibody.

2.3.5.3 Supplementary tests for brucellosis

In dairy herds, milk is an ideal medium to test as it is readily and cheaply obtained, test can be repeated regularly and give a good reflection of serum antibody. Milk from churns or the bulk tank can be screened to detect the presence of infected animals within the herd which can then be identified by blood testing. This method of screening is extremely effective and is usually the method of choice in a dairy herd.

(i) Milk Ring Test (MRT)

In MRT drop of haematoxylin stained antigen is mixed with a small volume of milk in a glass or plastic tube. If specific antibody is present in the milk it will bind to the antigen and rise with the cream to form a blue ring at the top of the column of milk (WHO, 2006). Its sensitivity is low compared to ELISA (Vanzini *et al.*, 2001). The milk ring test (MRT) is a simple and effective method, but can only be used with cow's milk. The milk ring test was first described in Germany by Fleischhauer (1937). It is an agglutination test conducted on fresh milk collected from dairy cows, but it does not work on pasteurized or homogenized milk (Fleischhauer, 1937).

(ii) Milk ELISA

Milk samples are tested undiluted after removal of the fat layer following centrifugation of milk samples. Briefly, 100 µl of samples and controls are added to antigen-coated plate and incubated at 25°C for 30 min. The plate is washed four times, and 100 µl of diluted (1X) antibody-peroxidase conjugate added to each well and incubated at 25°C for 30 min. The plate is washed again, and 100 µl of substrate solution is added to each well, and incubated at 25°C for 10 min. Then 100 µl of stop solution is added to each well, and the optical density (OD) is measured at 620 nm. The mean OD of negative controls for each plate should be < 0.50 and that of positive controls should be between 0.60–1.80. For interpretation of the test results, spontaneous potential values are calculated (Alton *et al.*, 1988).

The milk ELISA is far more specific than milk ring test (MRT). It is used to test bulk milk and is extremely sensitive and specific enabling the detection of single infected animals in large herd in most circumstances.

2.4 Treatment of Brucellosis

2.4.1 Livestock brucellosis

No practical effective treatment for brucellosis in livestock is known, and efforts are directed at control and prevention (Animal Health Australia, 2005). Treatment trials that have been undertaken have shown only partial success in eliminating the infection (Radostitis *et al.*, 2000). An attempt to use antibiotic such as penicillin and oxytetracycline causes L-transformation on the bacterial cell wall thereby possibly creating carrier animals, and thus affecting future serological detection (Bishop *et al.*, 1994).

2.4.2 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. The goal of medical therapy in brucellosis cases is to control symptoms as quickly as possible, to prevent complications and relapses. Multidrug antimicrobial regimens are the mainstay of therapy because of high relapse rates reported with mono therapeutic approaches (WHO, 2006). The risk of relapse is not well understood, as resistance is not a significant issue in treating brucellosis.

The World Health Organization recommends the following for adults and children older than 8 years:

(i). Doxycycline 100 mg PO bid and rifampin 600-900 mg/d PO: Both drugs are to be given for 6 weeks (more convenient but probably increases the risk of relapse).

(ii). Doxycycline 100 mg PO bid for 6 weeks and streptomycin 1 g/d IM daily for 2-3 weeks: This regimen is believed to be more effective, mainly in preventing relapse. Gentamicin can be used as a substitute for streptomycin and has shown equal efficacy (Roushan *et al.*, 2006).

(iii). Ciprofloxacin-based regimens have shown equal efficacy to doxycycline-base regimens.For Children younger than 8 years: The use of rifampin and trimethoprim-sulfamethoxazole (TMP-SMX) for 6 weeks is the therapy of choice. Relapse rate appears to be approximately 5% or less.

(iv). Pregnant women: Brucellosis treatment is a challenging problem with limited studies. The recommendation is a regimen of rifampin alone or in combination with TMP-SMX. However, TMP-SMX use by the end of pregnancy is associated with kernicterus. In patients with spondylitis, doxycycline and rifampin combined with an aminoglycoside (gentamicin) for the initial 2-3 weeks followed by 6 weeks of rifampin and doxycycline is usually recommended.

(v). Patients with meningoencephalitis may require doxycycline in combination with rifampin, TMP-SMX, or both. A brief course of adjunctive corticosteroid therapy has been used to control the inflammatory process, but studies are limited. Patients with endocarditis require aggressive therapy.

(vi). Aminoglycoside therapy in conjunction with doxycycline, rifampin, and TMP-SMX for at least 4 weeks followed by at least 2-3 active agents (without aminoglycosides) for another 8-12 weeks is preferred. Many other drugs have good *in vitro* activity against *Brucella*, including, but not limited to, chloramphenicol, imipenem-cilastin, and tigecycline+Gentamicin-loaded microparticles and immune-response stimulates may hold future promise.

2.5 Control of Brucellosis

2.5.1 Brucellosis control in livestock

Brucellosis is an infectious disease which has been controlled and eradicated in some countries in the world (Godfroid *et al*., 2004). In sub Saharan Africa animal health services delivered by the public sector have greatly decreased over the last twenty years due to various factors such as decreasing government budget particularly for operational cost of disease control. Thus, programmes that require coordinated surveillance information exchange and application of control measures are not implemented in many sub Saharan countries (McDermott and Arimi, 2000). An effective control of animal brucellosis requires the following basic elements:

(i) Surveillance to find all the infected animals and herds.

(ii) It also requires controlling the transmission of the infection to new animals or herds.

(iii) Eradication of the reservoir to eliminate the sources of the infection in order to protect susceptible animals or herds (Metcalf, 1986). The most effective control method in bovine brucellosis is vaccination at age of 3 month. The vaccines consist of a live suspension of a smooth intermediate attenuated strain of

B.abortus (strain 19). It fully protects 65-75% of the animals while remaining animals are at least partly protected Other vaccines include H38, B.suis 2, McEwan strain 45/20, Rev 1, and strain RBS 1 (McEwan and Samuel, 1955).

2.5.2 Brucellosis control in humans

In humans, brucellosis is a public health disease. From public health point of view the main sources of brucellosis is either food related or are dependent on contact with infected animals either in an occupational or recreational contact, local customs, habits and beliefs however, may impede the wide application of potential preventive measures to minimize brucellosis in rural areas in many developing countries (Corbel, 1997).

Usually person to person transmission of brucellosis is not a significant problem except through blood or organ transfer which should be subject to proper control (WHO, 2006). Air borne or contact infection through environmental contamination may be a signifant problem when infected animals pass through densely occupied areas for example on the way to market, so appropriate measure should be taken to address these problems. A key means of achieving this is through education of the population and especially those directly involved in the animal and food industries (WHO, 2006).

The development of an effective *Brucella* vaccine for use in humans would be an important step to controlling and probably eradicating brucellosis. However, the vaccine strategy is currently applicable only in control of livestock disease (WHO, 2006). Various preparations have been used, including the live attenuated *B. abortus* strains 19-BA and 104M used in the USSR and China, and in the cases of live vaccines, there were

potentially serious reactogenic (Corbel, 1999; Shang *et al*., 2002). Therefore, since vaccination is among the potential means of controlling brucellosis in humans then further research is required to discover vaccine preparation that will be safe for human, conveniently available and affordable especially to poor communities.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The present study was carried out in Morogoro Municipality (Appendix 1). The Municipal area is situated at latitude 5.7 to 10º S and longitude 35.6 to 39. 5° E at an elevation of 500 to 600 metres above sea level and is about 200 kilometres West of Dar es Salaam. The Municipality occupy an area of about 260 km² which is divided in 19 wards (NBS, 2007). According to different national census the human population of this Municipality has been growing very fast. For instance in 1967, 1978, 1988 and 2002 censuses there were 24,999, 74,114, 117,601 and 228,863 people, respectively. The number of households has also been increasing and in 1998 there were 26,706 households with an average size of 4.4 persons per households, by 2002 households had already increased to 54,207 with an average size of 4.2 persons per household (NBS, 2007 and URT, 1997).

Morogoro Municipality lies within Morogoro District; it is one of the six councils of Morogoro Region. Other councils are Morogoro rural, Kilosa, Kilombero, Ulanga and Mvomero. The municipal council has only one division, which is sub divided into 19 administrative wards and 274 streets as shown in Appendix 2.

3.1.1 Economic activities and farming practices of people in Morogoro Municipality

Economic activities in Morogoro Municipality are divided into five categories;

(i) Commercial undertaking wholesale and retail trading = 35%,

(ii) Livestock keeping and subsistence farming = 33%,

(iii) Office works = 16%,

(iv) Elementary occupations = 11%.

(v) Industrial production = 5%.

About 75% of the working force in the Municipality is engaged in agriculture related activities (NBS, 2007). Farming is largely carried out in the outskirts of the town and in the neighbouring district of Mvomero. The major crops cultivated include rice, maize, banana, cassava, fruits and vegetables. Livestock production systems practised in this area is smallholder dairy farms, pastoral and agro–pastoralism.

This study focused on smallholder dairy farms. There are an estimated a total of 6,981 dairy cows in the Municipality (Morogoro Municipal Livestock office May, 2011). The number of dairy cows in each ward were as follows; Mzinga (19), Mwembesongo (250), Kiwanja cha ndege (40), Bigwa (332), Sabasaba (6), Mafiga (36), Boma (61), Mazimbu (788), Mji Mkuu (4), Mji mpya (11), Kihonda (3,701), Kingo(0), Kingolwira (640), Mlimani (170), Sultani Area (3), Uwanja wa Taifa (3), Kichangani (307), Mbuyuni (290) and Kilakala (322).

3.1.2 Dairy production system

In this study a smallholder dairy farm is defined as a dairy unit keeping one to ten dairy cows and not more than 15 dairy cows. Majority of smallholder dairy farms keeping <15 dairy cows of different age, and were fed mainly native grass supplemented with varying amount of homemade concentrate mixture of cereal grains

i.e. maize bran and cotton seed cake or sunflower seed cake. The amount and type of supplement utilized varied from household to household. They keep different types of breeds such as crosses of Ayrshire, Friesian and Tanzania Short Horn Zebu.

3.2 Study Design

A cross sectional study was carried out from May 2012 to September 2012. Cluster sampling method (Bennet *et al.*, 1991) was carried out where sample collection involved two stage cluster sampling based on wards and streets. Lactating dairy cows were sampled according to cluster sampling methods (Bennet *et al.*, 1991) and household head was interviewed during administration of the structured questionnaire.

3.3 Sample size Estimation

Sample size estimation was based on brucellosis herd prevalence in cattle of 1-30% in the Northern Zone of Tanzania (Mtui-Malamsha, 2001; Minja, 2002; Swai et al., 2005). The sample size required was determined using the formula by Daniel (1999):-

$n_{=}z^2*p\ (1-p)/d^2$ where; $n_{=}$ sample size, $z_{=}$ statistic for a level of confidence, 95%, $p_{=}$expected prevalence of brucellosis, 30%, $d_{=}$ precision, 5%,

n is 314.07. To correct for the difference in design, the sample size is multiplied by the design effect (D) which is calculated using the formula below (Otte and Gum, 1997).

$D = \rho\ (n-1) +1$, Where n is average number of cattle in cluster (2), ρ is inter-cluster correlation coefficient (0.2). The design effect (D) is 1.2

Therefore the total sample required was nxD, which is 314.07X1.2 = 376.88≈377.

Considering different cluster levels, 13 wards were randomly selected from the list of 19 wards in Morogoro municipality, then 4 streets in each ward were selected, then 4

households selected in each street and then 2 lactating dairy cows selected in each herd. The final sample size (n) was 416 from the total number of dairy cows in Morogoro Municipality which is approximately 6,981 (Municipal Livestock office, 2011).

From the sample size calculation it was estimated to sample 416 dairy cows from 13 wards but due to cooperation from livestock keepers and extension officers (livestock field officers) it was possible to obtain one sample per cow for a total of 450 cows from the 13 wards.

3.4 Data collection

3.4.1 Primary data collection

Information about each herd and the type of animals kept was collected by means of a structured questionnaire (Appendix 3) which was administered by direct interview at all the selected herds on a single visit. The questionnaire was designed to comprise mostly of closed ended (categorical) questions to ease data processing, minimize variation, and improve precision of responses (Thrusfield, 2005). The questionnaire was filled up by interviewing the selected respondent knowledgeable about the household's dairy herd particularly the head of the household, but if head of household was absent other members like spouse, child, parents/parents in law or other specified member with knowledge of herd under investigation was interviewed. Important herd level data collected were location, type and herd size, history of vaccination and method of disposal of afterbirth. Significant animal level data recorded were breed, age, history of vaccination and breeding method (natural or artificial), pregnancy and lactation status. Other information on occurrence of reproductive events such as; history of abortion, retained placenta or

other reproductive disorder was also collected. Herd personnel's knowledge on awareness of brucellosis and its transmission, disposal of placenta, aborted materials and history of raw milk consumption were also recorded (Appendix 3). A total of 135 households were included in the study.

3.4.2 Identification of farmers and cows for sampling

The dairy cows included in the study were obtained by considering different cluster levels. Once the list of the owners and animals to be selected was ready the farm was visited. As most of dairy cows were not marked, the owner was asked to call out loud each animal by the given name. Then the animal with a temporary number corresponding to the selected number was identified.

3.4.3 Milk sample collection and handling

The udder was washed and dried with a clean towel and approximately 10mls of milk hygienically collected from each teat into a sterile bottle (Universal bottles) according to OIE guidelines (2000). The first stream of milk was discarded. Within six hours of collection the samples were screened using Milk Ring Test as described by Shafee *et al.* (2011). Milk samples were collected using adequate equipment and handled according to OIE requirements (OIE, 2000).

3.4.4 Blood sample collection and handling

Blood samples were collected from all dairy cows that had tested positive on MRT. Cows were adequately restrained manually before taking a sample. Approximately 10 mls of whole blood was drawn from the jugular vein using plain vacutainers tubes (Becton Dickson UK), (Fig1). Each tube was labelled using codes (number) describing the specific

animal and herd. The test tube was tilted on a table overnight at room temperature to allow clotting. On the following day the clotted blood in the tubes was centrifuged at 3000 rotation per minute for 10 minutes to obtain clear serum. The serum was decanted into Eppendorf tubes and stored at -20ºC until tested by using c-ELISA. Blood samples were collected using adequate equipment and handled according to OIE requirements (OIE, 2000). The samples were carefully collected and packed, avoiding possibility of leakage or cross contamination. Immediately after collection samples were transported to the laboratory and stored as recommended into (OIE, 2000). For transport the blood sample were packed in a cool box with ice packs and kept cool during transport from the place of collection (field) to the laboratory.

Figure 1: Blood sample collection from jugular vein of a cow

3.4.5 Laboratory analysis of samples

Milk samples from dairy cows were collected and tested for brucellosis antibodies. Blood samples were collected from cows that tested positive on milk and the blood samples tested for brucellosis antibodies. Two tests were performed: Milk Ring Test (MRT) as an initial screening test and followed by Competitive Enzyme Linked Immunosorbent Assay (c-ELISA) as a confirmatory test for the positive reactors in the first test (WHO, 2006). Both tests were carried out at Faculty of Veterinary Medicine (FVM) Laboratory at Sokoine University of Agriculture.

3.4.5.1 Milk ring test

Milk was tested for antibodies against *B. abortus* by (MRT). Milk samples and *Brucella* antigen were kept at room temperature for at least 30 minutes before testing; the test was performed according to Alton *et al.* (1988). Each milk sample was thoroughly mixed to disperse the cream and 1ml of whole milk dispensed into each test tube and one drop (0.03 ml or 30 µl) of MRT antigen was dispensed into each test tube and shaken gently to ensure that the antigen and milk were thoroughly mixed. Test tubes were incubated for one hour at 37ºC. A strongly positive reaction was indicated by formation of a dark blue ring above a white milk column. The test was considered negative if the colour of the underlying milk exceeds that of the cream layer and the cream layer was the normal cream layer (Fig. 2).

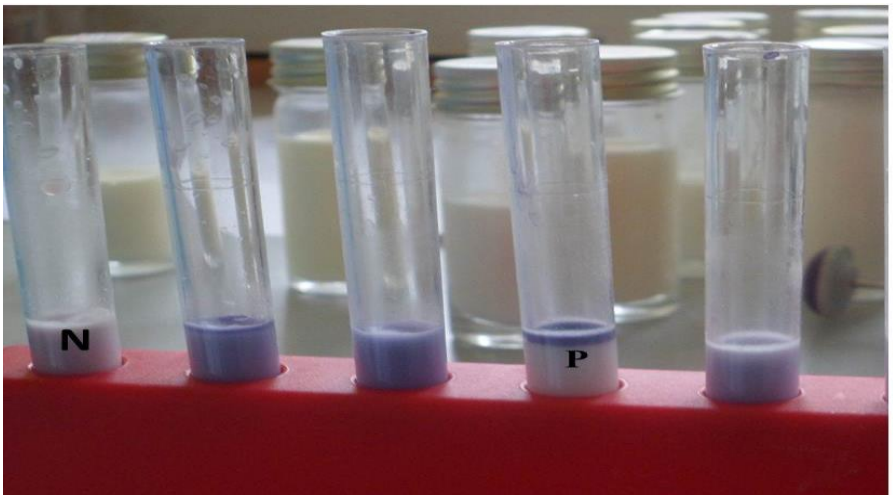

Figure 2: P- Positive MRT sample (Reactor) and N – Negative MRT sample (Non-reactor)

3.4.5.2 Competitive enzyme linked immunosorbent assay (c-ELISA)

The positive and negative sera were tested again for antibody against *Brucella spp*. by the competitive Enzyme Linked Immunosorbent Assay (c-ELISA) as described by Veterinary Laboratory Agency (VLA), New Haw Addlestone Surrey KT15 3NB United Kingdom. The conjugate solution was prepared and diluted to working strength. Twenty microlitre of each test serum sample was added per well. Sixteen wells on column 11 and 12 were left for controls. A 20 microlitre of negative controls was added to wells A11, A12, B11, B12 C11 and C12 A 20 µl of the positive control was also added to wells F11, F12, G11, G12 H11 and H12. No serum was added to remaining wells and this was to act as conjugate controls (Appendix 4). A hundred microlitre of prepared conjugate controls were dispensed into each well. This gave a final dilution of 1/6. The plate was then vigorously shaken on the micro titre plate shaker for two minutes in order to mix the serum and conjugate solution. The plate was covered with a lid and incubated at room temperature ($21^0C \pm 6^0C$) for 30 minutes on rotary shaker, at 160 revolutions per minute. After incubation the contents of plate was

shaken out and rinsed five times with washing solution and then thoroughly dried by tapping on absorbent paper towel. The microplate reader was switched on and the unit was allowed to stabilize for ten minutes. Before the unit was used, the substrate and chromogen solution were prepared by dissolving one tablet of urea H_20_2 in 12 ml of distilled water. When dissolved the OPD tablet was added and mixed thoroughly. One hundred microliter (100µl) of the prepared solution was added to each well.

The plate was left at room temperature for a minimum of 10 minutes and maximum of 15 minutes. The reaction was slowed down by addition of 100 µl stopper solution to all wells and condensation on the bottom of plate was removed by absorbent paper towel. Photometer was adjusted at 450 nm. A positive negative cut-off was calculated as 60% of the mean of the optical density (OD) of the four conjugate control wells. Any test sample that gave OD equal to or below the value was be regarded as being positive.

3.5 Statistical analysis

Data from the questionnaires and laboratory results were stored in computer, using Microsoft Excel spread sheet program 2007. Descriptive statistics for the animal and herd level explanatory variables (grazing system, herd size, breeds and breeding methods) examined in the study were developed using Epi-Info version 7.097. Statistical significance were determined at 95% CI at critical probability ($P<0.05$).

CHAPTER FOUR

4.0. RESULTS

4.1. Prevalence of Brucellosis in Smallholder dairy Farms in Morogoro Municipality

A total of 450 dairy cows in 13 wards were sampled. The proportions of positive reactors to MRT were 29.3% of the milk samples; and c-ELISA were 18.4% of the serum samples. The overall prevalence of brucellosis in smallholder dairy farms in Morogoro Municipality was as shown in Table 3:

Table 3: Overall herd Prevalence of brucellosis in Morogoro Municipality based on MRT and c-ELISA (n=450)

Test	Total samples	Negative Reactors	Positive Reactors	Prevalence
MRT	450	318	132	29.30%
c-ELISA	450	367	83	18.40%

The prevalence of brucellosis based on MRT in the 13 wards was highest in Kiwanja cha Ndege Ward (66.7 %) and lowest in Mafiga (7.6%). Based on c –ELISA test, the prevalence was highest in Kiwanja cha Ndege Ward at 44.4 % and lowest in Boma ward at 9.1% as shown in Table 4.

Table 4: Prevalence of brucellosis based on MRT and c-ELISA in 13 wards of Morogoro Municipality (n=450)

		MRT			c-ELISA		
Ward	Sample	Positive	Negative	Percentage	Positive	Negative	Percentage
Mlimani	52	13	39	25	9	43	17.3
Mafiga	13	1	12	7.6	0	13	0
Sabasaba	1	0	1	0	0	1	0
Boma	22	6	16	27.2	2	21	9.1
Mzinga	10	1	9	10	0	10	0
K/Ndege	9	6	3	66.7	4	5	44.4
Bigwa	33	9	24	27.2	7	26	21.2
Mbuyuni	8	4	4	50	2	6	25
Magadu	88	17	71	19.3	10	78	11.3
Kingolwira	49	19	30	38.7	12	37	24.4
Kichangani	82	32	50	39	24	58	29.2
Mazimbu	37	11	26	29.7	5	32	13.5
Kihonda	46	13	33	28.2	8	38	17.3
Total	**450**	**132**	**318**		**83**	**367**	

4.2. Social Characteristics of the Respondents

A total of 135 selected households were visited and all the household heads in the selected household interviewed.

4.2.1. Gender, age, education level, and experience of dairy cattle keeping of the respondents

Of the 135 respondents, 79.6% were male and 20.4% were female (Table 5). Meanwhile 14.6% of the respondents had professional training in various careers, 28.3% were graduates, 36.0 % secondary school leavers and 21.1 % primary school. Fifty percent (50%) of the respondents had 3 to 10 years experience as smallholder dairy farmers, 46.2% had 10 – 20 years experience and only 3.8% had less than three years experience (Table 5). The majority of respondents (50.1%) were aged 46 to 65

years, while 42% were aged between 20 – 45 years. The remaining 7.6% were aged above 66 years (Table 5).

Table 5: Respondent's gender, age, education level, and livestock keeping experience in Morogoro Municipality

Variable (n=135)	Percent
Gender of respondents	
Male	79.6
Female	20.4
Age in years	
20-45	42
46-65	50.1
66-85	7.6
Education level	
Primary	21.1
Secondary	36
Graduate	28.3
Professional	14.6
Livestock keeping experience in years	
Up to 3	3.8
3-10	50
10-20	46.2

4.2.2 Herd size, age in years and breeds of cattle kept in the study area

The households herd size of 1-15 dairy cattle were the majority (63.9%), 21.7% had herd size of 16-30 dairy cattle and only 14.4% had herd size of more than 30 dairy cattle. The prevalence of brucellosis among the age group using MRT test showed that cattle older than 3 years had 78.6%, followed by those between the age of 2-3 years (19.1%) and 1-2

years (2.3%). The c-ELISA test showed a prevalence of 2.4% for 1-2 years, 18.1% for 2-3 years and 79.5% for cattle older than 3 years. Crossbreeds were the most popular genotype reared by 68.7% of the 135 households, while 19.3% of the households reared Ayrshire breed and only 12.0% reared Friesians.

4.3. Smallholder dairy Herd Characteristics in the Study Area

4.3.1 Animal health management practices

There were no herds vaccinated against Brucellosis. However, 31.8% of herds were vaccinated against other diseases such as Foot and Mouth Disease (FMD), Rift Valley Fever (RVF), East Coast Fever (ECF), Contagious Bovine PleuroPneumonia (CBPP), Anthrax, and Lumpy Skin Disease (LSD). Only 19.8% of the herds were bred using artificial insemination, while 92.4% used natural breeding. Records keeping for vaccination and other routine treatment were reported from 72.8% of the respondents. Majority of the respondents (68.3%) reported to have access to public veterinary services while 31.7% of the respondents reported to get veterinary services from other farmers. Reported common diseases and disorders that affected cattle other than brucellosis were as shown in Fig 3. The most common ones were mastitis, Infertility, ECF and FMD.

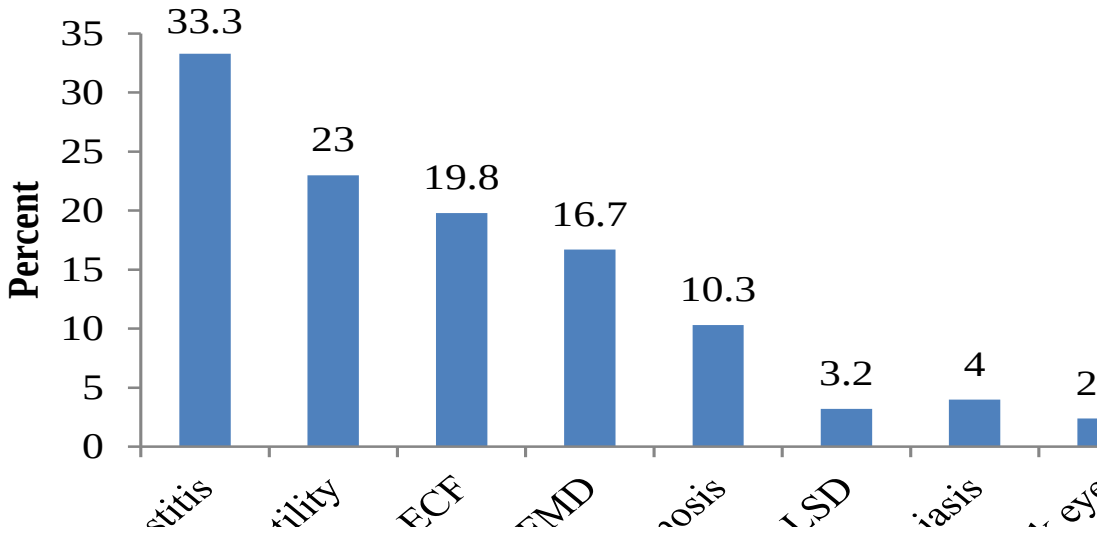

Figure 3: Other common diseases and disorders affecting dairy cattle in Morogoro Municipality

4.3.2 Cattle management systems

The management systems practiced by the livestock keepers were free range grazing (extensive) system in 45.8% of households, zero grazing (intensive) system in 36.1% of households and mixed grazing system in 18.1% of the households. Most herds (56.4%) that were housed had closed type houses (roofed house) while 43.6% were keeping cattle in open house i.e house without roof.

4.3.3 Milk production

At the time of visiting the households, milk production per day varied, with 49.4% of cows produced 1-10litres of milk per day, 41% produced 11-20litres and 9.6% produced >20 liters of milk per day. Percentage of milking cows in the households was 96.4% and 3.6% dried cows.

4.3.4 Persons responsible for daily management activities for dairy cows in Morogoro Municipality.

The present study revealed that the daily care of cattle were undertaken by employed attendants, head of households, relatives living in the household, wives and children in 77, 7.8, 6.5, 5.3 and 3.1% of the responding households, respectively. The proportion of assistance given by other members of households to calving cows was as shown in Fig 4 below.

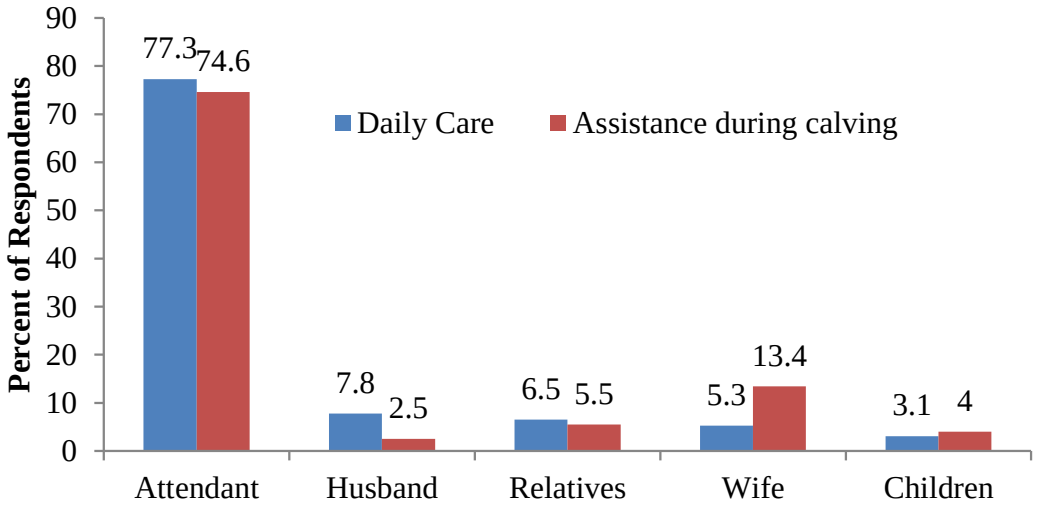

Figure 4: Persons responsible for daily care and assistance during calving in smallholder dairy farms in Morogoro Municipality

4.4. Factors and practices associated with occurrence of brucellosis in a particular household in Morogoro Municipality.

Among factors considered in the questionnaire form, history of abortion (p=0.00) and herd size (p=0.04) were significantly associated with findings of an animal testing

positive for brucellosis in a household. Other practices like raw milk consumption (p=0.65), breeding methods (p=0.87) and grazing system (p=0.07) were not significantly associated with findings of an animal testing positive for brucellosis (Table 6).

Table 6: Factors and practices associated with occurrence of brucellosis in a household in Morogoro Municipality

Variable	Percent	P value
Factor/Practice		
Raw milk consumption		
Yes	72.2	
No	27.8	0.65
Breed		
Ayrshire	19.3	
Friesian	12.0	
Cross breed	68.7	0.16
Calf feeding		
Bucket feeding		
Yes	59.6	
No	40.4	
Calf sucking	59.6	
Yes	40.4	0.32
No		
Breeding methods		
Natural services		
Yes	92.4	
No	34.7	
Artificial insemination	19.8	0.16
Yes	80.2	
No		
Herd size		
1-15 cows	63.9	
16- 30 cows	14.5	0.04
>30 cows	21.7	
Grazing system		
Free range system	45.8	
Mixed system	18.1	0.08
Zero grazing system	36.1	
History of abortion		
Yes	34.9	0.00
No	65.1	

4.5 Knowledge on brucellosis and methods of placenta, aborted foetus and dead calves disposal in the study area.

4.5.1 Awareness of brucellosis and its transmission

Up to 78.9% of respondents interviewed had never heard of brucellosis and out of those responded to know the disease, 66.4% had no idea of the disease transmission from cattle to human (zoonosis). Due to low knowledge on brucellosis transmission from animal to human, people live close to animal house and share utensils like buckets as shown in Fig.5. In this particular household there was a cow that tested positive for brucellosis.

Figure 5: Example of households showing close proximity between human and animal houses in Morogoro Municipality.

4.5.2 Methods of disposing placenta, aborted foetuses and dead calves

About 35% of respondents admitted to have observed abortion in their herd. The most common method of disposing off placenta, foetuses and dead calves was by burying in the ground as reported by 80% of respondents. Other methods of disposal were as shown in Table 7.

Table 7: Methods of disposing placenta, aborted foetuses and dead calves

Variable	Percent
Buried	80.0
Disposal pit	9.5
Dog food	6.7
Throw to the bush	3.8

CHAPTER FIVE

5.0. DISCUSSION

The prevalence of bovine brucellosis in thirteen (13) wards of Morogoro Municipality was found to be 18.4% (95% CI) based on c-ELISA as a confirmatory test, after screening using MRT which showed 29.3% positive. The two tests showed degree of agreement; however the variation in prevalence by two tests could be due to false positives. The Milk Ring Test (MRT) has been described as a highly sensitive but not specific test while the Competitive Enzyme Linked Immunosorbent Assay (c-ELISA) is both a specific and sensitivity test and can eliminate cross reaction due to heterogeneous bacteria and can minimize false positives.

The results observed in present study agree with previous studies in different parts of Tanzania which fall in range of 1-30% (Kitaly, 1984; Otaru, 1985; Minga and Balemba, 1990; Swai, 1997; Mtui Malamsha, 2001; Minja, 2002; Shirima, 2005 and Karimuribo et al., 2007). The variation in prevalence of brucellosis reported in Tanzania is probably due to different livestock management systems used in areas where the studies were conducted. The 18.4% prevalence of brucellosis was only to the area of study Morogoro Municipality which represents part of the country. More studies should be conducted to provide comprehensive status of brucellosis in the entire country.

The prevalence was lower in young cattle screened in this study compared to the older ones. Usually young animals are protected by maternal immunity until when the immunity

disappears (Jordan, 1995). The herd sizes were categorized into small, medium and large. The results showed increases in prevalence with small herd size. The study showed that majority of households herd kept 1-15 dairy cows which is small herd size.

The movement of animals between herds has been established to be an important factor for Brucella species infection in other regions of the world (Al-majali et al., 2009; Kabagambe et al., 2001; Muma et al., 2007b; Omer et al., 2000). The practice of mixing of cattle either through grazing or sharing of water point is an important factor for transmission of brucellosis (Al-majali et al., Muma et al., 2007b).

The prevalence could not reflect the past or present exposure to *Brucella* organisms because vaccination against brucellosis using *Brucella abortus* S19 was previously practiced only in state owned dairy farms and this stopped in 1980s due to resource constrains (Shirima, 2005). However positive tests for *Brucella* antibodies does not necessarily mean that the animals have current or active infection at the time of sampling but that it may be a result of past infection resulting in a "self-limiting disease".

None of the respondents have reported their herds vaccinated against brucellosis although some (19.8%) reported that they vaccinate cattle against other diseases such as Foot and Mouth Disease, Lumpy Skin Disease, East Coast Fever, Contagious Bovine Pleural Pneumonia and Anthrax. This is due to the nature of the disease that doesn't exhibit clinical symptoms except the abortions which sometimes they occur once and subsequent gestations may just generate calves that may be born weak or healthy.

The possible explanation for the relatively high and variable prevalence within the study might be those related to transmission of disease between herds due to the proximity between herds in the communal grazing areas and water points as well as purchasing of infected animals. The prevalence rate in this study is higher compared to those observed by Swai (1997) and Mdegela *et al.* (2004) in smallholder dairy cows in both Iringa and Tanga regions probably due to management systems used in the areas where this study was conducted.

From the result of this study majority of respondents interviewed had never heard of brucellosis and had no idea of the disease transmission from cattle to human. Lack of knowledge as was featured in most of the respondents in study area is likely to contribute to increase the disease incidence. Failure to know the disease and the transmission mode may lead to poor management of aborted fetus and after birth contents as well as all other precaution that might have been taken to reduce transmission rate.

The study considered several factors which could be related to the occurrence and prevalence of *brucella* infection which include; abortion, herd size, poor disposal of aborted material, consumption of raw milk, vaccination, veterinary services, and lack of knowledge on the transmission of brucellosis in cows. However only the herd size and history of abortion in a herd shown to be statistically major factors associated with finding a positively testing cow in a herd. Poor disposal of aborted materials such as feeding to dogs, throwing away to the bush and mixing with manure all these subjects other animals coming into contact with these materials hence increase spread of the disease.

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The present study reveals that bovine brucellosis is a problem of concern in smallholder dairy farms in Morogoro Municipality with a prevalence of 18.4%. This is a threat to the public health and social economic wellbeing at household, and also to animal life. The major factors to brucellosis occurrence were herd size and abortion. So, care should be taken while handling the suspected animal cases and consuming raw milk. Findings from this study may be a base for responsible authorities to design and institute control measures of the disease in the area of study.

6.2 Recommendations

The following recommendations have been made to improve the farm management and animal health management practices in smallholder dairy farms and to reduce the transmission of brucellosis:

i) It is therefore recommended that, the best way of reducing the prevalence of bovine brucellosis is to carry out effective education campaign aimed at clearly explaining the factors and mode of transmission of the diseases from the animal to animal and animal to human, awareness on the economic and public health impact of the disease should also be provided.

ii) Further epidemiological studies are needed to establish factors responsible for the prevalence and occurrence of brucellosis in our country, evaluate the socio

economic losses caused by the disease and to carry out molecular studies to confirm the possible cross infection to human in Tanzania.

iii) The prevention of human brucellosis is dependent on control of the disease in domestic livestock. This can be achieved by elimination of infected animals and mass vaccination of healthy ones; this will render individual coming in contact with animals a lower risk and help produce *Brucella* free animal products.

iv) As there is no effective and organized brucellosis control programme in our country, the ultimate control would be achieved through a special programme aimed at public health education about the disease and associated risk factors, maximum cooperation between Ministry of Health and Social welfare and Ministry of Livestock Development and awareness to the physicians to include brucellosis in their immediate differential diagnosis especially in the high risk group areas.

v) Routine screening of animals or surveillance for brucellosis is important that would help to detect positive cases and measures taken to reduce the risk of transmission and proper measures to be taken on time.

vi) Since brucellosis is known to have a multiplicity of agents and hosts, it is crucial to learn more about the prevalence of the disease in other domestic and wild including vermin species and in all other categories of cattle kept in study area not only in dairy cows.

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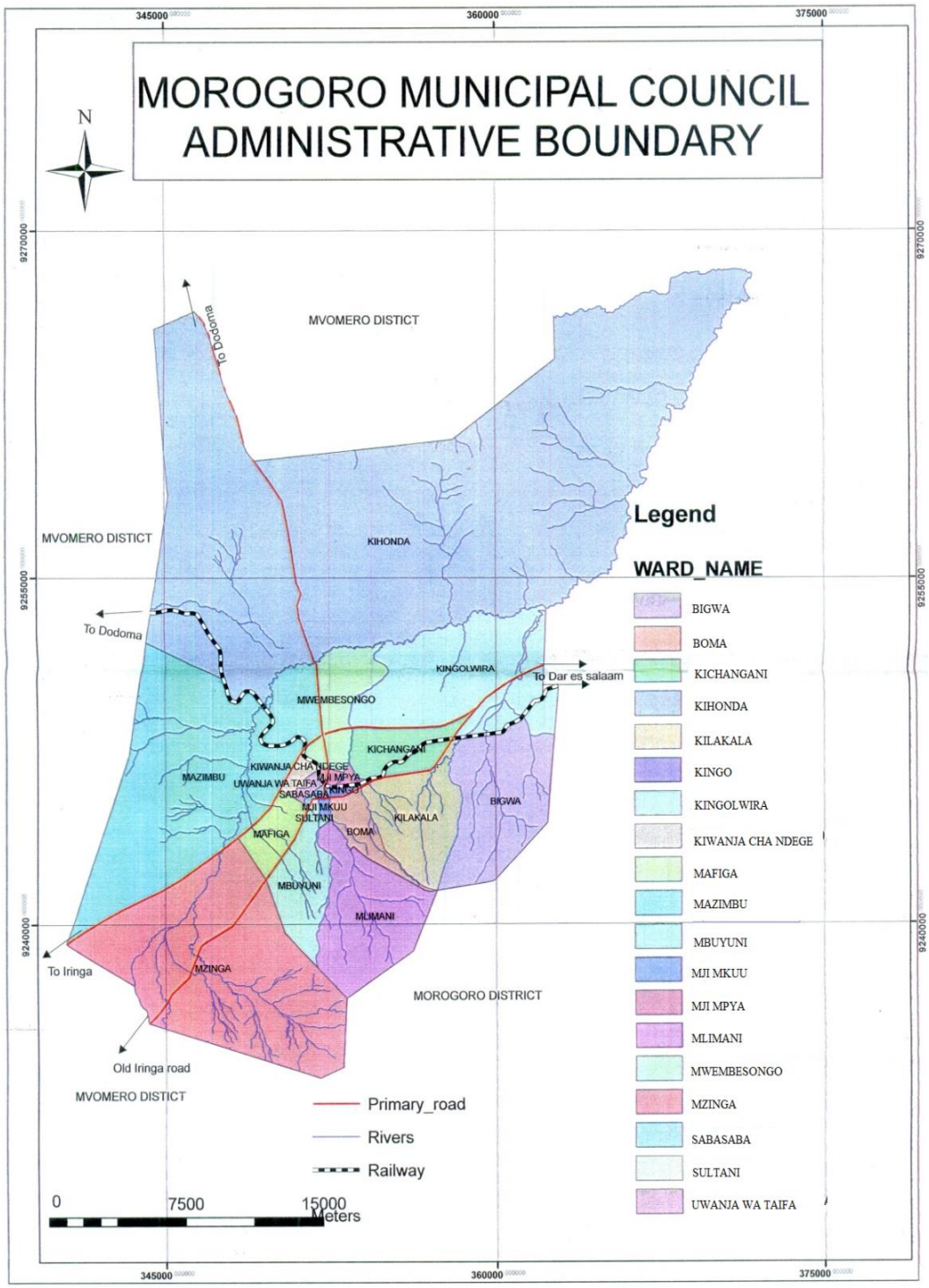
APPENDICES

Appendix 1: Map showing location of study area in Morogoro municipality.



[Source: Morogoro municipal director's office, 2012]

Appendix 2: List of wards and number of streets in Morogoro Municipality

Ward	Number of Streets
Bigwa	9
Boma	15
Kichangani	11
Kihonda	20
Kilakala	14
Kingo	17
Kiwanja cha Ndege	13
Mafiga	15
Mazimbu	32
Mbuyuni	13
Mji Mkuu	07
Mji Mpya	12
Mlimani	14
Mwembesongo	19
Mzinga	15
Sabasaba	12
Sultani Area	10
Uwanja wa Taifa	11
Total	**274**

[Source: Morogoro municipal director's office, 2012]

Appendix 3: Prevalence of bovine brucellosis in smallholder dairy farms in Morogoro municipality, questionaire form

PART A: GENERAL CHARACTERISTICS

A. 1. LOCATION

1.1. Name of Head of Household_____

1.2. Sex _____

1.3. Age _____

1.4. Village: _____

1.5. Ward: _____

1.6. District: _____

1.7. Highest education Level: Primary (P)

Secondary (S)

Graduate (G)

Professional (PR)

1.8. Experience year: Up to 3 (1)

>3-10 (2)

>10-20 (3)

A. 2. ANIMAL MANAGEMENT SYSTEM

2.1. Operation type: Free range (FR)

Zero grazing (ZG)

Mixed (M)

2.2. Housing: Open house (OH)

Close house (CH)

2.3. Herd size (i.e. total number of dairy animals on farm):1-15dairy cows. (1)

16-30 (2)

>30 (3)

Number of milking cows: _____

Number of dried-off cows: _____

Number of suckling calves: _____

2.4. Calf suckling system used, specify below ONLY if there is a suckling calf:

Bucket feeding (**Y**)es or (**N**)o_____

Residual calf suckling (**Y**)es or (**N**)o_____

Other, specify:_____

2.5 Who takes daily Care: (**H**)usband, (**W**)ife, (**C**)hildren, (**A**)ttendant, (**R**)elative, (**O**) Other specify _____

2.6. Which activity ((**F**)eeding/(**M**)ilking) _____

2.7. Who assist calving cows /heifers (H)usband, (W)ife, (C)hildren (A)attendant, (R)elative, (O)thers specify

2.8. How are placenta, aborted fetus, and dead calves disposed off? _____

PART B: ANIMAL- LEVEL INFORMATION

B. 3. ANIMAL INFORMATION

3.1. Cow name: _____

3.1.1. Cow ID_____

3.1.2. Age years: 1-2years (1)

2-4yrs (2)

>4yrs (3)

3.2. Breed: (A)yrshire

(F)riesian

(C)ross breed.

3.3. Number of calving (Parity)_____

3.4. Date last calving_____

3.5. Milk production per day: Up to 10litres (1)

>10-20lts (2)

>-20lts (3)

3.6. Consumption of raw /cuddled milk in house hold (Y)es or (N)o _____

3.7. Breeding method:

3.7.1. Natural service (Y) es or (N) o_____

3.7.2. Artificial Insemination (Y) es or (N) o_____

3.8. Reproductive disorders:

3.8.1. Abortion (Was there any expulsion of dead fetus at any time of pregnancy in your herd) (Y) es or (N) o_____

If YES at what stage of pregnancy…….

3.8.2. Placenta retention with normal calving (Y) es or (N) o_____

3.8.3. Placenta retention with abortion/stillbirth (Y) es or (N) o_____

3.9. Other(s) disease disorder: _____

PART C: ANIMAL HEALTH MANAGEMENT

4.1. Any Vaccination program followed? (**Y**) es or (**N**)o_____

4.2. Last vaccination (Year)_____

4.3. Which type of vaccination done? _____

4.3.1. Brucellosis vaccination done? (**Y**)es or (**N**)o_____

4.3.2. Last vaccination: _____

4.4. Records keeping for vaccination and other routine Treatment (**Y**)es or (**N**)o_____

4.5. Veterinary services: Public service (extension service) (**Y**)es or (**N**)o_____

Private services (**Y**)es or (**N**)o_____

From other farmers (**Y**)es or (**N**)o_____

4.6. Awareness of Brucellosis disease before:

Seen similar condition in the past (**Y**)es or (**N**)o_____

If yes, When Month and Year ----------

Local name of the disease …….

4.7. Are you aware that Brucellosis can be transmitted from cattle to human?

(**Y**)es or (**N**)o_____ If Yes How?.....

Appendix 4: A sketch showing Microtitre plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	T	T	T	T	T	T	T	T	T	T	NC	NC
B	T	T	T	T	T	T	T	T	T	T	NC	NC
C	T	T	T	T	T	T	T	T	T	T	NC	NC
D	T	T	T	T	T	T	T	T	T	T	C	C

C= Conjugate PC=Positive control

T=Test sample NC=Negative control

ABCDEFGH= Stands for rows 1 2 3…12=Stands for columns.