ASSESSMENT OF MICROBIAL QUALITY OF RAW COW'S MILK AND ANTIMICROBIAL SUSCEPTIBILITY OF SELECTED MILK-BORNE BACTERIA IN KILOSA AND MVOMERO DISTRICTS, TANZANIA

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ABSTRACT

Milk is an important source of nutrients to human and animals, but due to its high water activity and nutritional value it serves as an excellent medium for growth of many kind of microorganisms under suitable conditions. The present cross sectional study was conducted to assess microbial quality of raw cow milk and to determine antimicrobial susceptibility of the selected common milk-borne bacteria in Kilosa and Mvomero districts, Morogoro region. A total of 56 respondents were interviewed and subsequently, milk samples were collected for laboratory analysis including microbial quality assessment and antimicrobial susceptibility tests. Results showed that, majority of small-scale livestock keepers were males with no formal education, managing their cattle in dirty environments, practicing extensive grazing system on communal grazing area and treat sick animals themselves. Common antimicrobial agents used were antibiotics. Several factors were observed to predispose milk to microbial contamination. Furthermore, results indicated that 33.9% of respondents consume milk from animals that are under medication, 94.6% of them did not adhere to withdrawal periods, 76.8% of respondents consume raw milk while 78.6% consume milk products made from raw milk. Generally, 85.7% of milk samples had significantly ($P < 0.05$) higher total bacterial count than the recommended level of 2.0×10^6 cfu/ml by EAC standards. Isolated bacteria included *Listeria* spp., *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Pseudomonous aeruginosa* and *Proteus* spp. All isolates were resistant to ampicillin but susceptible to gentamycin, and 91.2% of isolates showed multi-drug resistant to more than two antibiotic drugs. This study concludes that the quality of raw cow milk was poor; unhygienic practices and poor animal husbandry at farm level predispose farmers, consumers and the public to risk of contracting milk-borne infections and associated bacterial resistances. It is recommended that veterinarians, extension officers and all stakeholders should play their roles in order to ensure safe quality milk delivery to consumers.

DECLARATION

I, Happy Brown Kanyeka, 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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LIST ABREVIATIONS AND SYMBOLS

Abbreviation	Descriptive meaning
%	Percent
&	and
<	Less than
>	Greater than
≤	Less or equal
≥	Greater or equal
µg/ml	microgram per millitre
®	Registered trade mark
ATCC	American Type Culture Collection
CI	Confidence interval
cfu/ml	Colony forming unit per millitre
EAC	East African Community
EAS	East African Community standard
EDTA	Ethylenediaminetetraacetic acid
e.g.	For example
et al	and others
FAO	Food and Agriculture Organization
FBDs	Food-borne diseases
g	gram
g/ml	gram per millitre
GPS	Global Positioning System
ILRI	International Livestock Research Institute

ISO	International Organization for Standardization
MDR	Multi-drug resistance
MPEE	Ministry of Planning, Economy and Empowerment
NCTC	Public Health England
^{o}C	Degrees Celsius
OIE	Office International des Epizooties
pH	Hydrogen ion concentration
PHCT	Population and Housing Census, Tanzania
SFFF	Safe Food, Fair Food
spp.	Species
SUA	Sokoine University of Agriculture
USA	United States of America
TSHZ	Tanzania short-horned Zebu
SUA	Sokoine University of Agriculture
SAS	Statistical Analysis System
TBS	Tanzania Bureau of Standards
TBC	Total bacterial count
TCC	Total coliform count
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Milk is an important source of nutrients to human and animals. It is meant to be the first and the only food for the offspring of mammals as is almost complete food (Pandey and Voskuil, 2011). Almost 87% of milk is composed of water and the remaining part comprises total solids (carbohydrates, fat, proteins and minerals) contained in a balanced form and digestible elements for building and maintaining the human and animal body. Other milk ingredients include immuno-globulins which protect the newly born against a number of diseases (Pandey and Voskuil, 2011). Milk has a complex biochemical composition and its high water activity and nutritional value serves as an excellent medium for growth and multiplication of many kinds of microorganisms when suitable conditions exists (Parekh and Subhash, 2008).

Milk meant for human consumption must be free from any pathogenic organisms (Bertu *et al.*, 2010). Microbial contamination in milk may cause milk-borne diseases to humans while others are known to cause milk spoilage. Many milk-borne epidemics of human diseases are spread through milk contamination. Sources of microbial contamination in milk include primary microbial contamination from the infected or sick lactating animal. The secondary causes of microbial contamination occurs along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipments and water supplies used in sanitary activities. Other secondary sources of microbial contamination occur during milk handling, transportation and storage. There is tertiary microbial contamination which occurs mainly due to re-contamination of milk after being processed due to unhygienic conditions and/or poor or improper handling

and storage of milk during consumption (Parekh and Subhash, 2008). The quality of milk is determined by its composition and overall hygiene. However, consumption of contaminated food like milk may lead to food-borne diseases (FBDs).

The WHO has described FBDs as illnesses of an infectious or toxic nature caused by, or thought to have been caused by the consumption of food and water (Adams and Motarjemi, 1999), which conceivably represents the most common health problem of recent days, thus reducing significantly economic productivity (Mukhola, 2000). It is estimated that up to a third of people in developed countries are affected by FBDs (WHO, 2009). FBDs are caused by the consumption of foods exposed to hazards that may be biological or pathogenic (e.g. viruses, bacteria, parasites), chemical (e.g. heavy metals and toxins), and others physical (e.g. glass fragments, bone chips) (Schmidt *et al.*, 2003). Etiological information suggests that the frequency of occurrence from microbial or pathogenic origin is by far higher (WHO, 2009). According to the WHO, 62% of all human pathogens are zoonotic (Taylor *et al.*, 2001). This is in aggrement with the OIE that 75% of all emerging human diseases originate from animal reservoirs (Vallat, 2007). Consequently, animal sourced foods have been found guilty for the majority of FBDs (De Buyser *et al.*, 2001) and incidences increase with increasing access to such foods especially without adequate hygiene, inspection for safety or satisfactory heating to kill pathogens (McCrindle, 2008).

Specifically, human may be infected with milk-borne pathogens through consumption of infected raw or unpasteurized milk and milk products (Bertu *et al.*, 2010). Sometimes consumption of contaminated or spoiled milk and dairy products may cause milk-borne diseases in humans. Indeed, FBDs are a serious threat to people in Africa, responsible for 33-90% cases of deaths in children (Flint *et al.*, 2005). Although milk and milk products are a minor constituent in most diets but contaminated milk are responsible for up to 90%

of all dairy related diseases of humans (De Buyser *et al*., 2001). A study by Shirima *et al.* (2003) documented several pathogens resulting to milk-borne zoonotic diseases including brucellosis, tuberculosis and enterotoxaemia. The risk of infection by milk-borne zoonotic diseases is one of the reasons for public health regulations, which discourages the informal milk markets and consumption of raw or unpasteurized milk (Kang'ethe *et al.*, 2000).

Pathogenic microorganisms commonly isolated from milk and milk products pose a serious threat to human health. Some of these pathogens include *Escherichia coli, Staphylococcus aureus, Salmonella* spp., *Listeria moncytogens, Brucella abortus, Mycobacterium* spp., *Campylobacter* spp., *Leptospira* spp., *Clostridium* spp., *Pseudomonas aeruginosa* and *Proteus* spp. (Shirima *et al.*, 2003; Al-Tahiri, 2005; Donkor *et al*., 2007; Lei *et al.*, 2008; AlAll *et al.*, 2012). Raw milk is known to be a major vehicle that serves as means of transmission of these milk-borne pathogens to humans.

On the other hand, raw milk apart from being potential carrier of pathogens can also cause serious health risk to consumers due to antimicrobial residues (Omore *et al.*, 2005; Kivaria *et al.*, 2006a). Antibiotic residues are remnants or small amounts of antimicrobial drugs or their active metabolites which remain in milk after treating lactating cows (Shitandi, 2004; Syit, 2008). Antimicrobial agents especially antibiotics are normally used in dairy cattle for treatment and prevention of bacteria and associated infections (Syit, 2008; Nonga *et al.*, 2010; Sharma *et al.*, 2011). Inappropriate use of these antibiotics by small-scale livestock keepers at farm level may lead to various bacterial pathogens developing resistance to most commonly used antibiotics which in turn increases bacterial resistant to almost all existing antibiotics (Kivaria *et al.*, 2006b; Sharma *et al.*, 2011). The outcome of random use of antimicrobial agents in animals is expansion of antimicrobial resistant bacteria that may be transferred from animals to humans through contact, contaminated

environment or milk and milk products (Syit, 2008; Sharma *et al.*, 2011). The side effects associated with antibiotic residues in milk include the risk of allergic reactions to sensitive people, increased selection of resistant bacterial pathogens towards commonly used antibiotics for human illnesses and inhibition of starter cultures used in production of different milk products (Aboge *et al.*, 2000; Shitandi, 2004; Kurwijila *et al*., 2006). Presence of antibiotic residues in milk may be the result of failure to observe the mandatory withdrawal periods, incorrect dosage levels and/or illegal or extra-label use of drugs (Syit, 2008).

Despite the existence of milk quality control measures and regulations at different points before processing and consumption, majority of milk such as over 75% of milk marketed in many developing regions (including East Africa) is sold raw or unpasteurized through informal channels (Bertu *et al.*, 2010; Oliver and Murinda, 2011). Milk is consumed raw at household and/or village level especially to pastoral and agro-pastoral communities who do not believe that milk could be a potential source of human infections and they are not ready for any kind of treatment (Bertu *et al.*, 2010). This poses health risks to consumers and the general public especially for those who consume raw or unpasteurized milk and milk products. Concerns about human health risks from the market pathways need to be addressed in the context of consumer practices, such as boiling, to reduce or eliminate potential infection by milk-borne health hazards without discouraging the markets through which the majority of smallholders' dairies and livestock keepers sell their milk (Kang'ethe *et al*., 2000).

This study has quantified and identified some bacteria species that are common contaminants of milk from small-scale livestock keepers as suggested by the SFFF II project. It further attempted to establish the risk factors for microbial contamination of

milk at farm level. The study further determined the susceptibility of the bacterial isolates to commonly used antibiotics in veterinary and human practices. Information that have been obtained from this study will be useful in knowing the status of microbial contamination in milk, the common species of bacteria involved and their susceptibility to antibiotics. Also the public will be enlightened and educated on the importance of knowing the microbial quality of milk they consume and this can give the evidence-based advice on the likely dangers of consuming raw or unpasteurized milk.

1.2 Problem Statement and Justification

Raw milk is an important vehicle for the transmission of milk-borne pathogens to humans, as can be easily contaminated during milking and handling (Addo *et al.*, 2011). Being highly perishable commodity and highly nutritious food, milk serves as an ideal medium for the growth and multiplication of various microorganisms (Parekh and Subhash, 2008). Poor or improper handling of milk can exert both a public health and economic constraints thus requiring hygienic vigilance throughout the milk value chain (Swai and Schoonman, 2011). Although fresh milk from cattle may possess temporary germicidal or bacteriostatic properties, growth of microorganisms is inevitable unless it is processed or well stored (Swai and Schoonman, 2011). The main health concerns associated with milk include tuberculosis caused by *Mycobacterium bovis* and *M. tuberculosis* and brucellosis caused by *Brucella* spp. (Al-Tahiri, 2005). In some parts of the world including developing countries like Tanzania, milk is still a significant source of these infections and other FBDs (Shirima *et al.,* 2003). It also applies in developed countries for example it was reported in England and Wales that there are yearly outbreak of food poisoning from *Salmonella* and *Campylobacter jejuni* in milk not receiving heat treatment or imperfectly pasteurized, and also *S. aureus* be isolated from most samples of raw milk and may be found in unheated or lightly heated dairy products (Al-Tahiri, 2005). Therefore,

microbiological assessment of milk is essential to establish the degree of contamination and recommend some corrective measures (Parekh and Subhash, 2008).

On the other hand, antimicrobial residues (antibiotics and other anti-bacteria's) in milk cause bacterial resistance to common antibiotics (Omore *et al.*, 2005). Records indicates an increasing incidence of antimicrobial resistance amongst the commonly isolated bacteria, though not quantified (Kivaria *et al.*, 2006b). Furthermore, limited information is available on susceptibility of bacterial isolates in Tanzania, thus hindering the choice of appropriate antibiotics for veterinary use (Kivaria *et al.*, 2006b).

1.3 Objectives of the Study

1.3.1 General objective

This study aimed at assessing microbial quality of raw cow's milk and determined antimicrobial susceptibility of the selected common milk-borne bacteria isolated in Kilosa and Mvomero districts in Morogoro region.

1.3.2 Specific objectives

i) To assess the total bacterial load of raw cow milk at farm level,
ii) To isolate selected common bacteria species contaminating raw cow milk from small-scale livestock keepers,
iii) To establish the possible risk factors for microbial contaminations of raw cow milk at farm level and
iv) To determine antimicrobial susceptibility of the common milk-borne bacteria isolated from raw cow milk.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition and Composition of Milk

Milk is a yellowish-white non-transparent liquid secreted by the mammary glands of all mammals. It is the primary source of nutrition and sole food for offspring of mammals before they are able to eat and digest other types of food. It contains in a balanced form of all the necessary and digestible elements for building and maintaining the human and animal body (Pandey and Voskuil, 2011). The main composition of milk is water (87 – 88%); the remaining part is total milk solids which include carbohydrates, fat, proteins and ash or minerals. This composition is not constant, the average percentages of milk components vary with species and breeds of animal, season, feeds, stage of lactation and health and physiological status of a particular animal (Pandey and Voskuil, 2011). Sometimes the composition might even change from day to day, depending on feeding and climate, but also during milking the first milk differs from the last milk drops (Pandey and Voskuil, 2011). Moreover, milk is an excellent source of high quality protein, vitamins, minerals such as calcium and phosphorus. Fresh milk has a pleasant soft and sweet taste and carries hardly any smell.

2.2 General Overview of Milk Production and Safety regulation in Tanzania

The population of cattle in Tanzania is estimated to be 22.8 million, of which, about 98% are indigenous cattle known as Tanzanian Shorthorn Zebu (TSHZ) (Swai and Karimuribo, 2011; Ministry of Livestock, 2012). These TSHZ cattle are small in body size and have poor milk production, mainly kept by either pastoralists and/or agro-pastoralists. The dairy industry and milk production system are divided into two systems which are traditional system dominated by TSHZ cattle with production of 70% milk of which 90% is

consumed at home and 10% contributes commercial sector (Mutagwaba, 2005). The other system is commercial dairy farming system dominated by smallholder's dairy farmers who keep mainly improved dairy cattle and contributes about 30% of all milk produced and marketed in Tanzania (Swai and Karimuribo, 2011).

In Tanzania the safety and quality of milk and milk products is regulated by the Tanzania Dairy Board, although the marketing of milk from most smallholder, pastoral and agro-pastoral producers mainly follows informal or traditional market channels (Kurwijila *et al*., 2006). Constraints in milk marketing such as poor infrastructure, collection centers, storage equipments, transport facilities and poor organization in dairy sector limit optimal productivity of milk at farm level. In addition, lack of processing of milk into better different final products further minimizes the accrued income from milk production and the high prevalence of factors that contribute to poor milk quality, then milk testing and quality controls are essential components of milk production (Mdegela *et al*., 2009).

2.3 Sources of Microbial Contamination in Milk

Milk is sterile when it is in the udder of a health animal but becomes contaminated with bacteria mainly during and/or after milking (Karimuribo et al., 2005; Makerere University, 2011). Milk from subclinical mastitic cows usually contains aetiological agents but milk from non-mastitic cows is often contaminated from extraneous dirt or poor quality water (Kivaria *et al.*, 2006a). Microbial contamination in milk comes from milk itself as it can be naturally contaminted or comes from infected or sick animal, human, environment, water and equipments used for milking and storage of milk. These sources of contamination include disease-causing organisms (pathogens) shedding in milk, infected udder and/or teats, animal skin, faecal soiling of the udder, contaminated milking and storage equipments and water used for cleanliness. Other bacterial sources are from air,

milkers, handlers, drugs or chemicals used during treatment of animal and from water used for adulteration by unscrupulous and unfaithful workers/sellers which may be contaminated and may cause additional health problems (Karimuribo *et al.*, 2005; Swai and Schoonman, 2011). Exposure of milk to these sources or conditions may lead to increased microbial contamination and affect its quality. Although, sometimes re-contamination may occur after processing and is mainly due to unhygienic conditions, poor or improper handling of milk during consumption (Parekh and Subhash, 2008). In general qualiy of milk may be lowered when it is contaminated by a number of factors such as adulteration, contamination during and after milking, presence of udder infections, mastitis (inflamation of mammary gland) disease and drugs residues used for treatement of disease which is considered to be public health concern and one of the most important causes of economic losses in the dairy industry worlwide including Morogoro and Tanzania at large (Karimuribo *et al.*, 2005; Syit, 2008; Mdegela *et al*., 2009).

2.4 Milk-borne Infections and Pathogenic Microorganisms

Various bacteria may have access to milk and milk products from different sources and cause different types of milk-borne illnesses. Sometimes milk and milk products may carry microorganisms or their toxic metabolites (poisons/toxins). Some of these microorganisms are pathogenic and cause illness to humans while others cause spoilage in milk rendering it unsuitable (unsafe) for human consumption (Kivaria *et al.*, 2006a; Parekh and Subhash, 2008; Bukuku, 2013). Many milk-borne epidemics of human diseases are spread through consumption of contaminated milk (Parekh and Subhash, 2008). Few examples of the known milk-borne diseases are bovine tuberculosis, brucellosis, anthrax, listeriosis, salmonellosis, leptospirosis, Q fever, campylobacteriosis and *E. coli* O157:H7 as an emerged new milk-borne bacterial pathogen reported recently with a very serious health effects (Sivapalasingams *et al*., 2004). These are zoonotic

diseases which are transmitted to consumers and pose a risk to public health. To protect consumers and public health against these milk-borne infections it require proper hygienic milking and milk handling procedures.

Common bacteria reported to be isolated from milk include *Staphylococcus* spp., *Listeria* spp., *Salmonella* spp., *E. coli* spp., *Campylobacter* spp., *Mycobacterium* spp., *Brucella* spp., *Coxiella burnetii, Yersinia* spp., *Pseudomonas aeroginosa* and *Corynebacterium ulcerans.* Others are *Proteus* spp., *Leptospira* spp., *Clostridium* spp., *Streptococcus* spp, *Klebsiella* spp., *Enterobacter* spp. and *Bacillus* spp. (Shirima *et al.*, 2003; Sivapalasingams *et al.*, 2004; Al-Tahiri, 2005; Donkor *et al*., 2007; Parekh and Subhash, 2008). All these are pathogenic bacteria that pose serious threat to human health and contribute up to 90% of all dairy related diseases (De Buyser *et al*., 2001; Sivapalasingams *et al.*, 2004; Donkor *et al*., 2007).

Therefore, proper milking, cleaning and sanitizing procedures of equipments and environments are essential tool to ensure quality of milk. Many countries have implemented laws and regulations concerning the composition and hygienic quality of milk and milk products to protect both the consumers and the public health (Pandey and Voskuil, 2011). Unfortunately, these laws and regulations are not often adhered in developing countries making milk-borne diseases a higher health risk to public. This is examplified by over 75% of milk marketed in many developing countries is sold raw/unpasteurized through informal channels (Bertu *et al.*, 2010; Oliver and Murinda, 2011). Some studies show that a big percentage of people in Tanzania especially in rural areas consume raw milk (Mullins, 1993; Kurwijila *et al.*, 1995) which predisposes them to the risk of contracting zoonoses, and other milk-borne diseases.

2.5 Hygiene, Handling and Microbial Quality of Raw Milk

Milk is a perishable product and an ideal medium for the growth of a wide variety of bacteria (Parekh and Subhash, 2008). When it is secreted from a healthy udder, raw milk contains only a very few bacteria of about 500 to 1,000 bacteria per milliliter (Omore *et al.*, 2005; Pandey and Voskuil, 2011). After milking enviromental contamination occurs, which in turns increases the total bacteria count up to 50,000 per ml or may even reach several millions bacteria per milliliter (Pandey and Voskuil, 2011). That count level indicates a very poor hygienic standard of milk during milking and handling or milk of a diseased animal. The presence of coliform bacteria particulary *E. coli* in raw milk is an indicator of feacal contamination which implies poor hygienic conditions and unsanitized environment since these bacteria are of faecal origin.

In developing countries like Tanzania, most of the milk is produced by smallholder farmers dominated by local herds of cattle (Pandey and Voskuil, 2011). Their milking units are widely distributed throughout in rural areas with a poor infrastructure, while most of the markets and customers are in urban areas. Therefore, the need for good hygienic practices and a streamlined collection, handling and transport system is important but has been always a challenge (Pandey and Voskuil, 2011). However, milk contains a natural inhibitory system or temporary germicidal or bacteriostatic properties which prevents a significant rise in the bacteria count during the first 2 - 3 hours (Swai and Schoonman, 2011; Pandey and Voskuil, 2011). If the milk is cooled to 4ºC within this period immediately after milking, it maintains nearly its original quality and remains safe for processing and consumption. Temperature of storage and time since milking are also important in determining milk quality, as these influence the rate at which the bacteria will increase in number (Omore *et al.*, 2005). To prevent a too high multiplication of bacteria,

the milk has to be produced as hygienic as possible and should be cooled or heated at the earliest (Pandey and Voskuil, 2011).

2.6 Prevention and Control of Microbial Contamination in Milk

Prevention and control of microbial quality of milk is through elimination of organisms from human carriers by general improvements in water supplies, public health education, personal and environmental hygiene. Also can be achieved through proper boiling or pasteurization of raw milk before processing and consumption. Pathogenic organisms from the lactating animals can be controlled through improvements in animal husbandry and maintanance of good animal practices, and those from the environments and equipments can be prevented by adhering to general hygienic practices and environmental cleanliness.

Generally, microbial contamination in milk can be minimized through adherence to effective good hygienic practices at farm level; and in order to protect the public against milk-borne infections it is important to screen milk which is informally taken to the market. The lack of awareness of milk-borne infections in many developing countries and consumption of raw milk predispose small-scale livestock keepers, consumers and the general public at risk of contracting these infections (Mosalagae *et al.*, 2011).

2.7 Antimicrobial Residues, Bacterial Resistance and their Effects to Animals and Humans

Antimicrobial agents particularly antibiotics are veterinary drugs used in dairy cattle for treatment and prevention of various diseases. Also they are used to improve feed efficiency, increase milk production or as growth promoters (Syit, 2008; Sharma *et al.*, 2011). Antibiotic use sometimes occur in response to several challenges that face the

livestock industry that include high level of stress, diseases, poor animal genetic potential, poor management, poor nutrition and drought (Mellau *et al*., 2010). Furthermore, misuse and incorrect applications of antimicrobials and antibiotics deposit noticeable residue in tissues of animals, particularly when the milk is harvested and marketed within the withdrawal period of the drug. The rampant and indiscriminate uses of antibiotics among the small-scale livestock keepers increase possibility of antibiotic resistant bacteria that may be transferred from animals to humans and leads to various chronic diseases to the users of milk and milk products.

Because of limited extension services and poor animal health delivery systems in Tanzania, the pastoralists and agro-pastoralists buy veterinary drugs from veterinary shops and treat by themselves. Katakweba *et al.* (2012) reported that a lot of drugs such as oxytetracycline are used abusively to treat and protect cattle against various diseases. When such drugs are administered by non-professionals correct dosages are unlikely to be observed as well as withdrawal period for products like milk that may lead to antimicrobial residues. The antimicrobial residues such as antibiotics and other anti-bacteria's can be found in milk as leftovers after the drugs have been administered in animal. These residues in milk are often due to farmers failing to adhere to the specified milk withdrawal periods after antibiotic use to sick lactating cows, illegal or extra label use of drugs and incorrect dosage levels and route of administration (Shitandi, 2004: Kivaria *et al.*, 2006a; Kurwijila *et al.*, 2006; Syit, 2008). Since boiling or pasteurization does not destroy antibiotic residues, this chemical hazard may pose a more serious long-term health risk to public (Omore *et al.*, 2005).

Commonly used antimicrobial agents particularly antibiotics in farm level are of different groups or classes. These include the penicillins, tetracyclines, aminoglycosides, beta-

lactams, sulphonamides, macrolides, and phenicols (Bukuku, 2013). These antibiotics may be used singly or sometimes in combination when treating cattle. Studies have been done in Tanzania (Kivaria *et al.*, 2006b; Katakweba *et al.* 2012) and elsewhere such as in Kenya (Aboge *et al.*, 2000; Shitandi, 2004) and found that these antibiotics are extensively used for treatment of different livestock diseases.

The presence of antimicrobial drug residues in milk above the maximum allowable limits and when taken into body can result to undesirable health safety effects to human being. Among the effects include allergic reactions (hypersensitivity reactions) in sensitive persons, occurrence of resistant strains of bacteria or selection of resistant bacteria that do not respond well to treatments of commonly used antibiotics for human illnesses, specific tissue damage, toxicity and carcinogenic effects. Also drug residues inhibit starter cultures used in production of cheese, fermented or cultured milk and other dairy products (Aboge *et al.*, 2000; Shitandi, 2004: Kivaria *et al.*, 2006a; Kurwijila *et al.*, 2006; Syit, 2008). Heat treatment of milk such as boiling and pasteurization destroys or eliminate pathogenic microorganisms but have limited or variable effects on drug residues (Shitandi, 2004; Kurwijila *et al.*, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The present study was conducted in two districts namely Kilosa and Mvomero both located in Morogoro region (Fig. 1). Morogoro region lies between latitude 5^0 58" and 10^0 0"to the South of the Equator and between longitude 35^0 25" and 35^0 30" to the East of Greenwich. The region is bordered by seven regions, Dodoma and Iringa regions on the Western side, Tanga and Manyara regions on the Northern side, Coast/Pwani and Lindi regions on the Eastern and Ruvuma region on the Southern side. According to the 2012 National census, Morogoro region had a population of 2 218 492 people (PHCT, 2012) and total area of 70 799 km^2 of which 2 240 km^2 is covered by water (MPEE, 2007).

Kilosa district had a population of 438 175 people (PHCT, 2012) and land area of 14 245 km^2 while Mvomero district had a population of 312 109 people (PHCT, 2012) and land area of 7 325 km^2 with insignificant water areas in both districts (MPEE, 2007). The cattle population is estimated to be 215 040 cattle in Kilosa district (MPEE, 2007; Ndanu *et al.*, 2012), while in Mvomero district is estimated to be 172 827 cattle (MPEE, 2007). Morogoro region was chosen for the study since it is among the regions in Tanzania with many livestock keepers especially Kilosa and Mvomero districts. In addition the SFFF II project operates in these districts within a region.

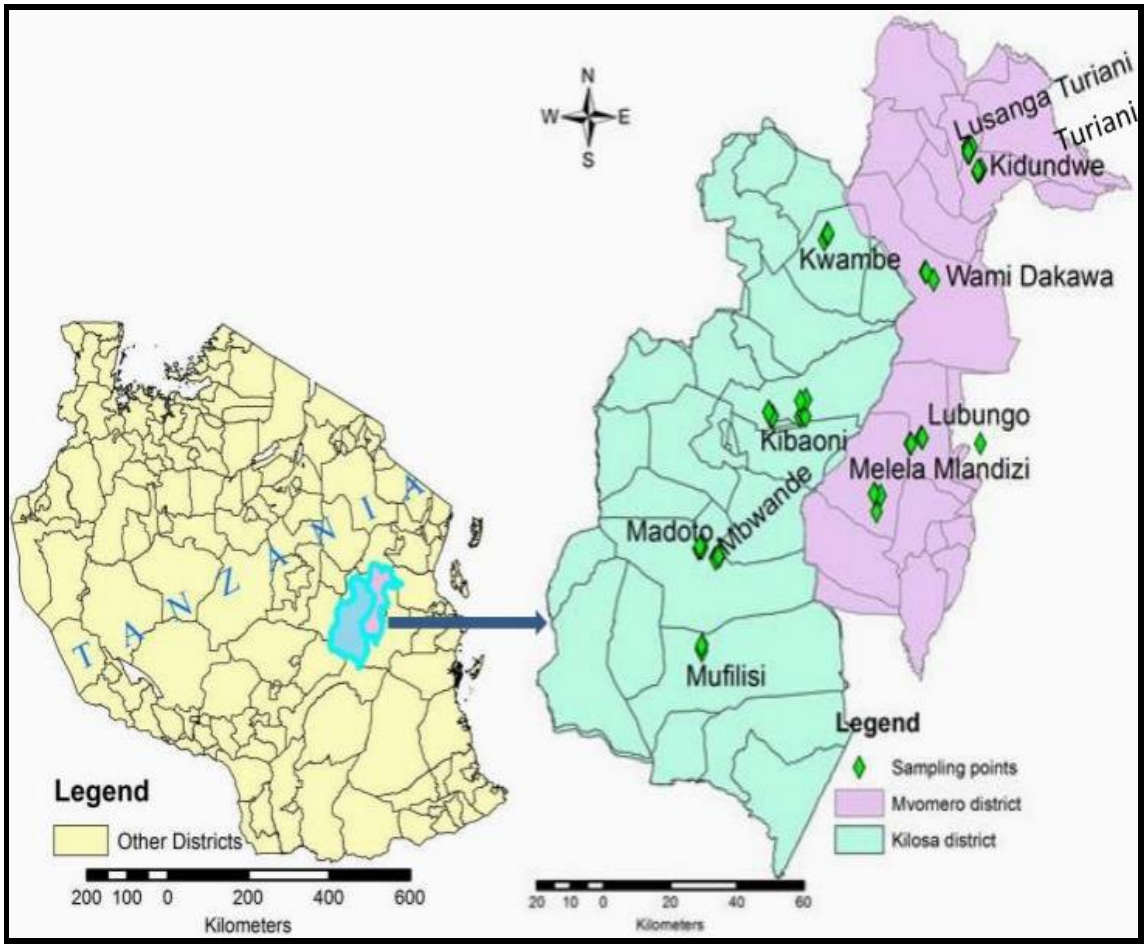

Figure 1: A map of study area showing two districts and location of villages in each district. Insert is a map of Tanzania showing location of Morogoro Region.

3.1.1 Farming systems practiced by people in the study area

The livestock keeping and production systems in the study area are of three categories namely pastoral, agro-pastoral and smallholder dairying. Pastoral system mainly keep indigenous cattle called Tanzanian short-horn zebu (TSHZ), while agro-pastoralist also keep indigenous cattle as well as cultivate crops. Other animals kept by these livestock keepers are goats, sheep, poultry and dogs for security purposes. They both practice a free grazing system, using communal grazing land and share water points but in most cases cattle and small ruminants are grazed separately. These animals are kept as source of

income, meat, milk, draught power for agro-pastoralist as well as for traditional and cultural activities such as dowry, celebration, gifts, prestige and symbol of wealth.

On the other hand, smallholder dairy farming system was observed to be practiced at Mvomero district whereby indigenous cattle (TSHZ) and improved/exotic breeds of cattle are kept. They also practice free grazing on communal grazing as well as in-door grazing. In Kilosa district the farming systems observed practiced mostly are pastoral and agro-pastoral farming systems while in Mvomero district both three farming systems were observed practiced.

3.2 Study Design

The present study employed a cross-sectional study design to establish the magnitude of microbial contaminants in raw/unpasteurized cow milk and the commonly isolated milk-borne bacteria. Farmers involved in the study were small-scale pastoralists, agro-pastoralists and small-holder dairy farmers in which they were randomly selected and visited once. The study units were smallholder farmers with lactating cows where raw cow milk was collected and questionnaires were administered at the same time.

3.3 Study Animals and Population

The study animals were lactating cows from small-scale farmers in the two districts. The target farmers were the pastoralists and agro-pastoralists who normally keep traditional cattle specifically TSHZ which are managed under free range grazing system. Nevertheless, in both districts some farmers practice dairy farming with improved breeds (crosses of Friesian, Ayrshire and Jessey) especially in urban and peri-urban areas. The traditional cattle rarely get veterinary services although some practices tick control through use of acaricides (Nonga *et al.*, 2012).

3.4 Selection of Study Districts, Villages and Households

The two districts were been selected purposely because they have many small-scale livestock keepers who practice different farming systems (pastoralist, agro-pastoralist and smallholder dairying) and supply milk to the local community and nearby milk processing plants. Five villages in each district were purposely selected by SFFF II project mostly based on the number of livestock keepers and accessibility. With the help of Livestock Extension Officers (LEO), households with cattle in the selected villages were identified and 10 household were selected to be used for study based on inclusion and exclusion criteria stated in section 3.5.

3.5 Inclusion and Exclusion Criteria

The study inclusion criteria for the household were: must be a smallholder farmer both men and women with lactating cows during the study, willing to participate in the study, ready to give the required information through questionnaires and availability of milk at the time of data collection. The exclusion criteria were: those who were not around, unwilling to participate in the study, unable to give the asked/required information and absence of milk at the time of data collection. Also those who had no time for questionnaires interviews were excluded.

3.6 Sample Size Determination

A formula by Kothari (2004) for unknown population (i.e. $n = Z^2SD^2/e^2$) was used to calculate the sample size for this study. Where Z, is the estimated standard variation at 95% confidence interval (CI) which was considered the point of the normal distribution corresponding to the level of significance (Z=1.96). Standard deviation (SD) was estimated at 0.15 or 15% and e, is the estimated error and was considered at 0.05 or 5%.

Therefore, the sample size ‘n’ was calculated as:

$n = \frac{(1.96)^2 \times (0.15)^2}{(0.05)^2} = 34.6$ approximately n = 35 samples per each district (35 x 2)=70

Based on the above formula 70 milk samples were supposed to be collected, but during the time of sample collection it was a dry season, therefore many cattle were not lactating thus, 56 milk samples were collected (32 milk samples in Kilosa District and 24 milk samples in Mvomero District).

3.7 Ethical Consideration

A research permit to conduct this study was provided by the Vice Chancellor of SUA and permission to conduct research was obtained from Ward Executive Officer (WEO) (verbally). Before going to the field communication was made with the Village Livestock Extension Officer and accepted the study to be conducted to his/her area of work. Farmers participated in the study on voluntary basis. Verbal consent was obtained from each of the selected heads of households of small-scale livestock keepers after explaining the purpose and importance of the study prior to commencement of interviews and sampling. Households who willingly agreed to participate in the study signed the consent form which abided with the rules and regulations of research. The consent form was prepared by ILRI (Appendix 2). All the information collected from the participants and the laboratory results obtained after milk sample analysis were kept under the custody of the researcher as confidential and the study participants were anonymized.

3.8 Data Collection

Two types of data were collected which are sociological and laboratory based data.

3.8.1 Sociological data collection

Structured questionnaire (Appendix 3) was used to collect information from smallholder livestock keepers with lactating cows. The questionnaire was made with pre-coded response choices (closed-ended questions) with a few open-ended questions. Also, the

questionnaire was used to collect sociological information on possible risk factors for microbial contaminations in milk. The information collected include bio-data information, animal health and management, general health status of cattle and common diseases affecting herds, treatment and medication of animals, occurrences of mastitis, milk production, types and practices of milking and milk handling, sanitary measures during milking, utensils used for milking, milk storage and storage conditions, uses of milk (for selling or domestic purposes), habit of drinking raw milk and milk products, issues of antibiotic usage and compliance to drug withdrawal period.

3.8.1.1 Pre-testing of questionnaires

Prior to start of data collection, pre-testing of questionnaire was done at Mtipule village (not included in the study) located in Mvomero District where 10 livestock keepers were involved. The aim was to check the clarity, sequence and applicability of the questions and estimate the duration of time for each questionnaire. After testing of the questionnaire, it was revised and arranged in a better chronology. The revised version of the questionnaire that was used in the pilot study was translated into ‘Kiswahili’, the National language that is clearly understood by majority of Tanzanians.

3.8.1.2 Administration of questionnaires

The questionnaire was administered through face to face interview conversation. While administering questionnaires, direct observation on general cleanliness and hygienic conditions and practices with regard to milk were also done and noted. Upon finishing of the administration of questionnaires, milk samples were collected for laboratory analyses. Sometimes milk was sampled first before administering questionnaires because some farmers wanted to boil the milk for home use or sell to milk vendors.

3.8.2 Laboratory data collection

3.8.2.1 Sampling of milk

Milk samples were collected directly from the storage containers used by corresponding farmers in the visited households. Approximately 50 ml of milk was aseptically collected and put into a sterile screw capped falcon tubes. All samples were drawn from pooled containers containing milk that were milked on that particular day which is either consumed at household level, sold to the public or both.

3.8.2.2 Milk sample handling

All samples were coded with random numbers for identification and stored in a cool box with ice packs during field work. Thereafter, the samples were transported to the Department of Animal Science and Production at SUA and were stored at -20°C until analyses.

3.9 Laboratory Analysis of Milk Samples

Analyses were carried out in the Public Health Research Laboratory in the Faculty of Veterinary Medicine, SUA. Two kinds of laboratory analyses of milk samples were performed. First was analysis for microbial quality of raw milk which involved establishing the total bacterial counts (TBC) and isolation of some common milk-borne bacteria namely Enterobacteriaceae (specifically *E. coli* and *Salmonella* spp.), *S. aureus* and *L. monocytogenes*. Secondly was determination of antimicrobial susceptibility of the isolated milk-borne bacteria.

3.9.1 Determination of microbial quality of raw cow milk

3.9.1.1 Media preparation and storage

All the media used in this study were prepared according to manufacturer's instructions. Details of the preparations and handling of different types of media used is hereby shown:

3.9.1.1.1 Nutrient Agar (NA)

The medium NA (Laboratorios Conda, S.A. Cat.1060.00, Lot 005251) is composed of 5 g/l Gelatin peptone, 3 g/l Beef extract, 15 g/l Bacteriological agar and final pH of 6.8 ± 0.2 at 25°C. The medium was prepared according to the manufacturer's instructions whereby 23 g of the powdered medium was suspended into 1 litre of distilled water, mixed well and left on the bench to stand until the mixture is uniform. Then the mixed solution was heated with gentle agitation and boiled until completely dissolved. The medium solution was sterilized in the autoclave at 121°C for 15 minutes then allowed to cool to 45^{o}C and poured onto sterile Petri dishes. The plates were left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37°C to check for sterility and to dry the condensed vapour on the plate cover.

3.9.1.1.2 Buffered Peptone Water (BPW)

The medium (BPW powder, Oxoid® Ltd., Basingstoke, Hampshire, England, CM0509, Lot 1442805) is composed of 10 g/l Peptone, 5 g/l Sodium chloride, 3.5 g/l Di-sodium phosphate and 1.5 g/l Potassium di-hydrogen phosphate. The medium was prepared according to manufacturer's instructions whereby 20 g of the powdered medium was dissolved in 1 litre of distilled water. The culture medium was mixed well and each 10 ml were dispensed into capped test tubes. Then, the test tubes were sterilized by autoclaving at 121°C for 15 minutes and cooled to 25°C before use. All the unused prepared media were being stored under refrigeration temperature.

3.9.1.1.3 Violet Red Bile Glucose (VRBG) Agar

The VRBG medium (Oxoid® Ltd., Basingstoke, Hampshire, England, CM0485, Lot 1437851) is composed of 3 g/l Yeast extract, 7 g/l Peptone, 5 g/l Sodium chloride, 1.5 g/l Bile salts No. 3, 10 g/l Glucose, 0.03 g/l Neutral red, 0.002 g/l Crystal violet, 12 g/l Agar

and final of pH 7.4 ± 0.2 at 25°C. It was prepared according to the manufacturer's instructions whereby 38.5 g of the powdered medium was suspended into 1 litre of distilled water. The medium was boiled for 1 minute with frequent agitation to dissolve completely. No further sterilization is necessary. Then, was mixed well and placed into water bath set at 48ºC for use within 3 hours from preparation time.

3.9.1.1.4 MacConkey Agar

The medium (Oxoid® Ltd., Basingstoke, Hampshire, England, CM0007, Lot 1367351) is composed of 20 g/l Peptone, 10 g/l Lactose, 5 g/l Bile salts, 5 g/l Sodium chloride, 0.075 g/l Neutral red, 12 g/l Agar and final of pH 7.4 ± 0.2 at 25°C. The medium was prepared according to the manufacturer's instructions whereby 52 g of the powdered medium was suspended into 1 litre of distilled water. The medium was boiled to dissolve completely followed by sterilization by autoclaving at 121°C for 15 minutes and cooled to below 45°C and poured onto sterile Petri dishes. The plates were left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37°C to check for sterility and to dry the condensed vapour on the plate cover.

3.9.1.1.5 Brain Heart Infusion (BHI) Broth, Porcine

BHI broth (Sigma-Aldrich, Co., USA, Pcode 101350135, Lot BCBF8661V) is composed of ≥ 4.5% Amino N, ≥ 11% Total nitrogen N, ≤ 15% Residue on ignition, ≤ 6% Loss on drying and final pH of 6.5 – 7.5 at 25°C. The medium was prepared according to the manufacturer's instructions where 2 g of the powdered medium was dissolved in 100 g of distilled water (2% in water, solubility) and mixed well. Each 5 ml of the medium were transferred to capped test tubes, sterilized by autoclaving at 121°C for 15 minutes and cooled ready for use. All the prepared but unused BHI tubes were stored under refrigeration temperature.

3.9.1.1.6 BD BBL™ Coagulase Plasma, Rabbit with EDTA

BBL Coagulase Plasma, Rabbit with EDTA (lyophilized 10 x 15 ml rabbit plasma with 0.15% ethylene di-amine tetra-acetate and 0.85% sodium chloride, approximately) contains dry natural rubber (Becton, Dickson and Company, Sparks, MD 21152 USA, Cat.240826 Lot 3326034). The medium was prepared by reconstituting the powder with 15 ml of sterile distilled water followed by gentle shaking. Then, each 0.2 ml was dispensed into eppendorf tubes for coagulase test.

3.9.1.1.7 Rappaport-Vassiliadis (RV) Enrichment Broth

RV enrichment broth (Oxoid® Ltd., Basingstoke, Hampshire, England, CM0669 Lot 1452981) is composed of 5 g/l Soya Peptone, 8 g/l Sodium chloride, 1.6 g/l Potassium di-hydrogen phosphate, 40 g/l Magnesium chloride, 0.04 g/l Malachite green and final pH of 5.2 ± 0.2 at 25°C. The medium was prepared according to the manufacturer's instructions by weighing 30 g of the powdered medium, added into 1 litre of distilled water and heated gently until completely dissolved. Then, 10 ml of the medium were dispensed into capped test tubes followed by autoclaving at 121^0C for 15 minutes and cooling to about 25°C prior to use.

3.9.1.1.8 Muller Kauffmann Tetrathionate-Novobiocin (MKTTn) Broth

MKTTn broth (Oxoid® Ltd., Basingstoke, Hampshire, England, CM1048 Lot 1443832) is composed of 4.3 g/l Meat extract, 8.6 g/l Enzymatic digest of casein, 2.6 g/l Sodium chloride, 38.7 g/l Calcium carbonate, 30.5 g/l Sodium thiosulphate (anhydrous), 4.78 g/l Ox-bile, 0.0096 g/l Brilliant green and final pH of 8.0 ± 0.2 at 25°C. The medium was prepared according to manufacturer's instructions by suspending 89.5 g of the powdered medium into 1 litre of distilled water, mixed well, boiled and then cooled to below 45°C.

Immediately before use 20 ml of iodine-iodide solution were added, prepared by dissolving 25 g of Potassium iodide in 10 ml of distilled water, adding 20 g of iodine and then diluting to 100 ml with distilled water and one vial of Novobiocin Supplement (SR0181E) was reconstituted per 250 ml of medium as recommended. The medium was well mixed and each 10 ml were aseptically dispensed into sterile capped test tubes ready for use.

3.9.1.1.9 Fraser Broth Base

The medium (Oxoid® Ltd., Basingstoke, Hampshire, England, CM0895 Lot 1431937) is composed of 5 g/l Proteose peptone, 5 g/l Tryptone, 5 g/l 'Lab-Lemco' powder, 5 g/l Yeast extract, 20 g/l Sodium chloride, 12 g/l Di-sodium hydrogen phosphate, 1.35 g/l Potassium di-hydrogen phosphate, 1 g/l Aesculin, 3 g/l Lithium chloride and final pH of 7.2 ± 0.2 at 25°C. To make a full Fraser broth, the medium was prepared according to the manufacturer's instructions by adding 28.7 g of the powdered medium into 500 ml of distilled water and mixed well to dissolve completely. The medium was then sterilized by autoclaving at 121°C for 15 minutes and cooled to below 50°C. The contents of 1 vial of SR0156E (Fraser Selective Supplement) was reconstituted as directed in the product insert and aseptically added. The medium was well mixed and each 10 ml were aseptically dispensed into sterile capped test tubes and stored under refrigeration.

3.9.1.1.10 Mueller-Hinton (MH) Agar

The medium (Oxoid® Ltd., Basingstoke, Hampshire, England, CM0337 Lot 744451) is composed of 300 g/l Beef, dehydrated infusion, 17.5 g/l Casein hydrolysate, 1.5 g/l Starch, 17 g/l Agar and final pH of 7.3 ± 0.1 at 25°C. The medium was prepared according to manufacturer's instructions whereby 38 g of the powdered medium was suspended into 1 litre of distilled water, mixed well and brought to boil to dissolve the medium completely.

Then, the medium was sterilized by autoclaving at 121°C for 15 minutes, cooled to below 45°C and poured into sterile Petri dishes. The plates were left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37°C to check for sterility and to dry the condensed vapour on the plate cover.

3.9.1.1.11 Normal saline solution

The solution was prepared by dissolving 0.85 g of Sodium chloride (Sigma-Aldrich, Co., USA, Cat. S5886, Lot SLBC3215V) into 100 ml of sterile distilled water, mixed well and sterilized by autoclaving at 121°C for 15 minutes and cooled to below 45°C, the solution was ready for use.

3.9.1.2 Ready-to-use media

A number of the already prepared plated media were received from E & O Laboratories Ltd, Scotland which was being kept under refrigeration temperature all the time before being used. The media included: Plate Count agar (PCA), Nutrient agar (NA), MacConkey agar with salt, Baird-Parker (BP) agar, Glucose agar, Xylose Lysine Desoxycholate (X.L.D.) agar, Listeria Oxford agar, Colorex *Listeria* agar added with *Listeria* selective supplement and *Listeria* differential supplement, Sheep Blood agar base added with 5% sheep blood and packs of 5 L each Fraser broth (Half-Strength) added with SR0166E (Half Fraser Selective Supplement).

3.9.1.3 Laboratory procedures

3.9.1.3.1 Preparation of control isolates

About 500 ml of raw cow milk was collected from Magadu farm at Sokoine University of Agriculture (SUA) as a control sample. The sample was sterilized by boiling, cooled and placed in a sterile bottle. Part of the sample was inoculated with reference strains of

Escherichia coli ATCC® 2262-79 (DEC9B), *Salmonella enterica* ATCC® 13076, *Staphylococcus aureus* NCTC 6571/ATCC® 9144 and *Listeria monocytogenes* NCTC 13372/ATCC® 7644 (TCS biosciences). A sterile pipette was used to transfer 10 ml of the milk sample into a sterile beaker containing 90 ml of Half-Fraser broth for cultivation of *L. monocytogenes.* Also, another 25 ml of the milk sample was transferred into a sterile conical flask containing 225 ml of Buffered Peptone water (BPW). The suspension solution (with BPW) was well mixed and serial dilutions were prepared as described sub-section 3.9.1.2.2.1. Detection and enumeration of the desired microorganisms was done and the respective bacterial colony on the media was stored and used for comparison (colonial morphology) against the test samples. The reference strains were sub-cultured and stored in sterile condition in a refrigerator at 2 – 8°C for use in the entire microbial analysis of milk samples.

3.9.1.3.2 Total Bacterial Count (TBC)

Determination of total bacterial count (TBC) was done by using ISO 4833-1:2013 protocols.

3.9.1.3.2.1 Sample preparation, inoculation and incubation

A total of 10 sterile test tubes were dispensed with 9 ml of sterilized BPW. Samples were removed from the freezer and thawed at room temperature. Using a sterile pipette 25 ml of the milk sample was transferred into a conical flask containing 225 ml of Buffered Peptone water (BPW) and mixed well. Ten-fold serial dilution of the inoculums from 10^{-1} to 10^{-10} was done into sterile BPW solution using disposable sterile pipettes tips. One millitre of the prepared inoculum was transferred into test tube containing 9 ml of BPW (10^{-1} dilution). Then using another sterile pipette, 1 ml of the resulting dilution was transferred into a second test tube containing 9 ml of BPW (10^{-2} dilution). The procedure

was repeated for further dilutions up to 10^{-10} dilution and in the last dilution 1 ml of inoculum was discarded as shown in Fig. 2. The dilutions were mixed using a vortex mixer for 5 – 10 seconds.

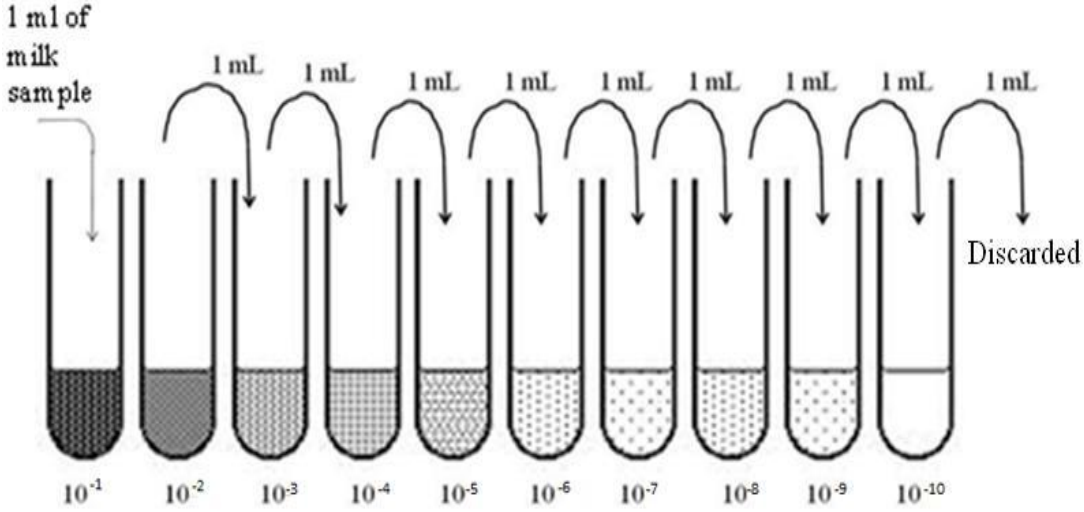

Figure 2: Serial dilutions of milk samples in 10 test tubes containing 9 ml of BPW each

The already prepared PCA plates were removed from the refrigerator, kept at room temperature and labeled prior to inoculation. From each dilution (starting with the last dilution), two sterile PCA plates were each inoculated with 0.1 ml of the test sample. With the aid of sterile swab the sample was spread on the media surface and the plates were allowed to dry with their lids on for about 15 minutes. The plates were inverted and incubated at 30ºC under aerobic condition for 72 hours to allow bacterial growth.

By using a new sterile pipette for each dilution, the procedure was repeated as above with further dilutions up to the first dilution and for the remaining test samples, where only consecutive critical dilution steps were chosen for the inoculation on plates. From the results it was learnt that, critical dilutions of 10^{-3} to 10^{-5} were the best for countable range of less than 300 colony forming units per plate (cfu/plate).

The dilutions were also used for detection and enumeration of Enterobacteriaceae and *S. aureus*, and the remained initial sample suspensions in the conical flasks were used in the initial preparation for isolation and presumptive identification of *Listeria* spp. and *Salmonella* spp.

3.9.1.3.2.2 Counting of bacterial colonies

After the incubation period, bacterial colonies on the culture plates were countered manually. Two critical dilutions per each sample were counted. A plate was divided into quarters using a marker-pen and colony forming units were counted on at least two critical dilution plates by the aid of colony counter. Two consecutive plates with less than 300 colonies were considered for record (ISO 4833-1:2013).

3.9.1.3.2.3 Expression of results

The countable bacterial colonies from two consecutive plates of each sample were converted into colony forming units per millilitre (cfu/ml) using a formula given by ISO 7218:2007(E):

$$N = \frac{\sum C}{V \times (n_1 + 0.1\, n_2) \times d}$$

Where; N = number of bacterial colonies counted, C = sum of colonies identified on two consecutive dilution steps, where at least one contained 10 colonies, V = volume of inoculum on each dish/plate, in ml and d = dilution rate corresponding to the first dilution selected (the initial suspension is a dilution).

3.9.1.4 Identification of common bacteria in cultures

3.9.1.4.1 Detection of *Salmonella* spp.

Identification of *Salmonella* spp. in milk samples was done by using ISO 6579:2002 protocols and involved the following stages:

Stage 1: Pre-enrichment in a non-selective medium

As described in part 3.9.1.3.2.1 above, the remained initial sample suspensions in the conical flasks were used in the initial preparation for isolation and presumptive identification of *Salmonella* spp. BPW was used as a pre-enrichment liquid medium and the initial suspensions was incubated at 37ºC for 18 hours.

Stage 2: Enrichment in a selective liquid medium

Briefly 0.1 ml of the culture obtained in stage 1 was inoculated in a test tube containing 10 ml of RVS broth. Also, about 1 ml of the same culture was inoculated in a test tube containing 10 ml of MKTTn broth. The inoculated MKTTn and RVS broths were incubated at 37ºC and 42ºC respectively for 24 hours.

Stage 3: Plating out and identification

Using a sterile loop RVS broth culture obtained in stage 2 was inoculated onto XLD and MacConkey agar plates (already prepared plated media). The same was done using the culture obtained in the MKTTn broth (stage 2). The plates were inverted and incubated at 37ºC for 24 hours. After incubation period, the plates were examined for typical colonies of *Salmonella* and atypical colonies that may be *Salmonella*. Typical colonies of *Salmonella* grown on XLD agar are red colonies with black centers due to xylose fermentation, lysine decarboxylation and production of H_2S gas.

The procedure was repeated as above with further dilutions up to the first dilution and for the remaining test samples. Each dilution a new sterile pipette was used. Only consecutive critical dilution steps were chosen for the inoculation on plates.

Stage 4: Confirmation of *Salmonella*

Suspected *Salmonella* colonies from each XLD agar plate was confirmed using *Salmonella* Test kit (Oxoid® Ltd., Basingstoke, Hampshire, England, Ref DR1108A, Lot

1272360) which is a rapid latex agglutination test for the presumptive identification of *Salmonella*. The test is performed within a drawn circle.

Parallel with the test samples, positive and negative controls were prepared and used. For a positive control, a drop of *Salmonella* latex reagent was mixed with a smooth suspension of a known *Salmonella* spp. on the reaction card and observed for agglutination within two minutes. As for a negative control, a drop of *Salmonella* latex reagent was mixed with a drop of normal saline on the reaction card and observed for agglutination within two minutes.

For the test sample cultures, a drop of 0.85% isotonic saline was placed on the reaction card. Using a sterile loop the suspected colony was emulsified in a drop of normal saline and then, a drop of *Salmonella* latex reagent was added to the saline suspension and mixed well with a clean sterile loop. Thereafter, the suspension mixture was examined for agglutination within two minutes along with positive and negative controls. If agglutination observed was indicated positive reaction.

3.9.1.4.2 Detection of *Pseudomonas aeruginosa* and *Proteus* spp.

Based on morphological features on MacConkey and Blood agar plates, and Gram staining (Cheesbrough, 2000), *Proteus* spp. and *P. aeruginosa* were identified. On Gram stain they appeared Gram-negative bacteria, rod shaped and pale to dark red in colour. On culture plates *Proteus* spp. colonies appeared as large, circular and smooth, with yellowish to pinkish colour which had translucent shining black centers. Further, *Proteus* colonies observed to have swarming characteristic. *P. aeruginosa* appeared large, round and convex in shape, rough colonies with creamy white or pale yellowish colour with opaque zones at the end having distinctive grape like odor. Different sugars were also used as

biochemical tests for *Proteus* spp and *P. aeruginosa*. Other tests included motility, coagulase and oxidase reactions (Quinn *et al.* 1994; Isenberg, 1998).

3.9.1.4.3 Detection of *Listeria* spp.

Identification of *L. monocytogenes* in milk samples was done by using ISO 11290-1:1996 protocols through the following stages:

Stage 1: Primary enrichment in a selective liquid enrichment medium

Test samples were primarily enriched in a selective liquid enrichment medium with reduced concentration of selective agents (Half Fraser Broth). Using a fresh sterile pipette 10 ml of the test sample was transferred into a sterile beaker containing 90 ml of Half Fraser broth and the inoculum was incubated at 30ºC for 24 hours.

Stage 2: Secondary enrichment in a selective liquid enrichment medium

Test samples were again enriched in a selective liquid enrichment medium with full concentration of selective agents (Fraser Broth). After 24 hours, using a fresh sterile pipette 0.1 ml of the culture obtained in stage 1 was transferred to a sterile test tube containing 10 ml of Fraser broth. The inoculated test tube was incubated at 37ºC for 48 hours (sub-culturing).

Stage 3: Plating out and identification

The cultures obtained in stage 1 and 2 above were further inoculated onto two different selective solid media which were Oxford and Colorex *Listeria* agars. Using a sterile loop the culture obtained in stage 1 (primary enriched culture incubated for 24 hrs) was inoculated onto the surfaces of Oxford and Colorex *Listeria* agar plates (both are already prepared plated media). The plates were inverted and incubated at 37ºC for 24 hours and

for an additional 24 hours (48 hours). The same procedure was repeated for the culture obtained in stage 2 (secondary enriched culture in the test tube incubated for 48 hrs). After incubation period the plates were examined for the presence of colonies presumed to be *Listeria* spp. Typical colonies of *Listeria* spp. grown on Oxford agar are small, brown-green to dark-brown surrounded by black halos due to hydrolysis of aesculin present in the medium. While for *L. monocytogenes* and *L. ivanovii* grown on Colorex *Listeria* agar are blue-green colonies with well-defined edges surrounded by opaque, white halos, as the medium contains lecithin substrate, which differentiates these bacteria from other *Listeria* spp.

By using a new sterile pipette for each dilution, the procedure was repeated as above with further dilutions up to the first dilution and for the remaining test samples, where only consecutive critical dilution steps were chosen for the inoculation on plates.

Stage 4: Confirmation of *Listeria*

Presumed *Listeria* colonies obtained in stage 3 each from Oxford and Colorex *Listeria* cultured agar plates were used for confirmation through the following biochemical tests:

1. Haemolysis Test: The Sheep Blood agar plates (already prepared plated media) were inoculated with cultures obtained in stage 3 to determine the hemolytic reactions. Using a sterile loop an isolated colony from each cultured plate was inoculated and stabbed on single space on the Sheep Blood agar plate. Also, a control culture was stabbed at the same time. The plates were inverted and incubated at 37ºC for 24 hours. After incubation period, the plates were examined for haemolysis in light and compared with controls. If β-haemolysis appeared it was considered a positive reaction.

2. CAMP Test: This term (CAMP test) describes the synergistic reaction of diffusible substances produced by microorganisms growing adjacent to each other on Sheep Blood

agar medium, which results in an enhanced zone of haemolysis in the medium. Using fresh and separate sterile loops, known cultures of *Staphylococcus aureus (S. aureus)* and *Rhodococcus equi* (*R. equi*) were streaked in single lines onto Sheep blood agar plate parallel and completely opposite to each other. Then, using other fresh and separate sterile loops, several presumed isolated test colonies or cultures were streaked in single lines on the same plate but at right angles to the two known cultures, so that the test cultures and known cultures (*S. aureus* and *R. equi* cultures) were about 1 – 2 mm apart. Parallel with the test samples, control cultures were streaked as well. The plates were inverted and incubated at 37ºC for 24 hours. After incubation period, the plates were examined for haemolysis. An enhanced zone of β-haemolysis at the intersection between the test culture and each of the known cultures was considered as a positive reaction.

3. Oxidase Test: The test was performed as described by Oxoid® Ltd., Basingstoke, Hampshire, England, Ref MB0266A, Lot 1284539.The presumed and well-isolated colonies were stickled and streaked onto the moistened oxidase detection strips using a sterile plastic loops, and then the strips were observed for colour change within 10 seconds. If the deep blue or purple colour appeared, was confirming a positive reaction.

4. *Listeria* Test kit: The *Listeria* Test kit (Oxoid® Ltd., Basingstoke, Hampshire, England, Ref DR1126A, Lot 1239689) use the principle of rapid latex agglutination test for the presumptive identification of *Listeria*. The test is performed within a drawn circle. Along with the test samples, positive and negative controls were prepared and used. For a positive control, a drop of *Listeria* latex reagent was mixed with a smooth suspension of the positive control antigen or known *Listeria* spp. on the reaction card and observed for agglutination within two minutes. As for a negative control, a drop of *Listeria* latex reagent was mixed with a drop of normal saline on the reaction card and observed for agglutination within two minutes.

For the test sample cultures, a drop of 0.85% isotonic saline was placed on the reaction card. Using a sterile loop the suspected colony was emulsified in a drop of normal saline and then, a drop of *Listeria* latex reagent was added to the saline suspension and mixed well with a clean sterile loop. Thereafter, the suspension mixture was examined for agglutination within two minutes along with positive and negative controls. Observation of agglutination it was an indication of positive reaction.

3.9.1.4.4 Detection and enumeration of Coagulase positive Staphylococci (CPS) (*S. aureus*)

Identification of *S. aureus* in milk samples was done by using ISO 6888-1:1999 protocols through the following stages:

Stage 1: Preparation of initial suspension and serial dilutions

The dilutions were prepared for initial suspensions as described in part 3.9.1.3.2.1 above.

Stage 2: Inoculation, incubation and isolation

Two sterile Baird-Parker (BP) agar plates (already prepared plated media) were removed from the refrigerator kept at room temperature and labeled prior to inoculation. Using a sterile pipette, 0.1 ml of the test sample was transferred from the last dilution to each of the two media plates. Then, a fresh sterile swab was used to spread the sample on the surface of the media. The plates were allowed to dry with their lids on for about 15 minutes. Thereafter, the plates were inverted and incubated at 37ºC for 24 hours. After 24 hours, all typical and atypical colonies present were counted and the plates were re-incubated at 37ºC for a further 24 hours (48 hrs). After that, again, all typical and atypical colonies present were counted. Typical colonies grown on BP agar are black or grey, shining due to reduced action of tellurite, convex shaped and surrounded by a clear zone. After incubation for at least 24 hrs, an opalescent ring (due to proteolysis) in contact with

colonies may appear in the clear zone. Atypical colonies are shining black colonies with or without a narrow white edge, the clear zone and opalescent ring are absent or hardly visible.

By using a new sterile pipette for each dilution, the procedure was repeated as above with further dilutions up to the first dilution and for the remaining test samples, where only consecutive critical dilution steps were chosen for the inoculation on plates.

Stage 3: Confirmation of CPS (*S. aureus)*

Confirmation of CPS was done by sub-culturing selected typical and/or atypical colonies obtained in stage 2 on BHI broth and by Coagulase test as biochemical test for identification of *S. aureus.*

1. Sub-culturing onto Brain-Heart Infusion (BHI) Broth: Using a sterile loop, an inoculum was picked from the surface of each selected colony and transferred into a sterile test tube containing 5 ml of BHI broth and incubated at 37ºC for 24 hours.

2. Coagulase Test: Coagulase is a protein enzyme produced by microorganisms, among them is *S. aureus.* The enzyme protease converts fibrinogen to fibrin resulting to blood clotting. The BBL Coagulase Plasma, Rabbit with EDTA was used for the test. From incubated test tube containing BHI broth, 0.1 ml of each test culture was aseptically added to 0.2 ml of the rabbit plasma in eppendorf tube and incubated at 37ºC for 4 – 6 hours. After the incubation period, eppendorf tubes were examined for clotting and if the test was negative, tubes were re-examined again at 24 hours of incubation. The test was considered to be positive if the clot occupied more than half of the original volume of the liquid. Parallel with test samples, controls were performed simultaneously. As a positive control,

0.1 ml of sterile BHI broth inoculated with a known *S. aureus* was added to 0.2 ml of rabbit plasma in eppendorf tube and incubated. Also, for a negative control 0.1 ml of sterile BHI broth was added to 0.2 ml of rabbit plasma and incubated without inoculation.

Stage 4: Counting and calculation of CPS

Counting of colonies: After the incubation period, plates containing less than 300 colonies at two successive dilutions were selected for counting. Manual counting was done and the condition was at least one of the plates must contain at least 15 colonies.

Calculation of CPS: For each plate, the number of identified CPS was calculated according to the following equation:

$$a = \frac{b_c}{A_c} \times C_c + \frac{b_{nc}}{A_{nc}} \times C_{nc}$$

Where; a = the number of identified CPS in plate,

A_c = the number of typical colonies submitted to the coagulase test,

A_{nc} = the number of atypical colonies submitted to the coagulase test,

b_c = the number of typical colonies, which have been shown to be coagulase positive,

b_{nc} = the number of atypical colonies, which have been shown to be coagulase positive,

C_c = the total number of typical colonies seen on the plate and

C_{nc} = the total number of atypical colonies seen on the plate.

From the two successive dilutions, the number of identified CPS present in the test sample was calculated using the same formula applied for the determination of total bacterial count (part 3.9.1.3.2.3 above) and expressed as colony forming units per millilitre (cfu/ml).

3.9.1.4.5 Detection and enumeration of Enterobacteriaceae

Identification of Enterobacteriaceae in milk samples was done by using ISO 21528-2:2004 protocols through the following stages:

Stage 1: Preparation of initial suspension and serial dilutions

The dilutions were prepared for initial suspensions as described in part 3.9.1.3.2.1 above.

Stage 2: Inoculation, incubation and isolation

Two sterile petri dishes were labeled prior to inoculation. About 10 ml of the Violet Red Bile Glucose (VRBG) agar at 44 - 47ºC was poured into each petri dish. Using a sterile pipette, 1 ml of the test sample was transferred from the last dilution to each of the two petri dishes. The inoculum and the medium were carefully mixed by rotating the petri dishes and allowed to solidify by leaving the petri dishes standing on the horizontal surface of the working bench. After solidification of the mixture, a covering layer of about 10 ml of the VRBG agar was added onto petri dishes to prevent spreading growth and to achieve semi-anaerobic conditions, and then allowed to solidify again. Thereafter, the plates were inverted and incubated at 37ºC for 24 hours. After incubation period, the plates were examined for typical and atypical colonies of Enterobacteriaceae. Typical colonies are pink to red or purple, with or without precipitation haloes or colourless mucoid colonies, with a diameter of 0.5 mm or more.

By using a new sterile pipette for each dilution, the procedure was repeated as above with further dilutions up to the first dilution and for the remaining test samples, where only consecutive critical dilution steps were chosen for the inoculation on plates.

Stage 3: Confirmation of Enterobacteriaceae

Confirmation of Enterobacteriaceae was done by sub-culturing selected typical and/or atypical colonies obtained in stage 2 and by biochemical tests as follows:

1. Sub-culturing of suspected colonies: Using a fresh and separate sterile loop, five suspected colonies were selected at random from each plate and streaked onto MacConkey agar for differentiation of Enterobacteriaceae. The plates were inverted and incubated at 37ºC for 24 hours. After 24 hrs, a well-isolated colony was selected and streaked onto Nutrient agar (NA) plates for biochemical confirmation. Again, the plates were inverted and incubated at 37ºC for 24 hours.

2. Biochemical Tests: Well-isolated colonies were selected from each NA plate and used. Oxidase and Glucose fermentation tests were performed on each selected colony as follows:

- **Oxidase Test:** The test was performed as described by Oxoid® Ltd., Basingstoke, Hampshire, England, Ref MB0266A, Lot 1284539. The presumed and well-isolated colonies were stickled and streaked onto the moistened oxidase detection strips using a sterile plastic loops, and then the strips were observed for colour change within 10 seconds. Appearance of deep blue or purple colour, was a confirmation for an oxidase positive reaction.

- **Glucose Fermentation Test:** Using sterile loops, selected colonies which were negative on Oxidase test were stickled and stabbed into tubes containing glucose agar and then, the tubes were incubated at 37ºC for 24 hours. After incubation period, the tubes were examined for colour change. If a yellow colour develops throughout the tube and sometimes with gas production, it was regarded as a positive reaction (Fig. 3).

Figure 3: Yellow tubes are positive reaction of Enterobacteriaceae for glucose fermentation test with production of gas while the purple tubes are negative reaction.

Stage 4: Counting and calculating the number of Enterobacteriaceae

Counting of colonies: After the incubation period, plates containing less than 150 characteristic/typical colonies were selected and counted manually.

Calculation of Enterobacteriaceae: From the two successive dilutions, the number of identified Enterobacteriaceae present in the test sample was calculated using the same formula applied for the determination of total bacterial count (part 3.9.1.3.2.3 above) and expressed as colony forming units per millilitre (cfu/ml).

3.9.1.4.6 Detection of *E. coli*

Stage 1: Culturing of milk samples

All test samples that showed positive bacterial growth during Enterobacteriaceae count and those suspected as *E. coli* colonies during detection of *Salmonella* were removed from the refrigerator, thawed at room temperature and used for detection of *E. coli*. Petri dishes with MacConkey agar media were labelled and divided into two equal halves. A sterile loop was dipped into a thawed milk sample and streaked onto MacConkey agar plates as a

differential media for identification of *E. coli*. Then, the plates were inverted and incubated at 37ºC for 24 hours. After incubation period, the plates were examined for typical and atypical colonies. Typical colonies of *E. coli* grown on MacConkey agar are dry, medium in size, pink in colour and appeared singular or in groups. Atypical colonies were small red colonies in singular or group form.

Stage 2: Sub-culturing of presumed *E. coli* colonies

The presumed well-selected typical and atypical colonies were again sub-cultured in the same media (MacConkey agar) and under the same conditions in order to get pure colonies of *E. coli.* After the next 24 hrs of incubation, well-isolated colony was selected and sub-cultured further onto Nutrient agar (NA) so as to be used for biochemical confirmation.

Stage 3: Confirmation of *E. coli*

Tests such as Gram staining and biochemical reactions like oxidase and indole tests were performed to well-isolated colony from nutrient agar plates to confirm the presence of *E. coli* in the test samples. Parallel with the test samples, controls were used which was known *Escherichia coli* (ATCC® 25922) and *Pseudomonas aeruginosa* (ATCC® 27853).

- **Gram staining technique:** The Gram staining of the bacterial colony was done on a sterile glass slide as described by (Cheesbrough, 2000). A drop of normal saline was placed on a glass slide and loop full of well-isolated bacteria colony was added and made a smear which was dried in air and fixed by gently flaming. A fixed smear was covered with crystal violet stain for about 2 minutes then, rapidly washed with slowly running tap water and again the smear was covered with Lugol's iodine for about 2 minutes and washed again with tap water. Thereafter,

acetone-alcohol was used to decolorize the fixed smear and washed for the third time. Then, the fixed smear was covered with counter stain neutral red that stayed for about 2 minutes then washed off with running tap water. The slide with smear was placed on a draining rack for the smear to dry. A drop of oil immersion was added on the smear and examined under the light microscope with 100X objective to visualize the morphology of the bacteria. Gram positive bacteria appeared spherical or cocci in shape with pale to dark purple colour while Gram negative bacteria appeared rod or coccobacilli with pale to dark red colour.

- **Oxidase Test:** The test was performed as described by Oxoid® Ltd., Basingstoke, Hampshire, England, Ref MB0266A, Lot 1284539. The well-isolated colonies were stickled and streaked onto the moistened oxidase detection strips using a sterile plastic loops, and then the strips were observed for colour change within 10 seconds. If the deep blue or purple colour appeared, was confirming an oxidase positive reaction.

- **Indole Test:** Peptone water was prepared and about 3 ml of it was dispensed in bijou tubes using a sterile pipette. Then, fresh sterile loops were used to pick a well-isolated colony of bacteria and inoculated into bijou tubes, thereafter, the tubes were incubated at 37ºC for 48 hours. After incubation period, 0.5 ml of Kovac's Indole Reagent (Loba Chemie Pvt. Ltd, Lot LM01131303) was added to the inoculated bijou tubes. The tubes were subjected to gentle shaking and examined for red colour in the surface layer within 10 minutes (Cheesbrough, 2000). A red ring on top of the tube (Fig. 4) indicated indole positive reaction.

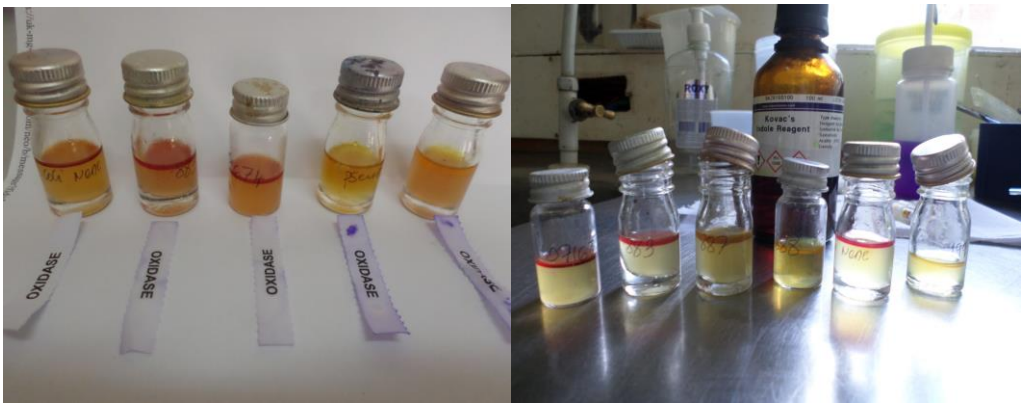

Figure 4: Red rings are positive reactions of *E. coli* to indole test and for oxidase tests blue or purple colour on test strips are positive reactions

3.9.2 Determination of antimicrobial susceptibility of common milk-borne bacteria

For assessment of antimicrobial susceptibility tests, the isolated milk-borne bacteria namely *L. monocytogenes, L. ivanovii, L. innocua, S. aureus*, *E. coli*, *Salmonella* spp., *P. aeruginosa* and *Proteus* spp. previously stored in 20% glycerol were incubated for sometimes to activate the microorganisms then, the inocula were streaked onto Nutrient agar (NA) plates, the plates were inverted and incubated at 37ºC for 24 hours. After 24 hours the microorganisms were tested for their susceptibility to a panel of seven commonly used antibiotics in veterinary and human practices. The discs used (Oxoid® Ltd., Basingstoke, Hampshire, England) were impregnated with antibiotics and their corresponding concentrations in brackets were as follows; Tetracycline (TE: 30 μg), Gentamycin (CN: 10 μg), Ciprofloxacin (CIP: 5 μg), Amoxycillin/Clavulanic acid (AMC: 30 μg), Nalidixic acid (NA: 30 μg), Ampicillin (AMP: 10 μg), and Amoxicillin (AML: 10 μg). These antibiotics were selected based on availability and usage at farm level.

Antibacterial susceptibility test was performed on Muller-Hinton (MH) agar by agar disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2007) guidelines. Well-isolated direct colonies were suspended into bijou tubes containing 2 ml

of sterile normal saline and the suspensions adjusted to a turbidity equivalent to a 0.5 McFarland standard using Vitek colorimeter (Lenexa, Kansas, USA). Sterile cotton-tipped swabs were then dipped into the suspensions to transfer the inocula onto MH agar plates and spread evenly on the entire surface to produce a confluent lawn of bacterial growth. After drying the plates with inocula for few minutes, antibiotic discs were placed over inoculated plates using disc dispenser (Oxoid® Ltd., Basingstoke, Hampshire, England) and sterile forceps. Thereafter, the plates were inverted and incubated under aerobic conditions at 37°C for 24 hours. After incubation period, the plates were examined for zones of inhibition around the discs. Diameters of inhibition zones around the discs were measured in millimeter (mm) using a metal caliper, and the results were recorded and classified as resistant (R), intermediate (I) and sensitive (S) according to the general guidelines prepared by (CLSI, 2007). Standard reference strains of *Staphylococcus aureus* (ATCC® 29213), *Escherichia coli* (ATCC® 25922) and *Pseudomonas aeruginosa* (ATCC® 27853) were used as quality control organisms in antimicrobial susceptibility determination.

3.10 Data Management and Analysis

Quantitative questionnaire data were analyzed using a logistic regression SAS interpretation. Descriptive statistics; particularly frequencies, percentages, means and counts from multiple responses analysis were used to determine distributions and magnitudes of variables among the respondents. Relationship between different practices as risk factors for microbial contamination in raw milk was computed against TBC and TCC and statistical significance was established at 95% confidence interval and critical p-value of 0.05.

Laboratory based data were entered in Microsoft Excel data sheet and imported to Epi Info™ Version 7 (Centre for Disease Control, Atlanta, USA) software for analysis. The Chi-square and confidence intervals was used to compare proportions at 5% level of significance. Descriptive statistics was used to compute means, standard deviations, median and range. Analysis of variance (ANOVA) was adopted to compare differences in means of continuous variables.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic Characteristics and Distribution of Respondents

The study involved 56 respondents who had lactating animals during the time of data collection and who had accepted to participate in the questionnaire interviews. Demographic information of the respondents is detailed in Table 1. The results show that most respondents (71.4%) were male with the age of 15-20 years and majority (50%) had no formal education. It was also established that most respondents (85.7%) were the owners of cattle who practiced extensive grazing system (78.6%). However, 69.6% of the respondents were agro-pastoralists while up to 53.6% of respondents purely depended on livestock as their sole source of income.

Table 1: Socio-demographic characteristics of respondents

Demographic information	Category	Number (%) of respondents in the study districts		
		Kilosa (n=32)	**Mvomero (n=24)**	**Total (N=56)**
Gender	Male	25 (78.1)	15 (62.5)	40 (71.4)
	Female	7 (21.9)	9 (37.5)	16 (28.6)
Age (years)	15 - 20	11 (34.4)	10 (41.7)	21 (37.5)
	21 - 30	6 (18.8)	3 (12.5)	9 (16.1)
	31 - 40	7 (21.9)	4 (16.7)	11 (19.6)
	41- 50	0 (0.0)	0 (0.0)	0 (0.0)
	> 50	7 (21.9)	6 (25)	13 (23.2)
	Don't know	1 (3.1)	1 (4.2)	2 (3.6)
Level of education	No formal education	18 (56.3)	10 (41.7)	28 (50.0)
	Primary school	11 (34.4)	11 (45.8)	22 (39.3)
	Secondary school	1 (3.1)	1 (4.2)	2 (3.6)
	College education	2 (6.3)	2 (8.3)	4 (7.1)
Ownership of cattle	Owner and herding cattle	26 (81.3)	22 (91.7)	48 (85.7)
	Employed as herder	6 (18.8)	2 (8.3)	8 (14.3)
Types of grazing systems	Extensive grazing	31 (96.9)	13 (54.2)	44 (78.6)
	Intensive/zero grazing	0 (0.0)	6 (25)	6 (10.7)
	Semi-intensive grazing	1 (3.1)	5 (20.8)	6 (10.7)
Practicing both animal and crop farming	Yes	20 (62.5)	19 (79.2)	39 (69.6)
	No	12 (37.5)	5 (20.8)	17 (30.4)
Cattle as the only income source	Yes	21 (65.6)	9 (37.5)	30 (53.6)
	No	11 (34.4)	15 (62.5)	26 (46.4)

4.2 Animal Health and Management System

In both districts animal health and management systems are similar as presented in Table 2. The result shows that 53.6% of animal floor is covered with manure since majority (64.3%) of livestock keepers leave the manure on floor of animal house. It was shown that 85.7% of livestock keepers graze animals on communal grazing where animals from different herds and sometimes including with wild animals come in contact. On communal grazing animals share water sources and pastures which can be one among the means of disease transmission. Furthermore, results showed that treatment and medication of animals is mainly (71.4%) performed by farmers themselves, although sometimes they call veterinarians, animal health worker or village extension officers and rarely seek advice concerning animal health care and management from veterinary officers.

Some of the common diseases that affect cattle were mentioned by respondents and included foot and mouth disease (FMD), trypanosomosis, East Coast fever, respiratory or pneumonia disease, lumpy skin disease (LSD), anaplasmosis, wounds, abortions, unspecified diarrhoea and mastitis. In both districts different types of veterinary drugs were observed to be used for treatment of animals, the few mentioned are oxytetracycline, penicillin dihydrostreptomysin (Pen-strep), penicillin, diminazene aceturate (Berenil®), buparvaquone, parvaquone, tylosin, albendazole and different kinds of acaricides like cypermethrin.

Table 2: Animal health and management systems practiced by respondents

Parameter assessed	Category	Number (%) of respondents in the study districts		
		Kilosa (n=32)	Mvomero (n=24)	Total (N=56)
Type of animal house floor	Covered with manure	20 (62.5)	10 (41.7)	30 (53.6)
	Concrete	0 (0.0)	7 (29.2)	7 (12.5)
	Earthed floor	12 (37.5)	7 (29.2)	19 (33.9)
Manure disposal	Use on own farm	8 (25.0)	12 (50.0)	20 (35.7)
	Left on floor of animal house	24 (75.0)	12 (50.0)	36 (64.3)
Type of grazing areas	Communal grazing	32 (100)	16 (66.7)	48 (85.7)
	Zero grazing	0 (0.0)	7 (29.2)	7 (12.5)
	Privately owned grazing area	0 (0.0)	1 (4.2)	1 (1.8)
Animals become in contact with other herds	Yes	27 (84.4)	16 (66.7)	43 (76.8)
	No	5 (15.6)	8 (33.3)	13 (23.2)
Animals become in contact wild animals	Yes	7 (21.9)	5 (20.8)	12 (21.4)
	No	25 (78.1)	19 (79.2)	44 (78.6)
Source of drinking water for animals	Local rivers	23 (71.9)	9 (37.5)	32 (57.1)
	Local wells or boreholes	9 (28.1)	10 (41.7)	19 (33.9)
	Tap water	0 (0.0)	5 (20.8)	
Supplementary feed	Yes	2 (6.2)	15 (62.5)	17 (30.4)
	No	30 (93.8)	9 (37.5)	39 (69.6)
Ability to recognize mastitis in animals	Yes	19 (59.4)	20 (83.3)	39 (69.6)
	No	13 (40.6)	4 (16.7)	17 (30.4)
Practicing tick control	Yes	32 (100)	23 (95.8)	55 (98.2)
	No	0 (0.0)	1 (4.2)	1 (1.8)
Routine vaccination and treatment of animals	Yes	7 (21.9)	10 (41.7)	17 (30.4)
	No	25 (78.1)	14 (58.3)	39 (69.6)
Who provide treatment and medication of animals	Veterinarian	7 (21.9)	6 (25)	13 (23.2)
	Animal health worker or village extension officer	0 (0.0)	3 (12.5)	3 (5.4)
	Farmers themselves	25 (78.1)	15 (62.5)	40 (71.4)
Seek advice on animal management from veterinary officers	Yes	2 (6.2)	5 (20.8)	7 (12.5)
	No	30 (93.8)	19 (79.2)	49 (87.5)

4.3 Milk Production and Possible Factors for Microbial Contamination of Raw Cow Milk at Farm Level

The results on milk production and possible factors for microbial contamination are summarized in Table 3. Milking process primarily is done mostly by women (69.9%) using hands in the animal house and by means of stripping (pulling the teats). The animal houses were always dirty full of cow dung or dusts. The people milking sometimes wash their hands with cold water and rarely wash the udder and/or teats. During the due process of milking, the person milking uses the milk as lubricant of the teats. Plastic containers (buckets) and calabashes are used during milking (Fig. 5). It was found that all the produced milk was stored in plastic containers, bottles, calabash and aluminium cans under room temperature before selling or other home uses. Generally, it was observed that the person involved with milking was not clean, the milking environments and utensils were also unhygienic. All these gave possibilities for microbial contaminations in the milk.

During data collection it was a dry season in the study areas; in both districts therefore, it was difficult to estimate the average milk production per day. In addition, calves were left to suck milk from the dams before milking commences. The obtained milk normally are used for consumption at household and some are sold to neighbours, community members, milk vendors and to the near-by milk processing plants.

Several practices undertaken at farm level as were depicted from the respondents were thought to predispose raw milk to microbial contaminations which included type of animal house floor, cleanliness of the animal house, milking sick animals, milking animals with udder problems, dirty milking personnel, not washing hands before milking, not washing udder and/or teats before milking, dirty milking utensils, use of poor quality water for

cleanliness (hands and milk equipments), milk storage under shade, not covering milk during storage, type of storage containers used and milk storage duration under room temperature (Table 3).

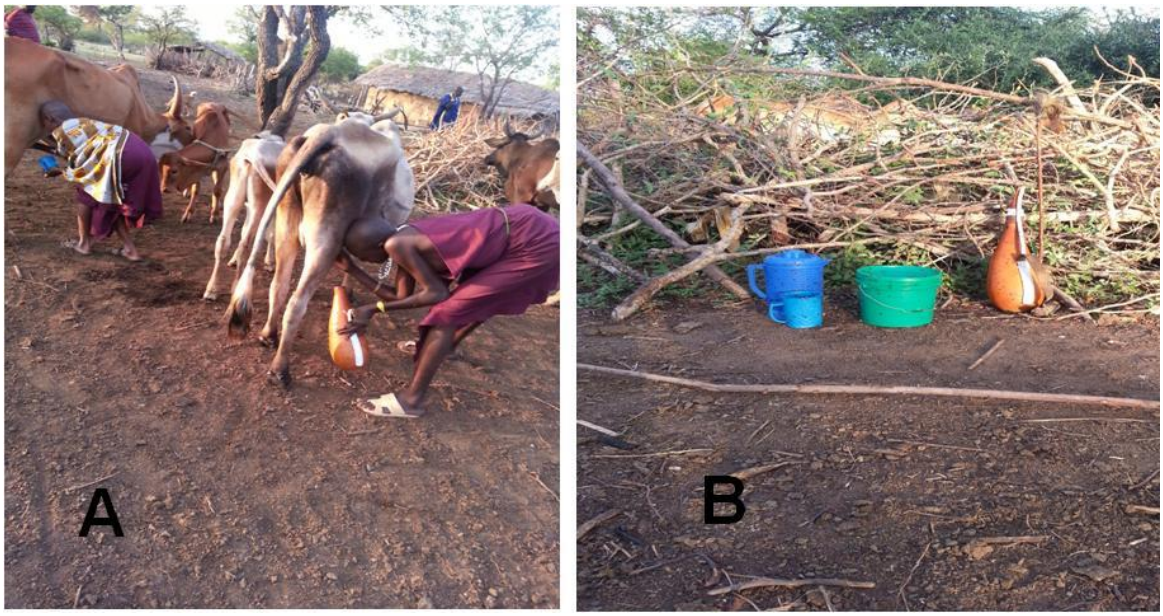

Figure 5: Practices that may predispose milk to microbial contaminations. Note that women doing hand milking of cows into a calabash and plastic cup in the dirty animal house (A) and milk storage containers used were calabash and plastic utensils (B).

Table 3: Practices that can predispose raw milk to microbial contamination at farm level (N=56)

Variable assessed	Category	Number (%) of respondents
Type of animal house floor	Covered with manure	30 (53.6)
	Concrete	7 (12.5)
	Earthed floor	19 (33.9)
Cleanliness of animal house	Dirty	53 (94.6)
	Clean	3 (5.4)
Milking sick animals	Yes	18 (32.1)
	No	38 (67.9)
Milking animals with udder problems	Yes	18 (32.1)
	No	38 (67.9)
Washing hands before milking	Yes	31 (55.4)
	No	25 (44.6)
Cleaning of teats before milking	Not cleaning	53 (94.6)
	Cleaning	3 (5.4)
Use water for cleanliness (hands and milk equipments)	Yes	56 (100)
	No	0 (0.0)
Milk storage under shade	Yes	39 (69.6)
	No	17 (30.4)
Covering of milk during storage	Not coverd	24 (42.9)
	Covered	32 (57.1)
Type of storage containers	Calabash	35 (62.5)
	Plastic bottles	18 (32.1)
	Glass bottles	1 (1.8)
	Metal cans	2 (3.6)
Milk storage duration under room temperature	< 2 hours	39 (69.6)
	Btn 2-6 hours	13 (23.2)
	Btn 6-12 hours	3 (5.4)

4.5 Use of Milk from Mastitis Animals and those under Treatment

The results indicated that all respondents reported udder disease (mastitis) as among the disease of cattle in their herds, different medications are given to such animals as has been indicated under 4.2 above. However, the milk from animals with udder problems was reported to be mostly used for feeding calves (75%). Similarly, milk from animals under medications was mostly given to calves (53.6%) but some relatively large number of respondents (33.9%) reported to consume the milk. Generally, majority of the farmers (96.9% in Kilosa district and 91.7% in Mvomero district) reported not to adhere to withdrawal periods after medications or treatments of animals since majority were even not aware about it. Also, some of the respondents were not aware of the associated health effects of antibiotic residues in milk.

Table 4: Use of milk coming from treated animals and habit of milking animals with udder problems

Parameter assessed	Category	Number (%) of respondents in the study districts			
		Kilosa (n=32)	Mvomero (n=24)	Total (N=56)	P-value
Milk from animals with udder problems	Feed calves	29 (90.6)	13 (54.2)	42 (75)	0.0001
	Consume	2 (6.3)	6 (25)	8 (14.3)	
	Discard	1 (3.1)	5 (20.8)	6 (10.7)	
	Sale	0 (0.0)	0 (0.0)	0 (0.0)	
Milk from treated animals	Feed calves	15 (46.9)	15 (62.5)	30 (53.6)	0.0001
	Consume	15 (46.9)	4 (40.6)	19 (33.9)	
	Discard	1 (3.1)	4 (12.5)	5 (8.9)	
	Sale	1 (3.1)	1 (4.2)	2 (3.6)	
Use of antibiotics and not adhering to withdrawal periods	Not adhering	31 (96.7)	22 (91.7)	53 (94.6)	0.9213
	Adhering	1 (3.1)	2 (8.3)	3 (5.4)	

4.6 Practices Related to Animal Management that may Predispose Small-scale Livestock Keepers to Infections

It was observed that some traditional ways practiced by respondents in the study area could predispose them to infectious agents from animals. Table 5, results indicate that majority of respondents assist animals during calving difficulties and other reproductive problems, and majority (87.5%) do not use protective gears during assistance of animals (Fig. 6). The reasons mentioned were that protective gears like gloves are not available, there is no problem with the touching of calving animal with its after birth and they are used to assist animals during calving with bear hands.

Furthermore, results showed that 76.8% of respondents consume raw milk while 78.6% consume milk products such as yoghurt, fermented milk, ghee, butter and cheese made from raw milk. The reasons mentioned by the respondents were that raw milk had no health problems, they have been using it for many years and for the fermented milk; respondents believed that fermentation process would kill microbes if the milk had microbial contaminations.

Table 5: Observed practices that may lead to health effects of respondents

Parameter assessed	Category	Number (%) of respondents in the study districts		
		Kilosa (n=32)	Mvomero (n=24)	Total (N=56)
Assisting animals during parturition	Yes	31 (96.7)	18 (75.0)	49 (87.5)
	No	1 (3.1)	6 (25.0)	7 (12.5)
Assisting animals with any reproductive problem	Yes	30 (93.8)	17 (70.8)	47 (83.9)
	No	2 (6.3)	7 (29.2)	9 (16.1)
Use of protective gears during assisted parturition/retained placenta	Yes	4 (12.5)	3 (12.5)	7 (12.5)
	No	28 (87.5)	21 (87.5)	49 (87.5)
Consumption of raw milk	Yes	31 (96.7)	12 (50.0)	43 (76.8)
	No	1 (3.1)	12 (50.0)	13 (23.2)
Comsumption of milk products made from raw milk	Yes	30 (93.8)	14 (58.3)	44 (78.6)
	No	2 (6.3)	10 (17.9)	12 (21.4)

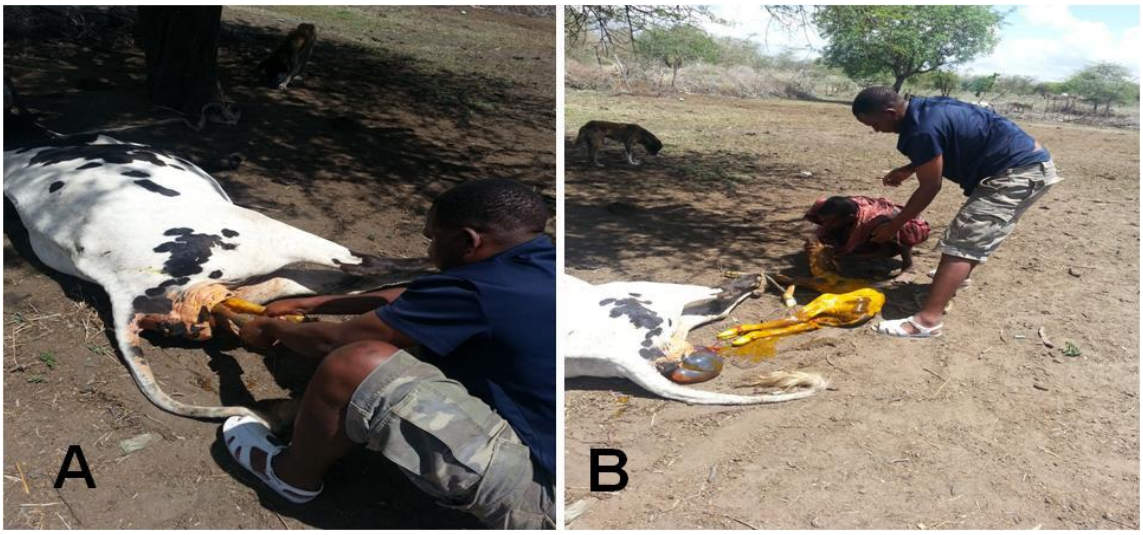

Figure 6: Livestock keepers assisting cow during parturition. Note that a livestock keeper is assisting to pull out the calf with bare hands (A). In (B) the livestock keepers are managing the calf and the placenta with bear hands.

4.7 Microbiological Quality of Raw Cow Milk

4.7.1 Total Bacterial Counts

A total of 56 milk samples from Kilosa and Mvomero districts were cultured for total bacterial count (TBC). It was found that 87.5% (n=49) of the samples had bacteria growth while seven samples (12.5%) had no growth and these were omitted in the subsequent data analysis. The results showed that the TBC ranged from 2.73 x 10^4 to 1.66 x 10^8 cfu/ml. Mean TBC was (2.41±4.1) x 10^7cfu/ml which is higher than the maximum recommended level of 2.0 x 10^6 cfu/ml (EAS 67:2007). The overall results indicated that 85.7% of all the milk samples handled had higher TBC than the maximum recommended level of 2.0x10^6cfu/ml as given by East Africa Community (EAC) standards. This implied that, raw cow milk from both districts had poor microbiological quality. Comparison of TBC between districts showed that more milk samples from Kilosa district had higher TBC (94.7%) than those which were sampled from Mvomero district (80%). However, the difference was not statistically significant (P = 1.0352).

4.7.2 Enumeration of Enterobacteriaceae

A total of 49 milk samples (those used in the TBC analysis) were cultured for Enterobactiaceae count and later for isolation of *E. coli*. Out of those, 21 (42.9%) milk samples showed growth of Enterobactiaceae and the mean value was calculated based on that. The results showed that the total coliform count (TCC) ranged from 0.0 to 4.0 x 10^7 cfu/ml. The mean value was (4.89±9.29) x 10^6 cfu/ml which was significantly higher ($p = 0.0328$) than the maximamun recommended level of 5.0 x 10^4 cfu/ml (EAS 67:2007).

4.7.3 Target bacteria isolates in the milk samples

A total of 49 milk samples that showed bacterial growth during total bacterial count were cultured with the purpose of isolating *Staphylococcus aureus, Salmonella*, *E. coli* and *Listeria monocytogenes*. The results showed isolation rate of *Staphylococcus aureus* (5/49), *Salmonella* spp. (1/49), *E. coli* (2/49) and *Listeria* spp. (35/49). Three species of *Listeria* were identified namely *L. monocytogenes (*62.9%), *L. innocua* (22.9%) and *L. ivanovii* (14.3%). Other bacteria isolated were *Proteus* spp. (1/49) and *Pseudomonous aeruginosa* (1/49). From the results it was further observed that 10.2% of milk samples had more than one target bacteria.

4.8 Antimicrobial Susceptibility test of Isolated Milk-borne Bacteria

A total of 45 bacterial isolates from milk culture samples that were confirmed positive for *Staphylococcus aureus* (n=5), *Salmonella* spp. (n=1), *E. coli* (n=2), *Listeria* spp. (n=35), *Pseudomonous aeruginosa* (n=1) and *Proteus* spp. (n=1) were subjected to antimicrobial susceptibility test against seven antibiotics from different antibiotic classes that are used for veterinary and human health practices.

From the results (Table 6), it was observed that all bacteria isolated were resistant to ampicillin but were all sensitive to gentamycin. Furthermore, results showed that 95.6% of bacterial isolates were resistant to amoxycillin, followed by nalidixic acid (80%), amoxycillin/clavulanic acid (31.1%), tetracycline (24.4%) and one (2.2%) isolate was resistant to ciproflaxicin only.

Table 6: Resistance patterns of isolates to antibiotics

Bacteria isolated	**Number (%) of resistant bacteria**						
	TET	**CIP**	**GEN**	**NA**	**AMC**	**AMP**	**AML**
S. areus (n=5)	5 (100)	0 (0.0)	0 (0.0)	5 (100)	5 (100)	5 (100)	5 (100)
Salmonella spp. (n=1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	1 (100)
E. coli (n=2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	2 (100)	2 (100)
L. monocytogenes (n=22)	5 (22.7)	0 (0.0)	0 (0.0)	17 (77.3)	4 (18.2)	22 (100)	20 (90.9)
L. ivanovii (n=5)	0 (0.0)	0 (0.0)	0 (0.0)	5 (100)	0 (0.0)	5 (100)	5 (100)
L. innocua (n=8)	1 (12.5)	1 (12.5)	0 (0.0)	8 (100)	1 (12.5)	8 (100)	8 (100)
P. aeruginosa (n=1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	1 (100)	1 (100)	1 (100)
Proteus spp. (n=1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	1 (100)	1 (100)
Total (N=45)	11 (24.4)	1 (2.2)	0 (0.0)	36 (80)	14 (31.1)	45 (100)	43 (95.6)

Key: TET: Tetracycline, CIP: Ciprofloxacin, GEN: Gentamycin, NA: Nalidixic acid, AMC: Amoxycillin/Clavulanic acid, AMP: Ampicillin, AML: Amoxycillin.

Multi-drug resistance (MDR) was also observed on bacterial isolates (Table 7), whereby 2 (4.4%) isolates were resistant to two antibitics and 41 (91.2%) were resistant to more than two antibiotic drugs. In addition results showed that 2 (4.4%) isolates were resistant to only one antibiotic.

Table 7: Multi-drug resistance patterns of isolates to antibiotics

Antibiotic resistance patterns	Total (N=45)	Bacterial isolates (%)							
		S. aureus (n=5)	*Salmonella* spp. (n=1)	*E. coli* (n=2)	*L. monocytogenes* (n=22)	*L. ivanovii* (n=5)	*L. innocua* (n=8)	*P. aeruginosa* (n=1)	*Proteus* spp. (n=1)
AMP	2 (4.4)	0 (0.0)	0 (0.0)	0 (0.0)	2 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AMP/AML	2 (4.4)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (20)	0 (0.0)	0 (0.0)	0 (0.0)
TET/AMP/AML	1 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
NA/AMPL/AML	24 (53.3)	0 (0.0)	0 (0.0)	0 (0.0)	13 (59.1)	4 (80)	7 (87.5)	0 (0.0)	0 (0.0)
AMC/AMP/AML	4 (8.9)	0 (0.0)	0 (0.0)	2 (100)	1 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)
TET/NA/AMP/AML	2 (4.4)	0 (0.0)	0 (0.0)	0 (0.0)	2 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
NA/AMC/AMP/AML	2 (4.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)
TET/AMC/AMP/AML	1 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TET/NA/AMC/AMP/AML	6 (13.3)	5 (100)	0 (0.0)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TET/CIP/NA/AMC/AMP/AML	1 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)

Key: TET: Tetracycline, CIP: Ciprofloxacin, GEN: Gentamycin, NA: Nalidixic acid, AMC: Amoxycillin/Clavulanic acid, AMP: Ampicillin, AML: Amoxycillin.

CHAPTER FIVE

5.0 DISCUSSION

The overall purpose of this study was to assess the bacterial quality of raw cow milk and determine antimicrobial susceptibility of the selected common milk-borne bacteria isolated in Kilosa and Mvomero districts in Morogoro region. This was due to the fact that milk produced in Tanzania by the informal sector is not regulated by any agency and such milk may pose a health hazard due to contamination with pathogens. Generally, findings showed that, there are several practices undertaken at farm level such as type of animal house floor, not washing hands and udder/teats before milking, milking sick animals and those with udder problems, water used for cleanliness (hands and milk equipments), type of storage containers used and milk storage duration under room temperature predispose raw milk to microbial contaminations. Apart from that, it was observed that there are traditional ways practiced by small-scale livestock keepers which includes consumption of raw milk and milk products, assisting calving and other reproductive problems without using protective gears predispose livestock keepers to health risks though they believe that there is no problem with such practices. Bacteriologically, high number of milk samples handled had higher TBC than the maximum recommended level given by EAC standards which implied that raw cow milk from both districts had poor microbiological quality. For the first time in Tanzania, this study isolated *Listeria* species (71.4%) in particular *L. monocytogenes* (62.9%) in raw cow milk that suggested existance of the bacteria that has been causing unnoticiable milk-borne diseases. The other bacteria isolated were *S. aureus*, *E. coli*, *Salmonella* spp., *P. aeruginosa* and *Proteus* spp. The isolated bacteria showed resistance to many antibiotic tested with multi-drug resistance being common implying indiscriminate use of antibiotics to the extent of developing resistant bacteria strains.

5.1 Sociological Study

The current study established that most of the cattle were extensively grazed as it was expected and majority of the people purely depended on livestock as their sole source of income. A study conducted by Karimuribo *et al*. (2005) in Dodoma rural and Mvomero districts reported the same that most pastoralist depends solely on livestock keeping for their livelihood. It is common to most pastoral and agro-pastoral communities to extensively graze animals in communal grazing where they share pastures and water sources for drinking with different herds, a practice which predisposes livestock vectors especially ticks and diseases. This was exemplified by a number of livestock keepers reported to use acaricides in the control of ticks but still tick-borne diseases like East Coast fever were common. Indeed, Maasai and Sukuma pastoralists own big herds of cattle that sometime shift away from their settlement to remote areas together with their animals in search for grazing areas and water for their animals "*transhumance"*. In so doing, their animals come in contact with other domestic and wild animals which may again facilitate acquisition of diseases.

This study further observed that most small-scale livestock keepers managed their cattle in dirty animal houses that are full of cow dung and may have implications on sources of pathogens for mastitis and other diseases to animals. Meanwhile, such dirty environments are also likely to be sources of milk contaminations. Similar observations have been reported by Shija (2013) in Tanga and Bukuku (2013) in Arusha, Tanzania. Under traditional livestock keeping system, it is somehow a challenge to have clean animal houses. During the survey, it was noted that accumulation of cow dung in animal houses was a tradition of pastoralists. Interestingly, direct physical observation realized that even the farmers themselves were also not clean, even their household utensils and home environments. These also included persons involved with milking activities, unhygienic

milking environments and milking utensils. All these possibilities predisposed milk to microbial contaminations at household level.

It was realized that a number of veterinary drugs are used but were dominated by antimicrobials in particular antibiotics being mostly administered by farmers themselves. With this in mind, proper indication, dosage and route of administration of drugs may be questionable. This habit predominates in many pastoralists and agro-pastoralists communities as it was also reported by Karimuribo *et al*. (2013) and Shija (2013) in Morogoro and Tanga regions respectively. The reasons for this observed practice could be due to ignorance on drug use, limited veterinary and/or extension services and uncontrolled availability of veterinary drugs in livestock markets without any restrictions. Furthermore, majority of the farmers rarely seek consultancy concerning animal health care and management from veterinary officers. Similar observation was reported by Karimuribo *et al*. (2005; 2013) and Shija (2013) in farmers from other parts of Tanzania.

It was further found that factors that were likely sources of microbial contamination in milk include hand milking in a dirty animal house, unclean milking utensils in particular calabash, not washing udder and/or teats before milking, milking sick animals especially those with udder problems, reckless milking personnel, not washing hands before milking, use of poor quality water for cleanliness (hands and milk equipments), not covering milk after milking and prolonged milk storage under room temperature. It was observed that majority of farmers do not comply with good milking practices and general sanitation. The general hygiene at milking time is known to affect the numbers of microorganisms in the milk. It is recommended that before milking, the animal house should be cleaned; the udder and/or teats should be washed and dried. The personnel and the equipments should be clean. Indeed, the hand milking practiced by animal attendants could result in microbial

contamination of the milk. These practices could have contributed to the observed high microbial load in the milk. Previous studies in Tanzania had similar observations (Karimuribo *et al*., 2005; Mdegela *et al*., 2009; 2013; Swai and Schoonman, 2011; Shija, 2013).

Surfaces such as milking equipments and hands coming in contact with milk if not clean enough may cause milk contaminations. During the current study, calabashes and plastic containers were the major utensils for collection and storage of milk. Narrow necked calabash containers which were commonly used are not easily washed especially in the bottom and inner corners thus may lead to sticking of milk residues. In such a situation, microorganisms can rapidly build up in potentially nutritious milk residues of storage containers consequently contaminating the milk on subsequent uses. Similar observations were also reported by Kivaria *et al.* (2006a); Bukuku (2013) and Shija (2013) who observed high microbial load in milk which was correlated with narrow necked plastic containers used in handling of milk.

Animal diseases like mastitis are sources of high microbial load that shorten shelf life of milk. During this study, farmers reported occurrences of animal diseases including mastitis that apart from causing high microbial load in the milk, disease increases the use of veterinary drugs that may lead to veterinary drug residues in milk. Worse enough some livestock keepers reported to use raw cow milk from animals that are under treatment and/or sick animals including those with mastitis. A study by Kivaria *et al.* (2006a) reported high microbial load in milk from mastitic animals in Dar es Salaam region of Tanzania. However, in contrast with this current study, a study by Mosalagae *et al*. (2011) in Zimbabwe reported that most farmers interviewed dispose-off milk coming from sick cows. Differences in results may be due to differences in levels of knowledge about

animal diseases and the likely consequences that may emanate from consumption of contaminated milk. Therefore, animal disease preventive measures should be instituted to farms to control diseases like mastitis in milking herds and a continuous public health education to the community.

Moreover, majority of the farmers reported not to adhere to drug withdrawal periods after medication or treatment of animals because of unawareness on drug residues and the associated health effects. This has a serious impact since the consumers keep on ingesting low doses of drugs like antibiotics in form of residues leading to development of antibiotic resistant strains as a consequence. Other human health problems that may result due to exposure to drug residues like antibiotic include allergic reactions in sensitive people, toxicity, and carcinogenic effects (Shitandi, 2004; Kurwijila *et al.*, 2006; Katakweba *et al.*, 2012). Other studies in Tanzania (Katakweba *et al*. 2012; Bukuku, 2013) reported high level of awareness on drug withdrawal periods with farmers though sometimes they did not comply with it. Non compliance to withdrawal periods being related to fear of losses from disposal of milk and milk products. This lack of knowledge could be contributed by low level of education observed to most of respondents in this current study. Others include poor knowledge on animal husbandry, inadequate information of different issues related to human and animal health services due to remoteness.

It was further realized that a number of practices related to animal managements and eating habits could predispose the livestock keepers to zoonotic infections. These practices included assisted calving, removal of placenta with bare hands, consumption of raw milk and milk products made from raw milk. The reasons could be the same lack of knowledge as described above. Elsewhere despite of livestock keepers being aware of the risk of contracting zoonotic infections and milk-borne diseases, the general public still consume

raw milk (Shirima *et al*., 2003; Karimuribo *et al*., 2005; Mosalagae *et al*., 2011). A study by Shirima *et al*. (2003) highlighted several zoonotic diseases that are common in pastoral, agro-pastoral and smallholder dairy which include tuberculosis, brucellosis, anthrax and FMD. Therefore, more public health education is needed at different levels along the food production chains (farmers, transporters, processors and consumers) to safeguard the public from health problems emanating from animals.

5.2 Microbiological Quality of Milk

Total bacterial count was used as an important indicator of the microbial quality of the raw milk. From the results of this study, it was found that the majority (85.7%) of the milk samples had higher TBC than the maximum recommended level of 2.0 $x10^6$cfu/ml as given by East Africa Community standards (EAS 67:2007), suggesting unfitness for human consumption especially for those with habit of consuming raw milk and milk products made from raw milk. The implication from these results is that, raw cow milk from both Kilosa and Mvomero districts is of poor microbial quality. Presence of high total bacterial load in raw milk indicates contamination possibly from lactating cows, milking equipments, storage containers, unsatisfactory hygiene/sanitation practiced at farm level, unsuitable storage condition, unclean udder and/or teats, poor quality of water used for cleanliness and dirty hands of milkers. Generally, it further indicates the degree level of hygiene practices in the whole milk production process and reflects the time elapsed since milking and/or processing at ambient temperature (Bukuku, 2013; Shija, 2013). From the observed practices involved in the whole chain of milk production, handling, storage, local processing and consumption, during this study the observed high TBC was expected. The results in this current study are inline with those done in raw milk by Karimuribo *et al*. (2005); Kivaria *et al.* (2006a) and Schoder *et al.* (2013) in Tanzania and elsewhere e.g. Al-Tahiri, (2005) in Jordan, Parekh and Subhash (2008) in India and

Addo *et al.* (2011) in Ghana which reported higher bacterial count above recommended level by standards in most of the samples that were tested . Therefore, based on these results and for the health safety of consumers, more food safety education should be given to producers, handlers and consumers. It is also emphasized that raw milk should be treated either by boiling before being consumed.

Interestingly, bacteria in the family Enterobactiaceae was detected in 42.9% of the milk samples analysed. It is known that presence of Enterobactiaceae in particular coliforms suggests faecal contamination which is normally associated with poor hygiene in milking and milk handling as has been discussed before. The mean total coliform count (TCC) found was (4.89±9.29) x 10^6 cfu/ml which was significantly ($p < 0.05$) higher than the maximamun recommended level of 5.0 x 10^4 cfu/ml (EAS 67:2007). Nevertheless, *E. coli* as the faecal coliforms was detected only in two milk samples (9.5%) out of 21 positive samples. This is contrary to several reports which reported high *E. coli* as coliform bacteria in Tanzania (Mdegela *et al*., 2009; Bukuku, 2013; Shija, 2013) and elsewhere (Al-Tahiri, 2005; Parekh and Subhash, 2008). The results are inline with other studies (in Tanzania) which also showed small percent like 4.1% reported by Kivaria *et al.* (2006b) and 6.3% by Karimuribo *et al*. (2005) and Kivaria *et al.* (2006a) and that of 2.1% in Ghana by Donkor *et al.* (2007). Although *E. coli* is an indicator bacteria of faecal contamination and was detected in small rate in this study the observation can not be ignored since there are several strains known to be highly pathogenic with a potential of causing illness to consumers. Some strains of *E. coli* are verocytotoxigenic like enterohaemorrhagic type of *E. coli* O157:H7 that are known to cause haemorrhagic colitis (John *et al.* 2001; Leclerc *et al.,* 2002). A study by Schoder *et al.* (2013) reported occurence of *E. coli* O157:H7 in raw milk from traditional cattle farms in Tanzania. A similar study by Lupindu (2014) isolated a highly pathogenic *E. coli* O157:H7 in cattle manure in Morogoro urban and peri-urban

areas. *Escherichia coli* O157:H7 is known to cause a deadly diarrhea in humans and consumption of contaminated raw milk is reported to be among important routes of transmission of these pathogenic bacteria (Kivaria *et al*., 2006a; Bukuku, 2013). Therefore, observation of *E. coli* in milk is a serious public health falt and need prompt attention in correcting the problem.

Surprisingly, for the first time in Tanzania, *Listeria* spp. (71.4%) was isolated in raw milk in this study being dominated by *L. monocytogenes* which is a pathogenic bacterium. The three species of *Listeria* isolated were identified as *L. monocytogenes*, *L. innocua* and *L. ivanovii*. The reasons for this high prevalence of *Listeria* spp. in the study area could be due to unhygienic practices during milking and poor milk handling. Also it could be due to environmental contamination with feacal and infected animal wastes as the results showed that the animal house floor and milking procedures was done in unhygienic manner. A study by Schoder *et al.* (2013) in regions around Dar es salaam and Lake Victoria, Tanzania did not isolate pathogenic specie *L. monocytogenes* in tested raw milk samples. It is known that the source of *L. monocytogenes* in raw milk is mostly the gastrointestinal tract of animals and the environment, skin of the teats, in particular (Harvey and Gilmour, 1992; Sanaa *et al.*, 1996). Shedding of *Listeria* into milk due to chronic mastitis (O'Donnell, 1995) is less frequent. The findings from this study are similar to previous studies that reported presence of *Listeria* spp. in raw milk elsewhere such as Nigeria (Yakubu *et al.*, (2012), India (Sharma *et al.*, 2012) and in Egypt (AlAll *et al.*, 2012). Therefore, the presence of *Listeria* spp. in raw milk is a public health concern because of the ability of *Listeria* spp. to survive even in different milk processing methods to the final product. The uniqueness of *Listeria* spp. to grow up at a low temperature, survive osmotic stress and mild preservation treatment indicates potential risk of milk-borne infection to

consumers. Sharma *et al.* (2012) reported *L. monocytogenes* as a milk contaminants, and a threat to consumers and the general public (AlAll *et al.*, 2012).

Several other bacteria were detected in the milk samples though at low isolation rate such as *S. aureus* (5/49), *Salmonella* spp. (1/49), *Proteus* spp. (1/49) and *P.s aeruginosa* (1/49). The possible sources of these bacterial contaminations in milk could be mastitic cows. Many studies conducted in different areas implicated *S. aureus* as the common mastitis causing organism in lactating cows (Kivaria *et al*., 2006a; Mdegela *et al*., 2009). According to Kivaria *et al*. (2006a), consumption of milk contaminated with *S. aureus* can be a health hazard because the main threat is based on the fact that about 10% of mastitis staphylococci are known to be producers of enterotoxins which are heat stable toxins. Some reports have associated *S. aureus* with gastroenteritis through these entorotoxins (Bukuku, 2013). Since *S. aureus* are contagious and common colonizer of teat end and teat canal, the use of therapy such as dry cow and post-milking teat disinfectants can be of great value in controlling the mastitis disease in lactating cows (Mdegela *et al*., 2009). Unfortunately, these control measures were not observed to be used by most of small-scale livestock keepers in the study area.

Other bacteria isolated in this present study such as *Salmonella* spp., *Proteus* spp. and *P. aeruginosa* also have been reported to occur in raw milk in Tanzania and elsewhere (Al-Tahiri, 2005; Karimuribo *et al*., 2005; Kivaria *et al.*, 2006b; Donkor *et al.*, 2007; Schoder *et al.*, 2013). *Salmonella* species are important bacteria known to cause food poisoning through consumption of contaminated milk and milk products (Al-Tahiri, 2005; Kivaria *et al*., 2006a). As described earlier that presence of these contagious pathogens in developing countries may be related to poor animal houses and poor milking hygiene practices by most small-scale livestock keepers (Karimuribo *et al*., 2005; Mdegela *et al*., 2009; Sharma

et al., 2011). *Pseudomonas* spp. is also a known causative of chronic mastitis in animals and may be shedded in milk. This study isolated *Pseudomonas* spp. in 9.5% of the milk samples suggesting that they had come from mistitic cows. Furthermore, from the results it was observed that 10.2% of milk samples had more than one target bacteria. Isolation of these pathogenic bacteria from milk samples must be regarded as a public health hazard. Therefore, emphasis on the importance of proper boiling or pasteurizing milk has to be put to the general public .

5.3 Antimicrobial Susceptibility Test of Isolated Milk-borne Bacteria

From the results of this study it was found that many bacteria isolates were resistant to all or most of the commonly used antibiotics. This finding potentially implies a serious problem. The possible reason for this high prevalence of bacterial resistant could be due to indiscriminate uses of antibiotics. Penicillin, penicillin-streptomycin, ampicillin and oxytetracycline for example were observed to be frequently used antibiotics. This indiscriminate use of antibiotics might be linked with low knowledge on animal health and husbandry, limited extension services and rampant uncontrolled availability of antibiotics even in livestock markets. Other reasons for development of resistant bacteria to antibiotic could be inappropriate use of the antibiotics in cattle, wrong dosage and routes of administration, arbitrary drug combinations and the acquisition of mobile genetic characteristics (Katakweba *et al.,* 2012; Sharma *et al.,* 2012; Yakubu *et al.,* 2012). Other studies have reported a number of resistant bacteria to commonly used antibiotics in livestock production in Tanzania (Mdegela *et al.,* 2004; Nonga and Muhairwa, 2009; Katakweba, 2014). Elsewhere Yakubu *et al.* (2012) reported high resistance rates in bacteria isolates to different kinds of antibiotics used in animals in Nigeria. The presence of antimicrobial resistant pathogens has an important public health implication especially

in developing countries like Tanzania where there is a widespread and uncontrolled use of antibiotics among livestock keepers.

Furthermore, multi-antibiotic resistance (defined as lack of susceptibility to at least two antibiotics from different classes) was also observed in several bacteria isolates with 91.2% of the bacterial isolates showing multi-antibiotic resistance patterns. This means that many of the antimicrobial agents that are used in livestock production are not suitable any more. There are several factors which might account for the observed multi-antibiotic resistance, this include antibiotic concentration, long-term exposure, organism type, antibiotic type and host's immune status. Nevertheless low-level, long-term exposures to these antibiotics remain to have a greater selective potential for development of resistance than short-term, full-dose therapeutic use (Shitandi, 2004). Other factors relate to under-dosing, incomplete treatment of animals and/or the long period of inappropriate use of antibiotics, since in Tanzania these are dispensed without a prescription (Kivaria *et al.*, 2006b; Katakweba *et al.*, 2014). Generally, antimicrobial resistances to animals are influenced by dosage being given (low/high), timing, frequency of use, type and frequent change of types of antibiotics. Therefore, based on the findings that majority of small-scale livestock keepers has tendency of treating animals themselves and rarely seek advices from veterinary or extension officers; the inappropriate use of veterinary drugs increases the risk of resistant bacteria in herds, which do not respond well to the antimicrobial agents in use and this will lead to chronic diseases.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the findings of this study it is concluded that:

1. Milk produced by small-scale livestock keepers from Kilosa and Mvomero districts are of poor quality, hazardous for human consumption and can be a potential source of milk-borne infections.
2. Poor milking procedures, milk handling practices including the surrounding environment and treatment practices has greater influence on the microbial contamination of raw milk and contributes to zoonotic pathogens.
3. Consumption of raw milk and milk products made from raw milk can result into health problems. This is supported by evidence of pathogenic bacteria isolated in this study. This raises a public health concern about safety of milk to consumers.
4. Paucity of veterinary or extension services and remoteness of small-scale livestock keepers contributes to low flow rate of information or knowledge about good animal health and management systems.
5. The study observed high resistance rate shown by some bacteria to commonly used antibiotics in livestock which was likely due to indiscriminate uses.

6.2 Recommendations

Based on the conclusions above, it is therefore recommended that:

1. Routine assessment of milk quality produced by small-scale livestock keepers and consumed by the general public has to be mandatory in order to safeguard the public from milk-borne zoonotic infections which may radiate through consumption of unsafe milk and milk products.

2. Strictly hygienic measures should be applied during milking and milk handling practices, achievable by educating small-scale livestock keepers especially pastoralist and agro-pastoralist communities on good animal husbandry practices.
3. The behaviour of consuming raw milk and milk products made from raw milk should be discouraged. Milk stakeholders have to play their roles in educating the general public on likely public health consequences associated with such behaviour.
4. Veterinary and/or extension officers and associated stakeholders have to make periodic surveillance visit to small-scale livestock keepers and create awareness, advice or conduct training on good animal health and management systems. Also, habit of treating animals by farmers themselves should be strongly discouraged so as to minimize exposure of milk consumers to antibiotic residues.
5. More research work has to be conducted in different parts of Tanzania with the aim of quantifying the magnitude of *L. monocytogenes* as it may be present in small-scale livestock keepers' communities and developing resistance to antimicrobial agents.

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APPENDICES

Appendix 1: Informed consent form for farmers

Farm code:……

Written Informed Consent "What is Killing My Cow?"

Information to be explained to participants

Hello, my name is _____ and my assistants' names are_____. We are from the Sokoine University of Agriculture (SUA) and the International Livestock Research Institute (ILRI - Kenya). We want to talk to you about why we are here today and ask if you would like to participate in our study. Please feel free to stop us and to ask us questions at any time.

Through discussions with farmers in Tanga and Morogoro regions over the last year, we have found that farmers would like to know what diseases are affecting cattle. There are many sicknesses that cause cattle to get skinny, produce less milk and sometimes die. Some of these diseases are preventable if vaccines are used and some can be treated. I is important to know what diseases are affection Tanzanian cattle, so that government services and development groups can prevent and treat them.

Today, we are inviting you to participate in a study to find out what diseases are affecting your cattle. We would like to ask some questions about your farm and your animals. We will also examine your farm and your animals in several ways. We would like to look at your cattle from a distance and more closely and we would like to take blood and milk samples from one, two or three of them. Milk samples will be processed at SUA in Morogoro and ILRI in Nairobi and blood will be processed at one laboratory in Germany and at ILRI, in Nairobi.

This study is funded by various sources, including the Irish Department of Foreign Affairs and Trade (IrishAid), the Germany Society for International Cooperation (GIZ) and the Consultative Group on International Agricultural Research (CGIAR).

If you wish to participate, we ask that you identify for us one to three animals you feel are unwell, to be examined closely and to collect blood and milk samples. The rest of the herd will only be examined at a distance.

We will need to restrain these animals. Firstly we will take a milk sample from individual animals and later from the household. We may need to use ropes to lower some animals to the ground. We will then take a blood sample from the neck vein. We will make every effort to be very clean and only cause very minimal discomfort. In this way, there is very little risk to your animals. We should only need to spend half an hour on your farm.

With the assistance of the veterinarians in the team, we will give you information about any diseases your cattle might have today. We would also like you to tell us how you most like to find out information about cattle keeping generally and when we combine all of the results from Tanga and Morogoro, we will make every effort to bring the information back to you, in the way most people prefer. This should occur in the next 12 months.

When we take the samples, we will give them a number and nobody will be given the results in a way that will identify you. The combined results of Tanga and Morogoro will be stored in such a way that no farmer will be identified. Other researchers and government bodies might look at the forms, to ensure we conduct the study properly. However, results will be kept private, according to the law. The information we get will be written in published studies but all personal details will be removed.

If you decide not to participate today, you will not be disadvantaged in any way. If you participate, you will not receive any money but you will have one veterinarian look at your animals and give some advice today, without having to pay.

Do you have any questions?

We are giving you a card today, of someone from the research team and someone from the ILRI ethics committee. If you think of any other questions or have any concerns about the study, please feel free to contact these people.

If you accept our invitation to participate, please sign here below:

"I consent to participate in the 'What is Killing My Cow' study today. I understand the information presented in this document and have been given the opportunity to ask questions."

(Please Print)

Participant Name: _____ _____

Address: _____ Signature (or other mark)

_____ _____

Phone # _____ Date

Witness (staff) Name _____

Witness Position Title _____

Witness Signature _____

Appendix 2: Questionnaire

The possible risk factors for microbial contaminations in milk at farm level

This questionnaire aims to find people's knowledge and awareness of risk factors that could lead to microbial contaminations of milk at farm level. It will take less than thirty minutes to complete this questionnaire. Please note that your answer is completely confidential and your name will not be included in any reports of these results. Your individual answer will not be shared with anyone.

1. Questionnaire number:……
2. Farm code:……...
3. Date of interview:……/……/20……...
4. GPS co-ordinates:……..…….
5. Region:……
6. District……
7. Village…….

PART A: RESPONDENT PARTICULARS (I will start asking you some personal questions)

1. How old are you?
   a) 15 – 20 yrs
   b) 21 – 30 yrs
   c) 31-40 yrs
   d) 41-50 yrs
   e) More than 50 yrs
   f) Don't know or prefer not to say
2. Sex of the respondent:
   a) Male
   b) Female
3. Level of education of the respondent
   a) No school education attendance
   b) School education ending primary school
   c) School education ending secondary school
   d) Religious schooling only
   e) School education ending higher education (i.e. college, university, etc.)

4. Are you the owner of this herd?
   a) Yes
   b) No (Go to question 13)

5. Do you own all the cattle in this herd?
   a) Yes
   b) No
   If no, how many different owners own this herd? Mention……..

6. Do you practice BOTH animal and crop farming?
   a) Yes
   b) No

7. For how long did you kept cattle?
   Mention (years)……

8. Is this herd/this employment your only source of income for your family?
   a) Yes (Go to question 10)
   b) No

9. What are other economic activities doing as source of income?
   Mention:……

10. What are the main roles of females in the farm?
    Mention:……

11. What are the main roles of males in the farm?
    Mention;…..……

12. How many working labour do you have which are not family members)
    a) Less than 3 people
    b) More than 3 people
    c) None

13. What is your role in the farm?(single choice) mandatory
    a) Herd care taker (*includes a family member, i.e. the son of the owner*)
    b) Manager (*normally refers to someone formally hired to MANAGE the herd*)
    c) Ordinary worker
    d) Other (specify)……

FARM MANAGEMENT PRACTICES (herd associated factors)

(I will now ask you some questions on how you manage your herd)

14. Do you raise beef cattle in the same herd?(mandatory)
    a) Yes
    b) No

15. What farming system are you practicing?(mandatory)(single choice)
    a) Extensive (never kept indoors)
    b) Semi-intensive (kept outdoors during day and kept indoors overnight)
    c) Intensive (primarily kept indoors – zero grazing)
    d) Other (specify)……

16. How many lactating cattle do you have?
    Mention…….

17. Do you practice transhumance/nomadism? (move with the herd in search of pasture/water)
    a) Yes
    b) No

18. Do you own/herd other animals apart from cattle?
    a) Yes
    b) No
    If yes, mention them ……

19. Where do you COMMONLY graze your cattle?(single choice)
    a) Open space - communal grazing fields
    b) Open space - private grazing fields
    c) Dumping sites
    d) Zero grazing
    e) Others (specify)……

20. How often does your herd graze in outside pastures DURING THE YEAR? (single choice)
    a) All year round
    b) Few months per year (seasonal)

21. Does this herd come into contact WITH OTHER HERDS (e.g. during watering or in communal pasture)? (mandatory)
    a) Yes
    b) No (skip to 23)

22. If yes how often do they come into contact with other herds?(single choice)
    a) Everyday
    b) Atleast once a week
    c) Atleast once a month
    d) Less often

23. Does this herd come into contact with wild game animals? (mandatory)
    a) Yes
    b) No (skip to 26)

24. Mention the wildlife/wild animals species does your herd come into contact with most frequently:……

25. How often does your herd come into contact with wild animals?(single choice)
    a) Everyday
    b) Atleast once a week
    c) Atleast once a month
    d) Less often

26. Are the cattle enclosed at night?
    a) Yes
    b) No

27. If your animals are enclosed, what type of animal house floor is in? (mandatory) (single choice)
    a) Covered with manure
    b) Concrete
    c) Earthed floor
    d) Others (specify)……

28. What is the water source for your cattle herd?(single choice)
    a) Tap water
    b) Local Rivers
    c) Water pans/flood water
    d) Local wells/boreholes
    e) Other (specify)……

29. What do you normally feed you animals (when out of pasture) (multiple choice)
    a) Hay
    b) Grass fodder
    c) Nothing because pastures are 100% available throughout a year
    d) Other (specify)……..

30. Do you supplement the feeding of your cattle?
    a) Yes (includes sometimes)
    b) No (go to question 32)

31. What form of supplementation do you give? (multiple choice)
    a) Sunflower seed cake
    b) Cotton seed cake
    c) Maize bran
    d) Brewers waste
    e) Others (specify) ……

32. Which breeding methods do you use in your farm?(mandatory)(single choice)
    a) Artificial insemination (go to 35)
    b) Bull
    c) Both

33. If you use bull for breeding, is the bull from your own herd?(single choice)
    a) Yes (go to 35)
    b) No

34. If No, where do you source the bull from?
    a) Neighbours
    b) Special breeders
    c) Relatives
    d) Others (specify)……

BIOSECURITY AND DISEASE CONTROL

35. How/where do you obtain your replacement animals from?(multiple choice)
    a) Own farm (breeding on farm)
    b) From breeding farms
    c) Animal market
    d) Neighbours
    e) Relatives
    f) Other (specify)…….

36. Do you have specific health criteria when selecting new animals for your herd?
    a) Yes
    b) No (go to 38)
    c) Not sure (go to 38)

37. If yes what are the specific health criteria?
    Mention them ……

38. Did you introduce any NEW animal(s) into your cattle herd IN THE PAST YEAR?
    a) Yes
    b) No (go to 43)

39. Was the new animal(s) tested for ANY diseases before introduction?
    a) Yes
    b) No
    If yes, mention the diseases tested with ……

40. Was the new animal(s) quarantined before introduction to the main herd?
    a) Yes
    b) No
    c) Not sure

41. Did you vaccinate THE NEWLY ACQUIRED animals before introduction to the main herd?
    a) Yes
    b) No (go to 43)

42. If yes above, which disease(s) did you vaccinate against?
    Mention the diseases tested with ……

43. Did you VACCINATE the rest of your cattle herd against infectious diseases in the past year?
    a) Yes
    b) No (go to 46)
    c) Don’t know/Not sure (go to 46)

44. If yes, who carried out the vaccination?
    a) Herd care taker (includes the owner if he works the farm)
    b) Veterinarian
    c) Animal health worker
    d) Village extension officer

e) Don't know /don't remember
f) Other (specify)……

45. If yes above, which disease(s) did you vaccinate against?
Mention the diseases vaccinated against:…..……

46. Did you seek VETERINARY ADVICE on management of your cattle in the past month? (Mandatory)
a) Yes
b) No

47. What are the most common cattle diseases that affect your herd? (Tick)
a) Lumpy disease
b) Mastitis
c) Helminthiosis
d) Anthrax
d) ECF
e) Brucellosis
f) Red water
g) Diarrhoea
h) Foot and mouth
i) Foot rot
j) Anaplasmosis
k) Respiratory diseases/pneumonia
l) Wounds
m) Plastic consumption/hardware disease
n) Others (specify)……

TICK-BORNE DISEASE KNOWLEDGE AND MANAGEMENT

48. Do you practice tick control in your farm?(Mandatory)
a) Yes
b) No (go to 51)

49. If yes , what method do you use?(check one- single choice)
a) Plunge dip
b) Hand spray
c) Pour on
d) Other (specify) ……

50. How frequently do you conduct tick control?(check one-single choice)
a) Several times a week
b) Once a week
c) Twice a month
d) Once a month
e) Less often (specify)……

51. Do you think that ticks transmit diseases from one animal to another? (Mandatory)
a) Yes
b) No
c) Don't know

INTERNAL PARASITES (KNOWLEDGE AND MANAGEMENT)

52. Do you know that cattle acquire worms (internal parasites)?
    a) Yes
    b) No

53. Do you treat your cattle for worms?
    a) Yes
    b) No (go to 55)

54. How often do you treat your cattle herd for worms (internal parasites)?
    a) Once a month
    b) Every three months (or less frequent)
    c) Less frequent
    d) Only if serious symptoms

REPRODUCTIVE DISORDERS

(I will now ask you a few questions on reproductive/breeding challenges (if any) that you've experienced in your herd)

55. Do you normally assist cattle during parturition?
    a) Yes
    b) No

56. And do you assist during any reproductive problem? (Mandatory)
    a) Yes
    b) No (skip to 59)

57. Do you use any protective gears such as gloves, masks, clothes when assisting with the parturition or abortion of animals or whilst handling placentas and aborted fetuses or when assisting any reproductive problem?
    a) Yes
    b) No

58. If no, why not?
    Explain reasons……

59. Do you separate cows during parturition from the rest of the herd? (Mandatory)
    a) Yes
    b) No

60. Do you disinfect or clean the site/boma after parturition?(Mandatory)
    a) Yes
    b) No (go to 62)

61. If yes, how do you disinfect or clean the site?(single choice)
    a) Cleaning with water
    b) Cleaning with water and soap
    c) Cleaning with water and disinfectant
    d) Other (specify)……

62. Have you experienced any abortions and/or stillbirth in your cattle herd in the past year? (mandatory)
    a) Yes
    b) No (skip to 64)

63. If yes, how many cattle were affected by any of these disorders?
    Mention:…….…….

64. Can you recall any other reproductive disorders in your herd in the last year? (multiple choice)
    a) Dystocia
    b) Metritis
    c) Weak calf
    d) Vaginal prolapsed
    e) Retained placenta
    f) Other (specify)……

65. If you have small ruminants in your herd, did they experience abortion and/or stillbirth in the past year? (mandatory) (only for those farmers that responded having small ruminants)
    a) Yes
    b) No
    If yes, mention type of small ruminants and how many affected……

66. Which other reproductive disorder did these animals experience?(multiple choices)
    a) Dystocia
    b) Metritis
    c) Weak kids/lambs
    d) Vaginal prolapsed
    e) Retained placenta
    f) Don't know
    g) Other (specify)…….……

67. What action(s) taken regarding to aborting animals in your herd? (Multiple choice-mandatory)
    a) Treat them yourself
    b) Call a veterinarian
    c) Separate them from the rest of the herd
    d) Sell the animal (alive)
    e) Slaughter them
    f) Do nothing and use again for breeding
    g) Others (specify)……

68. How do you dispose off the aborted materials (foetuses and placentas)? (single choice) (Mandatory) (Tick one as the most common way of disposing)
    a) Burning
    b) Dumping
    c) Burying
    d) Feed to the cats/dogs
    e) Put in the garbage
    f) Others (specify)……

MASTITIS RELATED QUESTIONS

69. How do you most commonly feed milk to your calves?(single choice)
    a) Bucket feeding
    b) Suckling from the dam
    c) Others (specify)……

70. Who primarily milks the lactating cows?(multiple choice)
    a) Myself
    b) Family member only
    c) External employees
    d) Both
    e) Others (specify)……

71. Is it primarily men or women who milk cows?
    a) Male
    b) Female
    c) Both

72. How much milk ON AVERAGE do you collect from this herd PER DAY?
    Mention:……Litres

73. List IN DETAIL all the steps undertaken when milking one of these cows, starting from the point of approaching the cow. Pay attention to not forget any steps
    List:……

74. You said that you do wash your hands, what do you use to wash your hands?
    a) Water only
    b) Water with soap
    c) Water with a disinfectant and soap

75. You said that you do clean udder and/or teats of the animal, what do you use to wash teats?(multiple choice)
    a) Warm water only
    b) Cold water
    c) Water with a disinfectant

76. Which milking technique do you use? (multiple choice)
    a) Hand milking
    b) Machine milking

77. If hand milking, which technique do you use?
    a) Stripping (Pulling the teat)
    b) Squeezing Action

78. Can you recognize if your cow has an infection/ a problem in the udder?
    a) Yes
    b) No (go to question 83)

79. If yes, how do you detect? (Tick)
    a) Change of colour of the udder/teats
    b) Udder feels warm than usual

c) Changed consistency of the udder
d) Changed size of the uddert
e) Presence of visible lesion on the udder
f) Udder veins are engrossed
g) Changed milk consistency and colour
h) Others (specify)……

80. If yes, do you milk animals with udder problem?
    a) Yes
    b) No (go to question 82)
    c) sometimes

81. If yes, when do you milk the animal(s), before or after the health ones?(options)
    a) before the health animals
    b) After the healthy animals
    c) Others (specify)……..

82. What do you do with the milk obtained from cows with udder infection?
    a) Discard
    b) Consume in the your household
    c) Sale to the market
    d) Feed to calves
    e) Others (specify)……

GENERAL ZOONOSES EXPOSURE PRACTICES

(I will now ask you some general questions on your behavior towards disease in animals and human)

83. What do you do with milk from YOUR cattle herd?(multiple choice- mandatory)
    a) Consume within the family
    b) Sell to milk vendors
    c) Sell to local businesses(restraurant , hotels schools)
    d) Sell to milk processing company
    e) Sell to neighbours and members of the community
    f) Other (specify) ……

84. Was the milk covered at household?
    a) Yes
    b) No
    c) Don’t know

85. Was the milk sample boiled?
    a) Yes
    b) No

86. What is the type of milk storage container used?
    a) Calabash (Wooden/Kibuyu)
    b) Plastic container
    c) Glass bottle
    d) Metal can
    e) Don’t know

87. Was the milk stored under shade?
    a) Yes
    b) No

88. At what time did the milk milked? (Milk storage duration)
    a) less than 2 hrs
    b) between 2 – 6 hrs
    c) between 6 – 12 hrs
    d) more than 12 hrs

89. When animal is sick, what do you do with the milk?
    a) Don't milk the animal
    b) Sell the milk
    c) consume it in the family
    d) use it for the calves
    e) Other (specify)……

90. And if the sick animal is treated with a medicine, what do you do with the milk?
    a) Don't milk the animal
    b) Sell the milk
    c) Milk the animal and discard the milk
    d) Consume it in the family
    e) Use it for the calves
    f) Other (specify)……

91. For how long do you discard the milk (withdrawal periods)……..…..days
92. Do you consume raw milk?(Mandatory-single choice)
    (Note: Raw being unprocessed milk, NOT BOILED, not pasteurized or homogenized)
    a) Always (regarded as yes)
    b) Sometimes (regarded as yes)
    c) No

93. Do you consume milk products made from raw milk?(Mandatory)
    a) Yes
    b) No

94. If yes, which ones?(multiple choice)
    a) Yogurt
    b) Fermented milk
    c) Ghee
    d) Cheese
    e) All milk products
    f) Others (specify)……

95. How do you handle an animal that is close to die or dies (dead one) on the farm?
    a) Bury (after exitus)
    b) Burn (after exitus)
    c) Slaughter and sale and/or eat the meat
    d) Others (specify)……

96. How do you dispose the manure from the herd?
    a) Do not dispose (Leave on animal house)
    b) Use in own crop farm
    c) Dispose by the road side
    d) Use it for biogas production
    e) Sale
    f) Others (specify).....

VETERINARY SERVICES

(I will now ask you some questions regarding veterinary services)

97. Who normally administer medication to your cattle most often?
    a) Myself
    b) Government veterinarian
    c) Extension officer
    d) Private veterinarian
    e) Farm employee
    f) Animal health worker
    g) Neighbor
    h) Others (specify)……

98. How easy is it to get animal health assistance?
    a) Very easy
    b) Easy
    c) Difficult
    d) Very difficult

99. What is the distance covered to the nearest veterinary office?
    a) <5kms
    b) >5kms

100. What is the distance to the nearest agro vet shop?
    a) <5kms
    b) >5kms

101. In your opinion, do you get quality services from veterinary service providers IN YOUR AREA?
    a) Yes
    b) No

102. How easy is it to get veterinary drugs?
    a) Very easy
    b) Easy
    c) Difficult
    d) Very difficult
    e) Depends on the vaccine (i.e. some are easily available, others are not)

103. How will you rate the service charge (PRICE) from government veterinary service providers?
    a) Expensive
    b) Cheap
    c) Reasonable

104. How will you rate the cost of veterinary drugs?
    a) Expensive
    b) Cheap
    c) Reasonable

105. Do you have any veterinary drugs at this moment in the farm?
    a) Yes
    b) No

106. If yes, can we see some of them? Interviewer to observe and record the following:
    Antibiotics – Yes / No (record name and quantity)……..
    Others……
    Is it Expired– Yes / No

MEDICAL SERVICES

107. When you seek healthcare where will you go first?
    a) Private clinic
    b) Religious prayers
    c) Government clinic or hospital
    d) Traditional healer
    e) A clinic owned by a non-governmental or faith based organization
    f) Stay home
    g) Other (specify) ……

108. In the last year how many times have you gone to a clinic or hospital TO GET TREATED YOURSELF?(single choice)
    a) Never
    b) Once
    c) More than once

109. What is the distance covered to the nearest clinic or hospital?
    a) <5kms
    b) 5-10 kms
    c) 10-25 Kms
    d) >25 Kms

Thank you* very *much for devoting your time to participate in this study