EPIDEMIOLOGY OF BRUCELLA INFECTION IN CATTLE IN URBAN AND PERI-URBAN AREAS OF SUMBAWANGA MUNICIPALITY, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN TROPICAL ANIMAL PRODUCTION OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2017

ABSTRACT

A cross sectional study was conducted to determine seroprevalence of *Brucella* infection in cattle in urban and peri-urban areas of Sumbawanga Municipality. All 19 wards of the Municipality were involved in the study where 13 villages and 26 neighbourhoods (*mitaa*) were randomly selected. To identify the potential risk factors associated with spread and transmission of the disease, questionnaires were administered to the heads of randomly selected 108 households. Blood samples were also collected from 354 cattle of all breeds available in the study area and screened for *Brucella* antibodies by Rose Bengal Plate Test (RBPT). Results showed that 5 (1.4%) of the serum samples were positive. Confirmatory test of the RBPT positive sera was done using competitive Enzyme Linked Immunosorbent Assay (c-ELISA). Only, 0.8% (95% CI: 0.2-2.7 %) of the serum samples were c-ELISA positive and herd level seroprevalence was 2.8% (95% CI: 0.6-7.9 %). Analysis of potential risk factors related with the occurrence of *Brucella* antibody seropositivity in the study area showed no statistical significant relationship between any predictor variables and c-ELISA seropositivity. However, this study gives evidence that brucellosis is prevalent in Sumbawanga Municipality at much lower rate than the reported range (1-30%) in Tanzania. The evidence obtained in this study should be used for development of policy and control strategies to institute appropriate prevention, control and eradication measures of the disease; and carry out more epidemiological studies so as to characterize the *Brucella* organisms prevalent in the study area.

DECLARATION

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

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ACKNOWLEDGEMENTS

Foremost thanks to the Almighty God for his blessings, goodness and mercy throughout the period of my work. I express my sincere thanks to all people who in one way or another have contributed to the success to this research work (Regional Administrative Secretary for Rukwa Region, Regional Medical Officer, Sumbawanga Municipal Director, Zonal Veterinary Center Sumbawanga Station, Municipal Livestock Officer and Ward Executive Officers).

My sincere thanks are extended to Prof. Lusato R. Kurwijila and Prof. Rudovick R. Kazwala, my supervisors and mentors, for giving me the light to realize this dissertation, for their constructive critiques, excellence inspiration and suggestions all along the way and for opening the world of professional and scientific studies to me.

Special thanks to Prof. Kimera S. I., Dr. Komba E. V. G., Dr. Mkupasi, E. M., Dr. Assenga, J., Mr. Reshola P. and Mr. Lawi S. who provided advice that has contributed to the successful completion of this work. May the Almighty Lord bless them.

Field work during sample collection could never have been successful without the full participation of the ward Executive officers (WEOS), Livestock field officers, livestock keepers and livestock attendants of the nineteen wards where I collected sociological data and blood samples for this study. My sincere thanks are extended to all of them.

My parents, your prayers and success wishes are highly appreciated since you are after God to me in this world. Dear Lord may you grant them with wisdom. It has not been possible to find space to mention all people, I extend my sincere thanks. To you all, I say "*asanteni sana".*

DEDICATION

This work is dedicated to my lovely wife Lulu Respich Maengo, all stakeholders in this study and my son and daughter Richard Respich Maengo and Vanessa Respich Maengo.

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LIST OF ABBREVIATIONS, SYMBOLS AND ACRONYMS

˚C	Degree Celsius
µl	Microliter
AHMP	Animal Health Movement Permit
AHVLA	Animal Health and Veterinary Laboratory Agency
AI	Artificial Insemination
BAPA	Buffered Acidified Plate test
CDC	Centre for Disease Control
c-ELISA	competitive Enzyme Linked Immunosorbent Assay
CFT	Compliment Fixation Test
CI	Confidence Interval
DB	Dairy breed
DCB	Dairy cross Breed
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
Fig.	Figure
FVM	Faculty of Veterinary Medicine
GPS	Global Positioning System
HPA	Health Protection Agency Centre for Infections
i-ELISA	indirect Enzyme Linked Immunosorbent Assay
IgA	Immunoglobulin A
IgG_1	Immunoglobulin G_1
IgG_2	Immunoglobulin G_2
IgM	Immunoglobulin M
km^2	Kilometer Square
LPS	Lipopolysaccharide

LZ	Local Zebu
mls	millilitres
MRT	Milk Ring Test
OD	Optical Density
OIE	Office International des Epizooties
O-PS	O-polysaccharide
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PPS	Probability proportional to size
RB51	*Brucella abortus* rough strain
RBPT	Rose Bengal Plate test
REA	Restriction Endonuclease Analysis
R-LPS	Rough-lipopolysaccharide
RVF	Rift valley fever
S19	Strain 19
SAT	Serum Agglutination Test
SE	Standard error
S-LPS	Smooth-lipopolysacchride
SPSS	Statistical Package for Social Science
SUA	Sokoine University of Agriculture
TSHZ	Tanzania Short Horn Zebu
UK	United Kingdom
USA	United State of America
UTM	Universal Transversal Mercator
VLA	Veterinary Laboratory Agency
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Tanzania is the third in Africa for having large population of livestock whereby cattle are predominant with an estimated population of 25 million followed by goats 16.7 million and sheep 8 million (Ministry of Livestock and Fisheries Development, 2016). Unlike the developed countries, the livestock industry in most African countries is not yet fully developed (USAID, 2004). Therefore, livestock products for example much of milk from the traditional sector is sold or consumed raw after natural fermentation (Karimuribo *et al*., 2007; Kilango *et al*., 2012; Gillah *et al.*, 2013). This poses a big risk of food borne diseases to humans such as brucellosis, tuberculosis and leptospirosis (Shija, 2013).

Brucellosis is one of the most common widespread zoonotic diseases globally, caused by several species of bacteria of the genus *Brucella* (Hegazy *et al*., 2011; Assenga *et al*., 2015). The listed high risk areas for the disease are the Mediterranean Basin (Portugal, Spain, Southern France, Italy, Greece, Turkey and North Africa), South and Central America, Eastern Europe, Asia, Africa, the Caribbean, and the Middle East (Kunda *et al*., 2005). It is a contagious infectious disease affecting various species of domestic animals, wildlife, marine mammals and humans (Galinska *et al*., 2013; Chitupila *et al*., 2015; Assenga *et al*., 2015). *Brucella abortus, B. melitensis* and *B. suis* are the species that have the highest impact on domestic livestock productivity and human health (Godfroid *et al*., 2011). Although these three species preferentially infect cattle, small ruminants and swine, respectively, cross-infections may be significant where mixed husbandry systems are practised or at the livestock-wildlife interface and some of them have zoonotic potential (Lopes *et al*., 2010; Godfroid *et al*., 2013). Goat for example, is the preferred host for

B. melitensis which is the most pathogenic *Brucella* species to humans (Nicoletti, 2010). *Brucella melitensis* is particularly common in the Mediterranean countries. It contains three biovars (biovars 1, 2 and 3) whereby, biovar 3 is predominant in the Mediteranean countries and the Middle East while, biovar 1 is predominat in Central America. This organism has been reported from Africa, India and Mexico in Northern America. Marine *Brucella* spp. (*B, pinnipedialis and B. ceti*) pose a zoonotic risk to human therefore, stranded marine mammals or their meat should be handled with caution (Matope, 2009).

In female animals the disease is characterized by abortion in the late pregnancy or birth of weak newborn, retained placenta, endometritis, infertility and reduced milk production. In males it causes orchitis and epididimitis with frequent sterility (Karimuribo *et al*., 2007; Jergefa *et al*., 2009). It therefore causes big economic losses to livestock farmers and the nation at large, lowers calving rate, abortion, reduced milk production and cost of replacement animals (Holt *et al*., 2011; Egaru *et al*., 2013; Chitupila *et al*., 2015).

Infection in animals may occur through ingestion of contaminated pastures, feedstuffs and water as well as licking infected placentae, foetus or uterine discharges from infected animals soon after abortion or delivery (Matope, 2009). Newborns may get infected through consumption of colostrums and milk from an infected dam. Transmission by natural mating in domestic ruminants is uncommon except where artificial insemination is practiced. Infected animals shed pathogens in the uterine discharges after abortion and subsequent parturition, also in the colostrums and milk (FAO, 2003; Matope, 2009). *Brucella* organisms have prolonged survival in both hot and cold environment particularly in moist conditions where they can survive for up to two years thereby putting animals and humans at risk (Lyimo, 2013). Pasture and animal houses can remain contaminated for several months. Survival of the organisms in manure, uterine discharges and milk can be

up to six months and eight months in aborted foetus in the shade (James, 2013). Cooking and pasteurization destroys *Brucella* (HPA, 2009).

Brucellosis has been eradicated in domestic animals in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand (Shirima, 2005) but has remained endemic in wildlife populations in some developed countries as evidenced by the presence of *Brucella* antibodies in American bison, wild boar and reindeer in France, Italy, Canada and Switzerland (Shirima, 2005). According to Rhyan *et al*. (2013), bovine brucellosis has been nearly eliminated from livestock in the United States but bison and elk in the Greater Yellowstone Area remain reservoirs for the disease.

The disease remains endemic in Africa, Asia, Middle East and Latin America due to lack of effective domestic animal health programs and appropriate diagnostic facilities both for livestock and humans as well as limited public awareness (WHO, 2006; John *et al*., 2010).

First laboratory confirmation of brucellosis in Tanzania was in 1928 and is currently considered endemic in most parts of Tanzania with varying prevalence (Karimuribo *et al*., 2007; Swai and Schoonman, 2010; Chitupila *et al*., 2015). Considerable number of studies on brucellosis carried out, had confirmed Tanzania to be among the countries with animal and human cases of brucellosis (James, 2013; Chota *et al*., 2016).

1.2 Problem Statement and Justification of the Study

Brucellosis is widespread throughout Tanzania and is a threat to food security, public health and causes big economic loses to livestock keepers and nation at large. Previous studies in other parts of Tanzania have demonstrated the occurrence of the disease in cattle with individual animal level seroprevalence of 1-30% in different management systems,

regions and zones (Karimuribo, 2007; Lyimo, 2013; Assenga *et al*., 2015). A recent study in Katavi-Rukwa ecosystem which is a major corridor for movement of livestock from northern regions to Rukwa indicated animal level seroprevalence of 6.8% in cattle (Assenga *et al*., 2015). There were huge influx of cattle from *Brucella* infected regions of Lake zone and Tabora where brucellosis seroprevalence ranged 2-22.5% (Kitaly, 1984) to Rukwa region, finally to Sumbawanga Municipality following the long dry season in the year 1974/75 onwards (Msanga *et al*., 2012; Rukwa Regional Commissioners' office, 2014). With all these, there has not been any research or confirmed report on the status of brucellosis in the study area. The extent of farmer's awareness, attitude and practices regarding brucellosis is not known. There is therefore a need to bridge the existing knowledge gap. The information obtained in this study will contribute important knowledge that may be used by the District and Regional Authorities to make decisions and develop strategies for prevention and control of brucellosis infection to both livestock and humans.

1.3 Objective

1.3.1 Overall objective

Investigation of epidemiology of *Brucella* infection in cattle population in urban and peri-urban areas of Sumbawanga Municipality.

1.3.2 Specific objectives

i) To determine the seroprevalence of *Brucella* infection in cattle in urban and peri-urban areas of Sumbawanga Municipality.

ii) To determine the association between potential risk factors and the prevalence of bovine brucellosis at herd level in urban and peri-urban areas of Sumbawanga Municipality.

1.4 Research Questions

i. What is the prevalence level of brucellosis in cattle population of Sumbawanga Municipality?

ii. What are the factors or practices associated with transmission and spread of brucellosis between and within herds of cattle in Sumbawanga Municipality?

1.5 Hypothesis

i) Null Hypothesis: Cattle population of Sumbawanga Municipality is free from *Brucella* infection.

ii) Alternative Hypothesis: Cattle population of Sumbawanga Municipality is *Brucella* infected.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and Nomenclature

Brucellosis had been given different names depending on whether it was humans or animal cases, geographical location of the incidence, mode of transmission, magnitude of outbreak or the person who had described the disease. Therefore, it has been commonly known as enzootic abortion or bovine contagious infection, epizootic abortion, infectious abortion, contagious abortion, slinking of claves, Bang's disease, and ram epididymitis (Tun, 2007). The disease is also named as "Mediterranean fever," "Malta fever," and "Undulant fever" in case of humans. It was then described in Denmark in cattle by Bang in 1897 (Lyimo, 2013). Brucellosis was first diagnosed in human by British scientist Sir David Bruce in 1887 when he isolated a causative organism from fatal cases and named it *Micrococcus melitensis* (Shirima, 2005). Alice Evans changed the genus and named it *Brucella* in honour of Sir David Bruce (Shirima, 2005).

2.2 Definition of the Disease

Brucellosis is an infectious, contagious zoonotic disease caused by bacteria of the genus *Brucella* (Tun, 2007). Infection is almost invariably transmitted by direct or indirect contact with infected animals or their products (WHO, 2006). It is considered as one of the most common global zoonoses (McDermott *et al*., 2013). According to OIE, brucellosis is the second most important zoonotic disease in the world after rabies (Abubakar *et al*., 2012). In animals, the disease primarily affects cattle, sheep, goats, swine and dogs and is characterized by abortion or infertility and also affects people and other animal species (Tun, 2007).

The disease causes severe illness in humans and economic losses in livestock (McDermott *et al*., 2013; Assenga *et al*., 2015). In humans, brucellosis is often easily misdiagnosed as other febrile syndromes such as malaria and typhoid fever, thereby resulting in mistreatments and underreporting (Adesokan *et al.*, 2013).

Brucellosis is an occupational disease affecting farmers, veterinarians, milkers, hunters and workers in meat industry and laboratories (Krausman and Cain, 2013; Adesokan *et al*., 2013). Consumption of raw milk and milk products from infected animals, working with livestock and livestock products are the main risk factors for the disease to humans (Karimuribo *et al*., 2007; Dean *et al*., 2012). Furthermore, lack of awareness of the disease to the livestock products consumers and farmers, unhygienic husbandry practices such as disposal of placenta and aborted materials are important risk factors influencing the transmission of the disease. Awareness of the risk factors has been helpful for policy makers to develop control strategies.

2.3 Aetiology of Brucellosis in Cattle

The disease in cattle is usually caused by *Brucella abortus* and less frequently by *B. melitensis* where cattle are kept together with infected sheep or goat (OIE, 2009). Occasionally, *B. suis* may cause a chronic infection in the mammary gland of cattle, but it has not been reported to cause abortion (Karimuribo *et al*., 2007; Lopes *et al*., 2010). According to Matope (2009), majority of cases of brucellosis in cattle worldwide are attributed to *B. abortus* biovar 1, while *B. abortus* biotype 2 has a worldwide distribution but considered less frequent than biotype 1.

2.4 Morphology and Characteristics of *Brucella*

Brucellae is a homogeneous group of small, non-motile, non-spore forming, non-encapsulated, gram-negative coccobacilli with straight or slightly convex sides and rounded ends, and facultatively intracellular bacteria, belonging to the α-2 subdivision of

the Proteobacteria (Chitupila *et al*., 2015; Mathew *et al*., 2015). They are aerobic bacteria, therefore no growth under strictly anaerobic conditions (Tun, 2007). *Brucella abortus, B. melitensis, B. suis* and *B. neotomae* may occur as either smooth or rough strains expressing smooth lipopolysaccharide (S-LPS) or rough-lipopolysaccharide (R-LPS) as major surface antigens, while *B. ovis* and *B. canis* are naturally rough strains (Shirima, 2005).

Currently genus *Brucella* has been found to include ten species (Galinska, 2013; Mugizi *et al*., 2015), named basing on their preferred natural host species: six classic species are (*B. abortus* biovar 1- 6 and 9); isolated from cattle and buffalos, (*B. melitensis* biovar 1 - 3); mainly isolated from goats and to a lesser extent sheep, (*B. suis* biovar 1 - 3); isolated from pigs, biovar 4 from reindeer and biovar 5 from small ruminants, (*B. canis*); isolated from dogs, (*B. ovis*); from sheep, (*B. neotomae*); isolated from desert wood rats. The recently described four species are two of marine origin (*B. ceti*); isolated from cetaceans (whales and dolphins) and (*B. pinnipedialis*); isolated from pinned marine mammals seal (Matope, 2009), others are (*B. microti*); had been isolated from a common vole *Microtus arvalis* (Scholz *et al*., 2008) finaly, *B. inopinata* which was isolated from a breast implant wound of a female patient (Galinska, 2013). However, *Brucella* is not species specific, therefore, individuals can be infected by more than one species although each has a preferred natural host (Azimi, 2012).

2.5 Epidemiology of Brucellosis in Animals

2.5.1 Distribution and prevalence of brucellosis in livestock

Brucellosis is widespread zoonoses in most parts of the world (Assenga *et al*., 2015). It is widespread in African countries although with varying prevalence (Karimuribo *et al*., 2007). Sampling techniques and differences in the sensitivity and specificity of the test used may be the cause of variation in prevalence. Other reasons of variation include location of farms as defined by ecology and management practices, distribution of animals

in the study area whether they were closely populated or rural animal population farms (Swai *et al.*, 2005). The disease is endemic in sub-Saharan Africa including Tanzania. The report of cross-sectional studies on prevalence of brucellosis in cattle in some African countries under different management systems is as shown in Table 1.

Table 1: Prevalence of antibodies to *Brucella* species in cattle in some African countries under different management systems

Country	Management system	Type of Test	Animal level Prevalence (%)	References
Zimbabwe	Semi-intensive	c-ELISA	3.6 - 12.6	(Matope, 2009)
Uganda	Semi-intensive	RBPT	14	(Miller *et al*., 2016)
		MRT	29	
Kenya	Extensive	ELISA	15	(Kadohira *et al*., 1997)
Sudan	Semi-intensive	c-ELISA	23.8	(Zein and Adris, 2015)
Kuwait	Semi-intensive	RBPT	7.1	(El-Gohary *et al*., 2016)
		BAPAT	7.25	
		CFT	7.04	
Ghana	Extensive	RBPT	6.6	(Kubuafor *et al*., 2000)
Ethiopia	Extensive	CFT	3.19	(Berhe *et al*.,2007)
Togo	Extensive	c-ELISA	7.3 – 9.2	(Dean *et al*., 2013)
Zambia	Semi-intensive	c-ELISA	6.0	(Muma *et al*., 2012)

Cross-sectional studies carried out in various regions and zones of Tanzania have shown the prevalence of the disease in cattle at varying levels in different production systems, the highest being in the Northern zone as shown in Table 2.

Table 2: Animal level prevalence of brucellosis in some study areas in Tanzania

Study area	Production system	Prevalence (%)	Serological Test used	References
Moshi	Intensive	12.2	SAT	(Swai *et al*., 2005)
District Arusha and Manyara	Extensive	5.7	c-ELISA	(Shirima, 2010)
Kibondo and	Extensive	9.4	c-ELISA	(Chitupila *et al*., 2015)
Kakonko	Extensive	1.9	c-ELISA	(Chitupila *et al*., 2015)
Lugoba	Extensive	12.3	SAT	(Weinhaupl *et al*., 2000)
Morogoro Municipality	Intensive	29.3 18.4	MRT c-ELISA	(Lyimo, 2013)
Tanga Municipality	Intensive extensive	10.5 20*	RBPT RBPT	(Swai and Schooman, 2010)
Iringa (small holder)	Intensive	0.6	RBPT	(Karimuribo *et al*., 2007)
Dar es salaam (20 Dairy farms)	Intensive	14.1	SAT	(Weinhaupl *et al*., 2000)
Rukwa-Katavi Ecosystem	Extensive	6.8	c-ELISA	(Assenga *et al.*, 2015)

* herd level seroprevalence

2.5.2 Source of infection and transmission of brucellosis

2.5.2.1 Source of new infection to the herds

Infection gets into herds either through introduction of infected animals from other herds (James, 2013) or sharing grazing grounds and water sources with animals from infected herds or wildlife.

2.5.2.2 Transmission of brucellosis in cattle

Animals of all age groups are susceptible to brucellosis but persists more in sexually mature animals (Matope, 2009). Horizontal transmission in cattle occurs as a result of ingestion of *Brucella* organisms in pastures, feedstuffs and water, licking infected placentae, foeti or uterine discharges from infected animals (Matope, 2009; Assenga *et al*.,

2015). *Brucellae* are also capable of penetrating the mucosa or skin of the throat, nose, conjunctiva, urogenital tract, and teat canal (Bishop *et al*., 1994; Shirima, 2005). Transmission by coitus is unlikely or uncommon, however, transmission through artificial insemination have been reported (Shirima, 2005; Matope, 2009). This is when *Brucella* infected semen is deposited in the uterus (Norman *et al*., 1998; Lyimo, 2013). Anatomically the epithelial lining of the uterus differs from that of vagina since, the mucosa of vagina is multi-layered therefore, seems to protect against infection following natural service while, uterine epithelium is more susceptible to bacterial infection and has cellular mechanisms for bacterial uptake that are absent in vaginal epithelium (Norman *et al*., 1998). Vertical transmission was proved by Plommet, who states that between 60 and 70% of the foetuses born to infected mothers carry the infection in pregnancy (Aparicio, 2013).

According to Makita *et al*. (2011), chronically infected cattle can shed lower numbers of organisms via milk and reproductive tract discharges, and can also vertically transmit infection to subsequently born calves, thereby maintaining disease transmission. Female calves can also be infected during birth when passing through the birth canal, or by suckling colostrums or milk from infected cows. However, most of these calves rid themselves of *Brucella*, but small percentage may continue to be infected until adulthood, remaining negative to diagnostic serological tests but aborting during their first pregnancy (Aparicio, 2013).

2.5.2.3 Transmission of brucellosis in humans

Brucellosis can be transmitted to humans through consumption of unpasteurized milk, undercooked or fresh meat and blood from infected animals and handling of aborted materials and live foetuses without using protective gear (Karimuribo *et al*., 2007). Transmission by contact is more likely to affect occupational groups such as farmers,

veterinarians, laboratory workers, butchers, hunters, milkers and inseminators (Matope, 2009; Lyimo, 2013) through broken skin, the conjunctiva or other membranes and inhalation of aerosols containing pathogens (James, 2013). The risk of transmission of brucellosis to humans and other animals can definitely be greatly diminished by diagnosis and control of infection in animals (Shirima *et al*., 2014).

2.5.3 Pathogenesis of Brucella infection in animals

Ingestion is the normal route of infection through contaminated pasture, feed and water, licking aborted foetus, infected placentas, and uterine discharges. Fully virulent *Brucellae* are highly invasive and capable of penetrating the mucosa or skin of the nose, throat, conjunctiva, urogenital tract, teat canal, and abraded skin (Tun, 2007). Having entered the body, *Brucella* organisms are carried by neutrophils and macrophages and localize in the regional lymph nodes (Shirima, 2005; Tun, 2007; Lyimo, 2013). *Brucella* organisms are capable of invading and surviving in both phagocytic and non-phagocytic cells and tend to localize in the rough endoplasmic reticulum. The bacteria are ingested by various local phagocytic cells and multiply in mononuclear and polymorph nuclear cells (Lyimo, 2013). This multiplication of *Brucella* organisms result into lymphadenitis and bacteraemia which may persist for several months (Shirima, 2005).

Brucella abortus has a predilection for the pregnant uterus, udder, testicle and accessory male sex glands, lymph nodes, joint capsules and bursae (Matope, 2009). Localization of *B. abortus* in the gravid uterus is due to the presence of sugar alcohol (erythritol) in the placenta, which has been found to be a strong growth stimulant of *B. abortus* organisms and depending on the severity of placentitis, abortion, premature birth or birth of a viable or non-viable calf may result (Bishop *et al*., 1994). The cause of abortion is not known exactly but it is believed to be due to the interference with foetal circulation due to placentitis, or the direct effect of endotoxins, or directly from foetal stress due to

inflammation of foetal tissues (Matope, 2009). According to Enright *et al*. (1984) *Brucella spp*. may stimulate the production of cortisol which causes low secretion of progesterone and an increase of oestrogen levels therefore induce a premature parturition.

Bacterial and host factors play role in the establishment of infection. Bacterial factors include size of the infective dose and virulence of the bacteria, animal factors are age, sex, innate resistance and reproductive status of the host animal (Shirima, 2005; WHO, 2006). Establishment of infection may also happen due to the existence of several host species and the potential of inter species transmission and maintenance of the disease as for the risky practice of mingling cattle, camel and small ruminants in the grazing lands. Calves born from seropositive dams are passively immunized via the colostrums and this interferes with vaccination and the antibodies declines into undetectable levels though few remain immune for a long time (Radostits *et al*., 2007).

2.5.4 Clinical manifestation

2.5.4.1 Brucellosis in livestock

The period between exposures to the first appearance of clinical disease is the common definition of incubation period to many diseases. With brucellosis, incubation period is variable and is defined as: - i) period between exposure and abortion or (ii) the period between exposure and before the first serological evidence of infection can be detected (Bishop *et al*., 1994; Shirima, 2005). The incubation period in cows varies according to the time at which infection occurred and may take 14-180 days depending on the size of the infective dose, age, sex, stage of gestation and innate immunity of the animal (Shirima, 2005; Lyimo, 2013). Clinical findings are dependent upon the immune status of the herd or flock. The major clinical signs though not pathognomonic are late term abortion, retained placenta, metritis and reduced milk production (Karimuribo *et al*., 2007; Megersa *et al*., 2011). Infected dams usually abort only once, and subsequent gestations may bear

calves that are weak or healthy (Lyimo, 2013). In males it causes orchitis and epididimitis with frequent sterility (Karimuribo *et al*., 2007; Jergefa *et al*., 2009).

2.5.4.2 Brucellosis in humans

Brucellosis in human is an acute, sub-acute or chronic form of illness with common clinical features including loss of appetite, muscular pain, lumber pain and loss of weight (Minas, 2007; James, 2013). *Brucella* infection causes focal lesions in bones, urogenital tract and other organs. Other reported complications are arthritis, sacroiliitis, spondylitis and central nervous system disorders. The disease causes abortion in pregnant women in the first and second trimester. In male it can result to epididymo-ochitis (James, 2013).

2.6 Diagnosis of Brucellosis in Animals and Humans

Clinical diagnosis of brucellosis in either animals or human is particularly difficult and has never been straight forward (Shirima, 2005; Matope, 2009; Lyimo, 2013). Abortion in the third trimester of gestation in bovine suggests brucellosis but other causes of abortion such as Rift valley fever (RVF), salmonellosis, leptospirosis and listeriosis should be put into consideration since they could cause abortion "storms" in cattle (Matope, 2009).

Diagnosis in general fall into two categories:-

i) Isolation and characterisation of disease causing organisms and

ii) Detection of specific antibody in serum or milk which is the most practical diagnosis of brucellosis (WHO, 2006; Godfroid *et al*., 2010).

Diagnosis may target different goals including; screening or prevalence studies, confirmatory diagnosis, certification or disease surveillance (Godfroid *et al*., 2010). According to Matope *et al*. (2010), the choice of which test to use in brucellosis surveillance programmes especially in developing countries, depends on several factors

that include specific objectives of the programme, cost of setting up the test, technical competence and application adaptability of the technique.

2.6.1 Isolation and characterization of disease causing organisms

2.6.1.1 Culture methods

Identification and isolation of *Brucella* spp. by culture is considered to be the "gold standard" and conclusive evidence of *Brucella* infection since it involves recovery of *Brucella* organisms from the patient (Alton *et al*., 1975; Lyimo, 2013). The method is not common in routine diagnosis of the disease (Bax *et al*., 2007). It involves taking appropriate samples. Suitable specimen for culture in animals are foetal membranes, uterine discharges, milk, colostrums or blood from infected animals, liver and spleen from the aborted foetus. The most suitable specimen for isolation of *Brucella* organisms are the supra mammary lymph nodes. Others are retropharyngeal or prescapular lymph nodes (Poester *et al.*, 2010; Lyimo, 2013).

A wide variety of culture media for growing *Brucella* spp. is commercially available (Poester *et al*., 2010). Depending on the requirements and preference, a liquid broth or solid agar medium can either be made from the powder media. For instance, a broth or biphasic medium is preferred for culturing blood and other body fluids while solid agar medium is suitable for other specimens as it facilitates recognition of colonies and discourages bacterial dissociation (Alton *et al*., 1975; Poester *et al*., 2010). In case the material for culture is contaminated, selective media that has antimicrobials incorporated may be required. The intention is to discourage the growth of fast growing microbes that may overwhelm the agar media and suffocate the growth of the desired *Brucella* organisms (Poester *et al*., 2010).

According to Alton *et al*. (1988), growth of *Brucella spp*. on media may appear within one to two weeks. However, for the culture to be discarded as negative, four to six weeks must elapse so as to declare that it is actually negative. Appearance, shape, colour and outline distinguish strains of *Brucella* organisms. Smooth strains such as *B. abortus* and *B. melitensis* appear transparent and yellow with a shiny surface when observed in transmitted light with their colonies being convex with a circular outline and a diameter between 0.5 to 1.0 mm (Poester *et al*., 2010). Additional testing and observation is required including; colony morphology, staining and biochemical tests such as urease, catalase and oxidase in order to comfirm that the organism belongs to the genus *Brucella*.

In the culture method, great care should be taken due to the considerable risk to health of laboratory personnel therefore, necessitating its culture to be carried out in bio-safety level 3 laboratories by highly qualified personnel (Mathew *et al*., 2015). The culturing process is very useful in the diagnosis of brucellosis although it has very low specificity and the results depend more on individual laboratory skills such that even highly experienced laboratories report low isolation rates between 20-50% (Gall and Nielsen, 2004; Poester *et al*., 2010). Furthermore, *Brucella* organisms are slow growing therefore time-consuming and cumbersome. Even with known positive samples, it may also be unsuccessful (Ray, 1979).

2.6.1.2 Microscopy

Smears of placental cotyledon, uterine discharges or foetal stomach contents are stained using Ziehl-Neelsen (stamp's staining) or Kosters' method to look for the presence of aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology for evidence of brucellosis. It is important to know that *Coxiella brunetii* or *Chlamidia* may superficially resemble *Brucella* in smears after staining (Bishop *et al*., 1994; WHO, 2006).

2.6.1.3 Molecular techniques

Molecular techniques identify the organisms and they may detect organisms directly in clinical specimens in short time. These techniques include Polymerace Chain Reaction (PCR), Restriction Endonuclease Analysis (REA) and Restriction Endonuclease and Hybridisation analysis which have been used for diagnosis and epidemiological studies of the disease (Shirima, 2005; Lyimo, 2013). These have high sensitivity and specificity. However, these techniques are not widely used due to their high cost therefore, not yet in routine clinical use (Nimri, 2003; James, 2013). They are more appropriate for differential diagnosis rather than for establishing prevalence.

2.6.2 Detection of specific antibody

In general, brucellosis in both animals and humans is diagnosed by serological methods (WHO, 2006). According to Nielsen (2002), serological tests detect antibodies produced against lipopolysaccharides (LPS) of both smooth and rough *Brucella* spp. The smooth species; *B. abortus*, *B. melitensis* and *B. suis* which contain the O-polysaccharide (OPS) as part of the lipopolysaccharides (LPS) are diagnosed serologically using either a whole cell antigen or smooth- lipopolysaccharide (S-LPS) prepared by chemical extraction, while the rough species; *B. canis* and *B. ovis;* which contain no detectable OPS, are mainly diagnosed using rough-lipopolysaccharides (R-LPS) or protein antigens.

Various serological tests are used to detect specific antibody in serum and milk following infection. These tests remain the most practical diagnosis of *brucellosis* (WHO, 2006; Lyimo, 2013). These include: Serum Agglutination Test (SAT), Complement Fixation test (CFT), Rose Bengal Plate test (RBPT), Buffered Acidified Plate test (BAPA), Enzyme Linked Immunosorbent Assay (ELISA) and Milk Ring Test (MRT) which is used for testing animals only (Radostitis *et al*., 2007). Meanwhile, there has been no report on its use for humans diagnosis.

2.6.2.1 Serum agglutination test (SAT)

This technique has been used widely for *Brucella* diagnosis for decades in several countries. The sensitivity and specificity of SAT for detection of *Brucella* antibodies were established to be 81.5% and 98.9% respectively (Godfroid *et al*., 2010). However, it has shown some limitations including failure to differentiate natural infections from the effect of vaccination, failure to detect *Brucella* antibodies following abortion or during early infection (Shirima, 2005; Lyimo, 2013) and negative results during chronic stages of the disease (Shirima, 2005; Matope, 2009; Lyimo, 2013). Detects IgM, and IgG_2 but often IgG_1 fail to agglutinate, so false negative may occur (Matope, 2009). Due to low sensitivity and specificity is advised to be used in the absence of alternative technique (Swai, 1997; WHO, 2006; Lyimo, 2013).

2.6.2.2 Complement fixation test (CFT)

This technique has high sensitivity (90 – 91.8%) and specificity (99.7 – 99.8%) therefore regarded as the definitive test for the detection of *brucellosis* in animals and humans (Matope, 2009; Godfroid *et al*., 2010). This test is relatively insensitive to antibodies produced in response to vaccination with the living attenuated vaccine (for *B. abortus, B. melitensis*) whilst being highly sensitive and specific in animals naturally infected with brucellosis (Tun, 2007). However, it is a complex method to perform requiring good laboratory facilities and well trained personnel (WHO, 2006).

2.6.2.3 Rose bengal plate test (RBPT)

This test has been used for screening livestock, wildlife and human population in several countries (Omer *et al*., 2001; Shirima, 2005). According to the EU requirements, the Rose Bengal antigen needs to be standardized and buffered to pH 3.65 (Tun, 2007). Its specificity is 70-99% and sensitivity is 63-97%. Immunoglobulins detected are IgG_1 and

IgG_2. It is a simple spot agglutination where 30 µl of stained antigen is mixed with equal volume of serum on a plate and rocked for 4 minutes. Any resulting visible agglutination signifies a positive reaction (WHO, 2006; Lyimo, 2013). It is capable of detecting early infection due to its ability to detect presence of IgG_1, which is actually produced early after exposure. False negative results are rare; they are obtained during early stages of the infection or immediately after abortion. False positive reactors are normally due to the presence of IgM as a result of S19 vaccination, colostral antibodies in young stock, cows tested at the end of lactation period and cross reaction with other bacteria as well as laboratory errors (Bishop *et al.*, 1994; Lyimo, 2013). Rose Bengal Plate Test technique demand minimum equipments therefore, is an excellent test for screening large number of sera samples (Blood and Radostitis, 1990). RBPT positive reactors are recommended to be retested for confirmation by other test such as CFT or SAT (Tun, 2007). ELISA has been validated to be an excellent confirmatory test for detecting *Brucella* antibodies in most mammalian species (Assenga, 2015).

2.6.2.4 Buffered acidified plate antigen test (BAPA)

This test is used for screening livestock, wildlife and human population. It is recognized by OIE as a screening test for cattle, bison and swine to detect immunoglobulins IgG_1 and IgG_2. The specificity and sensitivity are 65-99% and 70-99% respectively (Hennager, 2013). It is a simple spot agglutination where 80 µl serum and 30 µl of antigen are dispensed onto a clear glass plate and mixed with a stirrer, 10 – 12 minutes incubation time is needed while rocking. Any resulting visible agglutination signifies a positive reaction (Hennager, 2013).

2.6.2.5 Enzyme linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a serological technique used to detect antibodies against infectious agents in a sample. ELISA tests offer excellent sensitivity

and specificity. Two types of ELISA test are recommended for the purpose of international trade of livestock (OIE, 2004; Matope *et al*., 2010), these are indirect enzyme-linked immunosorbent assay (i-ELISA) and competitive enzyme-linked immunosorbent assay (c-ELISA). Test by ELISA is fairly simple to perform, equipments needed are minimum and commercially available in kit form (Munir *et al*., 2008; Lyimo, 2013). The sensitivity and specificity of c-ELISA for serological test is 95.2% and 99.7% respectively (Godfroid *et al*., 2010). It has a capacity to detect all antibody isotopes, IgM, IgG_1, IgG_2 and IGA (Matope, 2009). In comparison to other ELISA methods, c-ELISA is more robust and easy to perform. Competitive ELISA was developed and validated to reduce the shortfalls of low specificity of i-ELISA. It has the ability to differentiate vaccinated animals from naturally infected ones or those infected with cross-reacting organisms. It is also used in areas with low disease prevalence (Matope *et al.*, 2010; Lyimo, 2013). For these reasons, c-ELISA was chosen as a confirmatory serological test. On the other hand, i-ELISA has assays that can be used to test both serum/plasma and milk samples for antibodies to *B. abortus* and *B. melitensis.*

2.6.2.6 Milk ring test (MRT)

The milk ring test (MRT) technique was developed by Fleischner in 1937 (Matope, 2009). Sensitivity and specificity of MRT is 88.5% and 77.4 % respectively (Godfroid *et al*. (2010). It is a recommended screening test used to monitor brucellosis using bulk tank milk, (OIE, 2004) but pooling of milk samples can easily affect its sensitivity. It is simple and effective agglutination test carried out in fresh cow's milk. It does not work on pasteurized or homogenized milk and not suitable in sheep and goats due to the high fat content of their milk (Shirima, 2005; Lyimo, 2013). Availability of milk allows the test to be repeated regularly and give a good serum antibody. It has a capacity to detect IgM, IgG_1, and IgA (Matope, 2009). This test is performed by adding 30µl of antigen to 1 ml of whole milk that had been stored for at least 24 h at 4 ℃. The height of the milk column in

the tube is at least 25 mm. If specific antibody is present in the milk, it will bind to the antigen and rise with the cream to form a blue ring at the top of the white milk column indicating positive reaction (WHO, 2006; Al-Mariri and Haj-Mahmoud, 2009). The test is considered to be negative if the colour of the underlying milk exceeds that of the cream layer. False positive reactions may occur if testing:- i) colostrum or milk at the end of the lactation period and milk from cows suffering from a hormonal disorder or mastitis (Mohamand *et al*., 2014); ii) Cows vaccinated by *Brucella abortus* S19 vaccine at adult age because they tend to exhibit persistent positive milk ring test (Radostitis *et al*., 2006) as one of the disadvantages of milk ring test.

2.7 Treatment of Brucellosis in Livestock

Treatment of animals is normally not undertaken and treatment trials that have been performed have shown only partial success in eliminating the infection (Radostits *et al.,* 2007; Matope *et al*., 2011). *In vitro* treatment of *Brucella abortus* have been found to be sensitive to gentamycin, kanamycin, tetracyclines and rifampin.

However, the effectiveness of these antimicrobials *in vivo* have not been comprehensively evaluated (Matope, 2009). Some problems have been reported to be associated with the treatment of brucellosis. For instance, the use of antibiotics such as penicillin and oxytetracycline causes L-transformation on the cell wall thereby possibly creating carrier animals and affecting future serological detection (Bishop *et al.,* 1994; Shirima, 2005). Owing to the fact that treatment has shown partial success, efforts are directed at control and prevention (Animal Health Australia, 2005; Lyimo, 2013).

2.8 Control and Eradication

Several countries through control and eradication programmes have been successful in eliminating brucellosis (WHO, 1997). It is highly dependent on national strategies, priorities and policies (Bishop *et al.,* 1994). Multidisplinary approach, starting from

household to national level forms a good base of control of brucellosis (James, 2013). These strategies include those for prevention of the spread of disease between animals; monitoring of uninfected and suspected herds and zones, combined approach of systematic vaccination and test and slaughter in situations where 1-5% of animals are infected and test and slaughter alone in cases where the prevalence is less than 1%, strict control of movement of infected and suspected animals; strategic vaccination of herds of cattle at early age (3 to 10 months) and providing specific education and training programmes (Shirima, 2005; James, 2013; Lyimo, 2013).

2.8.1 Control by vaccination

The most effective control method in bovine brucellosis is vaccination at early age between 3 to 10 months of age using *B. abortus* strain S19 (Tun, 2007; Matope, 2009; Lyimo, 2013). The licensed vaccine preparations currently in use are those containing smooth *B. melitensis* Rev.l; rough *B. abortus* strain RB51; rough *B. melitensis* strain M111 and smooth *B. abortus* strain S19 (Shirima, 2005). According to Matope (2009), B. *abortus* S19 vaccine has been the most widely used vaccine in the control of bovine brucellosis. The recommended vaccines are live therefore, should not be given to pregnant cattle because they may cause abortion.

2.8.2 Control program on a herd basis

The level of infection present and general immune status of the herd determines the brucellosis control strategies at herd level. According to Matope (2009), test and disposal of positive reactors may not match during an abortion storm because the spread of infection occurs at faster rate than disposal is possible. The recommended control measures include :- i) Isolation of infected animals. ii) Hygienic disposal of aborted

foetuses, placentas and uterine discharges. iii) Routine testing of the herd as well as screening animals for *Brucella* antibodies before introducing them into the herds.

Other important management practice at farm level is subsequent disinfection of the contaminated surfaces. *Brucella* species are readily killed by most commercially available disinfectants including 70% ethanol, 2-3% caustic soda, 2.5% hypochlorite solutions, 3% formalin, 20% freshly slaked lime suspension, or 2% formaldehyde solution and isopropanol to mention but a few (Silbereisen *et al*., 2015).

2.8.3 Control in trade animals

Cattle trade is both for slaughter and rearing. In Tanzania, initial official stage of livestock trade starts at the livestock markets. From the market, cattle are transported through the stock routes in lorries and railways. Along the way, there are insufficiently manned check points for inspection of animals and the animal health movement permits (AHMP) at the starting point. In the livestock markets and check points is where important procedures such as inspection of documents and testing of diseases could be done if well organized (FAO, 2002). All trade animals are supposed to be screened for brucellosis whereby, only those animals free from brucellosis are eligible to be issued movement permits which also shows their health status in general.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The study was carried out in urban and peri-urban areas of Sumbawanga Municipality which is located to the South-West of Tanzania in Rukwa region (Fig. 1), lying between latitudes 7˚48'and 9˚31' South of equator and between longitudes 30˚29' and 31˚49' East of Greenwich. Sumbawanga Municipality covers an area of 1 329 km^2. Administratively the Municipality is divided into 19 wards, 24 villages and 121 neighbourhoods (*mitaa*). Ten wards out of 19 have urban characteristics, six wards are purely rural and the rest 3 have partly urban and rural characteristics (semi-urban) respectively. In the context of this study, "urban" is part of Sumbawanga Municipality areas characterised by small surveyed land plots and has neighbourhoods (*mitaa*) as its lowest administrative structure. The 10 urban wards were Chanji, Kizwite, Mafulala, Katandala, Mazwi, Msua, Izia, Majengo, Malangali and Sumbawanga Asilia. "Rural" in Sumbawanga Municipality represent the six peri-urban wards with rural characteristics namely Pito, Ntendo, Mollo, Kasense, Matanga and Senga which are made up of villages with large agricultural land area. "Semi-urban" is the intermediate location between Sumbawanga urban and peri-urban area. Have some urban and rural characteristics with moderately large land for agriculture. In this study, the three wards with semi-urban characteristics were Momoka, Lwiche and Milanzi. Administratively they are composed of villages and neighbourhoods (*mitaa*) showing that they are in transition.

According to 2012 Population Census, the human population is 29 793 of which 100 734 are males, 109 059 are females. Average temperatures fluctuate from 13˚C (June and July) to 27˚C (October to December). Relative Humidity is 51.5 – 75.2% and the average annual

rainfall is 800 to 1 200 millimetres. The altitude ranges between 1000 and 2 461 metres above the sea level (Sumbawanga Municipal Director's 2014/15 Agriculture Annual report personal communication, 2016).

Economic activities in Sumbawanga Municipality include:- subsistence farming and indigenous cattle keeping (76%), dairying (10%), trading (wholesale and retail) (10%), salaried employment (3%), industrial and factories (1%) (Sumbawanga Municipal Director's 2014 Investment profile, personal communication, 2016).

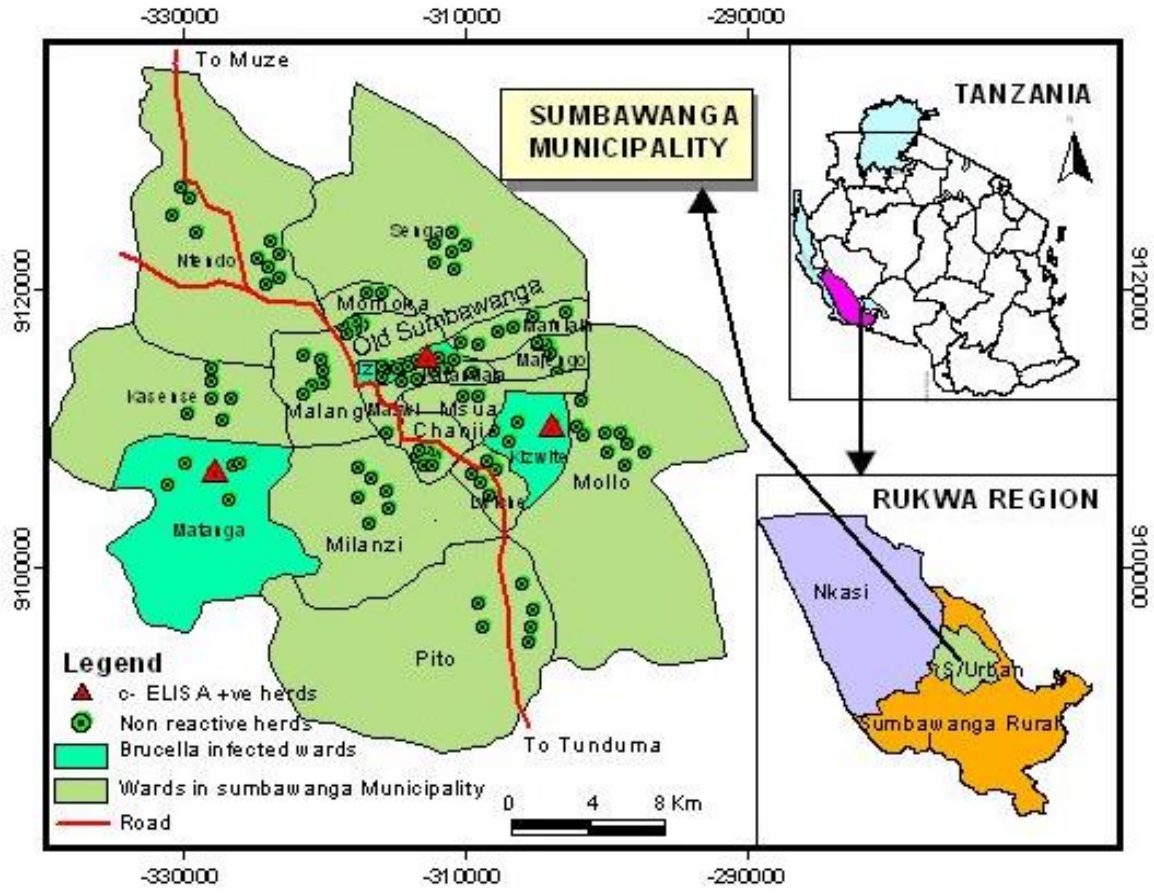

Figure 1: Map of Sumbawanga Municipality showing ward boundaries and distribution of animal *Brucella* antibodies.

Source: National Bureau of Statistics, 2012 Population and Housing Census, modified

The Municipality has a total of 25 909 cattle, of which 1 362 are dairy. The breeds of dairy cattle available in the study area include Friesian, Ayrshire and their crosses with short horn zebu. Other livestock include goats (14 297), sheep (634), pigs (5 395), chicken (67 794), donkey (818), ducks (4 810), turkey (541), guinea fowls (560) and 6 118 dogs (Sumbawanga Municipal Livestock Officer's 2014/15 Livestock and Fisheries Annual report personal communication, 2016).

The predominant farming system is mixed crop-livestock farming (agro-pastoral). Majority of cattle from different herds are allowed to intermingle in communal grazing areas and water points together with small ruminants (Fig. 2) while a few cut and carry system rely on un-developed plots, open spaces, the nearby river side's in Sumbawanga town and peri-urban areas. The herd size is categorized into smallholder, medium and large scale according to number of cattle (1-10, 11- 49 and 50 and above, respectively).

Figure 2: Cattle and small ruminants mixed in communal grazing land of peri-urban area of Sumbawanga Municipality

Dairy cattle and their crosses are kept in Sumbawanga urban and semi-urban where milk is marketable. Milk sold in the year 2014/15 was 3 049 995 litres (Sumbawanga Municipal Livestock Officer's 2014/15 Livestock and Fisheries Annual Report personal communication, 2016). In the peri-urban areas Ufipa cattle ecotype is dominant. They are kept purposely for draft work and social cultural values, manure, cash, milk, meat and breeding priorities (Msanga *et al*., 2012). Three management systems are practised including; extensive (free range), semi-intensive and intensive (zero grazing). This study area has been selected because no research on brucellosis has ever been carried out in any District of the Rukwa region.

3.2 Study Design and Sample Size Estimation

A cross-sectional study of bovine brucellosis was adopted in the Survey. Sample size was determined using the formula by Thursfield (1995):-

$n = Z^2 * P (1-P)/d^2$.

Where; n = sample size, Z = statistic for a level of confidence 95%, which is conventional, *Z* value is 1.96, P = estimated prevalence of brucellosis 15%, d = precision level of 0.05, resulted n = 196.

There was no previous study on brucellosis that was conducted in the study area therefore, an average of 15% prevalence was considerd based on the findings (1-30 %) of other studies carried out in other parts of Tanzania specifically, the findings of a recent study in the nearby districts by Assenga *et al*. (2015) in Katavi-Rukwa ecosystem who reported individual animal level prevalence of brucellosis been 6.8% in cattle. Therefore, it was assumed that 15% of cattle in the infected herd will have brucellosis. The original sample size (n) was multiplied by the design effect (D = 1.8), calculated using the formula by Otte and Gumm (1997):- $D = 1 + (b-1)\ \rho$.

Where; b (9) is the average number of samples per cluster and ρ (rho) 0.1, is the rate of homogeneity, equivalent to intra-cluster correlation coefficient in single stage cluster sampling. Rho is a measure of variability between clusters compared to the variation within cluster (Otte and Gumm, 1997).

The required sample size of cattle to be bled was n x D (196 x 1.8) = 352.8 ≈ 353. On average, 3 cattle per household and 3 households per village or administrative street were to be sampled. Eventually, 354 cattle were bled.

3.3 Village, Neighbourhoods and Animal Selection Procedures

Study animals were obtained using multistage sampling. The first stage involved selection of villages and neighbourhoods (*mitaa*) then households were randomly selected from them. Finally, individual animals within selected household were the sampling unit for primary data collection. All cattle breeds aged one year and above of both sexes available in the selected household herds was eligible for inclusion in the study. Age classes of animals were determined by dentition based on the number of pairs of permanent incisors as adopted from Shirima (2005). In this study, cattle ≤ 3 years old were categorized as young and those > 3 years old were categorized as adults.

The multistage sampling frame comprised of a list of 24 villages and 121 neighbourhoods (*mitaa*) in the study area. Only 18 villages and 56 neighbourhoods (*mitaa*) had cattle owning households. Names of 18 villages were listed on small pieces of paper then pick and replacement technique was applied for the selection so as to provide equal probability for each village to be selected until 13 villages were sampled while, selection of 26 neighbourhoods (*mitaa*) out of 56 was done by using a table of random numbers. A list of cattle keeping households for each selected village and neighbourhoods (*mitaa*) was

obtained from the Sumbawanga Municipal livestock keepers register. Names of household heads were assigned into a table of random numbers whereby, a total of 117 names were selected with average of 3 households per village or neighbourhood (*mtaa*). Selected households had a total of 2 374 cattle population whereby, 354 cattle were sampled from 108 respondent households. Depending on the size of the herd, simple random sampling and systematic random sampling techniques were employed in small and medium to large herds respectively. Selected cattle were identified by using ear tags.

3.4 Data Collection

3.4.1 Questionnaire design

To obtain animal-level and farm-level data, a pre-tested close ended questionnaire (Appendix 1) was administered through interviewing the head of each household or other knowledgeable member of the family who had taken care of cattle for not less than three years. Questions were designed to gather information about the farmer and his/her herd. Information collected about the farmer included age, sex, education level, livestock farming experience (years) and farmers' knowledge or awareness about brucellosis in cattle. Herd level information such as herd size, management system, breed of cattle and breeding method, history of vaccination against brucellosis and abortions, method of disposal of afterbirth and aborted materials, grazing system and cattle movements between and within the area. The involved households/farmers were identified by different codes.

Variables considered as potential risk factors for brucellosis in the study area were based on the reported risk factors in the reviewed literature such as mixing of herds of cattle and flocks of small ruminants in the grazing ground and water points, sharing of bulls, improper disposal of placenta and aborted foetal material, introduction of animals with unknown health status into herds and herd size. (Chitupila *et al*., 2015; Swai *et al*., 2010;

Egaru *et al*., 2013; Chimana, 2011; Matope, 2011). Same management practises were observed to be done by the farmers in the study area.

3.4.2 Brucellosis prevalence data

3.4.2.1 Blood sample collection and handling

Blood samples were collected from 354 cattle selected from 108 households. The herds were from 3 management systems (77 extensive, 24 intensive and 7 semi- intensively managed herds). Animals were restrained using crash and nose lead or casting ropes and nose lead for the safety of both, practitioners and the animals (Fig. 3). Approximately, 5 mls of whole blood was collected by jugular vein puncture from each animal using sterile vacutainer needle into plain vacutainer tube (BD Vacutainer Belliver Industrial Estate, Plymouth. PL6BP. UK.). By the use of Global position system (GPS), a grid position of each study household was obtained by employing Universal Transverse Mercator (UTM) coordinate system (easting and northing) measured in metres (Appendix 2).

Figure 3: Blood sample collection from the jugular vein of cattle

Each tube was labelled using codes (letter and number) describing the specific ward, herd and cattle. Tubes with blood samples were carefully packed upright in a cool box, avoiding possibility of leakage or cross contamination and transported to Mazwi health centre laboratory in Sumbawanga Municipality where they were kept at room temperature overnight to allow clotting. Tubes with clotted blood samples were centrifuged at 3 000 rpm for 5 minutes to obtain serum. About 2 mls of serum were decanted into Eppendorf tubes with codes corresponding to the vaccutainer tubes. Sera samples were stored at -20ºC in Rukwa Regional Hospital laboratory in Sumbawanga town till when they were transported in cool box with ice cubes to the Faculty of Veterinary Medicine (FVM) laboratory at Sokoine University of Agriculture for serological analysis.

3.4.2.2 Laboratory analysis of samples

Before testing, sera were allowed to equilibrate to room temperature (25-27°C). Screening of the sera samples for brucellosis was done by using Rose bengal plate test (RBPT) and eventually, reactors to RBPT were confirmed by Competitive enzyme linked immunosorbent assay (c-ELISA). Rose bengal plate test (RBPT) was performed as described by Alton *et al*. (1975) and as recommended by OIE (2009). Briefly, 30 µl of the test serum and 30 µl of RBPT antigen were placed alongside on the glass plate and mixed thoroughly using a stirrer over the entire surface of the teardrop spot and rocked for 4 minutes. Any evidence of visible agglutination was regarded positive and those with no agglutination as negative (Fig. 4).

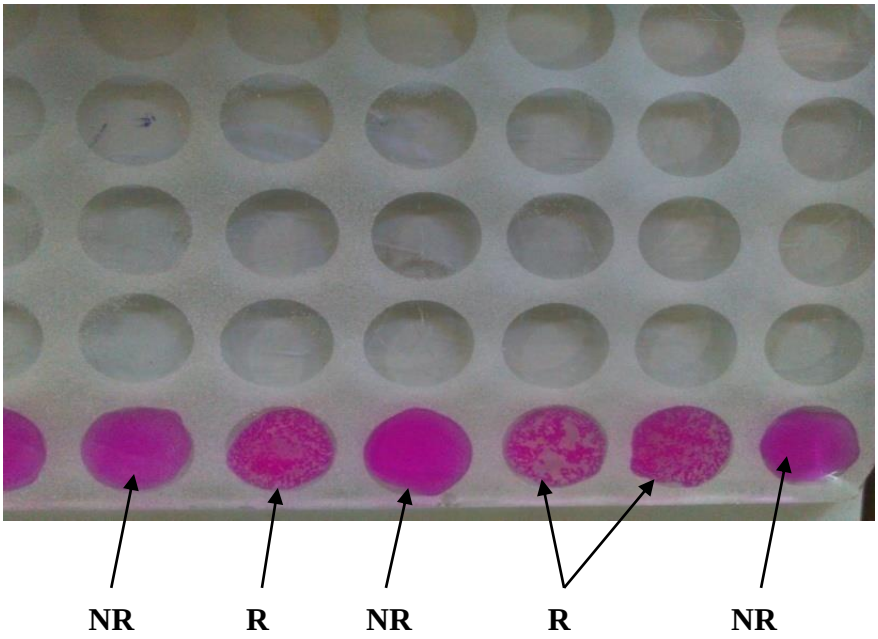

Figure 4: Appearance of RBPT results (R - reactive and NR - non-reactive samples)

All RBPT reactive sera samples were further tested for antibody against *Brucella* spp. by the c-ELISA for confirmation. ELISA test, employed a test procedure and interpretation of results as described by the Animal Health and Veterinary Laboratory Agency (AHVLA), New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom, according to the recommendations from the World Health Organization for animal health (OIE, 2009). The optical density (OD) was measured at 450 nm using ELISA reader Multiscan RC Version 6.0 (Thermo lab system, Helsinki, Finland). The positive/negative cut off was calculated as 60 % of the mean OD of four conjugate control wells. A test sample giving an OD equal to or below this value was regarded as positive. Validation of c-ELISA test was carried out with positive and negative controls as per manufacturer's instructions. A weak positive ELISA standard serum was used as reference sera as recommended by OIE. Animals positive to both RBPT and c-ELISA were regarded to be *Brucella* seropositive and a herd with at least one animal testing positive to c-ELISA was considered *Brucella* infected. Only c-ELISA positive test results were considered in the statistical analysis.

3.5 Statistical Analysis

Microsoft Office Excel® 2007 (Microsoft Corporation, One Microsoft Way, Redmond, 98052-7329, USA) was used to check, clean, store data and generate graphs. Laboratory, farm and animal level data were analysed by using the Statistical package for social science (IBM-SPSS) version 21.0. Logistic regression was performed to determine relationship between predictor variable such as age, sex, and breed of animals, respondent's socio-demographic characteristics and management practices with *Brucella* sero status. A p-value < 0.05 at 95% CI was considered significant.

3.6 Ethical Considerations

Approval for the protocols of this research was obtained from the Deputy Chancellor of the Sokoine University of Agriculture REF NO. SUA/ADM/R.1/8 dated 23 October, 2015. Permission to carry out the study in the Municipality was given by Sumbawanga Municipal Director by the letter Ref. NO. SMC/D.50/25/176 dated 13/11/2015 (Appendix 3). Request of doing this study at the household level was conveyed to the livestock farmers through Ward Executive Officers, Ward Extension Officers and village leaders. Willingness of the livestock keepers to participate in the study was sought before commencement of the study. Explanation of the purpose, objective and importance of the research was done to the leaders and the selected farmers asking for permission and willingness to participate or withdraw their consent if they think otherwise. Information from each participant was confidentially treated. Questionnaires were labelled using codes instead of names therefore, no one else could link data to any respective respondent.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic Characteristics of the Respondents

The characteristics of the respondents assessed were age, sex, education level and their livestock farming experience. The study found that, amongst the respondents, higher percent was in age group 36-53, most of the respondents were males (80.6%), primary education was dominating and the larger proportion of respondents had more than ten years cattle rearing experience. The results observed are summarized in Table 3.

Table 3: Socio-demographic characteristics of the respondents

Variable	Frequency (%) n=108	95%CI
Age in years		
18 – 35	29 (26.9)	18.8-36.2
36 – 53	44 (40.7)	31.4-50.6
54 – 71	35 (32.4)	23.7-42.1
Sex of respondents		
Male	87 (80.6)	71.8-87.5
Female	21 (19.4)	12.5-28.2
Education level		
Non formal	12 (11.1)	5.9-18.6
Primary	67 (62.0)	52.2-71.2
Secondary	18 (16.7)	10.2-25.1
Higher	11 (10.2)	5.2-17.5
Cattle keeping experience (years)		
1 – 3	6 (5.5)	2.1-11.7
4 – 10	34 (31.5)	22.9-41.1
More than 10	68 (63.0)	53.1-72.1

CI = Confidence Interval

4.2 Livestock Herd Characteristics

The types of cattle kept in the study area are the indigenous breeds, crosses of indigenous Tanzania short horn zebu (TSHZ) and improved breeds and the dairy breeds with different

herd sizes. Incidences of abortion were also reported. In some herds (43.5%), cattle are mixed with sheep and goats. Results are shown in Table 4.

Table 4: Livestock herd characteristics

Variable	Frequency (%) n=108	95%CI
Cattle breed category		
Local zebu	60 (55.6)	45.7-65.1
Dairy breed	6 (5.5)	2.1-11.7
Dairy cross breed	42 (38.9)	29.7-48.8
Cattle herd size		
1 to 10	66 (61.1)	51.3-70.3
11 to 25	26 (24.1)	16.4-33.2
More than 25	16 (14.8)	8.7-22.9
Sheep/goats in herds of cattle		
Mixed cattle with sheep/goats	48 (43.5)	34.9-54.3
Not mixed cattle with sheep/goats	60 (56.5)	45.7-65.1
Sheep/goats herd size		
1 – 10	34 (31.5)	22.9-41.1
11 – 25	11 (10.2)	5.2-17.5
More than 25	4 (3.7)	1.02-9.2
Abortion in cattle	22 (20.4)	13.2-29.2

CI = Confidence Interval

4.3 Knowledge, Attitude and Practices Regarding Brucellosis

Different levels of knowledge, attitude and practices were found to exist in the study area as reported bellow and summarized in Table 5.

4.3.1 Respondents knowledge and awareness of brucellosis (n=108)

The results show that, only 38% of the respondents had knowledge or awareness about brucellosis in cattle, majority of respondents (62%) had no knowledge (Table 5). The major source of knowledge was other farmers (27.7%). The rest acquired from the Extension Officers (5.6%), reading books (5.1%) and through media (3.7%). It was also found that, 44.4% of the respondents consume raw milk.

4.3.2 Person taking care of the animals

The study found that large proportion of the cattle herds (44.4%) are cared for by hired labour while 37.9% was by family members and the remaining 17.6% by owners (Table 5). The proportions of respondents who assist animals on calving were 25% while 75% do not. Among those who assist calving (n=27), 14 (51.8%) do not wear protective gears while 13 (48.2%) protect their selves.

4.3.3 Breeding methods practiced in the study area

Majority of the respondents (95.4%) breed their cattle by natural mating while only 4.6% practiced both natural mating and Artificial Insemination (AI) (Table 5). Sharing of bulls is common to most of livestock farmers in this study area whereby 97.2% of the respondents share bulls while 2.8% do not.

4.3.4 Handling of manure

Majority of the respondents (91.7%) reported to have piled manure outside the *boma* then took to the field for crop farming (Table 5). The rest (2.8%) piled outside the *boma* and left it there, 3.7% is used for production of biogas then to the field, 0.9% took out to dry then returned as cattle bedding while one respondent (0.9%) collected and sold the manure.

4.3.5 Grazing practices

Grazing systems are illustrated in Figure 5. However, the dominating grazing system is free range whereby 71.3% of respondents graze animals in the communal grazing lands. It was also found that cattle and small ruminants from different herds intermingle in the communal grazing areas and water points with dogs being used as guardians (Fig. 2).

Among the respondents, 71.3% mix herds of cattle and small ruminants in the communal grazing grounds and water points while 27.8% did not.

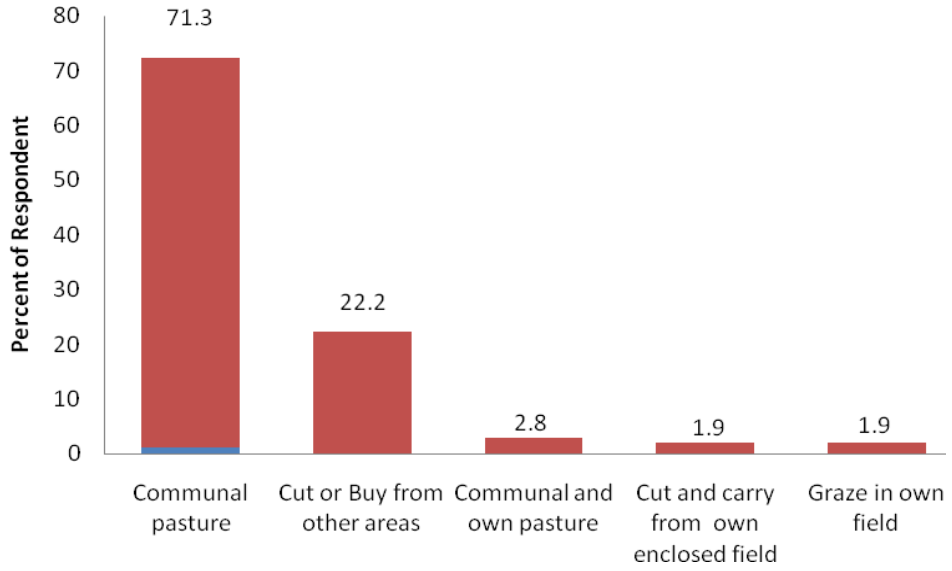

Figure 5: Cattle grazing systems in Sumbawanga Municipality (n = 108)

4.3.6 Source of cattle introduced into herds for replacement or addition

Large proportion of respondents (55.6%) introduced cattle into their herds from different sources while the rest (44.4) replaced animals within their herds (Fig. 6).

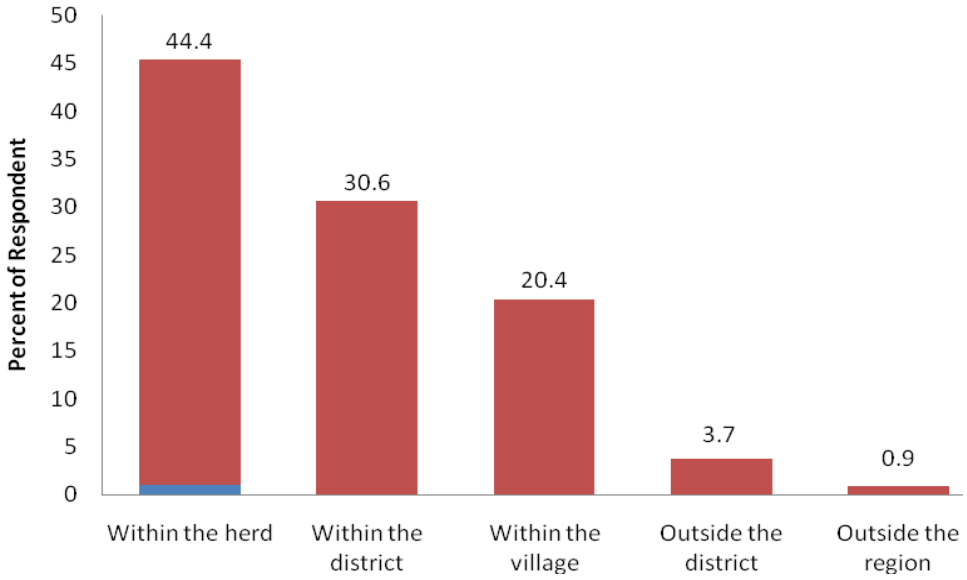

Figure 6: Replacement of animals into herds and sources (n = 108)

4.3.7 Incidences of abortion and methods of disposal

History of abortions was reported by 20.4% of the respondents (n = 22). The higher incidences were reported by 15.7% of the respondents to have happened in 2014/15 while the rest 3.7% were in 2013/14 and 0.9% in the year 2012/13.

Most of the abortions 14.8% occurred in the cattle *boma* and 5.6% in the grazing areas. The stages of gestation when abortions occurred were reported by the respondents to be 13.9% in the second, 4.6% the third and 1.9% in the first trimesters respectively. Different methods were used to dispose abortus whereby 13% of the respondents buried in the soil, 2.8% had been leaving them in the grazing area, 2.8% feed them raw to dogs, 0.9% threw into the bush while 0.9% reported to have been fed to dogs after cooking.

4.3.8 Retained placenta and methods of disposal

Cases of retained placenta were reported by 25.9% of the respondents. The disposal methods for placenta were different among the respondents. The proportion that buried placenta was 50.9% followed by those who left it in the grazing areas 21.3% and 20.4% reported to have fed dogs in raw form. Others (5.6%) were thrown into the bush and 0.9% fed dogs after cooking. One respondent (0.9%) had a herd of male cattle only (Fig. 7).

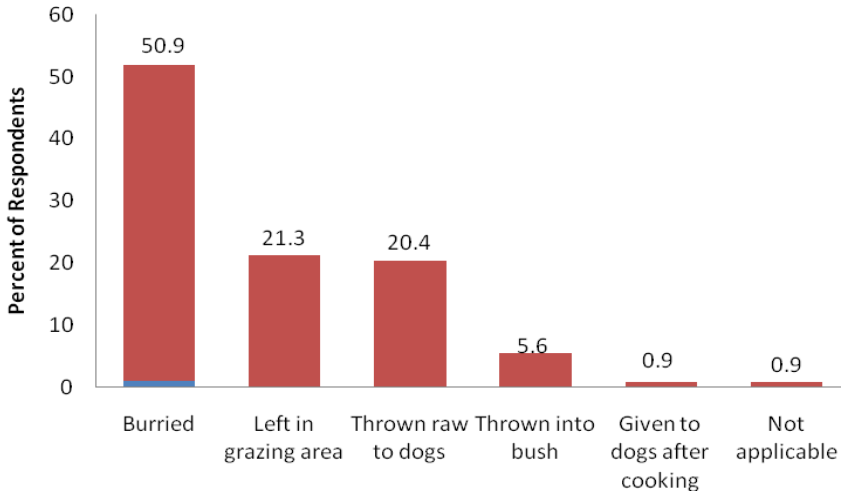

Figure 7: Methods of disposal of placenta in Sumbawanga Municipality (n = 108)

4.3.9 Bucket feeding

In the study area a proportion of livestock keepers (33%) feed milk to calves by using buckets whereby some of them (9.9%) reported to have been feeding calves with milk from different cows rather than relying on their mothers' milk only.

4.4 Presence of Wild Animals in the Grazing Land

Of the 108 interviewed respondents, 24.1% reported to have been seen wild animals in the grazing areas at different times. Dikdik were occasionally seen by 14.8% of respondents while 1.8% has seen them often. Impala were occasionally seen by 9.3% and often by 0.9% of the respondents while Thomson gazelle were occasionally (0.9%) seen in the communal grazing lands.

Table 5: Knowledge, attitude and practices regarding brucellosis

Variable	Frequency (%) n=108	95% CI
Persons who care animals		
Hired labour	48 (44.4)	34.9-54.3
Family member	41 (37.9)	27.9-46.9
Owner	19 (17.6)	10.9-26.1
Assist animals during calving	27 (25.0)	17.2-34.3
Assist without Protective gears	14 (51.8*)	7.3-20.8
Consumption of raw milk	50 (44.4)	36.7-56.1
Wild animals in grazing areas	26 (24.1)	16.4-33.3
Sharing communal grazing and water points:-	78 (72.2)	62.8-80.4
Sharing bulls	105 (97.2)	89.5-98.5
Knowledge of brucellosis in cattle	41 (38.0)	28.8-47.8
No knowledge of brucellosis in cattle	67 (62.0)	49.4-66.0
Source of knowledge		
Other farmers	23 (21.4)	14.0-30.2
Extension officer	9 (8.3)	3.9-15.2
Reading books	5 (4.6)	1.5-10.5
Media (radio, Television, Newspaper)	4 (3.7)	1.0-9.2
Breeding method		
Natural mating	103 (95.4)	89.5-98.5
Natural mating and AI	5 (4.6)	1.5-10.5
Abortions in cattle	22 (20.4)	13.2-29.2
Bucket feeding	36 (33)	28.2-38.2
Feeding milk from other cows	35 (9.9)	7.1-13.6
Disposal of abortus:-		7.3-20.8
Buried	14 (12.9)	0.6-7.9
Left in the grazing area	3 (2.8)	0.6-7.9
Thrown raw to dogs	3 (2.3)	0.0-5.1
Given to dogs cooked	1 (0.9)	0.0-5.1
Thrown into bush	1 (0.9)	
Handling of manure:-		
Collected out then to field	99 (91.7)	84.8-96.1
Use for biogas	4 (3.7)	1.0-9.2
Collected out of *boma*	3 (2.8)	0.6-7.9
Dried out then returned for bedding	1 (0.9)	0.0-5.1
Collected and sell to people	1 (0.9)	0.0-5.1

CI = Confidence Interval

* = based on n = 27

4.5 Animal Level Characteristics

A total of 354 cattle with different characteristics (sex, age and breed) were randomly sampled for blood samples collection. These animals were obtained from 108 randomly selected households in the three management systems whereby 77 were extensive, 24 intensive and 7 semi-intensive households/farms. Sampling results are shown in Table 6.

Table 6: Characteristics of the sampled cattle (n = 354)

Variable	Category	Frequency	%
Sex	Female	235	66.4
	Male	119 *	33.6
Age	Young	106	29.9
	Adult	248	70.1
Breed	Indigenous cattle	196	55.4
	Dairy cross breed	125	35.3
	Dairy breed	33	9.3

* (46 breeding bulls, 40 young intact males of 1 to 2 years old and 33 castrates)

4.6 Prevalence of Brucellosis

4.6.1 Prevalence of brucellosis in cattle in the study area

Among the 354 tested sera samples, the proportions of reactors to RBPT were 1.4% and 0.8% were confirmed positive by c-ELISA test. Among the 19 wards, 3 were found with evidence of exposure to *Brucella* pathogen while the rest 16 were not. These were Kizwite and Izia in the urban, and Matanga in peri-urban area (Fig. 1). The three exposed cattle were adult indigenous cattle, all from the extensive management system. Only three herds were found infected, therefore, herd level seroprevalence was 2.8% (95% CI: 0.6-7.9%). Individual animal level seroprevalence in urban was 1.3% while in peri-urban was 0.5%. The overall animal level seroprevalence of brucellosis in cattle in Sumbawanga Municipality is shown in Table 7.

Table 7: Herd and animal level seroprevalence of brucellosis based on RBPT and c-ELISA

Test	Total Samples	Positive Reactors	Herd level Prevalence (n=108)	Individual level Prevalence % (n=354)
RBPT	354	5	4.6	1.4
c-ELISA	354	3	2.8	0.8

4.6.2 Prevalence of brucellosis based on various predictor variables

4.6.2.1 Prevalence based on herd characteristics

Considering animal level risk factors (age, sex and breed), the infected cattle were adults of both sex but prevalence in male 1.7% (n=120) was higher than female 0.4% (n=234). The 3 seropositive cattle were local breed (short horn zebu) 1.5% (n=196) all were found in small scale herds (1-10 cattle herds). Cattle kept together with small ruminants had higher prevalence 1.2% (n=161) while, those without small ruminants were less infected 0.5% (n=193). Cattle in herds with history of abortion had higher prevalence 1.2% (n= 83) than those without history of abortion 0.7% (n=271).

4.6.2.2 Prevalence of brucellosis based on management practices

Based on management practices, nine predictor variables were investigated. The detailed results are summarised in Table 8.

Table 8: Proportion of each variable category in respect to prevalence of brucellosis based on cattle management practices

Variable	Proportion of respondents in each investigated variable n=108 (%)	Proportion of sampled animals in each investigated variable (n=354) (%)	Proportion of positive cases in each investigated variable (n=3)	Individual level seroprevalence for each investigated variable (%)
i) Introduction of cattle into herds (source)				
Within the herd	48 (44.4)	153 (43.2)	0	0.0
Within the village	22 (20.4)	71 (20.1)	0	0.0
Within the district	33 (30.6)	99 (28.0)	1	1.0
Outside the district	5 (4.6)	31 (8.7)	2	6.5
ii) Cattle grazing system				
Communal pasture (extensive)	77 (71.3}	242 (68.4)	3	1.2
Own field/paddocks	2 (1.9)	25 (7.1)	0	0.0
Communal and own pasture	3 (2.8)	11 (3.1)	0	0.0
Cut and carry from own field	2 (1.9)	3 (0.8)	0	0.0
Cut or buy from other areas	24 (22.2)	73 (20.6)	0	0.0
iii) Mixing herds in grazing				
Yes	79 (73.1)	250 (70.6)	3	1.2
No	29 (26.9)	104 (29.4)	0	0.0
iv) Disposal of placenta				
Buried	55 (50.9)	173 (48.9)	0	0.0
Left in the grazing ground	23 (21.3)	81 (22.9)	0	0.0
Thrown raw to dogs	22 (20.7)	71 (20.1)	2	2.8
Thrown into bush	6 (2.1)	18 (5.1)	0	0.0
Given to dogs after cooking	1 (0.9)	8 (2.2)	0	0.0
Not applicable (herd of males)	1 (0.9)	3 (0.8)	1	33.3
v) Disposal of aborted foetal materials				
None	86 (79.6)	271 (76.6)	2	0.7
Buried	14 (12.9)	43 (12.1)	1	2.3
Left in the grazing ground	3 (2.8)	7 (2.0)	0	0.0
Thrown raw to dogs	3 (2.8)	24 (6.8)	0	0.0
Thrown into bush	1 (0.9)	3 (0.8)	0	0.0
Given to dogs after cooking	1 (0.9)	6 (1.7)	0	0.0
vi) Handling of manure				
Pile outside then to the field	99 (90.4)	326 (92.1)	2	0.6
Use for biogas	4 (3.7)	12 (3.4)	0	0.0
Piled outside the boma	3 (2.8)	9 (2.6)	0	0.0
Dried then returned for bedding	1 (0.9)	3 (0.8)	1	33.3
Piled out then sold to farmers	1 (0.9)	4 (1.1)	0	0.0
vii) Mixed cattle with sheep/goat				
Yes	47 (43.5)	161 (45.5)	2	1.2
No	61 (56.5)	193 (54.5)	1	0.5
viii) Breeding method				
Natural mating	103 (95.4)	336 (89.3)	3	0.9
Both (natural and AI)	5 (4.6)	15 (10.7)	0	0.0
ix) Share bulls				
Yes	105 (97.2)	331 (93.5)	3	0.9
No	3 (2.8)	23 (6.5)	0	0.0

4.7 Relationship Between Various Factors and Brucellosis Seropositivity

4.7.1 Relationship between socio-demographic characteristics of farmers and herd characteristics with brucellosis seropositivity

Socio-demographic characteristics of farmers and herd characteristics in relation to c-ELISA seropositivity in the study area were investigated. However, among the investigated predictor variables by univariate model, none qualified to be included in step two Logistic regression (Multivariate model) since neither farmers' socio-demographic characteristics nor herd characteristics had statistical significant relationship with brucellosis seropositivity in the study area as shown in Table 9.

Table 9: Relationship between farmers' socio-demographic characteristic and herd characteristics with brucellosis seropositivity

Variable	Coefficients	SE	P-value
i) Respondents' education			
Non formal	19.2	15924.1	1.0
Primary school	- 0.4	11934.9	1.0
Secondary	16.4	14426.4	0.9
ii) Knowledge about brucellosis			
Yes	0.5	1.5	0.8
iii) Cattle keeping duration (years)			
1 to 3	-19.2	4851.1	0.9
4 to 10	17.8	6231.9	0.9
iv) Herd characteristics			
Herd size			
1 to 10	17.7	4851.1	0.9
11 to 25	0.8	1.7	0.6
Cattle breed			
Local Zebu	-17.0	5482.6	0.9
Dairy breed	2.6	13991.9	1.0
History of Retained placenta			
Yes	2.9	27555.6	1.0
History of Abortion			
Yes	- 0.9	1.5	0.5

4.7.2 Farm management practices

Practices considered to be related to *Brucella* seropositivity in the study area were investigated. However, among the investigated predictor variables by univariate model, none qualified to be included in step two Logistic regression (Multivariate model) since

the results did not show statistical significant relationship between the predictor variables and *Brucella* seropositivity in the study area as summarised in Table 10.

Table 10: Relationship between farmers' management practices and brucellosis seropositivity in the study area

Variable	Coefficients	SE	P-value
i) Disposal of placenta			
Thrown raw to dogs	-0.7	15164.0	1.0
Given to dogs after cooking	1.1	37363.4	1.0
Thrown into bush	-0.8	25576.4	1.0
Burried	1.4	19562.7	1.0
ii) Source of cattle introduced into herds			
Within the village	0.7	69049.2	1.0
Within the District	0.9	65749.7	1.0
Outside the District	-19.3	71064.7	1.0
iii) Handling of manure			
Pilled outside the boma	22.9	46250.7	1.0
Dried outside then used for cattle bedding	-34.2	49991.9	1.0
Collected outside and taken to field	4.0	36945.6	1.0
Collected and sold to people	3.9	55425.4	1.0
iv) Grazing system			
Communal grazing	-0.8	75562.7	1.0
Communal and own field	-1.2	75243.9	1.0
Cut and carry from own field	-2.4	54233.0	1.0
v) Mixing herds in grazing area			
Yes	1.2	69618.3	1.0
vi) Water points			
Share communal water	2.6	56886.6	1.0
Own water points	2.2	64638.3	1.0
vii) Breeding method			
Natural mating	2.0	25550.7	1.0
ix) Share bulls			
Yes	1.8	37482.0	1.0
x) Keeping Goats/sheep			
Yes	1.0	11778.2	1.0
xi) Wild animals in grazing area			
Yes	0.1	15216.4	1.0
xii) Method of disposal of abortus			
Thrown raw to dogs	0.8	51302.6	1.0
Given to dogs after cooking	-1.0	51480.2	1.0
Thrown into bush	1.4	63671.1	1.0
Burried	14.4	51147.9	1.0

CHAPTER FIVE

5.0 DISCUSSION

5.1 *Brucella* Seroprevalence

The findings of this study show that, there was evidence of cattle exposure to *Brucella* species pathogen in Sumbawanga Municipality of Rukwa region however, at much lower rates than has been reported elsewhere in Tanzania which range from 1-30% (Shirima, 2005; Lyimo, 2013; Chitupila *et al*., 2015; Assenga *et al*., 2015). Individual animal and herd level seroprevalence of brucellosis in cattle was found to be 0.8% and 2.8% respectively based on OIE recommended c-ELISA confirmatory test results. The sero prevalence observed could be associated with natural field exposure to *Brucella* pathogen as none of the respondents had reported their cattle to have been vaccinated against brucellosis.

Similarly, low prevalence of brucellosis has also been reported at 0.6% in smallholder cattle of Iringa (Karimuribo *et al*., 2007). Low percent of cattle introduced into the study area from the infected districts may be one of the reasons for low prevalence of brucellosis in the study area. This observation agrees with the findings of other studies (Swai *et al*. 2005; Shirima, 2005; Matope, 2009) that mode of acquisition of animal has impact on the spread and transmission of brucellosis whereby, homebred animals showed low seropositive results while, higher seropositivity to *Brucella* antibodies was associated with introduction of infected cattle from outside the herds. In the study area 3/4 of restocked cattle were from within the herds and in the same District.

Stall feeding which is practised by few farmers in the study area may be another contributing factor to low prevalence since it minimises the risk of exposure of zero grazed cattle to *Brucella* pathogen. This is similar to the observations by Swai (2010).

However, the “cut and carry” feeding system works better where fodder is taken from own enclosed field as observed from a few respondents in the study area therefore, concurs to other study (Karimuribo e*t al.*, 2007) that, it was found to lower spread and transmission of disease.

The dominating agro-pastoral farming system with small herds of cattle in the study area may also be the reason to the low prevalence observed unlike the higher prevalence in pastoral system therefore, results are consistent to other study findings in the country (Shirima, 2005; Karimuribo *et al.*, 2007). In agro-pastoral farming system like in this study area, animal movements are restricted by small land sizes available for grazing therefore, lead to low herd to herd and animal to animal contacts resulting to low spread and transmission of the disease.

With regard to the age of cattle, in this study there were no positive reactors among the young cattle. Seropositive animals were all adult. This agrees with the studies carried out in other parts of Tanzania (Swai and Schoonman, 2010; Chitupila *et al*., 2015; Assenga *et al*., 2015) and elsewhere (Degefu *et al.*, 2011; Egaru *et al*., 2013). Brucellosis is essentially a disease of sexually mature animal where the long exposure years is one of the essential factors which influence susceptibility of *B. abortus* infection (Chimana *et al*., 2010; Egaru *et al*., 2013). This finding could be due to the fact that sex hormones and sugar alcohol (erythritol) stimulate growth and multiplication of *Brucella* organisms and the concentration of secreted hormones tend to increase with age and sexual maturity (Ferede *et al.,* 2011).

The study also found that among the 3 exposed animals, 2 were males while 1 was female cattle. The higher prevalence in males may be influenced by the small number of tested males (120) compared to females (234). However, the results concur to other studies

(Chimana *et al*., 2010; Mai *et al*., 2012) who reported higher *Brucella* seroprevalence in bulls than cows. The findings are contrary to Assenga *et al*. (2015) and Chitupila *et al*. (2015) who reported significantly higher *Brucella* seroprevalence in female than male cattle. In this study area, as had been found by Msanga *et al*. (2012) male cattle are preferred and valued more for providing draft power for crop farming. For this reason, they are also retained longer in the herds and may be are subjected to prolonged exposure to *Brucella* pathogens if any.

With regard to the herd size, in this study, all 3 seropositive cases were found in small herds (≤ 10 cattle) n = 66. These findings are similar to other studies (Chitupila *et al*., 2015; Lyimo, 2013), reported significantly high *Brucella* seroprevalence in small herds. Contrary to these study findings, other studies (Swai and Schoonman, 2010; Al-Majali *et al*., 2009) had reported higher prevalence in large herds where contact between animals in the herd is high. The current observation might be influenced by higher proportion of the sampled small scale herds (n = 66) than the medium (n = 26) and large scales (n = 16) respectively in the study.

5.2 Awareness of Brucellosis and Risky Practices

Lack of knowledge and awareness on brucellosis is an open door for spread and transmission of brucellosis. Knowledge of a disease is a primary step for development of strategies for control and eradication measures. Majority of livestock keepers had no knowledge and awareness about brucellosis and its zoonotic potential. The findings are in agreement with the observations of other studies (Lyimo, 2013; Karimuribo *et al*., 2007 and Chitupila *et al.,* 2015) that, lack of knowledge may highly contribute to transmission and spread of brucellosis to both animals and humans. Since large proportion of livestock keeper had primary education and above, means that they are more likely to understand

and adopt knowledge if they are sensitized. However, the study did not find statistical significant relationship between farmers' knowledge and *Brucella* seropositivity in the study area.

Improper disposal of placenta and aborted foetal material as observed to be done by nearly half of the respondents are among the practices which were considered to be the potential risk factors associated with *Brucella* seropositivity in the study area. However, there were no statistical significant relationship between these unhygienic practices and *Brucella* seropositivity in the study area. The findings of this study are contrary to other studies in Tanzania and elsewhere (Chitupila *et al.*, 2015; Egaru *et al.*, 2016) who reported those practices as risk factors for transmission and spread of brucellosis. As a measure to maintain the current status of brucellosis in the study area, farmers should bury or burn placenta and aborted foetal materials.

The study had found high herd to herd interaction with inclusion of small ruminants in the communal grazing areas and the available water points. This concentration of cattle on the scarce pasture and water points was considered to cause contamination of the environment with aborted foetal materials and uterine fluids from infected normal calving. However, the current study found no statistical significant relationship between mixing of herds and *Brucella* seropositivity in the study area. These findings are contrary to observations of other studies in Tanzania and elsewhere (Chitupila *et al.*, 2015; Matope, 2011; Chimana, 2011; Egaru, 2013) who reported mixing of herds in communal grazing grounds and water points as risk factors for spread and transmission of brucellosis. The difference in this agro-pastoral study area may also be due to the fact that, in practice, only a few adjacent herds with low number of cattle share communal grazing grounds and water points therefore, the mixed herds in the grazing grounds originate from the same area with

minimum freedom of movements because of crop farming hence, risk of spread and transmission of disease if any, is highly minimized.

In the study area, natural mating and sharing of bulls was found to be the dominating breeding method and practice respectively. One could have expected high prevalence of brucellosis in the area resulting from natural breeding and sharing of bulls. May be this is not the case because among forty six tested breeding bulls none was c-ELISA seropositive. However, the reported observations in the country and elsewhere show that transmission of brucellosis through natural mating is uncommon, unlikely or epidemiologically non-significant (Norman *et al.*, 1998; Shirima, 2005; Matope, 2009). Therefore, more and long term research studies are required so as to establish the extent of risk since natural breeding and sharing of bulls are the major practices in the study area and elsewhere in the country.

Handling of manure by piling outside the *boma* to dry then using it for animal bedding as was found in the study area was thought to be a potential risk factor for transmission of the disease to both cattle and humans through contact and aerosol. However, in this study there was no significant relationship between manure handling and c-ELISA seropositivity may be because the data shows very low magnitude of exposure. Furthermore, according to Aiello and Moses (2010), *Brucella* organisms can survive up to six months in manure particularly in moist condition which is not the case in Sumbawanga Municipality where they experience long dry season of about six to seven months per annum extending from May to mid-November. This dries manure to the extent that no enough moisture to support survival of *Brucella* organisms.

As has been observed in the results of this study, no one amongst the predictor variables had shown statistical significant relationship with *Brucella* seropositivity in the study area. May be, this is influenced by the observed very low magnitude of infection (3/354) which statistically had indicated lack of variability by very large standard errors. However, management practices reported as risk factors in Tanzania and elsewhere are the same as those practised in the study area such as, mixing of herds of cattle and flocks of small ruminants in the grazing ground and water points, sharing of bulls, improper disposal of placenta and aborted foetal materials and introduction of animals with unknown health status into herds, to mention a few. With this observation, prompt control strategies to maintain the current low infection are required for the disease not to spread further in the study area.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From this study, the following conclusion can be made:

i) There was an evidence of low (0.8%) exposure of cattle to *Brucella* pathogens in Sumbawanga Municipality.

ii) None of the investigated potential risk factors had statistical significant association with *Brucella* seropositivity in the study area. This implies there was a low level of transmission of brucellosis in this study area as compared to other parts of the country.

6.2 Recommendations

From the study the following recommendations are made:-

i) This was cross-sectional study; longitudinal study should be carried out in this study area to determine the trend of the disease in the area and Rukwa region at large.

ii) Findings from this study may provide baseline information that might guide the design of strategies for prevention and control measures against brucellosis in the area.

iii) Further studies at molecular level to characterise and identify *Brucella* species prevalent in the study area are called for.

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APPENDICES

Appendix 1: Questionnaire for study on Risk factors associated with spread and transmission of brucellosis in cattle in Sumbawanga Municipality

I. General information

1. Number of respondent _____Phone number_____

2. Village: _____

3. Date of interview: Year: _____ Month: _____Day: _____

4. Position of the person interviewed _____

   1 = Head of household?

   2 = Other member.

5. Age of the respondent: _____ year group.

   1 = 18 – 35

   2 = 36 – 53

   3 = 54 – 71

6. Sex of the respondent_____

   1 = Male

   2 = Female

7. Highest level of education completed by the respondent. _____

   1 = No formal education

   2 = Primary education,

   3 = Secondary school education

   4 = Higher education.

8. Which Breed and number of cattle do you have in your household.

a) 1= Local zebu (LZ) _____

1 = 1- 10

2 = 11 – 25

3 = more than 25

b) 2= Dairy breed (DB) _____

1 = 1- 10

2 = 11 – 25

3 = more than 25

c) 3 = Cross breed (zebu with dairy) - DCB_____

1 = 1- 10

2 = 11 – 25

3 = more than 25

9. Do you have goats? _____

1 = Yes

2 = No

If yes how many_____

0 = none

1 = 1- 10

2 = 11 – 25

3 = more than 25

10. Do you have sheep? _____

1 = Yes

2 = No

If yes how many _____

1 = 1- 10

2 = 11 – 25

3 = more than 25

11. How long have you been keeping cattle? _____

1 = 1 – 3 years

2 = 4 – 10 years

3 = more than 10 years

II. Knowledge of practices predisposing humans to brucellosis

1. Who is primarily responsible for looking after the animals?_____

1 = Owner

2 = Family member

3 = Hired labour

2. Do you help animals in calving/ kidding/lambing?_____

1 = Yes

2 = No

3. Do you wear any protective gear when helping birthing animals?_____

1 = Yes

2 = No

4. Do you happen to drink raw milk? _____

1 = Yes

2 = No

5. Do you consume other products made from raw milk (butter, cheese)?_____

1 = Yes

3 = No

III. Risk factors for spread of brucellosis among livestock and wild animals to livestock

1. What type of feeding / grazing system do you practice? _____

1 = Communal pastures

2 = Own fields / paddocks

3 = Communal and own pasture grazing

4 = Cut and carry from own enclosed field.

5 = Cut or buy from other areas

2. Are the cattle herded with sheep and goats? _____

1 = Yes

2 = No

3. During grazing in communal pastures does your livestock mix with other livestock from different herds? _____

1 = Yes

2 = No

4. Have you ever observed wild animal grazing in your village communal pastures?_

1 = Yes

2 = No

5. Where do your animals drink water? _____

1 = Shared/Communal watering points

2 = Own watering points

3 = Own and communal watering points

6. Do your livestock share drinking water points with wild animals? _____

1 = Yes

2 = No

7. Do you buy livestock for replacement or addition into your herd? _____

1 = Yes

2 = No

8. If yes, where did you buy your animals? _____

1 = Within the village

2 = Within the district

3 = Outside the district

4 = Outside the region

0 = none

9. Do you practice bucket feeding to calves? _____

1 = Yes

2 = No

10. If yes, do you feed calves the milk from cows other than their mothers? _____

1 = Yes

2 = No

11. Which breeding method do you practise in your farm? _____

1 = Natural mating

2 = Artificial Insemination

3 = Both (Natural mating and Artificial Insemination)

12. Do you share bulls with other herds? _____

1 = Yes

2 = No

IV. Livestock farmer's knowledge of brucellosis in livestock

1. Have you ever heard about brucellosis in livestock? _____

1 = Yes

2 = No

2. If yes, from which source? _____

1 = Extension Officer

2 = Other farmers

3 = Media (Radio, Television, News paper)

4 = Livestock production books

0 = None

3. If yes, do you know how is it transmitted? _____ (if yes, ask how).

1 = Yes

2 = No

4. Did any of your animals abort? _____

1 = Yes

2 = No

5. If yes, when did it occur? (year) _____

0 = None

1 = 2014/2015

2 = 2013/2014

3 = 2012/2013

6. Where did it occur? _____

0 = none

1 = In the boma

2 = Outside the boma

3 = In the grazing areas

7. At what stage of gestation did abortion occur _____

0 = none

1 = First trimester (0 - 3 months)

2 = Second trimester (4 - 6 months)

3 = Third trimester (7 - 9 months)

8. What methods of disposing aborted foetus did you use? _____

0 = none

1 = Thrown raw to dogs

2 = Given to dogs after cooking

3 = Thrown into bush

4 = Buried

5 = Left in the grazing areas

9. Do you experience cases of retained placenta in your animals?_____

0 = none

1 = Yes

2 = No

10. Which method do you normally use to dispose placenta? _____

0 = none

1 = Thrown raw to dogs

2 = Given to dogs after cooking

3 = Thrown into bush

4 = Buried

6 = Left in the grazing areas

11. Have your animals ever been tested for Brucellosis? _____

1 = Yes

2 = No

12. If yes, which year _____

1 = 2003 – 2005

2 = 2006 – 2008

3 = 2009 – 2011

4 = do not remember

13. If yes, what measures were taken to the brucellosis positive animals? _____

1 = Still within the herd

2 = Sold for slaughter

3 = Sold to another farmer

14. Have your animals ever been vaccinated against brucellosis? _____

1 = Yes

2 = No

15. If yes, when vaccination was carried out? _____

1 = 2002 – 2004

2 = 2005 – 2008

3 = 2009 – 2012

4 = 2013 - 2015

16. Do you have cows that have given birth in 2014 but now fail to conceive?____

1 = Yes

2 = No

17. If yes how many? _____

0 = none

1 = 1 to 5

2 = 6 to 10

3 = more than 10

18. Why do you think this problem happens? _____

Reason

0 = none

1 = Lack of breeding males in the herd

2 = Problem from previous parturition.

3 = Recently mounted

4 = Failure to detect heat on time

5 = Animals are too old

7 = The animal do not show clear heat signs

8 = Lack of money to hire a male for service on time

19. What did you do with such animal? _____

0 = none

1 = Just left in the herd

2 = Slaughtered at home

3 = Sold

4 = Given as gift

5 = Given out as dowry

20. How was manure handed in 2014? _____

1 = Collected outside the boma/ house

2 = Taken out to dry and returned as bedding

3 = Collect outside and taken to field

4 = Collect and sell to people

5 = Use for biogas

6 = Use for burning

7 = Use to plaster pots and storage bins

V. Wildlife in the area

1. How frequently do you see the following wildlife species in the grazing grounds?

   i) Dikdik _____

      1 = often

      2 = occasionally

      3 = Never

   ii) Impala _____

      1 = often

      2 = occasionally

      3 = Never

   iii) Thompson Gazelle _____

      1 = often

      2 = occasionally

      3 = Never

Appendix 2: Blood sample collection register

WARD CODE	NUMBER AND NAME OF FARMER		GRID POSITION (UTM coordinate system) meters	CODE	CATTLE ID No.	SEX	AGE	BREED	SAMPLE CODE	CATTLE IN THE HERD
A	01	LULU NACHAN	347195 E 9118864 N	01	643	F	AD	DCB	A0101	6
				02	1728	F	AD	DB	A0102	
				03	1729	M	Y	LZ	A0103	

KEY: E = East, N = North, F = Female, M = Male, AD = Adult, Y = Young, DCB = Dairy cross breed, DB = Dairy breed, LZ = Local zebu

Appendix 3: Permission letter from Municipal Director

HALMASHAURI YA MANISPAA YA SUMBAWANGA

Simu Na: +25 25 -2802163

Nukushi Na: +255 25 2802163
Barua pepe:msumbawanga@yahoo.com

S.L.P-187

SUMBAWANGA

REF. NO. SMC/D.50/25/176

13/11/2015

MAKAMU MKUU WA CHUO,
CHUO KIKUU CHA SOKOINE,
S.L.P 3000,
MOROGORO.

YAH: KUKUBALIWA KUFANYA UTAFITI NDUGU MAENGO RESPICH

Rejea barua yako yenye kumbukumbu namba **SUA/ADM/R.1/8** ya tarehe **23 October 2015** iliyohusu kuomba kibali cha kufanya utafiti katika Halmashauri ya Manispaa ya Sumbawanga.

Kwa barua hii ofisi ya Mkurugenzi wa Manispaa ya Sumbawanga inapenda kukutaarifu kuwa ndugu ***MAENGO RESPICH*** amekubaliwa ombi lake la kufanya utafiti katika Halmashauri ya Manispaa ya Sumbawanga kuanzia **November 2015** mpaka **September 2016** kama alivyoombewa kufanya utafiti huo unaohusu **"uchunguzi wa uwepo wa ugonjwa wa ng'ombe kutupa mimba (Brucellosis) wenye madhara kwa binadamu, pia kutathmini visababishi vya uwepo na usambaaji wa ugonjwa huo kwa ng'ombe"**

Ofisi itakuwa tayari kutoa ushirikiano kadri atakavyohitaji.

Ofisi inamtakia Utafiti mwema

Johaness.C.Rugalabamu
Kny. Mkurugenzi wa Manispaa
SUMBAWANGA

Nakala:-
Bw, Maengo Respich,
Mtafiti.