PREVALENCE AND RISK FACTORS FOR BRUCELLOSIS TRANSMISSION IN GOATS IN MOROGORO, TANZANIA

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

2017

ABSTRACT

Brucellosis is a zoonotic disease that causes a great socio-economic as well as a public health concern worldwide. Limited studies have been conducted to determine the prevalence of brucellosis in small ruminants kept in the pastoral and agro-pastoral grazing systems of Tanzania. This study was conducted to determine the prevalence of *Brucella* infections and risk factors for its transmission to human in Morogoro region, Tanzania. A cross sectional study was conducted in nine wards of three Districts of Morogoro Region. Blood samples from 478 goats were collected and screened for brucellosis by serological tests. Real-time Polymerase Chain Reaction (qPCR) was used to detect the *Brucella* DNA. Structured questionnaire was administered to assess the awareness of brucellosis, occurrence of the disease in goats and managerial practices associated with Brucellosis transmission in the community. Out of 478 goat sera, one (0.2%) serum had positive reaction to both RBT and iELISA tests. Eighteen (66.7%) of the 27randomly selected samples were positive for *Brucella* spp on qPCR, and *Brucella abortus* was the only species detected in all infected goat sera. One (2.2 %) of the respondents was aware about human brucellosis while seven (15.2%) were aware of livestock brucellosis. Twenty six percent admitted to consume raw milk, 11 % consumed raw blood while 80.4% reported to have observed abortion cases in goats in their herds, 28.3 % observed cases of retained fetal membranes, and 42 % had no proper means of disposing animal fetal membranes. This study has indicated the presence of *B. abortus* infection in goats in Morogoro region. Transmission of the infection is likely to occur due to low community awareness about transmission, prevention and control of the disease. Therefore, there is a need for formulating and implementing disease control measures such as raising public awareness in prevention of brucellosis in the study area.

DECLARATION

I, HUSNA AYUB KASSUKU, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and have neither been nor concurrently submitted for a higher degree award in any other university.

....

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The above declaration is confirmed;

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ACKNOWLEDGEMENTS

Foremost, I would like to thank Allah, the most gracious and the most merciful for his endless blessings throughout this study. Without whom all this would have not been possible. I extend my heartfelt appreciations to my supervisors; Dr. Isaac Kashoma and Dr. Ernatus Mkupasi for their contributions, positive criticism and patience throughout my research. Deep appreciations also goes to the goat owner's in the three districts for their willingness to participate in this study. I wish to express my sincere gratitude to One Health Central and Eastern Africa (OHCEA) for funding this research. This support is deeply appreciated. Special thanks to Prof. Robinson Mdegela, Dr. Doreen Ndossi and Mr. Ulimbaga Kajobile who made sure that the funds were available on time. Their hard work is highly valued. Sincere gratitude to Mrs. Suzan Hamisi, Mr. Richard Kuziganika , Mr. Simon Kahesa, Mr. Francis Banzi, Mrs. Yustina Njau, Mrs. Maua Gurumo, Mr. Deusdedit Mabagala and Mr. Albert Manyesela for their field assistance and perseverance throughout the preparatory stages as well as the field work. Last but not least, I wish to thank Mr. Philemon Mkuchu, Mr. Godwin Minga, Mr. Shaban Motto and Miss Proskovia Kagaruki who provided technical assistance during laboratory sample analysis. Dr. Zachariah Makondo and Dr. Ray Kayaga whose contribution and assistance led to the successful completion of this study.

DEDICATION

To my father, Ayub A. Kassuku; separated by death, together by love, my mother Tatu S. Kassuku, for her continued love and support throughout my academic life, my siblings; Ahmed, Ashura, Fatma and Khadija for their moral support, but also to Ilham Liban, my late niece who lost a battle to a large PDA and whose fighting spirit gave me the motivation to go on.

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LIST OF ABBREVIATIONS AND SYMBOLS

Ab	Antibody
Ag	Antigen
CFT	Complement Fixation Test
Cq	Cycle value
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation
iELISA	Indirect Enzyme linked immunosorbent assay
IGM	Immunoglobulin M
LPS	Lipopolysaccharide
MAT	Microscopic Agglutination Test
mRBPT	Modified Rose Bengal plate test
MRT	Milk Ring Test
OHCEA	One Health Central and Eastern Africa
OIE	World Organisation for Animal Health
PDA	Patent Ductus Arteriosus
pH	Hydrogen ion concentration
qPCR	Real time PCR
SAT	Serum Agglutination Test
TVLA	Tanzania Veterinary Laboratory Agencies
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Livestock play a substantial role in supporting livelihood to many pastoral and agro-pastoral communities in the sub-Saharan Africa (Mangen *et al*., 2002). They are also used for draft power in cultivation and traction (Manhica, 2010). In these communities, livestock are valuable assets not only as a direct source of income source but also as an insurance for survival during times of crisis and a store of wealth in many societies (Pollot and Wilson, 2009). Goats belong to a group of small ruminants that offer sustenance to a large number of small scale livestock keepers. The goat population globally is approaching 500 million with the number steadily increasing more so in the developing countries with little improvement in productivity (Pollot and Wilson, 2009). However, Tanzania's overall goat herd population was approximately 16 million as per the 2013 FAO statistics documented by FAO (FAOSTAT, 2015). Goats are reported to be among the valuable livestock in the third world countries. This is due to their ability to tolerate and produce in harsh conditions hence offering a food security and a low cost income source (Anonymous, 1991). In spite of that, diseases such as brucellosis limit the goat's productivity and is of public health concern. The disease is reported to be inadequately controlled in sub-Saharan Africa causing a great socio-economic impact through lowered productivity due to abortion and reduced production (Mangen *et al*., 2002).

Brucellosis is a contagious bacterial zoonotic disease that has been ranked second in importance to rabies worldwide (WHO, 2006; Adugna *et al*.,2013). It is caused by *Brucella,* a Gram negative bacterium. Three species of major concern are *B. abortus*,

B. suis and *B. melitensis* being less host specific. Farmed animals that host *Brucella* organisms include cattle, goats, sheep, pigs, camels and horses (Raddostits *et al*., 2000). Additionally, several wildlife species such as fox, hare, wildebeest and African buffaloes are affected by the *Brucella* spp. In animals, the pathogens cause abortion and sterility, while in human, it is associated with febrile like symptoms that are recurrent (Kassahun, 2003; Aparicio, 2013). In human, clinical resemblance of brucellosis with other febrile diseases like malaria, arthroses and typhoid fever mask its diagnosis (Ducrotoy *et al*., 2017).Similarly, the disease in animals presents with variable clinical picture which limits its diagnosis basing on clinical signs (Ducrotoy *et al*., 2017).

Efforts have been made to control and eradicate the disease in developed countries (Fensterbank, 1986). It has been reported to be eradicated in some countries like Japan, Australia, Israel, Europe as well as Canada (Greening *et al*., 1995).The disease however, is still reported to be prevalent in many parts of the world especially in developing countries. Inadequate diagnostic facilities and poor infrastructures limits the control efforts in poorly resourced countries (Lyimo, 2013; Tsegay *et al*., 2017). The limited knowledge and poor health facilities also impedes control of the disease (Lopes *et al*., 2010; Lyimo, 2013).

The Gold standard test of *Brucella* infection diagnosis requires isolation of the causative agent from various tissues from infected animals (WHO, 2006; Wareth *et al*., 2014). *Brucella* antibodies can be demonstrated by using a serological approach (Mantur *et al*., 2006). The available serological tests include Rose Bengal Plate Test (RBPT), Enzyme Linked Immunosorbent Assay (ELISA), Serum Agglutination Test (SAT) and Complement Fixation Test (CFT). Nevertheless, the diagnostic approach

that offers certainty is DNA detection and isolation of *Brucella* organisms through the polymerase chain reaction (Godfroid *et al*., 2010).

Brucellosis is endemic in Tanzania with cases reported both in humans and livestock. A prevalence of 1% - 30% has been reported in cattle in various livestock keeping systems and geographical locations of the country. Most of the studies that have been conducted in Tanzania focused on bovine *Brucella* sero-prevalence. Asero-prevalence of 20.5% in human population in Morogoro (James, 2013) and 0.6% in Katavi-Rukwa ecosystem has been reported (Assenga *et al*., 2015). Also, Swai and Schoonman, (2009) reported a 5.5% prevalence of human brucellosis in a study conducted in Tanga while Mtui-Malamsha (2001) reported a sero-prevalence of 4.6% in the Babati and Hanang districts. A study conducted in the Serengeti ecosystem reported a 24% overall prevalence in Buffaloes (Fyumagwa *et al*., 2009).

In neighboring countries, a disease sero-prevalence of 5.8% among cattle keepers and 9% in unpasteurized milk customers of Mbarara and Kampala districts respectively in Uganda were reported (Nasinyama *et al*., 2014) and 17% in Kiboga District- Uganda (Tumwine *et al*., 2015) while Nanyende (2010) documented a prevalence of 17% in human in a study conducted in northern Turkana district in Kenya. Likewise a 4.8% prevalence has been documented in Addis Ababa (Kassahun, 2003) and a 24.1% in Abuja among abattoir workers (Aworh *et al*., 2011). This indicates that the community is at high risk of acquiring the disease if control measures are not taken.

1.2 Problem statement and justification of the study

High sero-prevalence percent of brucellosis in human reported in Morogoro is alarming and suggesting a need for immediate and coordinated control strategies (James, 2013). The source of the infection in reported human cases in Morogoro is not clearly described. Probably small ruminants contribute to the reported prevalence. Despite that no study has been carried out to assess the status of brucellosis in small ruminants in pastoral and agro-pastoral grazing systems around Morogoro, municipality. WHO (2006) has reported *B. melitensis* to be among the causation of cross-species infection and being associated with acute cases of brucellosis in human. Limited information about the status of the infection in small ruminant and possible risk factors especially in Morogoro, made it imperative to conduct prevalence surveillance of the disease in goats and assess risk factors for disease transmission in such communities to establish effective disease control strategies. Communities in pastoral and agro-pastoral areas are most likely to be infected with brucellosis due to close proximity with their livestock, and little knowledge about disease transmission mechanisms. Therefore this study gathered current information about disease status in goats in pastoral and agro-pastoral communities and possible risk factors for its transmission. This information will be useful in planning strategies for the control of the disease.

1.3 Main objective of the study

The study was conducted to establish the prevalence and risk factors associated with brucellosis transmission in goats in pastoral and agro-pastoral production systems in Morogoro region, Tanzania.

1.3.1 Specific objectives

The specific objectives were:

i). To determine the sero-prevalence of *Brucella* spp infection in goat herds in pastoral and agro-pastoral production systems in Morogoro Rural, Morogoro Urban and Mvomero districts

ii). Molecular detection of *Brucella* species affecting goats in pastoral and agro-pastoral production systems in Morogoro Rural, Morogoro Urban and Mvomero districts

iii). To identify the risk factors for brucellosis transmission in pastoral and agro-pastoral communities in Morogoro Rural, Morogoro Urban and Mvomero districts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Brucellosis definition

Brucellosis is a contagious disease of both human and animals species caused by microorganisms from the genus *Brucella* (Chitupila *et al*., 2015).The disease was founded by Sir David Bruce in 1887, who isolated the causative agent from a spleen of a departed British military soldier and named it *Micrococcus melitensis*. *Brucella abortus,* one of the important bacterial species was described in Denmark by Fredrick Bang in 1897 in cattle (Poester *et al*., 2010) and in 1914 by Traum in pigs from the aborted fetus of infected animals in both cases (Shirima, 2005). Brucellosis is a zoonotic disease in animals also referred to as Contagious abortion or Bang's diseases and it transmitted to human through the infective fluids and membranes from infected animals (WHO, 2006). In humans, the disease is a chronic, febrile and debilitating, while in livestock is characterized by reproductive complications (Radostits *et al*., 2000).

2.2 Morphollogical and Biochemical Charecteristics of *Brucella* organisms

Brucella organisms are small, cocco-bacillary or short rods, Gram negative, non-motile, non-spore forming bacteria. The organisms are sensitive to sunlight and some disinfectants but on favorable conditions they can survive up to two years (James, 2003). Moisture has seemed to be of great importance in their survival. Other factors that affect the endurance of the pathogen in the environment include the pH level, the nature of the substrate and the temperature (WHO, 2006; James, 2013).Among the *Brucella* species that causes brucellosis in animals are *B. melitensis* in sheep and goats, *B. abortus* in cattle, *B. ovis* in sheep, *B. neotomae* in desert rats and *B. suis* in pigs (Raddostits *et al*., 2000; Cloeckaerta *et al*., 2001; Shirima, 2005).However, the

species are reported to be less host specific (WHO, 2006). The disease in human is commonly caused by *B. suis*, *B. canis*, *B. abortus*, and *B. melitensis* infection (Shirima, 2005). However, *B. melitensis* is reported to be the most pathogenic species in human (WHO, 2006).

2.3 Virulence characteristics

The brucellosis causing bacterium being intracellular have developed mechanisms of growing inside macrophages. Their cell wall is resistant to the phagocytic enzymes hence increases their chances of survival (Mugabi, 2012). They have lipopolysaccharides (LPS) that are crucial for the integrity of its cell structure and also in its functions. Their lipopolysaccharides are classified into smooth and rough. *B. canis* is said to have a rough LPS while *B. suis*, *B. melitensis* and *B. abortus* have a smooth LPS. Apart from that they have an ability to restrict phagosome maturation process which is an essential process in destruction of an engulfed pathogen (Mugabi, 2012). This is due to the presence of the smooth LPS which prevents the formation of phygosome-lysosome fusion. Serological techniques alone have faced challenges to detect *Brucella* due to the reason that organisms under the genus *Brucella* have the smooth LPS like other Gram negative bacteria which results into false positives (WHO, 2006; Mugabi, 2012). Therefore, knowing the specific *Brucella* species that has elicited antibodies production requires the use of PCR.

2.4 Disease Distribution

Brucellosis is considered the most-wide spread zoonosis affecting a vast number of species such as bovines (domestic and wild), caprines, ovines, swine, camelids, canines, human beings as well as marine animals (WHO, 2006). Factors associated with the endemicity nature of brucellosis in developing countries include increased

animal keeping and production, poor farm husbandry practices and some socio-cultural aspects (Lyimo, 2013). Animal to animal transmission is aggravated by a large flock density which results into a close contact crucial for a successful transmission. Furthermore, due to a large number of animals per herd the rate of environmental contamination through shedding of the infective organisms is intensified (WHO, 2006). Most developed countries have invested plentyof resources to eliminate the problem through testing and culling of the infectedanimals (Mugabi, 2012). The approach is however not feasible in the developing countries because of lack of resources to compensate the farmers for the livestock that needs to be culled after a screening exercise (Godfroid *et al*., 2011). Occurrence of brucellosis in animals in Tanzania and some African countries has been summarized in Appendix II.

2.5 Risk factors for brucellosis transmission

2.5.1 Risk factors for transmission of brucellosis in livestock

Shirima (2005) reported that among the risk factors of *Brucella* infection in a disease free herd is the introduction of a new animal that has already been infected. Breed of an animal has also been reported to be among the risk factors. Goats are reported to be highly susceptible to *Brucella* as compared to sheep. Furthermore, age has been reported as another factor. The disease affects all age groups, however it remains persistent in sexually matured animals (Adugna *et al.*, 2013). Additionally, sex of the animal has also been reported to be a risk factor in the transmission of the disease. Male animals are reported to be less susceptible to the infection due to the absence of a compound known as erythritol (Adugna *et al*., 2013). *Brucella* organisms are strict on their hosts but cross-infection between one animal species and the other has been documented (WHO, 2006). The transmission is intensified by a large flock size

which increases inter-animal interaction. This is another risk factor to the disease transmission (Adugna *et al.*, 2013). Pregnant animals are highly vulnerable and shed the bacteria in the environment for a long time posing a threat to other animals in the herd. Furthermore, Shirima (2005) reported that animals are mainly exposed to the infection through ingestion of contaminated feed and water with the bacteria from aborted fetus, fetal membranes and uterine discharges. Inhalation of aerosols containing the bacteria has been reported as another route of exposure. Additionally, sexual transmission of brucellosis is reported to be important in small ruminants and porcine, however it plays a very little role in the epidemiology of the disease in cattle (WHO, 2006). Besides that, *Brucella* spp infection in wild animals can be natural in the susceptible group. However, it can be a spillover of infection between infected and susceptible wildlife and livestock species which poses a threat to the livestock populations especially in the livestock-wildlife interface areas (Davis *et al*., 1990; Fyumagwa *et al*., 2009).

2.5.2 Risk factors for transmission of brucellosis in humans

Brucellosis infection extends to human as it has been reported (WHO, 2006). The infection can be acquired through a direct contact with the infected animal or their infective materials. The infective discharges are such as birth fluids and membranes that pose a potential hazard (Shirima, 2005). Such transmission occurs as an occupational risk especially in people that are involved in the livestock sector such as abattoir personnel, veterinarians and animal health attendants. The transmission can also be through contaminated food of animal origin such as dairy products. Urban population is exposed to the pathogenic organism through ingestion of dairy goods from raw or poorly prepared infected milk (WHO, 2006; Mugabi, 2012). Meat has not been a frequent source of infection to human because in most communities it is

not eaten raw, however *Brucella* organism have been reported to be in low concentrations in such tissues (WHO, 2006) . Furthermore, there are rare cases of person to person transmission. Surgeries involving tissue transplantation and blood transfusion are reported to be transmission routes if the donor is infected (WHO, 2006). Tackling the risk factors will halt the disease transmission.

2.6 Clinical Presentation of Brucellosis

2.6.1 Brucellosis in Livestock

In livestock, *Brucella* organism causes abortion which usually occurs during the second half of gestation, stillbirths and weak calves (Shirima, 2005). The placenta may be retained and milk yield may be decreased. After the first abortion subsequent pregnancies are generally normal (OIE, 2008). However, lactating animals may shed the organism in milk and uterine discharges. Infertility occurs occasionally in both sexes, due to metritis or orchitis (Shirima, 2005). Systemic signs do not usually occur in uncomplicated infections, and deaths are rare except in the foetus or new-born. Infections in non-pregnant female are usually asymptomatic (OIE, 2008).

2.6.2 Brucellosis in Human

The disease is known as a great imitator with incubation duration of up to a month although one to three weeks is more common (WHO, 2006). It presents with an acute or sub-acute, life threatening, febrile illness usually manifested by an intermittent or remittent fever accompanied by malaise, anorexia and prostration, and which, in the absence of specific treatment, may persist for weeks or months to a chronic state with grave complications (Xavier *et al.*, 2010; Lyimo, 2013). The disease is often misdiagnosed due to its resemblance to other febrile disease conditions such as

typhoid fever and malaria which poses a great concern in human practice (WHO, 2006; James, 2013).

Bone and joint involvement are the most frequent complication of brucellosis occurring in up to 40% of cases. A variety of syndromes have been reported including sarcoilitis, spondylitis, peripheral arthritis, osteomyelitis, bursitis and tenosynovitis (OIE, 2008; Xavier *et al.*, 2010). Gastrointestinal signs including anorexia, nausea, vomiting, diarrhoea and constipation occur frequently in adult but less often in children. Most people with undulant form recover completely in three to twelve months with proper treatment. Some of the infected individuals may become chronically ill (OIE, 2008).

2.8 Disease Diagnosis

2.8.1 Clinical diagnosis

This form of diagnosis is based on the clinical picture presented by an infected animal. It is not definitive because it lacks specificity. The clinical resemblance with other diseases intensifies the need for further examination and confirmation from other diagnostic approaches. Among the major signs is abortion for pregnant animals especially at late gestation period (≥ 5 months) as well as placenta retention (Shirima, 2005; OIE, 2008). However, cases of hygroma, orchitis and epididymitis have been reported in infected male animals (OIE, 2008)

2.8.2 Laboratory diagnosis

Diagnosis of brucellosis can be accomplished through direct methods (DNA detection/ tissue culture) and indirect methods (serology) (Smits and Cutler, 2004; Godfroid *et al*., 2010). The aim of diagnosing brucellosis using laboratory techniques

is usually to be able to sort out infected animals in the herd. Direct microscopic examination of smear from vaginal discharges, placental cotyledons and fetal gut contents is documented by WHO (2006) and Kaltungo *et al*. (2014) to be one of the possible method used. However it is important to take into account some other bacteria such as *Coxiella burnetti, Chlamydophila abortus and Chlamydia psittaci* that appear similar and could lead to a misdiagnosis. Tissue culture is another method of diagnosis but in most cases takes longer time (4 to 30 days) as compared to the serological and molecular techniques (Kaltungo *et al*., 2014). This diagnostic approach is complicated by a possibility of contamination. Additionally it requires a well-established laboratory with highly trained personnel to avoid the risk of an outbreak (Anonymous, 2001).

Different serological testing methods such as Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Complement Fixation Test (CFT) are recommended in testing of animals (WHO, 2006; Lyimo, 2013). Serological diagnostic tests are usually combined strategically to avoid false results. Furthermore, other advanced tests include Enzyme Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR) and PCR-ELISA (Morta, *et al*., 2003; Lyimo, 2013). Rose Bengal Plate Test and ELISAs are usually used for screening purposes while CFT and PCR are used for confirmation of a *Brucella* infection.

2.8.2.1 Serological tests

A detection of *Brucella* antibodies is another method of diagnosing the disease, however, they are best coupled with other tests. They are commonly used for screening animals at herd level. Majority of serological tests are highly sensitive but less specific (Acharya *et al*., 2017). It is important to take into account the

vaccination history and the disease background before inferring serological test results due to their limitations to the tests (WHO, 2006). Among the tests used are Enzyme linked immunosorbent assay (ELISA), Complement fixation test (CFT), Rose Bengal plate test (RBPT), Serum agglutination test (SAT), Rivanol Precipitation Test and Buffered Acidified Plate Antigen Test (BAPA) (Lyimo, 2013).

Rose Bengal Plate Test is a useful test for detection of *Brucella* infected animals at a herd level. Its simplicity has made it a preferred test in the screening of animals to determine herd prevalence. The test uses a principle that immunoglobulin M (IgM) antibody's capability to bind to antigens is greatly reduced when the pH is low (acidic) (WHO, 2006). It falls in a collection of tests that are also recognized as the buffered *Brucella* antigen tests. It is a spot on test in which a stained antigen and antibodies are mixed on a glass plate. Positive reaction results into agglutinations (Rubegwa, 2015; Ducrotoy *et al*., 2017).

Enzyme Linked Immunosorbent Assay tests are considered to be more sensitive and provide more specificity than RBPT (WHO, 2006). However, there are cases that the tests fail to detect animals that were previously picked by RBPT to be positive. The test's specificity is only marginally to that of CFT and RBPT. Serum Agglutination Test is a simple and low-cost test in the diagnosis of Brucellosis. Nevertheless, due to its unsatisfactory sensitivity and specificity it has been discouraged in the presence of other diagnostic tests (WHO, 2006).

ELISAs are classified into competitive and indirect ELISAs (iELISAs) groups of which most iELISAs employ a purified smooth lipopolysaccharide antigen. The iELISAs are more sensitive but less specific as compared to cELISAs (Godfroid *et*

al., 2010). Complement fixation test (CFT) is considered definitive for the detection of animals and humans that are infected with Brucellosis with high specificity and sensitivity. It is recommended by OIE for international trade purposes. The tests permits the recognition of anti-*Brucella* antibodies that triggers the complement (Godfroid *et al*., 2010). It is comprised of proteins that undergo lysis when triggered by an antigen–antibody reaction (Tsegay *et al*., 2017). The test findings are usually not compromised by other reactions. It is used to confirm the serum samples that have tested positive by RBPT (James, 2013). However, WHO (2009) reports that the test is complex and requires trained personnel in an appropriate laboratory facility. ELISAs are tests that are increasingly replacing CFT (Godfroid *et al*., 2010). Other serological tests that are used in the diagnosis of Brucellosis are such as Serum Agglutination test (SAT), Buffered Acidified Plate Antigen Test, Milk ring test, Rivanol Precipitation Test and Milk iELISA (James, 2013).

2.8.2.2 Culture and isolation

Brucella organism isolation and identification from various tissues remains the most reliable means of diagnosis. The selection of tissue to culture is usually reliant on the type of clinical signs that the animal is presenting (Poester *et al*., 2010). The tissues however require careful and proper handling to prevent contamination but preserve the organisms as well. Among the preferred tissue from which samples for brucellosis diagnosis are acquired in animals are such as fetal membranes, hygroma fluids, vaginal discharges, aborted fetus, milk and semen. For cases that a postmortem is conducted then the preferred tissues are the udder, uterus (late term/ early post calving) and the reticulo-endothelial system tissues (Poester *et al*., 2010; Al-Garadi *et al*., 2011).

Media selection depends on the type of tissue sample collected. Fluid tissue sample handling such as blood requires broth or a biphasic media. In this case Castaneda which is a two phase system and more suitable especially if asepsis was ensured. It comprises both solid and liquid phase one blood culture bottle. Moreover, for other tissue samples solid media are used with 2.5% agar. Bacto Tryptose, Triptic soy, Triptcase soy and Tryptone soya are among the basal media that are used in the diagnosis of *Brucella* species through culturing. However, a selective media is recommended due to the risk of contamination of the tissue samples collected. Among those are Morse, Farrell's and Kuzda medium. It usually takes 3-4 days for the organisms to grow on culture media after inoculation however a period of 8-10 days should pass before the media is considered negative (OIE, 2008; Poester *et al*., 2010). Furthermore, there is an additional requirement of carbon dioxide 5-10% as well as an ideal temperature of 36-38°C in primary isolation by most of the *Brucella* wild strains which are fastidious (Poester *et al*., 2010).

2.8.2.3 Molecular Tests

Among the documented molecular tests for brucellosis are Restriction Endonuclease Analysis (REA), Restriction Endonuclease and Hybridisation and Polymerase reaction. The tests are reported to use lesser time in diagnosis as compared to other tests such as microbiological culture but also to be highly specific and sensitive (Shirima, 2005). PCR assays are reported to have an ability of detecting pathogen DNA in both active and passive infections (Klein, 2002). This is a shortfall because distinguishing between previous and present infection becomes difficult unless isolation is done as the *Brucella* DNA may be present in both situations.

2.9 Treatment in human and animals

Brucellosis is among the zoonotic diseases that are neglected hence its endemic nature. The most recommended treatment protocol in human cases is a combination between an amino glycoside and doxycycline for a period of a month followed by a combination of rifampicin and doxycycline for a period of about two months (Kassahun, 2003). Treatment in livestock is usually not undertaken due to the possibility of having carrier animals that will continue to contaminate the environment and hence expose both the public and other animals to the risk of acquiring the infection (Shirima, 2005). Furthermore, trials undertaken on a possible treatment approach to eliminate the *Brucella* organism in infected animals have so far lead to a limited success (Lyimo, 2013).

2.10 Prevention and Control

The best control strategy involves building up of the public awareness about the disease and the potential risks associated (Ragan *et al*., 2013). Another strategy is screening and early detection of the infected animals (Ragan *et al*., 2013). However, increasing the resistance of the vulnerable animals as well as inhibiting their exposure to the infective agents are things among which the control of the disease is largely depending upon (Ragan *et al*., 2013). Vaccination of replacement animals 3 to 4 months of age yearly has been recommended in the resourceful countries as a means of control. Mass vaccination has been recommended in poor resource countries with high prevalence of the disease where test and slaughter approach is not applicable and where tagging of animals for distinct identification is not possible (Blasco *et al*., 2016).

There are a number of vaccines that have been recommended by OIE in an effort to control and eliminate brucellosis from the herds at a national level. The live attenuated vaccines are such as the non-smooth *B. abortus* strain RB51 vaccine, smooth *B. abortus* strain 19 vaccine and smooth *B. melitensis* strain Rev.1 vaccine. It is however, crucial for the vaccines to be appropriately handled due to the health risks that it can cause to the handlers (WHO, 1997; Ragan *et al*., 2013). Despite the benefits that such vaccinations bring in the control of the disease there is a risk of impairing with some of the diagnostic tests. Vaccinated animals can result to titers that may be misjudged as positive case (Ragan *et al*., 2013). Persistence of antibodies in about 2% of young animals vaccinated by using *B. abortus* strain 19 vaccine as well as Rev 1 has been reported. However, this problem can be prevented by reducing the doses of the vaccine (Fensterbank, 1986; Blasco *et al*., 2016). *Brucella abortus* strain 19 in cattle and *B. melitensis* Rev 1 in goats and sheep coupled with test and slaughter has resulted in successful control of brucellosis in many developed countries. These two vaccines are reported to be the most competent vaccines (Blasco *et al*., 2016).

South Africa is the only country that has managed to actively control bovine brucellosis in Africa. However, it is reported to be prevalent in most sub-Saharan countries (McDermott and Arimi, 2002). In other African countries, vaccinations are either partially conducted or not done at all. Lesotho reports to have managed to control the disease in 1997 (McDermott and Arimi, 2002). Some vaccination control programs were documented in Namibia and Zimbabwe (McDermott and Arimi, 2002). A different study by Mangen *et al*. (2002) documented that the vaccination coverage in the sub-Saharan countries has been tremendously poor. Vaccination against brucellosis in small ruminants in Tanzania has not been documented.

However, there are some reports of vaccination in cattle that was initiated in the early 1980's in the dairy farms both parastatal and government owned (Shirima, 2005).

2.11 Goat Farming Systems in relation to brucellosis transmission

There are four known systems which are pastoral, extensive agro-pastoral, semi-intensive and Intensive goat farming system (Smith *et al*., 2002). Pastoralism is the type of goat keeping system in which goats are grazed freely in search for pastures and water. They are characterized by keeping a large number of livestock. The owners however are sometimes moving in search for pastures and water hence do not have a permanent homestead. In situations that a pastoralist keeps small ruminants and cattle they are then grazed together (Smith *et al*., 2002). This encourages interspecies and between herd transmission of brucellosis but also environmental contamination with the infectious agent (Smith *et al*., 2002).

In the semi-intensive system, small herds of indigenous or dairy goats are reared in a cut and carry system and sometimes by grazing or tethering near the household. Goat keepers perform crop cultivation just like in the agro-pastoral system and the residues are fed to the animals. The livestock's exposure to the infectious agent is reduced as compared to the pastoral farming system. Extensive agro-pastoralism this is the type that goats are reared freely. The goat keepers are characterized by engaging into crop production with permanent settlements. Goats graze freely but return at the end of the day to the premises where they have a permanent housing structure or "Boma". With this type of farming system different herds with different species of livestock meet in the grazing areas.

The intensive system of goat keeping is more or less like semi-intensive with an exception of that it involves strictly indoor goats that are supplied with feed through a cut and carry system (Smith *et al*., 2002). In such a farming system the livestock are not in contact with other herds hence chances of inter herd transmissions are reduced.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study Area

The present study was conducted in Morogoro region involving three districts, namely; Morogoro Rural, Morogoro Urban and Mvomero from November 2016 to July 2017. Morogoro region is situated between 5°58' and 10°0'S and 35°25' and 35°30' E. It has a population of 2,218,492 people (NBS, 2013).In each district three wards were selected which were; (Morogoro Rural): Mikese, Ngerengere and Gwata; (Mvomero): Dakawa, Mangae and Nyandira; Kihonda, Bigwa and Mkundi (Morogoro Urban). Study districts and wards were purposively selected based on the presence of pastoral and agro-pastoral communities keeping goats apart of other animals. The region has a total of 441,406 goats however the number in study districts were 51,161; 35,935and 4,300 goats for Mvomero, Morogoro Rural and Morogoro urban, respectively (Anonymous, 2014).

3.2 Study animals

The criterion used to select study animal was that, a female goat should be greater than six months old and have at least kidded once. The reason behind that was because male animals are less susceptible to brucellosis due to the absence of a compound known as erythritol which increase in amount with the age of the animal (Adugna *et al*., 2013; Assenga *et al*., 2015). The compound is responsible for an increase in the multiplication of the organisms (Assenga *et al*., 2015).

3.3 Study design

A cross-sectional study design was adopted where a multistage cluster sampling method as described by (Bennet *et al*., 1991) was carried out to select wards and later

villages in each study districts. The wards in each district were selected based on the number of goats kept. From a list of wards that had goat populations representative wards were selected using a simple random technique and the study villages were selected from the selected wards. Households rearing goats were primary sampling units. The list was obtained from the village administrative office.

At the household level, goats that met the selection criteria were selected by using a simple random approach to get a desired sample size. The quantitative part of this research involved blood sampling while the qualitative part involved administering of a questionnaire to animal owners or attendants to assess their awareness about the disease and associated risk factors.

3.4 Sample size estimation

Sample size was obtained using a level of confidence of 95% with expected error of 3%. An estimated prevalence of 6% (Shirima, 2005), based on systematic sampling for estimation of the disease prevalence and detection of *Brucella* was used. The sample size was calculated using a formula by Daniel (1999) and Naing *et al*. (2006).

$N=Z^2*P\ (1-P)/d^2$,

Where by n= sample size; Z= test statistic; P= expected prevalence;

In this case (Shirima, 2005); d = precision; Z= 1.96; P= 6%; d= 0.03. Therefore N = 241

The acquired sample size was corrected by multiplying with the design effect due to the multistage sampling technique used (Nain g*et al*., 2006). Design effect calculation as indicated by Bennet *et al*. (1991):

(D) =1+ (b-1) roh,

Where by b=Average number of animals in a cluster; b=9 goats as indicated by Safari *et al*. (2008); Roh= 0.09 (Otte and Gumm, 1997)

Therefore, =1 + (9-1)0.09 = 1.72 = 415 goats.

Corrected sample size =sample size (N)*Design effect (D) = 241x1.72=414.1~415 goats

3.3 Blood sample collection and handling

Blood samples were collected and handled according to OIE guidelines (OIE, 2008). The samples were carefully collected and packed to prevent possible leakage and hence cross contamination. From each goat, a total of 10 mls of blood sample was collected from jugular vein into a sterile plain vacutainer tube. Vacutainer tubes were then marked according to animal's identities including owner's name and geographical location (village, ward and district). Samples were packed in a cool box with ice packs and kept cool during transportation to the laboratory. Immediately after arriving at the laboratory, vacutainer tubes were tilted over night at room temperature to obtain serum samples. Serum samples were separated into labeled vials and stored at –20^{o}C ready for RBPT, iELISA and PCR tests.

3.4 Laboratory Analysis of Samples

Biosafety measures were ensured throughout sample analysis. Assays were performed in a biosafety cabinet where applicable (for example DNA extraction and PCR assays) (WHO, 2004). All working surfaces were cleaned and disinfected using 10% sodium hypochloride and 70% alcohol. Laboratory work comprised three tests; Rose Bengal Plate Test (RBPT) which was used for screening purposes, whereas indirect ELISA (iELISA) was performed as a confirmatory test and Polymerase Chain Reaction (PCR) assays was used for detection of *Brucella* species. The RBPT

and iELISA were carried out at the department of Veterinary Medicine and public health, at SUA while iELISA and PCR assays were conducted at the Tanzania Veterinary Laboratories Agency (TVLA) in Dar es Salaam.

3.4.1 Rose Bengal plate test

The RBPT was carried out for screening *Brucella* antibodies in goats as described by Kassahun (2003). Antigen (Ag), control sera and test sera were removed from the refrigerator half an hour before the test to attain room temperature. Briefly 25 µl of test serum was dispensed on a white plate followed alongside by an equal amount of RBPT antigen. Serum samples were mixed thoroughly with the antigens using clean applicator stick to produce a circular zone of approximately two centimeters in diameter. The mixtures on a glass plate were then agitated by using a rocker and results recorded within 4 minutes. Results were interpreted according to Nielson and Dunkan (1990), "0" as negative result (no agglutination), "+" (Barely perceptible agglutination), "++" (Fine agglutination and some clearing) and "+++" (Course clumping, definite with clearing).

3.4.2 Indirect Enzyme-linked immunosorbent assay

Briefly, a Priocheck® *Brucella* Ab 2.0 kit was used following manufacturer's instruction. The microtiter plate had wells pre-coated with purified extract of the LPS of *Brucella*. The reconstitution of diluting buffer, control sera, washing fluid, conjugate, chromogen substrate and stopping solution was based on the manufacture's instruction. The optical density (OD) was measured at 450nm using an ELISA microtiter plate reader. The cut-off point for positive result in iELISA was based on the conjugate controls. The mean OD_{450} for the negative control and

positive control as indicated by the manufacturer were supposed to be < 0.2 and ≥ 1.00 respectively, for the test run to be considered valid.

3.4.3 Polymerase chain reaction (PCR)

3.4.3.1 DNA extraction by Spin protocol

DNA extraction by using a spin protocol was used on serum samples. A Qiagen DNA extraction kit was used according to manufacturer's instruction whereby20 µl QIAGEN Protease were pipetted into the bottom of a micro-centrifuge tube and 200 µl of a sample were added. 200 µl Buffer AL were added to the mixture. A pulse-vortex was used to mix the components in the micro-centrifuge for 15 sec. The mixture was then incubated at 56°C for 10 min. 200 µl ethanol (96–100%) were added to the sample, and mixed again by pulse-vortexing for 15 sec. The mixture was then centrifuged briefly to remove the drops on the inside of the lid. The mixture was then applied into the QIAamp Mini spin and centrifuged at 8000 rpm for 1 min. QIAamp Mini spin column was placed in a clean collection tube while the filtrate was discarded. 500 µl Buffer AW1 were added to the QIAamp Mini spin column and centrifuged at 8000 rpm for 1 min. The QIAamp Mini spin column was placed in a clean collection tube and the tube containing the filtrate was discarded. 500 µl Buffer AW2 were added to the QIAamp Mini spin column and centrifuged at 14000 rpm for 3 min. The QIAamp Mini spin column was placed in a new collection tube and centrifuged at full speed for 1 min. The QIAamp Mini spin column was placed in a clean microcentrifuge tube and 200 µl Buffer AE was added. The mixture was then incubated at room temperature (15–25°C) for 1 min, and then centrifuged 8000 rpm for 1 min.

3.4.3.1 PCR analysis

Polymerase Chain Reaction analysis was carried out according to the manufacturer of the *Brucella* genus Genesig® standard kit. Briefly, 25 µl PCR reactions consisted of 22.5 µl master mix that contained 2.25µl of each primer (forward and reverse primers), 4.875 µl of PCR grade water, 0.625 of probe and 12.5 of 2K Dynamo color flash master mix (Enzyme). The remaining 2.5µl was a prepared DNA template. The reactions followed the following steps: the samples were kept at 37ºC for 15 minutes followed by enzyme activation at 95^{o}C for 2 min. This was then followed by 50 cycles of annealing and extension at 95^{o}C for 10 sec. Data acquisition was done at 60^{o}C for 60 sec.

3.5 Questionnaire survey

A semi structured questionnaire (Appendix I) was designed and used to obtain information from the owners or attendants of the sampled goat herds. Relevant herd data about animal owners, consumers as well as the animals were gathered. Acquired information included handling of aborted materials, animal herd structure, goat products consumption, goat diseases and treatment approach, grazing strategies, public awareness about the disease, attitude and perception about brucellosis in the study area and other managerial practices that might perpetuate the transmission of the disease.

3.6 Data analysis

Data from the questionnaire survey and laboratory results were stored in a computer, using Microsoft Excel spreadsheet program 2013. Descriptive statistics for districts and animal level variables were analyzed using SPSS version 20. Statistical significance were determined at 95% CI at a critical probability ($P<0.05$).

3.3 Ethical approval

This research was conducted based on the recommendations of Sokoine University of Agriculture code of conduct for research ethics as indicated on section 2.2 of the article (Anonymous, 2012). Ethical clearance to conduct this study was granted by the college of Veterinary Medicine and Biomedical Sciences, SUA and from District Executive Directors. The ward livestock extension officers and farmers were informed of the study and their verbal concert was sought prior to commencement of data collection.

CHAPTER FOUR

4.0 RESULTS

4.1 Serology

4.1.0 Rose Bengal Plate test

A total of 478 goats; Morogoro Rural (178), Morogoro Urban (51) and Mvomero district (249) were screened for *Brucella* infection using a RBPT. The collected samples per ward were as presented in Table 1.The test detected only one (0.2%) positive sample. The RBPT reactor was among of 100 samples (0.1%) collected from Dakawa ward in Mvomero district.

Table 1: Rose Bengal plate test result of collected goat serum samples

Ward	Number of serum samples	RBPT +ve samples	Prevalence (%)	Ward prevalence (%)
Mangae	100	0	0	0
Dakawa	99	1	0.2	1.0
Nyandira	50	0	0	0
Ngerengere	61	0	0	0
Gwata	49	0	0	0
Mikese	68	0	0	0
Kihonda	5	0	0	0
Bigwa	35	0	0	0
Mkundi	11	0	0	0
Total	**478**	**1**	**0.2**	**11.1**

4.1.2 Confirmation of RBPT positive sample by *i*ELISA

One out of 92 representative samples which were subjected to iELISA was positive for brucellosis (Table 2). This sample was confirmed by iELISA after testing positive in a RBPT. The iELISA microtiter plate has a capacity of 96 samples of which positive and negative controls were duplicated. The remaining 92 wells accommodated the test samples. Since only one sample needed confirmation, the remaining wells were filled with other samples selected randomly out of the total 478 samples collected.

Table 2: Indirect Elisa test results of the randomly selected goat serum samples including a RBPT positive sample

Ward	Samples tested	iELISA test result	Prevalence (%)
Nyandira	14	0	0
Dakawa	17	1	0.2
Mangae	13	0	0
Ngerengere	11	0	0
Mikese	10	0	0
Kihonda	7	0	0
Mkundi	1	0	0
Bigwa	6	0	0
Gwata	13	0	0
Total	92	1	0.2

4.2 Molecular detection of *Brucella* spp.

Due to financial constraints only 27 samples were tested by qPCR. Eighteen samples (66.7%) were positive for the presence of *B. abortus* DNA. Detailed distribution of

qPCR samples is summarized in Table 3. The negative and positive control samples (Cq values: 18.51 and 20.24) amplification graphs are as seen in the figure 1 and 2 below.

Table 3.Results of a qPCR analysis by individual districts

District	Ward	Number of tested samples	Positive samples
Mvomero	Mangae	3	2
	Dakawa	9	5
	Nyandira	0	0
Sub total		**12**	**7**
Morogoro Rural	Ngerengere	6	5
	Gwata	6	5
	Mikese	3	1
Sub total		**15**	**11**
Morogorourban	Kihonda	0	0
	Misongeni	0	0
	Mkundi	0	0
Sub total		**0**	**0**
Total: 3 districts	**9 wards**	**27**	**18**

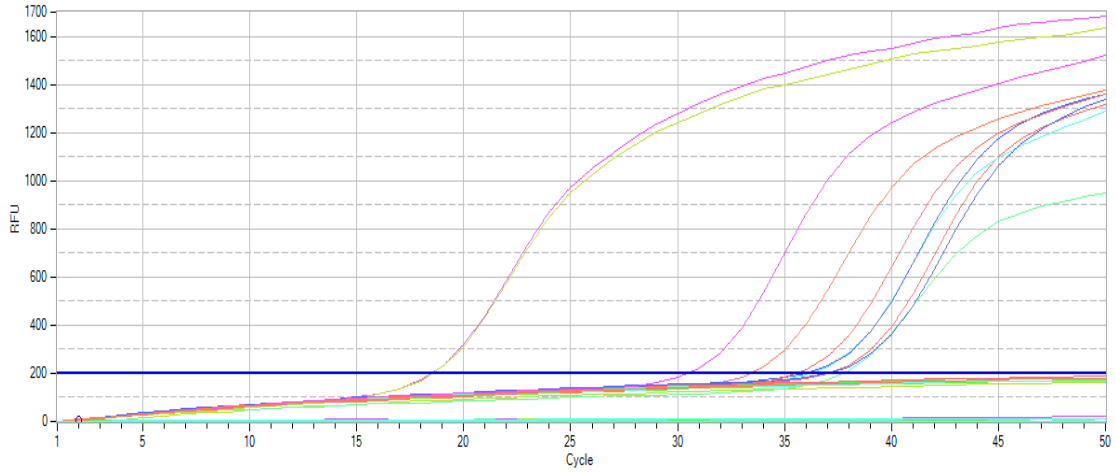

Figure 1: A graph output from a qPCR machine showing amplification curves corresponding to 12 samples ran (positive control: Cq value = 18.51)

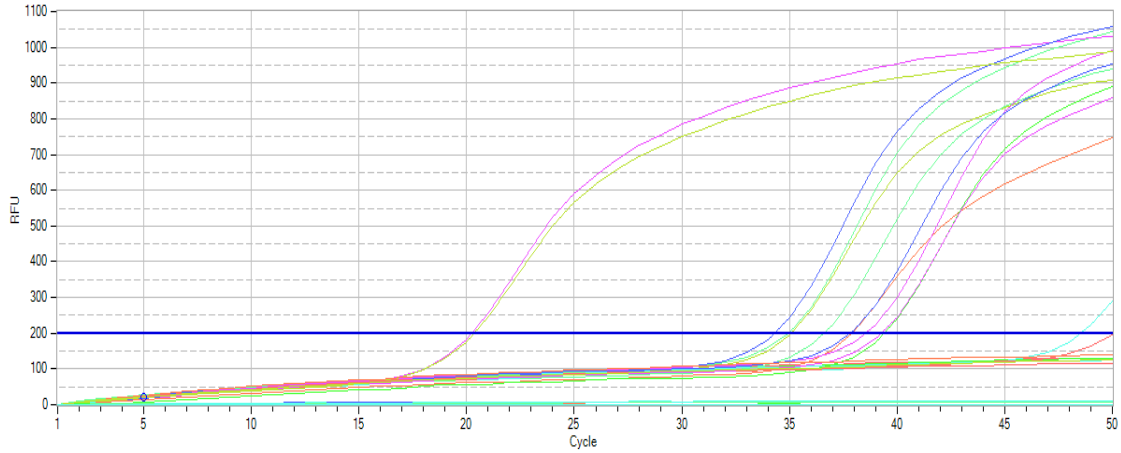

Figure 2: A graph output from the qPCR machine showing amplification curves corresponding to 15 samples ran (Positive control: Cq = 20.24)

4.3 Questionnaire results

A total of 46 (35 males and 11 females) respondents were interviewed (Table 4) during this study and their distribution district wise was as following; 50 % (n=23) were from Mvomero, 39.1% (n=18) from Morogoro Rural and 10.9 % (n=5) from Morogoro Urban district. The responses were as summarized in Table 4. Additionally, the respondent's herds had mixed species. Some had just goats and sheep while others had cattle as well. Respondents had in general little knowledge about brucellosis transmission risk factors both in human and animals (Table 4).

Table 4: Respondent's response to questionnaire survey

Criterion	Description	Response frequency	Percent
Grazing area	Individual grazing area	2	4.3
	Communal grazing area	44	95.7
	subtotal	**46**	**100**
History of fetal membrane retention	Yes	13	28.3
	No	33	71.7
	subtotal	**46**	**100**
Goat disease treatment	Veterinary	34	73.9
	Farmer	12	26.1
	subtotal	**46**	**100**
History of abortion cases	Yes	37	80.4
	No	**9**	19.6
	Subtotal	**46**	**100**
Fetal membrane handling	Buried	17	34
	Dog fed	9	18
	Thrown	21	42
	Goat to feed	3	6
	Subtotal	**50**	**100**
Goat products consumption	Meat	37	61.7
	Milk	16	26.7
	Raw blood	7	11.7
	Subtotal	**60**	**100**

CHAPTER FIVE

5.0 DISCUSSION

The present study established a 0.2% sero-prevalence of brucellosis in goats by serology as presented in Table 1 and 2. The sero-prevalence result observed in this study is lower as compared to other studies conducted in Tanzania and other parts over the years. Assenga *et al*. (2015) reported a 1.6% sero-prevalence in goats in Katavi region, 6% was reported by Shirima (2005) in some of the northern regions of Tanzania and 0.5% sero-prevalence was reported by Temba (2012) in a study conducted in Mikumi-Selous ecosystem. Such a dissimilarity in sero-prevalences could be due to the difference in sampling strategy, diagnostic techniques and the sample size. Additionally, the weather differences may have contributed to the observed differences as it has been reported that prolonged periods of dry and hot weather negatively affect the survival and transmission of the bacterium (Ducrotoy *et al*., 2017). Also risk factors differences between geographical zones could also account for the differences (Assenga *et al*., 2015).

Studies conducted elsewhere reported prevalence of 13.6% in Ethiopia, 2.8%, in Nigeria and 14.5% in Egypt being higher than the findings in this study (Adugna *et al*., 2013; Ogugua *et al*., 2014; Sharifi *et al*., 2015). However, the finding from this study was comparable to that reported in Spain which was 0.1% in goats (Sharifi *et al*., 2015).

It has been reported that the sensitivity of RBPT in goats and sheep serum samples in brucellosis diagnosis is low especially when using the commercial antigens in which standardization conditions favors diagnosis in cattle (Anonymous, 2001). This has

likely contributed to the low positive samples detected contrary to the PCR results. Real time PCR has been reported to be sensitive and specific hence recommended for diagnosis of brucellosis despite of being expensive (Doosti and Dehkordi, 2011).

Furthermore, PCR detects DNA as opposed to the detection of antibodies by serological tests. The fluctuation of the antibody titers during the course of *Brucella* infection is likely to affect the serological diagnostic tools performance. Rajala (2016) and Wareth *et al*. (2015) reports that antibodies (humoral response) takes time to build up to detectable amounts therefore the serological tests might miss early infection (<14 days). Therefore, latently infected animals do not become seropositive which as a result are not picked up by serological tests. Furthermore, in a chronic infection the antibody titers are no longer noticeable hence the animals usually tests seronegative (Gwida *et al*., 2011; Rajala, 2016). Such seronegative animals are likely to give false negatives that could not be picked up in early or chronic stages of *Brucella* infection. Serological tests therefore underestimate the magnitude of the infection in the screened animals. This is likely the reason why PCR detected more *Brucella* positive samples (circulating *Brucella* DNA) as compared to serological tests (RBPT and iELISA) used in this study. Similar observations have been documented in camels (Gwida *et al*., 2011), cattle (Rajala, 2016) and goats and sheep (Wareth *et al*., 2015). It is however, important to remember that PCR detects *Brucella* DNA but it fails to distinguish an active against a passive infection.

Results obtained from this study confirmed *B. abortus* infection in 18 (n=3.8%) serum from among the tested samples. These findings are in agreement with a study by McDermott and Arimi (2002) that documented an infection of *B. melitensis* to be less common in sub-Saharan African countries in sheep and goats. To the best of my

knowledge there are no molecular studies so far that have confirmed the presence of *B. abortus* DNA in goats in Tanzania. This is therefore a new contribution in the brucellosis research area in Tanzania.

Regarding evaluation of transmission risk factors, questionnaire analysis yielded a number of valuable information (Table 4). Among the respondents, only a small proportion (15.2%) had heard about brucellosis. Moreover, among the 15.2% (n=7) only 2.2 % (n=1) were aware that the infection in animals can be transmitted to humans. The findings on limited knowledge and awareness of the livestock keepers and abattoir workers on brucellosis is in agreement with those documented by the previous studies (Swai *et al*., 2010; Lyimo , 2013; Hassan-Kadle, 2015; Tsegay *et al*., 2017). The public awareness of brucellosis and its zoonotic implications is generally poor which then put them at a risk of acquiring the infection. It shows that many people in the studied area lack the understanding about the disease regarding its epidemiology as well as economic and public health importance. As a result of this there are a number of losses that they incur without their knowledge due to the endemic nature of the disease in most African countries including Tanzania.

Lack of movement control of animals may increase the spread of infection. This was revealed by 15.2% (n=7) of respondents who trade their animals among farmers with no prior screening measures. This therefore coupled with a lack of awareness of the disease epidemiology is a cultural practice that may contribute in the transmission of the infection from one goat herd to another.

About half of the respondents (57.1%; n=26) were practicing goat farming for socio-cultural purposes while a 42.9 % (n=20) reported to keep for commercial purposes.

This shows that many people in the three studied districts depends on goats among other livestock for their sustenance. Respondents pointed out that goats serve as an insurance when there is an urgent need for money be it for paying school fees or medical costs for a sick family member/relative and sometimes as a source of meat on special social and religious occasions like Christmas, Easter and Eid celebrations as also reported elsewhere (Pollot and Wilson, 2009).

Among the goat products consumed include meat, milk and blood. Sixty three percent (n=29) of the respondents report to be consuming meat, 26 % (n=12) consume milk and 11 % (n=5) raw blood (Table 4). Meat is largely consumed as compared to milk and raw blood. The Maasai community use raw blood for medicinal purposes particularly in treatment of anemia as it was reported by some of the respondents during questionnaire interviews in this study. This is among the many traditional practices in the pastoral community that may lead to brucellosis transmission in human. It has been reported that raw or partially cooked meat and milk as well as raw blood are significant epidemiological factors that predisposes human to brucellosis (Bashahun *et al*., 2016). Such socio-cultural practices need to be considered in planning for disease control strategies. Amongst the respondents that reported to be consuming raw blood reported that there was a cultural belief that it cures anaemia. Milk was reported to be consumed especially in Nyandira ward where Norwegian goats are kept for milk production purposes. Many respondents, however, keep local goats for meat production.

In addition, eating barbecued meat in auction markets especially in Dakawa and Melela wards of Mvomero district was reported by some of the respondents. In most instances meat is not well cooked before consumption due to the settings as well as a

limited time. A study conducted by James (2013) in Mikumi-Selous ecosystem of Morogoro documented that cultural delicacies of consuming raw/ undercooked meat, raw blood and unpasteurized milk as among the potential risk factors for brucellosis transmission to human. Rubegwa (2015) reported similar findings.

Thirty seven (80%) of the respondents had the history of occurrence of abortion cases in their goat herds while a 28.3 % (n=13) reported cases of retained fetal membranes. All these are among the signs of brucellosis in livestock although a definitive test is necessary. The high abortion cases reported in the study area may be associated with brucellosis. Similar observation was seen at the National Livestock Research Institute headquarters in Mpwapwa that experienced abortion storm in cattle. The outbreak was later on confirmed to be Brucellosis (Shirima, 2014). With respect to fetal membrane handling, 42 % (n=19) of the respondents reported to have no proper disposal approach, 34 % (n= 16) bury or throw in septic pits, 18 % (n=8) leave the membranes for dogs to feed on in a raw form while 6 % (n=3) leave the membranes in the grazing areas (Table 4). Fetal membranes from infected animals are reported to be among the organs that are highly infective (Shirima, 2005). Lack of a proper disposal plan means risk of environmental contamination leading to disease transmission to other animals and human beings.

Moreover, 73.9 % (n=34) of the respondents reported to be seeking veterinary assistance when the goats are sick while 26.1 % (n=12) handle the cases themselves. This practice poses a public health threat since many of them are not aware of the zoonotic implication of brucellosis. As a result they could spread the infection in various farms due to bacterial contamination of the surroundings because necessary precautions and procedures are not followed. These findings concur with those of

Rubegwa (2015) in a study conducted in Kibaha district that showed a lack of zoonotic implications awareness of brucellosis displayed by the agro-pastoral communities.

Furthermore, communal grazing is being practiced greatly as reported by 95.7 % (n=44) of the respondents (Table 4). Shirima (2005) documented contaminated feed and water to be one of the sources of transmission in animals. The risk of infection transmission through the infective material from one herd to the next in the common grazing grounds as well as water sources increases as opposed to individual grazing areas where there is a restriction of inter-herd contact. Similar findings have been reported by Karimuribo *et al*. (2007) and Rubegwa (2015). During the questionnaire survey none of the respondents reported vaccination program against brucellosis in their livestock. This clearly indicates that the disease has been neglected in the study area despite its public health significance. Therefore, the fact that there is a limited knowledge on brucellosis in farmers as well as the poor control measures in place means there is a great risk of brucellosis transmission.

This study has established presence of *Brucella* infection in goats in study area and existence of possible transmission risk factors. This suggest health risk to animal handlers and consumers of animal products especially milk if preventive measures are not put in place.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Results from this study have indicated a presence of brucellosis in goats in the region specifically in the study areas. The serological approach did not yield much information as compared to the molecular approach in brucellosis diagnosis in goats. However, the study has disclosed that a large proportion of people in the region lack awareness of the disease which predisposes them to the infection and causing direct and indirect economic losses.

6.2 Recommendations

From study findings, it is recommended that more intensive studies to be conducted on brucellosis in small ruminants to acquire more epidemiological data for evidence based control strategies. Furthermore, farmer's awareness creation and sensitization programs on epidemiology of the disease and its zoonotic implications are important. Nonetheless, there is a need for formulation and implementation of control strategies in the road towards brucellosis control, prevention and eventually eradication.

REFERENCES

Acharya, K. P., Niroula, N. and Kaphle, K. (2017). Review of brucellosis in Nepal. *Epidemiology and Health39* [http://doi.org/ 10.4178/ epih. e2017018] site visited on 7/11/2017.

Adugna, W., Tessema, T. S. and Keskes, S. (2013). Sero-prevalence of small ruminants' brucellosis in four districts of Afar National Regional State. Northeast Ethiopia. *Journal of Veterinary Medicine and Animal Health* 5(12): 358–364.

Al-Garadia, M. A., Khairani-Bejo, S., Zunita, Z. and Omar, A. R. (2011). Detection of *Brucella melitensis* in blood samples collected from goats. *Journal of Animal and Veterinary Advances* 10(11): 1437-1444.

Anonymous, (1991). World Animal Review: a Quarterly *Journal on Animal Health*, Production and Products. Rome: FAO. [lib.ugent.be/en/ catalog /ser01:000 226674] site visited on 20/6/2017.

Anonymous, (2001). Brucellosis in sheep and goats (*Brucella melitensis*). Scientific Committee on Animal Health and Animal Welfare. 89. pp. 11-35.

Anonymous. (2010). Rose Bengal Test; Standard Operating Procedure. EU Reference laboratory for Brucellosis. [https:// sites. anses. fr/en/system /files/private/ Brucellosis EURL_ RBT_SOP _2010_v0.pdf] site visited on 10/5/2017.

Anonymous, (2012). Code of conduct for research ethics. Sokoine University of Agriculture. [http://drpgs.suanet.ac.tz/index.php? option=com_ phoca download&view=category&download=1:code-of-conduct-for-research-ethics&id=1:ethics&Itemid=186.] site visited on 2/1/2017.

Anonymous. (2014). Environmental and social management framework. [http://www. kilimo.go.tz/index.php/en/resources/view/expanding-rice-production-project-erpp-environmental-and-social-management] site visited on 23/7/2017.

Aparicio, D. E. (2013). Epidemiology of brucellosis in domestic animals caused by *Brucella melitensis, Brucella suis and Brucella abortus*. *Small Ruminant Research* 32(1): 53-60.

Assenga, J., Matemba, L., Muller, S., Malakalinga, J. and Kazwala, R. (2015). Epidemiology of *Brucella* infection in the human , livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. *Biomedical Central Veterinary Research* 11: 189-199.

Aworh, M. K., Okolocha, E., Kwaga, J., Fasina, F., Lazarus, D., Suleman, I., Poggensee, G., Nguku, P. and Nsubuga, P. (2013). Human brucellosis: seroprevalence and associated exposure factors among abattoir workers in Abuja, Nigeria. *The Pan African Medical Journal* 16: 103-111.

Bashahun, D., Michael, G., George, N. and Gelelcha, B. (2016). Seroprevalence of human brucellosis community awareness and practices on its zoonotic importance in Jimma town and Chora Botor district, Ethiopia. *Journal of Zoonotic Diseases* 1(1): 58-64.

Bennet, S., Woods, T., Liyanage, W. and Smith, D. (1991). A simplified general method for cluster-sample surveys of health in developing countries. *World Health Statistics Quarterly* 44: 98-106.

Bertu, W., Ajogi, I., Bale, J., Kwaga, J. and Ocholi, R. (2010). Sero-epidemiology of brucellosis in small ruminants in Plateau State, Nigeria. *African Journal of Microbiology Research* 4(19): 1935-1938.

Blasco, J. M., Moreno, E. and Moriyón, I. (2016). Brucellosis vaccines and vaccine candidates.[https://www.researchgate.net/publication/302098529_ Brucellosis_vaccines_and_vaccine_candidates]site visited on 24/7/2017.

Cloeckaerta, A., Vergera, J., Grayona, M., Paquetb, J., Garin-Bastujic, B., Fosterd, G. and Godfroid, J. (2001). Classification of *Brucella spp*. isolated from marine mammals by DNA polymorphism at the omp2 locus. *Microbes and Infection* 3: 729−738.

Chitupila, G. Y., Komba, 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(6). [http://www.lrrd.org/ lrrd27/6/komb27118.html] site visited on 20/8/2017.

Daniel, W.W (1999). Biostatistics: A Foundation for Analysis in the Health Sciences. 7th edition. New York: John Wiley & Sons. European Commission. 782 pp.

Davis, D. S., Templeton, J. W., Ficht, T. A., Williams, J. D., Kopec, J. D. and Adams, L. G. (1990). *Brucella abortus* in captive bison. I. Serology, bacteriology, pathogenesis and transmission to cattle. *Journal of Wildlife Diseases* 26: 360-371.

Doosti, A. and Dehkordi, P. G. (2011). Application of real-time PCR for identification and differentiation of *Brucella abortus* and *Brucella melitensis* in cattle. *Bulgarian Journal of Veterinary Medicine* 14(2): 109-115.

Ducrotoy, M., Bertu, W. J., Matope, G., Cadmus, S., Conde-Álvarez, R., Gusi, A. M.,Welburn, S., Ocholi, R., Blasco, J. and Moriyón, I. (2017). Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. *Acta tropica* 165: 179-193.

FAOSTAT. (2015). Statistical Database of the Food and Agriculture Organization of the United Nations on agriculture, nutrition, fisheries, forestry, food aid, land use and population. [http://faostat.fao.org] site visited on 5/11/2017.

Fensterbank, R. (1986). Brucellosis in cattle, sheep and goats: diagnosis, control and vaccination. *Revue scientifique et technique (International Office of Epizootics)* 5(3): 605-618.

Fensterbank, R. (1987). Comprehensive report. Brucellosis in cattle, sheep and goats: diagnosis control and vaccination (6): 9-35.

Fyumagwa, R., Wambura, P., Mellau, L. and Hoare, R. (2009). Seroprevalence of *Brucella abortus* in buffaloes and wildebeests in the Serengeti ecosystem: A threat to humans and domestic ruminants. *Tanzania Veterinary Journal* 26(2): 62–67.

Greening, W. A., Forman, A. and Nunn, M. (1995). Exotic Disease of Animals. Australian Government Publishing service,Canbern, Australia. 306pp.

Godfroid, J., Nielsen, K. and Saegerman, C. (2010). Diagnosis of Brucellosis in Livestock and Wildlife. *Croatian Medical Journal* 51(4): 296-305.

Gwida, M., El-Gohary, A., Melzer, F., Tomaso, H., Rösler, U., Wernery, U., Wernery, R., Elschner, M., Khan, I., Eickhoff, M., Schöner, D. and Neubaue, H. (2011). Comparison of diagnostic tests for the detection of *Brucella* spp. in camel sera. *Biomedical Central* Research Notes 4: 525-531.

Hassan-Kadle, A. A. (2015). A Review on Ruminant and Human Brucellosis in Somalia. *Open Journal of Veterinary Medicine* 5: 133-137.

James, L. (2013). Studies on human brucellosis in the Mikumi- Selous ecosystem, Morogoro, Tanzania. Sokoine University of Agriculture. pp. 6-37.

Kaltungo, B. Y., Saidu, S. N. A., Musa, I. W. and Baba, A. Y. (2014). Brucellosis: A Neglected Zoonosis, *African Journal of Medicine and Medical sciences* 4(12): 1551-1574.

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

Kassahun, J. (2003). Seroepidemiological study of Brucellosis in humans and Dairy cattle in Addis Ababa. Addis Ababa university. pp 1-23.

Klein, D. (2002). Quantification using real-time PCR technology: applications and limitations. *Trends in Molecular Medicine* 8(6): 257-260.

Lopes, L. B., Nicolino, R. and Haddad, J. P. A. (2010). Brucellosis-Risk factors and Prevalence. *Veterinary Science Journal* 4: 72-84.

Lyimo, B. (2013). Prevalence of bovine brucellosis in smallholder dairy farms in Morogoro municipality,Tanzania.Sokoine University of Agriculture, Morogoro, Tanzania. pp.1-57.

MacMillan, A. P., Greiser-Wilke, I., Moennig, V. and Mathias, L. A. (1990). A competition enzyme immunoassay for brucellosis diagnosis. *Deutsche Tierarztliche Wochenschr* 97: 83-85.

Mangen, M.-J., Otte, J., Pfeiffer, D. and Chilonda, P. (2002) Bovine brucellosis in Sub-Sahran Africa: Estimation of Sero-Prevalence and Impact on Meat and Milk Off take Potential. Livestock Policy Discussion Paper No. 8, FAO Livestock Information and Policy Branch, AGA. 52pp.

Manhica, A. D. P. (2010). The prevalence of brucellosis in cattle, sheep and goats in Maputo Province, Moçambique (PhD Thesis). University of Pretoria. pp 1-2.

Mantur, B. G., Biradar M. S., Bidri, R. C., Mulimani, M. S., Veerappa, K., Kariholu, P., Patil, S. B. and Mangalgi, S. S. (2006). Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. *Journal of Medical Microbioliology* 55 (7): 897 - 903.

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

Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J. and Skjerve, E. (2011). Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. *Tropical Animal Health and Production* 43(3): 651- 656.

Mellau, L., Kuya, S. and Wambura, P. (2009). Seroprevalence of brucellosis in domestic ruminants in livestock- wildlife interface: A case study of Ngorongoro Conservation Area, Arusha, Tanzania. *Tanzania Veterinary Journal* 26(1): 44-50.

Morta, P., Queipo-Ortuno, J. M., Reguera, M. J., Garcia-Ordonez, A. M., Cardenas, A. and Colmenero, D. J. (2003). Development and evaluation of a PCR-ELISA assay for diagnosis of human brucellosis. *Journal of Clinical Microbiology* 41: 144-148.

Mtui- Malamsha, N. (2001). Epidemiology study of brucellosis in humans and animals in Babati and Hanang district of Tanzania. Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania. 51pp.

Mugabi, R. (2012). Brucellosis epidemiology, virulence factors, control and molecular targets to prevent bacterial infectious diseases.North Dakota State University of Agriculture and Applied Science. pp. 10-12.

Naing, L., Winn, T. and Rusli, B. N. (2006). Sample Size Calculator for Prevalence Studies. [http://www.kck.usm.my/ppsg/stats_resources.htm] site visited on 24/7/2016.

Nanyende, D. (2010). Estimation of the prevalence of brucellosis in humans and livestock in northern Turkana District, Kenya. University Of Nairobi. 53pp.

Nasinyama, G., Ssekawojwa, E., Opuda, J., Grimaud, P., Etter, E. and Bellinguez, A. (2014). *Brucella* sero-prevalence and modifiable risk factors among predisposed cattle keepers and consumers of un-pasteurized milk in Mbarara and Kampala districts, Uganda. *African Health Science* 14(4): 790-796.

NBS, (2013). 2012 Population and housing census: Population Distribution by Administrative Areas. [http://ihi.eprints.org/1344/] site visited on 4/4/2017.

Nicolettie, P. (1980). The epidemiology of bovine brucellosis. In: Brady, C.A., and Conelius, C.A., (eds.), Advance in veterinary Science and Comparative Medicine. New York, Academic Press Inc. pp. 69-98.

Nielson, K. and Dunkan, R. (1990). Animal brucellosis: In Bovine brucellosis. Manual of standards for diagnostic tests and vaccines. 3rd edition. CRC. Pres Inc., Florida, USA. pp. 252-265.

Ogugua, A. J., Akinseye, V. O., Ayoola, M. C., Oyesola, O. O., Shima, F. K., Tijjani, A. O. and Moriyon, I. (2014). Seroprevalence and risk factors of brucellosis in goats in selected states in Nigeria and the public health implications, *African journal of medicine and medical sciences* 43(1): 121-129.

OIE, (2008). Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). (1) 6th ed. pp. 3-34.

Omer, K., Skjerve, E., Holstad, G., Woldehiwet, Z. and MacMillan, P. (2000). Prevalence of antibodies to *Brucella* spp. in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry systems. *Epidemiolology and Infection* 125: 447-453.

Otte, M. and Gumm, I. (1997). Intra-cluster correlation coefficients of 20 infections calculated from the results of cluster-sample surveys. *Preventive Veterinary Medicine* 31: 147-150.

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

Pollot, G. and Wilson, T. (2009). Sheep and goats for diverse products and profits. FAO. Rome. [http://www.drcsc.org/VET/library/Animal/Bk--Sheep%20and%2 0Goads%20Rearing.pdf.] Site visited on 30/4/2017.

Raddostits, O., Gay, C., Blood, D. and Hinchcliff, K. (2000). Veterinary medicine;A textbook of the diseases of cattle, sheep,pigs,goats and horses. 9th ed.Saunders Ltd., Edinburgh, UK. pp. 867-881.

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.

Rajala, E. (2016). *Brucella* in Tajikistan - Zoonotic risks of urbanized livestock in a low-income country.Swedish University of Agricultural Sciences. pp. 39-40.

Rubegwa, B. (2015). Seroprevalence and risk factors of bovine brucellosis in dairy and traditional cattle herds in Kibaha district of Tanzania. The University of Zambia. pp. 27-29.

Safari, J., Mtenga, L. A., Eik, L. O., Sundstøl, F. and Johnsen, F. H. (2008). Analysis of three goat production systems and their contribution to food security in semiarid areas of Morogoro, Tanzania. *Livestock Research for Rural Development*. 20:74. [http://www.lrrd.org/lrrd20/5/safa20074.htm] Site visited on 20/6/2017.

Sharifi, H., Mashayekhi, K. and Tavakoli, M. M. (2015). Risk facts of small ruminant brucellosis: a crosssectional study in Southeast Iran 2012. Human and Veterinary Medicine. *International Journal of the Bioflux Society*. 7(1): 42-45.

Shirima, G. M. (2005). The epidemiology of brucellosis in animals and humans in Arusha and Manyara regions in Tanzania. Thesis for Award of PhD Degree at University of Glasgow, UK. pp. 4-25.

Shirima, G. M., Cleaveland, S., Kazwala, R. R., Kambarage, D. M., Nigel, F., McMillan, A., Kunda, J., Mfinanga, G, S. and Fitzpatrick, J. S. (2007). Sero-prevalence of brucellosis in smallholder dairy, agropastoral, pastoral, beef ranch and wildlife animals in Tanzania. *Bulletin of Animal Health and Production in Africa* 55: 13–21.

Shirima , G. M., Fitzpatrick, J., Kunda, J. S., Mfinanga, G. S., Kazwala, R. R., Kambarage, D. M. and Cleaveland, S. (2010). The role of livestock keeping in human brucellosis trends in livestock keeping communities in Tanzania. Short communication. *Tanzania Journal of Health Research*. 12(3). [http://www.bioline.org.br/pdf?th 10027] Site visited on 3/6/2017.

Shirima, G. M. and Kunda, J. S. (2016). Prevalence of brucellosis in the human, livestock and wildlife interface areas of Serengeti National Park, Tanzania, *Onderstepoort Journal of Veterinary Research* 83(1): 2219-0635.

Smith, T., Godfrey, S. H., Butter, Y. P. J. and Owen, E., (Eds.). (2002). Helping Smallstock Keepers Enhance their Livelihoods: Improving Management of Smallholder Owned Sheep and Goats by Utilising Local Resources: Proceedings of the Second DFID Livestock Production Programme Link Project (R7798) Workshop for Smallstock Keepers. Sokoine University of Agriculture, Morogoro, Tanzania. 8-10 January 2002. pp. 5-12.

Smits, H. L. and Cutler, S. J. (2004). Contributions of biotechnology to the control and prevention of brucellosis in Africa. *African Journal of Biotechnology* 12(3): 631-636.

Swai, E. S. and Schoonman, L. (2010). The Use of Rose Bengal Plate Test to Asses Cattle Exposure to *Brucella* Infection in Traditional and Smallholder Dairy Production Systems of Tanga Region of Tanzania. *Veterinary Medicine international* 837950 : 8.

Temba, P. B. (2012). Seroprevalence of *Brucella* species infection and associated risk factors in Wildlife-livestock interface. A case study of Mikumi-Selous Ecosystem, M.Sc. Dissertation. Tanzania: Sokoine University of Agriculture. 130pp.

Tsegay, A., Tuli, G., Kassa, T. and Kebede, N. (2017). Seroprevalence and risk factors of brucellosis in abattoir workers at Debre Zeit and Modjo export abattoir, Central Ethiopia. *Biomedical Central Infectious Diseases* 17: 101-108.

Tumwine, G., Matovu, E., Kabasa, J., Owiny, D. and Majalija, S. (2015).Human brucellosis: sero-prevalence and associated risk factors in agro-pastoral communities of Kiboga District, Central Uganda. *Biomedical Central Public Health* 15: 900-907.

Wareth, G., Hikal, A., Refai, M., Melzer, F., Roesler, U. and Neubauer, H. (2014). Animal brucellosis in Egypt. *The Journal of Infection in developing countries* 8(11): 1365-1373.

Wareth, G., Melzer, F., Tomaso, H., Roesler, U. and Neubauer, H. (2015). Detection of *Brucella abortus* DNA in aborted goats and sheep in Egypt by real‐ time PCR. *Biomedical Central Research Notes*.8: 212-216.

WHO (2004). Laboratory biosafety manual. [www.who.int/csr/resources/ publications/biosafety/ Biosafety7.pdf] site visited on 15/2/2017.

WHO (2006). Brucellosis in Humans and Animals. [www.who.int/csr/resources/ publications/Brucellosis.pdf] site visited on 20/6/2017.

Xavier, M., Tatiane, A., Paixão, T., Hartigh, A., Renée, M., Tsolis, R. and Santos, R. (2010). Pathogenesis of *Brucella* spp. *The Open Veterinary Science Journal* 4:109-118.

APPENDICES

Appendix 1: Questionnaire

Interviewers guide

1. Brief introduction of the Interviewer:
2. The aim of this study is to gather information on the occurrence and knowledge of Brucellosis infections in goats and sheep and husbandry practices surrounding small ruminants farming. Data will be collected from various selected animal owners for the understanding of the disease status and risk factors for better prevention strategies. The information collected will be kept confidential and names will not be revealed. The interviewee is not obliged to answer any question that is not comfortable with and can withdraw at any time. The community will benefit with this study through a feedback that will be sent to its local government officials that will be for future prevention and control strategies.
3. A brief explanation of how the interview will be conducted, how long it will take.
4. Explanation on the step by step conduction of the interview:
    i. Acquiring permission from the interviewee.
    ii. The purpose, the rights, advantages and privacy should be mad clear to the interviewee
    iii. Answers should be filled as indicated. Circle and write on the dotted lines where applicable.

Brucellosis status in goat herds

Date of the interview …… *(*dd/mm/yyyy)

Name of the interviewer ……..

I. Household information

Name of the herd/farm owner …….

Owner's phone number: ……

Age …….

Sex……...

a) Female

b) Male

Occupation…….

District……. .

Ward……..

Village……

Street /Hamlet…….

II. Herd information

1. Do you keep goats?(Circle correct answer)

    a) Yes

    b) No

2. For how long have you been keeping goats? .....

3. Why do you keep goats? (circle all correct answers)

    a) Home consumption

    b) Business

    c) Other purposes (specify)…….……

4. Breed of goats kept(choose all correct answers)
    a) Local
    b) Exotic
    c) Cross-breeds
5. How many of each of the following categories of goats do you have?
    a) Adults ……..
    b) Weaners ……..
    c) Kids ……
6. What type of farming system are you using?
    a) Intensive farming system
    b) Semi-intensive farming system
    c) Free range farming system
7. If the answer above is either (b) or (c) Where do you graze your herd?
    a) Communal grazing land
    b) Private grazing land
    c) Random grazing areas
8. Which water source do you use for your herd?
    a) Well
    b) Bore-hole
    c) River
    d) Tap
    e) Constructed dams
    f) Other: ……..

9. Are there herds of goats/sheep/cattle nearby?
    a) Yes(specify)…….
    b) No
10. What type of service do you animals receive?
    a) Natural service
    b) Artificial insemination
    c) Both methods
11. What type of parturition do the dams go through?
    a) Natural
    b) Assisted
12. Has your herd experienced abortion cases?
    a) Yes
    b) No
13. Has your herd experienced still birth?
    a) Yes
    b) No
14. Has your herd experienced weak kids?
    a) Yes
    b) No
15. How often has the cases in (12) above been occurring?
    a) Always
    b) Almost always
    c) Sometimes
    d) Rarely
    e) Never
    f) Cannot remember, do not know

16. How often has the cases in (13) above been occurring?

    a) Always
    b) Almost always
    c) Sometimes
    d) Rarely
    e) Never
    f) Cannot remember, do not know

17. How often has the cases in (14) above been occurring?

    a) Always
    b) Almost always
    c) Sometimes
    d) Rarely
    e) Never
    f) Cannot remember, do not know

18. At what age do the cases occur?

    a) Early term (less than 4 months)
    b) Late term (greater than 4 months)
    c) I do not know

19. Has the herd experienced cases of retained fetal membranes?

    a) Yes
    b) No

20. Do you handle fetal membranes?

    a) Yes
    b) No

21. How are the fetal membranes handled? …….

22. Do you trade animals with other herds?

    a) Yes

    b) No

23. How is the herd replacement done? .....

24. Do you treat your animals when they get sick?

    a) Yes

    b) No

25. What are the common livestock diseases in your area?

    i. ……..
    ii. ……..
    iii. ……..
    iv. ……..
    v. ……..

i. Information on animal products consumption.

26. How often do you slaughter goats?

    a) At least once a month

    b) More than once a month

    c) None in a year

27. For what purpose do you slaughter goats?

    a) Household consumption

    b) Commercial purpose

    c) Ritual purpose

    d) Others (Specify)……..

28. What kind of animal products do you consume?

a) Milk

b) Meat

c) Blood

d) Others (Specify)…….

29. In which form are the products consumed?

a) Raw

b) Locally processed

c) Industrial processed

d) Others (Specify)…….

ii. Information on brucellosis

30. Have you heard about a disease known as **Brucellosis (local name)**?

a) Yes

b) No

31. Do you have an idea of how livestock acquire the disease?

a) I don't know

b) Suckling of contaminated milk

c) Mating

d) Drinking contaminated water

e) By coming in contact with aborted fetal membranes

f) Others (Specify) ……

32. Do you have an idea of how humans acquire the infection?

a) I don't know

b) By drinking raw milk

c) By eating raw meat

d) By drinking contaminated water

e) By handling aborted fetal membrane

f) Others (Specify)……

33. Have you experienced the disease in your herd?

a) Yes

b) No

34. What were the symptoms seen? .....

How did you confirm that it was brucellosis (local name)?

35. How have you been handling the case?

a) Seek veterinary assistance

b) Local treatments

c) Others (specify) …….

iii. Human brucellosis

36. Have you visited the hospital in the past six month?

a) Yes

b) No

37. If yes, what were the symptoms?

a) Undulant fever (1) Yes (2) No

b) Joints and back pain (1) Yes (2) No

c) Chills (1) Yes (2) No

d) Headaches (1) Yes (2) No

e) General body weakness (1) Yes (2) No

f) Weight loss (1) Yes (2) No

g) Constipation (1) Yes (2) No

h) Dry cough (1) Yes (2)

i) Others (Specify)……

38. Have you ever consumed raw milk?

a) Yes

b) No

39. If yes in (33) above how long ago did you consume raw milk?

a) Last week

b) Last month

c) Last six months

d) Last year

e) Others (Specify)……..

40. Have you ever consumed raw meat?

1. Yes
2. No

41. If yes in (35) above how long ago did consume raw meat?

a) Last week

b) Last month

c) Last six months

d) Last year

e) Others (Specify)……..

THIS IS THE END OF THE QUESTIONNAIRE

THANK YOU SO MUCH FOR YOUR COOPERATION

Appendix 2: **Prevalence of brucellosis in animals in some of African countries.**

S/N	Country	Area	Animal species	Test	Prevalence	Author and year
1	Tanzania	Ngorongoro conservation area	Cows and does	RBPT and MAT	14.2% and 11.9%	Mellau *et al*., 2009
		Serengeti	Buffaloes and wildebeest	RBPT and cELISA	24% and 17%	Fyumagwa *et al*., 2009
		Arusha and Manyara	Cattle and sheep		4.9% and 6.5%	Shirima *et al*., 2010
		Babati and Hanang	Goats and Catle	SAT and RBPT	4.3% and 5.7%	Mtui-Malamsha, 2001
		Kibondo and Kakonko	Cattle	RBPT andcELISA	5.6%	Chitupila *et al*., 2015
		Iringa	Cattle	RBPT and SAT	1.5-17.9%	Karimuribo *et al*., 2007
		Tanga	Cattle	RBPT and SAT	0.6-3.6%	Karimuribo *et al*., 2007
		Katavi-Rukwa	Goats	RBPT, cELISA and PCR	1.6%	Assenga *et al*., 2015
		Kibaha	Cattle	RBPT and cELISA		
		Arusha and Manyara	Goats	RBPT and cELISA	6%	Shirima, 2005
2	Kenya	Baringo county	Cattle	RBPT and cELISA	9.2%	Philemon, 2016
		Northern Turkana	Cattle and Goats	RBPT and cELISA	11% and 13%	Nanyende, 2010
3	Ethiopia	North-East	Goats	mRBPT and CFT	13.6%	Adugna *et al*., 2013
		South-East	Cattle	RBPT and CFT	3.5%	Megersa *et al*., 2011
		Addis Ababa	Cattle	RBPT, SAT and CFT	10%	Kassahun, 2003
		Borana	Cattle and	RBPT and	10.6% and 1.9%	Megersa *et al*., 2011

			goats	CFT		
4	Nigeria	Oyo, Benue, Borno and Sokoto states	Goats	RBPT and cELISA	2.8%	Ogugua *et al*., 2014
		Plateau state	Goats	RBPT and SAT	16.1%	Bertu *et al*., 2010
5	Eritrea	Asmara, southern highlands, western and Eastern lowlands	Cattle	RBPT and CFT	8.2%	Omer *et al*., 2000