ASSESSMENT OF MICROBIOLOGICAL HAZARDS ALONG THE MILK VALUE CHAIN IN KILOSA AND MVOMERO DISTRICTS, TANZANIA

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ABSTRACT

The consumption of raw milk is a common practice among pastoral and agro-pastoral communities of Tanzania. This behaviour predisposes consumers to the risk of contracting milk-borne and zoonotic diseases. This study was carried out to assess milk quality based on identification of bacterial contaminants indicated by total viable count (TVC), total coliform count (TCC) and contamination with *Brucella* and *E .coli* 0157: H7 microorganisms. The study was carried out along the milk value chain (MVC) in Kilosa and Mvomero Districts of Morogoro Region in Tanzania. A total of 109 milk samples were collected along the MVC from farmers (54), milk vendors (31), milk collection centres (6) and milk selling points (18). Collected milk samples were subjected to TVC, TCC and polymerase chain reaction (PCR) to identify the presence of microorganisms in the milk. Laboratory findings indicate that milk from Kilosa district had significantly ($p=0.015$) higher TVC than milk from Mvomero district. The TVC varied significantly ($p=0.00$) along the MVC in the two districts. Using PCR, the overall prevalence of *Brucella* was 17.1% (n=82 out of 109), with the prevalence of 25.8% and 11.8% recorded in Kilosa and Mvomero districts, respectively. The *E. coli* 0157:H7 was neither isolated nor detected in all 109 milk samples processed. Such findings suggest that milk marketed along the MVC is contaminated with *Brucella* organisms, thus posing public health risks to consumers. It is recommended that concerted efforts should be made to safeguard health of consumers through adopting various interventions that would reduce risks at each node along the MVC in the study area.

DECLARATION

I, Ernesta Joseph, 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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DEDICATION

This work is dedicated to Almighty God who gave me strength and made me what I am today, my parents Mr & Mrs. Joseph Mallya for nursing me with affection, unreserved assistance and for their dedicated encouragement in my academic carrier, my husband Mr. Constantine Kimolo and to my beloved son John for their love and prayers.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABREVIATIONS AND SYMBOLS

µl	Microliter
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
COMESA	Common Market for Eastern and Southern Africa
DNA	Deoxyribonucleic acid
EAC	East African Community
ELISA	Enzyme-linked immunosorbent assay
MVC	Milk value chain
ml	Milliliter
PCR	Polymerase chain reaction
SPSS	Statistical Package for Social Science
SUA	Sokoine University of Agriculture
TCC	Total coliform count
TVC	Total viable count
URT	United Republic of Tanzania

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Milk is one of the most nutritious food; it is also the most perishable product known to favour growth of several microorganisms (Hempen *et al*., 2004). The liquid nature makes milk a highly vulnerable food to microbial contamination and an efficient vehicle for transmission of diseases to humans (Kivaria *et al*., 2006). It is an excellent culture and protective medium for certain microorganisms, particularly bacterial pathogens (Hempen *et al*., 2004). The presence of food-borne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal (Oliver *et al*., 2005).

Traditionally, raw or unpasteurized milk has been a major vehicle for transmission of pathogens (Mubarack *et al*., 2010). Some of the microbial contaminants of milk cause milk spoilage while others are pathogenic to humans which may cause milk-borne and zoonotic diseases (Yirsaw, 2004). Pathogenic organisms in milk can be derived from the cow itself, from human handlers and/or from the environment (Hempen *et al*., 2004). Pathogenic organisms commonly isolated from cow's milk include *Staphylococcus, Streptococcus, Mycobacterium, Brucella, Escherchia* and *Corynebacterium* (Yirsaw, 2004). These bacteria pose a serious threat to human health, and constitute about 90% of all dairy- related diseases (Donkor *et al*., 2007b). Some of these foodborne pathogens like *Listeria, Campylobacter, Yersinia* and *Condria ruminantia*, can cause life-threatening diseases to humans and animals (Lei *et al*., 2007). Diseases that commonly spread from the milk to human beings are tuberculosis, brucellosis, salmonellosis, listeriosis, campylobacteriosis, yersinoses and Q-fever (Yirsaw, 2004).

In the dairy sector, zoonotic pathogens such as *Brucella* and *E. coli* may be present in dairy animals, raw milk, milk products, and the farm environment but are often difficult to diagnose (Mosalagae *et al*., 2011). *E. coli* 0157:H7 is a causative agent for haemorrhagic diarrhea and kidney damage, especially in young and old individuals with weak immunity, and is highly acknowledged as an important animal origin food-borne zoonosis (Omore *et al*., 2004). Ingestion of contaminated raw unpasteurized milk is considered as the most possible way of contracting milk-borne zoonoses such as brucellosis (Mosalagae *et al*., 2011). Human brucellosis on the other hand is a severely debilitating disease that requires prolonged treatment which results to considerable medical expenses in addition to loss of income due to loss of working hours (John *et al*., 2010). Therefore detection of microbial pathogens in milk is the solution to the prevention and recognition of problems related to healthy and safety (Velusamy *et al*., 2009).

The use of molecular-based diagnostic methods provides an alternative approach for identification of specific pathogens in milk (Lei *et al*., 2007). Polymerase chain reaction (PCR) involves DNA analysis and can be superior to culture for detecting the main pathogens in food samples and results can be obtained at relatively shorter times compared to most conventional method (Lopez-Campos *et al*., 2012). In spite of advantages of using PCR for detection of microbes, it is expensive and complicated, requiring skilled workers to carry out the test (Velusamy *et al*., 2009).

Conventional methods for the detection and identification of microbial pathogenic agents mainly rely on specific microbiological and biochemical identification (Velusamy *et al*., 2009). Conventional detection methods of milk-borne pathogens are common and may seem to be cheap but they are cumbersome, time consuming, invariably non-specific and

sometimes inaccurate (AlAll *et al*., 2012). Conventional culture methods remain the most reliable and accurate techniques for food-borne pathogen detection. Traditional methods, to a large extent, depend on using suitable culture media, where the culture and colony counting methods involve counting of bacteria. (Ge and Meng, 2009; Velusamy *et al*., 2009). Selective media are used to enhance the growth of the target organism(s) and suppress the growth of the rest (Ge and Meng, 2009). Although the culture based methods are found to be standard microbiological techniques to detect the single bacteria, amplification of the signal is required through growth of a single cell into a colony (Velusamy *et al*., 2009). Therefore PCR is used for more advanced techniques for accurate diagnosis of brucellosis that can overcome the draw backs of traditional diagnostic techniques (Moussa *et al*., 2010).

1.2 Problem Statement and Justification

Consumption of raw milk is a common practice in Tanzania particularly among pastoral and agro-pastoral communities who keep traditional livestock with limited or no diseases control programme such as those found in Kilosa and Mvomero districts. Such behaviour of consumption of raw milk predisposes consumers to risk of contracting zoonotic and other milk-borne diseases. Milk supply is high in Kilosa and Mvomero districts where some of it is consumed at home while some is sold to the milk processing factories such as Shambani Milk Enterprises in Morogoro, Tanga fresh factory in Tanga Region and DESA milk factory of Dar es Salaam. Due to the fact that milk is a perishable commodity, poor handling can exert both a public health and economic toll, thus requiring hygienic vigilance throughout the production to consumer chain.

Most of the milk consumed in rural areas is consumed raw. It is also known that even in town, despite the fact that most people use milk for tea or coffee or feeding children for

which the milk is boiled, there are a lot of people who prefer drinking sour milk prepared from raw un-boiled milk. It was observed that most people prefer consumption of raw to boiled milk and they associate their preference with the good taste of raw milk (Karimuribo *et al*., 2005). This tradition therefore poses public health risk to consumers in relation to milk-borne diseases. The consumption of raw milk has been recognized as a major cause of food borne diseases (Oliver *et al*., 2005). The informally marketed raw milk in Kilosa and Mvomero districts could be an important source of infection with a wide range of bacteria if effective control measures including improved hygienic handling of milk along the milk value chain and milk pasteurization are not practiced. Educational efforts should be aimed at making the rural population aware of the health risks associated with consumption of raw unpasteurized milk as well as reducing the potential for contamination during harvesting of milk which will result in a reduction of food borne pathogens in raw milk.

The rapid and sensitive nature of PCR gives a chance of testing multiple microorganisms in a short time for accurate detection (AlAll *et al*., 2012). Therefore rapid and effective detection and identification of food borne pathogens is important not only in controlling and investigating food-borne diseases but also in improving food safety and management in the food industry (Oh *et al*., 2009).

1.3 Objectives

1.3.1 General objective

To carry out assessment of microbiological hazards that pose risk to consumers of milk produced in Kilosa and Mvomero districts, Tanzania.

1.3.2 Specific objectives

1. To assess milk quality along the milk value chain based on total viable counts and total coliform counts.
2. To identify factors influencing contamination of milk with microorganisms along the milk value chain.
3. To identify zoonotic pathogens (*Brucella abortus* and *E.coli* 0157:H7) present in milk produced by pastoral and smallholder farmers.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Milk Production System in Tanzania

The dairy industry in Tanzania has a great potential for improving the living standards of the people, and contributing towards reduction of poverty through improved nutrition, arising from consumption of milk and incomes raised from sale of milk and milk products (Njombe *et al*., 2011). The bulk of milk produced in the country originates from traditional cattle and is consumed at the household level as well as marketed to reach other consumers (TAMPA, 2011). Total annual milk production in Tanzania is currently estimated at 1.65 billion litres (Njombe *et al*., 2011).

Milk production in Tanzania is out under the traditional and commercial improved dairy production systems and about 70% of the milk produced comes from the traditional sector (indigenous cattle) kept in rural areas, while the remaining 30% comes from improved dairy cattle mainly kept by smallholder producers (Njombe *et al*., 2011). Smallholder farmers in pastoral and agro-pastoral production systems account for about 99% of the total livestock population and support the livelihoods of approximately 80% of the population (Swai and Schoonman, 2010). Smallholder dairy farming in Tanzania is a relatively recent undertaking, initiated and developed either through bilateral agency support or through private farmers buying dairy cattle to supplement their incomes (Karimuribo *et al*., 2005). In Tanzania a farmer operating less than 50 heads of cattle is considered to be smallholder (Tulahi, 2010). Smallholder farmers are still the leading keepers for dairy cattle in Morogoro region whereby Kilosa district is reported to have most organizations involved in dairy production, followed by the Mvomero district which was having the largest proportion of dairy crossbred cows in the region making 49.72 %

of all dairies in Morogoro (Morogoro profile, 2007). The dairy cattle are kept by smallholder farmers and few medium and large scale farms. The indigenous cattle are kept by traditional livestock keepers in the pastoral and agro-pastoral systems (Njombe *et al*., 2011).

2.2 Factors that Influence Food Safety in Dairy Production System

In many countries of sub-Saharan Africa, significant post-harvest milk losses are incurred along the supply chain, largely due to lack of adequate markets and spoilage (Lore *et al*., 2005). Milk is mainly produced by indigenous cattle which are widely distributed in different areas including remote villages where the road infrastructure is poor and having inadequate provision of utilities such electricity (Njombe *et al*., 2011). Its quality is influenced significantly by adulteration, spoilage from poor storage and contamination during and after milking (Mdegela *et al*., 2009), stocking and transport method, poor cooling/refrigeration conditions leading to the growth of bacteria (Pistocchin *et al*., 2009; Afzal *et al*., 2011). These problems contribute to inefficiency in milk collection and addition of cost for milk collection as well as milk processing. Non-existence of producer societies not only makes collection and marketing of raw milk difficult but also discourage introduction of innovations (Njombe *et al*., 2011).

2.3 Concepts of Food Safety and Risk Analysis

Zoonotic diseases associated with dairy farming can be transmitted from animals to humans through various routes including ingestion of cow-derived foods such as milk, beef as well as milk and beef products. Possible means of transmission may also include direct contact as a consequence of contamination of wider environment due to spread of organic wastes/effluents from dairy farms (Andreoletti *et al*., 2009). A primary route of pathogen transmission in milk is faecal contamination during milking (Oliver *et al*.,

2009). As regular inhabitants of the intestine, enterococci may serve as indicators for faecal or soil contamination and implies a risk that other enteric pathogens may be present in the milk (Kivaria *et al*., 2006). Due to the low dose of *E. coli* O157:H7 needed to cause infection, sensitive and rapid detection methods for *E. coli* O157:H7 in food samples are necessary in order for the food industry to ensure a safe supply of foods (Lopez-Campos *et al*., 2012). Unpasteurized milk and processed dairy foods from infected animals have been considered a source of *Brucella* infection for the general population (John *et al*., 2010).

Informal milk markets involve milk sale through unregulated channels (Donkor *et al*., 2007b). It account for over 80% of milk sales in most of sub-Saharan African countries, and earlier studies have shown that consumers enjoy convenient delivery and lower prices from such informal markets (Donkor *et al*., 2007a). Informal milk marketing is the dominant channel through which milk produced by smallholder farmers reaches consumers in urban centres in Tanzania (Kilango *et al*., 2012). Informal dairy industry in Tanzania plays a dominant role in milk marketing, handling over 80%-90% of all milk sold (Swai and Schoonman, 2011). Unhygienic handling of milk at the farm influences spoilage of milk at the processor level (Lore *et al*., 2005). Since there is little or no quality control for milk handling practices in the informal channels, there is potential for presence of zoonotic pathogens, adulterants and antimicrobial drug residues in informal markets and these are of public health risks to consumers (Swai and Schoonman, 2011; Kilango *et al*., 2012).

Most developing countries lack affordable testing methods to monitor for food-borne hazards and to ensure that highly perishable products remain safe from a health standpoint, as the product moves through the value chain (Narrod *et al*., 2011). In

developing countries such as Tanzania, outlets for the purchase of milk are numerous but most operate under unsanitary conditions and are not adequately monitored or regulated; such conditions pose risk to food-borne zoonoses (Swai and Schoonman, 2011). In milk, risk factors leading to the growth of pathogenic bacteria occur throughout the value chain: at the farm, at the collection center and at the consumption stage of the chain (Narrod *et al*., 2011).

2.4 Food Quality Control Systems in Dairy Production Systems

Proper management of dairy farm operations is not only essential in animal welfare terms but also significantly reduces the likelihood of dairy cows transmitting food-borne zoonotic diseases to humans (Andreoletti *et al*., 2009). To protect public health against milk-borne infections, there are regulations that require proper hygienic handling of milk and its pasteurization (Donkor *et al*., 2007a). Developing uniform regulations including microbial standards for raw milk to be sold for human consumption, labelling of raw milk, improving sanitation during milking, and enhancing and targeting educational efforts are potential approaches (Oliver *et al*., 2009). Many countries have milk quality regulations, including limits on the total number of bacteria in raw milk, to ensure the quality and safety of the final product (Worku *et al*., 2012). Tanzania Food, Drugs and Cosmetics Acts 2003, states that; 'Milk from diseased dairy animals not to be used for human consumption'. The total bacterial counts of cooled raw milk, produced under good hygienic conditions, should be lower than 10 000 bacteria/ml, and if the bacterial counts of milk increase significantly, e.g. to over 3 million/ml this could lead to significant degradation of fat, protein or lactose causing off-flavours and would significantly reduce the flexibility the processor has with respect to storage and use of milk (Oliver *et al*., 2005). A harmonized trade standards agreed by EAC and COMESA Member States

recognize three grades of milk and set upper limits on total bacteria count in processed products and raw milk (EAC, 2007).

The maximum level agreed /recognized grades of pasteurazed milk include 30 000 cfu/ml for total plate count, 10 cfu/ml for total coliforms and absent for *Escherichia coli* while the standard set for raw milk for total plate count includes, grade I 0r A <200 000 cfu/ml, grade II or B > 200 000-1 i000 000, grade III or C > 1 000 000-2 000 000. For coliform plate count grades are; very good 0-1 000, good 1 000-50 000 (EAC, 2007).

2.5 Milking Practices that Can Affect Milk Quality

Quality deterioration of milk starts just after milking, when it is carried out under unhygienic conditions (Afzal *et al*., 2011). Milking hygiene influences the overall hygienic status of the farm including cleanliness of premises, animals, equipment and personnel which, in turn, determine the level of the risk of contamination of raw milk (Andreoletti *et al*., 2009). The major practices and factors that affect the quality of milk at the farm are animal mishandling, unhygienic milking, transportation equipment's and poor storage conditions (Yirsaw, 2004; Kurwijila, 2006; Pistocchin *et al*., 2009; Afzal *et al*., 2011). Microorganisms adhere to surfaces of the milking equipment and milk residues remain in the equipment after the cleaning cycle (Vissers and Driehuis, 2008). Milk drops left on the surface of milking equipments act as excellent media for the growth of a variety of bacteria that can then contaminate the milk of subsequent milking (Afzal *et al*., 2011; Worku *et al*., 2012). All such practices results in poor quality of milk in terms of its compositional and bacterial load. Microorganisms may contaminate milk at various stages of milk procurement, processing and distribution (Yirsaw, 2004; Lore *et al*., 2005). The use of soap and good quality water for cleaning the equipment could be expected to remove milk remains, including microorganisms, thereby affecting the microbial quality

of the milk (Kivaria *et al*., 2006). Processed milk must be handled hygienically to avoid post-processing contamination (Kurwijila, 2006).

2.6 Methods of Detecting Microbiological Hazards in Milk

Numerous technologies have been developed to enumerate the total and groups of microorganisms and to detect and identify specific pathogens and toxins present in foods (Ge and Meng, 2009). Polymerase Chain Reaction technology has successfully shortened analysis time and has been widely applied for the detection of food-borne pathogens (AlAll *et al*., 2012). The rapid increase in the number of copies of the target sequence that can be achieved with PCR-based methods makes them ideal candidates for the development of faster microbiological detection systems (Lopez-Campos *et al*., 2012). Methods linking PCR detection to samples enriched for pathogen proliferation (usually overnight) are available for the majority of food-borne pathogens. Other method like ELISA which is antibody-based assay, is useful for detecting pathogens and toxins in food (Ge and Meng, 2009).

Traditional culture methods for detecting bacterial pathogens in food are based on the incorporation of food sample into a nutrient medium in which the bacteria can multiply, thus providing visual confirmation of their growth (Lopez-Campos *et al*., 2012). It relies primarily on direct plating methods and biochemical tests which are time-consuming, labour-intensive, and expensive due to the necessity of separate cultivation of each target species (Oh *et al*., 2009). It provides essential benefits, such as diluting the effects of inhibitors, allowing the differentiation of viable from non-viable cells, and allowing for the repair of cell stress or injury that may have resulted during food processing, therefore it would be difficult to completely eliminate enrichment culture from the process of pathogen detection in foods (Lopez-Campos *et al*., 2012).

2.7 Qualitative Methods for Assessing Quality and Safety of Milk along the Milk Value Chain

Qualitative procedures are used when it is not necessary to know the amount of a microorganisms present in a sample but only its presence or absence (López-Campos *et al*., 2012). The detection of pathogen- specific DNA via PCR addresses the issues of presence of the microbes without the need for culture (Oh *et al*., 2009). Qualitative detection tests are used if information concerning the presence of an organism in a specified quantity of food is required and sensitive quantitative detection is usually achieved by traditional culture methods (López-Campos *et al*., 2012).

There are four simple tests for milk quality:sight-and-smell (organoleptic) test, clot-on-boiling test, alcohol test and lactometer test. These tests are routinely carried out at milk collection points to ensure that only milk of acceptable quality is received (Lore *et al*., 2006). Organoleptic test is the simplest test as it requires only use of the senses of smell and sight (Kurwijila, 2006). The milk quality is judged by the use of a person's senses view, smell, and taste if necessary. (Pandey and Voskuil, 2011). Milk which contains objectionable smell or particles or has an abnormal colour can easily be detected (Kurwijila, 2006).

Clot on boiling is quick and simple test which allows one to reject milk that has developed high acidity or colostral milk that has a very high percentage of whey proteins, which do not withstand heating at high temperatures (Kurwijila, 2006). If the milk is sour or if the milk is abnormal (colostrum or mastitis milk) the milk will clot and does not pass this test (Pandey and Voskuil, 2011).

Alcohol test is carried out when acid levels are high enough, the addition of an equal amount of 68 per cent alcohol to milk will lead to further dehydration and destabilization of casein and cause the milk to clot (Kurwijila, 2006). In case there is any reason to suspect that milk is sour, the alcohol test is used for rapid determination of an elevated acidity of milk (Pandey and Voskuil, 2011). The alcohol test can detect milk whose pH is 6.4 or lower (Kurwijila, 2006). Moreover, the lactometer test serves as a quick method to determine adulteration of milk by water (Pandey and Voskuil, 2011). The test is based on the fact that the density of whole milk ranges from 1.026 to 1.032 g/ml, therefore adding water to milk lowers its density, while addition of solids increases the density of milk (Kurwijila, 2006).

To confirm the identity of the desired microorganism in qualitative tests, various bacteriological, biochemical and/or serological tests need to be carried out with pure cultures obtained from these presumptive colonies (López-Campos *et al*., 2012). Culture methods depend on using suitable media to detect specific microorganisms, which often are a small proportion of the total microorganisms present in food. Selective media are used that enhance the growth of the target organism(s) and suppress the growth of the rest (Ge and Meng, 2009). The detection of pathogenic bacteria is a fundamental objective of food microbiology ensuring food quality, regarding this, PCR technology has successfully shortened analysis time and has been widely applied for the detection of food borne pathogens (AlAll *et al*., 2012)

2.8 Conclusion from the Literature Review

Hygienic milk production, proper handling and storage of milk, and appropriate heat treatment can reduce or eliminate pathogens in milk (Kurwijila, 2006). Establishment of a well coordinated milk collection network could be a kick start towards successful milk

processing and marketing (Njombe *et al*., 2011). One best way to prevent raw milk–associated food-borne illness is for consumers to refrain from drinking raw milk and from consuming dairy products manufactured using raw milk (Oliver *et al*., 2009). Improved detection methods with better sensitivity and speed will be a valuable tool in defining the problems and outlining solutions to ensure the safety and quality of our food supplies (Ge and Meng, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Kilosa and Mvomero districts which are dominated by pastoral and agro-pastoral communities. Kilosa District is presently divided into nine divisions, 37 wards and 164 villages; its population was estimated to be 438 175 in the year 2012 (URT, 2012). It is situated between latitude 6° 00" and $7^0$50" South of equator and longitude 35° 00" and $36^0$59" East of Greenwich.

Kilosa district is divided into three ecological zones which are the flat plain, the plateau and mountainous or upland zone (URT, 2012). In Kilosa district, this study was conducted within the flat plain ecological zone in five wards including Kimamba, Rudewa, Madoto, Dumila and Magomeni (Figure 1). The altitude of this area ranges from 300 m to 600 m above sea level, with an average rainfall between 700 mm and 1200 mm per annum. The average annual temperature of this zone is 18^0C. The zone is densely populated due to its suitability for agriculture and livestock keeping. Most cattle are indigenous kept by agropastoralists and few farmers keep cross-bred cattle.

Mvomero district is located between latitude 6º 13" and $6^0$46" South of equator and longitude 37º53" and $37^0$64" East of Greenwich. It has a population of 312 109 (URT, 2012). It is bordered to the north by Tanga region, to the northeast by Pwani region, to the east and southeast by Morogoro rural district and Morogoro Urban district and to the west by Kilosa district. The district varies greatly in its topography and climate. Mountains and highlands are located in the northwest, lowland rainforest in the north and central areas, and drier woodlands in the south.

In Mvomero district, this study was conducted in five villages belonging to three wards, include Diongoya, Mtibwa and Dakawa (Fig. 1). This area receives bimodal rainfall with a long wet season from March to May and a short wet season from October to December. The rainfall ranges between 600 and 1200 mm per annum. The animals kept are also indigenous Tanzania shorthorn Zebu cattle and crossbred cattle. The study area was selected by MoreMilkIT project, and for the purpose of this study, villages were selected from the list of identified villages by the project based on availability of various actors along the milk value chain.

3.2 Study Design and Selection of Villages

An observational cross-sectional study design was adopted whereby questionnaire administration and sampling was done once. Study villages in Kilosa and Mvomero districts were purposively selected from the list of MoreMilkIT project, based on the availability of farmers, vendors, milk selling centres and collection centres. The selected villages included Madizini, Manyinga, Wami Sokoine, Wami Dakawa and Wami Luhindo in Mvomero district, and Mbwade, Twatwatwa, Dumila, Kimamba A and B and Manzese/Uhindini in Kilosa district. Since the study was conducted at time when milk was insufficient, all households that had lactating cows during field visit were included in the study. Samples were collected from all lactating cows in a households, at the collection centres milk were collected from the cooling tanks while for Vendors samples were collected from vendors milk container at the selling centre and at the collection centres. In the selling centre sample collected was the boiled and raw milk from the owner of the selling unit.

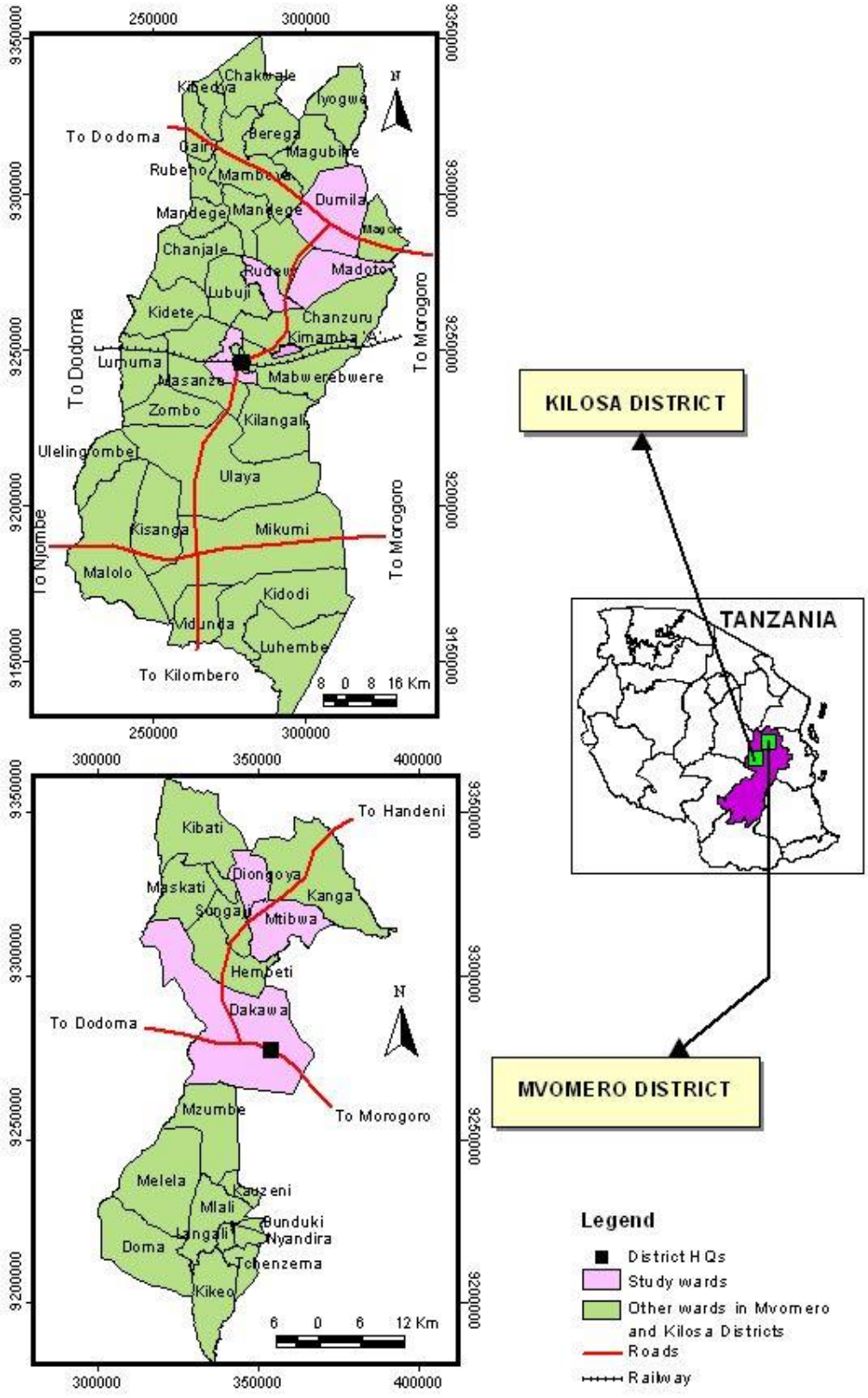

Figure 1: A map of Kilosa and Mvomero districts showing the study area.

3.3 Sample Size Determination and Sampling

The sample size was obtained as described by Fisher *et al*. (1991) using the following formula: $N = z^2pq/d^2$.

Where;

N= the desired sample size

z = the standard normal deviate, usually set at 1.96 which corresponds to the 95 percent confidence level.

p = proportion in the target population estimated to have a particular characteristics (which was approximated to be 0.5)

q = 1.0-p

d= degree of accuracy desired set at 0.1

$N = (1.96)^2(0.5)(0.5)/0.1^2$

=96

Using this formula, the required sample size was 96.

3.4 Data Collection

3.4.1 Questionnaire survey to identify factors influencing milk quality

A standardized questionnaire with closed and open ended questions was administered by face to face interview to collect information on risk factors influencing milk quality from 52 farmers, 22 vendors and 16 operators of milk selling centres in Kilosa and Mvomero Districts (Table 1). The questionnaire captured participant's knowledge on hygienic practices during milk handling along the milk value chain, habit and practices such as washing of milk equipments, hand washing before and after milking, sources of water used for household activities and means of milk transport to the selling and collection centres (Appendix 1-3).

3.4.2 Sample collection, transport and storage

Sample collection was done in early morning around 6-7 a.m. by using sterile containers into which approximately 50 ml of bulk milk taken from the storage container was sampled. In case there was more than one container of milk, sample was taken from each of the container. After sampling, the milk samples were stored in a cool box packed with ice packs and thereafter transported to field-based refrigerator and stored at 4^0C for a maximum of four days before being transported to the laboratory for analysis. Storage refrigerators were used for temporary storage of milk sample collected in remote areas of Kilosa and Mvomero districts. The samples collected at Dumila and Dakawa were transported immediately to Sokoine University of Agriculture (SUA) laboratory at the Faculty of Veterinary Medicine, where the samples were stored at -80^{o}C before analysis.

3.5 Laboratory Sample Processing

3.5.1 Total viable count and Coliform count

Milk samples were processed to quantify total viable count and coliform count. All procedures were carried out in biosafety cabinet to ensure sterility. Briefly, ten-fold serial dilutions of each sample from 10^{-2} to $10^{-5}$for raw milk and 10^0 to 10^{-3} for pasteurized were prepared in normal saline (BDH AnalaR®, BDH Limited Poole England) which was prepared according to the manufacturer's instructions. The wide range of dilutions was selected due to expected wide variation in bacterial counts. By using disposable pipette, 1,350 µl of normal saline was pipetted into each tube. Then 150 µl of milk was pipetted into the first tube and serial dilution was carried out by transferring 150 µl of the diluents to each tube. From 10^{-2} to 10^{-5} dilution of raw milk and each dilution of pasteurized milk, a 1000 µl of milk sample was placed onto sterile petri dish followed by the addition of 15-20 ml of sterilized of MacConkey agar (HiMedia Laboratories PVT. LTD, Mumbai, India) for total coliform count and Nutrient agar (Oxoid LTD, Basingstoke, Hampshire,

England) for total viable count, whereby media were prepared according to manufactures instruction. The sample and agar were then mixed and left to solidify after which the plates were incubated in inverted position at 37^0C for 24 hours to allow bacterial growth. Enumeration of bacterial was done by taking consecutive plates with countable number of colonies and counted manually.

3.6 Calculations of Mean Number of Colonies

The mean numbers of colonies were calculated from two successive dilutions as described by ISO 7218:2007(E), using the formula:

$$N = \frac{\sum C}{(Vx1)(1xd)} \qquad (1)$$

Where by $\sum c$ = is the sum of the colonies counted on the two dishes retained from the two successive dilutions.

V=volume of inoculums placed in each dish in millilitres.

d=is the dilution corresponding to the first dilution retained

3.7 Microbial Contaminants of Milk

The microbial contaminants of milk was defined by total viable count (TVC) and total coliform count (TCC), which are colony forming units (CFU) per ml of the milk sample based on bacteriological counts of raw and pasteurized milk samples' quality was graded as acceptable or unacceptable according to East Africa Standard specification (EAC, 2007) (Table 1).

Table 1: Microbiological limits

Type of milk	Bacteriological grade	Milk grade	Cfu/ml
Raw milk	Total plate count	I or A	<200 000
		II or B	>200 000-1 000 000
		III or C	>1 000 000-2 000 000
	Coliform plate count	Very good	0-1 000
		Good	1 000-50 000
Pasteurized milk	Total plate count	Maximum level	30 000
	Coliform plate count	Maximum level	10

3.8 Polymerase Chain Reaction

Molecular analysis was carried to investigate the presence of *E. coli* 0157:H7 and *Brucella abortus* using uniplex PCR. Milk collected from the two districts were analysed for the presence of *E. coli* 0157:H7 and *Brucella abortus*.

3.8.1 Extraction of DNA from milk samples

DNA extraction from milk samples was carried out at the Genome Science Centre of the Faculty of Veterinary Medicine. Milk samples were boiled for 30 minutes to precipitate proteins. Protein precipitates were pelleted by centrifugation at 17 000 g for 5 minutes and the supernatant used for DNA extraction using the QiAmp kits (Qiagen, Maryland, USA) according to the manufacturer's instructions. DNA was also isolated from a known isolate of *E. coli* 0157:H7 and *B. abortus* isolates (kindly obtained from the Faculty of Veterinary Medicine). DNA extraction from *E.coli* 0157:H7 and *B. abortus* bacteria cultures was done by boiling the isolates at 80^0C for 30 minutes in a thermal cycler (Applied Biosystems) followed by centrifugation at 17 000 g for 5 minutes. The pellet was discarded and the supernatant containing DNA was used for optimizing PCR and served as a positive control for all PCRs performed.

3.8.2 Amplification

Polymerase chain reaction was used for the amplification of the 16S-23S rDNA of *B. abortus* and the hlyA gene of *E. coli* 0157:H7 using BRU-P5/BRU-P8 and 0157- 3/ 0157-4 primers respectively (Table 2).

Table 2: Primer sequences for the detection of *Brucella abortus* and *E. coli* 0157:H7

Organisms	Primer sequence 5'-3'	Target gene	Expected fragment size
B. arbortus	F: BRU-P5 TCGAGAATTGGAAAGAGGTC	16S-23S	726 bp
	R: BRU-P8 GCATAATGCGGCTTTAAGA	16S-23S	
E. coli 0157:H7	F: 0157-3GTAGGGAAGCGAACAGAG	hlyA	361 bp
	R: 0157-4 AAGCTCCGTGTGCCTGAA	hlyA	

Note: F-forward primer and R-reverse primer

The reagents for PCR were prepared by pipetting appropriate volumes of 2x reaction buffer, RNase free water, primers, DNA polymerase and template in tubes as presented in Table 3.

Table 3: Composition of a single PCR reaction for *B. abortus* and *E. coli* 0157:H7

Reagent	Volume (µl)
2x Reaction Buffer	12.5
RNase Free water	7
Forward Primers	1
Reverse Primers	1
Taq polymerase enzyme	0.5
Template	3

PCR for both *B. abortus* and *E. coli* was performed in a total volume of 25 µl and all amplifications were done in a thermal cycler machine (Aplied Biosystems, USA). PCR

for the detection of *B. abortus* was performed as previously described by Rijpens *et al* (1996). The cycling conditions included an initial incubation at 95^{o}C for 1 minute to denature the template and activate the DNA *taq* polymerase. Then 30 cycles each consisting of denaturation for 15 seconds at 95^{o}C, annealing at 55^{o}C for 30 seconds and extension for 1 minutes at 72^{o}C. The last stage included a final extension step for 10 minutes at 72^{o}C. The amplification conditions for *E. coli* 0157:H7 were performed as previously described by Wang *et al*. (1997). Briefly, the amplification started by one cycle at 95^{o}C for 10 minutes followed by 35 cycles at 95^{o}C for 30 seconds, 50^{o}C for 30 seconds, 72^{o}C for 30 seconds and a final extension at 72^{o}C for 10 minutes. Negative controls in both *B. abortus* and *E. coli* 0157 reaction mixtures contained sterile distilled water in place of template DNA.

3.8.3 Agarose gel electrophoresis

Agarose gel was prepared by mixing 1.5g of agarose powder with 100 ml of 0.5x TBE buffer to obtain a 1.5% concentration of the agarose gel. Agarose was dissolved by heating the solution on a hot plate followed by cooling. A volume of 1µl of gel red (Biolithenix, USA) solution was added to every 100 ml of cooled molten agarose before casting and solidification. Separation and analysis of PCR products was carried out by adding 5 µl of amplicons to 1 µl of loading dye (Promega, USA). The products were run on 1.5% agarose stained with gel red for 30 minutes at a constant voltage of 50V. Imaging of separated PCR product was done using a gel doc machine (BioDock-It^{TM} Imaging System, USA).

3.9 Data entry and Analysis

Data collected through questionnaire and those obtained from laboratory analysis were coded and analyzed by SPSS version 20. Laboratory data, obtained from TVC and TCC

were first transformed into natural log to normalize them before analysis for statistical difference of means using analysis of variance, whereby the analysis of variance was used to examine the differences in variable along the milk value chain in the two districts. Descriptive statistics were computed to get frequencies and proportions of different variables. Chi-square test was used to establish statistical differences between proportions of different variables of which were compared for statistical significance at a critical probability of $P<0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Milk sampling and administration of questionnaire

A total of 109 samples were collected from Kilosa and Mvomero Districts whereby 49 samples were from Kilosa and 60 samples from Mvomero district. Milk samples were collected from farmers, vendors, selling centres and at the collection canters while questionnaire administration involved farmers, vendors and selling centres only (Table 4).

Table 4: Sources of data collection for questionnaire survey (n=90) and milk samples (n=109) in Kilosa and Mvomero district

Source	Kilosa Milk samples (Questionnaires)	Mvomero Milk samples (Questionnaires)	Total Milk samples (Questionnaires)
Farmers	10 (10)	44 (42)	54 (52)
Milk vendors	27 (18)	4 (4)	31 (22)
Milk selling centers	8 (6)	10 (10)	18 (16)
Milk collection centers	4 (-)	2 (-)	6 (-)
Total	49 (34)	60 (56)	109 (90)

4.2 Respondent Characteristics

The majority of respondents (62.2%) were from Mvomero district as shown in Table 5. Most of respondents (62.0%) were males. Farmers constituted the majority of actors along the milk value chain who participated in this study. Large percentage (44.4%) of cattle owned was crossbred. It was also observed that only 16.5% of the interviewed farmers had attended the training on basic hygiene practices (Table 5).

Table 5: Characteristics of interviewed participants (n=90)

Variable	Category	Number	Percentage
District	Mvomero	56	62.2
	Kilosa	34	37.8
Type of actor	Farmers	52	58.0
	Vendors	22	24.0
	Selling centres	16	18.0
Sex	Male	56	62.0
	Female	44	48.0
Type of cattle owned	Indigenous	21	38.9
	Crossbreed	24	44.4
	Both breed	9	16.7
Attended training on milk hygienic practices	Yes	14	16.5
	No	71	83.5

4.3. Microbiological Contaminants of Milk Produced by Pastoral and Smallholder Farmers in Kilosa and Mvomero Districts

The microbiological contaminants of milk produced were total bacteria and coliform bacteria count. The microbiological contaminants were examined across the two districts (Kilosa and Mvomero) as well as along the milk value chain.

4.3.1 Bacteria contaminants across the districts

The findings indicated that there was no significant difference in terms of coliform counts between Kilosa and Mvomero (Table 6). For total viable counts, Kilosa District had significantly ($p=0.015$) higher TVC than Mvomero district. Based on EAC milk standard (2007), 54% of the milk produced in Kilosa and Mvomero district had TCC above the recommended standards, where by Kilosa district had higher percentage (72.7%) of TCC than Mvomero district (39.3%).

In comparison with TVC, 50% of the milk produced in both districts had TVC above the recommended standard whereby each district had 50% of its milk with bacteria load beyond the recommendations.

Table 6: Bacterial contaminants of milk identified

	District	Number	Mean ± std. deviation	P value
Natural log TVC	Mvomero	29	9.73±3.277	
	Kilosa	22	12.03±3.183	0.015
Natural log TCC	Mvomero	25	7.20±4.090	
	Kilosa	20	8.66±4.995	0.289

4.3.2 Bacteria contaminants along the milk value chain

When bacteria counts were examined along the milk value chain, it was found that there was significant difference in terms of number of bacteria at different nodes. The results indicated that for both total bacteria counts and coliform counts the difference along the chain was highly significant (p=0.000) (Table 7). The result indicated that the number of TVC and TCC was increasing progressively from the farmer's level to vendors' level and up to collection centres. However the number of both TVC and TCC declined at selling centres.

Table 7: Microbiological contamination along the milk value chain

	Level	Number	Mean ± std.deviation	P value
Natural log TVC	Farmer	22	9.72±3.078	
	Vendor	15	12.18±2.821	
	Collection centre	6	14.56±1.593	
	Selling centre	8	7.88±2.670	0.000
Natural log TCC	Farmer	23	8.98±2.956	
	Vendor	7	12.23±2.392	
	Collection centre	2	14.68±4.327	
	Selling centre	13	2.44±1.149	0.000

Figure 2 shows trends of TVC and TCC along the milk value chain. The figure shows that number of bacteria counts increases from the farmer level up to collection centre level, but goes down at selling centre level.

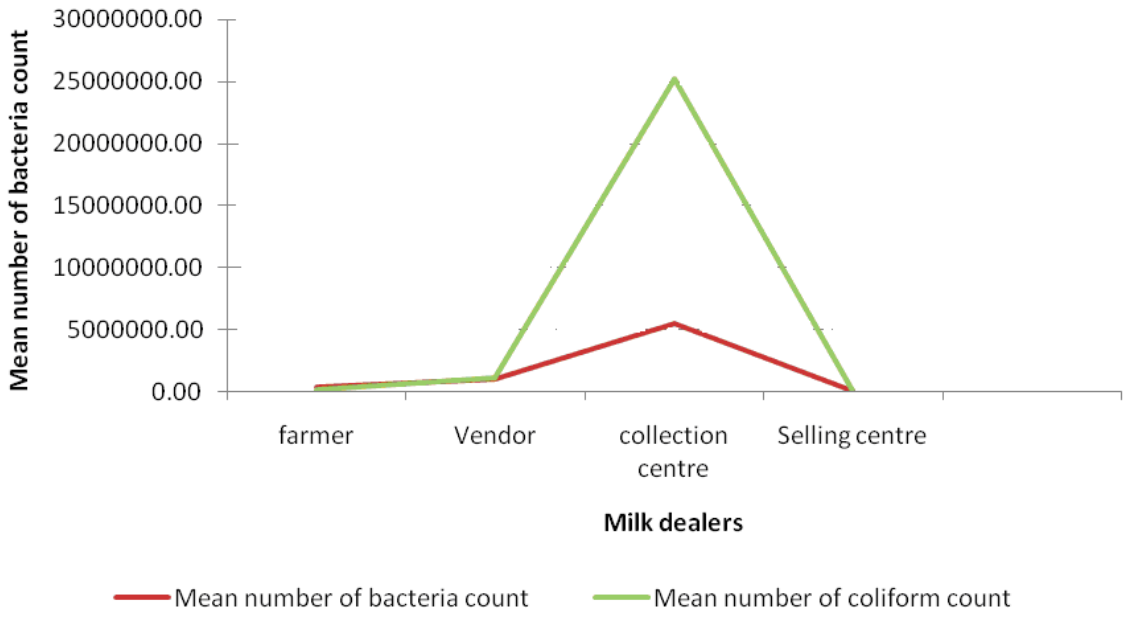

Figure 2: Mean number of bacteria count along the milk value chain.

4.4 Factors Influencing Contamination of Milk with Bacteria along the Milk Value Chain

A number of factors which could influence milk contamination along the milk value chain were assessed. These included sources of water used to wash milking utensils, water used to wash the udder or teats and knowledge of farmers and vendors on hygienic practices during milk handling. The findings show that tap water had significant ($p=0.02$) influence on milk contamination with coliform bacteria. It was also observed that normal water used to wash the udder (not used for drinking) significantly ($p=0.005$) influence the number of coliform count in milk. On assessment of the participants attendance to training on milk hygiene the findings showed that those who did not attend had significantly ($p=0.02$) higher bacterial counts than those who did not attend training.

Table 8: Factors influencing large number of total bacterial count in Milk

	Variable	Level	Mean ± std. deviation	P value
Natural log TCC	Source of water	Tap water	9.11±4.07	0.02
		dams	9.07±1.15	
		Underground shallow wells	6.40±3.35	
	Water used to wash teats/udder	Warm water	6.76 ±1.395	
		Water used for drinking	8.91 ± 3.143	
		Water not suitable for drinking	9.91±2.843	0.005
Natural log TVC	Attended training	Yes	13.28±1.07	
		No	10.22±3.22	0.02

Other factors which influenced milk contamination were place for milking and material used to dry teats/udder after washing. Milking at kraal significantly (p=0.052) influenced total coliform count with large percentage (83.3 %) of milk graded not as acceptable. When investigated on material used those who do not dry the teats after washing had influence on total bacteria count with large percentage (70%) been not in acceptable level at a significance of p=0.005.

Table 9: Factors influencing milk contamination with coliform bacteria

Variable	Level	Acceptability of milk microbial level	Percentage No. (%)	P value
Place for milking (TCC)	Kraal	Yes	1 (16.7)	0.052
		No	5 (83.3)	
	Cattleshed/banda	Yes	7 (77.8)	
		No	2 (22.2)	
	Outside kraal/banda	Yes	3 (37.5)	
		No	5 (62.5)	
Material used to dry udder (TVC)	Clean dry cloth	Yes	0 (0.0)	
		No	2 (100)	
	Do not dry	Yes	6 (30.0)	
		No	14 (70.0)	0.050

4.5 *B. abortus* and *E. coli 0157:H7* Present in Milk

It was found that none of 109 milk samples examined for presence of *E.coli* 0157: H7 was positive for this organism. For the case of *B. abortus*, 14 (17.1%) of the 82 samples examined were contaminated with this bacterium. Milk produced in Kilosa district had higher prevalence (25.8%) of *Brucella* compared with the milk produced in Mvomero district (11.8%). However, such difference was not statistically significant (p=0.092).

4.5.1 Prevalence of *Brucella* along the milk value chain

The prevalence of *Brucella* along the milk value chain was high at collection centres (50.0%) and vendors (30.3%) shown in Figure 3, whereby Kilosa district had (25.8%) higher prevalence of *Brucella* than Mvomero district (11.8%). There was no statistical significance (p=0.06) of the prevalence of *Brucella* along the milk value chain.

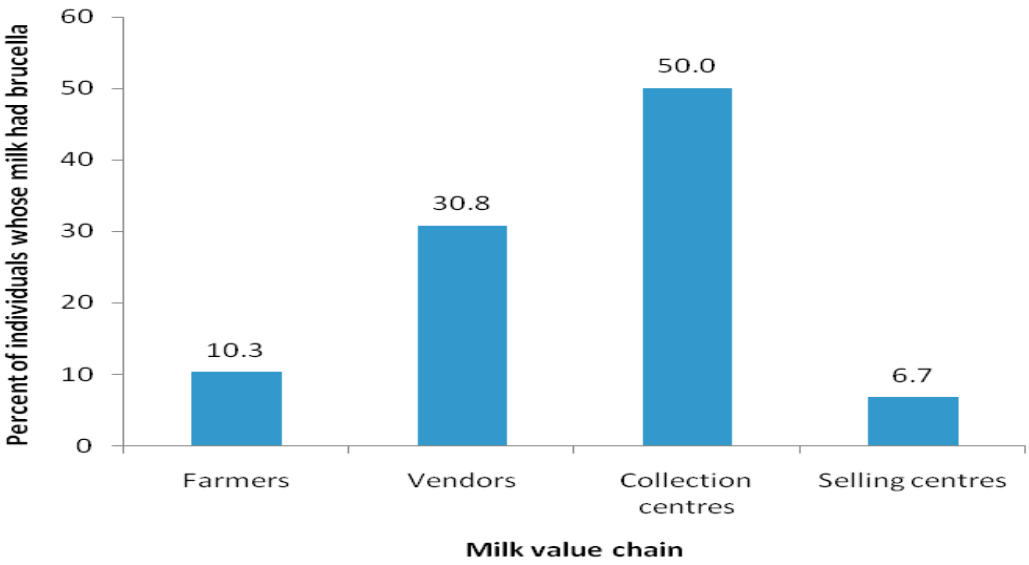

Figure 3: Trends of milk contaminated with *Brucella* along the milk value chain.

4.5.2 Detection of B. abortus in milk by PCR

Positive amplicons showed a band size of 726 base pair relative to the marker (lane M) whereby Lane 18 was positive control, Lane 19 negative control and Lane 1-17 were samples of which 1,3-8,10,11,13,15-17 were positive with band size of 726 base pair relative to the marker (Figure 4) and 2,9,12,14 were negative.

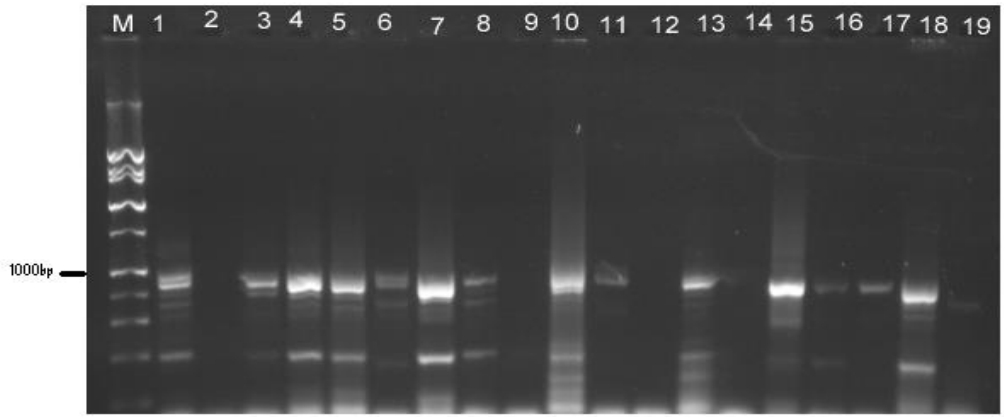

Figure 4: Agarose gel showing the detection of *B.abortus* 16S—23S rDNA by PCR using BRU P5/P8 primer set. Lane M, 1000 bp DNA lader marker.

CHAPTER FIVE

5.0 DISCUSSION

In the present study, Kilosa and Mvomero District were characterized based on the type of cattle owned, type of actor and trainings attended by the respondents on milk hygiene practices. It was found that, in Kilosa, the majority of farmers keep indigenous cattle while in Mvomero, most farmers kept crossbred cattle. Actors along the MVC included farmers, vendors, collection centres and selling centres in both districts. The majority of participants had not attended training on milk hygiene practices.

The study found that there were poor hygienic practices of washing of udder before milking whereby Kilosa district had higher percentage of farmers with poor hygiene practice during milking than Mvomero district which result to contamination of milk during milking and handling. Due to the use of unclean water for cleaning milk equipments, unclean personnel hands and insufficient washing of udder might have contributed to the increased bacteria counts (Worku *et al*., 2012). Personal hygiene constitutes a significant preventive measure alongside the use of potable water (Kivaria *et al*., 2006). Teats are often not washed prior to milking because of the belief that allowing the calves to drink milk before manual milking cleans the teats (Addo *et al*., 2011).

The study showed that large percentage of farmers milks their cows in the kraal. This is similar with study conducted by Mosalagae *et al.* (2011), most of the farmers' milk cows in open kraals, which constitutes one of the direct methods of milk contamination. A typical kraal consists of an enclosure of varying size surrounded by wooden or bamboo sticks with a bare ground covered with thick layer of fresh and dried cow dung (Addo *et al*., 2011).

The study showed that there was low level of knowledge on hygienic practices along the milk value. The low level of knowledge observed in this study is influenced by inadequacy of information due to remoteness of study areas, poor extension and lack of health programmes to educate disadvantaged communities such as the pastoralists (Karimuribo *et al*., 2005; Mosalagae *et al*., 2011). However, people with less than a high school education are more likely to consume raw milk than those who had completed high school, suggesting that level of education may influence a person's choice to consume raw milk (Olive *et al*., 2005).

The present study showed that the quality of milk in study areas was poor based on high values of TVC and TCC observed in milk samples. A study by Karimuribo *et al*. (2005) showed that most farmers (94.5%) reported to ferment milk from raw unboiled milk. This habit can predispose consumers to the health risk of contracting milk-borne zoonoses as most of them use raw fermented milk (Mosalagae *et al*., 2011). The improper milking hygiene practices by farmers may be due to poor or lack of knowledge on proper hygiene practices which may result to lowering the quality of milk (Karimuribo *et al.,* 2005).

The results indicated that the number of bacteria counts and coliform counts was increasing progressively from the farmer's level to vendors' level and up to collection centres. The presence of food-borne pathogens in bulk tank milk is directly linked to fecal contamination that occurs primarily during the harvesting of raw milk, however, some food-borne pathogens can cause mastitis in which case the organism can be directly excreted into milk (Oliver *et al*., 2005). Hence, this contributes to high bacteria counts in milk which is reported to increase milk contamination with *Staphylococcus* species which may result to udder infection (Mdegela *et al.,* 2004). Introduction of raw milk contaminated with food-borne pathogens into processing plants represents an important

risk of post-pasteurization contamination that could lead to exposure of consumer to pathogenic bacteria (Oliver *et al*., 2005).

This study has revealed that there were significantly increase of contamination along the milk value chain. Contamination occurs during milk handling due to lack of cooling facilities and absence of any test to screen for abnormal milk (Worku *et al*., 2012). When milk is produced under poor hygienic conditions and is not cooled, the main contaminants such as *Lactobacilli* organisms are produced which cause rapid souring (Mosalagae *et al*., (2011). A study by Lore *et al.* (2005) showed that poor handling of milk at the farm and long distances to market result in significant losses due to spoilage.

In this study milk contamination with *Brucella* was recorded in Kilosa and Mvomero Districts, whereby the prevalence was higher in Kilosa than in Mvomero District. This is similar to study conducted by Swai and Schoonman (2010) that the prevalence of brucellosis is higher in indigenous cattle than in crossbred kept by smallholder dairy farmers. The differences between traditional and crossbred animals are possibly attributed to increased contacts of infected herds/animals and non-infected ones in the indigenous traditional production system, as a result of communal grazing and watering, which become more apparent and acute during the dry period.

The findings of this study on milk contamination with *Brucella* are comparable to the study conducted by Mdegela *et al.* (2004) which showed that there was *Brucella* contamination of milk by 1% in Kibaha and 1.9% in Morogoro. Milk sample collected from vendors in both districts had higher bacterial count compared to those samples from the farmers and at the selling points.

This was similar to the study done by Arimi *et al.* (2005) which showed that the risk of exposure to *B. abortus* varies by bulking if milk is consumed raw. A study by John *et al.* (2010) showed that brucellosis occurs widely in livestock keeping populations in Tanzania where by 7.7% prevalence has been reported in northern Tanzania.

The results of the present study indicate that milk in Kilosa and Mvomero were not contaminated with *E.coli* 0157:H7. This was similar to study conducted by Swai and Schoonman (2011). The absence of *E.coli* 0157:H7 can be explained by the fact that the bacterium is not shed in milk but arises from contamination arising from sick handlers. Though in this study *E.coli* 0157: H7 was not detected in milk tested, the practise of drinking raw milk could expose the communities to milk-borne zoonoses.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

It can be concluded that raw marketed milk in the study area is of poor bacteriological quality and hazardous for human consumption. This highlights the need to implement good hygiene practices and effective monitoring from production through the delivery chain to consumer.

Cattle owners should be aware of milk-borne zoonoses that are prevalent in their areas and the risks they pose and how they are transmitted for them to make informed decisions on their control. Creation of awareness about brucellosis to farmers, milk vendors and milk consumers is also useful in order to reduce the health hazards associated with milk consumption.

It is recommended that concerted efforts should be made to safeguard health of consumers through adopting various interventions that will reduce risks at each node along the milk value chain in the study area. Further studies are needed for detection and quantification of health risks associated with *Brucella* infections in production animals as well as in humans.

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APPENDICES

Appendix 1: Questionnaire for farmers

1. Household identification

Date of survey(DD/MM/YYY	
Head of Household Name	
Number of Household	
District name:	
Ward name:	
Village Name:	
Name of survey respondent:	
Gender of respondent (M =1, F=2)	
Relationship of survey respondent to household head (Code a)	
Role (code b)	

Code a	**Code b**
a)Respondent relationship	**b) Role**
1=household head	1=Owner
2= spouse	2=Manager
3= other family member	3=Worker
4= Casual labourer	4=Other (specify)
5= other non-family member	

2. Cattle keeping

Type of cattle owned: (I)indigenous/ (C)crossbred/Other	Housing system for cattle:	Feeding system for cattle

Housing system	**Feeding system for cattle**
1=Cattle shed/banda 2=Kraal 3=both indigenous and cross bred 4=Others(specify)	1= Pasture grazing 2= Cut and carry (zero grazing) 3= Partly grazing partly cut and carry 4= Tethering 4=Others (Specify)

3. Water availability and uses

Where do you get water for household activities	Do you wash the udder/teats of the cattle before milking? Y=1, N=2, If yes, what is washed? Teat =1 or whole udder = 2	If yes which water do you use to wash the teats?

Where water is obtained for household activities	**Water used to wash the teats**

1= Water tap 2= Underground shallow wells 3= Stream 4= Surface running water 5= Drainage system 6= Others(specify) Dams	1= Water used for drinking 2= Water not suitable for drinking 3= Water with soap 4= Water with disfectant 5= Others (specify)

4. Treatment of the udder/teats after washing

After washing the teats /udder, do you dry it? Y=1 ,N=2	**If Yes what materials is used**	**Do you apply teat lubricants=1,N=2**	**If Yes, type of lubricants used**
Type of material used		**Type of lubricants used**	
1=Clean dry cloth 2=Any cloth 3=Other (specify)		1=Commercial petroleum 2=Milk itself 3=Cooking oil 4=Other(specify)	

5. Milking practices

At what time do you milk your cattle?	Where do you milk your cow	Do you put all the milk from different cow in one container? Y=1,N=2	If Yes how many cows of which its milk has been kept together in one container	Do you sieve your milk? Y=1,N=2	What is used for sieving milk

Time of milking	**Where the cow is milked**	**What is used for sieving**
1=5 am and 5pm 2=6 am 3=7 am and 7 pm 4=6 am and 6 pm 5= Others specify	1=In the cattle shed/banda 2=In the kraal 3=Others(specify)	1=Cloth 2=Metal sieve 3=Plastic sieve 4=Others

5.1 How many of your cows have blind quarters [ ]

5.2How many of your cows have had mastitis (unusual looking milk or inflamed udder) in the last 12 months?

5.3 When you milk cows do you use any of the following practices?

Milk safety practices	Do you practice? Y/N Y= 1 N=2	How often 1=Every day 2=At every milking 3=Weekly 4= Other
Cleaning of animal shed		
Feeding off the ground in a trough or pot while milking		
Cleaning of the hands before milking		
Cleaning of hands after milking		
Cleaning of the milking equipment before milking/keeping milk		

6. Milk spoilage

Have experienced milk spoilage Y=1,N2	If yes how often does milk get spoiled (*frequency per month)*?	What is done with milk which cannot be sold at the market because it is spoiled?	If consumed at home what form is milk consumed?

How often does the milk get spoiled	**What is done with the spoiled milk**	**What form is milk consumed**
1=1 2=2 3=3 4=0thers (specify)	1= consumed at home 2= Given to domestic animals such as dogs 3= Poured 4=Others (specify)	1= Fresh 2= Fermented 3=Other (specify)

Thank you very much!

Appendix 2: Questionnaire for milk vendors

1. **Background information**

Date of survey(DD/MM/YYY	
Name of survey respondent	
Gender of respondent (M/F)	
District name:	
Ward name:	
Village Name:	

2. Water sources

Where do you get water for household activities	Do you wash the milking equipment before keeping the milk?Y/N	If yes which water do you wash the equipment?

Where water is obtained for household activities	**Water used to wash the teats**
1 = Water tap 2= Underground shallow wells 3= Stream 4= Surface running water 5= Drainage system 6= Others(specify)	1= Water used for drinking 2= Water not suitable for drinking 3= Water with soap 4= Water with disfectant 5= Others (specify)

3. Milk handling

Do you sieve milk?Y/N	If yes what do you use for sieving?	What type of vessels used in handling milk?	How long does it take to get milk to the market/selling point?

What is used for sieving	**Vessels used in milk handling**	**Distance taken to get milk to the market**
1=Cloth 2=metal sieve 3=Plastic sieve 4=Others (specify)	1=Plastic bucket /Plastic containers 2=Aluminium can 3=Others (specify)	1=less than 1hour 2=2 hours 3=3 hours 4=Others (specify)

4. Milk transportation

Mode of milk transport used to reach the client	How do you protect the milk from being spoiled by the weather condition during transportation?

Mode of milk transport	**How milk is protected from spoilage during transportation**
1= On foot 2= Using bicycle 3= Using public transport 4= Using own car 5= Others (specify)	1=By using aluminium can 2= Adding ice bars in milk 3= Others (specify)

5. Milk spoilage

Have experienced milk spoilage Y=1,N=2	If yes how often does milk get spoiled?	What is done with milk which cannot be sold at the market because it is spoiled?	If consumed at home what form is milk consumed?

How often does the milk get spoiled	**What is done with the spoiled milk**	**What form is milk consumed**
1=1 2=2 3= 3 4= Other (specify)	1= consumed at home 2= Given to domestic animals such as dogs 3= Poured 4=Others (specify)	1= Fresh 2= Fermented

7. Milk quality

Do ever test for milk quality?Y=1,N=2	How often do you test for milk quality?	Which aspect of quality do you test for?

How often milk quality is tested	**Aspect of quality tested**
1= Every time when milk is purchased 2= Once per week 3= Once per month 4= Others (specify)	1= Water 2= Density 3= Others (specify)

8. Have you attended any training about hygienic practices in milk value chain? Y/N

Thank you very much!

Appendix 3: Questionnaire for milk selling centers

1. **Household identification**

Date of survey(DD/MM/YYY	
District name:	
Ward name:	
Village Name:	
Name of survey respondent:	
Gender of survey respondent (M=1,F=2)	

2. Water sources

Where do you get water for household activities	Do you wash the milking equipment before keeping the milk? Y=1,N=2	If yes which water do you wash the equipment?

Where water is obtained for household activities	**Water used to wash the equipments**
1= Water tap 2= Underground shallow wells 3= Stream 4= Surface running water 5= Drainage system 6= Others(specify)	1= Water used for drinking 2= Water not suitable for drinking 3= Water with soap 4= Water with disfectant 5= Others (specify)

3. Milk handling and transportation

Do you sieve milk?Y=,N=2	If yes what do you use for sieving?	What is the mode of milk transport used to reach the client	How do you protect the milk from being spoiled by the weather condition during transportation?

What is used for sieving	**Mode of milk transport**	**How milk is protected from spoilage during transportation**
1=Cloth 2=metal sieve 3=Plastic sieve 4=Others (specify	1= On foot 2= Using bicycle 3= Using public transport 4= Using own car 5= Others (specify)	1=By using aluminium can 2= Adding ice bars in milk 3= Others (specify)

4. Milk spoilage

Have experienced milk spoilage Y=1,N=2	If yes how often does milk get spoiled? (Frequency per month)	What is done with milk which cannot be sold at the market because it is spoiled?	If consumed at home what form is milk consumed?

How often does the milk get spoiled	**What is done with the spoiled milk**	**What form is milk consumed**
1=1 2=2 3= 3 4= Other (specify)	1= consumed at home 2= Given to domestic animals such as dogs 3= Poured 4=Others (specify)	1= Fresh 2= Fermented 3=Other (specify)

Thank you very much!