

**ASSESSMENT OF *BRUCELLA ABORTUS* AND ANTIMICROBIAL RESIDUES
IN RAW CATTLE MILK IN BUKOMBE DISTRICT, TANZANIA**

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
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ABSTRACT

A cross sectional study was carried out in January 2017 with the aim of estimating the seroprevalence of *Brucella* circulating antibodies, detection of *Brucella abortus* in raw cattle milk, assess the risk factors for infection and establish the presence and levels of antimicrobial residues in raw milk in Bukombe district, Tanzania. A total of 221 blood samples from purposively selected lactating cows in 17 villages were collected and analysed for *Brucella* circulating antibodies using Rose Bengal Plate Test and c-ELISA. Also 221 raw milk samples were collected from the same cows and analysed for antimicrobial residues using Delvo SP® test and subsequently, Tetracyclines (Chlortetracycline, Tetracycline and Oxytetracycline) were analysed and quantified in 10 Delvo test positive milk samples using HPLC. Milk samples of seropositive animals (n=3) were analyzed by PCR to detect *bcs31* gene of *B. abortus* using B4/B5 primer sets. The overall animal seroprevalence of brucellosis was 1.4% (n=219) and herd seroprevalence was 3.8% (n=52). One milk sample was confirmed to contain *bcs31* gene of *B. abortus*. The proportion of antimicrobial residue contaminations in milk was 11.6%. Tetracyclines were confirmed and quantified in 9/10 of selected Delvo positive raw milk samples. Tetracyclines concentration was 6.1 ± 5.8 ug/l with the mean concentrations of oxytetracycline and tetracycline being 7.7 ± 5.3 ug/l and 9.6 ± 16.9 ug/l respectively which were all below the recommended MRL of 100 µg/l. Interestingly, 70% of the quantified samples had oxytetracycline and only 40% had tetracycline. It is concluded that Bovine brucellosis is present in Bukombe district albeit at low prevalence (1.4%). Also detection of antimicrobial residues in raw milk samples (11%) may pose risk to consumers especially children who frequently drink milk. It is recommended that veterinarians in cooperation with other stakeholders and farmers to observe good livestock practices.

DECLARATION

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

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Date

The declaration above is confirmed by;

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DEDICATION

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations	Descriptive meaning
AOAC	Association of Official Analytical Chemists
B4	Forward primer
B5	Reverse primer
Bp	base pair
BQ	Black quarter
C.L	Confidence Limit
C18	Column 18
C8	Column 8
CAC	Codex Alimentarius Commission
c-ELISA	Competitive Enzyme Linked Immuno-Sorbent Assay
CFT	Compliment Fixation Test
COSTECH	Commission of Science and Technology
CTC	Chlortetracycline
DED	District Executive Director
DNA	Deoxy ribose Nucleic Acid
DVO	District Veterinary Officer
DVS	Director for Veterinary Services
EDTA	Ethylene diamine tetraacetic acid
FAO	Food and Agriculture Organization of the United Nations
FMD	Foot and Mouth Disease
FPIA	Fluorescence Polarisation Immunoassay
GARP	Global Antibiotic Resistance Partnership

GDP	Gross Domestic Product
HPLC	High Performance Liquid Chromatography
IDF	International Dairy Federation
kDa	Kilo Dalton
LC	Liquid Chromatography
LC-MC/MC	Liquid Chromatography-tandem Mass Spectrometry
LC-MS	Liquid Chromatography with Mass Spectrometry
LOD	Limit of Determination
LOQ	Limit of Quantification
LSD	Lumpy Skin Disease
MJNUAT	Mwalimu Julius K. Nyerere University of Agriculture and Technology
MRL	Maximum Residue Limit
MRT	Milk Ring Test
OD	Optical Density
OHCEA	One Health for Central and East Africa
OIE	Office International des epizooties
PBP	Penicillin Binding Proteins
PCFIA	Particle-Concentration Fluorescence Immunoassay
PCIA	Particle-Concentration Immunoassay
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
REA	Restriction Endonuclease Analysis
SAT	Serum Agglutination Test
SPE	Solid Phase Extraction

SUA	Sokoine University of Agriculture
TBE	Tris Boric EDTA
TCs	Tetracyclines
TSHZ	Tanzania Short Horn Zebu
TTC	Tetracycline
UHT	Ultra Heat Temperature
UK	United Kingdom
UPLC-MC	Ultra-performance Liquid Chromatography-Mass Spectrometry
URT	United Republic of Tanzania
USA	United States of America
WHO	World Health Organization
κ	Kappa

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Livestock sector in Tanzania grows at a rate of 3.4% and contribute to about 3.8% to the Gross Domestic Product (GDP). Of the 3.8% GDP contribution by livestock sector 40% comes from beef, 30% from dairy industry and 30% from other livestock sources (Chaussa, 2013). The dairy industry is among of the potential sectors in Tanzania whose value is to reduce poverty and improve people's livelihoods. It does so by improving nutrition through milk consumption and trading of milk and milk products. In Tanzania, dairy cattle is considered of dairy category when producing an average of 2000 litres of milk per lactation where as others are dual purpose indigeneous that produce an average of 300-500 litres of milk per lactation (Njombe *et al.*, 2011; URT, 2011). However 70% of the milk produced in Tanzania comes from indigenous cattle kept in rural areas and the remaining 30 % is from improved dairy cattle kept by smallholder farmers. Tanzania has 21.3 million cattle of which about 680, 000 are dairy cattle cross breeds of Friesian, Ayrshire, Jersey and Tanzania Shorthorn Zebu (TSHZ). Milk production has increased from 814 million litres in 2000/01 to 1.65 billion litres in 2009/10 (URT, 2010; Njombe *et al.*, 2011).

Indigenous cattle are reared under traditional farming system namely; pastoral and agro-pastoral systems. Most of the dairy cattle are reared under the smallholder farming and a few are reared under medium and large scale farming. Due to remoteness and poor infrastructure milk collection and marketing in pastoral and agro-pastoral settings is done with difficulties. Henceforth, most of the milk is consumed locally. A few of the traditional producers residing in peri urban areas travel long distances by motorcycles to

market their milk produce. Nevertheless, plenty of the milk is sold raw informally and little is available for processing (URT, 2010; URT, 2011; Engida, 2015).

Milk is nutritionally important throughout the world used as human food and therefore, quality and safety are the priority to safeguard consumers (Dey and Karim, 2013). Among the milk-borne zoonoses that may result from consuming unpasteurised milk and milk products include tuberculosis, brucellosis, enterohemorrhagic colitis, listeriosis, Q fever, staphylococcal food poisoning, campylobacteriosis and salmonellosis (Mekuria *et al.*, 2014). Some of these milk-borne zoonoses have been reported in humans in Tanzania which calls for more concerted efforts to control the infections in livestock otherwise the rate of infections may keep on increasing (Mbugi *et al.*, 2012; James, 2013; Bukuku *et al.*, 2015, Chuma *et al.*, 2016).

Brucellosis is a potential worldwide zoonotic disease known to cause non-specific signs to infected humans. This scenario may confuse the diagnosis of the disease with other febrile illnesses like malaria, typhoid fever and rheumatic fever. The situation is even worse in poor resource countries like Tanzania where diagnostic facilities are insufficient. Nevertheless, surveys done in Tanzania show that the prevalence of brucellosis in cattle ranges between 2% and 90% (Mahlau, 1967; Swai, 1997; Weinhäupl *et al.*, 2000; Swai *et al.*, 2005; Karimuribo *et al.*, 2007; Temba, 2012; Chitupila *et al.*, 2015; Assenga *et al.*, 2015). The factors for persistence of brucellosis in livestock are based on the grazing system, lack of test and slaughter policy and lack of practical disease control programmes. The prevalence of brucellosis in some of the pastoral and agro pastoral communities in Tanzania ranges between 0.7% and 58.1% (Kunda *et al.*, 2005; Kunda *et al.*, 2007; Swai, 2009; Mellau *et al.*, 2009; James, 2013). A study by Mellau *et al.*, (2009) reported the increase of human brucellosis from 35.6% in 2004 to 58.1% in 2005 in livestock-wildlife

interface in Serengeti ecosystem which mostly is inhabited by pastoralists whose animals interacts with wildlife. Humans are infected through contacts and consumption of raw unpasteurized milk, raw meat and blood (Tumwine *et al.*, 2015; Adesokan *et al.*, 2016; Tadesse, 2016). The current study estimated the prevalence of *Brucella* infection and established the circulating *Brucella* species in indigenous cattle in Bukombe District, Tanzania.

The uses of antibiotics in farm animals is a common practice in Tanzania for treatment of diseases, sometimes are used for prophylaxis and growth promoters in livestock (Katakweba *et al.*, 2012). Uses of antibiotics in Tanzania and other developing countries are not controlled; farmers can access any kind of these medicines over the counter without prescriptions (Katakweba *et al.*, 2012). Apart from indiscriminate uses of antibiotics in farm animals, farmers do not abide to drug withdrawal periods before they can harvest the food of animal origin like milk. Improper veterinary uses of antibiotics, as well as inadequate knowledge of the necessary withdrawal time, can easily make the drugs or their derivatives appear in the marketed animal products such as milk, meat and eggs. The occurrences of antibiotic residues in raw milk in Tanzania have been reported to range between 0 and 36% (Karimuribo *et al.*, 2005; Kurwijila *et al.*, 2006; Kaale *et al.*, 2008; Mdegela *et al.*, 2009; Rwehumbiza *et al.*, 2012; Bukuku *et al.*, 2015). The 36% occurrence is very high which implies that the community has been consuming milk with antibiotic residues. The outcome of this is the occurrences of antibiotic residues in food which otherwise have a number of effects which may include emergence of antibiotic resistance in bacteria and allergies in children. Nevertheless, Food and Agricultural Organization and World Health Organization (FAO/WHO) and the European Union (EU) have recommended a maximum residue limit (MRL) of 100 µg/l for tetracycline, oxytetracycline and/or chlortetracycline (singly or in combination) in milk (Applegren *et*

al., 1999; EU, 2009; CAC, 2012). Different antibiotics have also different set residue limit.

Antibiotic resistance is an emerging public health problem worldwide as so many bacteria have multidrug resistance against the commonly used antibiotics in humans and animals (Katakweba *et al.*, 2012). The use of antibiotics in dairy industry has led to existence of resistant genes in human pathogens making treatment of infectious diseases a problem worldwide. The antimicrobial resistance develops because of suboptimal exposure of the drug residues to the pathogens in milk (Karimuribo *et al.*, 2005; Kunda, 2015). The current study assessed the antibiotic residues in raw milk from indigenous cattle in Bukombe District, Tanzania.

1.2 Problem Statement and Justification

Brucellosis and antimicrobial residues are of concern in Tanzania as they threaten public and animal health, mainly attributed to inadequate veterinary and public health services. Both threats in the small holder dairy farming have been attributed to several factors including poor compliance to good animal husbandry, drug withdrawal periods, milk hygiene, personal hygiene, proper milk handling and storage. It is imperative to establish the status of brucellosis exposure in lactating animals as one of the important milk-borne zoonoses pathogens in Tanzania. Irrational use of antibiotics in animals by extensional officers and farmers themselves without proper compliance to drug withdrawal periods prompted to establish the status of antimicrobial residues in Bukombe.

Bukombe as one of the district in Geita region has many livestock keepers who supplies high proportion of milk to the urban community but its quality in terms of milk-borne zoonoses and antimicrobial residues is not documented. It has been reported in some

traditional cattle herds to experience abortions here and there. Some incidences of still births had also been observed in some pregnant cows in various herds of traditional cattle farmers, all of these events gave a suspect of brucellosis in the traditional cattle in Bukombe (Mashaka, C.S. personal communication, 2016). Therefore, this study aimed to estimate the prevalence of *Brucella* exposure and assess the possible risk factors for exposure. Also studies on antimicrobial residues in milk are limited in Tanzania and the reports available mostly reported qualitative information on antibiotic residues in milk. The current study has reported both qualitative and quantitative data for antimicrobial residues in raw milk from indigenous cattle in Bukombe district.

1.3 Overall Objective

The purpose of this study was to estimate the prevalence of *Brucella* circulating antibodies and responsible species in lactating cows and establish antimicrobial residues in raw milk in Bukombe district for the purpose of safeguarding the general public especially milk consumers.

1.4 Specific Objectives

- i. To establish the risk factors of *Brucella abortus* infection in cattle
- ii. To estimate the seroprevalence of brucellosis in lactating cattle
- iii. To establish the qualitative and quantitative levels of antimicrobial residues in raw milk
- iv. To establish the occurrence of *Brucella abortus* in raw milk using diagnostic polymerase chain reaction.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Milk and Milk Composition

Milk is the product of all mammals which is highly nutritious used as a primary source of nutrition and food for offspring of mammals before they are able to eat and digest other types of food. It is a balanced diet in which all the necessary and digestible elements for building and maintaining the human and animal body are available (Pandey and Voskuil, 2011). The main composition of milk is water (87 – 88%); and the remaining part is total milk solids which include carbohydrates, fat, proteins and ash or minerals. The composition of milk is not constant, its average percentages vary with species and breeds of animal, season, feeds, stage of lactation and health and physiological status of a particular animal (Kanyeka, 2014). Moreover, milk is an excellent source of high quality protein, vitamins, minerals such as calcium and phosphorus (Kanyeka, 2014). When milk comes from a healthy animal, it is always sterile when in the alveoli of mammalian udder but contaminations starts when is in the teat canal. Contaminations further increases as milk is in contact with the milking personnel, teat cups of milking machine, the surfaces of milk handling and storage facilities and many other possible sources of contaminations (Solomon *et al.*, 2013).

2.2 Milk Production in Tanzania

Dairy cattle is considered to be a main milk producer than other livestock in Tanzania. Dairy cattle in Tanzania has the capacity of producing milk averaging 2000 litres and dual purpose cattle produces an average of 400 litres (Njombe *et al.*, 2011; Swai and Karimuribo, 2011; Leonard *et al.*; 2016). An estimate of 1.853 billion litres of milk is produced annually in Tanzania (Wassena *et al.*, 2015). About 70% of milk in Tanzania is

produced by the traditional cattle and 30% from the improved breeds. The improved breeds are mainly crosses of Friesian, Jersey, and Ayrshire with Tanzania Shorthorn Zebu (TSHZ). Herd size is used by the cattle keepers as a factor of increasing milk production instead of milk production per lactating cow (Mbiha, 2008; Kurwijila *et al.*, 2012). A great amount of milk is consumed locally at the production sites and about 10% of the milk produced enters informal and formal market channels. Due to the remoteness and poor infrastructure, collection of milk and marketing constitute the largest bottlenecks. Nevertheless, some milk producers travel over long distances by bicycle to urban centres for selling their milk produce. Dairy industry is an important source of income for smallholder dairy farmers and it is a fundamental source of animal protein for various households in Tanzania (Njombe *et al.*, 2011; Kailembo, 2013).

2.3 Milk Collection, Processing and Milk Products

Milk collection centres in Tanzania are undertaken by farmer's groups, processors or few traders. Despite of milk collection done in places with surplus milk, the seasonal availability of milk discourages setting of milk collection centres and processing plants. The exercise of milk collection is expensive and inefficient because a considerable amount of milk is produced by the traditional cattle in rural areas where cold chain, transport and communication services are inadequate. Tanzania has few dairy producer associations which entail the process of milk collection and marketing difficult as well as discouragement of innovations (URT, 2011; Joseph, 2015). Dairy processing in Tanzania is achieved by small and medium scale dairy plants. They have capacity of processing 500 to 30000 litres of milk per day. The processing capacity at national level is 410,500 litres per day whereas the capacity utilization is about 30% which indicate inadequate availability of raw milk (Fussi, 2010; URT, 2011; Kadigi, 2013). The dairy plants in

Tanzania processes yoghurt, cheese, ghee, butter, fermented milk and pasteurised milk (Kadigi, 2013; Urassa and Martin, 2013).

2.4 Brucellosis

2.4.1 Definition and aetiology

Brucellosis is a highly infectious bacterial zoonotic disease caused by *Brucella* species of the genus *Brucella*. It is a primary reproductive disorder characterized by abortions, infertility as well as low milk production in animals (Yohannes *et al.*, 2012). The most common zoonotic *Brucella* species are *B. abortus*, *B. melitensis* and *B. suis*. *Brucella abortus* most frequently infect cattle and a few other animal species such as sheep, swine, dogs, camels and horses. (Kebede *et al.*, 2008). *Brucella* species are minute, Gram-negative, facultative intracellular, non-motile, non-spore forming, non-encapsulated and cocco-bacilli bacteria. There are 11 known genetically similar *Brucella* species with different host preferences. However six species are considered as classical *Brucella* species (Mathew *et al.*, 2015).

2.4.2 Epidemiology of brucellosis

Brucellosis is a worldwide zoonotic disease. It is endemic in Africa, Asia, Middle East, Latin America, Mediterranean Basin and Caribbean regions. Brucellosis both in animals and humans is more prevalent in sub-Saharan Africa than any other continent or region (s). As far as sub-Saharan Africa has large pastoral communities and probably the demand for meat and livestock products may double by 2050, brucellosis will pose a tremendous animal and public health threat to the region. The prevalence of brucellosis in cattle has been documented at the herd level, within-herd level and individual animal level (Yohannes *et al.*, 2012; Racloz *et al.*, 2013). The prevalence of bovine brucellosis in sub-Saharan Africa has been estimated to be 16.2% (Racloz *et al.*, 2013). Estimation of the

general prevalence of brucellosis for a country or region can sometimes be compromised by variations within the areas based on culture and animal breeds, herd composition and size, and micro-climatic features. There are significant differences of brucellosis incidences between urban and rural settings, and between pastoral and nomadic settings (Yohannes, 2012; Racloz *et al.*, 2013). Factors that can influence prevalence include; production systems, agro-ecological zones, husbandry practices, contact with wildlife and management factors (Godfroid *et al.*, 2011).

Brucellosis is a sexually transmitted disease in animals. Humans can be infected by direct contact with infected animals, contaminated animal secretions, fetuses and via retained placentas. They can also acquire infection by indirect transmission through consuming animal products mainly unpasteurized dairy products such as cheese, cottage and butter. It is an occupational disease for farmers, veterinary surgeons, and workers within the meat industry (Bertu *et al.*, 2010). It is implicated as a major source of poor reproductive performance in production animals in most parts of the world and hence production losses to livestock farmers is inevitable as well as negative economic gain to the country at large (Chitupila *et al.*, 2015).

Brucellosis is endemic in Tanzania where the animal seroprevalence has been reported to range between 1 to 30%. Studies involving wild animals have indicated a seroprevalence ranging between 4.2% to 17% in buffaloes and 24% in wildebeest. The human seroprevalence has been reported to vary between 0.7 to 20.5%. The areas in Tanzania where brucellosis have been reported include the Lake Victoria zone, Arusha, Northern Tanzania, Western zone, Manyara, Morogoro and Tanga. The interaction between wildlife, livestock and humans contributes to its persistence in Tanzania (Bouley *et al.*, 2012; Assenga *et al.*, 2015; Chitupila *et al.*, 2015).

2.4.3 Clinical manifestations of brucellosis

Clinical signs of brucellosis in animals involve; abortions usually occurs in the third trimester, still birth, retained placenta(s), declined milk production, infertility, and carpal hygromas. The clinical symptoms of the disease in humans are not specific and can easily be confused with other fever causing diseases such as malaria, typhoid fever, rheumatic fever and arthroses (Bouley *et al.*, 2012; Assenga *et al.*, 2015; Tumwine *et al.*, 2015). A study in Narok, Kenya diagnosed flu-like patients by Rose Bengal Plate Test (RBPT) and 12% of the patients were detected having *Brucella* circulating antibodies. Another study in Kampala, Uganda, studied patients with clinical features of joint pain, general malaise, and/or constant headache and 73% of the patients were found to be suffering from malaria and 13.3% from brucellosis. The clinical symptoms in humans range from acute, sub-acute to chronic form of the disease. Clinically infected humans can present acute non-specific symptoms such as; undulating fever, headache, sweating, loss of appetite, muscular pain, lumbar pain, weight loss, joint pain, malaise, hepatomegaly and splenomegaly (Kunda *et al.*, 2007; Tumwine *et al.*, 2015). The acute phase can progress to chronic phase with relapses, development of persistent localized infection or a non-specific syndrome like the “chronic fatigue syndrome”. The chronic phase is normally associated with multi-organ complications in the patients. The complications may involve; cardiovascular, testicular, cutaneous, neurological, gastrointestinal, respiratory, hepatobiliary and ophthalmic complications (Corbel, 2006).

2.5 Diagnosis of Brucellosis

The presumptive diagnosis of brucellosis both in animals and humans is done by history taking and clinical features. The laboratory diagnosis involves serological tests, molecular techniques and primary isolation of the organism (Geresu and Kassa, 2016). The microbiological culture and isolation of the organism is considered to be a confirmatory

diagnosis. Primary isolation of *Brucella* species in the laboratory is considered to be a gold standard test. Nevertheless, it is difficult to isolate *Brucella* species in the laboratory; as it requires a special media, takes several weeks of incubation, and has low sensitivity. Molecular techniques such as Polymerase Chain Reaction (PCR), Restriction Endonuclease Analysis (REA) and Hybridization are useful in diagnosis of *Brucella* species as they have high sensitivity and specificity. Nonetheless, they are mostly used in studies rather than the routine laboratory diagnosis as they are expensive and unavailable in most laboratories. This fact gives a chance for the laboratory serological tests to be used frequently in laboratory diagnosis of brucellosis than other techniques. The serological tests for both in animals and humans include; Rose Bengel Plate Test (RBPT), Fluorescent Polarization Assay (FPA), Complement Fixation Test (CFT), Mercaptoethanol test and Enzyme Linked Immunosorbent Assay (ELISA). However, Milk Ring Test (MRT) is documented for use only in animals. The direct serological tests such as RBPT are known to have false positives and negatives. The screening needs to be complimented with other tests such as Competitive ELISA (c-ELISA) and CFT to eliminate the false positives and negatives (Shirima, 2005; John, 2010; Lyimo, 2013).

2.6 Treatment of Brucellosis

There is no effective drug for treatment of brucellosis in animals and therefore more resources are directed to the control and prevention of the disease in animals. Brucellosis in humans is treated by administration of effective antibiotics for a considerable length of time, the major target being to mask the symptoms thereby preventing complications and relapses (Corbel, 2006; Franco *et al.*, 2007; Lyimo, 2013). A well devised multi-drug antimicrobial therapy is a treatment protocol of choice in favor of mono-drug treatment protocol because it is known to quickly avoid the relapses. The triple drug therapy is considered to be a better cure than two drug therapy although it is traditionally restricted to

the patients with neuro brucellosis, endocarditis and abscesses (Bosilkovski, 2010; Alavi and Alavi, 2013; Lymo, 2013). The drugs used for treatment in humans include; doxycycline, streptomycin, tetracycline, rifampicin, gentamycin and trimoxazole. However, prolonged treatment of rifampicin in tuberculosis and brucellosis endemic areas is not recommended as it increases possibility of *Mycobacterium tuberculosis* resistance (Ariza *et al.*, 2007; Bosilkovski, 2010; Hashemi *et al.*, 2012; Temba, 2012; Aliva and Aliva, 2013).

2.7 Control of Brucellosis

The eradication of brucellosis has been successfully in the developed world whereas in the developing world remains to be a great challenge. It was eradicated in the developed world by instituting test and slaughter method of all infected animals and the livestock keepers were compensated by their governments for the induced encountered loss. In the developing world like Tanzania eradication by test and slaughter is difficult because it is much expensive in terms of resources and expertise (Rubach, 2013., Zamri-Saad and Kamarudin, 2016). Therefore the control of this zoonosis in the developing world is focused in prevention of transmission of the disease to humans. More effort is directed to control brucellosis in animal populations than in human populations because animals act as reservoirs for transmission of the disease in humans. The control in animals involves animal vaccination programs which significantly reduce the prevalence of the disease both in animals and humans. The commonly used vaccines Rev1 for sheep and goats, S19 and RB51 for cattle. Public education, pasteurization of milk, control of animal movements, and testing and isolation are other useful means for zoonotic control of brucellosis both in animals and humans (James, 2013; Ducrotoy *et al.*, 2017).

2.8 Antimicrobial Residues

2.8.1 Definition and sources

Antimicrobial residues are defined as small amount of drugs or their active metabolites which remain in animal products including milk after treating animals (Bukuku, 2013). Among the reasons for the presences of antimicrobial residues are limited extension services, limited knowledge among farmers and poor animal health delivery systems, that pushes farmers to buy veterinary drugs from veterinary shops and treat sick animals (Katakweba *et al.*, 2012). They can administer incorrect dosage using wrong route of administration or sometimes correct dosage but without observing drug withdrawal period (Shitandi, 2004; Kivaria *et al.*, 2006). Antimicrobial drugs are widely used for prevention and treatment of animal diseases; growth promoters or as prophylaxis in animal feeds. (Moyane *et al.*, 2013). About 80% of all production animals are treated or given antibiotics for a part or in most of their lives (Darwish *et al.*, 2013).

Antimicrobial drugs that are used to treat cattle can be grouped into five major categories which are beta-lactams (e.g: penicillins and cephalosporins), tetracyclines (e.g. oxytetracycline, tetracycline and chlortetracycline), amino glycosides (e.g: streptomycin, neomycin and gentamycin), macrolides (e.g: erythromycin and sulfanomides (e.g: sulfamethazines). Routes for administration of drugs include intramuscular, oral (food and water), topical; intra-mammary and intra-uterine infusions. Any route of treatment used to treat lactating cows can result into antibiotic residues in milk post treatment and therefore it is important that the animals has to be left for a specified period (withdrawal period) for the drugs to be cleared from the body (Khaniki, 2007).

Tetracyclines (TCs) are a class of antibiotics with broad spectrum activity. The common ones are oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC) and

doxycycline. They are most preferred by the livestock keepers because of their broad spectrum of activity and are less costly. Moreover TCs are often incorporated into animal feedstuffs at low doses as growth promoters and for prophylactic treatment in food producing animals, chicken and aquaculture (Granados-Chinchilla and Rodríguez, 2017, Jayalakshmi *et al.*, 2017). They are believed to promote animal growth and protect animals against infectious diseases.

Antimicrobial residues in foods of animal origin are regulated by the Codex Alimentarius Commission (CAC). The commission is responsible for setting of the international food standards. The organization has established safety limits for foods and requires antibiotic residue concentration not exceeding maximum residue limits (MRLs). The MRLs for TCs recommended by CAC in cattle, fish, poultry and sheep are stipulated in Table 1. The international MRLs standards in various animal products are usually met in the developed world, but the situation is different in the developing countries like Tanzania where the standard laws and regulations for antimicrobial residues in animal products are usually not reinforced (Aalipour *et al.*, 2013; Mubito *et al.*, 2014; CAC, 2015).

Table 1: The recommended MRLs of Tetracyclines by the CAC in animal foods

Species	Tissue	MRL (µg/kg)	CAC	Notes
Cattle	Muscle	200	26 th (2003)	
Cattle	Liver	600	26 th (2003)	
Cattle	Kidney	1200	26 th (2003)	
Cattle	Milk (µg/l)	100	26 th (2003)	
Fish	Muscle	200	26 th (2003)	Applies only to oxytetracycline
Giant Prawn (<i>Paeneus monodon</i>)	Muscle	200	26 th (2003)	Applies only to oxytetracycline
Pig	Muscle	200	26 th (2003)	
Pig	Liver	600	26 th (2003)	
Pig	Kidney	1200	26 th (2003)	
Poultry	Muscle	200	26 th (2003)	
Poultry	Liver	600	26 th (2003)	
Poultry	Kidney	1200	26 th (2003)	
Poultry	Eggs	400	26 th (2003)	
Sheep	Muscle	200	26 th (2003)	
Sheep	Liver	600	26 th (2003)	
Sheep	Kidney	1200	26 th (2003)	
Sheep	Milk (µg/l)	100	26 th (2003)	

Adapted from CAC, 2015

Legend: MRL- Maximum Residue Limit; CAC- Codex Alimentarius Commission

2.8.2 Status of antimicrobial residues in food in Tanzania

In Tanzania, various studies of antimicrobial residues in animal foods were done in beef, broiler meat, eggs, and milk (Nonga *et al.*, 2009; Mgonja *et al.*, 2017). Studies of antimicrobial residues in raw milk in different parts of Tanzania showed that, consumers ingest suboptimal levels of antimicrobial residues which have significance influence of antimicrobial resistance to the commonly used antibiotics (Ramadhani, 2015). However antimicrobial residues in milk vary from place to place, and therefore a general conclusion on the level of antibiotic residues in Tanzania may be uncertain. The presence of antimicrobial residues in milk may be caused by failure to observe the required withdrawal periods, illegal use of drugs, incorrect dosage levels and route of administration (Karimuribo *et al.*, 2005; Kurwijila *et al.*, 2006; Nonga *et al.*, 2009; Nonga *et al.*, 2010; Nonga *et al.*, 2013; Kimera *et al.*, 2015). Other factors could be; poor records of treatment

leading to failure identification of treated animals, lack of advice on withdrawal periods, off-label use of antibiotics, access to antibiotics to lay persons over-the counter, extended usage or excessive dosages of antibiotics, non-existence of restrictive legislation or their inadequate enforcement and lack of consumer awareness about the human health hazards associated with antimicrobial residues in animal-derived foods (Akinwumi *et al.*, 2012; Bukuku, 2013).

Tetracyclines (TCs) are among of the commonest antibiotics in livestock production in Tanzania and other African countries. Most livestock keepers prefer buying TCs because are broad spectrum antibiotics, cheap, readily available from veterinary shops and are easily accessed by farmers without any barriers (Nonga *et al.*, 2009; Nonga and Muhairwa, 2010; Katakweba *et al.*, 2012; Nonga *et al.*, 2013; Kimera *et al.*, 2015; Mgonja *et al.*, 2017). Free availability of these kind of antibiotics without control have influenced livestock owners to abuse them unnecessarily in animal feeds and prophylactic treatments. Moreover, TCs in Tanzania are commonly sold by the veterinary drug middlemen in the livestock auctions without prescriptions. Most of the studies of antimicrobial residues in milk as well as other animal foods were qualitative based and a few of the studies were quantitatively based specifically targeting oxytetracycline residues in beef (Katakweba *et al.*, 2012; Nonga *et al.*, 2013; GARP, 2015; Kimera *et al.*, 2015; Mgonja *et al.*, 2017).

2.8.3 Effects of antimicrobial residues

About 90% of antimicrobial use in most of developing countries is delivered to clients without prescription (Laxminarayan and Heymann, 2012). This led to misuse of antimicrobials in animals that may cause antibiotic residues in animal products which have impact in human health. The health hazards attributed to antibiotic residues in animal products includes; toxic effects, transfer of antibiotic resistant bacteria to humans,

immune-pathological effects, carcinogenicity (e.g: sulphamethazine, oxytetracycline, and furazolidone), mutagenicity, nephropathy (e.g: gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (e.g: chloramphenicol) and allergy (e.g: penicillin) (Nisha, 2008; Beyene, 2016). Antibiotic residues in milk have been known to cause allergies in humans and actually development of resistant bacteria in long term basis (Kivaria *et al.*, 2006; Forouzan *et al.*, 2014). Although studies indicated that, suboptimal doses of TCs promote antimicrobial resistance, allergic reactions in humans, risk of teratogenicity when administered in the first trimester of pregnancy and permanent discoloration of teeth in infants or children of less than 12 years old consuming contaminated milk whereas little has been done to control its applications (Aalipour *et al.*, 2013; Kebede *et al.*, 2014; Layada *et al.*, 2016; Granados-Chinchilla and Rodríguez, 2017).

2.8.4 Detection of antimicrobial residues in milk

For the assurance of food quality, detection methods for the antimicrobial residues in milk are commonly used worldwide. The methods are categorized in two major groups such as; screening and confirmatory assays. Examples of screening assays are Enzyme Linked Immunosorbent Assay (ELISA), biosensor and microbial inhibition tests whereas confirmatory assays are High Performance Liquid Chromatography(HPLC), Liquid Chromatography with Mass Specctrometry (LC-MS), Liquid Chromatography-tandem Mass Spectrometry (LC-MC/MC) and Ultra-performance Liquid Chromatography-Mass Spectrometry (UPLC-MC) (Karmi, 2014; Padol *et al.*, 2015; Jayalakshmi *et al.*, 2017).

2.8.4.1 Screening (Qualitative) assays

They work on the principle of the variable susceptibility of bacteria to different antimicrobials. The antimicrobial residue screening methods that are readily available use

different methods and test microorganisms. The commonly used qualitative assays are microbiological, enzymatic and immunological assays (Navratilova, 2008; Karmi, 2014; Padol *et al.*, 2015).

i. Microbial growth inhibition assays

The assays employ the use of the standard culture of the test microorganism in liquid or solid medium. Milk sample to be analysed is applied on the agar surface and the plates are incubated for diffusion of the sample into the medium, and if the sample contains inhibitor agents, inhibition of growth occurs of the tested microorganism. The positive test is indicated either by formation of a clear zone of inhibition around the disc or a change in the colour of medium. The commonly used test microorganisms are *Geobacillus stearothermophilus* var. *calidolactis*, *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea*, *Escherichia coli*, *Bacillus cereus* or *Streptococcus thermophiles*. The advantages of these assays is that they have a wide detection spectrum, simple to carry out, reliability and they are cheap and can be used for the screening of a large number of samples. However they have low sensitivity and specificity. Microbial inhibitor tests detect a wider range of antimicrobial substances, including β -lactam antibiotics, and give a result within 3 hours or less (Navratilova, 2008; Karmi, 2014; Padol *et al.*, 2015; Layada *et al.*, 2016).

ii. Receptor/Protein binding assays

The principle of these assays is a protein conjugating to an enzyme. The conjugate binds to the free β -lactam antibiotics that may be present in a milk sample. The assays are used for the detection of β -lactam antimicrobial residues in the bulk milk or milk from individual lactating animals. Antibiotic specific receptor proteins or penicillin-binding proteins (PBP) are incorporated in commercially available tests. The common ones are; Penzym test, Beta Star test, Charm Safe Level test, SNAP test, DELVO-X-Press test and Biacore analysis (Navratilova, 2008; Nikolić *et al.*, 2011; Padol *et al.*, 2015).

iii. Immunoassays

The immunoassays work on the principle of specific antibody-antigen reactions. Immunoassays are well known for their high sensitivity and specificity, simplicity and are less costly. They are useful in quantitative/semi quantitative assays of antimicrobial residues in milk. They can be used for screening antimicrobial residues in milk and preliminary identification of classes of antibiotics. The non-isotopic immunoassays include Enzyme Linked Immunosorbent Assay (ELISA), Fluorescence Polarisation Immunoassay (FPIA), (Particle-Concentration Immunoassay (PCIA), Particle- Concentration Fluorescence Immunoassay (PCFIA), and monoclonal-based immunoassays (Navratilova, 2008; Gaurav *et al.*, 2014; Layada *et al.*, 2016).

2.8.4.2 Confirmatory (Quantitative) assays

These are physico-chemical methods based on chromatographic principles of analysis. They are involved in isolation, separation, confirmation and quantification of specific analytical antimicrobial residues contained in foods including milk. They have high sensitivity and specificity, high precision and capable of quantifying specific analytes at low concentrations. The chromatographic methods used in antimicrobial residue quantification include High Performance Liquid Chromatography (HPLC), gas chromatography, ionic chromatography, size exclusion chromatography, superficial fluid chromatography and affinity chromatography (Aytenfsu *et al.*, 2016; Jayalakshmi *et al.*, 2017). Spectrometric methods are also used either alone or coupled to chromatographic or immunochemical methods such as ultraviolet-visible absorption spectrometry, absorption spectrometry in the near and middle infrared sections, fluorescence and chemiluminescence spectrometry, X-ray fluorescence spectrometry, atomic absorption spectrometry, atomic emission spectrometry, inductively-coupled plasma atomic emission spectrometry, nuclear magnetic resonance, mass spectrometry and mass spectrometry in

tandem. (Navratilova, 2008; Karmi, 2014; Kebede *et al.*, 2014; Padol *et al.*, 2015; Aytenfsu *et al.*, 2016).

2.8.5 Control of antimicrobial residues in foods

The control of antimicrobial residues in animal derived foods has been adapted from Darwish *et al.*, (2013) and IDF Fact sheet (2014).

The risk of antimicrobial residues in foods of animal origin can be controlled by abiding to two main pillars namely:

1. Avoiding antimicrobial residues in animal foods by adequate management practices and a prudent use of antimicrobials. It can be adhered by observing preventive measures to keep animals healthy and strictly prescribing withholding times in case of treatments.
2. Effective screening with suitable analytical methods and procedures to detect eventual contaminated batches throughout the food chain as early as possible and discarding.

The following are the control tips expounding the information contained in the above pillars:

- The effective prevention of infectious diseases and the adoption of strict hygiene standards.
- The use of alternatives to antibiotics, such as plant-derived antimicrobial substances and probiotics.
- The reduction of unnecessary prophylactic treatment in animals.
- Strict national legislation must be passed around the world to avoid the unnecessary use of antibiotics.
- National surveillance and monitoring of antimicrobial residues in foods.
- Updating of the MRLs in animal foods.

- Antimicrobial use in feed additives should be abandoned.
- Avoid the use of antibiotics for treatment of animals without a veterinarian's prescription.
- Strict observation of antimicrobial withdrawal periods.
- The avoidance of antimicrobials lacking clearly documented pharmacokinetics and pharmacodynamics properties.
- The heat treatment of meat, milk, and eggs may inactivate antimicrobial contaminants in animal foods.
- The freezing of foods of animal origin, may sometimes reduce antimicrobial contamination.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area, Animals and Design

Bukombe district is one of the five districts in Geita region whose geographical coordinates are 3° 28' 0" S, 31° 54' 0" E. It has a human population of 224, 542 and a cattle population of 427, 231 (URT, 2013). Most of cattle are Tanzania Short Horned Zebu (TSHZ) and Ankole breeds which are kept by agro-pastoralists who rarely get veterinary services and animal disease control programmes. The study design was cross-sectional where sampling was done once.

3.2 Farming Practices in the Study Area

The population in the study area is sole dependent on agriculture for livelihood. Although mining activities serves as an alternative source of income, agriculture is still a main source of income for the people in Bukombe district. Food crop production and livestock husbandry are the main agricultural activities undertaken in the study area. The livestock farming systems include agro-pastoralism, pastoralism and smallholder dairy farming. The main food producing animals kept in the area are indigenous and dairy cattle, small ruminants and chicken. Indigenous cattle are kept in rural areas where as dairy cattle are reared in peri-urban and urban areas. The traditional livestock keepers practice free range grazing system in savannah grass land where as smallholder dairy farmers practice zero grazing with supplementation of concentrates, vitamins and minerals. The traditional animal keepers mainly keep TSHZ and Ankole breeds. The agro-pastoralists graze their animals in open grass lands especially during the rainy season and feed their animals with crop residues during the dry season. The pastoralists are mobile in search of water and

pastures and prefer grazing in protected areas such as Kigosi game reserve and Bukombe forest reserve.

3.3 Ethical Consideration

Research was conducted according to the guidelines provided by the code of ethics of SUA, indicated in sub-section 2.2 of section 2. Research permit was provided by the Vice Chancellor of Sokoine University of Agriculture on behalf of the Commission of Science and Technology (COSTECH) (Appendix 3); and permission to do research in Bukombe District was provided by the District Executive Director (Appendix 4). Verbal consent was requested from each of the study farmers. Participation in the study was by voluntary basis. All the data that was collected from the respondents and the laboratory sample analysis was a confidential to the researcher.

3.4 Inclusion and Exclusion Criteria

The inclusion criteria of the farmers who were involved in this study included; willingness of the respondents to participate in the study, accessibility of the areas, respondents with at least two lactating indigenous cattle, respondents who admitted to be interviewed and availability of un-milked lactating cows during sample collection. Exclusion criteria involved; unwillingness to participate in the study, inaccessible areas, respondents without lactating indigenous cattle, unacceptance of the respondents for interviews, absence of un-milked cows during sample collection.

3.5 Sample Size Estimation

Sample size was estimated as proposed by Fisher *et al.* (1991).

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

Whereby; N= Sample size, Z= Confidence interval, P= Prevalence estimate, 1-P= Probability of no *B. abortus* in milk samples, d=Level of precision. Confidence level=95%, Z=1.96 and Precision=5%. The prevalence was estimated to be 15.2% (Mahlau *et al.*, 1967); substituting given values sample size approximated to 200 milk samples. At least two herds were selected from each selected ward and at least one herd was selected from each selected village in a ward. A herd size of at least 10 cattle with at least 1 lactating cow was a criteria for inclusion and exclusion in this study. A maximum number of 54 households were selected for blood and milk sampling.

3.6 Assessment of the Risk Factors for Brucellosis and Antimicrobial Residues in Milk

Structured questionnaires (Appendix 1 and 2) with closed and some open ended questions were administered to the heads of households along with sample collection from selected nine wards of Bukombe district (Figure 1). Among other variables, the questionnaires assessed the risk factors for brucellosis in the cattle herd and antimicrobial residues in milk. Biodata was taken from each individual lactating animal selected for study.

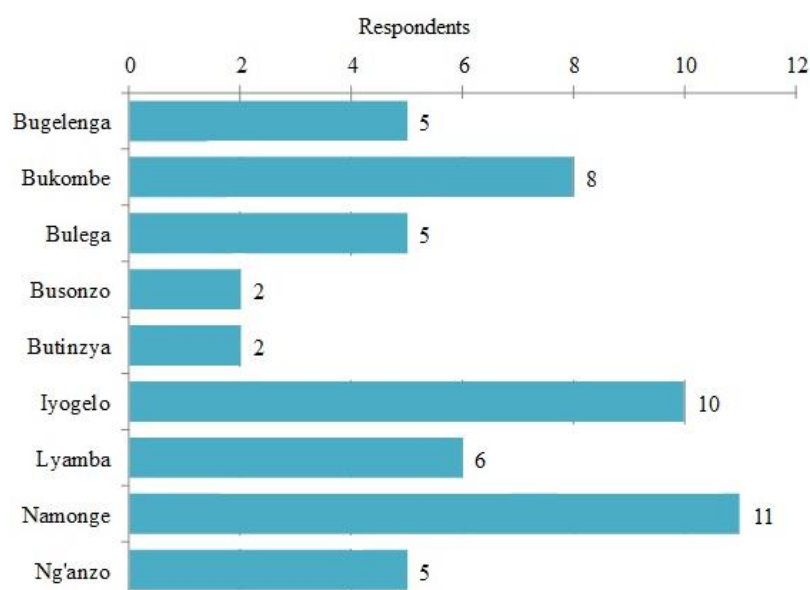


Figure 1: Distribution of respondents in selected wards

3.7 Sampling Procedures, Sample Collection and Handling

A total of 17 villages were purposively selected from Siloka and Ushirombo divisions for blood and milk sampling in January 2017 (Figure 2). The criteria for selecting villages was based on each village having at least 10 livestock keepers. Blood samples (n=221) and milk samples (n=221) were collected from 221 traditional lactating cows in 9 wards of Bukombe district (Figures 4 and 5).

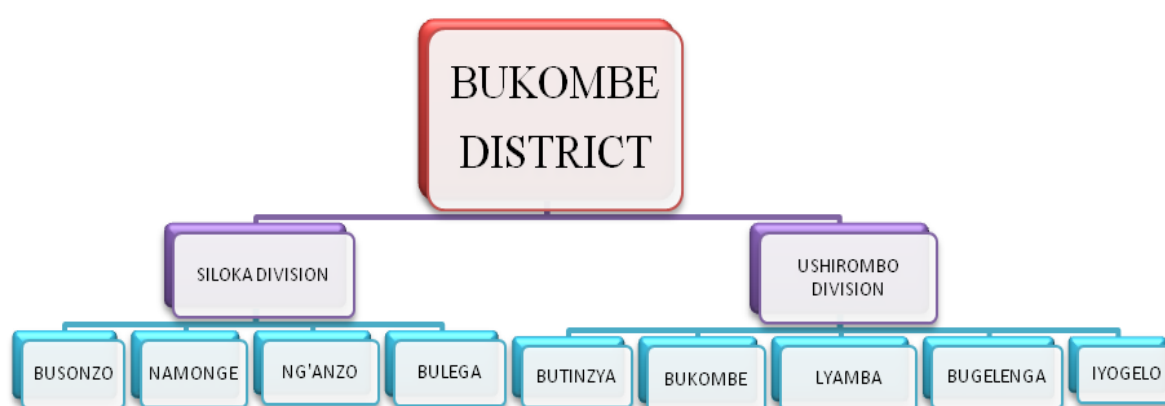


Figure 2: Villages selected for sampling in Siloka and Ushirombo divisions

At least two herds were purposively selected from each proposed ward. At least one village from each selected ward was included in this study. Each cow selected for sampling contributed one blood sample and one milk sample. Each milk sample was collected in a well labelled 15 ml falcon tube during milking from all quarters of a cow. To collect the blood sample, a cow was well restrained and blood sample was collected from jugular vein into a well labelled plain vacutainer tube (Figure 3). All samples were stored in cool boxes packed with ice packs during sample collection in the field. After field work of each day, milk samples were immediately stored in a freezer at -21 °C until analysis.



Figure 3: A researcher collecting blood sample via jugular vein

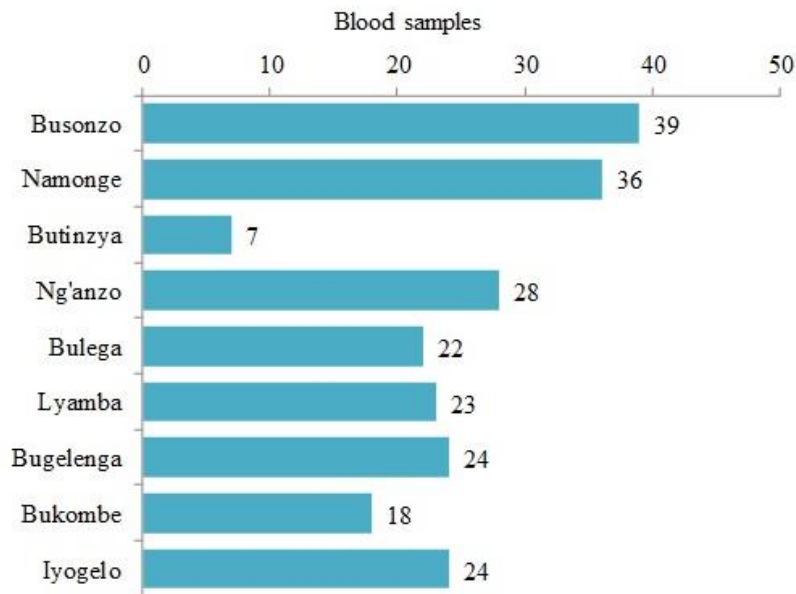


Figure 4: Distribution of blood samples in the study area

The samples in the vacutainer tubes was left to clot at room temperature overnight and centrifuged at 1 500 speeds for 10 minutes to obtain serum which was aliquoted into cryovials. All the samples (serum, n=221 and milk, n=221) were stored in a freezer at

-21 °C for 14 days before transportation to Sokoine University of Agriculture (SUA) for laboratory analysis.

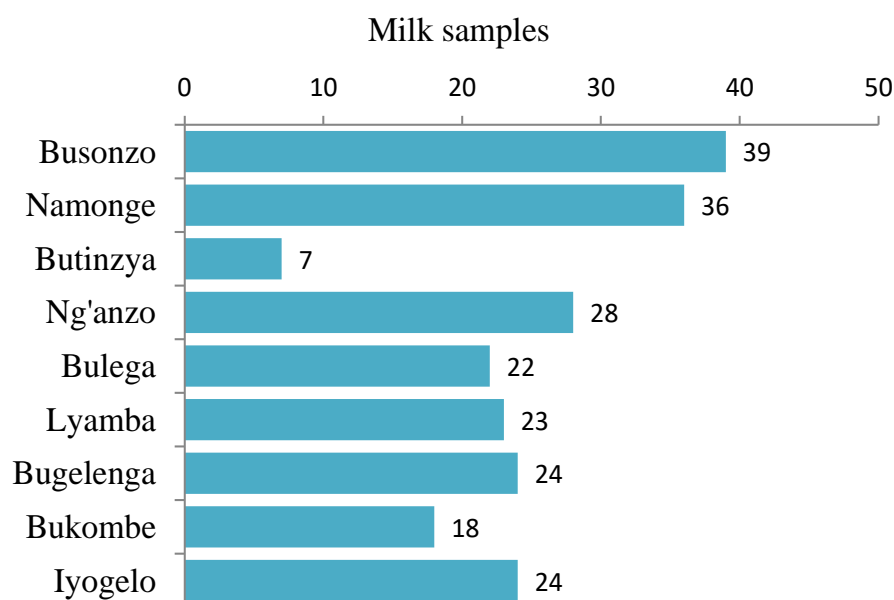


Figure 5: Distribution of milk samples in the study area

The samples (serum and milk) were transported to the Microbiology laboratory of SUA while stored in a cool box packed with ice packs. Some of the samples (serum, n=2 and milk, n=3) were damaged during transport to SUA. Samples (serum, n=219 and milk, n=218) at the Microbiology laboratory of SUA were further stored in a freezer at -21 °C until analysis.

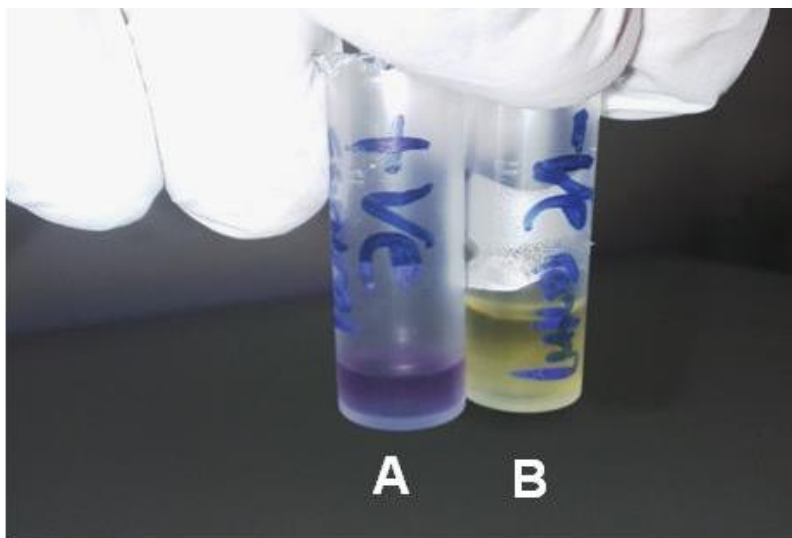
3.8 Laboratory Analysis of Samples

Laboratory analysis of samples was done at Microbiology laboratory of Sokoine University of Agriculture and Tanzania Food and Drugs Authority (TFDA) chemistry laboratory in Kinodoni Municipality, Dar es Salaam. These included qualitative and quantitative analysis of antimicrobial residues in milk. The collected sera samples were first screened for *Brucella* antibodies using Rose Bengal Plate Test (RBPT) and positive samples were confirmed by Competitive Enzyme Linked Immunosorbent Assay (c-ELISA), a test

which is recommended by OIE (2012). *Brucella abortus* DNA in raw milk was detected by Polymerase Chain Reaction (PCR). The details of the procedures are as shown in the subsequent subsections.

3.8.1 Qualitative assessment of antimicrobial residues in milk

A total of 198 milk samples were analysed by Delvo SP® test kit (Delft, Netherlands). Briefly, each 0.1 ml of milk sample was inoculated in a well labeled ampoule (Figure 6) with nutrient tablet. A negative and positive control was included in the analysis (Figure 6). The ampoules were incubated at $64 \pm 2^\circ\text{C}$ for 3 hours in the water bath as indicated by manufacturer's instruction. The test was negative when it turned to a partial yellow colour, means the milk tested did not contain antimicrobials or the antimicrobial concentration was below the sensitivity of the test. The test was positive when it turned to a complete purple colour, which means the milk tested contained antimicrobial residues at or above the detection sensitivity of the test. Milk samples with antimicrobial residues inhibited the growth of *Bacillus stearothermophilus* variant colidolacti (a test organism).



3.8.2 Quantification of Tetracyclines in raw milk by High Performance Liquid

Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) was done to establish the concentration of antibiotic in milk samples. HPLC analysis involved on Tetracyclines antibiotics (Chlortetracycline, tetracycline and Oxytetracycline) since questionnaire results showed that all respondents (n=54) were using the drugs in treatment of different health problems in cattle. The analysis involved samples of milk which tested positive in Delvo SP® test kit test. Due to limited resources, of the 23 positive Delvo tested raw milk samples 10 samples only were selected for confirmation and quantification by using HPLC-uv and the test was done according to (AOAC 995.04). The methods were done as described by Ghidini *et al.* (2003) and USA: CLG-TET2.04, 2011 with some modifications.

i) Testing principle

Partially thawed raw milk samples were extracted with McIlvaine Buffer/EDTA solution. After centrifugation, the extracts containing the Tetracyclines (TCs) was cleaned-up by passing through C18 SPE Cartridges. TCs were eluted from the cartridge with methanolic oxalic acid, evaporated and reconstituted with aqueous methanol and analysed by reverse phase High Performance Liquid Chromatography (HPLC-UV).

ii) Equipment and Apparatus

Analysis was carried out on a HPLC Model LC-20AT Prominence Liquid Chromatography series, serial No.C213346 015664 (SHIMADZU Corporation, Japan.), equipped with constant flow pump, degasser (DGU 20A₅ Serial L-20244 07326), column oven (CTO-10A VP Serial C 210447 06967), auto sampler (OPTIMAS- Spark) and computer software. Separations was carried out by BD5 Hypersil-Analytical C8 (125x 4

mm, particle size: 5 μ m, part number: 28205-124030 and serial number: 10058200) Column Agilent Technologies- USA and analyte was detected by PDA-Detector with variable wavelength UV detector (SPD-20A), CBM- 20A Prominence serial No L 202347 09807 (SHIMADZU Corporation Japan). Other equipments used were:

- a) Centrifugation during sample preparation and extraction was performed with a ROTOFIX 32A ® (ZENTAFUGEN)-Centrifuge made from Germany.
- b) Sonicator & Degasser used to degas mobile phase- Branson ® 3210E-DTH, UK
- c) Nitrogen drier used to dry sample during extraction process-Thermo Scientific heating module ® No. 8824
- d) PH Meter measuring \pm 0.05 unit,- JENWAY ® 3540 model-UK
- e) Analytical balance-Readability 0.0001g – OHAUS ® corporation-USA
- f) Mechanical shaker/Vortex mixer- SFI, Sturt Scientific- UK
- g) Clean-up Solid phase extraction (SPE) Cartridges-VARIAN Bond Elut SAX made from USA
- h) Disposable syringe filters-(0.45 μ m)-lot no. SF2350812, XIVA-SICHEM BIOTECH-Delhi India
- i) Sterile membrane filter- 0.45 μ m pore size, Cat no. 7141114 supplied from Japan

iii) Chemicals and reagents

- a) Methanol-HPLC grade, acetonitrile- HPLC grade and Water- HPLC grade was supplied from Fishers Scientific Limited- UK.
- b) Disodium hydrogen phosphate dihydrate, oxalic acid-dihydrate, citric acid-monohydrate, Sodium phosphate-dibasic anhydrous, Ammonium hydroxide was purchased from Fishers Scientific Limited, UK.
- c) Oxalic acid (Analytical grade) was supplied by Scharlau chemie S.A,
- d) EDTA, trifluoroacetic acid was purchased from Ps Park Scientific Limited-UK.

- e) Distilled and deionized water was obtained from TFDA Laboratory and
- f) Tetracyclines CRS was supplied from council of Europe (batch/lot no 4 a)

iv) Extraction solvents

- a) McIlvaine buffer (pH 4.0 ± 0.05)

The amount of 21.0 g of citric acid was taken and dissolved in 1 liter distilled water in a volumetric flask. In another flask, 28.4 g of (Na₂HPO₄) anhydrous disodium hydrogen phosphate was dissolved in 1 liter distilled water. McIlvaine buffer was then made by combining 1 liter citric acid and 625 ml of disodium hydrogen phosphate in a 2 liter volumetric flask. The pH was adjusted using 0.1M hydrochloric acid.

- b) McIlvaine buffer- EDTA solution (McIlvaine Buffer/0.1M EDTA)

On weekly basis McIlvane buffer was prepared as described by Ghidini *et al.*, (2003). To 1.625 liter McIlvaine buffer, 60.5 g of disodium ethylenediamine-tetracetate (EDTA dehydrate) was added and mixed until dissolved completely.

v) Elution solvent

Every day fresh methanolic oxalic acid solution was made by dissolving 1.26 g oxalic acid dihydrate in HPLC grade methanol in 1 liter volumetric flask and mixed until dissolved.

vi) Liquid Chromatography Mobile phase

Oxalic acid- Acetonitrile- Solution in a ratio of 75:25 was used (Ghidini *et al.*, 2003 and USA: CLG-TET2.04, 2011). The 0.005 M oxalic acid solution was prepared by weighing 5 mmol of oxalic acid and dissolved into a 1litre distilled water. The solution was also

filtered through 0.45 μm pore size membrane filter and degassed in the ultrasonic/degasser.

vii) Preparation of Tetracycline standards

a) Stock standards- (1000 $\mu\text{g/ml}$)

Stock standard was prepared by adding 108 ± 0.1 mg of Oxytetracycline (OTC), Tetracycline (TTC), and Chlortetracycline (CTC) reference standards to the separate 100 ml volumetric flasks and the flasks were filled to their volumes at room temperature.

b) Working standard- (25 $\mu\text{g/ml}$)

Working standard was prepared by first, preparing 100 $\mu\text{g/ml}$ from intermediate solution and then pipetting 2.5 ml of the prepared solution into 10 ml volumetric flask. Subsequently diluting with methanol to the volume and mixed thoroughly and stored in the refrigerator

c) TCs chromatographic standard solutions

The TCs chromatographic standard solutions- (25 $\mu\text{g/l}$, 50 $\mu\text{g/l}$, 75 $\mu\text{g/l}$, 100 $\mu\text{g/l}$, 125 $\mu\text{g/l}$, 150 $\mu\text{g/l}$ and 200 $\mu\text{g/l}$) were prepared from the TCs working standard solution (1000 $\mu\text{g/l}$) which was diluted to make the desired concentrations.

3.8.2.1 Sample preparation and extraction

i) Sample extraction

Homogenised, previously frozen raw milk samples (5.0 ± 0.1 ml test milk), showing no signs of souring or curdling was pipetted into 50 ml polypropylene centrifuge tube and centrifuged for 10 minutes at 4 000 rpm at approximately 15°C temperature to separate

cream. The contents were then mixed with 20 ml McIlvaine/EDTA solution and shaken for 10 minutes on flat bed shaker at high speed. The content of the tubes were then centrifuged for 10 minutes at a speed of 4 000 rpm at approximately 15°C. The supernatant was filtered through GFB filter paper after moistening with McIlvaine buffer-EDTA solution.

ii) Sample clean up by solid phase extraction

Solid phase extraction (SPE) cartridges were attached to an SPE vacuum manifold connected to 75 ml reservoir to each cartridge. The Bond Elut-SAX C18 cartridges were first conditioned with 10 ml methanol followed by 20 ml of HPLC- grade water, at approximately 1.5-2.5 ml/minute, vacuum was applied gently and the eluate was discarded.

iii) Elution step to remove residues from the cartridge

A total of 75 ml of reservoirs were connected to the cartridges and the filtered sample extracts were added to the SPE. Each flask was rinsed with approximately 4 ml McIlvaine/EDTA solution and the rinses were added to the specific reservoir. The sample extracts were drained through the columns by gravity. However for insufficient gravity for some slow samples vacuum was applied gently and stopcocks were adjusted to achieve a flow rate of 1.5-2.5 ml/min. It was followed by rinsing the side-arm flasks using 20 ml deionized water added to SPE reservoirs.

Draining of the deionized water was facilitated by 5-10 mm Hg vacuum. Thereafter, the cartridges were allowed to go dry and continued to draw air for approximately 2 minutes. The eluates were discarded. The 15 ml graduated centrifuge tubes were placed in the vacuum apparatus to serve as collection vessels. TCs residue test solutions were eluated

from the cartridges with 5.0 ml methanolic oxalic acid. A vacuum was initiated and elution continued and once the elution stopped vacuum was applied to evacuate the residue solutions from the cartridges. The tubes were removed from the vacuum manifold and mixed with a vortex mixer. The tubes containing methanolic eluates were placed in N-Evap with water bath and evaporated in a steam temperature at 40-50°C, facilitated by the gentle stream of nitrogen gas for approximately 1 hour and reducing volumes of the eluates to 1 ml. Finally the samples were reconstituted by the addition of 1 ml of methanol, mixed briefly by a vortex mixer and filtered through 0.45 µm syringe filtration cartridges into LC auto sampler vials and loaded into auto sampler. Finally, 200 µl of the solution was injected into the HPLC-UV system. A sample was analyzed at 365 nm wave length.

3.8.2.2 High performance liquid chromatographic analysis for tetracyclines

Using 0.45 µm pore size micro syringe filter, the test solution and the standards were filtered into the LC auto sampler vials and loaded into auto sampler. The analysis and quantification of the TCs residue in the extracts was done using a high-performance liquid chromatography (LC-20AT Model Shimadzu Prominence series -Japan) equipped with CBM-20A auto injector with sample cooler CTO-10AS VP and DGU-20A₅ on-line vacuum degassing solvent delivery unit, a constant flow pump and a variation wavelength UV detector set at 365 nm). The separation was done on BD5 Hypersil-analytical C8 (125x 4 mm, particle size:5 µ, part number:28205-124030 and serial number: 10058200) column with Oxalic acid-Acetonitrile solution, (75:25) as the mobile phase by gradient mode, the mobile phase flow-rate was 1.2 ml/min at room temperature and the sensitivity range was 0.08 ppm. The HPLC analysis was performed for 7 minutes in each sample.

To get the TCs concentration of a given sample, reference standards of known concentrations were used to determine the concentrations of the sample extract. For the

determination of TCs residues, the TCs standard solutions at different concentrations of 25, 50, 75, 100, 125, 150 and 200 $\mu\text{g/l}$ were injected in ascending order (Table 2, 3 and 4). The extract from each sample was then injected for HPLC analysis. The samples were considered to be positive for TCs if their retention time and peaks corresponded to that of the reference standards. The retention time of the standards was at 2.9, 3.4, 5.6 minutes for OTC, TTC and CTC respectively (Figure 7, 8 and 9).

i) Method development for HPLC analysis of raw milk samples

Prior to extraction of TCs in an unknown raw milk samples, the method was developed under the laboratory conditions suitable for analysis using HPLC. The developed method was later used to determine TCs residues concentration of the raw milk sample in the present study.

Table 2: OTC standard calibration curve

Point	Concentration ($\mu\text{g/l}$)	Mean Area	Area
1	25.0	23293.3	23293
2	50.0	68095.7	68096
3	75.0	91907.1	91907
4	100.0	155503.0	155503
5	125.0	189912.2	189912
6	150.0	268661.7	268662
7	200.0	324666.4	324666

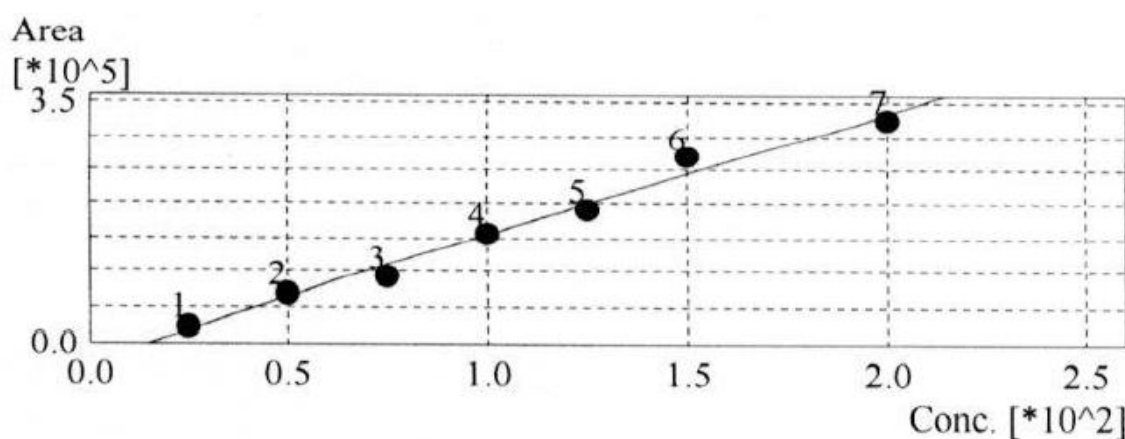
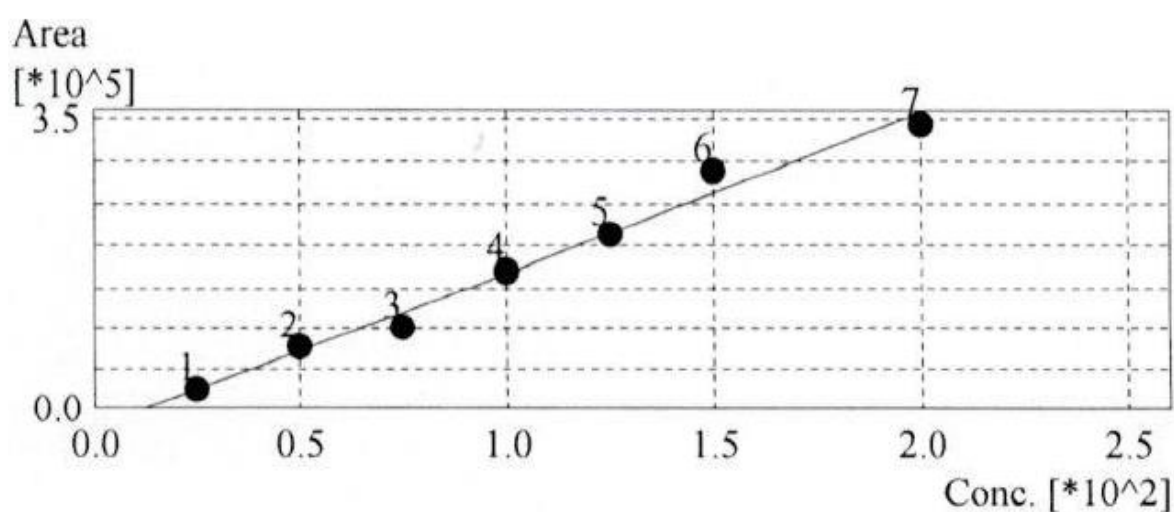


Figure 6: Calibration curve with seven concentration points of OTC standard

Table 3: TTC standard calibration curve

Point	Concentration ($\mu\text{g/l}$)	Mean Area	Area
1	25.0	24385.4	24385
2	50.0	76911.6	76912
3	75.0	101337.0	101337
4	100.0	169609.7	169610
5	125.0	213167.3	213167
6	150.0	288528.1	288528
7	200.0	343180.6	343181

**Figure 7: Calibration curve with seven concentration points of TTC standard****Table 4: CTC standard calibration curve**

Point	Concentration ($\mu\text{g/l}$)	Mean Area	Area
1	25.0	16421.9	16422
2	50.0	47882.3	47882
3	75.0	72664.9	72665
4	100.0	117798.8	117799
5	125.0	146613.3	146613
6	150.0	179097.1	179097
7	200.0	237460.2	237460

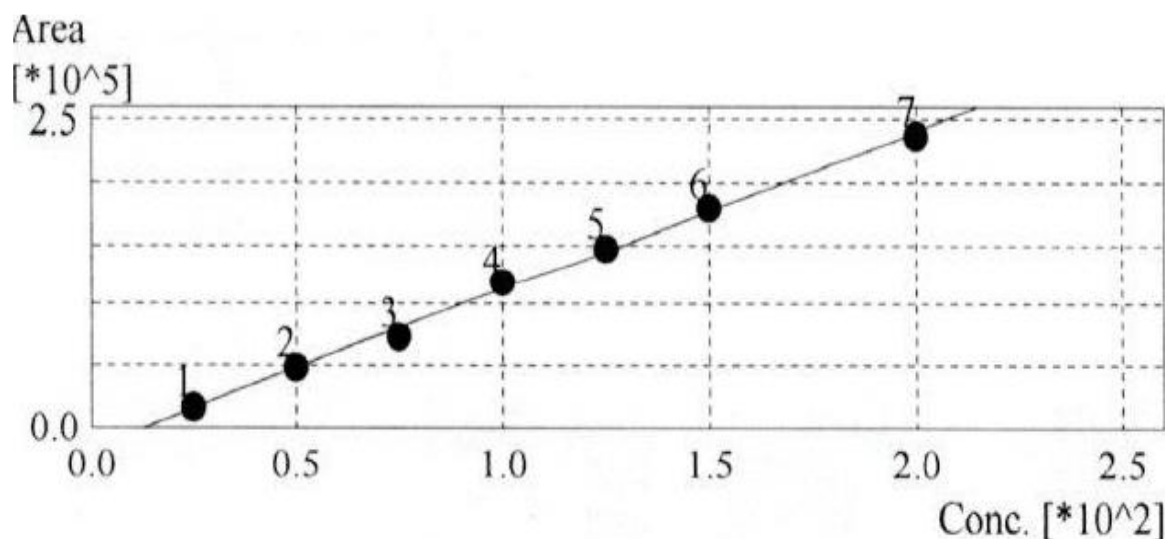


Figure 8: Calibration curve with seven concentration points of CTC standard

ii) Preparation of Tetracyclines standard solutions and injection in HPLC

One hundred and eight milligram of OTC, TTC and CTC standard solution were weighed separately, transferred into separate 100 ml amber volumetric flasks and dissolved in methanol at room temperature. The OTC, TTC and CTC stock standard solutions each 1000 $\mu\text{g/ml}$ was protected from light by using amber volumetric flasks and stored at -20°C and it was used to prepare the working TCs standard by serial dilutions.

The TCs intermediate stock solution-100 $\mu\text{g/ml}$ was prepared by diluting 10 ml of OTC, 10 ml of TTC and 10 ml of CTC stock standard solutions in 100 ml volumetric flask and then the flask was filled with methanol to its volume at room temperature and stored in a -20°C for three months. The intermediate stock solution was further diluted to get 25 $\mu\text{g/ml}$ of TCs working standard solution. It was done by diluting 2.5 ml of TCs intermediate stock solution in 10 ml volumetric flask and the flask was filled to its volume with methanol and mixed at room temperature. Working standard solution was used to prepare 1000 $\mu\text{g/l}$, then seven TCs chromatographic standard solutions at 25, 50, 75, 100, 125, 150

and 200 µg/l concentrations were prepared into separate 10 ml volumetric flasks containing 5 ml methanolic oxalic acid solution and mixed. Then distilled water was added to bring the volumetric flasks to final volumes.

Under gradient conditions, different standard concentrations of the prepared TCs standard solutions were injected in ascending order and the results were plotted automatically on the integrator (Figure 10). The machine was set to run for 7 minutes and TCs were detected at an average of 2.9, 3.4, 5.6 minutes as retention time of OTC, TTC, CTC respectively with the peak height increasing with increase in concentrations.

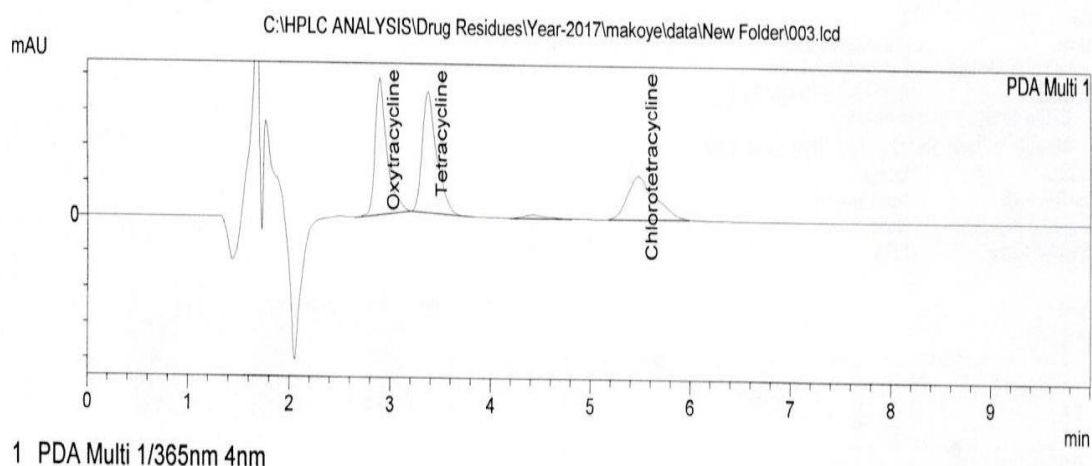


Figure 9: The chromatogram of mixed standard solution of TCs (104. 1 µg/l)

iii) The positive and negative control samples

A known negative and positive sample was used as quality control. Negative controls were those known to be free from TCs contamination. Positive controls were those known to be free from TCs that has been fortified (spiked) with a known quantity of TCs (Table 5). In this study, antibiotic free UHT milk (Brookside Dairy) was purchased from Dar es Salaam Shoprite super market. The negative control samples of UHT milk and fortified control

samples were treated the same way as the raw milk samples which underwent extraction, SPE clean-up and elution with methanolic oxalic acid.

Two hundred microliter (200 μ l) of the working solution of TCs standard (25 μ g/ml) was pipetted into 5 ml volumetric flask, followed by addition of antibiotic free UHT milk to the volume and mixed thoroughly by shaking for 10 seconds and left for 30 minutes before starting extraction procedure with McIlvaine buffer, solid phase extraction and elution from the cartridges. The control samples was run through the analytical column in a HPLC machine mobile phase conditioned with Oxalic acid- Acetonitrile solution in a ratio of 75:25 respectively. The column temperature and flow rate was maintained as the case for the TCs analytical standard solutions. The retention time of the control samples was compared with those of the TCs standard solution alone. During extraction blank samples eluted from SPE cartridge was included to check for the analytical column efficiency.

iv) LOD and LOQ of an analytical system

Limit of detection (LOD) and Limit of quantification (LOQ) depend on the noise and drift of the detection equipment. Absolute detector LOD can be determined by injecting a sample directly into detector. It is often expressed as minimum detectable level. However, the LOD depend on the oxygen content of the mobile phase, the injection system, peak broadening on the column and temperature differences among the system components. Taking these factors into account, the LOD was defined as 3 times the noise level and LOQ was defined as 10 times the noise level and in this study, it was determined by spiking free antibiotic UHT milk (Brookside brand) sample with 10 μ g/l. The recoveries of the TCs was calculated at six different concentration levels (25, 50, 100, 125, 150, 200

µg/l). The recovery rates were displayed at the averages of 86.6%, 86.5% and 84.4% for OTC, TC and CTC respectively. The mean recovery of the TCs was 85.8% (Table 5).

Table 5: Recovery and precision of OTC determined in spiked UHT milk sample

(n=6)

Fortification level (µg/l)	OTC (%)	TTC (%)	CTC (%)	Mean recovery (%)
25	87.74	90.75	90.324	89.605
50	88.992	94.04	92.622	91.885
100	88.826	86.314	81.039	85.393
125	84.111	88.11	79.76	83.994
150	82.562	80.334	81.08	81.325
200	87.287	79.273	81.51	82.687
Mean recovery (%)	86.586	86.470	84.389	85.815

The Mean recovery rate of the Tetracyclines was 85.8%

Legend: OTC- Oxytetracycline, TTC- Tetracycline, CTC- Chlortetracycline

3.8.3 The Rose Bengal plate test (RBPT)

RBPT antigen (Central Veterinary Laboratory, Weybridge, UK) was used to perform RBPT with 219 sera as described by Alton *et al.* (1988) and as recommended by OIE (2012). Briefly, 30 µl of the RBPT antigen was mixed with 30 µl of serum on the glass plate and through mixing was done by the help of the stirrer. The glass plate was then placed on the rocker for 4 minutes. Observation was done with the help of tilting the plate. A serum tested positive if it agglutinated with RBPT antigen and negatively tested if no agglutination was observed. Positive control was used to differentiate between positive and negative results.

3.8.4 Test of sera by Competitive Enzyme Linked Immunosorbent Assay(c-ELISA)

RBPT positive sera were retested by competitive enzyme linked immunosorbent assay (c-ELISA) as a confirmatory test of brucellosis. The laboratory protocol was used as

recommended by the manufacturer (Animal Health and Veterinary Laboratories Agency-AHVLA, New Haw, Addlestone, Surrey, KT153NB, UK). The spectrophotometer was adjusted at an absorbance of 450 nm. A positive negative cut-off was calculated as 60% of the mean of the optical density (OD) of the four conjugate control wells. Any test sample that gave OD equal to or below the value was be regarded as positive. Animals that were positive on c-ELISA were considered as *Brucella* seropositive. A herd was regarded as seropositive for brucellosis when at least one animal reacted positive for c-ELISA.

3.8.5 Detection of *Brucella abortus* in raw milk by Polymerase Chain Reaction (PCR)

The detection of DNA of *B. abortus* from milk was done by PCR as detailed in the subsequent sections.

3.8.5.1 *Brucella abortus* DNA extraction from milk samples

DNA extraction of *B. abortus* was done as explained by the manufacturer of Genomic DNA Purification Kit (Thermo Scientific USA). DNA quantification was with Nano-drop Thermo (Ferment as USA) and working aliquots of all extracted DNA samples were adjusted at same concentration level i.e. 50 ng/l. Stock DNA samples were stored at -20 °C freezer until the performance of PCR.

3.8.5.2 PCR for *Brucella abortus*

The *bcs31* gene of *B. abortus* encoding an immunogenic membrane protein of a 31 kDa (*B. abortus* antigen) was amplified as explained by Navarro *et al.* (2002) with few modifications in the total master mix and annealing temperature. The B4/B5 primers were used to amplify 223-bp fragment that contained the target gene (Table 6). These primers were obtained from Bioline, Inc., (Taunton, MA, USA), and were used as described by (Baily *et al.*, 1992).

Table 6: The primer sequences that were used to amplify *bcs31* target gene of *Brucella abortus* in milk samples

Primer used	5'-3' sequence	Target gene	Fragment	Reference
B4	5' TGG CTC GGT TGC CAA	<i>bcs31</i>	223bp	Baily <i>et al.</i> ,1992
	TAT CAA 3'			Baily <i>et al.</i> ,1992
B5	5' CGC GCT TGC CTT TCA	<i>bcs31</i>	223bp	Baily <i>et al.</i> ,1992
	GGT CTG 3'			Baily <i>et al.</i> ,1992

Legend: B4-Forward primer; B5-Reverse primer

Briefly, PCR was done in a final volume of 25 µl composed of 12.5 µl of 2X *Taq* DNA polymerase, (Invitrogen Carls bad, CA), 5.5µl of DNA free water, 1.0 µl of 10 µmol B4 (forward) primer, 1.0 µl of 10 µmol B5 (reverse) primer and 5 µl of DNA template. The PCR mixture was run up to 40 cycles of amplification in a thermal cycler (Step One PCR systems, Applied BioAsystems). The reaction mixtures were initially incubated at 93° C for 5 minutes. This was followed by 40 cycles with the settings of 90° C denaturation for 30 seconds, 64 °C annealing for 30 seconds, 72 °C extension for 1 minute and 72 °C final extension for 30 seconds. The last extension step was done at 72 °C for 10 minutes. DNA from *B. abortus* reference strain was used as a positive control and a negative control contained all of the components of the reaction mixture except DNA.

3.8.5.3 Preparation of agarose gel

Agarose gel was prepared by mixing 0.75 g of agarose powder in 50 ml of 0.5 × TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA, pH 8) in a Pyrex conical flask to obtain a 1.5% concentration of the gel. The mixture was completely dissolved by boiling on a hot plate while hand shaking the conical flask. Agarose solution was left to cool to a warmth temperature that could be touched by hand. This was followed by addition of 3 ml of ethidium bromide (2 ug/ml) and hand shaken accordingly. The mixture

was immediately poured into the horizontal electrophoresis casting equipment in the presence of a comb and was left out for about 20 minutes for solidification.

3.8.5.4 Loading of PCR products in agarose gel electrophoresis

A volume of 8 µl of the PCR products was mixed thoroughly with 1 µl of dark blue 6X loading dye (Promega, Madison, USA) on a laboratory parafilm. The PCR products were loaded in the wells of the agarose gel and 9 µl of a 50 kb molecular weight marker (Promega, Madison, USA) was loaded in a parallel track either side of the plate. The horizontal gel electrophoresis was accomplished at a voltage of 110V for 60 minutes. The DNA bands were visualized thereafter, under trans-illuminator UV light and a photograph was taken with a digital camera. After its use; the gel and gloves were handled careful and disposed in an incinerator.

3.9 Data Analysis

Data were entered into Microsoft Excel spreadsheet and analyzed using Epi Info™ Version 7 (Centre for Disease Control, Atlanta, USA). Logistic regression was used to assess the risk factors associated with transmission of brucellosis in cattle and occurrence of antimicrobial residues in raw cattle milk. Chi-square (χ^2) and confidence intervals were used to compare proportions of categorical variables like risk factors for brucellosis; a probability of $p < 0.05$ was considered to be statistically significant. Descriptive statistics like frequencies, means and standard deviation were estimated using Microsoft excel. Graphs and tables were drawn by using Microsoft Excel.

CHAPTER FOUR

4.0 RESULTS

4.1 Sociological Data

A total of 54 respondents were interviewed and the results are summarized in the subsequent sections of the results.

4.1.1 Demographic characteristics of the respondents

Table 7 summarizes the results of demographic characteristics of the respondents in Bukombe district. Most of the respondents had primary education (51.9%), 98.2% were males, 88.8% aged >40 years, 98.2% were fathers of households and none of them had ever attended training on livestock husbandry. The dominant ethnic group was Sukuma (90.7%) and all of the respondents (n=54) were agro-pastoralists.

Table 7: Demographic characteristics of the respondents in the selected wards in

Bukombe district (n=54)

Demographic information	Category	Number (%) of respondents
Sex	Male	53 (98.2)
	Female	1 (1.9)
Age	<40	6 (11.1)
	>40	48 (88.9)
Education	Informal	23 (42.6)
	Primary	28 (51.9)
	Secondary	1 (1.9)
	College	2 (3.7)
Ethnicity	Sukuma	51 (90.7)
	Sumbwa	2 (3.7)
	Ha	1 (1.9)
Position in the household	Father	53 (98.2)
	Mother	1 (1.9)
Occupation	Agro-pastoralists	54 (100)

4.1.2 Biodata of the selected study animals

A total of 221 lactating cows were involved in the study and their information is recorded in Table 8. Briefly, 58.3% sampled animals were TSHZ and 93.7% of the sampled animals had ≤ 5 years.

Table 8: The animal biodata of the sampled traditional lactating cows

Variable	Category	Number (%)
Breed	TSHZ	130 (58.3)
	Ankole	93 (41.7)
Age (years)	≤ 5	207 (93.7)
	> 5	14 (6.3)
Herd size	≤ 50	36 (66.7)
	> 50	18 (33.3)

Legend: TSHZ- Tanzania Short Horn Zebu

4.1.3 Factors considered to facilitate transmission of brucellosis in cattle

Table 9 showed 16 factors which were perceived by the respondents to facilitate transmission of brucellosis. The factors like repeated abortion, giving raw placenta and aborted foetus to dogs, communal grazing and watering points and grazing in protected areas in the advent of pastures scarcity had $\geq 40\%$ of responses as factors for transmission of brucellosis.

Table 9: Factors for transmission of brucellosis in cattle (n=54)

Factor	Number (%) of respondents
Repeated abortion	24 (44.4)
Still birth	1 (1.9)
Giving raw placenta and aborted foetus to dogs	41 (75.9)
Giving cooked placenta and aborted foetus to dogs	1 (1.9)
Burning of placenta and aborted foetus	1 (1.9)
Burying of placenta and cooked foetus	2 (3.7)
Throwing of placenta and aborted foetus in the bush	9 (16.7)
Buying or selling of animals	13 (24.1)
Own pasturing	1 (1.9)
Communal grazing and watering points	53 (98.1)
Own watering points	1 (1.9)
Contacting wild animals in grazing plains	16 (29.6)
Observing wild animals in the village grazing pastures	18 (33.3)
Grazing in protected areas in scarcity of pastures	31 (57.4)
Lack of vaccination	0 (0.0)
Livestock sharing watering points with wildlife	0 (0.0)

Table 10: Logistic regression of the risk factors of transmission of brucellosis in cattle

Term	Odds Ratio	95% C.L.		P-Value
		lower	Upper	
Abortion (Yes/No)	2.3848	0.1157	49.1425	0.5734
Raw (Yes/No)	0.2690	0.0134	5.3911	0.3906
Buying (Yes/No)	4.1502	0.2093	82.2933	0.3504
Contact (Yes/No)	0.0000	0.0000	>1.0E12	0.9664
CONSTANT	*	*	*	0.0413

Legend: C.L- Confidence Limit

4.1.4 Factors for the occurrence of antimicrobial residues in milk

Questionnaires (n=54) were administered to the livestock owners for assessment of the factors considered responsible for occurrence of antimicrobial residues in raw milk (Table 11). All respondents (n=54) admitted to use antibiotics in cattle for treatment of diseases. Of the respondents 100%, 98.1%, 11.1% used TCs, penicillin and tylosin, respectively for

the treatment of their animals. They also admitted to use anthelmintics. Of the respondents 59.3%, 13%, 1.9% used albendazole, ivermectin and mebendazole, respectively.

Table 11: Factors for the occurrence of antimicrobial residues (n=54)

Factor	Number (%) of respondents
	n=54
Buying of veterinary drugs from the agrovet only	33 (61.1)
Buying of veterinary drugs from both agrovet and auction	21 (38.9)
Education on the adverse effects of antibiotics by the veterinary practitioner	3 (5.6)
Knowledge on the effects of use of antibiotics in animals and humans	43 (79.6)
Observation of the withdrawal period	22 (40.6)

A number of respondents (79.6%) reported to have knowledge on the effects of use of antibiotics both in humans and animals. Most of the respondents bought antibiotics from agrovet alone or both the agrovet and livestock auction. It should be noted that 41% of the respondents admitted to have regular observation of drug withdrawal periods. Logistic regression analysis was done to the factors considered responsible for occurrence of antimicrobial residues in raw milk and none of these factors were statistically significant (Table 12).

Table 12: Logistic regression for the risk factors of the occurrence of the antimicrobial residues

Term	Odds Ratio	95% C.L.		P-Value
		lower	Upper	
Agrovet (Yes/No)	0.7738	0.2136	2.8024	0.6961
Education (Yes/No)	0.3945	0.0190	8.1725	0.5475
Knowledge (Yes/No)	0.3193	0.0663	1.5383	0.1547
Withdrawal (Yes/No)	1.5575	0.4117	5.8923	0.5139
CONSTANT	*	*	*	0.9532

Legend: C.L- Confidence Limit

4.1.5 Cattle diseases reported by farmers in Bukombe district

Respondents (n=54) reported occurrence of bovine diseases including Foot and Mouth Disease (FMD), Contagious Bovine Pleuropneumonia (CBPP), Blackquarter (BQ), Lumpy Skin Disease (LSD) and Helminthosis (Figure 11) . Among the diseases frequently reported diseases were FMD, CBPP and BQ.

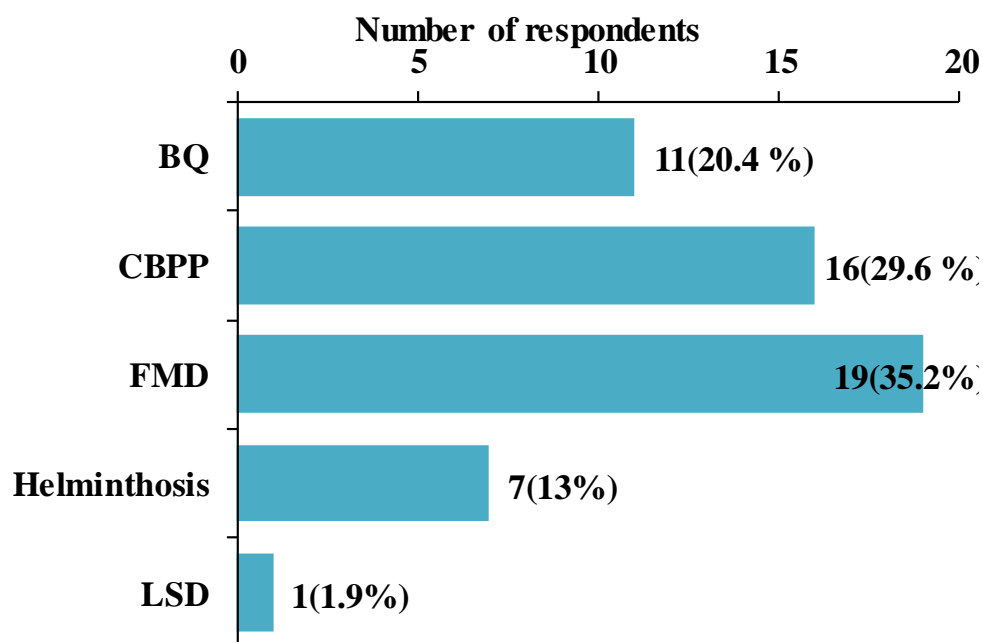


Figure 10: Major diseases reported by respondents

4.2 Brucellosis Results of Cattle in Bukombe District

4.2.1 Rose Bengal Plate Test (RBPT) and c-ELISA results

Of the 219 sera samples (3 samples were destroyed spontaneously) screened for brucellosis by RBPT, three samples (1.4%) were reactive against RBPT antigen (Figure 12). Two positive serum samples were from Namonge ward and one from Ng'anzo ward (Table 13).



Figure 11: Rose Bengal Plate Test Results

**Table 13: Distribution of Rose Bengal Plate Test Results of cattle in different wards
(n=219)**

Ward	Number of herds	Number of cattle tested	Positive serum samples (%) n=3	Negative serum samples n=216
Namonge	9	36	2 (5.6)	34
Ng'anzo	5	28	1 (3.6)	27
Busonzo	2	35	0 (0.0)	35
Butinzya	2	7	0 (0.0)	7
Bulega	5	24	0 (0.0)	24
Lyambamgongo	6	23	0 (0.0)	23
Bugelenga	5	24	0 (0.0)	24
Bukombe	8	18	0 (0.0)	18
Iyogelo	10	24	0 (0.0)	24

The overall animal seroprevalence of brucellosis in cattle was 1.4% as was confirmed by c-ELISA test (Table 14). The herd seroprevalence was 3.8%. There was a very good (perfect) kappa agreement between RBPT and c-ELISA as test diagnostic methods for brucellosis ($\kappa = 1$).

Table 14: Overall seroprevalence of brucellosis in cattle of Bukombe district based on RBPT and c-ELISA (n=219)

	RBPT	c-ELISA
Total	219	219
Negative sera	216	216
Positive sera	3	3
Cattle seroprevalence	1.4%	1.4%

Legend: RBPT- Rose Bengal Plate Test; c-ELISA- Competitive Enzyme Linked Immuno-Sorbent Assay

4.2.2 PCR results of *Brucella abortus* in milk samples

Three milk samples (two from Namonge and 1 from Ng'anzo wards) individually taken from seropositive animals against RBPT antigen were analysed for presence of DNA of *B. abortus*. One sample from Namonge ward was positive with PCR since a band of 223 bp was observed (Figure 13).

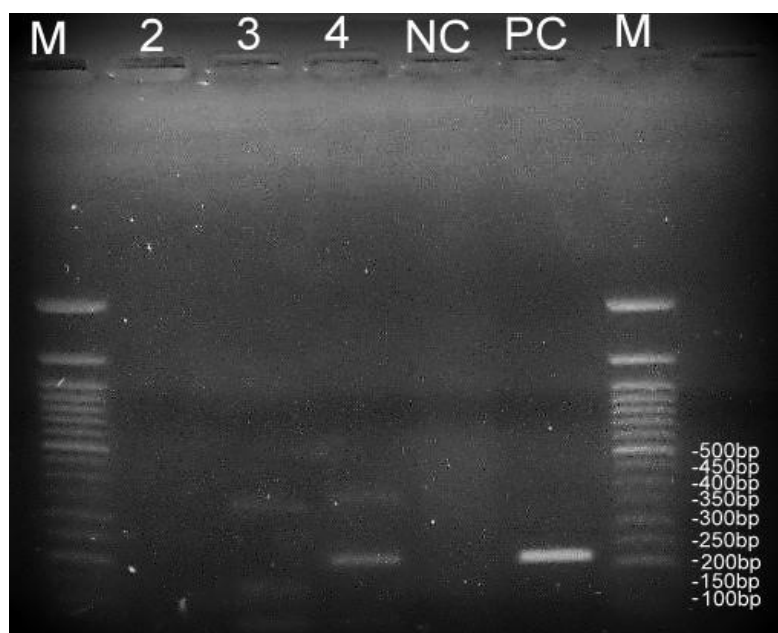


Figure 12: Gel electrophoresis photo displaying 223 bp fragment of *bcsf 31* gene in sample number 4. Lanes (left and right) is a 50 kb molecular weight marker; NC and PC are negative and positive control respectively. Lane 2, 3 and 4 are milk samples from RBPT seropositive cattle in Bukombe district. Note that a sample in lane 4 was positive for *Brucella abortus*.

4.3 Qualitative Analysis of Antimicrobial Residues in Milk

A total of 198 milk samples were analysed and 23(11.6%) were positive to Delvo test (Table 15). Although the herd prevalence of antimicrobial residues was 34.8%. Variations were noted among wards with highest at Busonzo (29.4%) and least at Iyogelo (4.3%).

Table 15: Delvo test results of 198 milk samples presented per ward

Ward	Number of herds	Number of milk samples tested	Positive milk samples	Negative milk samples
Busonzo	2	17	5 (29.4)	12
Lyambamgongo	6	23	5 (21.7)	18
Bugelenga	5	26	4 (15.4)	22
Butinzya	2	7	1(14.3)	6
Ng'anzo	5	24	3 (12.5)	21
Bukombe	8	16	1 (6.3)	15
Namonge	9	39	2 (5.1)	37
Bulega	5	22	1 (4.5)	21
Iyogelo	10	24	1 (4.3)	23

4.3.1 Qualitative analysis results of antimicrobial residues in milk

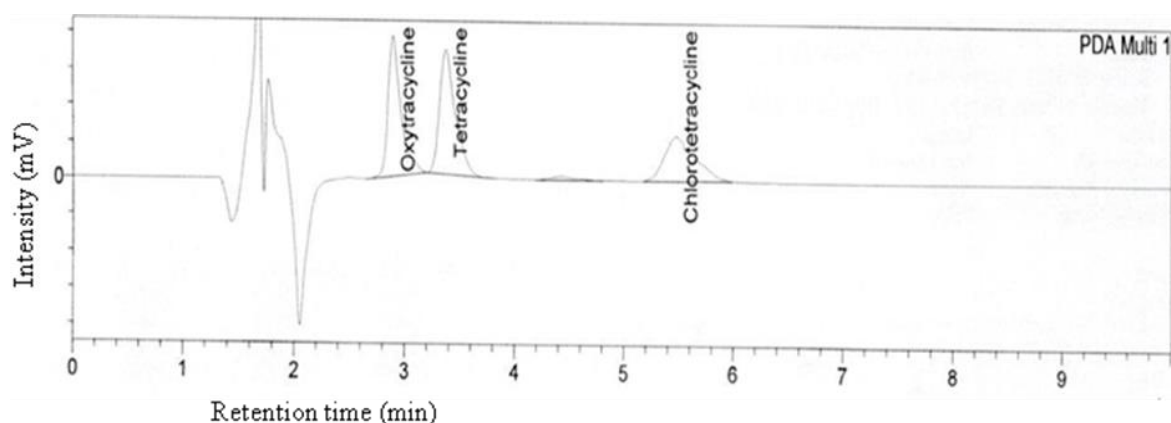
Among the selected 10 Delvo test positive milk samples, 9 were confirmed and quantified to have at least one detectable type of Tetracyclines by HPLC analysis (Table16 and Figure14). None of the quantified milk samples had measurable amounts of CTC (0.0%). The overall mean concentration of TCs in selected milk samples (n=10) was 6.1 ± 5.8 ug/l. The mean concentration of OTC and TTC were 7.7 ± 5.3 ug/l and 9.6 ± 16.9 ug/l respectively. Of importance, 70% of the quantified raw milk samples had OTC at varying concentrations and 40% of the quantified raw milk samples had TTC at varying concentrations. The recommended MRL for TCs cattle milk is 100 μ g/l (CAC, 2015).

Table 16: Concentration of Tetracyclines in selected raw milk samples (n=10)

Sample ID	OTC (ug/l)	TTC (ug/l)	CTC (ug/l)	Mean conc. (ug/l)
ED6	11.5	11.9	0.0	7.8
SM22	0.0	11.8	0.0	5.9
BD5	11.0	0.0	0.0	5.5
SM9	11.0	53.4	0.0	21.5
GF2	0.0	18.5	0.0	6.2
FF1	0.0	0.0	0.0	0.0
GA3	10.8	0.0	0.0	3.6
FD4	10.6	0.0	0.0	3.5
FD6	11.1	0.0	0.0	3.7
DB4	10.4	0.0	0.0	3.5
Mean conc. (ug/l)	7.7 \pm 5.3	9.6 \pm 16.9	0.0 \pm 0	6.1 \pm 5.8

Note: The recommended Maximum Residue Limit(MRL) for OTC, TTC and CTC is 100 μ g/l (CAC, 2015)

Legend: ID- Identity, OTC- Oxytetracycline, TTC- Tetracycline, CTC- Chlortetracycline

**Figure 13: A chromatogram indicating Tetracycline residues in raw cattle samples.**

Note that: the mean concentrations of Oxytetracycline was 7.7 μ g/l and that of Tetracycline was 9 μ g/l.

CHAPTER FIVE

5.0 DISCUSSION

The purpose of the current study was to estimate the prevalence of *Brucella abortus* infection in lactating cows, assess the risk factors for the infection and analyse the antimicrobial residues in raw milk in Bukombe district. It was generally found that most of the livestock keepers were agro-pastoralists who kept local breeds cattle under extensive management system. None of the factors considered being responsible for *Brucella* transmission in cattle and antimicrobial residues in milk were found to be significant ($p>0.05$). Seroprevalence of brucellosis in cattle was 1.4% while the herd prevalence was 3.8%. Of the three seropositive samples, only one was confirmed to be *B. abortus*. The proportion of antimicrobial residues contaminations in milk was 11.6% and the Tetracyclines concentration was 6.1 ± 5.8 ug/l. Although the observed seroprevalence of brucellosis in cattle was low, the nature of cattle farming gives possibilities for further spread and poses a threat to the general public who consumes milk. The milk consumers are possibly at stake from the effects of antimicrobial residues as supported by positive Delvo test results. However, consumers might be spared from hazardous effects of Tetracyclines residues in milk as the levels were below the recommended MRL in this study. This calls for deliberate control strategies of *Brucella* infection in cattle and antimicrobial residues in food of animal origin.

5.1 Sociological Study

The current study found that most of the livestock keepers were sukuma who mostly kept TSHZ and the animals were grazed and watered in communal areas. It was so, because the sukuma tribe is dominant in the lake zone and it is a leading tribe in traditional animal keeping in the lake zone. Interaction of cattle with wildlife was common, a practice that

can be the factor for the spread of brucellosis in domestic animals. It was demonstrated by the livestock keepers grazing their cattle around and sometimes in Kigosi game reserve and Bukombe forest reserve.

Among respondents, 44.4% reported problem of abortions in cattle and the raw placenta and aborted foetus were being fed to dogs. Possibly, brucellosis contributed to some of the observed abortions. A common practice of feeding raw placenta and aborted foetus to dogs observed in the study site can increase the transmission of brucellosis among the domestic animals. Similar observation has been reported by Temba (2012) in Mikumi-Selous ecosystem whereby frequent abortions was associated with *Brucella* seropositivity in cattle and the aborted foetuses and placenta were being fed to dogs. Public awareness on proper disposal of aborted foetuse and placenta materials is recommended.

Respondents reported a number of diseases that affected their cattle and this was among the reasons for frequent uses of veterinary drugs in particular antibiotics which they used to buy and administer to their animals themselves. The challenges with such practices are based on the fact that proper dosages, indications, route of administration and drug withdrawal periods are uncertain since 100% of the interviewed farmers bought and administered drugs to animals themselves. This gives more chance for making mistakes which may likely result to drug residues in food of animal origin like milk. This was probably due to low education and less knowledge of the farmers since majority (51.9%) had primary education and also had no training on animal husbandry. In the study by Midenge (2011) in Dar es Salaam and Bukuku (2013) in Arusha reported that among the reasons for indiscriminate uses of antibiotics were endemic livestock diseases and inadequate extension services at the grass route.

5.2 Seroprevalence of *Brucella* infection in Cattle

The current study estimated a seroprevalence of brucellosis in cattle to be 1.4% while the herd prevalence was 3.8%. It should be taken into account that the seroprevalence was based on lactating indigenous cows only. Studies of brucellosis in cattle in Tanzania range between 2% and 90% (Swai, 1997; Temba, 2012; Chitupila *et al.*, 2015; Assenga *et al.*, 2015). The animal sero-prevalence of 1.4% of brucellosis in lactating traditional cattle in Bukombe lies within the range of brucellosis studies done in several parts of Tanzania (Assenga *et al.*, 2015; Chitupila *et al.*, 2015). The seroprevalence of this study is in agreement with other studies from 0.94% to 21.88% in Kenya, 0.84% to 2.21% at Kwa Zulu Natal and 0% to 15% at magisterial districts in South Africa (Hesterberg, 2008; Chota *et al.*, 2016). It is slightly higher than 1% and 1.2% seroprevalence studies in Ethiopia and Iran respectively (Maadi *et al.*, 2011; Adugna *et al.*, 2013). It is slightly lower than 1.7%, 2.1% and 3.5% seroprevalence studies in Ethiopia (Asmare *et al.*, 2010; Yohannes *et al.*, 2012). It is argued that, the variation in animal sero-prevalence of brucellosis in Tanzania and probably other parts of the world could be contributed by variation in animal production systems in peri-urban, urban and rural settings. However, other factors could be inadequate law enforcement, uninformed farmers and unavailable tests (Karimuribo *et al.*, 2007; Lyimo, 2013). The sero-prevalence of 1.4% in this study probably gives a true exposure to *B. abortus* in traditional cattle of Bukombe because one of the milk sample of the seropositive lactating cattle was confirmed by PCR to have the *bcs31* gene encoding immunogenic membrane protein of 31 kDa of *B. abortus* (a band size of 223 bp). It is possible that brucellosis exists in low levels in some of the livestock keeping communities in Tanzania.

It was further observed that both RBPT and c-ELISA gave similar results. Therefore, with these results RBPT can be routinely used for screening of brucellosis without a need of c-

ELISA since it is costly and need more sophisticated equipment in better laboratories to perform it. PCR results indicated that out of the three blood samples that were positive to RBPT and c-ELISA test, only one was found to be *B. abortus*. This implies that the other two may be *Brucella melitensis* which is also reported to cause infection in cattle (Megid *et al.*, 2010). It is also possible that at the time of milk sampling *B. abortus* pathogen wasn't secreted into milk, for it is well known to be secreted intermittently in milk of lactating cattle and other animals (Hamidy and Amin, 2002; Capparelli *et al.*, 2009; Wareth *et al.*, 2014).

5.3 Antimicrobial Residues in Milk

The qualitative results of milk samples by Delvo test indicated that 11.6% of the tested samples had antimicrobial residues. At the herd level, it was found that 34.6% of the herds sampled had detectable levels of antimicrobial residues. The observed results are higher than the studies by Kivaria *et al.* (2006) and Mdegela *et al.* (2009) but lower than those reported by Rwehumbiza *et al.* (2012) and Karimuribo *et al.* (2015) in Tanzania. Elsewhere, there have been variable results on antimicrobial residues in milk (Addo *et al.*, 2011; Grădinaru *et al.*, 2011; Salman *et al.*, 2012; Aalipour *et al.*, 2013; Mangsi *et al.*, 2014; Ahlberg *et al.*, 2016; Layada *et al.*, 2016; Orwa *et al.*, 2017). The variation in proportions of milk with antimicrobial residues is probably due to different production system farmers awareness, available surveillance and monitoring systems.

The positive milk samples (11.6%) based on Delvo test is possibly due to traditional animal keepers and sometimes animal attendants administering antibiotics to their animals in incorrect dosages. These farmers actually are less educated and less knowledgeable (42.6% had informal education and 51.9% had primary education) on issues of veterinary drug administration at correct dosages and routes; and had not received any training on

animal husbandry. Therefore, these animal keepers can likely administer antibiotics at incorrect dosages and routes. Moreover, 59.3% of the respondents did not adhere to drug withdrawal periods resulting into drug residues in milk. Generally, all respondents admitted to use Tetracyclines and Penicillin as common antibiotics for treatment of various diseases in their traditional herds of cattle. This gives an insight of the kind of the antimicrobial residues to possibly have encountered in milk compared to others.

It was further observed that Namonge Ward had the highest number of animals but it was one of the wards with a few herds with antimicrobial residues in the milk detected by Delvo test. The reason for low antimicrobial residues is difficult to ascertain, however it can be argued that, Blackquarter is the rampant disease which in most cases is characterized by death of animals within 2 days after its onset and usually treatment with penicillin is not effective at all. Although it helps the ill animal recovery when it is earlier instituted at very high doses for up to seven days two times a day (Sultana *et al.*, 2008; Zahid *et al.*, 2012). A few treatment with penicillin is done if any in this ward as farmers are less knowledgeable about its rapid onset therefore, a delay of diagnosis that gives rare chances of the ill animals to survive.

Milk containing antimicrobial residues are unsuitable for human consumption, as the drug residues had the potential to cause allergic reactions to consumers (Katakweba *et al.*, 2012; Ndungu *et al.*, 2016), interfere with starter cultures in dairy processing such as yoghurt production and exert selective pressure to microbes for antimicrobial resistance.

Interestingly, 90% of the milk samples that were Delvo test positive were found to contain at least one or two types of tetracyclines residues which give more evidences on frequent uses of these types of antibiotics in traditional cattle production in Bukombe district.

However the quantified tetracyclines residues in the milk samples were below the recommended minimum residual limit (MRL) of 100 µg/l for tetracyclines in raw milk (Applegren *et al.*, 1999; EU, 2009; CAC, 2012). Although these findings comply with the set MRL for Tetracycline, the result does not exclude antimicrobial residues in milk as farmers are ill-informed about withdrawal periods and mechanisms are not in place for surveillance and monitoring.

The 1% negative results of the HPLC analysed milk samples gave an indication of false positives tests by the Delvo test kit. The false positives could be attributed by the accumulation of somatic cells counts and presence of natural inhibitors (Hillerton *et al.*, 1999). All in all, farmers need to be emphasized on the observation of drug withdrawal periods since antimicrobial residues and antimicrobial resistance is a global health agenda and to combat the problem need the concerted efforts (Bertu *et al.*, 2010; Huttner *et al.*, 2013). Brucellosis being a zoonotic and notifiable disease need attention by all responsible authorities to take care of its prevention and control in animals facilitated by a smart surveillance and monitoring program.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the above findings the following are the conclusions of the study;

- i. Bukombe has a wildlife domestic animal interface which is an accelerator for transmission of zoonotic diseases including brucellosis from wildlife to domestic animals.
- ii. Abortion cases seem to be among of the health constraints to cattle herds in Bukombe.
- iii. Most farmers either neglect or are unaware of abiding to drug withdrawal periods.
- iv. Bovine brucellosis is prevalent at low levels (1.4%) in Bukombe district.
- v. In this study RBPT and c-ELISA serological tests had a perfect agreement to each other, as they had displayed common results.
- vi. The PCR detection of *B. abortus* in one of the raw milk samples of *Brucella* seropositive animals, justified the risk of the consumers consuming raw milk.
- vii. The detection of antimicrobial residues in 11% of 198 raw milk samples by the Delvo test indicated the risk of consumers to allergies as well as a progressive development of antibiotic resistance.
- viii. The 90% confirmation of tetracyclines in quantified raw milk implies that the drug is commonly used cattle of Bukombe
- ix. The mean concentrations of tetracyclines in quantified raw milk were below recommended MRL (100 µg/l).

6.2 Recommendations

Based on the above conclusions the following are the recommendations;

- i. The District Veterinary Officer (DVO) in cooperation with the Director of Veterinary Services (DVS) should implement a continuous sero-surveillance of livestock herds. To pin point of whether the abortion cases appearing in the livestock herds here and there are brucellosis based or caused by other diseases.
- ii. Livestock households should be educated on the proper use of antibiotics and sensitized to follow recommended drug withdrawal periods.
- iii. Although brucellosis is available at low prevalence a mere control strategy should be designed to contain the disease as it is a risk for human brucellosis.
- iv. Livestock households should be educated on the global agenda of antimicrobial residues and antimicrobial resistance. They should conceptualize it, as a global emergency agenda that needs serious counteraction to rescue the current and future generation from failures of antibiotics.
- v. More representative raw milk samples from traditional lactating cows in Bukombe should be quantified for the TCs residues so as to have a profound evidence of their risk to the consumers.
- vi. The penicillin's residues should be quantified in raw milk from traditional lactating cows in Bukombe because they were reported by respondents as the second commonly used veterinary antibiotics.
- vii. The low levels of TCs in quantified raw milk samples possibly gives an expression of safe supply of raw milk in Bukombe in terms of TCs residues.

REFERENCES

- Aalipour, F., Mirlohi, M. and Jalali, M. (2013). Prevalence of antibiotic residues in commercial milk and its variation by season and thermal processing methods. *International Journal of Environmental Health Engineering* 2(3): 1-5.
- Addo, K.K., Mensah, G.I., Aning, K.G., Nartey, N., Nipah, G.K., Bonsu, C., Akyeh, M.L. and Smits, H.L. (2011). Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana. *Tropical Medicine and International Health* 16: 227-232.
- Adesokan, H.K, Alabi, P.I. and Ogundipe, M.A. (2016). Prevalence and predictors of risk factors for Brucellosis transmission by meat handlers and traditional healers' risk practices in Ibadan, Nigeria. *Journal of Preventive Medicine and Hygiene* 57: 164-171.
- Adugna, K.E., Agga, G.E. and Zewde, G. (2013). Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia. *Scientific and Technical Review* 32(3): 1-20.
- Ahlberg, S., Korhonen, H., Lindfors, E. and Kang'ethe, E. (2016). Analysis of antibiotic residues in milk from smallholder farms in Kenya. *African Journal of Dairy Farming and Milk Production* 3(4): 152-158.
- Akinwumi, O.A., Olatoye, O.I., Odunsi, A.A., Omojola, B.A. and Rafiu, A.T. (2012). Effect of antibiotic residues on the physical quality of beef. In: *Proceeding of the 4th International Conference on Agriculture and Animal Science*. 2012, Oyo state, Nigeria. 10 – 776pp.

- Alavi, S.M. and Alavi, L. (2013). Treatment of brucellosis: a systematic review of studies in recent twenty years. *Caspian Journal of Internal Medicine* 4(2): 636-641.
- Alton, G., Jones, L.M., Angus, R.D. and Verger, J.M. (1988). *Techniques for the Brucellosis Laboratory*. Institut National de la Recherche Agronomique. Paris France. 190pp.
- Applegren, L., Arnold, D., Boisseau, J., Boobis, A., Ellis, R. and Livingston, R. (1999). Evaluation of certain veterinary drug residues in food. *Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives* 1999; 888: 1–95.
- Ariza, J., Bosilkovski, M., Cascio, A., Colmenero, J.D., Corbel, M.J., Falagas, M.E., Memish, Z.A., Roushan, M.R.H., Rubinstein, E., Sipsas, N.V., Solera, J., Young, E.J. and Pappas, G. (2007). Perspectives for the treatment of brucellosis in the 21st century: The Ioannina recommendations. *PLoS Medicine* 4 (12): 1872-1878.
- Asmare, K., Asfaw, Y., Gelaye, E. and Ayelet, G. (2010). Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. *African Journal of Agricultural Research* 5(3): 257-263.
- Assenga, J.A., Matamba, L.E., Muller, S.K., Malakalinga, J.J. and Kazwala, R.R. (2015). Epidemiology of *Brucella* infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. *BMC Veterinary Research* 11: 1-11.
- Aytenfsu, S., Mamo, G. and Kebede, B. (2016). Review on Chemical Residues in Milk and Their Public Health Concern in Ethiopia. *Journal of Nutrition and Food Sciences* 6(4): 1-11.
- Baily, G.G., Krahn, J.B., Drasar, B.S. and Stoker, N.G. (1992). Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *The American Journal of Tropical Medicine and Hygiene* 95: 271-275.

- Bertu, W.J., Dapar, M., Gusi1, A. M., Ngulukun, S. S., Leo, S. and Jwander, L. D. (2010). Prevalence of Brucella Antibodies in Marketed Milk in Jos and environs. *African Journal of Food Science* 4(2): 62-64.
- Beyene, T. (2016). Veterinary Drug Residues in Food-animal Products: Its Risk Factors and Potential Effects on Public Health. *Journal of Veterinary Science and Technology* 7(1): 1-7.
- Bosilkovski, M., Krteva, L., Dimzova, M., Vidinic, I., Sopova, Z. and Spasovska, K. (2010). Human Brucellosis in Macedonia – 10 Years of Clinical Experience in Endemic Region. *Croatian Medical Journal* 51: 327-36.
- Bouley, A.J., Biggs, H.M., Stoddard, R.A., Morrissey, A.B., Bartlett, J.A. and Afwamba, I.A. (2012). Brucellosis among hospitalized febrile patients in Northen Tanzania. *American Journal of Tropical Medicine and Hygiene* 87(6): 1105-1111.
- Bukuku, J.N. (2013). Awareness of health risks as a result of consumption of raw milk in Arusha city and Meru district, Tanzania. A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Public Health and Food Safety of Sokoine University of Agriculture. Morogoro, Tanzania. 74pp.
- Bukuku, J.N., Nonga, H.E. and Mtambo, M.A.M. (2015). Assessment of raw milk quality and stakeholders awareness on milk-borne health risks in Arusha City and Meru District, Tanzania. *Tropical Animal Health and Production* 47(5): 927-932.
- CAC (Codex Alimentarius Commission). (2012). *Maximum Residue Limits for Veterinary Drugs in Foods*. Updated as at the 35th Session of the Codex Alimentarius Commission 2012. pp1-40.

- CAC (Codex Alimentarius Commission). (2015). *Maximum Residue Limits (MRL) and Risk Management Recommendations (RMRS) for Residues of Veterinary Drugs in Foods CAC/MRL 2-2015*. Updated as at the 38th Session of the Codex Alimentarius Commission July 2015, 1-41.
- Capparelli, R., Parlato, M., Iannaccone, M., Roperto, S., Marabelli, R., Roperto, F. and Iannelli. (2009). Heterogeneous shedding of *Brucella abortus* in milk and its effect on the control of animal brucellosis. *Journal of Applied Microbiology* 106: 2041-2047.
- Chaussa, K.S. (2013). Assessment of factors affecting performance of dairy cattle kept in smallholder farms in peri urban areas of Temeke Municipality. A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Management of Natural Resource and Sustainable Agriculture of Sokoine University of Agriculture, Morogoro, Tanzania. 114pp.
- Chitupila, G.Y., Komba, E.V.G. and Mtui-Malamsha, N.J. (2015). Epidemiological study of bovine brucellosis in indigenous cattle population in Kibondo and Kakonko Districts, Western Tanzania. *Livestock Research for Rural Development* (6): 1-12.
- Chota, A.C., Magwisha, H.B., Stella, B., Bunuma, E.K., Shirima, G.M., Mugambi, J.M., Omwenga, S.G., Wesonga, H.O., Mbatha, P. and Gathogo, S. (2016). Prevalence of brucellosis in livestock and incidences in humans in East Africa. *African Crop Science Journal* 24(1): 45 – 52.
- Chuma, I.S., Nonga, H.E., Mdegela, R.H. and Kazwala, R.R. (2016). Epidemiology and RAPD-PCR Typing of Thermophilic Campylobacters from Children Under Five Years and Chickens in Morogoro Municipality, Tanzania. *BMC Public Health* 16: 1-11.

- Corbel, M.J. (2006). Brucellosis in humans and animals. Produced by the World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and World Organisation for Animal Health. 89pp.
- Darwish, W.S., Eldaly, E.A., El-Abbasy, M.T., Ikenaka, Y., Nakayama, S. and Ishizuka, M. (2013). Antibiotic residues in food: the African scenario. *Japanese Journal of Veterinary Research* 61: 13-22.
- Dey, S. and Karimm, M.H. (2013). Study on physicochemical and microbial quality of available raw, pasteurized and UHT milk during preservation. *International Journal of Science Inventions Today* 2(2): 150-157.
- Dinki, N. and Balcha, E. (2013). Detection of antibiotic residues and determination of microbial quality of raw milk from milk collection centers. *Advances in Animal and Veterinary Sciences* 1(3): 80 – 83.
- Ducrotoy, M., Bertu, W.J., Matope, G., Cadmus, S., Conde-Álvarez, R., Gusi A.M., Welburn, S., Ocholi, R., Blasco J.M. and Moriyón, I. (2017). Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. *Acta Tropica* 165: 179–193.
- Engida, E., Guthiga, P. and Karugia, J. (2015). The Role of Livestock in the Tanzanian Economy: Policy Analysis Using a Dynamic Computable General Equilibrium Model for Tanzania. In: *Proceeding of International Conference of Agricultural Economists*. 8-14, August, 2015. Milan, Italy. 22pp.
- European Commission (EU). (2009). On pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Commission Regulation (EU). No 37/2010 of 22 December 2009, 1–76.

- Fisher, A.A., Liang, J.E. and Townsend, J.W. (1991). *Handbook for family operations research and design* 2nd edition. Population Council, New York, New York. 77pp.
- Forouzan, S., Rahimirad, A., Seyedkhoei, R., Asadzadeh, J. and Bahmani, M. (2014). Determination of antibiotic residues in the pasteurized milk produced in West Azerbaijan province, North West of Iran. *Journal of Coastal Life Medicine* 2(4): 297-301.
- Franco, M.P., Mulder, M., Gilman, R.H. and Smits, H.L. (2007). Humana brucellosis. *The Latent Infectious Diseases* 7: 775-786.
- Fussi, J.I. (2010). Strategies to increase milk deliveries to the Tanzanian milk processing industry, a case of ASAS Dairies Ltd in Iringa District. A Research Project submitted to Larenstein University of Applied Sciences in Partial Fulfilment of the Requirements for the Degree of Master in Agricultural Production Chain Management Specialization in Livestock Production Chains, Netherlands. 57pp.
- Gaurav, A., Gill, J.P.S., Aulakh, R.S. and Bedi, J.S. (2014). ELISA based monitoring and analysis of tetracycline residues in cattle milk in various districts of Punjab, *Veterinary World* 7: 26-29.
- Geresu, M.A. and Kassa, G.M. (2016). A Review on Diagnostic Methods of Brucellosis. *Journal of Veterinary Science and Technlogy* 7(3): 1-8.
- Ghidini, S., Zanardi, E., Varisco, G. and Chizzolini, R. (2003). Residues of β -lactam antibiotics in bovine milk: confirmatory analysis by liquid chromatography tandem mass spectrometry after microbial assay screening. *Food Additives and Contaminants* 20: 528–534.

- Global Antibiotic Resistance Partnership (GARP)—Tanzania Working Group. (2015). Situation Analysis and Recommendations: Antibiotic Use and Resistance in Tanzania. Washington, DC and New Delhi: Center for Disease Dynamics, Economics and Policy. 110pp.
- Godfroid, J., Scholz, H.C., Barbier, T., Nicolas, C., Wattiau, P., Fretine, D., Whatmore, A.M., Cloeckaert, A., Blasco, J.M., Moriyon, I., Saegerman, C., Muma, J.B., Al Dahouk, S., Neubauer, H. and Letesson, J.J. (2011). Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Preventive Veterinary Medicine* 102(2): 118-131.
- Grădinaru, A.C., Popescu, O. and Solcan, G. (2011). Antibiotic residues in milk from Moldavia, Romania. *Human and Veterinary Medicine International Journal of the Bioflux Society* 3(2): 133-141.
- Granados-Chinchilla, F. and Rodríguez, C. (2017). Tetracyclines in Food and Feedingstuffs: From Regulation to Analytical Methods, Bacterial Resistance, and Environmental and Health Implications. *Journal of Analytical Methods in Chemistry* 2017: 1-24.
- Hamidy, M.E. and Amin, A.S. (2002). Detection of Brucella species in the milk of infected cattle, sheep, goats and camels by PCR. *Veterinary Journal* 163(3): 299-305.
- Hashemi, S.H., Gachkar, L., Keramat, F., Mamani, M., Hajilooi, M., Janbakhsh, A., Majzoobi, M.M. and Mahjub, H. (2012). Comparison of doxycycline–streptomycin, doxycycline–rifampin, and ofloxacin–rifampin in the treatment of brucellosis: a randomized clinical trial. *International Journal of Infectious Diseases* 16: 247-251.

- Hesterberg, U.W., Bagnallb, R., Perrettb, K., Boschb, B., Hornerb, R. and Gummow. (2008). A serological prevalence survey of *Brucella abortus* in cattle of rural communities in the province of KwaZulu-Natal, South Africa. *The Journal of the South African Veterinary Association* 79(1): 15–18.
- Hillerton, J.E., Halle, B.I., Neaves, P. and Rose, M.D. (1999). Detection of antimicrobial substances in individual cow and quarter milk samples using Delvo test microbial inhibitor tests. *Journal of Dairy Science* 82(4): 704-711.
- Huttner, A., Harbarth, S., Carlet, J., Cosgrove, S., Goossens, H., Holmes, A., Jarlier, V., Voss, A. and Pittet, D. (2013). Antimicrobial resistance: a global view from the 2013 World Healthcare-Associated Infections Forum. *Antimicrobial Resistance and Infection Control* 2: 1-13.
- International Dairy Federation Factsheet (IDF Fact sheet). (2014). *Control and Detection of Antimicrobial Residues in Milk and Dairy Products*. Guide to Prudent Use of Antimicrobial Agents in Dairy Production. 2pp.
- James, L.W. (2013). Studies on human brucellosis in the Mikumi Selous Ecosystem, Morogoro, Tanzania. A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Public Health and Food Safety of Sokoine University of Agriculture Morogoro, Tanzania. 64pp.
- Jayalakshmi, K., Paramasivam, M., Sasikala, M., Tamilam, T.V. and Sumithra, A. (2017). Review on antibiotic residues in animal products and its impact on environments and human health. *Journal of Entomology and Zoology Studies* 5(3): 1446-1451.
- John, K., Fitzpatrick, J., French, N., Kazwala, R., Kambarage, D., Mfinanga, G.S., MacMillan, A. and Cleaveland, S. (2010). Quantifying Risk Factors for Human Brucellosis in Rural Northern Tanzania. *PLoS ONE* 5(4): 1-6.

- Joseph, A. (2015). Assessment of Microbiological Hazards along the Milk Value Chain in 2Kilosa and Mvomero Districts, Tanzania. A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Public Health and Food Safety of Sokoine University of Agriculture Morogoro, Tanzania. 57pp.
- Kaale, E., Chambuso, M. and Kitwala, J. (2008). Analysis of residual oxytetracycline in fresh milk using polymer reversed-phase column. *Food Chemistry* 107(3): 1289–1293.
- Kadigi, M.L. (2013). Factors influencing choice of milk outlets among smallholder dairy farmers in Iringa Municipality and Tanga City. A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Agricultural Economics of Sokoine University of Agriculture. Morogoro, Tanzania. 153pp.
- Kailembo, L.C. (2013). Linking rural smallholder milk producers to urban markets a case study of selected wards in Kibaha District, Tanzania. A research report submitted to Van Hall Larenstein University of Applied Sciences in Partial Fulfillment of the Requirement of Master in Agriculture Production Chain Management specializing in Livestock Chains, Netherlands. 52pp.
- Kanyeka, H.B. (2014). Assessment of Microbial Quality of Raw Cow's Milk and Antimicrobial Susceptibility of Selected Milk-borne Bacteria in Kilosa and Mvomero Districts, Tanzania. A dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Public Health and Food Safety of Sokoine University of Agriculture. Morogoro, Tanzania. 7pp.

- Karimuribo, E.D., Gallet, P.L., Ng'umbi, N.H., Matiko, M.K., Massawe, L.B., Mpanduji D.G. and Batamuzi, E.K. (2015). Status and factors affecting milk quality along the milk value chain: a case of Kilosa district, Tanzania. *Livestock Research for Rural Development* 27(3). [http://www.lrrd.org/lrrd27/3/kari_27051.html].
- Karimuribo, E.D., Kusiluka, L.J., Mdegela, R.H., Kapaga, A.M., Sindato, C. and Kambarage, D.M. (2005). Studies on mastitis, milk quality and health risks associated with consumption of milk from pastoral herds in Dodoma and Morogoro regions, Tanzania. *Journal of Veterinary Science* 6(3): 213 – 221.
- Karimuribo, E.D., Ngowi, H.A., Swai, E.S. and Kambarage, D.M. (2007). Prevalence of brucellosis in crossbred and indigenous cattle in Tanzania. *Livestock Research for Rural Development* 19(10). [<http://www.lrrd.org/lrrd19/10/kari19148.htm>].
- Karmi, M. (2014). Detection and Presumptive Identification of Antibiotic Residues in Poultry Meat by Using FPT. *Global Journal of Pharmacology* 8 (2): 160-165.
- Katakweba, A.A.S., Mtambo, M.M.A., Olsen, J.E. and Muhairwa, A.P. (2012). Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. *Livestock Research for Rural Development* 24(10). [<http://www.lrrd.org/lrrd24/10/kata24170.htm>].
- Kebede, G., Zenebe, T., Disassa, H. and Tolosa, T. (2014). Review on Detection of Antimicrobial Residues in Raw Bulk Milk in Dairy Farms. *African Journal of Basic and Applied Sciences* 6(4): 87-97.
- Kebede, T., Ejeta, G. and Ameni, G. (2008). Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district) *Revue de Médecine Vétérinaire* 159(1): 1-9.

- Khaniki, G.R.J. (2007). Chemical contaminants in milk and public health concerns: A review. *International Journal of Dairy Science* 2(2): 104-115.
- Kimera, Z.I., Mdegela, R.H., Mhaiki, C.J.N., Karimuribo, E.D., Mabiki, F., Nonga, H.E. and Mwesongo, J. (2015). Determination of oxytetracycline residues in cattle meat marketed in the Kilosa district, Tanzania. *Onderstepoort Journal of Veterinary Research* 82(1): 1-5.
- Kivaria, F.M., Noordhuizen, J.P. and Kapaga, A.M. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region, Tanzania. *Tropical Animal Health and Production* 38(3): 185-194.
- Kunda, B. (2015). Hygienic and compositional quality of raw milk produced by smallholder dairy farmers in Lusaka Province of Zambia. A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in One Health Analytical Epidemiology of Zambia University, Zambia. 46pp.
- Kunda, J., Cleaveland, S., Fitzpatrick, J., French, N., Kambarage, D.M., Shirima, G. and Kazwala, R.R. (2005). Brucellosis in Arusha and Manyara Regions, Tanzania: a challenge to public health. *Tanzania Medical Journal* 20(1): 28-32.
- Kunda, J., Fitzpatrick, J., Kazwala, R., French, N.P., Shirima, G., MacMillan, A., Kambarage, D., Bronsvoot, M. and Cleaveland, S. (2007). Health-seeking behaviour of human brucellosis cases in rural Tanzania. *BMC Public Health* 7: 315. DOI:10.1186/1471-2458-7-315.
- Kurwijila, L.R., Omore, A. and Grace, D. (2012). The Tanzania dairy industry overview. Sokoine University of Agriculture. 2-4pp.

- Kurwijila, R.L., Omore, A., Staal, S. and Mdoe, N.S.Y. (2006). Investigation of the risk of exposure to antimicrobial residue present in marketed milk in Tanzania. *Journal of Food Protection* 69: 2487 - 2492.
- Laxminarayan, R. and Heymann, D.L. (2012). Challenges of drug resistance in the developing world. *British Medical Journal* 344: 1-4.
- Layada, S., Benouareth, D.E., Coucke, W. and Andjelkovic, M. (2016). Assessment of antibiotic residues in commercial and farm milk collected in the region of Guelma (Algeria). *International Journal of Food Contamination* 3:1-16.
- Leonard, A., Gabagambi, D.M., Batamuzi, E.K., Karimuribo, E.D. and Wambura, R.M. (2016). A milk marketing system for pastoralists of Kilosa district in Tanzania: market access, opportunities and prospects. *International Journal of Agricultural Marketing* 3(1): 90-96.
- Lyimo, B.E. (2013). Prevalence of Bovine Brucellosis in Smallholder Dairy farms in Morogoro Municipality, Tanzania. A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Public Health and Food Safety of Sokoine University of Agriculture Morogoro, Tanzania. 86pp.
- Maadi, H., Moharamnejad, M. and Haghi, M. (2011). Prevalence of Brucellosis in Cattle in Urmia, Iran. *Pakistan Veterinary Journal* 31(1): 81-82.
- Mahlau, E.A. (1967). Further brucellosis surveys in Tanzania. *Bulletin of Epizootic Diseases in Africa* 15: 373 – 378.
- Mangsi, A.S., Khaskheli, M., Soomro, A.H. and Shah, M.G. (2014). Detection of antimicrobial drug residues in milk marketed at different areas of Sindh province *Journal of Agriculture and Veterinary Science* 7(5): 65-69.

- Mathew, C., Stokstad, M., Johansen, T.B., Klevar, S., Mdegela, R.H., Mwamengele, G., Michel, P., Escobar, L., Fretin, D. and Godfroid, J. (2015). First isolation, identification, phenotypic and genotypic characterization of *Brucella abortus* biovar 3 from dairy cattle in Tanzania. *BMC Veterinary Research* 11: 1-9.
- Mbiha, G.E. (2008). Analysis of the dairy value chain in the Dar es Salaam milk shed, Tanzania. A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Agricultural Economics of Sokoine University of Agriculture. Morogoro, Tanzania. 151pp.
- Mbugi, E.V., Katale, B.Z., Kendall, S., Good, L., Kibik, G.S., Keyyu, J.D, Godfrey-Fausset, P., Helden, P.V. and Matee, M.I. (2012). Tuberculosis cross-species transmission in Tanzania: Towards a One-Health concept. *Onderstepoort Journal of Veterinary Research* 79(2): 1-6.
- Mdegela, R.H., Ryoba, R., Karimuribo, E.D., Pire, E.J., Løken, T., Reksenc, O., Mtengetib, E. and Urio, N.A. (2009). Prevalence of clinical and subclinical mastitis and quality of milk in smallholder dairy farms in Tanzania. *Journal of the South African Veterinary Association* 80(3): 163 - 168.
- Megid, J., Mathias, L.A. and Robles, C.A. (2010). Clinical Manifestations of Brucellosis in Domestic Animals and Humans. *The Open Veterinary Science Journal* 4: 119-126.
- Mekuria, S., Regassa, A., Abebe, R., Fekade, A. and Dares, B. (2014). Bacteriological study collected from Hawassa smallholder dairy farms. *Advances in Biological Research* 8(5): 194-200.

- Mellau, L.S.B., Kuya, S.L. and Wambura, P.N. (2009). Seroprevalence of brucellosis in domestic ruminants in livestock-wildlife interface: A case study of Ngorongoro Conservation Area, Arusha, Tanzania. *Tanzania Veterinary Journal* 26(1): 44-50.
- Mgonja, F., Mosha, R., Mabiki, F. and Choongo, K. (2017). Oxytetracycline residue levels in beef in Dodoma region, Tanzania. *African Journal of Food Science* 11(2): 40-43.
- Midenge, B.Y. (2011). Awareness on recommended veterinary drugs withdrawal period among small scale dairy cattle farmers in Kinondoni Municipality. Dissertation for Award of MSc Degree at Muhimbili University of Health and Allied Sciences, Dar es salaam, Tanzania. 90pp.
- Moyane, J.N., Jideani, A.I.O. and Aiyegoro, O.A. (2013). Antibiotics usage in food producing animals in South Africa and impact on human: Antibiotic resistance. *African Journal of Microbiology Research* 7(24): 2990-2997.
- Mubito, E.P., Shahada, F., Kimanya, M.E. and Buza, J.J. (2014). Antimicrobial use in the poultry industry in Dar-es-Salaam, Tanzania and public health implications. *American Journal of Research Communication* 2(4): 51-63.
- Navarro, E., Escribano, J., Fernandez, J.A. and Solera, J. (2002). Comparison of three different PCR methods for detection of *Brucella* spp. in human blood samples. *FEMS Immunology and Medical Microbiology* 34: 147-151.
- Navratilova, P. (2008). Screening methods used for the detection of veterinary drug residues in raw cow milk - A review. *Czech Journal of Food Sciences* 26: 393-401.
- Ndungu, T.W., Muliro, P.S., Omwamba, M., Oosterwijk, G. and Jansen, A. (2016). Quality control of raw milk in the smallholder collection and bulking enterprises in Nakuru and Nyandarua Counties, Kenya. *African Journal of Food Science* 10(5): 70-78.

- Nikolić, N., Mirecki, S. and Blagojević, M. (2011). Presence of inhibitory substances in raw milk in the area of Montenegro. *Mljekarstvo* 61 (2): 182-187.
- Nisha, A.R. (2008). Antibiotic Residues - A Global Health Hazard. *Veterinary World* 1(12): 375-377.
- Njombe, A.P., Msanga, Y., Mbwambo, N. and Makembe, N. (2011). The Tanzania Diary Industries: Status, Opportunities and Prospects. In: *Proceedings of African Diary Conference and Exhibition*, 25-27 May, 2011. MovenPick Palm Hotel, Dar es Salaam, Tanzania. 19pp.
- Nonga, H.E. and Muhairwa, A.P. (2010). Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* isolates from free range domestic duck (*Cairina moschata*) in Morogoro municipality, Tanzania. *Tropical Animal Health and Production* 42(2): 165-172.
- Nonga, H.E., Mariki, M., Karimuribo, E.D. and Mdegela, R.H. (2009). Assessment of Antimicrobial Usage and Antimicrobial Residues in Broiler Chickens in Morogoro Municipality, Tanzania. *Pakistan Journal of Nutrition* 8(3): 203-207.
- Nonga, H.E., Simon, C., Karimuribo, E.D. and Mdegela, R.H. (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from small holder poultry keepers in Morogoro. municipality, Tanzania. *Zoonoses Public Health* 57(5): 339-344.
- Nonga, H.E., Sungura, K.H. and Ngowi, H.A. (2013). Assessment of veterinary drug use and determination of antimicrobial residues in broiler chicken meat in Urban district, Zanzibar, Tanzania. *Tanzania Veterinary Journal* 28(2): 26-29.

- OIE (Office International des epizooties). (2012). Bovine brucellosis. In. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. [<http://www.oie.int/en/international-standard-setting/terrestrial-manual/>] site visited on 20/7/2017.
- Orwa, J.D., Matofari, J.W., Muliro, P.S. and Lamuka, P. (2017). Assessment of sulphonamides and tetracyclines antibiotic residue contaminants in rural and peri urban dairy value chains in Kenya. *International Journal of Food Contamination* 4: 1-11.
- Padol, A.R., Malapure, C.D., Dimple, V.D. and Kamdi, B.P. (2015). Occurrence, Public Health Implications and Detection of Antibacterial Drug Residues in Cow Milk. *International Journal of Environmental Science and Technology* 10: 7-28.
- Pandey, G.S. and Voskuil, G.C.S. (2011). *Manual on Milk Safety, Quality and Hygiene*. Golden Valley agricultural Research Trust, Zambia. 52pp.
- Racloz, V., Schelling, E., Chitnis, N., Roth, F. and Zinsstag, J. (2013). Persistence of brucellosis in pastoral systems. *Revue Scientifique et Technique* 32(1): 61-70.
- Ramadhani, R. (2015). A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Public Health and Food Safety of Sokoine University of Agriculture. Morogoro, Tanzania. 76pp.
- Rubach, M.P., Halliday, J.E.B., Cleaveland, S. and Crumpa, J.A. (2013). Brucellosis in low-income and middle-income countries. *Current Opinion in Infectious Diseases* 26(5): 404–412.
- Rwehumbiza, J.M., Ryoba, R. and Karimuribo, E.D. (2012). TVA Conference on Contribution of the Veterinary Profession to the Improvement of Human Health. In: *30th TVA Conference on Contribution of the Veterinary Profession to the Improvement of Human Health*, 2012. pp 1–73.

- Salman, A.M., ElNasri, H.A. and Osman, I. A. M. (2012). Detection of antibiotic residues in milk using delvotest kit and the disc assay methods in Khartoum State, Sudan. *Journal of Veterinary Medicine and Animal Production* 3(2): 3-15.
- Shirima, G.M. (2005). The epidemiology of brucellosis in animals and humans in Arusha and Manyara regions in Tanzania. Thesis submitted for the degree of Doctor of Philosophy of University of Glasgow, United Kingdom. 257pp.
- Shitandi, A. (2004). Risk factors and control strategies of antibiotic residues in milk at farm level in Kenya. Thesis for Award of degree of Doctor of Philosophy (PhD) at the Swedish University of Agricultural Sciences, Uppsala, Sweden. 306pp.
- Solomon, M., Megersa, M., Muhie, Y., Gebremedin, D. and Keskes, S. (2013). Bacteriological quality of bovine raw milk at selected dairy farms in DebreZeit Town, Ethiopia. *Comprehensive Journal of Food Science and Technology Research* 1(1): 1-8.
- Sultana, M., Ahad, A., Biswas, P.K., Ashiqur Rahman, M.A. and Barua, H. (2008). Black Quarter (BQ) Disease in Cattle and Diagnosis of BQ Septicaemia Based on Gross Lesions and Microscopic Examination. *Bangladesh Journal of Microbiology* 25(1): 13-16.
- Swai, E. M. (1997). Studies on the prevalence of bovine brucellosis and reproductive performance in small-scale dairy cattle herds in Dar es Salaam and Morogoro regions. Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania. 67pp.
- Swai, E.S. and Schoonman, L. (2009). Human Brucellosis: Seroprevalence And Risk Factors Related To High Risk Occupational Groups In Tanga Municipality, Tanzania. *Blackwell Verlag Journal* 4(56): 183-187.

- Swai, E.S., Karimuribo, E.D., Schooman, L., French, N.P., Fitzpatric, J.L., Kambarage, D.M. and Bryant, M.J. (2005). Description, social-economic characteristics, disease management and mortality dynamics in smallholder's dairy production system in coastal humid region of Tanga, Tanzania. *Livestock Research for Rural Development* 17(4). [<http://www.lrrd.org/lrrd17/4/swa17041.htm>].
- Swai, S. and Karimuribo, E.D. (2011). Smallholder dairy farming in Tanzania Current profiles and prospects for development. *Outlook on Agriculture* 40: 21-27.
- Tadesse, G. (2016). Brucellosis Seropositivity in Animals and Humans in Ethiopia: A Meta-analysis. *PLOS Neglected Tropical Diseases* 10(10): 1-30.
- Temba, P.B. (2012). Seroprevalence of a *Brucella abortus* and other *Brucella species* in Wildlife-Livestock interface: a case study of Mikumi-Selous Ecosystem. A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Public Health and Food Safety of Sokoine University of Agriculture Morogoro, Tanzania. 130pp.
- Tumwine, G., Matovu, E., Kabasa, J.D., Owiny, D.O. and Majalija, S. (2015). Human brucellosis: sero-prevalence and associated risk factors in agro-pastoral communities of Kiboga District, Central Uganda. *BMC Public Health* 15: 1-8.
- Urassa, G. and Martin, G. (2013). A baseline study on the implementation of the vat rate of zero percent in the dairy sector in Tanzania. Tanzania Milk Processors Association (TAMPA). 71pp.
- URT (United Republic of Tanzania, Ministry of Livestock and Fisheries Development). (2010). Livestock sector development strategy. 79pp.

- URT (United Republic of Tanzania, Ministry of Livestock and Fisheries Development). (2011). Livestock sector development program. 114 pp.
- URT (United Republic of Tanzania, Ministry of Livestock and Fisheries Development). (2013). Basic data for Livestock and Fisheries. 135pp.
- USA: CLG-TET2.04. (2011). United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science. 13pp.
- Wareth, G., Melzer, F., Elschner, M.C., Neubauer, H. and Rosler, U. (2014). Detection of *Brucella melitensis* in bovine milk and milk products from apparently healthy animals in Egypt by real-time PCR. *Journal of Infection in Developing* 8(10): 1339-1343.
- Wassena, F.J., Mangesho, W.E., Chawala, A., Laswai, G.H., Bwire, J.M.N., Kimambo, A.E., Lukuyu, B., Sikumba, G. and Maass, B.L. (2015). Effects of season and location on cattle milk produced and producer milk prices in selected villages of Tanga and Morogoro Regions, Tanzania. *Livestock Research for Rural Development* 27(10): 1-6.
- Weinhäupl, I., Schöpf, K.C., Khaschabi, D., Kapaga, A.M. and Msami, H.M. (2000). Investigations on the prevalence of bovine tuberculosis and brucellosis in dairy cattle in Dar es Salaam Region and in zebu cattle in Lugoba area, Tanzania. *Tropical Animal Health and Production* 32: 147 - 154.
- Yohannes, M., Mersha, T., Degefu, H., Tolosa, T. and Woyesa, M. (2012). Bovine Brucellosis: Serological Survey in Guto-Gida District, East Wollega Zone, Ethiopia. *Global Veterinaria* 8 (2): 139-143.

- Zahid, U.N., Kumar, S.M., Randhawa, S.S., Randhawa, S.S. and Hassan, M.N. (2012). Black Quarter in crossbred dairy cattle- A Case Report. *Veterinary World* 5(12): 767-770.
- Zamri-Saad, M. and Kamarudin, M.I. (2016). Control of animal brucellosis: The Malaysian experience. *Asian Pacific Journal of Tropical Medicine* 9(12): 1136–1140.

APPENDICES

Appendix 1: Checklist template for the study of risk factors of brucellosis

1.0 General information on interview

[1.1.] Geographical position

[1.2.] Name of interviewee

[1.3.] Village.....

[1.4.] District.....

[1.5.] Date of interview: Year..... Month..... Day.....

[1.6.] Time of beginning of interview.....

[1.7.0.] Ethnicity

[1.7.1.] ☐ Maasai [

1.7.2.] ☐ Barbaig

[1.7.3.] ☐ Sukuma

[1.7.4.] ☐ Other (specify).....

2.0 General information on interviewee/respondent

[2.1.] Age of the respondent:.....years old

[2.2.0.] Sex of the respondent:

[2.2.1.] ☐ Male? [2.2.2.] ☐ Female?

[2.3.0.] Position of the respondent in household

[2.3.1.] ☐ Head of household

[2.3.2.] ☐ Wife of head of household

[2.3.3.] ☐ Child

[2.3.4.] ☐ Parents or parents in law of head of household

[2.3.5.] ☐ Other (specify).....

[2.4.0.] Which number of animals do you have in your household? *(please cross and fill in a number if there are any:)*

[2.4.1.] ☐ Female (*adults, born before 2015*) [2.4.1.2] ☐ Female (*juveniles, born in 2016*)

[2.4.3] ☐ Male (*adults, born before 2015*) [2.4.4] ☐ Male (*juveniles, born in 2016*)

[3. 0.] Risk factors for brucellosis transmission in livestock

[3.1.0] Who is primarily responsible for looking after the animals?

[3.1.1] ☐ Owner/family member [3.1.2] ☐ Hired care taker [3.1.3] ☐ Both [3.1.4] Others specify.....

[3.2.0] Do you receive any Veterinary services?

[3.2.1.] ☐ No [3.2.2.] ☐ Yes

[3.3.0] Do you help in the birthing of animals?

[3.3.1.] ☐ No? [3.3.2.] ☐ Yes?

[3.3.2.0.] If yes, does it happen, that you have to break the umbilical cord?

[3.3.2.1.] ☐ No? [3.3.2.2.] ☐ Yes? If yes, how?

[3.4.0] Do you keep baby animals in one house?

[3.4.1.] ☐ Yes? [3.4.2.] ☐ No?

[3.5.0] Do your calves drink milk from the same container?

[3.5.1.] ☐ No..... [3.5.2.] ☐ Yes...

[3.6.0] Have you sold or buy any animals in the last twelve month

[3.6.1] ☐ No [3.6.2] ☐ Yes

[3.7.0] If yes, where did you sell your animals, or where did the buyers come from?

[3.7.1.] ☐ Within the neighbouring villages

[3.7.2.] ☐ Within the town.....

[3.7.3.] ☐ Outside the region.....

[3.7.4.] ☐ All of the above (3.7.1, 3.7.2 and 3.7.3.) [3.7.5.] ☐ Others Specify.....

[4.0.] Risk factors for spread of brucellosis between livestock and wildlife animals

[4.1.0] What type of feeding / grazing system do you practice? [

4.1.1.] ☐ Grazing from communal pastures.....

[4.1.2.] ☐ Grazing from own fields / paddocks.....

[4.1.3.] ☐ Communal and own pasture grazing.....

[4.1.4.] ☐ Others (state).....

[4.2.0] During grazing in plains do your animals come in contact with wildlife animals?

[4.2.1] ☐ Yes [4.2.2] ☐ No

[4.3.0] When there is scarcity of pasture can you graze in wildlife protected areas

[4.3.1] ☐ Yes [4.3.2] ☐ No

[4.4.0] Have you ever observed wild animals grazing in your village communal pastures?

[4.4.1.] ☐ Yes [4.4.2.] ☐ No

[4.5.0] If Yes, how frequently do you see the following wildlife species in the grazing grounds?

[4.5.1] Often [4.5.2] Occasionally [4.5.3] Never

[4.6.0] Where do your animals drink water?

[4.6.1] ☐ Shared/Communal watering points [4.6.2] ☐ Own watering points

[4.7.0] Do your livestock share drinking water points with wild animals?

[4.7.1.] ☐ Yes [4.7.2.] ☐ No

[4.8.0] If yes do your animals share drinking water with wild animals simultaneously?

[4.8.1] ☐ Yes [4.8.2] ☐ No [4.8.3] ☐ Not always

[5.0]Which method is used to dispose raw placenta and aborted foetus?

[5.1.] ☐ Thrown raw to dogs

[5.2.0] ☐ Given to dogs after cooking [5.2.1.] ☐ Buried [5.2.2.] ☐ Burned [5.2.3.] ☐

Thrown in the bush [5.2.4.] [5.2.5.] ☐ Others (specify) e.g.....

[5.3.0] Have you noticed brucellosis cases in your herd?

[5.3.1.0] ☐ No? [6.3.2.] ☐ Yes?

[5.3.2.1.] If yes, when? Year..... Month.....

[6.0.] why do you think this problem happens?

[6.1.1.] ☐ ☐ Lack of breeding males in the herd

[6.1.2.] ☐ ☐ Problem from previous parturition or abortion.

[6.1.3.] ☐ ☐ The animal resent been mounted

[6.1.4.] ☐ ☐ Failure to detect heat on time

[6.1.5.] ☐ ☐ Animal are too old

[6.1.6.] ☐ ☐ Problem of getting male on time

[6.1.7.] ☐ ☐ The animal do not show clear heat signs

[6.1.8.] ☐ ☐ Lack of money to hire a male for service on time

[6.1.9.] ☐ ☐ Male animal is tired of service

[6.2.0.] Other (specify) e.g.....

Livestock movement

[7.0.] Contact with other animals

[7.1.1.] ☐ ☐ Often,

[7.1.2.] ☐ ☐ Occasionally,

[7.1.3.] ☐ ☐ Never

[7.2.0] Where do you keep your animals at night?

[7.2.1.] In the house with family members [7.2.2] Outside in the boma

[7.3.0] Do your animals stay with other animals during the night?

[7.3.1.] ☐ ☐ Yes [7.3.2.] ☐ ☐ No

[7.4.0.] If Yes, why do you mix with other animals? [7.4.1] ☐ ☐ For security reasons

[7.4.2.] ☐ ☐ Others (specify) e.g.....

[7.5.0] Did you acquire any new livestock in 2016?

[7.5.1.] ☐ ☐ Yes [7.5.2.] ☐ ☐ No

[7.6.0] If Yes; indicates the origin

[7.6.1] ☐ ☐ Other Village [7.6.2] ☐ ☐ Market [7.6.3] ☐ ☐ Other District

[7.7.0] Do you slaughter animals in your household?

[7.7.1.] ☐ Yes? [7.7.2.] ☐ No?

[7.8.0.] Do you process the skin of any animal in your household?

[7.8.1.] ☐ Yes? [7.8.2.] ☐ No?

Name of the interviewer.....

Time at end of interview

Appendix 2: A checklist to the livestock keepers on the use of veterinary antibiotics

Personal particulars of the respondent

1. Respondent biodata:

Name:

Sex:

Age:

Position in the family: Father..... Mother..... Daughter/son.....Others

(Mention).....Occupation:

2. Education level:

a. Non formal education.....

b. Adult education.....

c. Primary education.....

d. Secondary education (mention the level).....

e. Certificate level.....

f. Diploma level.....

g. Degree level.....

h. Others (mention)

Name of Participant_____ Date: _____ Region_____

3. What are the common problems associated with cattle

a.

b.

c.

d.

4. (If diseases are mentioned in question 5); what are the major diseases/disease symptoms

.....

5. Who treats your animals once they fall sick?

.....

6. What type of medications do you give to your animals?

.....

If antibiotics are mentioned in 6 above, mention the common antibiotic(s) you use in your animals.....

7. Where do you get the drugs to treat the clinical signs.....

8. Does the drugs treat the problem completely Yes.....No.....

9. If answered **No** what steps do the individuals take.....

10. Is there any veterinary or medical practitioner that visits you and explains the effect of use of antibiotics in animals and humans?

Yes.....No.....

11. If answered **No** what are the effects mentioned

.....

12. How many days do you observe drug withdrawal periods?

Yes..... NO.....

If **Yes** how many days?

.....

Appendix 3: Vice Chancellor's Letter

KIBALI CHA KUFANYA UTAFITI NCHINI TANZANIA

CHUO KIKUU CHA SOKOINE CHA KILIMO
OFISI YA MAKAMU WA MKUU WA CHUO
 S.L.P. 3000, MOROGORO, TANZANIA
 Simu: 023-2604523; 2603511-4; Fax: 023-2604651, MOROGORO

Kumb. Zetu : SUA/ADM/R.1/8

Tarehe 9 Novemba, 2016

Mkurugenzi Mtendaji wa Wilaya
Halmashauri ya Wilaya ya Bukombe
GEITA

UTAFITI WA WAALIMU NA WANAFUNZI WA CHUO KIKUU

Madhumuni ya barua hii ni kumtambulisha kwako Bw. Makoye Mhozya ambaye ni Mwanafunzi (MSc. Public Health & Food Safety) katika Chuo Kikuu cha Sokoine cha Kilimo (SUA). Huyu ndugu hivi sasa yuko katika shughuli za utafiti.

Chuo Kikuu cha Sokoine cha Kilimo (SUA) kilianzishwa na Sheria ("Universities Act No.5 of 2005") na Hati Idhini ("SUA Charter, 2007") ambayo ilianza kutumika Januari 1, 2007. Hati Idhini ilichukua nafasi ya Sheria Na.6 ya mwaka 1984. Moja ya majukumu ya SUA ni kufanya tafiti mbalimbali na kutumia matokeo ya tafiti hizo. Kwa sababu hiyo, waalimu, wanafunzi na watafiti wa Chuo hufanya tafiti mbalimbali katika nyakati zinazostahili.

Ili kufanikisha utekelezaji wa tafiti hizo Makamu wa Mkuu wa Chuo SUA amepewa mamlaka chini ya Hati Idhini ya SUA ya kutoa vibali vya kufanya utafiti nchini kwa waalimu, wanafunzi na watafiti wake.

Hivyo basi tunaomba umpatie mwanafunzi aliyetajwa hapo juu msaada atakaohitaji ili kufanikisha uchunguzi wake. Gharama za malazi na chakula chake pamoja na usafiri wake atalipia wenyewe kutokana na fedha alizopewa. Msaada anaohitaji zaidi ni kuruhusiwa kuonana na viongozi na wananchi ili aweze kuzungumza nao na kuwauliza maswali aliyo nayo.

Kiini cha Utafiti wa mwanafunzi aliyetajwa hapo juu ni: "Assessment of Brucella abortus and antimicrobial residues in raw milk in Bukombe, Tanzania".
 Sehemu anayofanyia utafiti huo ni Halmashauri ya Wilaya ya Bukombe. Ikiwa kuna baadhi ya sehemu ambazo zinazuiliwa, ni wajibu wako kuzuia zisitembelewe.

Muda wa Utafiti huo ni kuanzia tarehe **14/11/2016** hadi **31/5/2017**.

Wasalaam,

Prof. Gerald C. Monela
MAKAMU WA MKUU WA CHUO

Nakala: Mtafiti

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

Appendix 4: District director's permit**HALMASHAURI YA WILAYA YA BUKOMBE***(Barua zote ziandikwe kwa Mkurugenzi Mtendaji Wilaya)*

Simu: 028 2520704
 Fax : 028 2520728
 Barua Pepe: dedbukombe@yahoo.co.uk

(Unapojibu tafadhali taja)

Ofisi ya Mkurugenzi Mtendaji (W),
 S.L.P. 02,
BUKOMBE.

Kumb. Na. BDC/V.20/9/70

10/01/2017

Mwenyekiti/Afisa Mtendaji wa Kijiji

Kijiji cha

BUKOMBE

**YAH: KUMTAMBULISHA BWN. MAKOYE MHOZYA AMBAYE NI MWANAFUNZI
 WA SHAHADA YA UZAMILI (MSc. PUBLIC HEALTH AND FOOD SAFETY)**

Tafadhali husika na somo tajwa hapo juu.

Tafadhali mpokee na kumpa ushirikiano mtajwa hapo juu ambaye ni mwanafunzi wa Shahada ya Uzamili katika Chuo Kikuu cha Kilimo cha Sokoine (SUA) ambaye anakuja katika kijiji chako kwa ajili ya kufanya utafiti unaohusiana na magonjwa ya ng'ombe yanayosababisha kutupa mimba.

Utafiti huu umeanza tangu tarehe 14/11/2016 na utaendelea hadi 31/05/2017. Utafiti huu utahusisha mahojiano na wafugaji pamoja na kukusanya sampuli za damu na maziwa kutoka kwenye ng'ombe wanaokamuliwa.

Naomba umpe ushirikiano wako,

A handwritten signature in blue ink, appearing to read 'Sudi Mhanda Kundelya'.

Dr. Sudi Mhanda Kundelya
 Mkurugenzi Mtendaji (W)

BUKOMBE
 FOR DISTRICT EXECUTIVE DIRECTOR
BUKOMBE

Nakala:

Mkurugenzi Mtendaji (W),
 S.L.P. 2, **BUKOMBE**

- Aione kwenye jalada

Makoye Mhozya
 Chuo Kikuu cha Kilimo Sokoine
 S.L.P. 3015,
CHUO KIKUU, MOROGORO