

**SEROPREVALENCE AND RISK FACTORS OF BOVINE BRUCELLOSIS IN  
DAIRY AND TRADITIONAL CATTLE HERDS IN KIBAHA DISTRICT OF  
TANZANIA**

A dissertation submitted to the University of Zambia in partial fulfillment of the requirements of the degree of Master of Science in One Health Analytical Epidemiology (Msc OHAE)

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**DECLARATION**

I, Bachana Ammeckson Rubegwa do hereby declare that the contents of this dissertation submitted herein are my original work and have not been previously submitted to any University for the award of Master's Degree or any other qualifications.

Signature..... Date.....

## CERTIFICATE OF APPROVAL

This dissertation submitted by BACHANA AMMECKSON RUBEGWA, is approved as fulfilling the partial requirements for the award of the degree of MASTER OF SCIENCE IN ONE HEALTH ANALYTICAL EPIDEMIOLOGY (OHAE) of the University of Zambia.

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## ABSTRACT

Brucellosis is a highly contagious bacterial zoonotic disease that affects domestic animals, wildlife, humans and marine mammals. A cross-sectional epidemiological study was carried out to determine the seroprevalence and risk factors of bovine brucellosis in dairy and traditional cattle herds in Kibaha district of Tanzania. Forty nine cattle herds were selected by simple random sampling among traditional and commercial dairy herds. All sera samples were initially screened by Rose Bengal Plate Test (RBPT) antigen and those found positive were re-tested and confirmed using Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) test. A questionnaire was administered to cattle farmers in order to identify risk factors associated with *brucella* seropositivity while a data collection sheet was used to capture bio-data for all individual animals that were sampled. The agreement between the RBPT and c-ELISA tests results was assessed using the Kappa statistic test. The association between each categorical risk factor and the outcome variable (*brucella* seropositivity) was assessed using Pearson chi-square test while the logistic regression was performed to estimate the multiple effects of predictor variables on the outcome variable. A total of 388 cattle serum samples were collected from 46 traditional and 3 dairy herds. From these herds, 330 traditional and 58 dairy cattle were screened, comprising 366 females and 22 males. The overall individual cattle prevalence of *brucella* antibodies was 5.9% (95% CI: 3.6-8.3) while herd level prevalence was estimated at 34.7% (95% CI: 20.9 - 48.5) based on c-ELISA test results. There were no *brucella* antibodies detected in the dairy cattle herds. There was no significant difference in brucellosis prevalence between females (6.0%) and males (4.5%) ( $P=0.741$ ). On the other hand, history of abortion (OR=6.8;  $P=0.002$ ), breed type (OR=8.3;  $P=0.008$ ) and source of animal (OR=6.2;  $P=0.043$ ) were significantly associated with *brucella* seropositivity.

This study also revealed 85% (42/49) of agro-pastoral households were not aware of the zoonotic risks of bovine brucellosis. The study found a relatively higher prevalence of brucellosis in 46 traditional cattle herds compared to 3 dairy farm herds and identified three possible risk factors that should be considered in designing interventions. Annual vaccination of young female calves (3-6 months of age), raising community awareness on brucellosis transmission and zoonotic risks associated with brucellosis in animals and the use of RBPT test for detecting *brucella* infected herds are recommended in the study area.

## **DEDICATION**

I dedicate this work to my deceased parents, my late father Gabriel Rubegwa and my mother Sophia Gabriel who brought me on earth and grounded a firm foundation of my academic journey. Their contribution is enormous indeed, that I cannot quantify. My beloved wife Nezia Bachana; my children Junior and Ivan for being patient and providing moral support throughout my two years stay at the University of Zambia; my elder brother Gerald Chongera for his valuable contribution to my academic life; my sister Odes Mayabala and my uncle Patrick Mayamba for their spiritual support and encouragement in my academic life as well as the Almighty God for giving me good health.

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## LIST OF ABBREVIATIONS

CDC	Centres for Disease Control
c-ELISA	Competitive Enzyme-Linked Immunosorbent Assay
CI	Confidence Interval
CO <sub>2</sub>	Carbon Dioxide
DED	District Executive Director
DNA	Deoxyribonucleic Acid
EID	Emerging Infectious Disease
FAO	Food and Agriculture Organization
IgM	Immunoglobulin M
KEC	Kibaha Education Centre
<i>K</i>	Kappa
LPS	Lipopolysaccharide
mAb	Monoclonal Antibodies
MLDF	Ministry of Livestock Development and Fisheries
ml	Millilitre
OD	Optical Density
OIE	World Organization for Animal Health
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PI	Percent Inhibition
PSU	Primary Sampling Unit
RBPT	Rose Bengal Plate Test
SAT	Serum Agglutination Test
Spp	Species
SRS	Simple Random Sampling
SUA	Sokoine University of Agriculture
TSZ	Tanzania Shorthorn Zebu
UNZA	University of Zambia
URT	United Republic of Tanzania
WHO	World Health Organization

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background Information

Brucellosis is a highly contagious zoonotic bacterial disease of both public health and economic importance with worldwide distribution (OIE, 2009). The disease has been eradicated from most industrialized countries such as Finland, Norway, Sweden, Denmark, Germany, Australia and Netherlands (Acha and Szyfers, 2001). However, in the Mediterranean and countries in sub-Saharan Africa, the disease is still prevalent and it has been shown that the economic burden of brucellosis is greatest in low-income countries (McDermott *et al.*, 2013). In most sub-Saharan countries, cattle seroprevalence estimates have been observed to range between 3 and 15% (Muma *et al.*, 2006; Ghanem *et al.*, 2009; Jergefa *et al.*, 2009; Haileselassie *et al.*, 2010). In Tanzania, brucellosis prevalence in traditional cattle has been estimated to range between 12-15.2% at individual animal level (Weinhäuapl *et al.*, 2000, Swai *et al.*, 2005) and between 2.2-7.6% in commercial sector (Swai, 1997). Risk factors include age, sex reproductive status of the individual animal, occupational personnel such as laboratory workers, abattoir workers, farmers and veterinarians are at high risk of acquiring the infection (Colibaly and Yamego, 2000). The bacterium possesses non-endotoxin Lipopolysaccharide (LPS) which confers resistance to antimicrobial attacks and modulate the host immune response. These properties make LPS an important virulence factor for *brucella* survival and replication in the host (Radostits *et al.*, 2006).

## **1.2 Causative agent and host**

Brucellosis is caused by infection with bacteria of the Genus *Brucella*. *Brucella* are small gram negative bacteria, coccobacillus, non-spore forming and non-motile facultative intracellular organism (Walker *et al.*, 1999). *Brucella* organism is able to survive within macrophages because it has the ability to survive phagolysosome. Brucellosis affects a wide range of hosts that include domestic livestock and wild animals with serious zoonotic implications and causes huge economic losses to the livestock industry through abortion, loss in milk production, low fertility rates, and cost of replacement of animals (McDermott and Arimi, 2002). *B. melitensis*, *B. suis*, *B. abortus* and *B. canis* are the classic causative agents of the disease in humans. Although human brucellosis is the most common bacterial zoonotic infection worldwide, it is still a regionally neglected disease (Pappas *et al.*, 2006). It is the second most important zoonotic disease in the World after rabies (FAO, 2003). The WHO estimates that a quarter of human cases go unreported, yet half a million cases per year are recorded (WHO, 2006).

## **1.3 Transmission**

In cattle, transmission is by direct contact with *brucella* infected material or indirect through ingestion of contaminated fomites (Maurin, 2005). The spread of the disease from one herd to other and from one area to another is always due to the movement of an infected animal from infected herd into a non-infected susceptible herd (Radostits *et al.*, 2006). Humans are infected by ingestion of animal product that is contaminated with *brucella* or through breathing organism (inhalation). A low infectious dose often bacteria and the fact that *brucella* are easily transmitted to humans via aerosols make these bacteria most attractive for military researchers (Hoover and Friedlander, 2010).

In addition, fresh milk and dairy products prepared from unpasteurized milk such as soft cheeses, yoghurts and ice creams may contain high amounts of the bacteria and consumption of these is an important cause of human brucellosis (Bikas *et al.*, 2003). In countries where milk and dairy products are always pasteurized, brucellosis principally affects persons such as veterinarians, butchers, shepherds, meat inspectors (abattoir workers) and farm workers who are in close contact with animals and animal products (Dahouk *et al.*, 2007).

#### **1.4 Clinical signs and symptoms**

In cattle, the disease is mainly characterized by abortion, stillbirths, or birth of weak calves and lactating cows may show decrease in milk yield (Muma *et al.*, 2007a; Matope *et al.*, 2010). In highly susceptible non-vaccinated pregnant cows, abortion occurs after the 5<sup>th</sup> month of pregnancy. In bulls, orchitis and epididymitis are cardinal signs (Radostits *et al.*, 2006). It may also be manifested by unilateral or bilateral orchitis and sterility, while in all age groups, hygroma involving one or more leg joints may be observed, especially in chronic infections (OIE, 2008). The clinical manifestation of the disease in humans is characterized by febrile illness and is often difficult to diagnose solely from the clinical picture due to similarities to other febrile diseases such as Malaria and Typhoid fever (Shirima *et al.*, 2010). Common symptoms of brucellosis in humans include undulant fever with varying temperature from 37.8<sup>0</sup>C in the morning to 40<sup>0</sup>C in the afternoon, night sweats and weakness (Mantur and Mangalgi, 2007). Fever, chills, sweats, aches, lack of energy, joint and back pain, headache and loss of appetite are observed in majority of the patients (Gur *et al.*, 2003).

## **1.5 Economic importance**

Brucellosis causes major economic losses because of lower calving rates due to temporarily infertility or abortion resulting in a decreased milk production, increased replacement costs as well as lowered sale of infected cows. Major economic losses are categorized into; losses due to abortion in the affected animal population, diminished milk production, *brucella* mastitis and contamination of milk, culling and condemnation of infected animals due to breeding failure, endangering animal export trade of a nation, human brucellosis causing reduced work capacity through sickness, Government costs on research and eradication programs and loss of financial investments (Nuru and Schnurrenberger, 1975). Economic impacts exist beyond the cost of control, including the cost of hospital treatment, cost of drugs, direct decreases in household income due to reduction in livestock product sales, consumption impacts due to reduced food security, increased household vulnerability where livestock is used as a risk-coping mechanism and effects on household wealth which influence savings and gender equality (Birol *et al.*, 2010).

## **1.6 Dairy cattle management**

Tanzania livestock sector contributes 30% of the Livestock Domestic Product (GDP, URT, 2012). Most of dairy farmers keep cross breed cattle mainly Friesian and Ayrshire crosses. Semi-intensive system is the common management system in Kibaha dairy farms involving grazing on titled farm land. However, because of inadequate land, it is not uncommon to find dairy animals grazing close or in the communally owned land of which natural pasture is the main source of feed for such dairy cattle (Orodho, 2006). Limited supplementary feeds are provided during milking to lactating cows and sometimes to calves under one year.

In this sector, disease prevention and control are often well managed. Tick borne diseases are normally controlled using acaricides through dipping on weekly basis and vaccination is common against diseases such as Black Quarter (BQ), Lumpy Skin Disease (LSD) and sometimes Foot and Mouth Disease (FMD) and not against Brucellosis. Poor farm infrastructures like water troughs, paddocks, milking parlours, cattle sheds and feed storage facilities contribute to poor hygiene on farm and milk production hence increase the chance of transferring of zoonotic disease such as brucellosis and Tuberculosis (Gillah *et al.*, 2013). Natural breeding using farm bulls is the common breeding practice and artificial insemination is not often practiced. Milk produced from these farms is normally marketed to Kibaha town residents and more so in Dar es salaam city where the price of raw milk fetches better price than Kibaha town.

### **1.7 Traditional cattle management**

The TZS is the common type of indigenous cattle breed kept under the traditional sector. Farmers entirely depend on the natural pastures as source of feed through free grazing system in communally owned grazing land. Mixing of different cattle herds is very common during grazing, watering and sometimes during dipping. The majority of farmers benefit on bulls available in the communal gazing areas for breeding purposes. There are a number of cattle diseases reported in traditional cattle Attention on disease control is given to tick borne diseases which accounts for 77.1% of cattle health constraints (Kiswaga *et al.*, 2014), Contagious Bovine Pleuro Pneumonia (CBPP) vaccination and not against brucellosis. Open livestock market system is the major means of selling cattle for farmers to get basic life needs (money, food, clothes and fees) or for expansion of their herds. Inadequate availability of water and pasture is the main challenge to these farmers, especially during the dry season.

Consequently, most of the traditional farmers, keep on moving in different parts countrywide in search of water and pasture for their cattle. It could be this uncontrolled cattle movement which may encourage the spread and transmission of brucellosis and other infectious disease under this management system.

### **1.8 Diagnosis**

The diagnosis of brucellosis normally requires laboratory confirmation. This could be possible through direct demonstration of the causal organism using staining, immunoflorescent antibody, culture and isolation of bacteria and direct detection of antibodies using serological techniques like Rose Bengal Plate Test (RBPT) and c- ELISA (Quinn *et al.*, 2002).

### **1.9 Prevention and control**

Control of animal brucellosis in the developed world has been successfully achieved through a combination of mass vaccination, test and slaughter programs, effective disease surveillance and animal movement control while the disease in humans has majorly been controlled through milk pasteurization (McDermott and Arimi, 2002; Pappas *et al.*, 2006). In developing countries, these strategies have been ineffective due to inadequate financial resources to compensate farmers whose cattle are slaughtered during screening programs (Godfroid *et al.*, 2011). Control and eradication of the infection in animal reservoirs is the most rational approach for preventing human brucellosis and through proper handling and disposing of aborted fetus, fetal membranes and discharges as well as not to drink unpasteurized milk (Acha and Szyfers, 2001). In humans, treatment with Rifampicin at dosage of 600-900 mg daily combined with Doxycycline at 200 mg daily.

Both drugs are given in the morning as single dose and relapse is unusual after a course of treatment continued for at least 5 weeks (WHO, 2006).

Currently, in Tanzania test and slaughter policy is not practiced in the control and prevention of brucellosis. This is because of inadequate financial resources and breakdown of public veterinary services (TLP, 2006). However, the disease has not been sufficiently investigated and information relating to its prevalence magnitude, distribution and risk factors is scanty in the study area. This study was therefore initiated to look at the magnitude or levels of brucellosis prevalence in dairy and traditional cattle herds in agro-pastoral production system and investigate the distribution of risk factors associated with occurrence of *brucella* infection in Kibaha district. The study also looked at the level of awareness of people in the agro-pastoral communities on the zoonotic risk posed by *brucella* infected cattle.

## **1.2 Problem Statement and study justification**

### **1.2.1 Statement of the Problem**

Brucellosis has considerable impact on animal and human health, as well as wide socio-economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products (Maadi *et al.*, 2011). Livestock provides a lifeline for a large proportion of 95% of the world's rural population that live in the developing world (Hoffmann, 1999; Wadood *et al.*, 2009). The disease is important from the economic perspective because it is one of the most devastating trans-boundary animal diseases and also a major barrier for trade (Gul and Khan, 2007).

In Tanzania, Brucellosis has been reported to occur at the prevalence of 15.2% in Arusha (Mahlau, 1967), 12.2% in Kilimanjaro (Swai *et al.*, 2005), 12-14% in Eastern zone (Weinhäuopl *et al.*, 2000) and 15.2% in Southern zone (Otaru, 1985). A large proportion (85%) of Tanzania's population lives in rural areas with high levels of contact with livestock and their products (ESRF, 1995). Human brucellosis occurs widely in livestock keeping populations in Tanzania (Shirima, 2005), where prevalence estimated at 7.7% has been reported in northern Tanzania (Kunda *et al.*, 2007). Although livestock keepers might be aware of the risk of consuming raw milk or meat, the habit of consuming raw milk, blood or raw or undercooked meat is still a common practice, especially among rural communities (Kambarage *et al.*, 2003; Shirima *et al.*, 2003).

This could mainly be attributed to lack of knowledge of the zoonotic risks associated with the consumption of unpasteurized milk. Since the demand for milk is high in Dar-es-salaam city, milk from traditional cattle and dairy animals from Kibaha area also contribute significantly to milk marketed in the city. Hence, the need to conduct research on the prevalence of the disease in the area.

### ***1.2.2 Justification of the Study***

Brucellosis is considered by the FAO, WHO and the OIE as one of the most widespread zoonoses in the world (Schelling *et al.*, 2003). Studies on the economic production losses of bovine brucellosis are reasonably consistent across a range of production systems in Africa, with losses estimated at 6-10% of the income per animal (Camus and Landais, 1982; Domenech *et al.*, 1982; Perry *et al.*, 2002). The study area was selected because of high livestock densities and unconfirmed reports of brucellosis in cattle (RCO, 2014). The disease has not been sufficiently investigated in dairy and traditional cattle herds. Therefore, information relating to its prevalence magnitude, distribution and risk factors is not currently available. It was therefore important to know the status of the disease in the study area so that practical control strategies could be developed for future disease prevention and control in the study area.

### **1.3 Study Objectives**

#### **1.3.1 General Objective**

The main objective of this study was to determine seroprevalence and risk factors of bovine brucellosis in dairy and traditional cattle herds in Kibaha district of Tanzania.

#### **1.3.2 Specific Objectives**

The following were the specific objectives of the study.

- 1) To determine seroprevalence of brucellosis in traditional cattle herds in Kibaha district.
- 2) To determine seroprevalence of bovine brucellosis in dairy farm cattle in Kibaha district.
- 3) To identify risk factors that are associated with *brucella* seropositivity in traditional and dairy cattle in Kibaha district.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Disease aetiology

Brucellosis in cattle is usually caused by *B. abortus* and occasionally by *B. melitensis* and *B. suis*. *Brucella* are small, non-motile, non-sporulating, non-toxigenic, non-fermenting, facultative, intracellular, Gram-negative coccobacilli parasites that may, based on DNA homology, represent a single species (Grimont *et al.*, 1992; Moreno and Moriyon, 2002). The bacterium is 0.5-0.7µm in diameter and 0.6-1.5µm in length and it is oxidase, catalase and urease positive (Mantur *et al.*, 2007). It is partially acid fast bacteria because is not decolourized by 0.5% acetic acid in modified Zeihl Nielsen stain and thus appear pink in blue background (Walker *et a.*, 1999). *Brucella* may produce urease and may oxidize nitrite to nitrate. Species and biovars are differentiated by their carbon dioxide requirements, ability to use glutamic acid, ornithine, lysine and ribose, production of hydrogen sulfide, growth in the presence of thionine or basic fuchsin dyes, agglutination by antisera directed against certain lipopolysaccharide (LPS) epitopes and susceptibility to lysis by bacteriophage (Alton *et al.*, 1988). *Brucella* pathogens resist killing by neutrophils, replicate inside macrophages and in non-professional phagocytes and maintain a long lasting interaction with host cells (Donald *et al.*, 2002). Target cells and tissues include throphoblasts, fetal lung, macrophages and the male and female reproductive organs (Adams, 2002).

Historically, *brucella* are differentiated by host tropism, pathogenicity and phenotypic traits. A unique feature of this organism, unlike most pathogenic bacteria, is the lack of many classical virulence factors, such as exotoxins, capsule, flagella, fimbriae, plasmids, lysogenic phage, antigenic variation, cytolysins, pathogenic islands, or type I, II, or III

secretion systems, making characterization of pathogenic mechanisms in this organism highly challenging (Boschioli *et al.*, 2002).

*Brucella* virulence relies on the ability to survive and replicate in the vacuolar phagocytic compartments of macrophages. Many *brucella* virulent factor, such as Lipopolysaccharide (LPS) (Lapaque *et al.*, 2005), Type IV Secretion System (T4SS) (de Jong *et al.*, 2008) and the BvrR/BvrS two component system (Guzman-Verri *et al.*, 2002), have been identified to be critical in the intracellular process of *brucella* inside macrophages (Xiang *et al.*, 2006).

To date, ten species are recognized within the genus *Brucella*. The genus *brucella* consists of six classic species which are; *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. neotomae* and *B. canis*. The *B. melitensis* biovars (bvs) 1-3 (mainly isolated from sheep and goats), *B. abortus* bvs 1-6 and 9 (from cattle and other bovidae), *B. suis* bvs 1-3 (from pigs), bvs.4 (from reindeer) and bvs.5 (from small rodents), *B. canis* (from dogs), *B. ovis* (from sheep) and *B. neotomae* (from desert wood rats). This classification is based mainly on differences in pathogenicity and host preference (Oreno *et al.*, 2002).

In the last decade, several new marine species have been described including *B. pinnipedialis* (isolated from seals) and *B. ceti* (isolated from whales and Dolphins) (Foster *et al.*, 2007), *B. microti* (isolated from the common voles (*Microtus arvalis*) and red foxes (*Vulpes vulpes*) (Scholz *et al.*, 2008; Scholz *et al.*, 2009) and lastly *B. inopinata* (isolated from a human breast implant wound) is the only specie that has not been isolated from any animal reservoir (Scholz *et al.*, 2010).

## **2.2 Geographical distribution**

### **2.2.1 Global distribution of animal brucellosis**

Brucellosis is one of the most widely distributed diseases in the world caused by various species of the genus *Brucella* that infects domestic animals, wildlife and humans (OIE, 2009). It was first recognized as a disease affecting humans on the Island of Malta in the early 20th century (Gul and Khan, 2007). Although the distribution of brucellosis is worldwide, the disease is more common in countries with poorly standardized animal and public health programs (Capasso, 2002). Since cattle are found throughout the world, prevalence of brucellosis (0.85 to 23.3%) in cattle has been reported from a wide range of countries (Silva *et al.*, 2002; Refai, 2002; Cadamus *et al.*, 2006). *B.melitensis* biovar 3 is the most commonly isolated species from animals in Egypt, Jordan, Israel, Tunisia and Turkey (Refai, 2002).

### **2.2.2 Global distribution of human brucellosis**

Five species of *brucella* have known pathogenicity for humans Worldwide, these include; *B. melitensis*, *B. abortus*, *B. suis*, *B. canis* and *B. inopinata* (Godfroid, 2011). However, *B. melitensis*, *B. arbotus*, and *B. suis* are highly pathogenic for humans (OIE, 2011a) with *B. melitensis* being the most pathogenic for humans (Young, 1995).

Human brucellosis is known to be highly endemic in the Mediterranean basin, Middle East, Western Asia, Africa and South America (Pappas *et al.*, 2006). Countries with the highest incidence of human brucellosis include Saudi Arabia, Iran, Palestinian Authority, Syria, Jordan and Oman (Pappas *et al.*, 2005). Syria had the highest annual brucellosis incidence worldwide, reaching an alarming 1603 cases per million per year according to data from OIE (2004). In the United Arab Emirates, most cases are reported from Dubai a popular international travel destination, underlining the importance of the disease in the field of travel medicine (Refai, 2002).

The World Bank (2011) ranked Dubai and Abu Dhabi as being the second and third, most popular medical tourism destination in the region behind Jordan. Bahrain is reported to have no incidence of brucellosis (Refai, 2002).

### ***2.2.3 Africa and the sub-region distribution of brucellosis***

According to the OIE (2009) bovine brucellosis is a reportable zoonosis and is of considerable socioeconomic concern. Most African countries are of poor socioeconomic status, with people living with and by their livestock, while health networks and surveillance and vaccination programs are virtually non-existent in North Africa, Algeria has the tenth highest annual human brucellosis incidence worldwide (Mc Dermott and Arimi, 2002). In most low-income countries, there is much less public investment in veterinary and health services, with weaker surveillance and operational capacity. Such interventions are not feasible in many developing countries because of poor surveillance programs, limited institutional capacity and lack of funds for livestock holder compensation (Zinsstag *et al.*, 2007). According to data from OIE (2004) Cameroon, Ethiopia, Kenya, Nigeria, Tanzania and Uganda reported the existence of human cases of brucellosis, while in 2003 similar reports existed for Burkina Faso, Democratic Republic of Congo, Eritrea, Mali, Namibia, and Swaziland. Ghana, Togo and Chad are probably also endemic according to seroepidemiological studies. Brucellosis prevalence has been reported in Africa East 8.2%, West Africa, 15.5%, North Africa, 13.8% and South Africa 14.2% (McDermott *et al.*, 2013). However, the World Livestock Disease Atlas (World Bank, 2011), based on official reports to the OIE, has few data from tropical Africa and Asia and does not show these as the highest risk countries and the degree of under-reporting brucellosis is quite enormous.

#### **2.2.4 History of brucellosis in Tanzania**

The history of brucellosis in Tanzania began in 1927 when an outbreak of abortion in cows was reported in Arusha region (Mahlau, 1967; Shirima, 2005). The first report of human brucellosis in Tanzania was in 1935 (Wilson, 1936). Other reports were recorded from the Lake Zone and Western Regions in 1959, 1960 and 1961 (Mahlau and Hamond, 1962). Surveys have shown the disease to occur in cattle in various production systems, regions and zones, with seroprevalence varying considerably (Swai, 1997; Swai *et al.*, 2005; Shirima *et al.*, 2007). Mahlau (1967) isolated *B. melitensis* from aborting goats and *B. abortus* in aborting cows in Iringa and Arusha regions, respectively. Brucellosis has been reported to occur at the prevalence of 15.2% in Arusha (Mahlau, 1967), 12.2% in Kilimanjaro (Swai *et al.*, 2005), 12-14% in Eastern zone (Weinhäuupl *et al.*, 2000) and 15.2% in Southern zone (Otaru, 1985).

### **2.3 Host range and source of infection**

#### **2.3.1 Terrestrial animals**

Brucellosis is an infectious disease that affects a wide range of mammalian species and is regarded as the world's most common bacterial zoonotic disease (Pappas *et al.*, 2006). Almost all domestic species can be affected with brucellosis except cats which are resistant to *Brucella* infection (Gul and Khan, 2007). With respect to animal hosts, brucellosis prevalence in buffaloes has been reported from Egypt at 10.0% and Pakistan 5.05%; respectively. The disease has also been reported in the one-humped camel (*Camelus dromedaries*) and two-humped camel (*Camelus bactrianus*) as well as in a number of South American camelids-llama (*Lama glama*), alpaca (*Lama pacos*), guanaco (*Lama guinicoe*) and vicuna (*vicugne vicugne*) following contact with large and small ruminants infected with *B. abortus* or *B. melitensis* (OIE, 2009).

The organism infects mainly cattle, sheep, goats and other ruminants, in which it causes abortion, foetal death and genital infections (Nicoletti, 1980; Meador *et al.*, 1988). The manifestations of brucellosis in these animal hosts are similar to those of bovine brucellosis and can become epidemiologically important in sustaining infections in cattle where they share pasture and water holes (OIE, 2010; CDC, 2011 and Gomo *et al.*, 2012).

The main pathogenic species worldwide are *B. abortus*, responsible for bovine brucellosis, *B. melitensis*, which is the main etiologic agent of ovine and caprine brucellosis. These three *Brucella* species cause abortion (abortion storm) in naive heifers. *B. ovis* and *B. canis* are responsible for ram epididymitis and canine brucellosis; respectively. *B. suis* is responsible for swine brucellosis and the only species recognized to cause systemic and generalized infection in pigs which results in reproductive failure. Pigs can be infected with other *brucella* species, but the infection is self-limiting and restricted to regional lymph nodes at the point of entry (Deyoe and Manthei, 1975).

*Brucella abortus* and *B. suis* have also been isolated from a great variety of wildlife species such as bison (*Bison bison*), elk (*Cervus elaphus*), African buffalo (*Cyncerus caffer*), water buffalo (*Bubalus bubalus*), reindeer (*Rangifer tarandus*), feral swine and wild boars (*sus scrofa*), the red fox (*vulpes vulpes*) and the European brown hares (*Lepus aeropaeus*) (Davis, 1990). For this reason wildlife must be considered a potential reservoir for brucellosis in livestock (Davis, 1990; Godfroid, 2002).

### **2.3.2 Marine brucella spp**

Both *B. ceti* and *B. pinnipedialis* are smooth-type *brucella* equipped with all known virulence factors and, as with their terrestrial counterparts, have their preferred hosts but are able to infect other species (Guzman-Verri *et al.*, 2012).

These *brucella* spp have been found in whales, dolphins, seals and sea lions (Foster *et al.*, 2007). *Brucella ceti* and *B. pinnipedialis* isolated from marine mammals can also cause

human brucellosis (Foster *et al.*, 2007). Four human cases with *brucella* infections have been reported presumably of marine mammal origin (Whatmore *et al.*, 2008). *B. microti* is also known to be pathogenic to mammalian hosts (Jiménez de Bagüés *et al.*, 2010). *B. melitensis* has been isolated from Nile catfish (*Clarias gariepinus*) (El-Tras *et al.*, 2010). The potential impact of marine *brucella* in coastal areas where stranded cetaceans may come into contact with domestic animals, scavenging animals or humans should be investigated to avoid exposure to *brucella* infection. However, marine *brucella* have been identified in cases of brucellosis in humans who frequently consume raw seafood (McDonald *et al.*, 2006). Despite this, there is no report of marine brucellosis and the possible resulting infections in humans and domestic animals.

## **2.4 Brucellosis transmission dynamics**

### **2.4.1 Transmission of brucellosis in animals**

In cattle, transmission of *B. abortus* typically occurs through ingestion of live bacteria. It is transmitted among animals mainly through ingestion of contaminated feed and water and occasionally by inhalation of aerosols or by direct contact with infected materials (McDermott and Arimi, 2002; Maurin, 2005). Products of conception at the time of abortion may contain up to  $10^{10}$  bacteria per gram of tissue (Anderson and Cheville, 1986a). The most significant feature of bovine brucellosis epidemiology is the shedding of large numbers of organisms during the ten days after abortion or calving of infected cows and the consequent contamination of the environment (Abubakar *et al.*, 2012).

The disease is spread through contamination of placental material and vaginal discharges of aborting animal (Woodhead and Aitken, 1889).

Movement of infected cattle into a herd can result in transfer of the disease when cattle ingest the bacteria from aborted fetuses, placenta and discharges from cows that have aborted or contaminated pasture or water (Park *et al.*, 2005).

In cattle, venereal transmission is not a major route of infection under natural conditions but artificial insemination with contaminated semen is a potential source of infection (Rankin, 1965). The incubation period varies widely depending on exposure dose, previous vaccination, species, age, sex, stage of gestation and susceptibility (Nicoletti and Gilsdorf, 1997). Venereal transmission is an important route of spread in pigs (Alton, 1990). The transmission of brucellosis by ticks, fleas or mosquitoes from an infected herd to non-infected herd has never been proved (OIE, 2009).

#### **2.4.2 Transmission of brucellosis in humans**

The disease is mainly transmitted to humans through ingestion of contaminated animal products such as cheese and unpasteurized milk and by direct contact with infected animals through handling abortions, dystocia and parturitions (Shirima *et al.*, 2010). The source of naturally acquired brucellosis in humans is almost always from animal reservoirs, but very few cases of human to human transmission have been reported (Godfroid, 2011). The source of human infection resides always in domestic or wild animal reservoirs. The risk of contracting zoonoses from wildlife is higher in poor communities whose people and livestock interact with wildlife, commonly referred to as wildlife-livestock interface areas (Karesh *et al.*, 2005). Wildlife-livestock interfaces pose a challenge to human, animal and environmental health practitioners due to the complex and continuous cycle of disease transmission that such areas foster (Malama *et al.*, 2013). From the public health view point, brucellosis is considered to be an occupational disease for people who work with infected animals, particularly farm workers, veterinarians, ranchers, game hunters and meat packaging factory employees (OIE, 2011).

Human infection transmission typically occurs through three primary sources which include; consumption of unpasteurized dairy products consumed in or imported from a country where brucellosis is endemic, contact with meat or tissues of infected wild animals

and laboratory exposures. Infection may also occur by inhalation, conjunctival contamination, accidental ingestion, skin contamination especially via cuts and abrasion and accidental self-inoculation with S19 vaccine during field vaccination can lead to brucellosis transmission to handlers (WHO, 2006). *Brucella* are highly infectious in laboratory settings and numerous laboratory workers who culture the organism have become infected. It is a frequently reported laboratory acquired infection (Singh, 2009). However, the risk of transmission through intrauterine, breastfeeding, blood transfusions, bone marrow transplants and sexual contact still exists (Megid *et al.*, 2010). *Brucella* organisms can be shed in the milk of infected animals for variable length of time, but for many, it can be shed for the life of the infected animal (Merck Veterinary Manual, 2012). Although *brucella* agents can be transmitted directly and indirectly from its animal reservoir to humans, indirect transmission remains the highest overall risk and mainly occurs through the consumption of unpasteurized milk or dairy products (Godfroid *et al.*, 2005). Fresh milk and dairy products prepared from unpasteurized milk such as soft cheeses, yoghurts and ice creams may contain high amounts of the bacteria and consumption of these is an important cause of human brucellosis (Bikas *et al.*, 2003). Almuneef *et al.* (2003) and Makita *et al.* (2008) demonstrated that consumption of raw milk continues to be a major mode of exposure to the infections.

## **2.5 Risk factors associated with brucellosis**

The occurrence and spread of brucellosis in livestock could be attributed to several managerial factors such as farming system and practice, farm sanitation, livestock movement and sharing of grazing lands (Kadihire *et al.*, 1997; Omer *et al.*, 2000; Kabagambe *et al.*, 2001). Factors influencing prevalence of brucellosis include type of production system, agro-ecological factors, herd size, interaction with wildlife and management factors (Omer *et al.*, 2000; Muma *et al.*, 2007b; Matope *et al.*, 2010).

The outcome of infection in cattle also depends on the age, reproductive and immunological status of the individual animal and natural resistance, route of infection, infectious challenge and virulence of the infective strain (Carvalho Neta *et al.*, 2010). Sexually mature pregnant cattle are more susceptible to infection than sexually immature cattle of either sex (Teshager *et al.*, 2014). Susceptibility increases as stage of gestation increases (Radostits *et al.*, 2006). In some situations, interaction of cattle with wildlife in livestock-wildlife interface areas has been implicated because of sharing of the grazing land and water between wildlife and domestic animals (Pandey *et al.*, 1999).

In addition, laboratory workers handling *brucella* cultures, abattoir workers, farmers, animal attendants, stockmen, shepherds and veterinarians and inseminators are at high risk of acquiring the infection (Colibaly and Yamego, 2000; Radostits *et al.*, 2006). Movement of an infected animal from infected herd into a non-infected herd always facilitates the spread of the disease from one herd to the other or from one area to another (Radostits *et al.*, 2006).

In humans, consumption of raw milk continues to be the major mode of exposure in developing countries (Pappas *et al.*, 2006; Makita *et al.*, 2008; Swai and Schoonman, 2009). A survey conducted in Kenya showed that boiling of milk reduced the risk of exposure to *Brucella* (Arimi *et al.*, 2005). But those people living in rural communities are often unaware of the risk of infection from livestock and livestock products (Marcotty *et al.*, 2009).

Generally, it has been observed that increasing intensification of small and medium-sized livestock enterprises in which livestock movement is relatively uncontrolled is associated with increasing prevalence owing to increased livestock density and contact rates (Mc Dermott *et al.*, 2013).

In addition, risky practices in rural areas such as skinning of stillborn lambs and kids, as well as crushing the umbilical cord of newborn lambs and kids with teeth can also be contributing factors (Hussein *et al.*, 2005).

The antibody titers against *B. abortus* appear to be associated with age, as low prevalence in young stock has been reported than the adults (Ahmed and Munir, 1995). High prevalence of brucellosis among older cows might be related to maturity with the advancing age. However, some reports indicate that *Brucella* antibody titers are not associated with sex in some animal species such as the Kafue lechwe (*Kobus lechwe kafuensis*) (Muma *et al.*, 2006).

The tropism of *Brucella* to the male or female reproductive tract is thought to be by erythritol, which stimulates the growth of the organism, but *Brucella* has also been found in the reproductive tract of animals with no detectable levels of erythritol (Anonymous, 2007).

## **2.6 Pathogenesis of brucellosis**

The disease in cattle is usually caused by *B. abortus* and occasionally by *B. melitensis* and *B. suis*. The incubation period varies widely depending on exposure dose, previous vaccination, species, age, sex, stage of gestation, and susceptibility (Nicoletti and Gilsdorf, 1997). The most important port of entry of *B. abortus* are the nasal and oral mucosae from where organisms are drained into the regional lymph nodes. From these foci, bacteremia occurs and the organisms localize in other lymphoid tissues such as the spleen and the iliac, mesenteric and supramammary lymph nodes where they may induce a granulomatous reaction (Sotherland and Searson, 1990). Abortion is the characteristic sign of acute brucellosis and *brucella* bacteria can be shed in aborted tissues, reproductive tissues and discharges especially prior to, during, or soon after abortion or live birth (Rhyan *et al.*, 1994). Generalized infection affecting other target organs such as pregnant uterus and the udder

and their associated lymph nodes. Following invasion, *Brucella* are capable of surviving intracellularly within phagocytic or non-phagocytic host cells (Carvalho Neta *et al.*, 2010). Trophoblasts are placental cells that are targeted during infection of pregnant cows. LPS plays an important role in *Brucella* virulence because it prevents complement-mediated bacterial killing and provides resistance against antimicrobial peptides such as defensins and lactoferrin (Lapaque *et al.*, 2005). In pregnant cows, it produces a chronic infection replicating within the chorionic trophoblasts of the placenta resulting in placentitis, fetal death and abortion (Enright, 1990). *B. abortus* induces suppression of the transcription of pro-inflammatory mediators in trophoblastic cells at very early stages of infection (Carvalho Neta *et al.*, 2010). Chronic orchitis and fibrosis of the testicular parenchyma of infected bulls are frequently followed by impairment of semen production and partial or permanent infertility (Rankin, 1965). *Brucella* organisms highly reproduce in milk glands and uterus of pregnant and lactating animals. These organisms reside in chorionic epithelial cells, which cause necrosis in placental cotyledons. Abortion of fetuses in pregnant animals usually is due to intrauterine infection. In the uterus, infection causes varying degrees of placentitis (inflammation of the placenta) as a result of disturbed gaseous exchange between the dam and foetus and is aborted (Megid *et al.*, 2010).

## **2.7 Clinical manifestation of brucellosis**

### **2.7.1 Infection in cattle**

Brucellosis is characterised by late term abortion, infertility and reduced milk production as a result of retained placenta, endometritis and a varying degree of sterility in the males and cows (Radostitis *et al.*, 2000).

Cattle abortions due to *Brucella* usually take place at between six and eight months of gestation (Vandeplasseche, 1982; Enright, 1990). Usually abortion is experienced at the first gestation in the third trimester period.

Abortion is the characteristic sign of acute brucellosis and *brucella* bacteria can be shed in aborted tissues, reproductive tissues and discharges especially prior to, during, or soon after abortion or live birth (Rhyan *et al.*, 1994). Often, infected females will abort only once, although they may remain infected their entire life (Godfroid *et al.*, 2010). In the absence of effective intracellular microbicidal mechanisms, these tissues permit exuberant bacterial growth, which leads to foetal death and abortion.

In fully susceptible herds, abortion rates may vary from 30% to 80% (Karimuribo *et al.*, 2007), although in some cases, abortions may be more insidious. In cattle, brucellosis continues to contribute to huge economic losses associated with abortions, infertility and prolonged calving to conception intervals.

Infected bulls may develop systemic signs of infection, but the most significant lesion produced by *B. abortus* in males is orchitis, often associated with seminal vesiculitis and epididymitis. In bulls, *brucella* infections may also be characterized by unilateral or bilateral enlargement of testicles resulting in decreased sexual desire (libido) and infertility. Chronic orchitis and fibrosis of the testicular parenchyma of infected bulls are frequently followed by impairment of semen production, and partial or permanent infertility (Rankin, 1965).

Sometimes the disease is manifested by testicular atrophy resulting from fibrosis and adhesions (Musa *et al.*, 1990, Geering *et al.*, 1995).

Seropositive animals often have higher rates of abortion, stillbirth, infertility and calf mortality, as well as reduced growth and longer calving intervals (McDermott *et al.*, 2013).

### **2.7.2 Infection in humans**

Brucellosis in humans manifests as an acute or sub-acute febrile illness usually marked by an intermittent fever accompanied by malaise, anorexia and prostration and which in the absence of specific treatment may persist for weeks or months (WHO, 2006). The disease

is always caused by infection with *brucella* strain and diagnosis must be supported by laboratory tests which indicate the presence of the organism or a specific immune response to its antigen. Disease caused by infection with *B. abortus* is generally indistinguishable from that caused by *B. melitensis* and may be equally severe (Dokuzoguz *et al.*, 2005).

Human brucellosis is often misdiagnosed in developing countries (Paul *et al.*, 1995) resulting in under reporting of cases. The disease is acute in about half of the cases, with an incubation period of two to three weeks. In the other half, the onset is insidious, with signs and symptoms developing over a period of weeks to months from the infection (WHO, 2006).

Patients usually complain of non-specific symptoms such as fever, sweats, fatigue, malaise, anorexia, weight loss and muscle or joint aches and back pain. Acute brucellosis is characterized by non-specific systemic signs and clinical symptoms consistent with a flu-like or septicaemic illness such as fever, fatigue, malaise, weight loss, headaches, arthralgia, myalgia, chills and sweats (Poiester *et al.*, 2010). The major effect of zoonoses cause human illness, permanent disability and death (Zinsstag *et al.*, 2007).

## **2.8 Brucellosis diagnosis in cattle**

In most developing countries, surveillance of zoonotic diseases is not recognized as a “one-health” collaboration undertaking between veterinary medicine and human medicine. In addition, many countries lack diagnostic capacity and health infrastructure to diagnose the disease (Muma *et al.*, 2014) to diagnose the disease. Despite the vigorous attempts for more than one century to come up with a definitive diagnostic technique for brucellosis, diagnosis still relies on the combination of several tests to avoid false negative results (Poiester *et al.*, 2010). Several diagnostic methods have been used in the diagnosis of brucellosis, these include; clinical diagnosis, bacterial culture and isolation, serological tests and molecular based methods.

### **2.8.1 Clinical diagnosis**

In non-vaccinated herds or flocks, abortion is the most important symptom of the disease. Abortion occurs in cattle after the fifth month of pregnancy while it occurs in the last two months of pregnancy in sheep and goats (Megid *et al.*, 2010). However, brucellosis diagnoses based on abortion alone is not equivocal since many pathogens can induce abortion. The clinical picture is not pathognomonic, although herd history may be helpful. Clinical diagnosis of brucellosis in animals is complicated by paucity of reliable clinical signs. This makes clinical diagnosis unreliable unless there is strong epidemiological information. Retained placenta, metritis, hygroma, decreased milk production, permanent or temporary infertility, delay in reproductive seasons and increased lactation intervals can be cited as other symptoms of this disease. Symptoms such as fever, respiratory impairment, weight loss, diarrhea and limping may be observed in acute form of the disease (Megid *et al.*, 2010). Infection in pigs is characterized by abortion, stillbirths, decreased litter size, weak piglets, infertility, orchitis and epididymitis in males and focal abscess formation (Megid *et al.*, 2010).

Sheep and goats have similar clinical manifestation that observed in other species of animals. Abortion in goats occur most frequently in the third or fourth months of pregnancy (Radostits *et al.*, 2006).

### **2.8.2 Bacteriological culture and isolation**

If brucellosis is suspected in livestock or in wildlife because of positive serological results, attempts to isolate the organism are considered mandatory and should always be performed (Godfroid, 2002). Direct culture and isolation of *brucella* are usually performed on solid media. Some satisfactory basal media include Serum-Dextrose Agar (SDA), Glycerol Dextrose Agar, Tryptose Soy agar (TSA) and Columbia Agar (Alton *et al.*, 1988). SDA is usually preferred for observation of colony morphology.

All these basal media are used for preparation of selective media. The widely used selective medium is the Farrell's Medium (Farrell, 1974) which is prepared by the addition of six antibiotics namely; Polymixin B sulphate (5mg), Bacitracin (25mg), Natamycin (50mg), Nalidixic acid (5mg), Nystatin (100mg) and Vancomycin (20mg) to a basal medium to suppress the growth of organisms other than *brucella* (OIE, 2009). The choice of samples for cultural examination depends on the clinical signs observed. The most valuable samples for bacteriological culture include aborted fetus (stomach contents, spleen and lungs), fetal membranes, vaginal secretions (swabs), milk, semen and arthritis or hygroma fluid (OIE, 2009).

All samples should be cooled immediately after they are taken and transported to the laboratory in a most rapid way. Milk and tissue samples should be frozen if they are not to be cultured immediately (OIE, 2009). Vaginal swabs taken after abortion or parturition are an excellent source for the recovery of *brucella* and far less risky for the personnel than abortion material. The swab is then streaked on to solid media. Growth normally appears after 3-4 days, but cultures should not be discarded as negative until 8-10 days have elapsed. Growth of bacteria in culture media is an unequivocal proof of infection (OIE, 2009; Poiester *et al.*, 2010).

However, it is time consuming, low sensitivity and needs skilled personnel and well-equipped laboratory facilities (Bricker, 2002). Further, bacteriological culture is not an appropriate technique for routine screening owing to costs, difficulties and danger to personnel due to laboratory acquired infection. However, bacterial culture remains the gold standard and most reliable technique for the isolation of *brucella* spp.

If brucellosis is suspected in livestock or in wildlife because of positive serological results, attempts to isolate the organism are considered mandatory and should always be performed (Godfroid, 2002).

### **2.8.3 Serological tests**

For the control of brucellosis at National or local level, the RBPT as well as the c-ELISA are suitable screening tests (OIE, 2009). The currently available sero-diagnostic assays have been shown to be capable of enabling the sustained eradication of brucellosis. The commonly used serological tests include, RBPT, Milk Ring test (MRT), Serum Agglutination Test (SAT) and Complement Fixation Test (CFT). Others include the Card test (CARD), Rivanol test, Coombs test, Heat Inactivation Test (HIT) and Indirect Immune-fluorescent test (IFAT).

Serological tests are relatively easy to perform and provide a practical advantage in detecting the prevalence of *Brucella* infections. Although these screening tests are cheap and easy to perform, diagnostic material is relatively easily accessible, fast and highly sensitive most of the time lack specificity. The presence of *brucella* antibodies suggests exposure to *brucella* spp, but it does not necessarily indicate which *brucella* species induced production of those antibodies.

Therefore, confirmatory test such as c-ELISA is required to be both sensitive and specific, thereby eliminating some false positive reactions (Etman *et al.*, 2014).

#### **2.8.3.1 Rose Bengal Plate Test (RBPT)**

The classical RBPT is often used as a rapid screening test (Ruiz-Mesa *et al.*, 2005). It is a simple spot agglutination test using antigen stained with Rose Bengal and buffered to a low pH usually  $3.5 \pm 0.05$  (Morgan *et al.*, 1969). The antigen should be stored as recommended by the manufacturer but usually should not be frozen. It is simple to perform, rapid, homogeneous, sensitive and the sample (serum) is easily accessible and the consumables are cheap. Further, there is low equipment requirement and the assay is standardized (Nielsen and Ewalt, 2010).

### **2.8.3.2 Limitations of RBPT**

RBPT is usually interpreted in a subjective manner, there is no multiplex capability, it is prone to False Positive Serological Reactions (FPSRs) and false negative results may occur due to prozoning (Nielsen, 2002). The test has limited mobility because it does not work with whole blood or plasma. The positive predictive value of this test is low and a positive result is required to be confirmed by some other more specific test like c-ELISA. Although the serological tests have higher sensitivities as compared to culture techniques, their specificities are generally low (Al-Attas *et al.*, 2000).

RBPT has limitations in the diagnosis of chronic brucellosis because the test mainly detects IgM, yet the amount of IgM in serum of infected animals declines with time to levels below the sensitivity of this test (Walker, 1999). The most one being the issue of prozoning, where sera with high levels of antibody results in non-visible reactions with the RBPT antigen (Alton *et al.*, 1988).

Thus a strong *Brucella* positive serum may be classified as negative in contrast to results on other serological assays. The sensitivity of RBPT has shown to be 96.1% while its specificity was 99.3% (Etman *et al.*, 2014).

#### ***Test Protocol***

- i. Bring the serum samples and antigen at room temperature. Only sufficient antigen for the day's tests should be removed from the refrigerator.
- ii. Place 25-30ul of each serum sample on a white tile, enamel, or plastic plate.
- iii. Shake the antigen bottle well, but gently, and place an equal volume of antigen near each serum spot.
- iv. Immediately after the last drop of antigen has been added to the plate, mix the serum and antigen thoroughly (using a pipette tip for each test) to produce circular or oval zone approximately 2 cm in diameter.

- v. The mixture is agitated gently for 4 minutes at ambient temperature on a rocker or three-directional agitator.
- vi. Read for agglutination immediately after 4 minutes period is completed. Any visible reaction is considered to be positive.

The diagnosis of bovine brucellosis may be adversely affected by the presence of cross reactions that produce False Positive serological test results because of S19 Vaccination or other Gram negative bacteria that share similar epitopes such as *Brucella abortus* O-Chain polysaccharides (Al-Dahok *et al.*, 2006; Nielsen *et al.*, 2006).

Thus *Yersinia enterocolitica* 0:9, *Escherichia coli* 0157: H7, *Salmonella* group N (0:30), *Francisella tularensis* and *Vibrio Cholera* can react in serological tests for bovine brucellosis (Nielsen *et al.*, 2006; See *et al.*, 2012; Adone and Pasquali, 2013). Therefore, positive reaction should be investigated using suitable confirmatory tests and or epidemiological investigation.

RBPT appears to be adequate as a screening test for detecting infected herds or to guarantee the absence of infection in brucellosis free-herds (OIE, 2009).

#### **2.8.3.2 Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA)**

The competitive ELISA (c-ELISA) is usually carried out by selecting monoclonal antibodies (mAb) with slightly higher affinity for the antigen than most of the vaccinal /cross reacting antibody, but with lower affinity than antibody arising from infection (Munoz *et al.*, 2005; Poiester *et al.*, 2010). The specificity of the competitive enzyme assays is very high and is able to detect all antibody isotypes (IgM, IgG1, IgG2 and IgA (Nielsen, 2002). The c-ELISA has both high diagnostic specificity (100%) and sensitivity of 98.8% and it was observed to be the most specific test (Etman *et al.*, 2014). The highest specificity in case of c-ELISA is due to using specific monoclonal antibodies as a conjugate which has the ability to compete with other non-specific antibodies and attach to certain

specific epitopes on SLPS antigen (Godfroid *et al.*, 2010; Nielsen and Yu, 2010). The confirmatory test must demonstrate high level of diagnostic specificity and maintain effective sensitivity in order to decrease the number of false positive reactors to the minimal levels (Poester *et al.*, 1997). The c-ELISA is also capable of eliminating most reactions due to residual antibody produced in response to vaccination with S19. Internationally, the three OIE-ELISA standard sera should be used by National Reference laboratories to check or calibrate the test method in question (OIE, 2009). The assay should be calibrated such that the Optical Density (OD) of the strong positive OIE ELISA standard serum should be close to maximal inhibition (OIE, 2009).

#### ***2.8.3.2.1 Limitations of the c-ELISA***

The only limitation of the c-ELISA assay is that it is more complicated and more expensive to perform than the screening tests (Diaz *et al.*, 2011). The Weak positive OIE ELISA standard sera should give reaction that is moderate (moderate inhibition).

The negative serum and the buffer/mAb control should give reactions that are always less than the test population (OIE, 2009).

#### ***2.8.4 Molecular based Methods***

The Polymerase Chain Reaction (PCR) is a recent and promising technique that allows for rapid and accurate diagnosis of brucellosis without the limitations of conventional methodology (Baddour, 2012). Two major genetic targets are the *Brucella* gene BCSP31 and the 16S-23S rRNA operon (Debeaumont *et al.*, 2005; Navarro *et al.*, 2006). The practical advantage of PCR is that it is more specific and is able to detect *brucella* at species level. A Bruce- ladder multiplex PCR assay was also developed for identification and differentiation of *Brucella* spp and vaccine strains in a single step (Weiner *et al.*, 2011).

This PCR was further enhanced to identify the marine strains like *B. microti* and *B. inopinata*. However, it does not differentiate at the biovar level or below (Mayer-Scholl *et al.*, 2010). More recently, a multiplex PCR assay (suis ladder) was developed to differentiate among *B. Suis* biovars 1 to 5 (López-Goñi *et al.*, 2011).

## **2.9 Prevention and control of brucellosis**

Like all other bacterial diseases, brucellosis is a highly infectious and contagious disease with rapid intra and inter-herd spreading potential (Ahmad, 2005). The WHO has long been involved in brucellosis surveillance and control, including research and development of vaccines to prevent animal brucellosis (Munir *et al.*, 2010).

In the developed world, control of animal brucellosis has been successfully achieved through the combination of vaccination and test and slaughter programs and human brucellosis through milk pasteurization (McDermott and Arimi, 2002; Pappas *et al.*, 2006), coupled with effective disease surveillance and animal movement control. The disease has been eradicated in Japan, Canada, Australia, New Zealand and several Northern and Central European countries (OIE, 2011), yet it remains an uncontrolled problem in regions of high endemicity such as the Africa, Mediterranean, Middle East, parts of Asia and Latin America (Refai, 2002).

Many industrialized countries have successfully controlled or eliminated zoonotic diseases through costly public investment facilitating coordinated interventions, including test and slaughter, feed bans, mass vaccination of domestic animals and wildlife, health education and milk pasteurization. These are highly effective methods of eliminating zoonotic diseases which require important operational, legal and financial collaterals (Keusch *et al.*, 2009). In endemic areas, human brucellosis has serious public health consequences and in countries where eradication in animals is not feasible, prevention of human infection is

primarily based on raising awareness, food-safety measures, occupational hygiene and laboratory safety (WHO, 2006).

Modified live vaccines are available against *Brucella* spp. *B. abortus* S19, RB51 and *B. melitensis* Rev.1 are proven effective vaccines against *B. abortus* in cattle and against *B. melitensis* and *B. ovis* in sheep and goats, respectively (Elberg, 1996).

Many industrialized countries are able to control or reduce the risk of zoonotic diseases through public investment in preventative measures such as surveillance and compensation of farmers for culled stock in the event of an outbreak. For instance, in April 2001, the British government slaughtered and destroyed more than two million animals in England to stop the spread of Foot and Mouth Disease (Sobrino and Domingo 2001).

In developing countries, this strategy has been ineffective due to inadequate financial resources to compensate farmers whose cattle are slaughtered during screening programs (Godfroid *et al.*, 2011). Currently, there is no vaccine available for humans, pigs, or wildlife (Godfroid *et al.*, 2011; Godfroid *et al.*, 2013). In the absence of a human brucellosis vaccine, prevention of human zoonotic brucellosis depends predominantly on the control of the disease in animals (Godfroid *et al.*, 2005; Pappas *et al.*, 2006).

Control of brucellosis in developing nations is no longer a local problem but should be considered as global challenge since any global citizen can easily become exposed as they travel abroad (Al Dahouk *et al.*, 2005; Godfroid *et al.*, 2005).

Zoonoses control is unique in that effective interventions may lie outside the health sector because transmission often does not occur between humans, but only from animals to human like in brucellosis (Zinsstag *et al.*, 2005).

The global public health community now recognizes that control of diseases in animals is the principal means of reducing human exposure to the majority of Emerging Infectious

Diseases (EIDs) (Levings, 2012). Most infectious diseases are poorly controlled in both animals and humans in many sub-Saharan states. Some of the factors responsible include limited veterinary resources due to low budgetary allocations from central treasuries, insufficient diagnostic tools for rapid field detection of animal diseases; dysfunctional laboratories, poor surveillance systems as well as inefficient disease reporting systems and poor attitudes among public service providers (Muuka *et al.*, 2012).

Therefore, national control programs in low income countries are unlikely to succeed when national veterinary services are weak and livestock movements are uncontrolled. For example, in Tanzania, vaccination for bovine brucellosis using *B. abortus* S19 was previously practiced in State-owned dairy farms, but this stopped in 1980s due to resource constraints (Shirima, 2005). Animals owned by poor farmers in such contexts remain susceptible to a broad spectrum of diseases due to their owners' inability to meet the cost of disease prevention and production inputs (FAO, 2002).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Study area, district profile and population

The study was conducted in Kibaha district, one of the six districts of the Coast region of Tanzania, located at 6,77° South latitude, 3,92° East Longitude and 156 meters elevation above sea level. The district has three divisions, 13 wards and 25 villages (RCO, 2014) and covers an area of about 1,812 km<sup>2</sup>. Kibaha is located 40 km west of Dar-es-salaam and is bordered to the North by the Bagamoyo district, to the east by Dar-es-salaam, to the South by the Kisarawe district and to the West by the Morogoro region (Fig.3.1). According to the 2012 Tanzania National census, the population of the Kibaha district was estimated at 198,697 (URT, 2012). The main economic activity in the district is agriculture (crop and livestock production) whereby 68% of the population is engaged in agricultural activities. Food crops grown include cassava, rice, millet, legumes, maize and sweet potatoes. The most dominant domestic animals kept are cattle and goats. The district has an estimated cattle population of 52,820 comprising 48,500 indigenous / traditional cattle and 4,320 improved dairy cattle. The target population in the study area included cattle from dairy farms located in the district and traditional cattle herds kept under agro-pastoral production system in the district. Dairy cattle farms in Kibaha district consists of private owned farms and Institutional farms. Kibaha Education Centre dairy farm being an example of an institutional farm run by Kibaha Education Centre (KEC) which is a government parastatal organization. Milk produced from these farms is usually sold to KEC community and nearby people in Kibaha town and Dar-es-Salaam.

Currently, there is no specific reports on the enforcement on mandatory screening or testing of dairy animals against Brucellosis and Tuberculosis in Tanzania. This poses a serious public health risk to people consuming the milk produced from these farm animals in the study area.

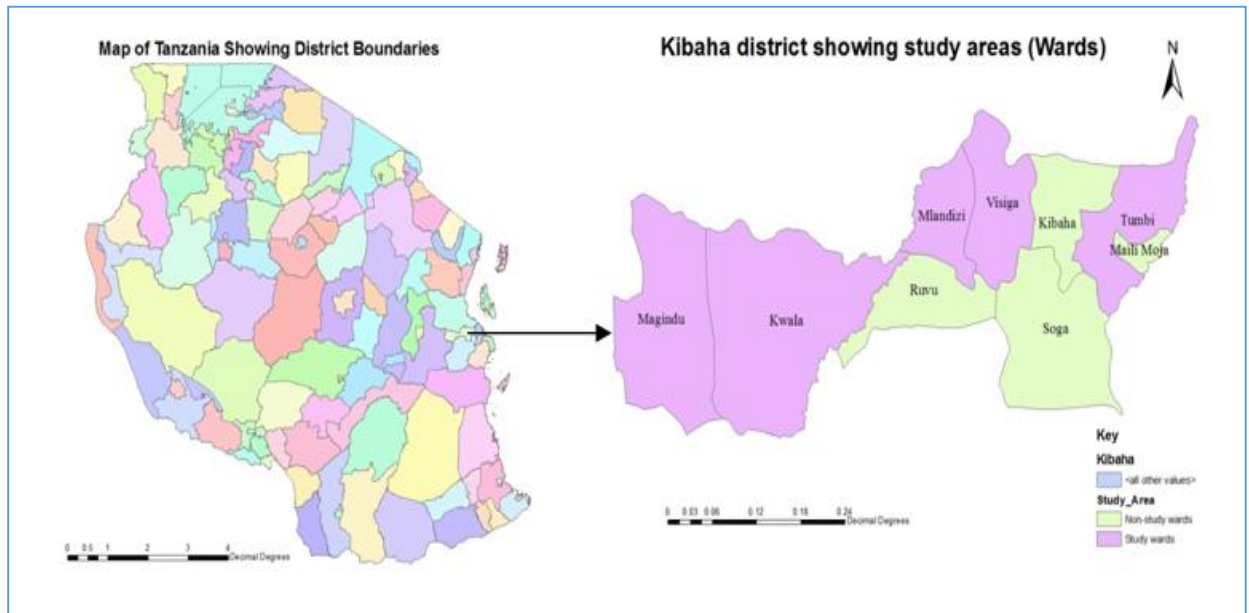


Figure 3.1 Map of Tanzania showing District boundaries and wards of Kibaha district.

### 3.2. Study design and timeframe

A cross-sectional study was conducted between October 2014 and February 2015 in Kibaha district of Tanzania.

### 3.3 Sampling frame

Administratively, the district has 3 divisions, 13 wards and 25 villages. The sampling frame comprised all 25 villages in the study area which were obtained from District Livestock Development and Fisheries Office (DLDFO).

The village was the study unit of interest because cattle in any particular village in the study area were epidemiologically considered to share a similar disease profile. Therefore, the sampling comprised a list of all villages in the study area.

### 3.4 Sample size determination

The sample size of independent sampling units (villages) were calculated on the assumption of 13% prevalence of bovine brucellosis in Eastern zone (Weinhäuapl *et al.*, 2000) and computed with expected precision of 10%.

The sample size was calculated according to a formula by Thrusfield (2005).

$$n = \frac{Z^2 \times P \exp (1 - P \exp)}{d^2}$$

Where  $n$  = Required sampling units.

$Z$  = Multiplier from normal distribution at 95% Confidence interval (1.96)

$P_{\exp}$  = Estimated (expected) prevalence 13% (0.13)

(1-P) = Probability of having no disease 87% (0.87)

$D$  = Desired absolute precision 10% (0.1)

$$n = \frac{1.96^2 \times 0.13(1-0.13)}{0.1^2}$$

$$n = \frac{3.8416 \times 0.1131}{0.01} = 43 \text{ villages (independent sampling units)}$$

To improve the approximation to the exact distribution, the sample size based on finite population was adjusted according to a formula by Dohoo *et al.* (2003).

$$n' = nx \frac{N}{N + n}$$

Where  $n = 43$  villages uncorrected sample size

$N = 18$  uncorrected number of agro-pastoral villages in the target population

(Independent study units) obtained from DLDFO.

$n'$  = Adjusted/corrected sample size taking into account the finite target population.

$$n' = 43 \times \frac{18}{18+43} =$$

$$n' = 43 \times 0.29 = 12.4 \approx 12 \text{ villages (adjusted sample size (independent study units))}$$

### 3.5 Sampling procedures

Random sampling procedure was applied for traditional cattle. Since there were few dairy herds, all commercial farms were sampled. Multistage sampling procedure was employed where by twelve villages were purposively selected from the 18 agro-pastoral villages in the district that predominantly keep cattle. The selected villages were regarded as Primary Sampling Units (PSU) and then from each village, a sample of five herds were randomly selected and considered as Secondary Sampling Units (SSU). Then simple random sampling (SRS) was used to obtain cattle to be sampled at each stage within each cattle herd in the traditional sector. However, in some villages less than five herds were sampled because some farmers had already shifted their cattle in far areas in search of water and pasture, hence were not accessed during sampling.

Dairy farms for sampling were purposively selected and they were few and all farms except one were included in the study. This was because the owner was outside the country so we were not allowed to take blood samples from this particular dairy farm.

Finally, a total of 49 cattle herds were screened to detect the presence of *brucella* antibodies. Out of these, 46 came from traditional cattle herds and three (3) herds were from dairy farms. Since farmers are not vaccinating their cattle against brucellosis, cattle sampled were those with no history of vaccination against brucellosis and only mature cattle with more than two years were only included and sampled in the study area.

To obtain a desired number of cattle for the study, the following procedure was followed during sampling;

- Twelve villages (independent sampling units) were purposively selected basing on number of cattle kept in those areas.

- From each village (PSU), five cattle herds were randomly selected (SSU); bringing the number of farmers to be sampled to 60.
- From each cattle herd, at least five cattle were to be randomly sampled bringing the total number of 300 cattle sampled from traditional cattle herds. However, we sampled a total of 330 cattle from traditional sector.
- In the commercial sector 3 farms out of 4 were sampled because one farmer declined and from these 3 farms, 58 cattle were sampled for this study.

Finally, a total of 388 cattle serum samples were collected and screened for brucellosis in the study area.

### **3.6. Blood sampling and data collection**

#### ***3.6.1 Blood sampling***

Simple Random Sampling (SRS) procedure was employed to obtain cattle to be sampled. Selected animals were properly restrained for easy blood sample collection. About 5 mls of blood was collected from the jugular vein of each selected cattle using plain vacutainer tubes and needle. After blood sample collection, labelling and recording of date and biodata for each corresponding vacutainer tube was done. Blood sample tubes were set tilted and allowed to clot overnight at room temperature. The sera samples were separated and removed from the clot and placed into a 2mls cryovial tubes.

Finally, serum samples were transported to Kibaha Education Centre Diary Farm (KEC) office where they were stored in a freezer at -20 °C until the sera were transported to the University of Zambia, School of Veterinary Medicine in the department of disease control for laboratory analysis.

### **3.6.2 Epidemiological data collection**

Epidemiological data for this study were collected using sample data collection sheet during blood sampling for individual animal level data and herd level data, respectively.

Individual animal data included age, sex, breed type, history of abortion; retained placenta and parity were recorded separately on the data collection sheet (Appendix pg.83).

Moreover, herd level potential risk factors like herd type, herd composition, herd size, contact with wildlife, source of animal, disease awareness and milk drinking habits were captured from 49 respondents using structured questionnaire (Appendix pg.79).

### **3.7 Laboratory analysis**

Laboratory analysis of sera samples was performed at the University of Zambia, School of Veterinary Medicine, Department of Disease Control and Public Health Laboratory. Sera were first screened by RBPT antigen and then confirmed using c-ELISA test.

#### **3.7.1 Rose Bengal Plate Test (RBPT)**

Sera samples were screened using Rose Bengal Plate Test (RBPT) protocol according to the procedures described by Alton *et al.* (1975) and the OIE (2009) as a screening test. The pink dyed *Brucella abortus* antigen was used to screen serum for the presence of antibodies to *brucella* species. Serum samples and *B. abortus* antigen were thawed and equilibrated at room temperature before performing a test. Equal volumes of 25µl of RBPT *brucella* antigen and 25µl of the test serum were placed on each well of the white tile plate.

Immediately after the last drop of antigen has been added to the plate, antigens and serum were thoroughly mixed using a pipette tip for each test to produce circular zone on each well.

The mixture on the plate was shaken gently for four minutes at ambient room temperature on a rocker. Thereafter, the degree of agglutination reactions was observed and recorded.

The sample was considered to be positive if agglutination formed and negative if no agglutination formed. All positive sera samples on RBPT were re-tested and confirmed by c-ELISA test.

### **3.7.2 Competitive Enzyme Linked Immunosorbent Assay (c-ELISA)**

The c-ELISA assay uses the mAb specific for one of the epitopes of *brucella* species. In the absence of anti-*brucella* antibody in the test serum (negative), the mAb binds to the polysaccharide epitope of the S-LPS antigen and is indicated by colour development. If the test serum contains *brucella* specific antibodies (positive) they compete with the mAb for the epitope sites and inhibit the mAb binding to the O-polysaccharide portion of S-SLP antigen and the subsequent colour formation. Serum from S 19 vaccinated cattle do not compete with the mAb because of their specificity and lower affinity, leading to negative reaction (OIE, 2009). In cattle, this assay is able to distinguish *brucella* infected animals, animals vaccinated with *brucella* strains 19 and animals infected with cross-reacting gram negative bacteria. These assays are internationally validated and are prescribed tests for International trade (OIE, 2009).

The c-ELISA, was performed according to *Brucella* (*Brucella*-Ab c-ELISA) SVANOVIR manufacturer (Biotech AB, Uppsala, Sweden) and OIE (2009) protocol. All reagents were equilibrated to ambient room temperature before use. All materials needed for the test which were not provided in the test kit were prepared in advance in the laboratory.

#### ***Test procedure.***

- i. 45 µl of Sample Dilution Buffer constitutes of proprietary synthetic, active and stabilizing component was added into each microplate well that was used for serum samples and serum controls.

- ii. Then, 5  $\mu$ l of serum controls, positive, weak positive and negative were added into each of the appropriate wells.
- iii. Then, 5 $\mu$ l of sample dilution buffer was added into two appropriate conjugate control well (Cc).
- iv. 5 $\mu$ l of test sample was added to each of appropriate well on the micro plate (with 96 well plates).
- v. Thereafter, 50  $\mu$ l of mAb solution was added into all wells used for controls and samples. The microplate was sealed using adhesive microplate sealing films and the reagents were mixed thoroughly for 5 minutes using a plate shaker. Then, the plate was incubated at room temperature for 30 minutes.
- vi. The micro plate was rinsed with PBS-Tween Buffer four times by filling up the wells at each rinse and pour into the tap water sink, emptying was accomplished by tapping the washed plate upside down on an absorbant paper to remove all remaining unbound materials.
- vii. Then, 100 $\mu$ l of conjugate Solution was added to each well. The micro plate was again sealed using thin polythene and incubated again at room temperature for 30 minutes.
- viii. Rinsing was again repeated as described above (step vi).
- ix. Thereafter, 100 $\mu$ l Substrate Solution using a multi-channel pippete was added to each well and incubated for 10 minutes at ambient room temperature (timing began after the first well was filled).
- x. After that, the reaction was stopped by adding 50 $\mu$ l of Stop solution to each well and mixed thoroughly (Stop solution was added in the same order as the substrate was added using multi-channel pippete).

- xi. Lastly, the Optical Density (OD) of the controls and samples was measured at 450 nm in a Microplate photometer (Humareader, Japan) to get the final OD. The OD was measured immediately after addition of Stop solution to prevent fluctuations in OD values.

The Mean OD values (for duplicate samples) were initially calculated. This was followed by calculation of Percent inhibition (PI) values for controls as well as samples using the following formula.

$$PI = 100 - \frac{(\text{Mean OD}_{\text{samples/controls}})}{(\text{Mean OD}_{\text{Conjugate control Cc}})} \times 100$$

The status (seropositivity) of the test samples were determined as follows, those test samples with  $PI < 30\%$  were considered as Negative and those test samples with  $PI \geq 30\%$  were considered positive. This threshold ( $PI \geq 30\%$ ) for determining seropositivity was based upon the manufacturer's recommendations.

### **3.8 Data analysis**

The data collected in the field on data collection sheet were entered into Microsoft Excel spread sheet. Data from laboratory analysis were also coded in an Excel spreadsheet.

These Data from Excel® Spread sheet were imported into STATA® version 12.0 (STATA Corp, College Station, Texas USA) to compute proportions of seropositive animals. All analyses were based on the c-ELISA serological test results. Two epidemiological parameters were generated namely individual animal prevalence and herd level prevalence. Individual animal prevalence was calculated by dividing the number of positive animals by c-ELISA by the total number of animals tested. Similarly, herd level prevalence was calculated by dividing the number of herds with at least one brucellosis positive animal (positive on c-ELISA) by the total number of all herds screened. Association between

categorical variables and the outcome variable (brucellosis seropositivity) was assessed by using Pearson chi-square test. The multiple effect between predictor variables and outcome variable was assessed by Odds ratio (OR) and 95% CI values in a Logistic Regression Model. For all analyses, a *p-value* of less than 0.05 at 95% CI was considered statistically significant. Finally, tables were used to present results generated from STATA software.

### **3.9 Ethical consideration**

Before blood sample collection in Kibaha district, permission was granted by the District Executive Director (DED) to access cattle farmers in the study area. Cattle used for this study were humanely handled and treated as per Tanzania Animal Welfare Act, No.19 of 2008 guidelines. Oral consent was sought from the farmers before commencing blood sampling from each farm or herd.

Permission to transport sera samples from Tanzania to Zambia was granted by the Director of Veterinary Services in the Ministry of Livestock Development and Fisheries in Dar-es-salaam through Permit No. 003991 of 27<sup>th</sup> January, 2015. Similarly, importation permit of the sera into Zambia was granted by the Director in the Department of Veterinary Services in the Ministry of Livestock and Agriculture Zambia through Permit No.16/2015/(VTHQ/8/3/20) of 19<sup>th</sup> January, 2015.

## CHAPTER FOUR

### RESULTS

#### 4.1 Descriptive results

Out of the 388 cattle serum samples collected, 330 were from traditional cattle herds and 58 samples were from 3 dairy cattle farms in the study area. The proportion of traditional cattle herds and dairy cattle sampled was 85.0% and 15.0%, respectively. The overall mean age of cattle sampled was 5.6 years (95% CI: 5.1 - 6.1) and the mean parity was 1.1 (95% CI: 1.1 - 1.2). 22 serum samples out of 388 cattle serum collected were positive using RBPT (5.7%; 95% CI: 3.6-7.9). Out of these 22 RBPT positive samples, 19 samples (86.4%) confirmed to be positive using c-ELISA test. Also because of high sensitivity of c-ELISA (98.8%) (Etman *et al.*, 2014), four serum samples that tested negative on RBPT test (used as a screening test) became positive using c-ELISA. Only serum samples that were positive on c-ELISA were considered positive to brucellosis in this study. Finally, individual animal seroprevalence of 5.9% (23/388) was estimated (95% CI: 3.6 – 8.3).

From table 4.1 below, the overall prevalence of the presence of *brucella* antibodies using RBPT and c-ELISA tests were estimated at 5.7% (95% CI: 3.4 -7.9) and 5.9% (95% CI: 3.6 - 8.3), respectively.

Table 4.1: Overall individual animal brucellosis seroprevalence based on RBPT and c-ELISA

Test Assay	Classification		Prevalence %	95% CI
RBPT	Negative	366		
	Positive	22	5.7	(3.6 - 7.9)
c-ELISA	Negative	365		
	Positive	23	5.9	(3.6 - 8.3)
Total		388		

From table 4.2 below, seroprevalence was observed to vary according to the study wards. 84 cattle samples were collected from Dutumi ward (Dutumi and Mwembengozi villages) and 14.3 % (12/84) were detected positive to the presence of *brucella* antibodies using c-ELISA test.

From Kwala ward (Msua village) a prevalence of 4.2% was observed (1/24). The same prevalence was recorded in Magindu ward (Magindu, Mizuguni and Lukenge villages) whereby 5/119 (4.2%) cattle were positive against the presence of *brucella* antibodies.

From Gwata ward (Gumba and Gwata) 103 samples were screened and 4.8% (5/103) cattle tested positive to *brucella* antibodies. Out of 58 samples collected from 3 dairy farms and screened for the presence of *brucella* antibodies, only 2 (3.5%) animals tested positive using RBPT as screening test. However, these samples became negative to the presence of *brucella* antibodies using c-ELISA test. Therefore, from the dairy animal herds no *brucella* antibodies were detected (Table 4.4).

Table 4.2: Sampling distribution of variables and brucellosis prevalence-individual animal

S/N	Variable	Level/Group	No. of cattle screened	Sampling (%)	No. of cattle positive	Prevalence %
1	Village source	Dutumi	84	10.6	12	14.3
		Kwala	24	6.2	1	4.2
		Magindu	119	11.3	5	4.2
		Gwata	103	5.4	5	4.8
		Mlandizi	20	5.2	0	0.0
		Tumbi	20	5.2	0	0.0
		Bungo	18	4.6	0	0.0
2	Herd type	Traditional cattle	330	85.1	23	6.9
		Dairy cattle	58	14.9	0	0.0
3	Sex of animal	Female	366	94.3	22	6.0
		Male	22	5.7	1	4.5
4	Parity (n=270)	< 5 calving	237	87.8	19	8.0
		≥ 5 calving	33	12.2	4	12.1
7	Breed type	TSZ	322	82.9	20	6.2
		Ankole	8	2.1	3	37.5
8	Age category	Improved breed	58	14.9	0	0.0
		< 5 years	248	63.9	18	7.2
		≥ 5 years	140	36.1	5	3.6
9	History of abortion	Yes	15	4.1	4	26.7
		No	351	95.9	19	5.4
10	Retained placenta	Yes	1	0.3	0	0.0
		No	365	99.7	23	6.3

NB Females=366, Males=22, Total=388

Table 4.3: Frequency and sampling distribution of variables at herd level-Kibaha district

S/N	Variable	Level/criteria	No. of herds screened	Sampling (%)
1	Traditional herds	Masai	33	67.3
		Mang'ati	8	16.3
		Sukuma	5	10.2
2	Dairy herds	Ruvu Dairy Farm	1	2.0
		Kibaha Ed.Centre	1	2.0
		Efatha dairy farm	1	2.0
3	Contact with wild animals	Yes	33	67.3
		No	16	32.7
4	Herd size	< 100 (small)	24	48.9
		≥ 100 (large)	25	51.1
5	Herd composition	Cattle only	20	40.8
		Mixed with other spp	29	59.2
6	Source of animal	Homebred	42	85.7
		Brought in	7	14.3
7	Disease awareness	Yes	5	10.2
		No	44	89.8
8	Milk drinking habit	Raw milk	34	69.4
		Boiled milk	15	30.6

Table 4.4 below indicated that Dutumi ward had the highest overall brucellosis herd seroprevalence of 12.2 % (95% CI: 12.0 - 36.9) with six herds testing positive out of 12 herds screened. Kwala ward had the lowest prevalence of 2.0 % (95% CI: 0.8 – 13.1). This could be attributed to limited sample size collected from this particular ward (Msua village). Magindu and Gwata wards had almost similar herd prevalence of 10.2% (Table 4. 4). On the other hand, commercial herds in all three Wards of Mlandizi, Tumbi (KEC) and Mkuza had zero seroprevalence (Table 4.4) and these dairy animals were not vaccinated against brucellosis. In this study, village was considered to be an independent epidemiological unit. This is because the disease profile in one epidemiological village is considered to be similar.

Table 4.4: Seroprevalence of brucellosis in Kibaha district at Ward level c-ELISA results

Herd Type	Ward name	No. of herds sampled	No. of cattle sampled	No. of positive herds	Ward/herd prevalence %	95 % (CI)
Traditional	Dutumi	12	84	6	12.2	(12.0 - 36.9)
	Kwala	3	24	1	2.0	(0.8 - 13.1)
	Magindu	18	119	5	10.2	(22.7 - 50.7)
	Gwata	13	103	5	10.2	(13.7 - 39.3)
Commercial	Mlandizi	1	20	0	0.0	(0.0 - 6.1)
	Tumbi	1	20	0	0.0	(0.0 - 6.1)
	Mkuza	1	20	0	0.0	(0.0 - 6.1)
Total		49	388	17	34.7	(20.9 - 48.5)

From table 4.5 below, seroprevalence of 6.0% was recorded in females as compared to males with 4.5% seroprevalence. However, the difference in seroprevalence between sexes was not statistically significant ( $P=0.741$ ) (Table 4.7).

Table 4.5: Seroprevalence of brucellosis in Kibaha district according to sex

Sex	No. cattle screened	No. cattle positive	Prevalence %	95% CI
Male	22	1	4.5	(0.0 - 12.9)
Female	366	22	6.0	(3.6 - 8.5)
Overall	388	23	5.9	(3.6 - 8.3)

Table 4.6 below indicated that a total of 49 herds were screened against the presence of *brucella* antibodies from 7 wards in Kibaha district. Out of these, 46 herds were from traditional cattle and 3 herds were from the dairy sector.

It was observed that 17 herds out of 49 screened against the presence of *brucella* antibodies had at least one animal tested positive to *brucella* antibodies. The overall herd level brucellosis seroprevalence was estimated at 34.7% (95% CI: 20.9 - 48.5) based on the c-ELISA test results.

Table 4.6: Herd level seroprevalence of brucellosis in Kibaha district

Test Assay	Classification	No. of herds	Prevalence %	95% CI
RBPT	Negative	32		
	Positive	17	34.7	(20.9 – 48.5)
c-ELISA	Negative	32		
	Positive	17	34.7	(20.9 – 48.5)
Total		49		

From table 4.7 below, the Cohen’s kappa statistic test was used to assess the level of agreement between the two tests. In this study, a Kappa statistic estimate was found to be 0.73 (95% CI: 0.46 – 1.00) and it was observed to be statistically significant (P=0.001). This indicated substantial agreement of the results obtained between RBPT and c-ELISA tests. This implies that RBPT test is as good as c-ELISA test particularly when investigating herd level brucellosis seroprevalence.

Table 4.7 Cohen Kappa statistic test of agreement between RBPT and c-ELISA tests

Agreement	Expected Agreement	kappa	Std. Error	Z	Prob>Z
87.76%	54.69%	0.7298	0.1429	5.11	0.001

## 4.2 Risk Factor Analysis (Univariate analysis)

Risk factors associated with *brucella* infections at animal and herd levels were evaluated using the Pearson Chi-square test. Each predictor variable was assessed on its association with the outcome variable. The P-values of predictor variables are shown in Table 4.8

Table 4.8: Distribution of brucellosis seroprevalence results by risk factors-Kibaha district

S/N	Risk factor	Level	No. of cattle/herds screened	Prevalence %	P-value
1	Sex	Male	23	4.5	0.741
		Female	365	6.0	
2	Herd type	Masai	33	32.2	0.702
		Mang'ati	8	33.3	
		Sukuma	5	50	
3	Age category	≤ 5 years	248	5.2	0.446
		> 5 years	140	7.1	
4	Breed type	TSZ	322	6.2	0.001
		Ankole	8	37.5	
		Improved breeds	58	0.0	
5	Parity category	≤ 5 calving	340	5.8	0.735
		> 5 calving	38	6.4	
6	Retained placenta	Yes	1	0	0.8
		No	365	6.3	
7	Communal grazing	Yes	46	36.9	0.23
		No	3	0	
8	Contact with wildlife	Yes	33	30.3	0.354
		No	16	43.7	
9	Herd composition	Cattle only	20	55	0.052
		Cattle and goats	9	22.2	
		Cattle and Shoats	17	17.6	
		Cattle, shoats and donkeys	3	33.3	
10	Herd size	≤ 100=small	24	41.7	0.315
		> 100=large	25	38	
11	Source of animal	Homebred	42	35.7	0.027
		Brought in	7	28.6	
12	History of abortion	Yes	15	26.7	0.001
		No	351	5.2	

The categorical variables sex and age category were tested for their association with *brucella* seropositivity and were found not to be significantly associated with the presence of *brucella* antibodies in both two cattle populations with  $P=0.741$  and  $P=0.702$ , respectively; (Table 4.8). The study showed that four variables were significantly associated with *brucella* seropositivity. These variables include; breed type ( $P=0.001$ ), herd composition ( $P=0.052$ ), source of animal ( $P=0.027$ ) and history of abortion ( $P=0.001$ ) in the study area.

It was also found that there was a significant association between breed type and the presence of *brucella* antibodies ( $P=0.001$ ) (Table 4.8). Ankole breed had the highest risk of animals tested positive to *brucella* compared to TSZ and improved cattle breeds.

The study also showed a significant association between cows with a history of abortion and *brucella* seropositivity ( $P= 0.001$ ) (Table 4.8). Four cows with a history of abortion tested positive to brucellosis (equivalent to 26.7 %) out of 15 cows with history of abortion screened for the presence of *brucella* antibodies.

The study also showed a significant association between source of animal and *brucella* seropositivity ( $P=0.027$ ) (Table 4.8).

It was also observed that the prevalence of *brucella* antibodies was observed to be higher in animals sourced from open livestock markets or introduced in a herd from other herds compared to cattle bred within the cattle.

Herd composition, meaning herding cattle together with other types of livestock in one herd was associated with *brucella* seropositivity ( $P=0.052$ ) (Table 4.8). Mixing cattle, sheep, goats and donkey together in one herd could increase chances of bovine brucellosis due to differences in infective *brucella* strains and host susceptibility.

Table 4.9 below indicated multivariable logistic regression results that were used to investigate the multiple effects of risk factors on brucella seropositivity at herd level. The model results showed that cows with history of abortion were 6.8 times more likely to become *brucella* positive compared to those cows with no history of abortion (OR=6.8, P=0.002, 95% CI: 1.9–23.9). It was also observed that, the odds of the disease was higher (OR=6.2) in those herds exposed to at least one animal bought from livestock market or other herds than those non-exposed herds to new bought animal from livestock market (OR=6.2, P = 0.043, 95% CI: 1.1 – 36.9). Also the odds of the disease was higher (OR=8.3) among the Ankole cattle breeds compared to TZS cattle and other improved breeds (OR=8.3, P=0.008, CI: 1.7 – 39.7).

Table 4.9: Results of the Logistic regression analysis for brucella seropositivity (n=388)

Variable	n	Odds ratio	95% CI	P-value
Breed type				
Ankole	8	8.3	1.7-39.7	0.008
TSZ	322			
History of abortion				
Yes	15	6.8	1.9-23.9	0.002
No	351			
Source of animal				
Brought in	7	6.2	1.1-36.9	0.043
Homebred	42			

## CHAPTER FIVE

### DISCUSSION

Seroprevalence of bovine brucellosis using c-ELISA test in Kibaha district was estimated at 5.9% at individual animal level and 34.7% at herd level.

Several risk factors that could be associated with brucellosis were determined at individual animal and herd level. Individual animal level factors include; age, sex, breed type, parity, history of abortion and retained placenta. Potential herd level factors to brucellosis were herd type, herd size, contact with wildlife, herd composition and source of animal.

Among the individual animal factors, breed type and history of abortion were significantly associated with the presence of *brucella* antibodies in traditional cattle. Likewise, herd composition and source of animal were factors associated with *brucella* seropositivity at herd level.

These results are similar to the findings of other investigators who reported significant association in animals with reproductive disorders like abortions and retention of placenta and repeat breeding (Thrusfield *et al.*, 2005; Aguiar *et al.*, 2007; Muma *et al.*, 2007a; Kiputa *et al.*, 2008). Although there could be other infections that are implicated as potential causes of abortion in cattle such as Bovine Viral Diarrhea (BDV), Bovine Genital Campylobacteriosis, severe stress and dietary deficiencies, brucellosis remains one of the major causes of abortion in cattle (Muma *et al.*, 2007a).

Breed type in Kibaha was observed to be significantly associated with *brucella* seropositivity. Cattle breeds screened for the presence of *brucella* antibodies were Ankole cattle breed (Long-horned cattle), TSZ from traditional sector and cross breed mainly from commercial sector.

Ankole breed was observed to be more likely to become *brucella* positive compared to TSZ. These results are similar to recent findings reported in Uganda where Ankole cattle breeds were significantly associated with *brucella* seropositivity compared to other breeds (Mugizi *et al.*, 2015). Haileselassie *et al.* (2010) also found similar results suggesting that there is breed susceptibility variation among cattle breeds. On the other hand, the findings contrast with Muma *et al.* (2007a) who reported no significant association among cattle breeds. The possible explanation of such variation could be that certain breeds of cattle (such as Ankole breed) is likely to be found in a particular management system (agro-pastoral production system) where by dairy farms would normally keep improved cross breeds. These findings also contrast with findings from Shirima *et al.* (2007) who observed higher prevalence of brucellosis in TSZ cattle than in cross bred cattle. However, real susceptibility differences could exist depending on the management system and availability of certain breeds of cattle in a particular area.

Source of animal was also an important risk factor in explaining herd *brucella* seropositivity. In the study area, there are several open livestock markets which operate on weekly basis. The agro-pastoral communities mainly depend on selling of cattle for their livelihood to get money for buying food, clothes and other basic needs. Traditionally, they normally purchase cattle from these open markets mainly for expansion of their original herds or receive cattle from other farmers in terms of dowry or bride price paid to their daughters. Since these animals were introduced into another herd prior-testing to brucellosis, there could be a major source of spreading the infection within and between cattle herds. This underscores the importance of providing diagnostic screening tests services at these open livestock markets for possible prevention and control of brucellosis in animals in the study area.

This is because there is no controlled movement of cattle and different herds are allowed to mix during grazing and watering which is very common practice among agro-pastoral tribes in the study area.

Mixing of cattle herds during grazing and at watering points increases the risk of brucellosis transmission among traditional cattle (Muma *et al.*, 2007a; Haileselassie *et al.*, 2010; Makita *et al.*, 2011). The results are also similar to findings by Karimuribo *et al.* (2007) who observed higher brucellosis prevalence in communally grazed cattle compared to those reared under semi or intensive production system.

Herding cattle together with other types of livestock such as goats, sheep and donkey was observed to be associated with *brucella* seropositivity. This observation agrees with findings from Swai and Schoonman (2010) who reported that mixed herding and frequent contact with small ruminants and cattle could also be contributing factors to the occurrence of brucellosis in Tanzania. The status of brucellosis prevalence in small ruminants was estimated at 6.5% (Shirima *et al.*, 2010) and 1.6% (Justine *et al.*, 2015) in Tanzania.

Livestock interaction with wildlife is another risk factor responsible for elevated *brucella* infection prevalence (Muma *et al.*, 2007b). Wildlife interaction variable was not associated with the occurrence of *brucella* seropositivity in the study area. However, cattle that were sampled and screened from Dutumi ward had the highest herd level brucellosis prevalence (50%) compared to other wards in the area. This could probably be attributed to its proximity to Kisarawe district which forms part of Selous Game Reserve interface area, where herds from this ward may be mixing with herds close to interface area. However, further study need to be done at the interface area to investigate the level of disease to establish a clear epidemiological link between wildlife and livestock.

The role of wildlife in the epidemiology of brucellosis in Tanzania also warrants attention because *brucella* seropositivity was detected in 41/103 (39.8% prevalence) buffaloes

sampled in Arusha region an area where livestock wildlife interactions are common (Shirima *et al.*, 2005). Similarly, 24% prevalence was observed in buffaloes and wildebeests in the Serengeti Ecosystem (Fyumagwa *et al.*, 2009).

Sex was not found to be significantly associated with *brucella* seropositivity in the study area. This observation is contrary to previous findings where higher *brucella* seroprevalence of 12.5% was observed in males than in females (Chimana *et al.*, 2010) in Zambia. This is also in contrast with other findings from Ferede *et al.* (2011), Degefu *et al.* (2011) and Din *et al.* (2013) who reported higher *brucella* prevalence in females compared to males in cattle. One of the major reason for lack of association between sex and *brucella* seropositivity was that there were inherently significantly more females than males screened for brucellosis in the study area. In addition, Hirsh and Zee (1999) reported that male animals are less susceptible to *brucella* infection due to absence of erythritol which stimulates the growth and multiplication of *brucella* in females. Generally, it is known that brucellosis is more prevalent in females than in males.

Age was categorized into two groups namely those cattle with < 5 years and cattle with  $\geq$  5 years of age. Although it was not significant risk factor, higher prevalence of the disease was observed in older cattle above 5 years compared to cattle with < 5 years. The results contrast with those reported by Matope *et al.* (2011) who observed decreased frequency of brucellosis with increasing age, with 2-4 years old cattle having higher odds of being seropositive to *brucella* compared to cattle with more than 7 years.

The reason could be that some older cows may not exhibit detectable antibody titres possibly due to latency, which is common in chronic brucellosis (Matope *et al.*, 2011).

This is contrary to what is generally reported that brucellosis is a disease of mature cattle and the risk of the disease to individual animal increases with an advancing age (Kubuafor *et al.*, 2000; Junaidu *et al.*, 2011).

Herd size seemed not be associated with brucellosis in the study area. These results contrasts with recent findings conducted in Uganda by Mugizi *et al.* (2015) and Haileselassie *et al.* (2010) who reported significant association between herd size and *brucella* seropositivity. The possible explanation for these variations could be due to differences in the management system from where samples were collected such as traditional agro-pastoralist against zero-grazing or tethering system in urban and peri-urban areas. Different criteria used in defining large or small herds was also different between these two production systems. Generally, it has been observed that large herds increase animal to animal contact (high contact rate) within the herd hence increased chances of disease transmission.

Generally, the overall results observed in this study with 5.9% individual animal prevalence in traditional cattle were more or less similar to previous findings done in Tanzania where 5.7% (Shirima *et al.*,2010), 6.9% (Karimuribo *et al.*, 2007) and recently findings of 5.6% (Chitupila *et al.*,2015) and 5.8% (Justine *et al.*, 2015), in Western Tanzania and Katavi-Rukwa ecosystem; respectively.

Results were similar to those reported outside Tanzania including; Ethiopia, 5.9% (Bedane *et al.*, 2012), Nigeria, 5.8% (Cadamus *et al.*, 2006), Sudan, 5.0% (El-Ansany *et al.*, 2001), Zambia, 6.0% (Muma *et al.*, 2012), Jordan, 6.5% (Al-Majali *et al.*, 2009) and recently in Uganda, 7.5% (Mugizi *et al.*, 2015).

However, these study results contrast with observations from other previously epidemiological surveys done by other researchers who recorded varying prevalence levels ranging from 12-15.2% (Swai *et al.*, 2005, Weinhäuupl *et al.*, 2000 and Otaru,1985) in Tanzania, 11-17% in Uganda (Mwebe *et al.*, 2010) and 14-28% in Zambia (Muma *et al.*, 2006).

Differences in these findings could be due to sampling techniques, variation of risk factors from one area to another, study size and diagnostic tests employed. In previous studies some investigators used RBPT as a screening tests while CFT was used as a confirmatory test, unlike in this study where c-ELISA was used as confirmatory test. However, it was difficult to make direct comparison of the results because of different study conditions that could influence the findings.

The observed herd level seroprevalence of 34.7% in Kibaha district was lower compared to 40% that was observed and reported in Zimbabwe (Matope *et al.*, 2011) under similar extensive production system, 42% in Ethiopia (Berhe *et al.*, 2007), 55.6% in Uganda (Bernard *et al.*, 2005) and 63% in Brazil (Aguiar *et al.*, 2007).

Despite using the RBPT as a screening test and c-ELISA as a confirmatory test, only results from the c-ELISA were used in the analysis. The choice of the c-ELISA test was influenced by its performance index such as high sensitivity, specificity and accuracy. Also c-ELISA has several diagnostic advantages such as better standardized, robust, specific applications, objective results, adapted to automatized procedures and commercially available as kits (Etman *et al.*, 2014).

Although RBPT is a very sensitive test having sensitivity of about 91% sometimes it gives false positive results because of S19 vaccination or other cross-reactions such as reaction with *Yersinia enterocolitica* 09 (Nielsen *et al.*, 1996). Due to the smooth nature of the strain S19 and the strong antibody response against the O-side chain, RBPT does not permit discrimination of infected from vaccinated animals.

According to the reports the bacteria *Yersinia enterocolitica* 0:9, *Escherichia coli* 0157:H7, *Salmonella* spp, *Francisella tularensis* and *Vibrio cholera* can react in serology test for bovine brucellosis (Al dahok *et al.*, 2006; Godfroid *et al.*, 2002).

Since cattle in the study area were not vaccinated against brucellosis probably because of lack of financial resources to vaccinate cattle country wide. The False Positives Serological Reactions (FPSR) which could exaggerate brucellosis prevalence levels using RBPT alone were taken care of by using c-ELISA which is capable of eliminating some reactions due to possibly *Y. enterocolitica* serotype 0:9 or other cross-reacting antibodies (OIE,2008). The c-ELISA is a rapid assay, faster and it can be automated and therefore the results was measured objectively. This is because c- ELISA test has been used to differentiate between vaccinated or infected animals in the field (Adams, 1990).

The c-ELISA sensitivity in this study based on 30% Percent Inhibition cut off value was 86.4%. This contrasts with Muma *et al* (2007a) who reported c-ELISA sensitivity of 98% and specificity of 99% in Zambian cattle.

The traditional cattle sub-sector in Tanzania consists of 97% of the country's total cattle population of about 23 million cattle, 15.6 million goats, 7 million sheep and 2.01 million Pigs (MLDF, 2014). RBPT test should continue to be used as a screening test for herd level brucellosis infection surveillance in traditional cattle herds of Tanzania especially in areas where farmers do not vaccinate cattle against brucellosis.

This can easily be implemented at livestock market level or at herd level by veterinary personnel and could ultimately help to prevent and control the spread of infection emanating from uncontrolled movement of cattle country wide prior to testing the presence of *brucella* antibodies in cattle herds.

Serum samples from three commercial farms were also part of this study which were collected from Ruvu dairy farm (DAFCO), Kibaha Education Centre dairy farm and Efatha dairy farm. There was no *brucella* antibodies detected from these 3 dairy farm animals. The difference in prevalence between traditional and dairy cattle herds could be explained

by differences in management practices such as disinfection, proper disposal of aborted materials, use of protective gears and controlled movement of dairy animals.

This is in contrary to what is normally practiced in the traditional sector where many cattle farmers do not isolate cows during parturition, or dispose well the placenta following calving. This results in extensive contamination of the environment exposing cattle to risk of contracting the infection through ingestion of contaminated pasture and water. Similarly, many cattle farmers in the traditional sector do not take measures to protect themselves against brucellosis and are quite willing to drink unpasteurized milk (Saleem *et al.*, 2010). The results of the study also indicated that majority (85%) of the farmers were not aware of the zoonotic risks of brucellosis. These results are similar to the previous findings of study conducted in Tanzania that assessed the knowledge and awareness of *brucella* infections among agro-pastoral communities (Kambarage *et al.*, 2003). Similar to another study done in Tanzania showed that even medical professionals especially those in rural areas had poor knowledge on the zoonotic risks of the disease (John *et al.*, 2008).

Lack of disease awareness indicated that the agro-pastoral communities in this area were at high risk of being infected with brucellosis because of their traditional habit of drinking raw milk, eating raw meat and blood (Shirima *et al.*, 2010; Swai and Schoonman, 2010; Kunda *et al.*, 2010). In Northern Tanzania (Arusha and Manyara regions) where Masai and Manga'ti tribes originate, the habit of drinking raw milk, blood and eating undercooked meat for the reason that it is tastier and increases energy has been reported as their traditional customs (Shirima *et al.*, 2010; Kambarage *et al.*, 2003).

As observed in this study, the majority (63%) of them drank raw milk. Milk is usually preserved by souring, which does not destroy *brucella* as they are preserved in the milk fat (Eze, 1978).

In contrast, people in commercial sector had sufficient knowledge on the zoonotic risks associated with brucellosis and better understanding of transmission mechanisms which is mainly through consumption of contaminated raw milk in humans and direct contact with *brucella* infected aborted materials.

This study also highlighted some of challenges encountered during sampling. Some of agro-pastoral farmers were not willing to allow us to take samples from their cattle herds because of misconception that the government would confiscate their cattle. Shifting of farmers with their cattle herds far away from their living homes was another challenge. This was because sampling was done during very dry season (October) therefore some farmers had already shifted their cattle herds in different areas in search of pasture and more so water for their cattle.

Lack of cooperation was also encountered where one of the dairy farm manager did not allow us to collect blood samples from his farm because the owner was outside the country. Lastly, we planned to sample 60 herds of cattle and we managed to sample only 49 cattle herds, this was because our financial resources were limited to allow us to conduct large study with bigger sample size in the study area especially in dairy farm animals.

## CHAPTER SIX

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

- Brucellosis seroprevalence was observed to be relatively higher in traditional cattle.
- There was no *brucella* antibodies detected in dairy farm animals in the study area.
- Risk factors that were significantly associated with brucellosis seropositivity were breed type, history of abortion and source of animal in the study area.
- The majority of people in agro-pastoral communities were not aware of the zoonotic risks associated with *brucella* infection.

#### 6.2 Recommendations

From the study findings the following preventive and control measures are recommended.

- Since farmers are not vaccinating cattle against brucellosis, vaccination of female calves at the age of 3-6 months using S19 should be implemented to control the disease.
- Increasing farmers' knowledge through intensified veterinary extension services, particularly among the agro-pastoral farmers would raise awareness about the transmission of brucellosis and the zoonotic risks associated with it for better future control of the disease in the study area.
- Dairy farm owners should maintain good husbandry practices into their farms to prevent and control the infection in dairy animals.
- RBPT test appeared to be an adequate screening test for detecting *brucella* infected herds (as for c-ELISA test results), therefore, it should be used to screen brucellosis and ascertain the absence of the disease at herd level.
- Absence of *brucella* seropositivity in dairy farm cattle does not reflect true picture of the absence of the disease hence a bigger study size with large coverage need to be conducted in the study area in future.

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## APPENDICES

### 1.0 Questionnaire.

Questionnaire format to interview selected cattle farmers in Kibaha district, Coast region Tanzania.

Herd ID.....

Name                      of                      farmer.....Ward.....  
Village.....

- 1) What type of livestock do you keep in your herd
  - a) Cattle
  - b) Sheep
  - c) Goats
  - d) Donkey
  
- 2) How many livestock do you have in your herd
  - a) Cattle
  - b) Goat
  - c) Sheep
  - d) donkey
  
- 3) Which breed of cattle do you keep
  - a) TZS
  - b) Ankole
  - c) Others specify
  
- 4).What was the source of your livestock to your herd
  - a) Bought from livestock market
  - b) Dowry
  - c) Others specify
  
- 5).What is the grazing management are you using
  - a) Communal gazing land
  - b) Private grazing
  - c) Others specify
  
- 6) If communal grazing, do you interact with other farmers cattle herds.
  - a) YES
  - b) NO
  
- 7) What is the source of water to your livestock?
  - a) Permanent river
  - b) Seasonal river
  - c) Charcoal dam or ponds
  - d) Others specify

- 8) Does water available to your livestock throughout the year.
- Yes
  - No
- 9) If No, when does water scarcity occurs
- During rainy season
  - Just after rain season
  - During dry season
- 10) Do you have enough pasture to feed your animals
- YES
  - NO
- 11) If yes in which season does forage scarcity occurs
- During rainy season
  - During dry season
  - Just after rainy season
- 12) Do you interact with wildlife during grazing?
- Yes
  - No
- 13) What type of wild animal are common in your grazing area.
- Buffalo
  - Thomson gazelle
  - Others
- 14) What are the major challenges of livestock keeping?
- Feed shortage
  - Lack of grazing land
  - Drought
  - Burning of forage
  - Livestock diseases
  - Veterinary drugs and vaccines
  - Others (Specify).....
- 15) What are the common diseases affecting your livestock
- .....
  - .....
  - .....
  - .....
- 16) Have you ever heard disease called brucellosis
- Yes
  - No
  - I don't know

- 17) Have you encountered this disease before into you herd.
- a) Yes
  - b) No
- 18) If yes what were the clinical signs of the disease
- a) .....
  - b) .....
  - c) .....
- 19) At which stage of pregnancy did it occur
- a) First trimester
  - b) Middle of pregnancy
  - c) Last trimester
- 20) Which type of livestock is affected most by brucellosis?
- a) Cattle
  - b) Sheep and goats
  - c) Others
- 21) Have you had a problem of retained placenta in cows during calving?
- a) Yes
  - b) No
- 22) Do you assist your animals during calving?
- a) Yes
  - b) No
- 23) If yes, how do you dispose the foetal membranes
- a) Burning
  - b) Burying
  - c) Throwing
  - d) Others specify
- 24) How do you control the disease in animal affected by brucellosis
- a) Treatment
  - b) Vaccination
  - c) Others specify .....
- 25) Have you ever vaccinated your cattle against brucellosis
- a) Yes
  - b) No
- 26) If No, what are the reasons for not vaccinating your cattle against brucellosis
- a) .....
  - b) .....
  - c) .....

27) Does brucellosis also affect humans

- a) Yes
- b) No
- c) I don't know.....

28) Do you boil milk before consumption?

- a) Yes
- b) No

Name of interviewer.....

Signature.....

Date.....

Thank you.

## 2.0 Data Collection Sheet



**The University of Zambia**  
**School of Veterinary Medicine**  
**Data collection sheet**

Herd owner (name).....

Village.....ward.....district.....

(a) Animal level data sheet

Animal ID	Sample ID	Age (yrs.)	Sex M/F	Breed type	No. parity (cows)	History of abortion Yes/No	Retained fetal membranes Yes/No	Homebred or brought in

(b) Herd level data sheet

Herd ID.....

Type of farming system Agro-Pastoral/Dairy	Herd size	Herd composition	Milk drinking habit (raw or boiled)	Communal grazing land Yes/No	Contact with wildlife Yes/No	Zoonotic awareness of disease Yes/No