EPIDEMIOLOGICAL STUDY OF BOVINE BRUCELLOSIS IN SMALLHOLDER DAIRY CATTLEIN LUSHOTO AND RUNGWE DISTRICTS, TANZANIA

RUTH LINDIZYANI MFUNE

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

2015

ABSTRACT

A cross-sectional study was conducted between November,2014 and March,2015 in Lushoto and Rungwe districts of Mbeya and Tanga regions respectively to estimate the seroprevalenceof bovine brucellosis in smallholder dairy cattle and the associated risk factors. Blood samples from 400 animals from Lushoto (n=172)and Rungwe (n=228) were tested for *Brucella* circulating antibodies using Rose Bengal Plate Test (RBPT), Lateral Flow Assay (LFA) and Competitive-enzyme-Linked Immunosorbent Assay (cELISA).Information regarding risk factors associated with Brucella seropositivity, smallholder farmers' knowledge, attitudes and practices (KAPs) were collected using structured questionnairesadministered to 400 smallholder households.All animals tested negative on RBPT and LFA tests, while the overall seroprevalence based on cELISA results,was 5.3%.Seropositivity was significantly higher in Rungwe (8.3%) than Lushoto (1.2%) (p=0.01).Although femaleshad higher seropositivity (5.8%)compared to males (2.7%), the difference was not statistically significance (p=0.28). Furthermore, risk of seropositivity was not associated to breeding method(Artificial Insemination or bull service), history of abortion herd size, vaccination against brucellosisand number of services before last conception. District (Rungwe) (OR=6.49, CI=1.46-28.76, p=0.01) was a significant risk factor that was associated with brucellosis seropositivity. Results further revealed that majority of farmers had poor knowledge and awareness of brucellosis and their practices are potential risk factors for disease transmission.Bovine brucellosis is prevalent in the study area; this calls for public health awareness programmes, and implementation of strict control measures to curb further spread of the disease in the smallholder production systems within and outside the study area.

DECLARATION

I,**Mfune Ruth Lindizyani**,do hereby declare to theSenate of Sokoine University of Agriculturethat this dissertation is my own original work within the period of registration and that it has neither been submitted nor being concurrently submitted for a higher degree award in any other institution.

_____ _____

Mfune Ruth Lindizyani Date

[MSc (Public health & Food safety) candidate]

The above declaration is hereby confirmed by

_____ _____

Prof S.I Kimera Date

(Supervisor)

_____ _____

Dr. H. Kiara Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

Firstly I thank the almighty God my Ebenezer for his love and faithfulness because without him I would be nothing.My postgraduate studies would not have been possible without the generous scholarship from INTRA-ACP mobility scheme,under its EU funded project “Enhancing Community of practice in One Health for infectious diseases through postgraduate training”. I am especially grateful to the project leader Professor D.M Kambaragefor his assistance.This research was a component of the Tanzania Dairy Genetics project.I would therefore like to thankallmembers of the projectespecially Dr. F. Mujibi for their invaluable technical and financial support throughout my research.I am extremely thankful and indebted tomy supervisors Prof. Kimera S.I (SUA) and Dr. Kiara H. (ILRI, Nairobi) for their valuable guidance and advicethroughout my research work and finally thesis writing. I would also like to thank Mr. Mkuchu P. (SUA, FVM) and Mr. Mwalukasa N. (SUA Library) for their assistance in laboratory and statistical analysis respectively.Lastly but not the least, I would like to thankmy family: my parents and my brother for their love and encouragements throughout my research work.

DEDICATION

To my only brother, Joshua: my dad, Mr. Webster Mfune and my beloved mum, Mrs. Grace Mfune who have contributed insurmountably to my success in so many ways.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

LIST OF APPENDIX

LIST OF ABBREVIATIONS

%	Percentage
AI	Artificial insemination
cELISA	Competitive Enzyme- Linked Immuno-Sorbent Assay
CFT	Complement Fixation Test
CI	Confidence interval
df	Degree of freedom
FAO	Food and Agriculture Organisation
FPA	Fluorescence Polarization Assay
FVM	Faculty of Veterinary Medicine
iELISA	Indirect Enzyme- Linked Immune-Sorbent Assay
IgA	Immunoglobulin Alpha
IgG	Immunoglobulin Gamma
IgM	Immunoglobulin Beta
ILRI	International Livestock Research Institute
LFA	Lateral Flow Assay
mAb	Monoclonal antibody

masl	Meters above sea level
mm	Millimeters
OD	Optical density
ODK	Open data collection kit
OIE	The Office International de Epizooties
PCR	Polymerase Chain Reaction
PP	Percentage positivity
RBPT	Rose Bengal Plate Test
RLPS	Rough Lipo-polysaccharides
SAT	Serum Agglutination Test
SUA	Sokoine University of Agriculture
SLPS	Smooth Lipo-polysaccharides
TSZ	Tanzania shorthorn zebu
WHO	World Health Organisation
χ^2	Chi-square

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Cattle production is the main component of livestock production in most farming systems in sub-Saharan Africa where it contributes significantly to national food security, generation of income and rural development (FAO, 2011). Tanzania's cattle populationranks third in Africa after Ethiopia and Sudanat 25 million(TLMI, 2015),98% of which are indigenous Tanzanian shorthorn zebu (TSZ) breed, and 2% areexoticand crossbred(NBS, 2007).The country has a total land area of 886,000 km², and anestimated human population of45million with a3% annual growth rate.More than 80% of Tanzania's population lives in rural areas and are engaged in subsistence agriculture as smallholder farmers.In 2009,the livestock sector contributed4% to the gross domestic product (GDP) and 30% of thiscame from the dairy industry which is dominated bysmallholder farmers (NBS, 2007).Poor productivity in smallholder dairy herds has beenattributedto poor feeds and feeding practices,poor breeding practicesas well as poor genetic potential. Other factors are poor infrastructure, lack of modern technologies and prevalent diseases and pests (Sikira *et al*., 2013; Swai *et al*., 1997).

Brucellosis is one of the prevalent zoonotic infectiousdiseasesof hugeeconomic and public health importance worldwidemainly due to abortions and calf losses, infertility and, decreased milk production that lengthens the average of intercalving period and prolongs the time between lactations. It is caused by bacteria of the genus *Brucella* and mostly affects sexually mature animals. The Food and Agriculture Organization

(FAO), theWorld Health Organization (WHO), and the Office International des Epizooties (OIE) consider brucellosis as one of the most widespread zoonoses in the world. The disease is common in developing countries while most developed countries such as Canada, Australia, New Zealand, Japan and some countries in Northern and Central Europehave managed to eradicate it (Schelling *et al*, 2003). Brucellosis still remains a serious problem in Africa possibly due to insufficient disease control programs and strategies. The disease has been reported in several countries such as Zambia (Muma *etal*., 2012), Nigeria (Bertu *et al*., 2012; Mai *et al*., 2012), Uganda (Magona *et al*., 2009) and Ethiopia (Kebede *et al*., 2008).

The first brucellosis report in Tanzania was in 1928 after an outbreak of abortions in cows in Arusha region (Kitalyi, 1984).Many studies havebeen carried out since then thatshow varying disease seroprevalence in cattle in various regions and production systems and continued spread.Studies in Arusha and Manyara regions (Shirima, 2005) found seroprevalence of 13.2% in pastoral 5.3% in agro pastoral and 2.3% in smallholder cattle.Other studiesin smallholder dairy farms foundseroprevalence of 12.2% in Moshi (Swai *et al*., 2005), 3.6% in Tanga (Karimuribo*et al*., 2007), 1.9% in Morogoro region and 1% in Coastal region (Mdegela *et al.,* 2004).

1.2 Problem Statement and Justification of the study

Rungwe and Lushoto are among the districts that have high potential for dairying. However, they do not have systematic surveillance systems in place to monitor disease occurrences following their collapse in the 1990s.These involved livestock multiplication units which were a major source of heifers for smallholder farmers and

led to low disease prevalence. Brucellosis is one of theprevalent zoonoses in Tanzania, and is characterized by late stage abortions,stillbirths, and retained placenta followed by endometritis which often leads to infertility. These result in increased number of services, reduced calving rates, long calving intervalsand consequently reduced reproduction followed by huge economic losses. Most smallholder farmers do not have adequate knowledge on brucellosis hence are at high risk.Furthermore, theirpoor practices and attitudes such as consumption of raw milk, undercooked meat and poor hygiene as well as handling of aborted materials without protective wearpave way to introduction of infectious diseases such as brucellosis and Tuberculosis which are zoonotic.

Several studies on Brucellosis have been carried outin pastoral and agro-pastoral production systems and parastatal farms. However,very few have been conducted in the smallholder dairy system. Furthermore, most of these studies have merely mentioned the potential risk factors without clearly identifying the risk factors responsible for transmission of brucellosis.This research is the first of its kind in Rungwe and Lushoto districts.

1.3 Objectives

1.3.1Main Objective

To establish the seroprevalence of bovine brucellosis, the associated risk factors in smallholder dairy cattle and the knowledge attitudes and practices of smallholder farmers in Lushoto and Rungwe districts, Tanzania.

1.3.2Specific Objectives

1. To estimate the seroprevalence of brucellosis in smallholder dairy cattle in Lushoto and Rungwe districts.
2. To identify the possible risk factors associated with the prevalence of brucellosis in smallholder dairy cattle in Lushoto and Rungwe districts.
3. To assess the knowledge, attitude and practices of smallholder farmers in relation to brucellosis in Lushotoand Rungwe districts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aetiology

Brucellosis is an infectious disease caused by gram negative, facultative intracellular bacteria of the genus *Brucella. Brucella* organismsare small, non-motile, non-sporulating aerobic coccobacilli 0.5-0.7µmwide and 0.6-1.5µm long (OIE Diagnostic Manual, 2012). Thereare six terrestrial *Brucella* species;*Brucella abortus, B. melitensis,B. ovis, B. suis, B.canis* and *B.neotomae* .The principle hosts include;cattle and bison(*B.abortus*), goats andsheep(*B.melitensis*), pigs(*B.suis*), dogs(*B.canis*) andrats(*B. neotomae*) (Radostits *et al*., 2000). Biochemically, *Brucella* organisms are able to oxidize some carbohydrates such as D-glucose and I-erythritol as well as certain amino acids likeL-asparagine and L-glutamic.Thesebacteria have surface antigens called lipopolysaccharide (LPS) which are either smooth or rough depending on whether they have or lack the surface exposed O-polysaccharides (O-PS) chain respectively (OIE Diagnostic Manual, 2012;Poester *et al*.,2013). These virulence factors are essential for the structural and functional integrity of the outer membrane. Studies indicate that *Brucella*species are genetically and immunologically related whilecross species infection is possible for example *B.abortus* and *B. melitensis* can both infect cattle concurrently*(*OIE Diagnostic Manual, 2012; Radostits *et al*., 2000).

2.2 Epidemiology of Brucellosis

2.2.1 Distribution and prevalence

Bovine brucellosis has been reported in nearly all countries worldwide except for New Zealand, Australia, Canada, Japan, Northern and Central Europe that are disease free(Diaz *etal*., 2013;OIE Diagnostic Manual,2012).Brucellosisstill remains a threat in Latin America, Mediterranean region, Western Asia and Africa. It is widespread in Africa andhas been reported in most African countries although with varying prevalence(McDermott and Arimi, 2002) as shown in table 1. Following the first reported outbreak of bovine brucellosis in Tanzania in 1927, many studies have been carried out which indicate presence of the disease in different regions and production systems across Tanzania with varying prevalence rates as shown in table 2.Brucellosis is an endemic disease in Tanzania and is of significant concern to the economy and food security of the country (Karimuribo *et al*., 2007).

Table 1: Prevalence of Bovine brucellosis by country and production system

Country	Production system	Prevalence (%)	References
Zambia	Intensive(smallholder)	6	Muma *et al*., 2012
Uganda	Intensive	3.3	Magona *et al*., 2009
	Extensive	34	
Kenya	Intensive and extensive	5.5-17.5	Delgado *et al*., 2001
Ghana	Extensive	2.93	Folitse*et al*., 2014
Nigeria	Extensive and intensive	15.9-45.1	Mai *et al*., 2012
Ethiopia	Intensive	11	Kebede *et al*., 2008

Table 2: Prevalence of Bovine brucellosis in selected regions and production systems in Tanzania

Region	Production system	Prevalence (%)	Reference
Morogoro	Smallholder	1.9	Mdegela *et al*., 2004
Tanga	Smallholder	3.6	Karimuribo *et al*., 2007
Arusha and Manyara	Pastoral	13.2	Shirima,2005
	Agro-pastoral	5.3	
	Smallholder	2.3	
Kilimanjaro	Smallholder	12.2	Swai *et al.,* 2005
Tanga	Smallholder	4.1	Swai and Schoonman,2010
	Pastoral	7.3	
Kigoma (Kibondo and Kakonko districts)	Agro pastoral	5.6	Chitupila *et al*., 2015

2.2.2 Transmission

Brucellosis is transmitted from one herd to another by infected or exposed animals. This is facilitated when a herd owner buys replacement cattle that are infected prior to purchase. In addition, the disease may spread when brucellosis-free herds mingle with wild animals or animals from an infected herd that hadrecently aborted or recently calved down and contaminated pastures and the environment through foetal fluids,placentae and vaginal discharges (Radostits *et al*., 2000;Bishop *et al*., 1994). Oral transmissionis the main route of transmission within and between herds through grazing on contaminated pastures, drinking contaminated water or licking each other.Studies show that calvesare predisposed toinfectionduring parturition as they pass through the birth canal and by suckling colostrum or milk from infected dams.

Although few calves born of seropositive dams get rid of the organisms, mostcalves are likely to develop congenital infections and test negative to serological tests but nevertheless abort during their first pregnancy (Diaz *et al*., 2013;Shirima, 2005).

The *Brucella* organisms stay hidden despite the seronegative test, a condition referred to as latent infection where an animal does not displayany signs of infection. The infection is nevertheless reactivated by pregnancy following production of erythritol which stimulates multiplication of theorganism (Islam*et al*., 2011;Radostits *et al*., 2000). This is notably important because latently infected animals usually go unobserved and serve as future sources of infection to susceptible herds.Bulls may retain infection for life; however they are not able to transmit it to cows by natural breeding even though their semen may harbor the organisms.The semen from infected bullscan be a source of infection if used in artificial insemination (Mai *et al*., 2012).

Brucellosis is a zoonotic disease and transmission to humans is hugely influenced by the disease prevalence in animals, the knowledge attitude and practices that predispose themto infection. Humans acquire the infection through consumption of contaminated milk, dairy products, blood and meat. This is an important source of infection especially in developing countries where disease control programs still remain a challenge.Occupational exposure is highamongpeople who directly handle infected animals and animal products, aborted materials and infected carcasses such as veterinarians, farmers, milkers and abattoir workers (John, K. *et al*., 2010;Corbel, 1997).Some studies have shown that laboratory workers are at high risk of infection

through accidental exposure to live *Brucella*vaccines (OIE Diagnostic Manual, 2012;McDermott and Arimi, 2002).

2.2.3 Risk factors associated with Brucellosis

Factors which influence the transmission of *Brucella sp*. can be grouped into three aspects namely; the host (animal), management practices and the biology of the pathogen itself. The susceptibility of the animalis influenced by age, sex and reproductive status.Susceptibility is higher in older and sexually mature pregnant animals than young sexually immature ones of either sex and it increases with the stage of pregnancy and vaccination status (Radostits *et al*., 2000). The animals are more susceptible to infection during the late stages of pregnancy, when the placental trophoblasts tend to produce high levels of erythritol which stimulates the growth and multiplication of the *Brucella* organism (Magona *et al*., 2009;Kebede *et al*., 2008;Radostits*et al*., 2000).Husbandry practices such as farming systems, breeding practices, herd sizes, type of housing and replacement stock all have an influence on the spread of the infection.A higher seroprevalence has been reported in animals kept under extensive systems(Magona *et al*., 2009)while McDermott and Arimi (2002) observed that large herd sizes and poor housing increased the risk of exposure to infection.

2.2.4 Pathogenesis

The pathogenesis of brucellosis is not clearly understood. The bacteria enter the body through ingestion, inhalation and penetrationthrough abraded skin or mucous membranes of the pharynx and alimentary tract. Following host invasion, *B.abortus* is

phagocytized by macrophages and neutrophils, where it is able to survive phagolysosome and replicate without being killed (Radostits *et al*., 2000;Corbel 1997).Thereafter, the organism is transported to regional lymph nodes, where it multiplies and causes lymphadenitis. *B.abortus* enters the circulatory system via the thoracic duct causing bacteremia which may last for months, resolves or becomes recurrent (Bishop *et al*., 1994).The bacteria are disseminated to different predilection sites such as; gravid uterus, udder, testicles, supramammary lymph nodes, spleen, male accessory sex glands and synovial structures (Poester *et al*., 2013;Radostits *et al*., 2000).In pregnant infected animals, *B.abortus* colonizes and replicates in the endometrium of gravid uterus and the chorionic trophoblasts of the developing foetus. *B.abortus* has a special affinity for erythritol, a sugar alcohol present in the placenta which stimulates growth and multiplication of the organism. Erythritol levels are high in placental and fetal fluids from about the 5^{th} month of pregnancy which causes severe ulcerative endometritis of the intercotyledonary spaces, invasion of the allantochorion, fetal fluids and placental cotyledons and destruction of the villi (Radostits *etal*., 2000;Bishop *et al*., 1994).The degree of placentitis determines the outcomes which can be; abortion during the last trimester, premature birth and birth of either a viable or non-viable calf.

The mechanisms of *Brucella*-induced abortions are not clearly understood. Some hypotheses suggest thatplacentitis or inflammation of foetal tissues disrupts foetal circulation resulting in foetal stress and death. *Brucella* endotoxins induce cortisol production which causes a shift from production of progesterone to estrogen production which results in endometrial production of prostaglandin F-2-alpha(PGF2α) and consequentlyinduced premature parturition (Poester *et al*., 2013).

2.2.5 Clinical manifestations

In cattle, *B.abortus* causes abortion in the third trimester, stillbirths, retained placenta and metritis. Metritis may be acute leading to septicemia and death or becomes chronic and result in infertility (Radostits *et al*., 2000; Bishop *et al*., 1994). Calves born weak may survive or die shortly after birth and milk production decreases. Incubation period of brucellosis varies depending on the infective dose, sex, age, immune status of the animal and stage of gestation (Radostits *et al*., 2000). Several definitions of the incubation period have been proposed. It canbe the period between exposure and first manifestation of the diseaseor between exposure and abortion .It can also be the periodbetween exposure and before the first serological detection of infection (Shirima 2005; Bishop, *et al.,* 1994). However, some animals may not fall in any of the categories defined. The abortion rate in infected animalsvaries from 30-70% depending on management practices, period of infection and susceptibility of the pregnant animal(Radostits *et al*., 2000). In bulls, the organism is localized in testis, epididymis and accessory glands. This causes acute orchitis, epididymitis, seminal vesiculitis and consequently infertility even though libido is retained. Hygromas on leg joints are common in long-term infections that may result into arthritis (Shirima 2005;Radostits *et al*., 2000; Bishop *et al*., 1994).

In humans, brucellosis has an acute or insidious onset,characterized by intermittent fever, backache, headache, anorexia, weight loss, weakness and arthralgia. It may last for several months or years if it is not properly treated (OIE Diagnostic Manual, 2012).

2.2.6 Immunity

Immunity is mediated by cellular immune response and the humoral (antibody) responses. The CD4+ and CD8+ T-cells produce cytokines (IFN-γ and IL-2) which activates the microbial killing activity of the macrophages. The antibody response is directed against the smooth lipopolysaccharide(S-LPS) on the *Brucella* organism and this is extensively used in the serological diagnosis of bovine brucellosis (Godfroid *et al*., 2010; Radostits *et al*., 2000). Animals that are naturally infected or vaccinated with *B.abortus* strain 19 vaccine are considered to have relative immunity against brucellosis and stay seropositive for long periods. Sexually immature and sexually mature but non pregnant animals become immune after infection but may not show clinical signs. Calves from positive reactor cows are passively immunized via colostrum, the half –life of colostral antibodies is about 22 days.

The antibody response to *B.abortus* consists of immunoglobulins IgM, IgG1, IgG2 and IgA with variations in the duration of the antibodies dependent on the sex, age, pregnancy stage and virulence of the organism. The first response to develop is IgM; itis detected at 5-7 days and reaches its peakat 13-21 days indicating acute infection. IgG1 follows shortly, and is first detected at 14-21 days, though its peak is reached at 28-42 days.IgG2 and IgA responses are produced laterin small inconsistent amounts. IgG1 responses persist after the peak stage and remain detectable over a long period (up to several years) whereas IgM gives rapidly induced responses that disappear after a few months. Serological tests that measure IgG1 are more suitable than those of IgM because IgM is the most cross reacting antibody resultingfrom exposure to other bacteria that are antigenically similar to*Brucella spp*. leading to false positive results (Godfroid *et al*., 2010;Radostitis *et al*., 2000;Bishop *etal*., 1994).

2.2.7 Diagnosis

It is very difficult to make a diagnosis based on clinical signs despiteabortions in the third trimesterbeing indicative of brucellosis; this is because other infectious diseases such as leptospirosis, Rift valley fever and Listeriosis can also cause abortion storms. Diagnostictests for bovine brucellosis are divided into three groups namely: tests for demonstration of *B.abortus* organisms, tests for immunoglobulins detection and those which depend on allergic reactions (OIE Diagnostic Manual, 2012; Godfroid *et al*., 2010).An 'ideal' diagnostic test should: detect infection early during the incubation period; not be influenced by the presence of 'non-specific' antibodies; detect carriers; and be able to differentiate between responses due to vaccination and those due to infection. Gall and Nielsen (2004)concluded that there is no single perfect test following a performance review of different serological tests for bovine brucellosis.

2.2.7.1 Tests to demonstrate*Brucella* organisms

(i) Culture and isolation

The choice of samplesin animalsdepends on the observed clinical signs withideal samples in clinical cases being aborted fetuses (spleen, stomach and lungs), colostrum, milk, vaginal discharge, semen and fluids from hygroma. In acute or chronic cases, the best samples are spleen, mammary glands and their lymph nodes, oropharyngeal and genital lymph nodes. Farrell medium is commonly used, having an antibiotic to inhibit growth of other bacteria. Growth is seen after 2-3 days and only considered negative after 2-3 weeks of incubation (OIE Diagnostic Manual, 2012; Godfroid *et al*., 2010). Culture method is time consuming and has many

limitations: *Brucella* organisms are fastidious slow growers, hence are easily overgrown by other bacteria which often lead to misdiagnosis. In chronic cases, cultures may fail to grow due to low levels of bacteria. Stamp's modified Ziehl Nelsen staining is used to identify *Brucella* organisms as they stain red against a blue background when examined under a light microscope. The colonies appear small, singly or paired coccobacilli. However, other organisms that cause abortions like *Chlamydia Coxiella* and *Norcardia spp* are also acid-fast and stain the same color (OIE Diagnostic Manual, 2012; Bishop, *et al.,* 1994).

Brucella species are highly pathogenic to humans, hence all infected tissues, cultures and potentially contaminated materials must be handled under appropriate containment conditions (OIE Diagnostic Manual, 2012; Nielsen and Yu, 2010).Consequently, laboratory personnel are highly at risk of contracting this zoonotic infection during thesehazardous procedures that require high security laboratory facilities (biological containment level 3) and highly skilled personnel.

(ii) Molecular methods

PCR based techniques have been developed in recent years and are in use as alternative diagnostic tests for brucellosis. They are based on the detection of specific sequences of *Brucella spp*., DNA in clinical samples (Radostits *et al*., 2000). PCR techniques have lower diagnostic sensitivity and higher specificity than culture methods hence best results are obtained when the two are combined.

2.2.7.2 Tests for detecting specific Immunoglobulins

There are variousstandardized and accepted serological testsfor detecting antibodies in humans and animals. These include; Buffered*Brucella* antigen tests (Rose Bengal plate test and buffered plate agglutination test), complement fixation (CFT), serum agglutination test (SAT), Indirect Enzyme-Linked Immunosorbent Assays (iELISA) and Competitive Enzyme- Linked Immuno-Sorbent Assay(cELISA) (OIE Diagnostic Manual, 2012).Others are the newly developed lateral flow assay and the fluorescence polarization assay. Studies indicate that the O-Polysaccharides of *Brucella* are antigenically similar to bacteria such as *Yersiniaenterocolitica* and others that elicit cross reactions similar to brucellosis serological reactions, resulting in false positive results and reduced specificity of the tests (Godfroid and Nielsen 2010; Nielsen *et al*., 2004).

(i) Serum agglutination test (SAT)

This standard tube agglutination test detects IgM and IgG2.It has good sensitivity but poor specificity due to cross reactions of IgMs with other bacteria and *Brucella* S19 vaccine.Despite being cheap and simple to perform, the test is considered inferior to other tests such as CFT and RBT and therefore discouraged for international trade purposes (OIE Diagnostic Manual, 2012; Gall and Nielsen 2004).

(ii) Buffered *Brucella* antigen tests

The Rose Bengal Plate Test (RBPT) and buffered acidified plate agglutination tests (BAPAT) are simple and rapid spot agglutination tests that are more sensitive than the SAT (OIE Diagnostic Manual, 2012).

The RBPT uses an antigen buffered at pH 3.65 whichreduces nonspecific reactions by preventing agglutination of IgMand enhances agglutination of IgG1(Díaz *et al*., 2011).The testinvolves mixing drops of serum and stained antigen and a positive reaction is signified by visible agglutination. It has been used in many studies as a screening test for brucellosis, followed by confirmatory tests (Mai*et al*., 2012;Swai *et al*., 2010)which increase proportion of infected animals that test positive. RBT is likely to give a few false positive results due to cross reactions with other bacteria, residual antibodies from *Brucella* S19 vaccination and colostral antibodies in calves. False negatives are rare but may be seen shortly after abortions or in early stages of incubation. Studies of various tests found that RBPT has high sensitivity (90-98%) and 86% specificity, thereby requiring more specific serological tests to confirm the diagnosis (Yu and Nielsen, 2010; Gall and Nielsen 2004).This test is able to eliminate too many false positive cows in herds where the prevalence of infection is low and also assures absence of infection in brucellosis free herds (Radostits *et al.*, 2000).

(iii) Complement Fixation Test (CFT)

This is a widely used confirmatory test following a positive agglutination test although it is gradually being replaced by the more stable ELISAs and FPAs(Gall and Nielsen, 2004).It is highly sensitiveand specific but like other serological tests, it can

give false positive results due to S19 *Brucella* vaccinations. Unlike SAT,CFT rarely shows non-specific reactions however,latest studies show that CFT is not very accurate, it is time-consuming, costly and technically challenging(Yu and Nielsen, 2010 ; Gall and Nielsen, 2004).

(iv) Enzyme Linked-immunosorbent Assay (ELISA)

These primary binding assays were developed as more specific and sensitive tests following the failure of conventional tests to differentiate *B.abortus* S19 vaccinated animals from the naturally infected ones.The high sensitivities of ELISA reduce chances of false positive results that could result in slaughter of negative animals. ELISAs are in two categories, the indirect and competitive ELISA (iELISA and cELISA).

Indirect ELISA is based on the specific binding of antibodies to an immobilized antigen, this is seen using a fluorescent or colorimetric reaction that is chemically or enzymatically-derived. Studies show that iELISA are highly sensitive with low specificity (Poester *et al*., 2010) and are regarded more as screening than confirmatory tests.The iELISA detects antibody of all isotypes unlike other tests, they are however unable to distinguish naturally infected animals from the *B.abortus* S19 vaccinated or those infected with cross reacting bacteria such as *E.coli* and *Yersinia enterocolitica* (Poester *et al*.,2010; Radostits *et al*., 2000).Competitive ELISAwas developed in order to overcome the shortfalls arising from iELISAand has greatly improved the sero-diagnosis of brucellosis. It employs the use ofmonoclonal antibody (mAb) specific for one of the epitopes of the *Brucella* O-PS of the SLPS chains. The mAb competes with low-affinity antibodies present in the test serum and has a higher

affinity for the antigen than the vaccine/cross reacting antibody (Poester et al., 2010). This test has high sensitivity and specificity compared to other tests and is able to distinguish vaccinated animals or animals infected with cross-reacting organisms from naturally infected animals.This helps to reduce false positive reactions and subsequent trace backs or slaughter of animals in a negative or healthy population (Gall and Nielsen, 2004; Corbel, 1997). Other studies found that specificity of cELISA was higher than that of CFT and iELISA in vaccinated cattle whereas in unvaccinated animals, specificity was equal to or greater than that of CFT and iELISA, with iELISA giving a higher rate of false positives (OIE Diagnostic Manual, 2012; Poester et al., 2010).Competitive ELISA is an excellent alternative assay for serodiagnosis of brucellosis as it is cheaper and easier to perform than other tests especially in developing countries (Mythili *et al.*, 2011).

(v) Lateral flow assay (LFA)

This is a rapid and simple qualitative immunochromatographic assay that detects antigen specific antibodies in serum samples. It is a simplified ELISA based on the binding of specific antibodies to an immobilized antigen on a test strip, which is then visualized using a secondary conjugated antibody(Smits *et al*., 2003).A comparative study by Barend *et al*.(2009) showed that LFA is as sensitive as RBT (87%) but has higher specificity (97%). The high specificity ensures a very good prognostic value of the LFA positive results when testing animals in areas of low disease prevalence.The assay does not require specificexpertise, equipment or electricity, and tests may be kept in stockwithout refrigeration(Posthuma-Trumpie *et al*., 2009). It is therefore a

very useful tool for initial screening to identify infected cattle in smallholder dairy herds, though not ideal for large scale screening.

(vi) Fluorescent polarization assay (FPA)

The FPA is a simple rapid validated test which is based on the principle that a molecule's rateof rotation in liquid correlates with its mass, the rate of rotation is inversely proportional to the size of the molecule. A small molecular weight subunit of OPS is labelled with fluorescein isothiocyanate and used as the antigen in a serum sample. Large fluorescent complexes are formed if*Brucella spp*antibodies are presentwhile the antigen remains uncomplexed if antibodies are absent (OIE Diagnostic Manual, 2012).The sensitivity and specificity of the FPA test are almost identical to cELISA while the specificity in S19 vaccinated animals is higher than that of iELISA. The FPA test is better than iELISA because it can eliminate some cross reactionsas well asdifferentiatebetween *B.abortus* S19 vaccinated and naturally infected animals (Poester *et al*., 2010;Gall and Nielsen, 2004;).

Brucella seropositivity indicates presence of infection although it does not indicate time of infection or stage of the disease, therefore the actual disease prevalence may be higher than what the diagnostic tests indicate (Smirnova *et al*., 2013;Godfroid *et al*., 2010).Following an animals' exposure to infection, the time at which serological tests are carried out has a huge impact on the results due to the kinetics of the immune response that are induced. In acute stage of infection, IgMs are produced whereas in chronic stage,IgG dominate while IgM are waned out. The production and loss of these antibodies, and their activity in different serological tests markedly

distinguishes between acute and chronic infections. In order to achieve high performance and specificity,most eradication programmes use a combination of conventional (agglutination) tests and ELISA in parallel. A positive agglutination test (IgM) does not indicate brucellosis if it is not accompanied by a positive IgG response by cELISA. In contrast,detectionof IgM by agglutination tests and IgG by cELISA suggests acute infection while presence of IgG alone indicates chronic infection (Smirnova *et al*., 2013).

2.2.8 Treatment

Treatment in animals is neither effective nor practical since *Brucella spp*. are facultative intracellular bacteria that can survive and multiply within macrophages.Followingexposure to antibiotics such as penicillin and oxytetracycline,*Brucella* undergoes L-transformation which hinders serological detection and results in carrier state animals (Bishop *et al*., 1994). Unsuccessful treatments have been reported because the drugs are unable to penetrate the cell membrane barrier due to the intracellular sequestration of the organisms in the lymph nodes, mammary glands and reproductive organs (Radostits*et al.,* 2000;Bishop *et al.,* 1994).

2.2.9 Prevention and Control

Bovine brucellosis has been successfully eradicated in some developed countries thoughit still remains a challenge in most developing countries including sub-Saharan Africawhere the animal health services have been gradually declining due to various factors (McDermott and Arimi, 2002).The effective control of brucellosis largely

depends on two principles: preventing the exposure of susceptible animals to infection, and increasing resistance of the population through vaccination (Ragan *et al.,* 2013).Many vaccines are available for use in spite of their potential health risks to handlers and interference with serological tests (Ragan *et al*., 2013).

Brucella abortus S19; a smooth strain is a live attenuated vaccine that is most widely used in cattle and remains a reference point for new vaccines such as RB51 a rough killed vaccine (OIE Diagnostic Manual, 2012).S19 vaccine is administeredonce to calves between 3 and 6 months old to confer long term immunity.It has however been associated with sterility problems in males, induced abortion in cows vaccinated in late pregnancy and it also interferes with serological tests (OIE Diagnostic Manual, 2012).RB51 vaccine induces immunity against infection and abortion and has been found to be superior to S19 if given as a single dose in adjuvant.It is however less preferred than S19 because it needs a booster adjuvant thus making it more expensive than S19.

In Tanzania S19 brucellosis vaccinations werepracticed inthe 1980's but were only confined to state-owned dairy farms. In traditional and smallholder dairy production systems vaccinations are not practiced (Shirima, 2005.)

Prevention of brucellosis in humans largely depends on its control and eradication in animals, good hygienic practices when handling animals and animal products and consuming processed dairy products. However these measures are undermined in most rural areas in African countries by local beliefs and practices such as drinking raw milk and assisting in calving with bare hands.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conductedin Lushoto and Rungwe districts fromthe northern coastal Tanga region and southern highland region of Mbeya respectively (**Fig. 1**).These two districts are considered to have potential for high milk production in Tanzania. Tanga region lies between latitudes 4º to 6º S and longitudes 38º to 39º E, whilst Mbeya region lies between Latitudes 7 º to 9 º S, and Longitudes 32 º to 35 º E. Lushoto districtis situated in the northern part of Tanga region within 4^0 25' – 4^0 55' S and 30^0 10' – 38^0 35' E.Rungwe Districton the other hand lies between 8°30' and 9°30' S and 33° and 34° E.

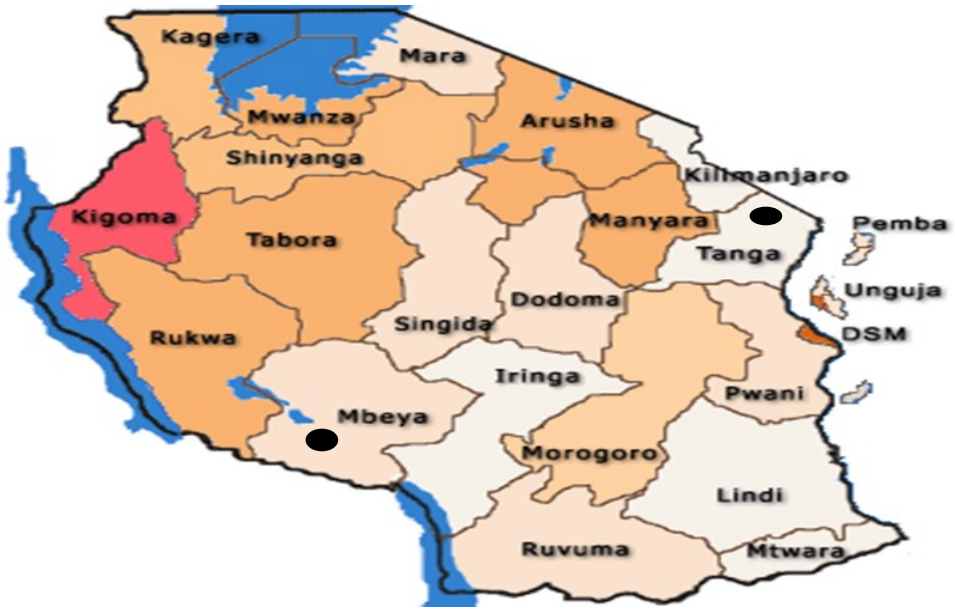

Figure 1: Map of Tanzania showing Rungwe and Lushotodistricts (black dots) in Mbeya and Tanga regions respectively in relation to surrounding regions (Source: http:// *www.tanzania.go.tz/census/census/maps*)

Lushotois one of the six administrative districts in Tanga region with an estimated human population of 437,073and 29,200 dairy cattle(NBS, 2007).The highlands in Lushoto cover about 75% (2,625 km²) of the total district area of 3,500 km²,with the Usambara Mountains taking over most of the highlandwhile the rest is lowland. Lushoto district experiences a short rainy season from October to December and a long rainy season from March to June. The highlands get an average of 800 – 2000mm rainfall per annum whereas the lowlands get about 500-800 mm per year.

Rungwe is one of the seven administrative districts in Mbeya region with a human population of 307,270(NBS, 2007). Rungwe district has a total land area of 2,211 km²which is mostly mountainous (Rungwe Mountains) andthereby greatly influencesthe climatic condition of the district (cold and rainy). Rungwe has a tropical climate and experiences rains throughout the year. The district has three distinctive agro-ecological zones which are: highland (2,200 m.a.s.l), midland (1,200 m.a.s.l) and lowland (800 m.a.s.l) with the highlands zone covering the western, central and eastern parts of Rungwe. The average annual rainfall ranges from 900mm in the lowland zones to 2,700mm in the highland zones. Rungwe is one of the leading districts in dairy production in the region and has a dairy cattle population of 26,137 (NBS, 2007).

Dairy farming is one of the major economic activitiesin both districts and it is greatly concentrated in the highlands where livestock are kept under intensive zero-grazing system. A smallholder dairy farm is defined as a dairy unit that has less than ten dairy animals of all ages and sex (Swai and Schoonman, 2010). The animals are watered daily, fed onnative grass and supplemented with homemade concentrates. The

animalsare confined in sheds that range from simple structures with a sandy floor and a thatched roof to those with a concrete floor and iron roofing sheets. Most farmers have 2–5 cowsthat give an average milk yield of 3–5 liters/cow/day up to a maximum yield of 6–10 liters/cow/day (Urassa *et al*., 2002). The types of cattle breeds kept include *Bos taurus*(Friesian, Ayrshire, Jersey, and Simmental) and crosses of these with *B.indicus* (Tanzania shorthorn zebu).

3.2 Study design

A cross-sectional study was carried out using stratified sampling procedure to select households and individual animals in Rungwe and Lushoto between November 2014 and March 2015.

3.3 Sampling procedure

This study was part of a larger genetics study that needed to use new born calves and lactating cows, therefore theselection criteria used inthe samplingwas an animal that was either a:lactating cow, breeding bull, in -calf heifer or an in-calf cow in the third trimester of pregnancy.Smallholder dairy households that had animals fitting this recruitment criterion were randomly selected by proportional sampling from each study district (172 in Lushoto and 228 in Rungwe) using a tentative list obtained from the district veterinary office.One dairy animal was selectedper household andthe household heads wereinterviewed using a detailed questionnaire to collect information on risk factors associated with brucellosis seroprevalence as well as their knowledge, attitudes and practices in relation to brucellosis (SeeAppendix 1).

3.4 Sample size estimation

Based on an estimated prevalence of 12.2 % (Swai *et al*., 2005), the sample size was calculated using the formula by Dohoo *et al*. (2003):-

$$n = \frac{Z^2 p(1-p)}{d^2}$$

Where;

n= sample size

z= 1. 96 (corresponding to 95% confidence interval)

p=estimated prevalence, 12.2%

d= precision, 5%,

Design effect (DE)=2

10% markup value =16.46 ≈17

The calculated minimum sample size for this study was 346.However,400 animals were sampled.Based on a list of eligible cattle population in each district, proportional sampling was used to sample 172animalsin Lushoto and 288 in Rungwe.

3.5 Questionnaire Design and Data Collection

Information on the household herd and particular animal was collected using a structured questionnaire having open and closed-ended questions (Appendix 1) using anelectronic open data collection kit (ODK) form. The questionnaire data was collected using a mobile device (Samsung tablet) and sentto a server where the data was aggregated and extracted in excel format. The interview was conducted on a single visit with a family member who was knowledgeable about the herd and took

about 30-40 minutes. The information collected included retrospective herd information over a period of one year.

The questionnaire captured important information on potential risk factors that are believed to influence the spread of *Brucella spp.* infections. These include herd and animal level data such as; age, animal breed, sex, breeding method, history of abortioncurrent reproductive status and vaccination history. In addition, the type of floor in the animal house grazing system practices and farmer's knowledge and awareness on bovine brucellosis, its transmission and symptoms, disposal of aborted materials and consumption habits were collected (Appendix 1).

3.6 Blood sample collection

Each animal was bled from the jugular vein using a sterile needle and a plain vacutainer. The animal'sname was recorded and in cases where the animal had no name; the farmer was requested to give it one. The animal was also ear tagged with a labelled plastic tag for identification while the vacutainer was pre-labelled with aunique bar code. The vacutainer tubes were set tilted overnight at room temperature to separate serum from the blood clot. The following day, serum was collected using a disposable plastic Pasteur pipette, dispensed into bar code labelled cryovial tubes. The cryovial tubes were stored at the district veterinary offices in the freezer at -20°C until transported to SUA for analysis.The ear tag number, vacutainer and cryovial labels for each animal were systematically entered into the ODKserver system.

3.7 Serological analysis

3.7.1 Rose Bengal Plate Agglutination Test (RBPT)

All serum samples were tested for *Brucella* antibodies using the PrioCHECK® *Brucella* Rose Bengal test kit from Prionics AG, (Switzerland) according to manufacturer's instructions. Equal volumes (25 μL) of the antigen and test serum were thoroughly mixed on each well of the plate using an applicator stick and the plate was gently hand rocked for four minutes.Thereafter the plate results were visually examined foragglutination and compared to a positive control.Any degree of agglutination was taken as positiveand absence of agglutinates was regarded to be negative. The results were recorded andthe plate was washed with water and methylated spirit thendried before re-use.

3.7.2 Lateral Flow Assay

The *Brucella abortus* antibody rapid test kit used in this study was obtained from Ubio biotechnology systems, India.The principle behind the test is that when a sample is added to the sample well with buffer, it migrates from the sample pad along the conjugate pad where any IgG present in the sample will bind to the colloidal gold conjugate. The sample migrates across the membrane until it reaches the capture zones where the antibody-antibody conjugate complex will bind to the immobilized *Brucella abortus* LPS antigen (on the test line) thereby producing a visible line on the membrane. If the antibody is not present in the sample, no reaction occurs in the capture zone and test line is not formed in the zone hence the sample migrates further along the strip until it reaches the control zone.

The test card was aseptically removed from the foil pouch and placed on a horizontal surface. Five microliters of serum sample was added to the Sample well "**S**", when it was fully absorbed, three drops of the provided diluent was added to the sample hole. The results were interpreted after fifteen minutes. The test was interpreted as positive if the color bands were at position **C** and **T** while a single color band at position **C** was regarded as negative.

3.7.3 Competitive ELISA(cELISA)

Sera were tested for *Brucella* antibodies using PrioCHECK® *Brucella*-Ab c-ELISA test kits (Prionics AG,Switzerland) whether they tested negative or positive on RBPT. The c-ELISA was performed according to the manufacturer's instructions. The kit was stored at 8 degrees Celsius and the reagents were equilibrated to room temperature before use. The serum samples were runin duplicates on 96 well polystyrene plates that were pre-coated with *Brucella spp*. lipopolysaccharides (LPS) antigen.The serum samples were pre-diluted in a dummy plate at a 1:10 ratio by adding 10 µl of serum to 90 µl of the Dilution Buffer in each well. On the test plate, 100 µl of positive controls was dispensed to wells A1 and B1 while 100 µl of the negative control was dispensed into wells C1 and D1. 90µl of Dilution Buffer was dispensed to the rest of the wells after which 10 µl of the pre-diluted sera was added to each of the corresponding wells of the test plate. The plate was shaken, sealed and incubated at 25ºC for 1 hour. The plate was emptied and washed 6 times with 300 µl of washing solution then dried by tapping firmly on absorbent paper towels.100µl of the diluted conjugate was dispensed into all wells on the plate, sealed and incubated at 25 ºC for 30 minutes. Thereafter, the plate was emptied, washed 6 times with

washing solution and dried by tapping firmly on absorbent paper towels.100 µl of the Chromogen (TMB) substrate was dispensed into all wells then incubated in the dark for 10 minutes at 25 ºC. The reaction was stopped by adding 100 µl of the stop solution to all wells and evenly mixed.The optical densities (OD) were measured at 450 nm in a microplate photometer(Multiskan™ RC, Version 6, Labsystems, Finland), and antibody titres were recorded asPercentage positivity (PP) defined as:

$$PP = \frac{\text{Mean OD of test sample}}{\text{Mean OD of positive control}} \times 100$$

Sera were classified as positive and negative according to the manufacturer'srecommendations. All sera with PP values >40% were classified as positive while those <40% were negative.

Validation criteria:

- The mean OD of the negative control must be ≤ 0.2
- The meanOD of the positive control must be ≥1.000

If the criteria were not met, the results were discarded and the test was rerun.

3.8 Statistical analysis

Data from questionnaires and laboratory results were recorded in Microsoft Excel® program 2007and later transferred to SPSS (statistical package for social scientists)

Version 18 for statistical analysis.Descriptive statistics, particularly frequencies were computed for proportions of positive animals using cELISA results and their seroprevalence recorded. Associations between explanatory and dependent variables were investigated in two steps. In the first step, associations between animal factors and brucellosis seropositivitywere individually investigatedin univariate analysis and significance was tested using thePearson's Chi- square test (χ^2). In the second step,variables with a significant association level of p-value <0.2 from univariate analysis were included in thefinal multivariate model. The model was constructed by a forward stepwise selection of variables. The model was evaluated for goodness of fit using the Hosmer-Lemeshow test.Explanatory variables were considered statistically significant at $p<0.05$ and 95% confidence interval (CI).

CHAPTER FOUR

4.0 RESULTS

4.1 Seroprevalence of bovine brucellosis in smallholder dairy cattle in Lushoto and Rungwe districts

A total of 400 dairy animalswere tested for the presence of antibodies to *Brucellaspp*. (seroprevalence) using RBPT, LFA and cELISA tests. The overall seroprevalence of bovine brucellosis in individual animals based on cELISA results was 5.3% (Table 3).For individual districts, Rungwe had a significantly higher seroprevalence (8.3%) than Lushoto(1.2%) (p=0.01) (95% CI) as shown in table 3**.**All animals were negative to RBPT and LFA tests for *Brucella*antibodies.

Table 3: The individual and overall seroprevalence (%) of bovine brucellosis in Lushoto and Rungwe districts

District	**N**	**RBPT**	**LFA**	**cELISA**	**χ^2**	**df**	**p-value**
		n (%)	**n (%)**	**n (%)**			
Lushoto	172	0(0)	0(0)	2(1.2)			
Rungwe	228	0(0)	0(0)	19(8.3)	10.1	1	0.01
Overall	400	0(0)	0(0)	21(5.3)			

N=Number of animals tested

n= number of positive animals

%= percentage of positive animals

ELISA seropositivity was higher in females(5.8 %) compared to males(2.7 %) (Table 4) but the difference was not statistically significant (p=0.28).Although small herd

sizes(1-5) had higher seropositivity(6.5%) compared to herds with 6-10 animals (2.8 %)the difference was not statistically significant (p=0.48) (Table 5).

4.2Possible risks factors associated with seroprevalence of bovine brucellosis in smallholder dairy cattle

Table 4: Seroprevalence of brucellosis in relation to sex in Lushoto and Rungwe districts

District	Sex	N	n(%)	χ^2	df	p-value
Lushoto	Female	132	1(0.8)	0.81	1	0.37
	Male	40	1(2.5)			
Rungwe	Female	194	18(9.3)	1.52	1	0.22
	Male	34	1(2.5)			
Overall	Female	326	19(5.8)	1.18	1	0.28
	Male	74	2(2.7)			

Table 5: Seroprevalence of brucellosis in relation to herd size in Lushoto and Rungwe districts

District	Herd size	N	n (%)	χ^2	df	p-value
Lushoto	1-5	160	2(1.25)	0.15	1	0.69
	6-10	12	0(0)			
Rungwe	1-5	204	18(8.8)	0.61	1	0.44
	6-10	24	1(4.2)			
Overall	1-5	364	20(5.5)	0.48	1	0.48
	6-10	36	1(2.8)			

Table 6: Seroprevalence of brucellosis in relation to history of abortions in Lushoto and Rungwe districts

District	Response	N	n (%)	χ^2	df	p-value
Lushoto	Yes	8	0(0)	0.09	1	0.75
	No	164	2(1.2)			
Rungwe	Yes	9	0(0)	0.85	1	0.35
	No	218	19(8.7)			
Overall	Yes	17	0(0)	0.98	1	0.32
	No	382	21(5.5)			

Table 7: Seroprevalence of brucellosis in relation to breedingmethods in Lushoto and Rungwe districts

District	Breeding method	N	n(%)	χ^2	df	p-value
Lushoto	Natural	152	2(1.3)	0.27	1	0.60
	Artificial	20	0(0)			
Rungwe	Natural	206	19(9.2)	2.21	1	0.13
	Artificial	22	0(0)			
Overall	Natural	358	21(5.9)	2.6	2	0.10
	Artificial	42	0(0)			

Table 8: Seroprevalence of brucellosis in relation to number of services before conception in Lushoto and Rungwe districts

District	# of services	N	n (%)	χ^2	df	p-value
Lushoto	1-2	148	1(0.7)			
	3-4	19	1(5.3)	3.14	2	0.2
	5-10	5	0(0)			
Rungwe	1-2	186	13(6.9)			
	3-4	34	5(14.7)	2.43	2	0.29
	5-10	8	1(12.5)			
Overall	1-2	186	14(7.5)			
	3-4	34	6(17.6)	4.83	2	0.08
	5-10	8	1(0)			

Univariate analysis (Pearson's Chi square test), showed that only breeding methods (Table 7), number of services before conception (Table 8), and handling of aborted materials without protective wear qualified for multivariate analysis ($P<0.20$). In multivariate analysis, only district (Rungwe) ($p=0.01$) showed a significant effect on brucellosis seropositivity. Animals in Rungwe were 6.49 times more likely to be seropositive than those from Lushoto. The Hosmer-Lemeshow test showed that the model fit the data;

($\chi^2=1.25$, df =8, $p=0.99$,Cox and Snell= 0.065,Nagelkerke R= 0.19,

-2 Likelihood=137.61)

4.3 Smallholder farmer's knowledge, attitudes and practices (KAPs) relating to brucellosis

Table 9: Smallholder farmer's knowledge, attitudes and practices (KAPs) relating to brucellosis

Variable	**Category**	**Lushoto n (%)**	**Rungwe n (%)**	**χ^2**	**p-value**
Know how it is transmitted	Yes	24(14%)	15(6.6%)	6.05	0.01
	No	148(86%)	213(93.4%)		
Brucellosis in local language	Don't know	162(94.2%)	226(99.1%)	8.21	0.004
	know	10(5.8%)	2(0.9%)		
Disease transmission between animals	Know	8(4.7%)	4(1.8%)	2.83	0.09
	Don't know	164(95.3%)	224(98.2%)		
Observed signs in cattle	Know	9(5.2%)	2(0.9%)	6.95	0.00
	Don't know	163(94.8%)	226(99.1%)		
Milk consumption behavior	Raw	1(0.6%)	39(17.1%)	29.74	0.00
	Boiled	171(99.4%)	189(82.9%)		
Wear protective clothes when handling aborted/afterbirth materials	Yes	77(44%)	28(12.3%)	53.44	0.00
	No	95(55.2%)	200(87.7%)		

Table 10: Gender, age, education level of dairy household heads in Lushoto and Rungwe

Variable	Lushoto (N=172)	Rungwe (N=228)
Gender		
Male	149(86.6%)	185(81.1%)
Female	23(13.4%)	43(18.9%)
Age(years)		
20-45	66(38.4%)	108(47.4%)
46-65	81(47.1%)	95(41.7%)
66-85	25(14.5%)	25(11%)
Education level		
No education	13(7.6%)	7(3.1%)
Primary	138(80.2%)	194(85.1%)
Secondary	17(9.9%)	18(7.9%)
Tertiary	4(2.3%)	9(3.9%)

CHAPTER FIVE

5.0 DISCUSSION

All 400 animals tested negative for *Brucella spp*. antibodies to RBPT and RFA tests whilst 21 animals tested positive to cELISA test. These results agree with findings by Smirnova *etal*.(2013) that LFA is as sensitive as RBPT (87%) but with higher specificity (97%) which gives it good predictive values in areas of low disease prevalence like Lushoto. Furthermore, a positive RBPT and cELISA test indicates acute infection stage whereas a positive cELISA test alone shows that the animal is in a chronic stage of infection (Godfroid *et al*., 2010;Smirnova *et al*., 2013). Moreover, cELISA test has a higher sensitivity and specificity than RBPT and LFA tests, unlike RBPT, it can eliminate cross reactions arising from heterogeneous bacteria such as *Yersiniaenterocolitica*which subsequently reduces false positives (Nielsen *et al*., 2004). These findings therefore indicate that the cELISAseropositive animals were most likely in a chronic state of infection.

Seroprevalence of bovine brucellosis

The study revealed that cELISA seropositivitywas significantly higher (p=0.01) in Rungwe (8.3%) than Lushoto (1.2%) whilst the overall was 5.3%. in the study area. Since brucellosis vaccinations were not carried out in the study areas, theobserved seropositivitywas due to previous exposure. Seropositivity in similar ranges(1-15%)has been reported in various studies done in smallholder animals in different parts of Tanzania (Shirima, 2005, 2.3%; Swai *et al*., 2005,12.2% ;Mdegela *et al*., 2004,1%; Karimuribo *et al.,*2007, 3.6%; Swai and Schoonman, 2010,4.1%).Other

countries have likewise reported similar seropositivity such as Zambia (Muma *et al*., 2012,6%), Kenya (Delgado *et al*., 2001, 5.5-17.5%), Ethiopia (Kebede *et al*., 2008, 11%) and Uganda (Magona *et al.,* 2009,3.3%). Furthermore, smallholderdairy herds are small-sized units of one to ten animals under zero-grazing system, fodder is cut and carried to the animals hence they have minimal contact with other animals (Swai and Schoonman, 2010).The 'cut and carry' method likely plays a role in transmission of disease particularly if the fodder is collected from areas used by indigenous traditional cattle especially during wet seasons. Rungwe and Lushoto are both highlands but are ecologically different.Firstly, Rungweexperiences more rains throughout the year than Lushoto. During rainy periods, environmental contaminations significantly create an enabling climate for *Brucella* organisms from foetal and other contaminated materialsto survive longerthan in dry periods, which leads to the spread of brucellosis(Bishop *et al*., 1994). Secondly, possible introduction of exposed animals could relatively be high in Rungwe compared to Lushotowhich explainsto some extent the higher seroprositivity rate observed in Rungwe than Lushoto. However, a well-designed study to capture all possible contributors for this difference is required.

Theoverall and individual district seroprevalenceswere higher in females(5.8%) thanmales (2.7%) although this was not statistically significant(p=0.1). Thismay have been influenced by the smallermale sample size (n=74) than females (n=326).Other relatedstudies found similar results (Shirima, 2005; Swai *et al*., 2005) while others (Mai *et al*., 2012) found a higher seroprevalence among males.Radostits *et al*. (2000) indicated that *Brucella* organism have a high affinity for erythritol, a sugar alcohol

found in placental and foetal fluids in females.Following infection, the elevated sugar levels stimulates growth and multiplication of the organism. Additionally,dairy production systemstend to keep males for shorter periods than females; hence they have lower chances of exposure.

A higher seroprevalence was observed in small herds than large herds in both Lushoto and Rungwe despite its statistical insignificance, which corroborates with other results (Lyimo, 2013). Contrary to this, other studies (Shirima, 2005; Kebede *et al*., 2008; Makita *et al*., 2011) have reported higher seroprevalences in large herds than small herds. The herd size seroprevalence in the small herds was higher in Rungwe (8.8%) than Lushoto (1.25%) nevertheless;it is worth noting that chances of contact between infected and non-infected animalsare higherin large herds than small ones.

The variations in the seroprevalences in Rungwe and Lushoto can be attributed to differences in management systems on the individual smallholder farms, characteristics of the animal population and the number of animals sampled per household. The tested animals had no history of brucellosis vaccination against *B.abortus*S19, which ruled out false positives due to vaccination.

Risk factors associated with brucellosis seroprevalence

District of origin (Rungwe) (p=0.01)was found to bea statisticallysignificant risk factor associated with brucellosis seropositivity. The odd of animals fromRungwe to be seropositive were 6.49 times more than those from Lushoto. However, a detailed study to investigate this difference between Lushoto and Rungwe is required.Although history of abortions was not significantly associated with

seropositivity as corroborated by other studies (Swai and Schoonman, 2010), Shirima (2005) reported an association between the two, and observed that brucellosis causes abortionstorms in newly infected herds.

Knowledge, attitudes and practices of smallholder farmers relating to brucellosis

A significantly high proportion of farmers in both Lushoto (94.2%) and Rungwe (93%) had poor knowledge of brucellosis with respect to its causes, symptoms and mode of transmission between animals or from animals to humans as well as the local name of the disease.The district veterinary offices do not provide adequate information regarding brucellosis as was markedly observed by over 95% respondents in both districts.Over 50% of the farmers handlefoetal and aborted materialsbarehanded (p=0.00) while very few households consume raw milk in both districts. This unhygienic practice underscores the risk of brucellosis transmission to farmers in the area.Additionally, over50% of the farmers in both districts allow newborn animals inside their houses whilea few either throw the animal afterbirth materials in garbage or feed to their dogs, a practice that is associated with *Brucella* seropositivity in some studies (Shirima, 2005).Proper disposal of aborted foetal and placental materials is cardinaland has been identified as one of the stringent measures in the control of brucellosis (Shirima, 2005).No vaccination history against brucellosis was observed in Lushoto except for a very low proportion in Rungwe, mainly because vaccination is not commonly practiced in both smallholder and pastoral systems in Tanzania (Karimuribo *et al*., 2007). Nevertheless, poor vaccination practices and introduction of animals from unknown sources are reportedly important brucellosis risk factors (Shirima, 2005; Swai *et al*., 2005).

Theattitudes and practices observed in the study areas clearly indicate that farmers hardly take precautionary measures in their management practices, thereby increasing the risk of disease transmission. Such findings are of great importance, considering the zoonotic and public health significance of the disease.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Although RBPT and LFA did not pick up seropositive animals, they will still remain the screening tests and cELISA as a confirmatory test. Under sero-surveillance programmes, diagnostic tests that detect IgM and IgG are inevitable in identifying individuals with early and chronic infections.

The present study shows that bovine brucellosis is prevalent in smallholder dairy cattle in Lushoto and Rungwe districts. Seropositivity was significantly higher in Rungwe (8.3%) compared to Lushoto (1.2%).The smallholder farmershave poor knowledge of brucellosis with reference to its cause, symptoms and mode of transmission in animals and from animals to humans. Most of their practices are potential risk factors in brucellosis transmission.

6.2 Recommendations

Based on the results of this study, it is pertinent to carry out further epidemiological studies to investigate*Brucella spp* responsible and the link between bovine and human brucellosis using molecular characterization.The existing knowledge gaps observed in knowledge and awareness of brucellosis can be reduced through public health awarenessprogrammes to educate the communities on brucellosis, its cause, mode of transmission associated risk factors and zoonotic implications.Preventive measures using proper hygienic practicessuch as the use of locally available protective wear when handling aborted materials or assisting in parturitionmust be

encouraged. The government should, through veterinary authorities implementstrategic control measures against brucellosis in smallholder dairy communities.

REFERENCES

Barend, M., Koterwas, B., Land, F., Handel, I. G., Tucker, J., Morgan, K. L. and Smits, H. L. (2009). Comparison of a Flow Assay for Brucellosis antibodies with the reference cELISA test in West African *Bosindicus*. *PLoS One 4*(4): 10-1371.

Bertu, W. J., Gusi, A. M., Hassan, M., Mwankon, E., Ocholi, R. A., Ior, D. D. and Smits, H. L. (2012).Serological evidence for brucellosis in *Bosindicus* in Nigeria.*Tropical Animal Health and Production44* (2): 253-258.

Bishop, G. C, Bosman, P. P. and Herr, S. (1994). Bovine Brucellosis: In: *Infectious Disease of Livestock with Special Reference to Southern African.* (Edited by Coetzer, J. A. W., Thompson, G. R. and Tustin, R. C.).*Oxford University Press*, UK. pp. 1053-1066.

Chitupila, G. Y., Komba, E. V. G. and Mtui-Malamsha, N. J. (2015).Epidemiological study of bovine brucellosis in indigenous cattle population in Kibondo and Kakonko Districts, Western Tanzania.*Livestock Research for Rural Development* 27: 118.

Corbel, M. J. (1997). Brucellosis: an overview. *Emerging Infectious Diseases3:* 213-221.

Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S. and Courbious, C. (2001). Livestock to 2020: the next food evolution. *Outlook on Agriculture* 30: 27-29.

Diaz, A. E. (2013). Epidemiology of brucellosis in domestic animals caused by *Brucellamelitensis*, *Brucella suis* and *Brucella abortus*. *Revue Scientifique et Technique (International Office of Epizootics) 32*(1): 43-51.

Díaz, R., Casanova, A., Ariza, J. and Moriyón, I. (2011). The Rose Bengal test in human brucellosis: A neglected test for the diagnosis of a neglected disease. *PLoS Neglected Tropical Diseases5*(4): 1–7.

Dohoo, I., Martin, W. and Henrik, S. (2003).*Veterinary Epidemiologic Research.*VER Inco Monitoba, Canada.pp 48.

FAO.2011*World Livestock 2011 – Livestock in Food Security.*Rome, FAO. http://www.fao.org/docrep/014/i2373e/i2373e00.htm site visited on 15/11/2015.

Folitse, R. D., Boi-Kikimoto, B. B., Emikpe, B. O. and Atawalna, J. (2014).The prevalence of Bovine tuberculosis and Brucellosis in cattle from selected herds in Dormaa and Kintampo Districts, Brong Ahafo region, Ghana. *Archives of Clinical Microbiology 5*(2):1-6.

Gall, D. and Nielsen, K. (2004). Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. *Revue Scientifique et Technique (International Office of Epizootics)23*(3): 989–1002.

Godfroid, J. and Nielsen, K., (2010). Diagnosis of brucellosis in livestock and wildlife. *Croatian Medical Journal 51*(4): 296-305.

Islam, M. A., Khatun, M. M. and Baek, B. K., (2011). Rats born to *Brucella abortus* infected mothers become latent carriers of *Brucella*. *The Journal of Infection in Developing Countries 6*(03): 256-261.

John, K., Fitzpatrick, J., French, N., Kazwala, R., Kambarage, D., Mfinanga, G. S. and Cleaveland, S. (2010). Quantifying risk factors for human brucellosis in Rural Northern Tanzania.*PLoS one5*(4):1-6.

Karimuribo, E. D., Ngowi, H. A., Swai, E. S. and Kambarage, D. M. (2007).Prevalence of brucellosis in crossbred and indigenous cattle in Tanzania. *Livestock Research for Rural Development* 19(10): 148-152.

Kebede,T., Ejeta, G. and Ameni, G. (2008). Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). *Revue de Medecine Veterinaire159*(1): 3-9.

Kitalyi, J. (1984). Bovine brucellosis in government, parastatal, and Ujamaa dairy farms in the central zone of Tanzania.In *Proceedings of the Tanzania Veterinary Association Scientific Conference* 2:172-183.

Lyimo, B. E. (2013). Prevalence of Bovine Brucellosis in smallholder dairy farms in Morogoro Municipality, Tanzania.Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania, pp.60.

Magona, J. W., Walubengo, J., Galiwango, T. and Etoori, A. (2009).Seroprevalence and Potential Risk of Bovine Brucellosis in Zero grazing and Pastoral Dairy systems in Uganda.*Tropical Animal Health and Production41*(8): 1765-1771.

Mai, H. M., Irons, P. C., Kabir, J. and Thompson, P. N. (2012). A large Seroprevalence survey of Brucellosis in cattle herds under diverse production systems in Northern Nigeria. *BMC Veterinary Research 8*(1): 1-144.

Makita, K., Fèvre, E. M., Waiswa, C., Eisler, M. C., Thrusfield, M. and Welburn, S. C. (2011). Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. *BMC Veterinary Research7*(1): 1-60.

McDermott, J. J. and Arimi, S. M. (2002). Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Veterinary Microbiology 90*(1): 111-134.

Mdegela, R. H., Kusiluka, L. J. M., Kapaga, A. M., Karimuribo, E. D., Turuka, F. M., Bundala, A. and Kambarage, D. M. (2004). Prevalence and Determinants of Mastitis and Milk-borne Zoonoses in Smallholder Dairy Farming Sector in Kibaha and Morogoro Districts in Eastern Tanzania. *Journal of Veterinary Medicine, Series B 51*(3):123-128.

Ministry of Livestock and Fisheries Development.(2015).Tanzania livestock modernization initiative (TLMI).Dar es Salaam, Tanzania:Ministry of Livestock and Fisheries Development.http://www.mifugouvuvi.go.tz/sitevisited on 20/09/15

Muma, J. B., Pandey, G. S., Munyeme, M., Mumba, C., Mkandawire, E. and Chimana, H. M. (2012).Brucellosis among smallholder cattle farmers in Zambia.*Tropical Animal Health and Production44*(4): 915-920.

Mythili, T., Rajendra, L., Bhavesh, T., Thiagarajan, D. and Srinivasan, V. A. (2011).Development and Comparative Evaluation of a Competitive ELISA with Rose Bengal Test and a Commercial Indirect ELISA for Serological Diagnosis of Brucellosis.*Indian Journal of Microbiology51*(4): 528–530.

NBS (2007).Tanzania National Household survey 2007, National Bureau of statistics, Ministry of State President's Office, Planning and Privatisation. http://nbs.go.tz site visited on 15/11/2014.

Nielsen, K. and Yu, W. L. (2010).Serological diagnosis of brucellosis. *Prilozi* 31(1): 65-89.

Nielsen, K., Smith, P., Widdison, J., Gall, D., Kelly, L., Kelly, W. and Nicoletti, P. (2004). Serological diagnosis of bovine brucellosis: A review of test performance and cost comparison. *Revue Scientifique et Technique (International Office of Epizootics)*23(3):989-1002

Office International des Epizooties (2012) *"Brucellosis,"* in *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals(*7th edition*)* World Organisation for Animal Health.http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-animals/ site visited 15/10/15

Poester, F. P., Nielsen, K., Samartino, L. E. and Yu, W. L. (2010).Diagnosis of Brucellosis.*Open Veterinary Science Journal* 4:46–60.

Poester, F. P., Samartino, L. E. and Santos, R. L. (2013).Pathogenesis and pathobiology of brucellosis in livestock. *Revue Scientifique et Technique (International Office of Epizootics)32*: 105-115

Posthuma-Trumpie, G. A., Korf, J. and Van Amerongen, A. (2009). Lateral flow (immuno) assay: Its strengths, weaknesses, opportunities and threats. A literature survey. *Analytical and Bioanalytical Chemistry 393*(2): 569-582.

Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W. (2000). *Veterinary Medicine.A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses.(*9th Edition). Harcourt Publishers Limited. London. pp 1963.

Ragan, V., Vroegindewey, G. and Babcock, S. (2013). International standards for brucellosis prevention and management. *Revue Scientifique et Technique (International Office of Epizootics) 32*(1): 189-198.

Schelling E, Diguimbaye C, Daoud S, Nicolet J, Boerlin P, Tanner M. and Zinsstag J. (2003). Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad.*Preventive Veterinary Medicine* 61(4): 279 – 293.

Serological relationship between cattle exposed to *Brucella abortus*, *Yersinia enterocolitica* O: 9 and *Escherichia coli* O157: H7. *Veterinary Microbiology 100: 25-30.*

Shirima, G. M. (2005). The epidemiology of brucellosis in animals and humans in Arusha and Manyara regions in Tanzania. Dissertation for Award of Doctoral degree, University of Glasgow, pp.288

Sikira, A. N., Ndanu, H., Laswai, G. and Nandonde, S. W. (2013). Rapid appraisal of dairy value chains in Morogoro and Tanga regions in Tanzania.pp10

Smirnova, E. A., Vasin, A. V, Sandybaev, N. T., Klotchenko, S. A., Plotnikova, M. A., Chervyakova, O. V. and Kiselev, O. I. (2013). Current Methods of Human and Animal Brucellosis Diagnostics, *Advances in Infectious Disease* (3): 177–184.

Smits, H. L., Abdoel, T. H., Solera, J., Clavijo, E. and Diaz, R. (2003). Immunochromatographic *Brucella*- Specific Immunoglobulin M and G Lateral Flow Assays for Rapid Serodiagnosis of Human Brucellosis. *Clinical and Diagnostic Laboratory Immunology10*(6): 1141–1146.

Swai, E. M. (1997). Studies on the prevalence of bovine brucellosis and reproductive performance in small-scale dairy cattle herds in Dar es Salaam and Morogoro regions.Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania. 67pp.

Swai, E. S. and Schoonman, L. (2010).The use of Rose Bengal Plate Test to assess cattle exposure to *Brucella* infection in traditional and smallholder dairy production systems of Tanga Region of Tanzania. *Veterinary Medicine International* 2010:1-8

Swai, E. S., Mshanga, D., Sanka, N. P. and Marandu, N. H. (2005).Prevalence of bovine brucellosis in smallholder dairying farming area, Moshi, Tanzania. *Bulletin of Animal Health and Production in Africa 53*(2): 97-105.

Urassa, J. K. and Raphael, E. (2002). The contribution of small scale dairy farming to community welfare: A case study of Morogoro Municipality. *Online at: http://www. fiuc.org/esap/MOROG/MOROG5/General/dairyfarming. pdf (accessed 2 March 2015)*.

APPENDIX

Appendix 1: Brucellosis questionnaire

District..... Ward..... Village….....

Farmer's name …… Identity number.....

Farmer information

Gender of farmer M □ F□ Age category (estimate) Youth□ Adult □ Elderly □

Age of farmer 20-45□ 46-65□ 66-85□

Role on the farm: Herdsman □ Farmer/owner □ Family member □Other (specify).....

Level of education…….

Animal information

1. Number of cattle in the herd……..
2. Sex of animal……
3. Type of animal……..

Vaccination

4. Have your cattle been vaccinated against brucellosis? Yes or no

5. If no, give reasons:

It's not important □ the vet did not show up□ Insufficient information □ I don't know□ I refused to vaccinate my animals□ other reasons□

6. Give Reasons for refusing vaccinations ……..

Breeding method

7. What breeding method is used on your farm?

Artificial insemination □ Natural breeding □ Both□

8. If natural breeding is used, is the male used on other farms? Yes □ No □

How many times was your cow serviced before last conception? .....

Knowledge and awareness relating to brucellosis

9. Do you get any information on brucellosis from the veterinary office?

Do you know how brucellosis is transmitted? Yes or no

10. Which other disease causes abortion in cattle?

11. Have you heard about a disease called Brucellosis? Yes □ No □

13. What is brucellosis called in your local language?

14. What are the symptoms in cattle?

15. Which animal transmits brucellosis infection to cattle?

Cattle □ Buffalo □ Mouse Goat □ Pig □Horse □ Bird □ Sheep □ Gazelle□

16. How is brucellosis transmitted between animals?

17. How is brucellosis transmitted to humans?

Practices relating to brucellosis

18. Do you consume the following livestock products?

Raw testicles □ raw liver □ raw blood □ fried placenta □

19. How do you consume your milk? Boiled□ Raw □

20. Do you allow newborn animals into your house? Yes □ No □

21. Have you assisted your cow in any difficult calving? Yes or no

22. (a) Do you wear any protective clothing when handling afterbirth/aborted materials?

23. (b)Specify the protective clothing ……..

24. How do you dispose of the afterbirth materials?

25. How many cases of abortion have you had in your herd?

26. How did you dispose of the fetal materials?