EVALUATION OF MICROBIAL CONTAMINATION ALONG THE MILK VALUE CHAIN IN TWO DISTRICTS OF TANZANIA

EMIL HYERA

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

2015

ABSTRACT

Milk is important as a valuable diet, but due to its nutritional value and perishable product it serves as an ideal medium for growth of various microorganisms under suitable conditions, hence it is a staple food in epidemiology linked to zoonotic pathogens. This study was carried out in two districts in Tanga region (Northern Tanzania) to estimate microbial load, isolate selected pathogens and establish their possible sources or entry along the milk value chain. A total of 114 respondents were interviewed and subsequently milk samples were aseptically collected for laboratory microbial analyses using the standard ISO procedures for Food microbial analyses — Horizontal methods. The results revealed poor practices and lack of formal training on milk hygiene among most of the actors. More than 90% of all handled milk samples had Total plate count (TPC) above the EAC maximum acceptable standard of $2.0x10^5$ CFU/ml. The overall mean coliform plate count (CPC) was $1.8x10^6 \pm 6.2x10^6$ CFU/ml, which indicated poor animal husbandry and hygiene practices. The values of TPC and CPC between independent variables were not statistically significant different ($P > 0.05$). In the samples, one contained CPS isolate counting to $5.1x10^5$ CFU/ml likely to cause staphylococcal poisoning. Isolated bacteria were *Escherichia coli, Staphylococcus aureus* and *Listeria spp.* including *Listeria innocua*, *Listeria ivanovii* and *Listeria monocytogenes*. Other microorganisms included *Klebsiella spp*., *Proteus spp., Staphylococcus spp*., *Enterococcus faecalis*, *Bacillus cereus* and *Pseudomonas spp*. In the identified pathogens, *L. monocytogenes* was most (42.1%) predominant. The quality of milk was poor; unhygienic practices, poor animal husbandry practices, organization of milk supply chains, dysfunction of the regulatory agencies and quality control structures predispose the public to risk of contracting milk-borne infections. Training on animal husbandry practices and public education on general milk handling and hygiene are recommended. Also, sector policies, organizational structures and support services and research into public health risks in milk must be focused.

DECLARATION

I, EMIL HYERA do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work that it has neither been submitted, nor being concurrently submitted for a degree award in any other institution.

_____ _____

Emil Hyera Date

(MSc. Tropical Animal Production candidate)

The above declaration is confirmed:

_____ _____

Dr. George Msalya Date

(Supervisor)

_____ _____

Prof. Esron D. Karimuribo Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

This study was co-funded by the Government of the United Republic of Tanzania (URT) through the Ministry of Livestock and Fisheries Development, German Department for International Cooperation (GIZ) and CGIAR CRP4 Agriculture for Nutrition and Health through the Safe Food Fair Food II (SFFF II) project, which is implemented in partnership between the International Livestock Research Institute (ILRI) and Sokoine University of Agriculture (SUA).

I am sincerely grateful to my supervisors Dr. George Msalya and Professor Esron Karimuribo for their brilliant supervision, corrective ideas and patience for the whole period of the study. You are excellent supervisors, thank you. My profound thanks go to SUA SFFF II project coordinator, Professor Lusato Kurwijila for allowing me to carry out a research on milk safety and sharing his broad experience. Thank you for your hospitality. I wish to record my deep sense of gratitude and indebtedness to Dr. Silvia Alonso and Ms. Tarni Cooper of ILRI, Nairobi whose sentiments guided me in the whole research work. Also, Dr. Alexandra Fetsch of Federal Institute for Risk Assessment (BfR), Germany, for provision of training on microbial analyses in milk and for the experimental control. Mr. George Makingi of the Faculty of Veterinary Medicine at SUA provided technical assistance in the laboratory. Thank you for kindness and for sharing your broad experience. Further thanks go to the DALDO office and extension officers of Handeni and Lushoto districts as well as project participants in the selected villages for devoting time and providing platform for the field work. Finally, I wish to thank my family in particular my parents Mr. Melchior Maurus Hyera and Mrs. Rose Thomas Hyera, my wife Agnes and our children Doreen and Davis who supported me morally and

materially throughout the study. Friends and many other people that I could not mention have contributed in various ways to the completion of this work, I thank you all.

DEDICATION

This work is dedicated to Agnes, my wife, partner, friend and lover and our beloved children Doreen and Davis, the light of my life. May you live to enjoy the paramount delights and desires of this work for which you bore little distress.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Descriptive meaning
®	Registered trade mark
AENOR	Asociacion Espanola de Normalizacion y Certificacion (Association for Standardization and Certification)
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
BHI	Brain Heart Infusion
BfR	Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment
BP	Baird-Parker
BSI	British Standards Institute
CBPP	Contagious Bovine Pleural Pneumonia
CEN	Comité Européen de Normalisation (European Committee for Standardization)
CFU/ml	Colony forming unit per millitre
CGIAR	Consultative Group on International Agricultural Research
CI	Confidence interval
CPC	Coliform plate count
CPS	Coagulase Positive Staphylococci
DALDO	District Agriculture and Livestock Development Officer
EAC	East African Community
EAS	East African Community Standard
ECF	East Coast Fever

EDTA	Ethylene di-amine tetra-acetic acid
EFS	Extensive Farming System
FAO	Food and Agriculture Organization of the United Nations
FMD	Foot and Mouth Disease
GDP	Gross Domestic Product
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit (German Federal Enterprise for International Cooperation)
ILRI	International Livestock Research Institute
ISO	International Organization for Standardization
MA	MacConkey agar
MoAC	Ministry of Agriculture and Co-operatives
NA	Nutrient agar
NADIS	National Animal Disease Information Services
OECD	Organization for Economic Co-operation and Development
OIE	Office International des Epizooties (World Organization for Animal Health)
PCA	Plate count agar
pH	Hydrogen ion concentration
PMO-RALG	Prime Minister Office – Regional Authority and Local Government
SAS	Statistical Analysis System
SFFF II	Safe Food Fair Food project phase II
SIFS	Semi-intensive/Intensive Farming system
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TB	Tuberculosis

TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
TPC	Total plate count
TSA	Tryptone Soya Agar
TSZ	Tanzania Short horned Zebu
UK	United Kingdom
URT	United Republic of Tanzania
USA	United States of America
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

There is a rapid increasing demand for livestock products in developing countries as a result of population and income growth as well as urbanization (Delgado *et al.*, 1999). Annual milk consumption increase in these countries averaged 3.5 to 4.0% between 1995 and 2005 (FAO, 2010a) and is expected to increase further by 2020 (Nene *et al.*, 1999). Therefore, if properly managed, the dairy sector could serve as a powerful tool for reducing poverty and creating wealth in developing countries (FAO, 2010a).

In Tanzania, out of 4.9 million agricultural households, 35% engage in both crop and livestock production while 1% consists of pure livestock keepers. Total annual milk production is estimated to be 1.65 billion litres. Of these 86% comes from the traditional sector, which is dominated by the native Tanzania Short Horn Zebu (TSZ) cattle. The dairy sub-sector forms one-third of the 4.6% Gross Domestic Product (GDP), which is contributed by the livestock sector. The sub-sector has therefore a big potential for improving standards of living of the majority of Tanzanian farmers through enhanced nutrition, increased income from sales of milk and milk products as well as reducing vulnerability (Njombe *et al.*, 2011).

Despite its importance, in many African countries marketing of milk is traditional and informal. In Tanzania these markets handle 80–90% of the locally produced milk (Staal *et al.*, 2000) and less than 10% of milk produced in the country is marketed as processed milk. In these systems of marketing, milk is commonly rural to rural and a few rural to direct sales to urban consumers (Kurwijila, *et al.*, 2003). These market pathways provide

social and economic benefits to smallholder producers, agents and consumers in terms of competitive market prices and creation of employment (Kang'ethe *et al*., 2000; Gopalakurup, 2002). Therefore, they are difficult to abolish. However, there are neither regulations nor control of products in these markets and the consumption of raw milk and milk products from such markets may cause health risks, although a few traders or consumers may take some precaution measures. On the other hand, being a nutritious food, milk serves as an ideal medium for the growth and multiplication of various microorganisms (Bonfoh, 2003; Parekh and Subhash, 2008). Moreover, milk is a highly perishable commodity and therefore poor handling of milk can exert both public health risks and economic losses. Thus milk requires hygienic handling all the way from production to consumption (Hayes *et al.*, 2001; Swai and Schoonman, 2011).

Taken together, the present state of milk handling and marketing may pose health risks to the public. These risks are linked to contamination of milk, growth and survival of harmful pathogens in the milk and increasing number of other micro-organisms caused by storage time and conditions such as temperature and humidity. The aim of this study was to review the present status of milk handling and marketing and to establish how these can contribute to the presence of harmful microbial pathogens in milk in the supply chain in two districts of Tanzania.

1.2 Problem Statement and Justification of the Study

Over 90% of the milk, which is consumed in Tanzania is from the informal markets (MoAC/SUA/ILRI, 1998). This milk is supplied by traditional livestock sector, which is mainly composed of indigenous animals. In the traditional sector there is evidence of inappropriate milking and poor handling of milk, which predispose milk to microbial contamination. Furthermore, because of the greater prevalence of tropical diseases among

livestock in the traditional sector, lactating and milking animals might have inborn pathogens in blood. These may shed harmful pathogens in milk and negatively affect the health of consumers of milk or milk products.

Preliminary results obtained in recent studies in Morogoro and Tanga regions in Tanzania (Kilango, 2011; Shija, 2013; Joseph, 2014) have shown that a number of harmful pathogens exist in milk within the farm and intermediaries. However, these studies highlighted little on the pathogenicity and sources of identified pathogens due to studies scope and limitations of the analytical methods employed. It was therefore worthy carrying out a study that will fill this information gap. In the present study, a multi-pathogens analysis was carried out with the aim of detecting additional pathogens in milk and providing information on the levels and sources of these pathogens and potential risks for public health. This study ultimately was aimed at identifying a set of suitable recommendations for improving hygiene of milk along the value chain.

1.3 Objectives

1.3.1 General objective

To identify pathogens in milk and proposing strategies of reducing the harmful microbial load in milk along the milk value chain in Tanzania.

1.3.2 Specific objectives

i) To estimate the microbial load in milk produced in two farming systems of Handeni and Lushoto districts

ii) To isolate selected milk-borne zoonotic pathogens along the milk value chain in the study area

iii) To establish the possible sources of milk microbial hazards in the Tanzanian milk value chain

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of the Importance of Dairy Production

FAO (2010b) estimated that 12 – 14% of the world population lives are sustained by dairy farming. World milk production is expected to increase by 153 Million tons between 2010 and 2020 of which 73% is anticipated to come from developing countries (OECD/FAO, 2011). In Tanzania, annual milk production has increased from 814 million litres in 2000/01 to 1.65 billion litres in 2009/10 of which over 86% comes from the traditional system, which is mainly composed of the indigenous stock dominated by TSZ cattle (Njombe *et al.*, 2011). These animals account for over 90% of the dairy ruminants in the country and elsewhere in Sub-saharan Africa and contribute significantly to the production of milk in the country (Ndambi *et al*., 2007). The dairy industry is developing with prospects for expansion and a greater contribution to the GDP and improvement of the welfare of the people (Njombe *et al.*, 2011). Less than 5% of the total milk produced in Tanzania is formally marketed whereas 70 – 80% is consumed or lost at farm level and only 15 – 25% passes through informal markets (direct sales, hawkers and small vendors) (RLDC, 2009).

2.2 Milk Value Chain

According to Kaplinsky and Morris (2000), the value chain describes the complete range of activities required to bring a product from conception to the delivery to final consumers, and the final disposal after use. It includes activities like design, production, marketing, distribution and support to the final consumer (Ruijter de Wildt *et al*., 2006). Milk value chains have several outlets through which milk products flow from the producer to the consumer, which impacts the quality of milk and transaction costs as well

as potential risk of contamination with pathogens. Hence, an understanding of functional market chains is an important first step towards understanding and dealing with milk safety risks (Kilango, 2011).

2.3 Milk Composition and Milk Quality

On average 87.4 % of the cow's liquid milk is water, 3.7% is milk fat (milk lipids or butter fat), 8.9% is solids-not-fat (SNF), 3.4% is protein (2.8% casein, 0.6% whey protein), 4.8% is lactose, 0.7% includes minerals (micronutrients such as Zinc, Iron and Copper as well as macronutrients such as Calcium, Phosphate, Magnesium, Sodium, Potassium, Citrate and Chlorine). This group also includes sulphate, bicarbonate, acids (citrate, formate, acetate, lactate and oxalate), enzymes (peroxidase, catalase, phosphatase and lipase), gases (oxygen and nitrogen) and vitamins A, C, D, Thiamine and Riboflavin (Nangwala, 1996; Tesha, 2010). In addition, milk is a good source of many other vitamins such as B6, B12, K, E, niacin, biotin, folates, and pantothenic acid (Goff and Hill, 1993). In general, milk has a high nutritional value and it is a good diet for the children (FAO, 2005). It provides nourishment and immunological protection (Bauman, 2004). However, if not handled properly, milk can be easily destroyed through contaminations and bacterial growth and becomes unfit for human consumption. Some of the microbial contaminants are responsible for milk spoilage while others are pathogenic with potential health effects to cause milk borne diseases (Kivaria *et al.,* 2006). Bacterial count in milk is influenced by the temperature at which milk is stored and the time that elapses since milking. Once the milk is cooled to 4ºC within 2 – 3 hours after milking, it preserves its original quality and remains safe for processing and consumption (Omore *et al.*, 2005). East African countries (EAC) have harmonized standards for some products including milk. Standards are reference points and tools for ensuring quality and safety. East African Standard (EAS 67) prescribes quality requirements for raw, normal cow's milk. It covers bacteriological

quality. It is important that all players in the milk value chain implement standard at their level of operation to protect the consumer (EAS, 2006). The quality classification for standard plate count per ml or g in raw milk as developed by the EAS is shown in Table 1.

Table 1: Microbial limits in raw milk

Bacteriological grade	CFU/ml
I or A	< 200 000
II or B	> 200 000–1 000 0000
III or C	> 1 000 000–2 000 000

Source: EAS (2006)

2.4 Sources of Microbial Hazards in the Milk Value Chain

Microbial contamination of milk in the value chain can originate from a diseased cow, unhygienic milking practice, poor personal hygiene, unsanitary utensils and/or milking equipment and water supplied in sanitary activities (Parekh and Subhash, 2008; Kilango, 2011; Lubote *et al*., 2014). A cow with an infectious disease can shed pathogens from its blood into the milk. Findings by Streeter *et al*. (1995) indicate that infected cows with clinical disease and subclinical infections shed *Mycobacterium avium* subspecie *Paratuberculosis* in both milk and faeces. Detectable levels of the organism were observed in milk from both clinically infected and asymptomatic carrier animals. Also, infected mammary quarters or cows and the environment, in which animals are kept, are known to be chief sources of bacteria that cause udder infections in a herd. Transmission of infectious bacteria to teats of uninfected mammary quarters or cows occurs mostly at milking (Kilango, 2011). Appropriate milking hygiene practices reduces the rate of new infections during milking (Robert, 1996). The use of pre- and post-milking teat disinfectants is an effective measure in reducing the risk of new infections. Pre-dipping reduces the resident teat skin bacterial population, which is the main source of infection

for the mammary gland. It can reduce new environmental streptococcal infections and *E. coli* by 50%. Post-dipping prevents the transmission of contagious bacteria such as *S. aureus* (NADIS, 2013). All individuals involved in the milk value chain should maintain hygiene and must be in sound health because microbes may drop from hands, clothing, nose and mouth, and from sneezing and coughing. It is important for them to be in good health to avoid becoming a source of infectious diseases (Kurwijila, 1998). Other bacterial sources are from air, drugs or chemicals used during treatment of animal and from contaminated water used for adulteration by unscrupulous and unfaithful workers/sellers may cause additional health problems (Karimuribo *et al.*, 2005).

2.5 Health and Economic Impact of Unsafe Milk

Food safety is an area of great concern in terms of public health management especially from an economic point of view (Mangwayana *et al*., 2000). Food-borne diseases due to microbial pathogens in milk are a serious threat to the health of millions of people (FAO, 2006). Raw milk continues to be a staple in the epidemiological literature linked to campylobacteriosis, salmonellosis, tuberculosis, brucellosis, hemorrhagic colitis, Brainerd diarrhoea, Q fever, listeriosis, yersiniosis, and toxoplasmosis to name a few (Plotter, 2002). These impose a substantial burden on health care systems and reduce economic productivity (FAO, 2006). Seventy percent of deaths among children under five years are linked to biologically contaminated food and water (Unnevehr and Hirschhorn, 2000). Also, unsafe milk and milk borne illnesses cause producers, vendors and wholesalers to earn a poor reputation, which may take time to overcome and consequential loss of income. These important players may also become prey of milk borne illness thus perpetuating the cycle of poverty (Nhachi and Kasilo, 1996; FAO, 2006). The sources of contamination are variable and can take place at any point in the milk production and marketing chain. The major milk borne pathogens of concern are zoonoses and

environmental coliforms of faecal origin. The latter are commonly introduced in milk due to poor handling at farm and along the value chain. Contamination of faecal bacteria in milk usually occurs through the use of contaminated water and unsanitary equipment (Kilango, 2011). Currently, there is limited scientific data to quantify the magnitude of the problem and to provide baseline data from which informed decisions can be made. More information is needed that will help improved regulatory policy decisions to be made. Scientific data will also help ensure more effective control when outbreaks occur (Mangwayana *et al*., 2000). There is evidence of many harmful bacteria being potentially linked with bovine milk contaminations (Table 2).

Table 2: Bacterial types commonly associated with bovine milk

Bacteria group and species	Outcome
Lactococci: *L. lactis-diacetylactis, L. lactis, L. cremoris*	Flavour production and fermentation
Lactobacillus: *L. lactis, L. bulgaricua, L. acidophilus, Leuconostoc lactis, Propionibacterium*	Acid production/fermentation
Pseudomonas, Bacillus cereus	Spoilage
Enterobacteriaceae	Pathogenic and Spoilage
Staphylococci: *Staphylococcus aureus* (*S. aureus*).	Pathogenic
Streptococcus: *Strep. agalactiae*	Pathogenic
Zoonotic *Brucella abortus*	Pathogenic
Zoonotic *Mycobacterium bovis*	Pathogenic
Coliforms: *Escherichia coli* (*E. coli*)	Some are zoonotic and pathogenic
Listeria: *Listeria monocytogenes* (*L. monocytogenes*)	Pathogenic; mainly in unpasteurised cheese

Source: O'Connor (1995)

2.5.1 *Enterobacteriaceae* in milk

The *Enterobacteriaceae* is a large family of gram-negative, rod shaped bacteria, which includes more familiar pathogens, such as *E. coli, Salmonella, Klebsiella, Shigella, Yersinia pestis* and other disease causing bacteria such as *Proteus, Serratia, Enterobacter* and *Citrobacter* (Brenner *et al*., 2005). Many members of this family are normal inhabitants of the large intestines of human, animals and insects, while others are found in soil, water and decaying matter. Some are enteric pathogens and others are urinary or respiratory tract pathogens. These organisms also are used as indicators of microbial quality and hygiene. As several of these organisms are potential pathogens, consumption of raw milk is considered highly risk (Anand and Griffiths, 2011). In recent years, there has been emergence of new milk borne bacterial pathogens with serious and even life-threatening complications such as enteric *E. coli* serotypes (Sivapalasingams *et al*., 2004). The enterohemorrhagic (EHEC) class is of most concern, due to its low infectious dose and its association with hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) (Keller and Miller, 2006; Vojdani *et al.,* 2008; Simforian, 2013). Verocytotoxigenic *E. coli* sero-groups may infect humans through consumption of infected raw unpasteurized milk and milk products, which have significant contribution to the reported cases of Shiga toxin producing *E. coli* (STEC) in humans (Baylis, 2009).

Also, *Salmonella* infections of food animals play an important role in public health and particularly in food safety, as food products of animal origin are considered to be the major source of human *Salmonella* infections (OIE, 2008). It has adapted to survive and recognize temperature and pH extremes, oxygen limitations, presence of bile salts, digestive enzymes, and competing micro flora. The hostile environment within the gastrointestinal tract is tolerated and serves as a signal to induce transcription of genes

required for host cell attachment and invasion (Ahlstrom, 2011). In contrast with other pathogens of the family, the reservoirs of *Salmonella* cover a greater variety of warm and cold blooded animal. *Salmonella* may be found in milk, and has been associated in milk borne disease. *Enterobacteriaceae* infections are among the most killing diseases of children in developing countries (Frey and Sherk, 2006). Moreover, gastrointestinal infections due to pathogenic *Enterobacteriaceae* in particular *Escherichia* and *Salmonella spp.* are significant causes of morbidity and mortality worldwide (Bisi-Johnson *et al*., 2011).

2.5.2 *Staphylococcus* aureus in milk

Staphylococcus aureus is a facultative anaerobic**,** Gram-positive coccus, which appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with haemolysis, when grown on blood agar plates (Ryan and Ray, 2004). It is a versatile pathogen of humans and animals and causes a wide variety of diseases ranging in severity from slight skin infection to more severe diseases such as pneumonia and septicemia. It is an important food-borne pathogen, which ranks as one of the most prevalent causes of gastroenteritis worldwide (Dinges *et al*., 2000). It survives in as much as 15% NaCl and can grow at pH = 4.2 – 9.3 and in temperatures ranging from 7 to 48.5ºC.

These characteristics enable *S. aureus* to grow in a wide variety of foods. The bacterium may occur in the milk of cows with clinical or sub-clinical mastitis or as the result of contamination by handlers. When toxigenic strains of this organism replicate to numbers exceeding 10^5 CFU/ml, they may produce staphylococcal enterotoxins that cause staphylococcal food poisoning (Hudson, 2010). The intoxication is characterized by

enteric responses such as diarrhea, abdominal cramps and vomiting within 1 – 6 hours of consumption of contaminated food. The bacterium is heat labile and does not compete well with other microorganisms. Contamination usually occurs when there is little competition from other microorganisms. Although Staphylococci are also commonly found in other materials including animal skins, water and soil, bacteria from food handlers and other human sources are considered as the most important contributing factors to intoxications associated with food (Kilango, 2011).

2.5.3 *Listeria* species in milk

The genus *Listeria* contains gram positive, non-spore forming, catalase-positive, oxidase-negative, and facultative anaerobic bacteria (Vázquez-Boland *et al*., 2001). It includes species such as *L. monocytogenes, L. seeligeri, L. welshimeri, L. innocua, L. ivanovii, L. grayi* and recently identified species, namely *L. marthii* and *L. rocourtia*. Two species are considered pathogenic with *L. monocytogenes* representing the only pathogen of public and veterinary health significance, while *L. ivanovii* is usually restricted to causing disease in ruminants, mainly sheep. These bacteria are considered as saprophytes as they can survive in soil, decaying vegetation, various food products and in both food and non-food associated environments. They survive in as much as 20% NaCl and can grow in bile salts, at pH = 4.3 – 9.0 and in a wide temperature range of 1 to 45ºC, hence they are capable of tolerating a variety of environmental stresses (Ahlstrom, 2011). The ability for cells to grow at refrigeration temperatures during shelf-life storage is a major concern for food safety (Vasquez-Boland *et al*., 2001). *L. monocytogenes* is one of the most important agent of food-borne disease. In humans, foodborne *L. monocytogenes* causes large outbreaks of Listeriosis, with a mortality rate of 9 – 44% (Clark *et al*., 2010). Possible reasons for the emergence of human food-borne Listeriosis as a major public health concern include major changes in food production, processing and distribution, increased

use of refrigeration as a primary preservation means for foods, changes in the eating habits of people towards convenience and ready to eat foods, and an increase in the number of people at high risk for the disease (Swaminathan, 2001). The risk of infection with *L. monocytogenes* is increased in the elderly, pregnant women, neonates, immunocompromised people and may lead to meningitis, sepsis and abortion (Fsihi *et al*., 2001). Listeriosis causes encephalitis, septicaemia and spontaneous abortion and stillbirth in domestic animals and is common among individuals who work with animals. Although *L. monocytogenes* has definite zoonotic potential, it is also an important environmental contaminant of public health significance (OIE, 2008).

2.6 Multi-Pathogens Analyses

Multi-pathogens analyses are detailed examinations of numerous microorganisms likely to be present in individual samples. These analyses use microbiological testing technologies for identification of hazards in the value chain for multi-pathogen risk assessment and/or risk management. Microbiological risk assessment in foodstuffs relies on classical microbial detection and quantification of indicator microorganism (Kilango, 2011). Food microbial analysis is essential for prevention and identification of problems related to public health and food safety (Kostić and Sessitsch, 2012). It is based on detection of microorganisms by visual, biochemical, immunological or genetic means. Traditionally, it is carried out using conventional culture and colony counting methods. These methods are based on enumeration and detection of microorganisms present in food. Enumeration of microorganisms is accurately estimated through the plate count method. This method relies on culturing dilutions of sample suspensions in the interior or on the surface of an agar layer. Individual microorganisms will grow and form individual colonies that can be counted (Blodgett, 2010). The method has sufficient sensitivity for direct testing (López-Campos *et al*., 2012). On the other hand, detection of microorganisms is done through

enrichment methods, which permit growth of target organisms to detectable levels and performing various biochemical and/or serological tests with pure cultures obtained from presumptive colonies to confirm the identity of the desired microorganisms (Betts and Blackburn 2009). Conventional culture and colony counting methods remain the most reliable and accurate techniques for food-borne pathogen detection with which other methods are compared. They are usually very sensitive. However, selection of the appropriate analytical method must consider the sensitivity of analysis, the time of detection and the specificity of the test (López-Campos *et al*., 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Handeni and Lushoto districts of Tanga region in Tanzania. These districts were earlier identified as project sites for the Safe Food, Fair Food (SFFF II) project. Selection of these districts was based on the assessment of rural production to rural or urban consumption, dairy farming practices, presence of milk collection centres, seasonality effects, and agro-ecosystems (Häsler *et al*., 2013). Tanga region is situated at the extreme north–east corner of Tanzania between longitudes 37º and 39º East and latitudes 4º and 6º South and is characterized by hot and humid tropical climate with rainy seasons experienced from March to April and November to December. The mean annual rainfall varies from 500 – 1400 mm with relative humidity ranging from 60 – 90% throughout the year.

In the Western plateau of Handeni district a hot and dry climate dominates while the other part, which is composed of the Usambara Mountains has temperate climate. Handeni is bordered by Kilindi district to the west, Korogwe district to the north, Pangani district to the East and Bagamoyo district (Coast region) to the South. The district is characterized by the extensive farming system (EFS) where livestock production is dominated by the pastoral farming system (Sikira and Ndanu, 2012). Lushoto district is bordered by the Republic of Kenya to the north, Muheza district to the east, Same district to the north–western and Korogwe district to the south. The semi-intensive/intensive system (SIFS) of livestock production is practiced in this district and is mainly characterized by smallholder production system dominated by agricultural activities with few herds of improved or crossbred cattle (Sikira and Ndanu, 2012). Five villages namely Kibaya, Masatu, Sindeni,

Kwediyamba and Konje in Handeni and another five villages namely Handei, Manolo, Mbokoi, Mwangoi and Kwang'wenda in Lushoto were selected for this study (Figure 1).

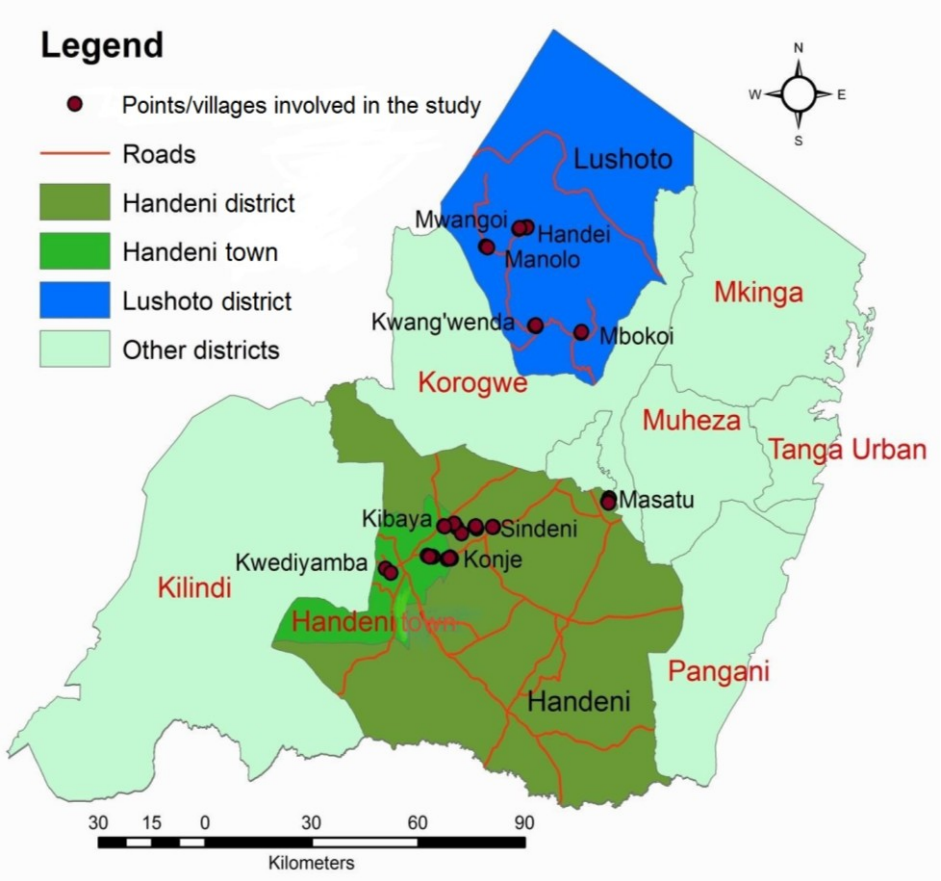

Figure 1: A map of Tanga region showing surveyed districts and villages involved in the study

3.2 Data Collection

In total, 54 households (from 10 villages) were available for the study. These were purposively obtained from a list of cattle keeping households in each village. Selection of the households was based on willingness of the respondent to participate in the study. Prior to a questionnaire interview and milk sampling, individual consent was asked and the respondent was requested to sign an informed consent (Appendix 1). In addition, 25

milk suppliers, 13 milk vendors, 18 restaurants and four milk collection centres were involved in this study making a total of 114 respondents.

3.2.1 Administration of questionnaire

A pre tested questionnaire (Appendix 2) was used to capture various information relevant for the study including sex, age, education and main occupation of farmers as well as their herd size; cow breed, measures practised to control zoonotic diseases, routine mastitis control practices, knowledge on health risks associated with consumption of milk, knowledge of factors affecting hygiene or quality of milk and their milk handling practices at different levels of the value chain. The question was administered by face-to-face method. Other information such as housing condition, milking methods and condition of milk storage equipment, cleanliness of vendors or milk servers, milk serving utensils and chilling/cooling facilities were verified through direct observation.

3.2.2 Collection and handling of milk samples

In total, 114 milk samples were collected in the study areas. Of these, 54 samples were taken from household containers and 60 samples were obtained from other actors of the value chain such as milk vendors/traders, restaurants and the collection centres. Milk was aseptically collected from bulked milk in the households or other nodes of the value chain using a sterile falcon tube to a total volume of 50ml for each sample. The samples were immediately placed in a cool box packed with ice at an appropriate temperature of −20°C. Later on the same day, samples were transferred to −20°C for storage until analysis.

3.3 Laboratory Analyses

Laboratory analyses were carried out in the Public Health Research Laboratory in the Faculty of Veterinary Medicine at SUA using conventional microbiological testing

methods. The methods involved enumeration and detection of microorganisms present in milk. Initially, enumeration of microorganisms was done using colony counting methods to establish the microbial load in milk. Then, detection of microorganisms was done using enrichment methods and performing various biochemical tests with pure cultures obtained from presumptive colonies.

3.3.1 Media preparation

3.3.1.1 Buffered peptone water

To obtain Buffered peptone water (BPW), 20 g of the BPW powder was dissolved in 1 litre of distilled water according to the manufacturer's instructions (OXOID® Ltd., Basingstoke, Hampshire, England). Original BPW powder is a mixture of 10 g/l peptone, 5 g/l sodium chloride, 3.5 g/l disodium phosphate and 1.5 g/l potassium di-hydrogen phosphate. Each 10 ml of the mixture was dispensed in new sterile test tube, sterilized by autoclaving at 121°C for 15 minutes and cooled to 25°C for serial dilutions.

3.3.1.2 Plate count agar

Ready-made sealed pre-poured petri dishes containing 2.5 g/l yeast extract, 5 g/l pancreatic digest of casein, 1 g/l glucose, 15 g/l agar and final pH 7.0 ± 0.2 at 25°C (E & O Laboratories Ltd., Burnhouse Bonnybridge, Scotland) were received from the supplier. These were stored in sterile condition at 2 – 8°C until time for culturing. The formula conforms to American Public Health Association (APHA) (Wehr and Frank, 2004) and Association of Official Analytical Chemists (AOAC) standard (Latimer, 2012).

3.3.1.3 Nutrient agar (NA)

Ready-made sealed pre-poured petri dishes containing 1 g/l 'Lab-Lemco' powder, 2 g/l yeast extract, 5 g/l peptone, 5 g/l sodium chloride, 15 g/l agar and final pH 7.3 ± 0.2 at

25°C were received from the supplier (E & O Laboratories Ltd., Burnhouse Bonnybridge, Scotland). These were stored at 2 – 8°C until when they were used as a basic culture medium.

3.3.1.4 Violet red bile glucose (VRBG) agar

This medium contains 3 g/l yeast extract, 7 g/l peptone, 1.5 g/l bile salts, 10 g/l glucose, 5 g/l sodium chloride, 0.03 g/l neutral red, 2 mg/l crystal violet and 12 g/l agar (OXOID® Ltd., Basingstoke, Hampshire, England). To obtain VRBG agar, 38.5 g of the powder was suspended in 1 litre of distilled water. The mixture was then boiled to allow a complete dissolution of the powder and was followed by sterilization through boiling with frequent agitation for 1 minute and cooling to 46 ± 2ºC. Then, bottles containing medium were placed into water bath at 48ºC for use within 3 hours.

3.3.1.5 MacConkey agar (MA)

Ready-made sealed pre-poured petri dishes containing 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 pH 7.4 ± 0.2 at 25°C were received from the supplier (E & O Laboratories Ltd., Burnhouse Bonnybridge, Scotland). These were stored at 2 – 8°C until when they were used. Because of a small number of imported ready-to-use MacConkey agar plates, additional preparation was done by dissolving 52 g of the powder in 1 litre of distilled water according to manufacturer's instructions (OXOID® Ltd., Basingstoke, Hampshire, England). The mixture was then boiled to allow a complete dissolution of the powder. This was followed by autoclaving at 121°C for 15 minutes before cooling the medium to 45°C and pouring on petri dishes.

3.3.1.6 Tryptophan broth

Tryptophan broth was made using 16 g of the powder reconstituted in 1 litre of distilled water according to the manufacturer's instructions (Sigma-Aldrich Company, USA.). The mixture was mixed thoroughly followed by heating with frequent agitation and boiling for one minute for complete dissolution. 3 ml of the medium were dispensed into new sterile test tubes. The tubes were closed with rubber stoppers and sterilized by autoclaving at 121°C for 15 minutes followed by cooling to 25°C ready for use.

3.3.1.7 Glucose agar

Ready-made and sealed 15 ml tubes containing glucose agar were received from the supplier (E & O Laboratories Ltd., Burnhouse Bonnybridge, Scotland). The media content was composed of 10 g/l tryptone, 1.5 g/l yeast extract, 10 g/l glucose, 5 g/l sodium chloride, 15mg/l bromocresol purple, 9g agar and Final pH 7.0 ± 0.2 at 25°C. These were stored at 2 – 8°C until analysis.

3.3.1.8 Baird-Parker agar

Baird-Parker agar was ready-made, pre-poured in petri dishes and sealed. The medium contained 10 g/l tryptone, 5 g/l` Lab-Lemco' powder, 1 g/l yeast extract, 10 g/l sodium pyruvate, 12 g/l L-Glycine, 5 g/l lithium chloride, 20 g/l agar and final pH 7.0 ± 0.2 at 25°C (E & O Laboratories Ltd., Burnhouse Bonnybridge, Scotland). Media plates were stored in sterile condition at 2 – 8°C until analysis.

3.3.1.9 Brain heart infusion, porcine broth

This medium is composed of 10 g/l peptone, 12.5 g/l dehydrated calf brain infusion, 5 g/l dehydrated beef heart infusion, 2 g/l glucose, 5 g/l sodium chloride, 2.5 g/l disodium hydrogen phosphate anhydrous and final pH 7.4 ± 0.2 at 25 °C. To obtain Brain heart

infusion (BHI) broth, 2 g of the powder was dissolved in 100 g of distilled water (2% in water) and mixed well as according to the manufacturer's instructions (Sigma-Aldrich Company, USA). Each 5 ml of the medium were then transferred to tubes, sterilized by autoclaving at 121°C for 15 minutes and cooled at 25 °C ready for use. All the prepared but unused BHI tubes were stored under refrigeration temperature until further analysis.

3.3.1.10 BD BBL™ Coagulase plasma, rabbit with EDTA

BBL Coagulase plasma, rabbit with EDTA (lyophilized 10 × 15 ml rabbit plasma with 0.15% ethylene di-amine tetra-acetic acid and 0.85% sodium chloride, approximately) contained dry natural rubber (Becton, Dickinson and Company, Sparks, USA). This medium was obtained after reconstituting the powder with 15 ml of sterile distilled water followed by gentle rotation. Each 0.2 ml of the medium was aseptically dispensed into new Eppendorf tubes for coagulase test.

3.3.1.11 Rappaport-vasiliadis soy (RVS) enrichment broth

This medium contained 5 g/l soya peptone, 8 g/l sodium chloride, 1.6 g/l potassium di-hydrogen phosphate, 40 g/l magnesium chloride and 0.04 g/l malachite green oxalate (OXOID® Ltd., Basingstoke, Hampshire, England). It was prepared according to the manufacturer's instructions by weighing 30 g of the powder and adding to 1 litre of distilled water. The mixture was heated gently until completely dissolved. Then, 10 ml were dispensed into capped test tubes followed by autoclaving at 115°C for 15 minutes and cooling to 25°C for use as a culture enrichment broth.

3.3.1.12 Müller Kauffmann tetrathionate-novobiocin (MKTTn) broth

MKTTn broth contained 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 sodium thiosulphate, anhydrous, 4.78

g/l ox bile and 9.6 mg/l brilliant green (OXOID® Ltd., Basingstoke, Hampshire, England). The medium was prepared as indicated by suspending 89.5 g in 1 litre of distilled water. The mixture was then mixed well, boiled and left to cool to 25°C. Immediately before use 20 ml of iodine-iodide solution prepared by dissolving 25 g of potassium iodide in 10 ml of distilled water, adding 20 g of iodine and diluting to 100 ml with distilled water were added. Also, one vial of Novobiocin supplement (SR0181E) was reconstituted per 250 ml of medium as recommended. The medium was mixed well and each 10 ml were aseptically dispensed into sterile capped test tubes for use as a selective enrichment broth.

3.3.1.13 Xylose lysine desoxycholate (XLD) agar

The contents of XLD agar were 3 g/l yeast extract, 5 g/l L-Lysine, 3.75 xylose, 7.5 g/l lactose, 7.5 g/l sucrose, 1 g/l sodium desoxycholate, 5 g/l sodium chloride, 6.8 g/l sodium thiosulphate, 0.8 g/l ferric ammonium citrate, 0.08 g/l phenol red and 12.5 g/l agar. Ready-made sealed pre-poured petri dishes were received from the supplier (E & O Laboratories Ltd., Burnhouse Bonnybridge, Scotland). These were stored at 2 – 8°C until time of culture.

3.3.1.14 Fraser broth base

Bags of 5 litres half strength hydrated Fraser broth added with half Fraser selective supplement (SR0166E) containing 0.5 g/l ferric ammonium citrate, 12.5 mg/l acriflavine and 10 mg/l nalidixic acid were received from the supplier (E and O Laboratories Ltd., Burnhouse Bonnybridge, Scotland). These were stored at 2 – 8°C until time of analysis. Fraser broth (OXOID® Ltd., Basingstoke, Hampshire, England) contained 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 potassium di-hydrogen phosphate, 1 g/l aesculin and 3 g/l lithium chloride. To obtain a full Fraser broth, the medium was

prepared as indicated by dissolving 28.7g of the powder into 500ml of distilled water and mixing well to dissolve completely. The medium was sterilized by autoclaving at 121°C for 15 minutes and cooled to below 50°C. The content of one vial of Fraser selective supplement (SR0156) reconstituted as directed in the product insert was aseptically added before use. The medium was mixed well and each 10 ml were aseptically dispensed into sterile capped test tubes and stored under refrigeration for use as an enrichment medium for the detection and isolation of Listeria.

3.3.1.15 *Listeria* Oxford agar

Listeria Oxford agar was pre poured in petri dishes and sealed (E & O Laboratories Ltd., Burnhouse Bonnybridge, Scotland). The contents of this medium were 39 g/l columbia blood agar base, 1 g/l aesculin, 0.5 g/l ferric ammonium citrate, 15 g/l lithium chloride, 2 g/l agar and *Listeria* selective supplement at a final pH 7.0 ± 0.2 and temperature of 25°C. Storage of the medium was at 2 – 8°C until analysis.

3.3.1.16 Colorex *Listeria* agar

Colorex *Listeria* agar (E & O Laboratories Ltd., Burnhouse Bonnybridge, Scotland) contained 18.5 g/l peptone, 4 g/l yeast extract, 9.5 g/l sodium chloride, 2 g/l sodium pyruvate, 15 g/l lithium chloride, 4 g/l maltose, 0.2 g/l X-glucoside chromogenic mix, 14 g/l agar, Listeria selective supplement and Listeria differential supplement at a final pH 7.2 ± 0.2 and temperature of 25°C. The medium was pre-poured in petri dishes and sealed. These were stored at 2 – 8°C until time of culture.

3.3.1.17 Sheep blood agar

Sheep blood agar is a non-selective medium with the addition of sheep blood used to isolate and cultivate fastidious microorganisms with clearly visible haemolytic reactions.

Ready-made sealed pre-poured petri dishes contained 14 g/l tryptone, 4.5 g/l peptone neutralized, 4.5 g/l yeast extract, 5 g/l sodium chloride, 12 g/l agar and Final pH 7.3 ± 0.2 at 25°C added with 5% sheep blood (TSA with 5% Sheep Blood). These were received from the supplier (E & O Laboratories Ltd., Burnhouse Bonnybridge, Scotland) and stored under refrigeration temperature until time of culture.

3.3.1.18 Glycerol medium

Glycerol medium was used for preservation and long term storage of the isolated colonies. This was prepared by mixing nutrient broth with glycerol solution (HiMedia laboratories Pvt® Ltd., Mumbai, India) according to the manufacturer's instructions. The medium was obtained by dissolving 13 g of nutrient broth in 1000ml of distilled water. The medium was heated to dissolve completely and autoclaved at 121°C for 15 minutes. Thereafter, the nutrient broth was mixed with 20% of Glycerol solution (Philip Harris Limited, Shenstone, England) and was dispensed into the cryovials for the inoculation of isolated colonies. For long term storage of the isolates, inoculated vials were stored at –20°C.

3.3.2 Initial suspension and serial dilutions

Initial suspension and serial dilutions were prepared according to the ISO 6887-1 rules (ISO, 1999). The samples were left to thaw at room temperature for 1 hour (Plate 1). Using a sterile pipette 25 ml of the sample were transferred into a conical flask containing 225 ml of BPW and mixed well. From the mixture 1 ml as the initial inoculum was transferred into a test tube containing 9 ml of BPW (10^{-1} dilution) using a fresh sterile pipette, which was followed by serial dilutions. The procedure was repeated up to 10^{-7} dilution and in the last dilution 1 ml of inoculum was discarded (Figure 2). The dilutions were mixed using a vortex mixer for 5 – 10 seconds. The dilutions were used in microbiological tests to detect and enumerate *Enterobacteriaceae* and *S. aureus* and to

estimate the microbial load in milk. The remaining initial suspension was used in the initial preparation for isolation and presumptive identification of *Salmonella spp*. Remains of the original sample was used in the initial preparation for isolation and presumptive identification of *Listeria spp.* All samples were passing through this preparatory step prior to microbiological tests.

Plate 1: Thawing of samples

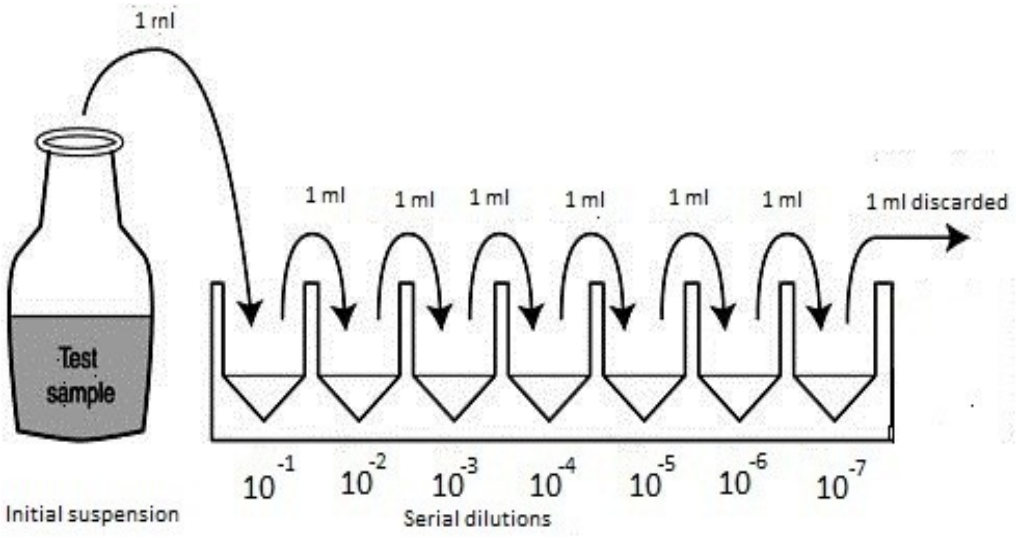

Figure 2: Procedure adopted for serial dilutions of milk samples

3.3.3 Control isolates

Five hundred millilitres of milk was obtained from Magadu dairy farm at Sokoine University of Agriculture (SUA) and were used as a control during the microbial analyses. The control sample was sterilized by boiling at 100°C and was placed in a clean sterile bottle. Part of the sample was inoculated with known bacterial strains of *E. coli* (*E. coli* 2262-79 DEC9B), *Salmonella* (*S. enterica* ATCC13076), *S. aureus* (*S. aureus* NCTC 6571/ATCC 9144) and *L. monocytogenes* (*L. monocytogenes* NCTC 13372/ATCC 7644). A fresh sterile pipette was used to transfer 10 ml of the inoculum sample into a sterile beaker containing 90 ml half strength Fraser broth for cultivation of *L. monocytogenes.* The mixture was sealed with an Aluminium foil and incubated at 30°C for 24 hours. Another 25 ml of the inoculum sample were mixed with 225 ml of BPW from serial dilutions were prepared after initial suspension. Thereafter, the ISO procedures were followed to detect and enumerate the desired microorganisms. The resulting colony morphology and colour were used to do comparisons against test samples. Known bacterial strains were cultured and stored in sterile condition at 2 – 8°C for use as a control in the entire microbial analysis. Confirmation of the identified colonies from each sample was done along with positive and negative control cultures.

3.3.4 Microbiological tests

For evaluation of milk quality, microbial contamination was assessed through estimating total plate count (TPC), coliform plate count (CPC) and Coagulase Positive *Staphylococci* (CPS) count in milk produced in farming systems of the study districts and testing the samples for presence of pathogenic bacteria. In this study, a few selected pathogens including *E. coli*, *Salmonella*, *S. aureus* and *L. monnocytogenes* were targeted.

3.3.4.1 Total plate count

Total plate count (TPC) was determined according to the procedure of ISO 4833-2 standard protocol. The protocol detects all viable microorganisms that can grow aerobically on plate count agar at an appropriate incubation condition of 30 ºC for 72 hours. Plate count agar (PCA) plates were placed at room temperature and labelling of the agar plates was clearly done prior to culturing. From the last dilution (10^{-7}) of milk sample, 0.1 ml was obtained for inoculation of PCA plates in duplicates. The sample was spread on the media surface using a fresh and sterile swab and the plates were allowed to dry with their lids on for 15 minutes. The plates were inverted and incubated at 30ºC under aerobic condition for 72 hours to allow microbial growth (ISO, 2013). The procedure was repeated as above with the other dilutions up to the first dilution and with the remaining test samples, where only consecutive critical dilution steps were chosen for the inoculation of petri dishes. Two consecutive plates with 15 to 300 colonies per plate were considered for record. TPC was determined by manual counting of colonies and was expressed as CFU/ml. The following formula was used in the final estimation of TPC:

$$N = \frac{\Sigma c}{[V \times (n_1 + 0.1n_2)] \times d} \quad \text{.....(1)}$$

Where; N = the number of bacteria,

Σc = the sum of colonies identified on two consecutive dilution steps,

V = the volume of inoculum on each dish, in millilitres,

n_1 = the number of dishes selected at the first dilution,

n_2 = the number of dishes selected at the second dilution,

d = the dilution rate corresponding to the first dilution selected.

3.3.4.2 Detection and enumeration of coliforms

Detection and enumeration of coliforms in the *Enterobacteriaceae* family was carried out according to the procedure of ISO 21528–2: 2004 standard protocol (ISO 2004) through the following stages:

Stage 1: Inoculation and incubation

By means of a fresh and sterile pipette, 1 ml of the test sample was transferred from the serial dilutions (10^{-7} – 10^{-1}) to the media on petri dishes in duplicates. About 10 ml of the Violet Red Bile Glucose (VRBG) agar at 46 ± 2ºC was poured into each petri. The inoculum was mixed with the medium by horizontal movements and allowed to solidify. After complete solidification of the mixture, a covering layer of about 15 ml of the VRBG agar was added to prevent spreading growth and to achieve semi-anaerobic conditions and then allowed to solidify again. Thereafter, the plates were inverted and placed in the incubator at 37 ºC for 24 hours. The procedure was repeated with the remaining test samples where only consecutive critical dilution steps were chosen for the inoculation of petri dishes.

Stage 2: Sub-culturing and identification of coliforms

Five suspected colonies were selected at random from each plate and streaked onto NA plates for biochemical confirmation. Colonies were also streaked onto MA plates in order to stain them for lactose fermentation and hence differentiation of *Enterobacteriaceae*. All plates were incubated at 37 ºC for 24 hours. Thereafter, MA plates were examined for lactose fermentation. Strong lactose fermenting bacteria such as *E. coli*, *Enterobacter* and *Klebsiella* species utilize lactose available in the medium, which produce sufficient acid to cause precipitation of the bile salts also available in the medium, resulting in a pink halo in the medium surrounding individual colonies or areas of confluent growth. Bacteria with

weaker lactose fermentation such as *Serratia* and *Citrobacter spp.* appear pink to red but are not surrounded by a pink halo in the surrounding medium. Lactose non fermenting bacteria such as *Salmonella*, *Proteus* and *Shigella* utilize peptone available in the medium, which forms ammonia that raises the pH of the agar resulting into the formation of colourless colonies on the medium and the agar surrounding the bacteria becomes fairly transparent (Allen, 2005).

Stage 3: Biochemical confirmation of coliforms

Isolated colonies were picked from NA plates for biochemical confirmation. An oxidase test and a glucose fermentation test were performed on each selected colony. In summary, well-isolated colonies were streaked onto oxidase strips (OXOID® Ltd., Basingstoke, U.K.) using sterile plastic loops. The strips were observed for colour change. A positive detection changes into purple colour within 10 seconds. As for glucose fermentation test, a positive reaction changes into yellow colour throughout the contents of the glucose agar tube and sometimes with gas production after 37 ºC incubation for 24 hours. Colonies that were oxidase negative and glucose positive were confirmed as coliform bacteria.

Stage 4: Colony counting and determination of coliform plate count

When all of the selected typical colonies were oxidase negative and glucose fermentation positive, the number of colonies was counted. In the other case the number was calculated as the percentage of oxidase negative and glucose fermentation positive colonies in relation to the total number of selected colonies. Coliform plate count (CPC) was determined using the same formula as that used for the determination of TPC and was expressed as CFU/ ml.

3.3.4.3 Detection and enumeration of Coagulase Positive Staphylococci

This was done according to the European Standard EN ISO 6888-1:1999/A1: 2003, which has the status of a British Standard (BSI, 2003).

Stage 1: Inoculation and incubation

Using a fresh and sterile pipette, 0.1 ml of the test sample was transferred from 10^{-7} – 10^{-1} diluents onto Baird-Parker (BP) agar plates. The sample was spread on the media surface using a fresh sterile swab. The plates were allowed to dry with their lids on for 15 minutes. The plates were then inverted and incubated at 37 ºC for 24 hours. After incubation period, all typical and atypical colonies present were counted and the plates were re-incubated at the same condition for another 24 hours. Thereafter, all typical and atypical colonies present were counted for a second time. The same procedure was used in the remaining samples where only consecutive critical dilution steps were chosen for the inoculation of petri dishes. To estimate Coagulase Positive Staphylococci (CPS, plates with 25 – 250 colonies were selected for reading. 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 sometimes with an opalescent ring due to proteolysis. Atypical colonies are shining black or grey with or without a narrow white edge; the clear zone and opalescent ring are absent.

Stage 2: Coagulase test

Coagulase test was used to test for the production of coagulase enzyme, which determines pathogenicity. An average of three colonies of each typical and atypical colonies were isolated for confirmation. Using a sterile loop an inoculum was removed from the surface of each isolated colony and transferred into a tube containing BHI broth and incubated at 37 ºC for 24 hours. Then 0.1 ml of each culture was aseptically added to 0.2 ml of the

rabbit plasma in Eppendorf tube and incubated at 37 ºC for 4 – 6 hours. Thereafter, the tubes were examined for clotting and if the test was negative, tubes were re-examined 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. As a negative control 0.1 ml of sterile BHI broth was added to 0.2 ml of rabbit plasma and incubated without inoculation. Also, for positive control, 0.1 ml of sterile BHI broth inoculated with a known strain of *S. aureus* was added to 0.2 ml of rabbit plasma and incubated.

Stage 3: Determination of number of Coagulase Positive Staphylococci

For each plate, the number of Coagulase Positive Staphylococci was calculated using the following equation:

$$a = \frac{b_c}{A_c} \times C_c + \frac{b_{nc}}{A_{nc}} \times C_{nc} \quad \text{.....(2)}$$

Where; a = the number of identified coagulase positive staphylococci,

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 shown to be coagulase positive,

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

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

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

As a weighted mean from the two successive dilutions, the number of identified CPS present in the test sample was calculated using the same formula as that used in the determination of TPC and was expressed as CFU/ml.

3.3.4.4 Isolation and identification of *Salmonella spp.*

Identification of *Salmonella spp.* in milk was done according to the ISO 6579:2002 standard protocol (BSI, 2002; BSI 2007) through the following stages:

Stage 1: Selective enrichment of initial cultures

For the selective enrichment of initial culture, the initial suspension was incubated at 37 ºC for 18 hours. Thereafter, 0.1 ml of the culture obtained was transferred to a test tube containing 10 ml RVS broth and another 1 ml of the culture was transferred to a test tube containing 10 ml MKTTn broth. The inoculated RVS and MKTTn broths were incubated at 42 ºC and 37 ºC respectively for 24 hours. The procedure was repeated with the remaining test samples.

Stage 2: Plating out and identification of *Salmonella*

RVS broth culture was inoculated to XLD and MA plates to isolate colonies and stain them for lactose fermentation and similarly for the culture obtained in the MKTTn broth. The plates were inverted and incubated at 37 ºC for 24 hours. After incubation the plates were examined for the presence of typical colonies and atypical colonies that may be *Salmonella* and the ability to ferment lactose on MA. Typical colonies of *Salmonella* are characteristically red with black centers on XLD agar due to xylose fermentation, lysine decarboxylation and production of Hydrogen Sulphide (H_2S). This is the primary differentiation of *Salmonella* from non-pathogenic bacteria. *Salmonella* H_2S negative variants grown on XLD agar are pink with a darker pink centre and Lactose positive *Salmonella* are yellow with or without blackening. Also, red colonies might occur with some *Proteus* and *Pseudomonas* species.

Stage 3: Confirmation of *Salmonella*

Identification of colonies from each XLD agar plate was confirmed using *Salmonella* test kit (OXOID® Ltd., Basingstoke, U.K.). This is a rapid latex agglutination test for the presumptive identification of *Salmonella* (Plate 2). As a negative control, a drop of Salmonella latex reagent was mixed with a drop of 0.85% isotonic saline within one circle on the reaction card and observed for agglutination for two minutes. Also, as a positive control, a drop of *Salmonella* latex reagent was mixed with a smooth suspension of a known *Salmonella spp.* within one circle on the reaction card and observed for agglutination for two minutes. For test cultures, a drop of 0.85% isotonic saline was added within one circle on the reaction card. Suspect colony was emulsified in the drop of saline and the suspension was observed for any agglutination. Thereafter, a drop of *Salmonella* latex reagent was added to the saline suspension. The mixing was done using a clean sterile loop and was examined for agglutination together with positive and negative control for two minutes.

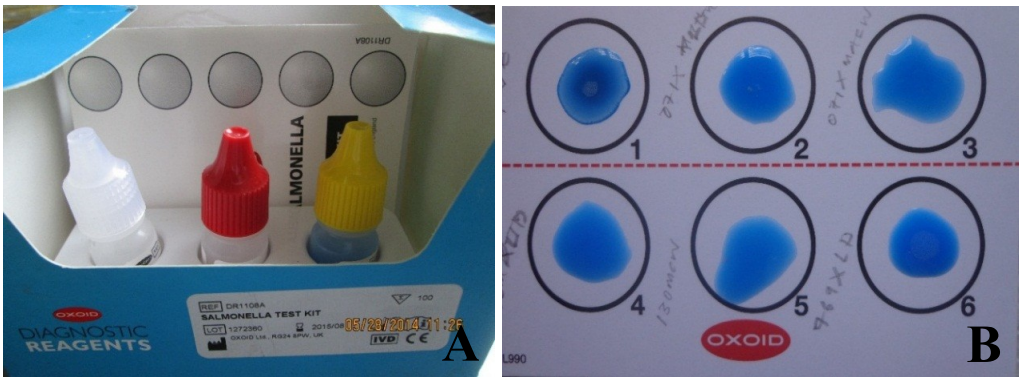

Plate 2: (A) *Salmonella* test kit and (B) Latex agglutination test for the identification of *Salmonella*

3.3.4.5 Isolation and identification of *Escherichia coli*

To isolate and identify *E. coli*, well stained colonies for lactose fermentation, which showed pink colour were picked from MA plates. These isolates were streaked on MA plates and incubated at 37^{o}C for 24 hours to obtain pure colonies. Thereafter, Gram stain test was carried out to characterize the available bacterial organisms by chemical and physical properties of their cell walls. The Gram stain protocol involves the application of a series of dyes that results in some bacteria staining purple and others pink. Biochemical analysis was performed to confirm the presence of *E. coli* using Indole production test.

Gram stain test: The Gram staining of the bacterial colony was done according to the procedure described by the supplier (Remel Inc., Santa Fe Drive, Lenexa, Kansas KS 66215 USA). Suspected colonies were smeared on glass slides and were allowed to air-dry completely. Firstly, these slides were fixed by passing through the flame of a Bunsen burner 3 – 4 times. Secondly, all slides were placed on a staining rack, overlaid with Gram Crystal Violet for one minute and washed thoroughly with water. Then, the slides were overlaid with Gram Iodine mordant for one minute and flooded with Gram Decolourizer for 10 – 30 seconds until the solvent streamed colourless from the slides. Thereafter, the slides were rinsed with water, overlaid with Gram Safranin for 30 seconds, rinsed with water and allowed to dry. Finally, specimens on slides were viewed under oil immersion (1000x) with a bright-field compound microscope (Plate 3). Bacteria that appeared spherical or cocci in shape with pale to dark purple stain were considered Gram-positive, and those that appeared rod or coccobacilli with pink or pale to dark red stain were classified as Gram-negative.

Plate 3: Microscopic examination of *E. coli* cells

Indole test: From the surface of each suspected colony an inoculum was obtained and transferred into a test tube containing tryptophan broth followed by incubation at 37 ºC for 24 hours. Also, positive and negative controls were prepared. To the broth culture, 0.5 ml of Kovac's reagent (Loba Chemie Pvt. Ltd. 107 Wode House Road, Mumbai, India) was added. The formation of a pink to cherry red color in the reagent layer on top of the medium, within seconds of adding the reagent, revealed positive indole test. The reagent layer remained yellow or slightly cloudy, when a culture was indole negative. The presence of a black coloration in the media after incubation indicated lack of H_2S in the media (Cappuccino and Sherman, 2002). Most strains of *E.coli, Proteus vulgaris*, *Klebsiella oxytoca, Citrobacter koseri* and *Providencia spp.* are indole positive. *Salmonella spp., Pseudomonas spp, Citrobacter freundii*, *Proteus mirabilis* and most *Klebsiella spp.* are indole negative (Winn Jr. *et al*., 2006).

3.3.4.6 Isolation and identification of *Listeria spp*

The presence or absence of *Listeria spp*. in test samples was determined by carrying out the tests in accordance with the ISO 11290-1:1997/A1:2005 protocol (AENOR, 2005) through three stages described hereafter.

Stage 1: Primary and secondary enrichment of test samples

In the enrichment stage, 10 ml of the test sample were transferred into a sterile beaker containing 90 ml half strength Fraser broth and the inoculum was incubated at 30ºC for 24 hours. Thereafter, 0.1 ml of the culture was transferred to a test tube containing 10 ml Fraser broth. The test tube was incubated at 37ºC for 48 hours. The procedure was repeated for the remaining test samples.

Stage 2: Plating out and identification of *Listeria*

In the plating stage, the primary enrichment culture previously incubated for 24 hours was inoculated to the surface of *Listeria* Oxford agar and Colorex (CX) *Listeria* agar. The plates were then inverted and incubated at 37ºC for 24 hours. Thereafter, the plates were examined for the presence of colonies. Then, the same plates were again incubated for another 24 hours after which they were examined for the growth of colonies. The procedure was also followed with the secondary enrichment culture. The plates were examined for the presence of colonies supposed to be *Listeria spp.* according to the explanation of media manufacturer (Biomed Diagnostics, Inc. White City, USA). All *Listeria spp.* grown on *Listeria* Oxford agar are characteristically small, brown-green to dark-brown surrounded by black zones due to hydrolysis of aesculin present in the medium. Besides, *L. monocytogenes* and *L. ivanovii* grown on CX Listeria agar are blue-green colonies with well-defined edges surrounded by an opaque, white halo, as the medium contains lecithin substrate, which differentiates these bacteria from other *Listeria spp.* Some strains of *Bacillus cereus* can also grow as blue colonies on CX Listeria agar, but they can simply be distinguished from colonies of *Listeria* since they are much larger with an irregular edge to the colony and very large white halo.

Stage 3: Confirmation of *Listeria*

Four tests namely haemolysis on a sheep blood agar, CAMP, oxidase and *Listeria* test kit were carried out to confirm the presence of *L. monocytogenes* and other *Listeria spp.* in test samples.

Haemolysis on a sheep blood agar: This test was carried out to confirm whether the isolates can destroy the cells and digest the haemoglobin. An isolated colony for each culture was plated and stabbed on one space of sheep blood agar plate. Also, a control culture was stabbed at the same time. The plates were inverted and incubated at 37 ºC for 24 hours. After incubation, the plates were examined in light for revealing signs of α or β-haemolysis in comparison with the control. Discolouration or darkening of the medium after growth indicated that the organism had demonstrated α-haemolysis. If the medium was cleared under growth, the organism was β-haemolytic. Unchange in the color of the medium indicated ɣ-haemolysis.

CAMP test: In the CAMP test synergistic reaction of diffusible substances produced by microorganisms growing adjacent to each other on sheep blood agar results in an enhanced zone of hemolysis in the region between the two cultures. Therefore, known cultures of *S. aureus* (*S. aureus* NCTC 6571/ATCC 9144) and *Rhodococcus equi* (*R. equi* NCTC 1621/ATCC 6939) were streaked in single lines across the sheep blood agar plate parallel and completely opposite to each other. Also, several isolated test strains were streaked in single lines on the same plate at right angles to the two cultures so that the test culture and *S. aureus* and *R. equi* cultures were about 1 – 2 mm apart. Moreover, control cultures were streaked simultaneously. The plates were incubated at 37 ºC for 24 hours while inverted. After incubation, the plates were examined for the contents. An enhanced zone of β-haemolysis at the intersection between the test strain and each of the *S. aureus*

and *R. equi* cultures was considered as a positive reaction. *L. innocua* did not haemolyse the medium. *L. monocytogenes* formed a weak enhanced and small less obvious rectangular zones of β-haemolysis between streaks of test strain and *S. aureus* whereas *L. ivanovii* formed a shovel shape hemolysis between streaks of test strain and *R. equi*.

Oxidase test: This test was undertaken to avoid false reactions that might occur to *Listeria* test kit with oxidase positive cultures. *Listeria spp*. are oxidase negative. Well-isolated colonies were streaked onto oxidase test strips using sterile plastic loops and were observed for colour change within 10 seconds.

***Listeria* test kit**: The kit (Oxoid® Ltd., Basingstoke, Hampshire, England) uses the principle of rapid latex agglutination test for the presumptive identification of *Listeria spp.* (Plate 4). This was performed to test the possibility of existence of rare strains of *L. monocytogenes*, which might not show β-haemolysis or a positive reaction to the CAMP test. *Listeria* latex reagent was used as a negative control whereby one drop was mixed with another drop of saline within one circle on the reaction card and was observed for agglutination for 2 minutes. As a positive control, a drop of *Listeria* latex reagent was mixed with a smooth suspension of the known *L. monocytogenes* within one circle on the reaction card and was observed for agglutination for 2 minutes. For smooth strains of the bacterial isolates, the test was performed by adding a drop of 0.85% isotonic saline within one circle on the reaction card. The suspected colony was emulsified in the drop of saline by using a sterile loop and the suspension was observed for any agglutination. Thereafter, a drop of *Listeria* latex reagent was added to the saline suspension and the mixture was mixed with a clean sterile loop. The suspension mixture was examined for agglutination within two minutes along with positive and negative control. In the case rough strains of the bacterial isolates, non-specific agglutination was demonstrated in normal saline alone.

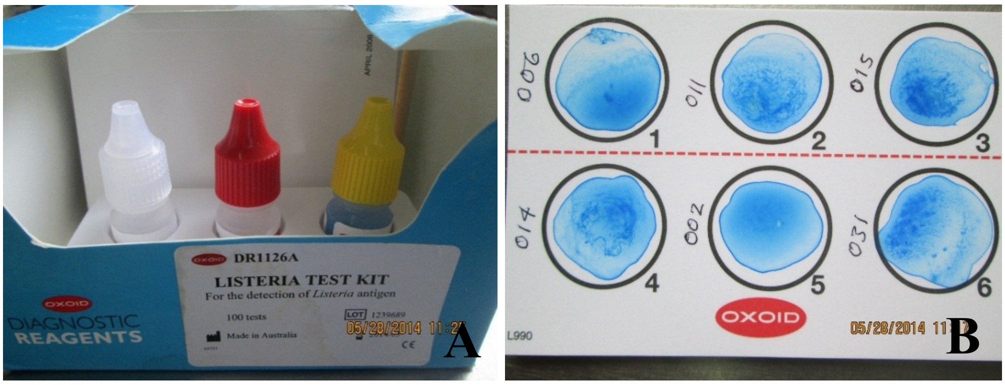

Plate 4: (A) Listeria test kit and (B) Latex agglutination test for the presumptive identification of *Listeria spp*.

3.3.5 Statistical analysis

Two types of analyses were performed in the present study. Firstly, questionnaire data were analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0. The proportions of categorical variables were computed and compared for statistical significance by Pearson's Chi-square test at a Confidence Interval (CI) of 95%. The difference was considered statistically significant at $P < 0.05$. Secondly, three dependent variables namely TPC, CPC and CPS counts were analyzed against independent variables using the General Linear Models (GLM) procedures of the Statistical Analysis System (SAS) version 9.1 for Windows (SAS Institute Inc. Cary, NC 27513, USA). Bacterial counts were normalized by exponential conversion. The counts were used to compute means, standard deviations; minimum and maximum CFU/ml. Independent variables were farming system (F), source (S) and form (T) of milk as shown in the model:

$$Y_{ijkl} = \mu + F_i + S_j + T_k + (FS)_{ij} + (FT)_{ik} + E_{ijkl} \quad \ldots\ldots(3)$$

Where; Y_{ijk} = observed value (TPC/*Enterobacteriaceae* count or CPS count),

μ = Overall mean,

F_i = effect of the i^{th} farming system,

S_j = Effect of the j^{th} source of milk (Household, vendor/trader, restaurant, kiosk or collection centre),

T_k = Effect of the k^{th} form of milk (Raw, boiled, freezed or fermented milk),

$(FS)_{ij}$ and $(FT)_{ik}$ = interactions,

E_{ijkl} = random residual error.

3.4 Consent and Ethical Consideration

Consent to conduct the study was sought from participants in the selected villages before beginning the study. The people involved in the study were informed about the background and objectives of the study, types of data required and collection procedure, how the information would be used and how the confidentiality of participants would be protected. Participation in the study was on voluntary basis. Data collected and results of laboratory microbial analysis of milk samples were under protective care of researchers as confidential.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Characteristics and Distribution of Respondents

Characteristics and distribution of household respondents are presented in Table 3. Of the total household respondents who participated in the study, 83.3% were males and 16.7% were females. Those aged above 45 years comprised 64.8% and the remaining proportion (35.2%) were under 45. Regarding their level of education, the majority (70.4%) had completed only primary education. Most of the respondents were households' heads; a few were either spouses or sons of the heads of the households. All respondents were obtained from villages indicated in Table 3. Regarding characteristics of other categories of respondents (suppliers, vendors and restaurateurs) followed a similar trend as household representatives with some few exceptions. Males constituted a large part of respondents in all categories. The majority of respondents in these categories were under 45 years of age. Regarding education, all respondents had completed primary education except 33.3% of owners of restaurants that had completed secondary education. The majority were heads of the households.

Table 3: Characteristics and distribution of respondents

Demographic information	Category	Households (N = 54)		Suppliers (N = 25)		Vendors (N = 13)		Restaurants (N = 18)	
		n	%	n	%	n	%	n	%
Sex	Males	45	83.3	22	88.0	13	100	13	72.2
	Females	9	16.7	3	12.0	0	0.0	5	27.8
Age	>45 years	35	64.8	6	24.0	3	23.1	8	44.4
	≤45 years	19	35.2	19	76.0	10	76.9	10	55.6
Education	Primary	38	70.4	25	100	13	100	12	66.7
	Secondary	1	1.9	0	0.0	0	0.0	6	33.3
	No formal	15	27.7	0	0.0	0	0.0	0	0.0
Position in the household	Head	39	72.2	13	52.0	7	53.8	10	55.6
	Spouse	9	16.7	4	16.0	0	0.0	6	33.3
	Son	6	11.1	8	32.0	6	46.2	2	11.1
Study villages in Handeni	Kibaya	9	24.3	0	0.0	1	12.5	4	50.0
	Masatu	7	18.9	2	15.3	2	25.0	0	0.0
	Sindeni	8	21.6	3	23.1	3	37.5	2	25.0
	Kwediyamba	3	8.1	5	38.5	2	25.0	1	12.5
	Konje	10	27.0	3	23.1	0	0.0	1	12.5
Study villages in Lushoto	Handei	5	29.4	2	16.7	1	20.0	1	10.0
	Manolo	3	17.7	2	16.7	0	0.0	2	20.0
	Mbokoi	2	11.7	5	41.6	0	0.0	3	30.0
	Mwangoi	3	17.6	1	8.3	2	40.0	3	30.0
	Kwang'wenda	4	23.5	2	16.7	2	40.0	1	10.0

EFS = Extensive farming system, SIFS = Semi intensive/intensive farming system

4.2 Farming Systems, Farm Management and Control of Diseases

Two farming systems namely Extensive farming system (EFS) and Semi intensive/ intensive farming system (SIFS) existed in the study sites at the time of this study. In the EFS majority of animals were TSZ as revealed by 86.5% of the respondents. Farmers in this system owned between 6 and 125 herds of cattle and the majority (75.7%) of farmers were milking between 1 and 10 cows collecting 2 – 20 litres of milk per household per day at the time of the survey. Natural mating was the common method where 10.8% of farmers were obtaining bulls for mating when their herds come into contact with other herds during communal grazing. On communal grazing, animals share water sources and pastures which can be one among the means of disease transmission in cattle herds. Herding was commonly done by males (83.8%). In the SIFS, it was observed that all animals in the participant households have exotic blood dominated by the Friesian x

Indigenous crosses. The total herd size ranged from 1 to 8 crossbred cows, 1 and 2 heifers and 1 and 2 calves. Milk collection was estimated at 2 – 7 litres of milk per cow per day. In an earlier study, Chang'a *et al*. (2010) showed that smallholder farmers in Tanzania were individuals that kept a small number of cattle indoors with the average herd consisting of 3 – 9 crossbred cows. Mating was natural at the time of this study in which breeding bulls were commonly shared between farmers in a village.

Regarding management, most of animals in the EFS were kept in simple shelters built of trees/logs "boma". A few animal houses were made of tree branches and/or woods, concrete floors and roofed with iron sheets. Floors were of poor drainage mainly of mud or earthen. A few farmers provided beddings. In the SIFS floors were mainly concrete and beddings. A similar situation was reported by Shija (2013). It is advised that cattle houses should be designed well and constructed properly in order to protect the health of animals due to the fact that on many instances, the animal sheds are breeding places for microorganisms, flies and mosquitoes, which may attack the animal, causing various kinds of infectious diseases (Sharma, 2009). Wilson *et al.* (1997) observed that poorly designed and unclean animal houses accelerate the transmission of mastitis, especially when milking practices are also poor. Besides, Ruegg (2003) reported that exposure to moisture, mud, and manure in cow housing areas can influence the rate of clinical mastitis thus influencing the quality of milk produced. This would probably be the causes of prevalence of a number of diseases in the study area. Major disease conditions reported to affect cattle in the two farming systems are shown in Figure 3.

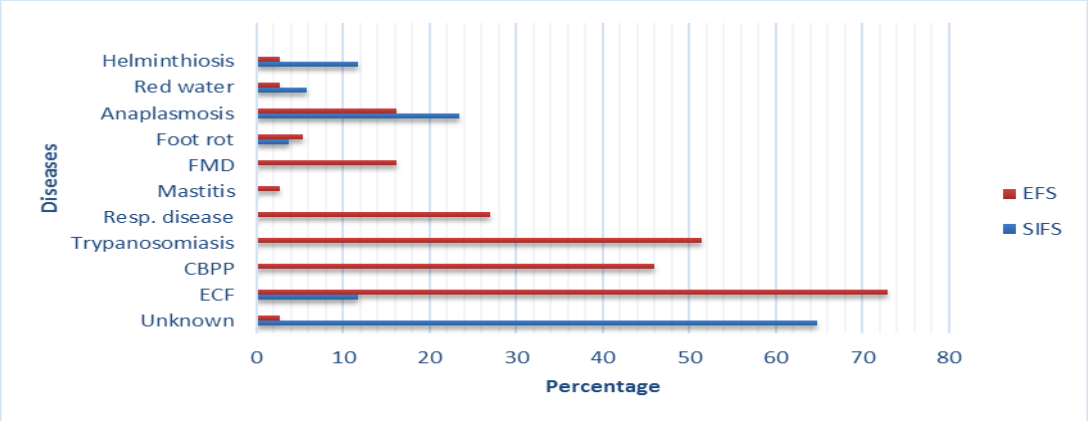

Figure 3: Prevalence of common diseases reported to affect cattle in the two farming systems

Regarding animal health, knapsack spraying and plunge dipping were practiced in both systems to control ticks and external parasites. 32.4% and 41.8% of the household respondents reported to give prophylactic treatment to cattle for helminthiosis in the EFS and SIFS, respectively. A few respondents reported to previously vaccinate cattle against Anthrax and Lumpy skin disease (LSD). Of the interviewed household respondents, 31.5% reported that their cattle herds had been vaccinated by Veterinary officers, 16.7% by animal health attendants and 7.4% by village extension officers. The rest did not vaccinate against diseases. In each village there was an animal health worker administering treatment to the livestock. However, most (68.5%) of the household respondents had complained about high price of veterinary drugs. There was no routine screening for TB and brucellosis. It is advised that all animals in cattle households including Dogs and Cats must be screened and vaccinated against infectious diseases on a regular basis. Also, the awareness on the importance of milk borne zoonoses as diseases of public health and economic concern should be improved through training of farmers (Minja and Latonga, 2003). General information on cattle management and types of animals owned is summarized in Table 4.

Table 4: Cattle management and types of animals owned

		EFS (N = 37)		SIFS (N = 17)	
Factor	Category	n	%	n	%
Cattle breed	TSZ	32	86.5	0	0.0
	Friesian cross	4	10.8	17	100
	Boran cross	1	2.7	0	0.0
Source of breeding bull	Own herd	30	81.1	1	5.8
	Herd contacts	4	10.8	0	0.0
	Neighbours	1	2.7	10	58.8
	Farmers' groups	0	0.0	5	29.4
Type of animal house	Tree/logs "boma"	23	62.2	0	0.0
	Shed	0	0.0	17	100
Floor design	Natural earth	14	37.8	13	76.5
	Deep liter	9	24.3	1	5.8
	Beddings	0	0.0	1	5.8
	Concrete	0	0.0	2	11.8
Prophylactic treatment	Helminthiosis	12	32.4	7	41.8
Disease vaccination	Anthrax	0	0.0	1	5.8
	LSD	4	10.8	2	11.8

4.3 Milking, Milk Handling, Consumption and Awareness on Milk Quality

During the survey, hand milking was the common method of milking practiced in all surveyed households. Safe hand milking steps have been highlighted by Kurwijila (1998) and Sharma (2009). It is important that before milking the hands should be washed using clean water and soap and dried well and fore-stripping should be done to discard the first few strokes of milking in order to avoid milk contamination by extraneous bacteria and allow a quick check for signs of clinical mastitis. Farmers are advised to use pre and post dipping in order to reduce the resident teat skin bacterial population and prevent the transmission of contagious bacteria respectively. The teats of the cow should be dried after washing to avoid milk contamination with water remaining on the teats. Moistening hand in milk, water or oil is not recommended and the technique of pulling teats in milking should be avoided as it can cause irreparable damage to the udder due to the fact that the udder is made by tissues and ligaments. However, most of the farmers were either skipping or not following at all some of the important steps during milking. Some (33.3%)

of them reported using crushes during milking. Only, 55.5% of the interviewed farmers reported that they were washing hands before milking and only 38.9% of them were cleaning the teats of cows before milking. The rest allowed the calf to suckle for a few minutes before milking in order to stimulate the flow of milk and this was regarded as cleaning of teats. All farmers were not fore-stripping on the quarters during milking. Very few (5.6%) farmers reported to dry cow teats after washing. Also, few (12.9%) farmers reported to apply teat-dip. The technique of pulling teats was commonly used by most farmers in hand milking.

It was observed in this study that most of the milk collected was consumed in the households. Whereas 35.2% of respondents reported consumption of actual raw milk, above half (53.7%) reported consumption of fermented raw milk and the rest were boiling the milk before consumption. Surprisingly, milk from sick animals was also reported to be consumed by family members. This was evidenced by consumption of this type of milk by 27.8% of the household respondents. While 42.6% did not milk sick animals, 14.8% were leaving the milk for calves, 9.2% were discarding the milk and the rest were selling the milk.

The behaviour of direct consumption of home produced products such as milk is common in many developing countries including Tanzania (Ndambi *et al*., 2007; RLDC, 2009). Farmers visited in the present study were practicing the same but what was surprising was the consumption of raw milk and milk from sick animals. The consumption of raw and/or raw fermented milk should be avoided as it can pose significant health risks to the consumers due to the fact that most of the milk produced and consumed in rural areas is handled un-hygienically. Moreover, consumption of milk from sick animals should be avoided as it may pose an unnecessary health threat to the consumers due to its possible

contamination with a variety of agents including pathogenic microorganisms. Also, these animals might have been treated with antibiotics and milk should therefore be discarded for the prescribed duration as it might have antibiotic residue and antibiotics, which not only affects the quality of the milk but also the health of consumers (Sharma, 2009).

Bacterial contamination in milk can come from several sources, such as the presence of animal excrement on the animal's skin, the milk containers and even the hands of the workers who milk the animals. The potential pathogens present in raw milk can be diverse, variable, and unpredictable (Oliver *et al*., 2009). It is therefore strongly advised that milk should be boiled before consumption (Claeys *et al*., 2012). Whenever possible, routine health checks for people handling milk should be conducted to ensure good quality milk. However, at the time of this survey there was no routine screening of health status of people who were handling milk. Another possible source of milk contamination can be equipment for handling and storing milk. In the present study 64.8% of the farmers were storing milk in plastic buckets. 12.9% were using metal containers and another 12.9% were using calabashes. However, metal containers were only observed in the SIFS while calabashes were found in the EFS. The storage containers were cleaned on daily basis. Upon direct observation, most (50%) of the milk storage equipment were found to be covered while 14.8% were uncovered. The rest vessels were unknown if they were previously covered. All farmers reported that they did not get formal training on milk handling and marketing. There were no cold storage facilities as milk was being stored at room temperature. This way of storage facilitates growth of microorganisms over time. Water that was being frequently used for watering cattle herds and for sanitary including washing hands, utensils and/or equipment was obtained mainly from unsanitary sources. This might also be causing health problems to the animals and contaminate the milk cratched plastic containers and traditional vessels made out of wood (e.g. calabash), clay

or animal skin are not easily cleanable. Aluminium vessels or stainless steel containers should be used due to the fact that they can't be easily scratched and they are easy to clean (Plate 5). FAO (1995) recommended that farmers' training is important in order to meet the demands of a modem dairy industry and the requirements of the market. It is advised that water for animals and sanitary activities should be obtained from portable sources. Responses on milking, milk handling, consumption and awareness on milk quality are presented in Table 5.

Plate 5: Equipment for handling and storing milk (A) Dirty plastic milking bucket in one of the surveyed households (B) Calabash; is not easily cleanable and (C) Aluminium can and stainless steel bucket; the best materials for milking

Table 5: Milking, milk handling, consumption and awareness on milk quality

Variable	Category	Responses (N=54)	
		n	%
Milking practices	Restraining cow in the milking crush	18	33.3
	Hand washing/disinfection	30	55.5
	Cleaning of cow teats	21	38.9
	Drying of teats	3	5.6
	Teat dip application	7	12.9
Hand milking technique	Squeezing action	2	3.7
	Stripping (Pulling the teat)	49	90.7
Milk consumption habit	Raw milk	19	35.2
	Raw fermented milk	29	53.7
	Boiled milk	6	11.1
Practice when milking cow is sick	Milk the cow and consume at home	15	27.8
	Don't milk the animal	23	42.6
	Leaving the milk for calf	8	14.8
	Discarding the milk	5	9.2
	Selling the milk	3	5.6
Containers used for milk storage	Plastic vessels	35	64.8
	Metal/Aluminium vessels	7	12.9
	Calabash	7	12.9
Milk handling at household	Covered	27	50.0
	Not covered	08	14.8
	Unknown	19	35.2
Source of water	Tap	7	12.9
	Local River	16	29.6
	Wells/boreholes	14	25.9
	Dam/flood water	9	16.7
	Dam/well	3	5.5
	Tap/dam	2	3.7
	Local River/well	1	1.8

4.4 Quality of Milk at the Supplier Node

Milk suppliers were identified as the agents who were bulking milk from farms and providing in bulky quantities to the retailers and milk collection centres. Findings of this survey have shown that 72% of the milk handled by suppliers is obtained from the farmers. A few received milk from own farms. When receiving milk, as large as 64% of

the respondents were not undertaking any quality check. Only 28% were checking milk using lactometers. The rest were pouring portions of milk to the ground and examined for adulteration. Plastic filters were normally used for removing solid matters when collecting milk from the farmers and plastic containers were commonly used for collection, storage and transportation of milk and 68% of the respondents were mixing milk from different sources. Milk transportation to final destinations was mainly (48%) done using bicycles, some (36%) on foot and the remaining 16% were using motorcycles. The process between collection and delivery was taking 2 – 5 hours. The milk was commonly stored at room temperature until completion of selling. According to Omore *et al*. (2005), pooling of milk from different sources without quality checks increases the risk of infection with milk-borne zoonoses especially among people who drink raw milk. Thus, bulked milk should be processed or screened for potential infections before selling. Also, storing milk at room temperature for a long time should be avoided as it facilitates growth of microorganisms thus reducing its safety (Omore *et al.*, 2005). The milk has to be produced as hygienic as possible and should be cooled or heated at the earliest to prevent a too high multiplication of bacteria (Pandey and Voskuil, 2011).

Also, results indicated that 88% of respondents were washing empty containers using soap and hot water, 56% were using washing powder. In general, 60% of containers were observed to be in dirty condition. Washing of hands was commonly done using soap and cold water and the water used for sanitary measures was mainly obtained from unsanitary sources.

4.5 Quality of Milk at the Vendor Node

Milk vendors were selling milk direct to consumers in streets. The main source of milk for the vendors was reported to be farmers (69.2%). All respondents understood that the

quality of milk was mostly related to cleanliness of containers and milking practice at farm level. However, only 23.1% of the interviewed vendors were checking the milk for adulteration using lactometers. Also, 23.1% were checking the milk for adulteration through pouring portions of it to the ground. All respondents filtered milk after receiving it from the farmers and before selling to consumers. Materials used by vendors for collection, storage and transportation of milk were plastic containers. Most (69.2%) vendors were mixing milk from different farms. The most common means of transportation was the bicycle (61.5%) followed by walking (23.1%) and use of motorcycle (15.4%). At this node of the value chain the time estimated from milk collection to sale was between 2 – 7 hours. However, milk was commonly stored at room temperature until completion of selling. Only 7.7% of the interviewed vendors were washing containers using soap and cold water. It was also observed that majority (76.9%) of respondents were using washing powder during cleaning of milk containers. Taken together, 92.3% of the milk containers were found to be in dirty condition. Washing of hands was commonly done using soap and cold water.

Although farmers discard milk on ground to check quality this practice has many errors and might not correctly check milk purity. Proven rapid methods such as lactometer and alcohol test can be used for checking the quality of milk at a low cost. Also, characteristically, plastic containers are unsuitable for milk handling. These can be easily scratched and are difficult to clean thus provide hiding places for bacteria. Moreover, plastic containers are poor conductor of heat and hence may hinder effective sanitization by heat (Addis *et al*., 2011) and based on makeup and design they are difficult to sanitize especially in the inner corners and bottom (Plate 6A). In such a situation, microorganisms can rapidly build up in milk residues in storage containers, and may subsequently contaminate the milk (Shija, 2013). Plastic containers may affect the quality of milk by

bringing in bad odour (Bukuku, 2013). Moreover, mixing milk from different sources in one container as evidenced by vendors in the present study is not a healthy practice. Single spoiled milk can spoil all bulked milk causing an economic loss. Also, storing milk at room temperature might have a significant influence on bacterial load. Temperature of storage is an important factor in determining milk quality as this influences the rate at which the bacteria will increase in number (Omore *et al.*, 2005). The use of washing powder might affect the quality of milk by bringing in smell in case the observed plastic containers are not rinsed properly.

Portioning of milk was found to be done by scooping using plastic jugs. However, the serving jugs were hanged naked in open air or immersed into large partially cleaned containers and touched by unsanitary handlers (Plate 6B and C). Mwangi *et al*. (2000) suggested that some practices in the informal markets, such as scooping of milk and use of plastic containers, could be improved by extension and training. This is recommendable due to the fact that most of the respondents in the present study had low formal education and were lacking training on milk handling.

Another aspect that was found to compromise the safety of milk at this node was the water used for cleaning and washing hands and vessels. In general, 53.8% of respondents reported using tap water. Some (23.1%) were using water from wells and the rest (23.1%) were using water from constructed dams and rain water. Water obtained from these sources was used for washing of hands, equipment and/or utensils. It is advised that water used in sanitary activities along the milk chain should always be obtained from clean sources as it can be easily polluted by environmental organisms.

Plate 6: Containers used by communities (A) Closed containers (B) Serving jug immersed in milk container and (C) Serving jug touched by unsanitary hands of a vendor while being used

4.6 Awareness of Restaurateurs on Milk Quality

Of all respondents, 72% reported receiving milk from suppliers, 16.7% obtained the milk from own farms and 11.1% were receiving milk direct from farmers. Only 50% of respondents were checking for the quality of milk at receiving, 38% were using visual and smell examination whereas 11.1% were boiling the milk to check for coagulation. Plastic containers were commonly used in handling of milk. Regarding washing, 88.9% washed using soap and hot water and of these 50% were using washing powder. 50% of the respondents reported using water from inferior sources such as wells, dams, rain water and Local River. Direct observation indicated that 27.8% of the selling points were not meeting an ordinary standard for milk collection standards. Milk received by restaurateurs was mainly used for tea making or was boiled fresh for customers. General handling practices of milk among the suppliers, vendors and restaurateurs are presented in Table 6.

Table 6: General handling practices of milk among the suppliers, vendors and restaurateurs

Parameter	Category	Suppliers (N = 25)		Vendors (N = 13)		Restaurateurs (N = 18)	
		n	%	n	%	n	%
Source of milk	Farmers	18	72.0	9	69.2	2	11.1
	Own farm	7	28.0	4	30.8	3	16.7
	Suppliers	0	0.0	0	0.0	13	72.2
Quality assurance	Lactometer	7	28.0	3	23.1	1	5.6
	Clot on boiling	0	0.0	0	0.0	2	11.1
	Pour on ground	1	4.0	3	23.1	0	0.0
	Visual and smell	0	0.0	0	0.0	7	38.8
	None	16	64.0	7	53.8	9	50.0
Pooling of milk	Yes	17	68.0	9	69.2	13	72.2
	No	8	32.0	4	30.8	5	27.8
Transportation	Bicycle	12	48.0	8	61.5	2	11.1
	Motorcycle	4	16.0	2	15.4	1	5.6
	On foot	9	36.0	3	23.1	14	77.7
	Vehicle	0	0.0	0	0.0	1	5.6
Milk containers	Plastic containers	25	100	13	100	15	83.3
	Thermos flask	0	0.0	0	0.0	2	11.1
	Glass bottle	0	0.0	0	0.0	1	5.6
Container cleaning	Hot water and soap	22	88.0	12	92.3	16	88.9
	Cold water and soap	3	12.0	1	7.7	2	11.1
	Bar soap	11	44.0	3	23.1	9	50.0
	Washing powder	14	56.0	10	76.9	9	50.0
Washing hands	Soap and cold water	25	100	13	100	18	100
Status of containers	Clean	10	40.0	1	7.7	14	77.8
	Dirty	15	60.0	12	92.3	4	22.2
Source of water	Tap	9	36.0	7	53.8	9	50.0
	Wells/bore holes	5	20.0	3	23.1	3	16.7
	Dam	2	8.0	1	7.7	3	16.7
	Rain water & dam	5	20.0	2	15.4	2	11.1
	Local River	4	16.0	0	0.0	1	5.5

4.7 Milk Handling Practices at the Collection Centres

The collection centres reported to receive between 1000 and 3000 litres of milk per day depending on the season. Based on direct observation, it was found that plastic buckets were commonly used to collect the milk before transferring into bulk tanks. Quality checks were done using lactometer and alcohol tests (Plate 7A). The accepted milk was cooled in bulk tanks until time of transportation to final destinations. Refrigerated vehicles were being used to transport bulked milk. One collection centre based in Bumbuli division

had no cooling facilities. Hence, bulked milk was being transported some distance to the cooling tank at another collection centre based in Lushoto town. Also, one collection centre was found in Handeni district. This was causing farmers and milk suppliers from far villages to travel for a long time to deliver their milk. Washing powder was commonly used in cleaning of equipment. Surprisingly, few storage plastic containers that were used by farmers and milk suppliers to deliver milk to the collection centres were found to be fitted using plastic bags (Plate 7B). Transportation of milk for a long period without cooling might influence bacterial load, which can spoil it. It is advised that before transportation milk should be cooled immediately to preserve its original quality safe for processing and consumption. Also, the collection centres should be placed nearby potential production areas to shorten milk delivery period. Fitting of milk storage containers with plastic bags must be avoided as it contaminates the milk making it unsafe for consumption.

Plate 7: (A) Milk quality check – Lactometer (B) Storage plastic container fitted with plastic bag

4.8 Bacteriological Quality of Milk Along the Value Chain

To evaluate the bacteriological quality a total of 114 milk samples were cultured for bacterial count. However, 73.7% of the samples, which had bacteria grown within the

range that can be counted as recommended by the ISO protocol were examined. These were evaluated for TPC, CPC and CPS count (Plate 8). The evaluations were done based on farming systems, sources and forms of milk samples as well as the interaction among these factors.

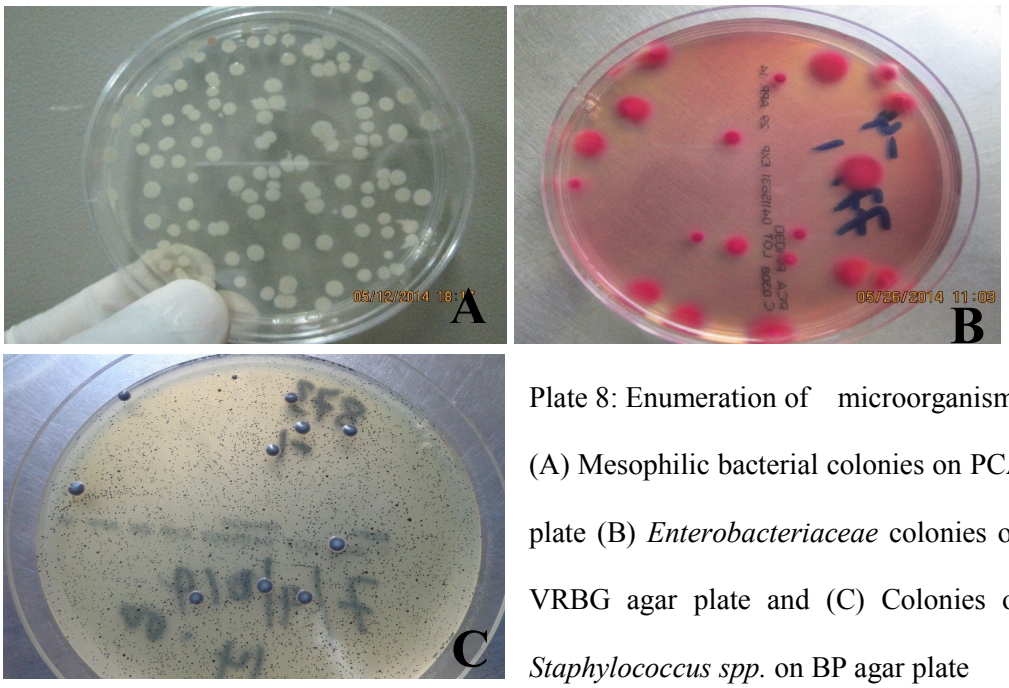

Plate 8: Enumeration of microorganisms (A) Mesophilic bacterial colonies on PCA plate (B) *Enterobacteriaceae* colonies on VRBG agar plate and (C) Colonies of *Staphylococcus spp.* on BP agar plate

4.8.1 Total plate count

An overall mean TPC of $1.7x10^7 \pm 7.8x10^7$ CFU/ml was obtained, which is higher than the EAC maximum recommended level. Standard deviation values were observed to be larger due to majority of deviations far from the mean. Mean TPC value for each node of the value chain is presented in Table 7. The present results indicate that milk from SIFS had greater TPC compared to that from EFS. Similarly, milk from households had greater TPC than the other nodes of the value chain. Regarding the form, raw milk had greater TPC compared to boiled milk. However, TPC values between farming systems and between the sources of milk were not statistically significant different ($P > 0.05$).

Table 7: Mean total plate counts in milk from selected nodes of the value chain

Factor	Variable	N	Mean CFU/ml	Std. Dev. CFU/ml	Min. CFU/ml	Max. CFU/ml	P-value
Farming system	EFS	40	6.4×10^6	1.3×10^7	3.4×10^4	7.0×10^7	0.6739^{ns}
	SIFS	40	2.8×10^7	1.1×10^8	3.6×10^4	6.4×10^8	
Source of milk	Households	30	4.2×10^7	1.2×10^8	3.4×10^4	6.4×10^8	0.2141^{ns}
	Suppliers	24	2.6×10^6	4.9×10^6	3.6×10^4	2.5×10^7	
	Restaurants	16	2.4×10^6	1.5×10^6	3.5×10^5	4.9×10^6	
	Street vendors	10	1.7×10^6	1.7×10^6	1.2×10^5	5.6×10^6	
Form of milk	Raw	71	1.9×10^7	8.3×10^7	3.4×10^4	6.4×10^8	0.9906^{ns}
	Boiled	9	2.2×10^6	1.5×10^6	3.5×10^5	4.5×10^6	

$^{ns}(P > 0.05)$

Regarding quality, the overall results indicated that more than 90% of all handled milk samples were above the EAC maximum acceptable standard of 2.0×10^5 CFU/ml in raw milk intended for further processing (EAS, 2007). This is an indication that most of milk produced in the study areas are of poor bacteriological quality. In earlier studies elsewhere in Tanzania (Kivaria *et al*., 2006; Rwehumbiza *et al*., 2013; Shija, 2013) as well as other African countries (Addo *et al*., 2011; Tassew and Seifu, 2011; Mosu *et al*., 2013) similar situation has been reported. Poor bacteriological quality of milk in the study area could be contributed by unhygienic milking, poor milk handling practices and poor animal management practices including unclean udder and teats caused by manure, soil, feed, personnel and water; unhygienic milking, unsanitary facilities and utensils and/or use of inferior water for washing and drinking as well as poor storage conditions, which needs attention of actors of the chain and the public (Khan *et al*., 2011).

4.8.2 Coliform plate count

Coliform plate count (CPC) was also evaluated in the present study. As shown in Table 8, mean values for CPC were greater in EFS than SIFS, in households than suppliers, restaurants, street vendors and in raw than in boiled milk. There was no statistical significance in the CPC mean values between farming systems, sources and forms of

milk, and interaction (P > 0.05). The overall mean CPC was $1.8x10^6 \pm 6.2x10^6$ CFU/ml, which was above the EAC maximum acceptable standard of 5.0 x 10^4 CFU/ml (EAS, 2007). Standard deviation values were also observed to be larger because of majority deviations far from the mean.

Table 8: Mean coliform plate counts in milk from selected nodes of the value chain

Factor	Variable	N	Mean CFU/ml	Std. Dev. CFU/ml	Min. CFU/ml	Max. CFU/ml	P-value
Farming system	EFS	25	$1.9x10^6$	$6.6x10^6$	$1.4x10^3$	$3.2x10^7$	0.9726^{ns}
	SIFS	18	$1.7x10^6$	$5.8x10^6$	$1.8x10^3$	$2.5x10^7$	
Source of milk	Households	17	$4.3x10^6$	$9.5x10^6$	$7.7x10^3$	$3.2x10^7$	0.3804^{ns}
	Suppliers	10	$5.2x10^5$	$1.3x10^6$	$1.4x10^3$	$4.2x10^6$	
	Restaurants	12	$1.3x10^5$	$9.9x10^4$	$1.4x10^3$	$2.5x10^5$	
	Street vendors	4	$2.1x10^4$	$2.8x10^4$	$1.4x10^3$	$6.2x10^4$	
Form of milk	Raw	35	$2.2x10^6$	$6.8x10^6$	$1.4x10^3$	$3.2x10^7$	0.9971^{ns}
	Boiled	8	$1.3x10^5$	$1.1x10^5$	$1.4x10^3$	$2.5x10^5$	

^{ns}(P > 0.05)

The above results indicate poor microbial quality of milk, which may be due to poor hygiene and improper handling of milk. Other factors include unhygienic environment and poor general milk handling (Shija, 2013). Bonfoh *et al*. (2006) reported that udder infection, water quality, hygiene behaviour in relation to hand washing, cleaning and disinfection of containers are key factors that contribute to such contaminations in non-industrialized milk production. Generally, CPC greater than 100 CFU/ml would indicate poor milking hygiene (Boor *et al*., 1998).

4.8.3 Coagulase Positive *Staphylococci* count

In the samples evaluated for bacteriological quality one contained CPS isolate. This sample had a CPS count of $5.1x10^5$ CFU/ml and was sampled from the EFS. CPS isolate obtained in the present study indicates a possible risk to Staphylococcal poisoning in milk in the study area. Literature suggest that CPS count above $1x10^5$ CFU/ml is enough for

the occurrence of milk staphylococcal poisoning (Nádia *et al*., 2012). Elsewhere Staphylococcal poisoning has been reported. Tebaldi *et al*. (2008) and Mattos *et al*. (2010) reported contamination of *S. aureus* above $1x10^5$ CFU/ml in refrigerated raw milk. In Brazil, Nádia *et al*. (2012) reported reasonably lower CPS counts of $1.1x10^3$ and $2.3x10^2$ CFU/ml in milk samples from two dairy herds.

4.9 Identification of Bacteria in Milk Along the Value Chain

To identify bacteria in milk, 114 milk samples were cultured to isolate pathogens particularly those, which are of public and veterinary interests. Identified bacteria were *Enterobacteriaceae* including *E. coli, Klebsiella spp*. and *Proteus spp*. as well as *S. aureus* and *Listeria* species including *L. innocua, L. ivanovii and L. monocytogenes*. All samples tested negative for *Salmonella*. Other microorganisms included *Staphylococcus spp*., *Enterococcus faecalis*, *Bacillus cereus* and *Pseudomonas spp.* Proportionate of bacteria isolated in milk along the value chain are shown in Table 9.

Table 9: Distribution of bacterial contaminants along the milk value chain

	Bacterial isolates																	
	Enterobacteriaceae		*E. coli*		*S. aureus*		*L. innocua*		*L. ivanovii*		*L. monocytogenes*		*Klebsiella spp.*		*Proteus spp.*		CNS		*B. cereus*	
Variable	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
EFS (N = 67)	27	40.3	5	7.5	1	1.5	10	14.9	1	1.5	34	50.7	6	8.9	4	5.9	37	55.2	1	1.5
SIFS (N = 47)	19	40.4	2	4.3	0	0.0	3	6.4	1	2.1	14	29.8	1	2.1	1	2.1	20	42.6	1	2.1
Total (N = 114)	46	40.4	7	6.1	1	0.9	13	11.4	2	1.8	48	42.1	7	6.1	5	4.4	57	50.0	2	1.8
P-value	0.9891^{ns}		0.4826^{ns}		0.4002^{ns}		0.1578^{ns}		0.7993^{ns}		0.0257*		0.1350^{ns}		0.3240^{ns}		0.1829^{ns}		0.7993^{ns}	
Households	18	15.8	3	2.6	1	0.9	6	5.3	1	0.9	25	21.9	0	0.0	5	4.4	28	24.5	1	0.9
Suppliers	10	8.8	3	2.6	0	0.0	3	2.6	0	0.0	9	7.9	4	3.5	0	0.0	14	12.3	0	0.0
Street vendors	4	3.5	1	0.9	0	0.0	2	1.8	0	0.0	6	5.3	1	0.9	0	0.0	4	3.5	0	0.0
Restaurants	12	10.5	0	0.0	0	0.0	1	0.9	0	0.0	7	6.1	2	1.8	0	0.0	10	8.8	0	0.0
Collection centres	2	1.7	0	0.0	0	0.0	1	0.9	1	0.9	1	0.9	0	0.0	0	0.0	1	0.9	1	0.9
Raw	38	33.4	7	6.1	0	0.0	12	10.5	2	1.8	47	41.2	7	6.1	5	4.4	51	44.7	2	1.8
Boiled	8	7.0	0	0.0	0	0.0	01	0.9	0	0.0	1	0.9	0	0.0	0	0.0	6	5.3	0	0.0

*^{ns}(P > 0.05), *(P < 0.05)*

Identification of these bacteria in the Tanzanian milk value chain suggests possible prevalence of a number of bacteria in the milk and supplement information already available. Although previous studies in Tanzania have indicated prevalence of pathogens in milk, findings of this study therefore share more promising evidence on the microbiological status of milk in the Tanzanian milk value chain. In the list of identified pathogens, *Listeria spp.* ranks first. These bacterial organisms were identified in above 50% of the cultured milk samples, which is a high prevalence. *L. monocytogenes* was more (42.1%) prevalent followed by *L. innocua* (11.4%) and *L. ivanovii* (1.8%). The isolates of *Listeria spp.* are shown on Plate 9.

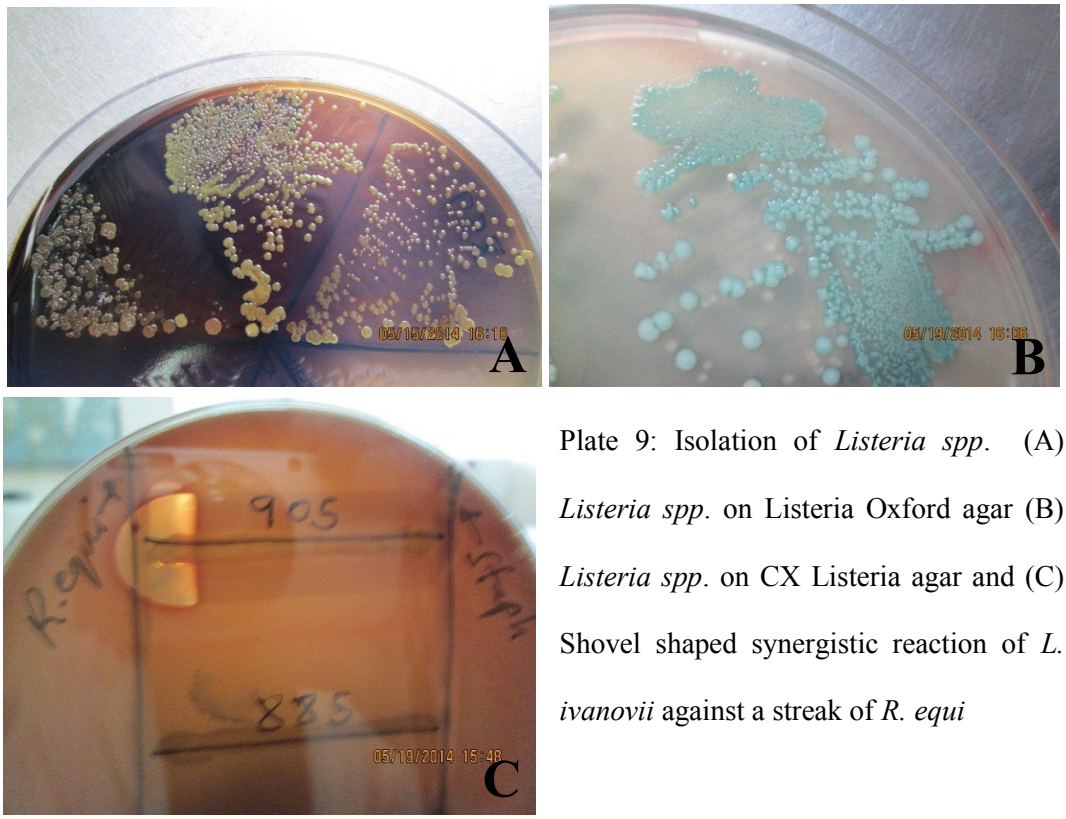

Plate 9: Isolation of *Listeria spp*. (A) *Listeria spp*. on Listeria Oxford agar (B) *Listeria spp*. on CX Listeria agar and (C) Shovel shaped synergistic reaction of *L. ivanovii* against a streak of *R. equi*

Second in the rank were *Enterobactericeae*, which were identified in 40.4% of the cultured milk samples presenting another reasonably high prevalence of members of this family in milk in the Tanzanian milk value chain. In this family *E. coli spp.* was further

identified, which was confirmed by indole test (Plate 10). This bacterium species was present in 6.1% of the samples. The presence of *E. coli* in milk indicates existence of enteropathogenic microorganisms, which are of public health concerns. There were no *E. coli* isolates in milk from street vendors and the collection centres.

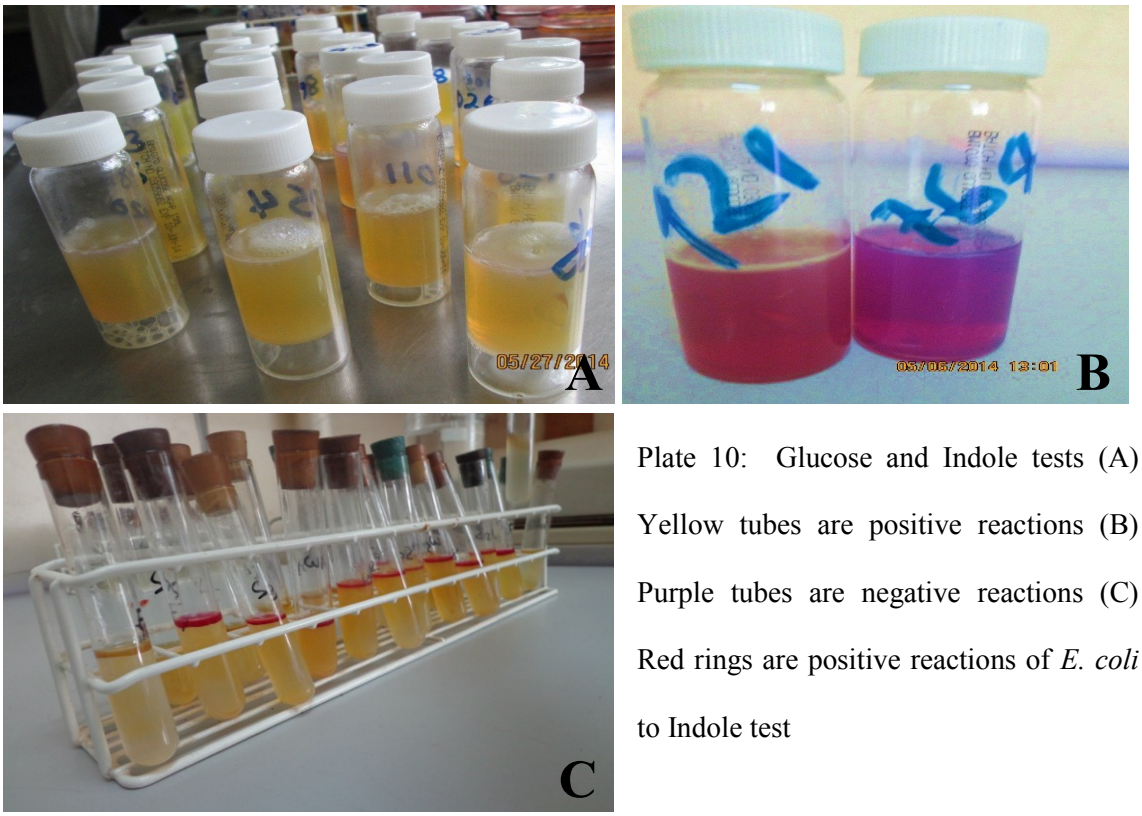

Plate 10: Glucose and Indole tests (A) Yellow tubes are positive reactions (B) Purple tubes are negative reactions (C) Red rings are positive reactions of *E. coli* to Indole test

Regarding CPS, which was confirmed using coagulase test, was in the lowest occurrence of 0.9% in milk obtained from the study area. There were more bacterial pathogens in milk from the EFS compared to the SIFS. However, no statistical significant difference ($P > 0.05$) was observed on the prevalence of bacterial organisms in milk between the two farming systems except for *L. monocytogenes*. Basing on the nodes of the milk value chain, results indicate that there was greater prevalence at households for all microbial organisms compared to other nodes. Also, a great umber was observed at the supplier and restaurateur nodes. There was low number of bacterial isolates in milk from the collection

centres. Based on Salmonella test kit, there were no *Salmonella* isolated from the milk culture. Also, regarding the forms of milk, there were more bacterial isolates in raw milk compared to boiled milk.

Prevalence of bacterial pathogens in milk value chain in the present study is challenging and needs attention of all actors in the chain. According to Matofali *et al*. (2013), as the the food goes through many handlers and middlemen and women, the risk of exposing the food to unhygienic environments, contamination and adulteration increases. *Enterobacteriaceae* includes member such as coliforms, which are often used as indicators of faecal contamination and other strains that commonly exist in the environment are used as hygiene indicator microorganisms because they are most commonly associated with manure or environmental contamination. Some genera such as *Klebsiella* and *Citrobacter* are psychrotrophic and may increase 100 to 1000 fold within 72 hours of milk storage at less than 7°C. In some circumstances such as consumption of unpasteurized or un-boiled raw milk, the presence of these bacteria and other enteric microorganisms in milk could result in spoilage and severe human disease. Members of the family *Enterobacteriaceae* have been considered a potent cause of foodborne outbreaks (Centinkaya *et al*., 2008). Hence, identification of factors that determine milk contamination is important in order to protect human health and improve the quality of milk produced and supplied along the value chain (Pantoja *et al.*, 2011). In one study in South Africa *Enterobacteriaceae* represented 46% of the isolates Nyenje *et al*., 2012).

Regarding *E. coli*, several strains of this bacterium species can cause severe diarrhea and vomiting in infants, and young children. However, the presence of the species of *E. coli* itself in milk, as a possible cause of food borne disease, is not significant as *E. coli* is normally a ubiquitous organism, yet the pathogenic strains if present could be harmful to

consumers (Ahmad *et al*., 2011). Percentages obtained in the present study were lower when compared with the prevalence of 65% and 73% reported by Thaker *et al*. (2012) and Ahmad *et al*. (2011) in India and Pakistan respectively. Omore *et al.* (2001) isolated *E. coli* O157:H7 in 1% of the samples in milk markets survey in the Kenyan highlands. Also, Kang'ethe *et al*. (2007) isolated *E. coli* O157:H7 from cattle faeces in urban and peri-urban settings of Nairobi, Kenya. In Tanzania, *E. coli* O157:H7 has not been isolated in raw milk (Swai and Schoonman, 2011; Shija, 2013). Similarly, Addo *et al.* (2011) reported negative results on *E. coli* O157:H7 in raw milk in Ghana. Some other verocytotoxigenic *E. coli* serotypes such as the EHEC serogroups O26, O111 and O103 may be pathogenic in both humans and animals (OIE, 2008). Further studies should be conducted to investigate presence of EHEC class in milk and completely eliminate the occurrence of toxins produced by *E. coli* (Swai and Schoonman, 2011).

Although *Salmonella* was not isolated in milk in the present study, it is the most common foodborne pathogen worldwide (Forshell and Wierup, 2006). Thus, there should be vigilance in maintaining standard hygiene and periodic screening for food contamination against this bacterial organism. Elsewhere in South Africa (Nyenje *et al*., 2012) and Zimbabwe (Mhone *et al*., 2012) there has been similar findings. However, Vigano *et al*. (2007); Dagmar *et al*. (2013) and Lubote *et al*. (2014) reported *Salmonella* prevalence of 11%, 10.1% and 37.3% respectively, in bovine milk samples in Tanzania. Besides, studies reports from USA by Van Kessel *et al*. (2004) and Karns *et al*. (2005) showed that 2.6% and 11.8% of bulk tank milk samples were culture positive for *Salmonella*.

On CPS, pathogenicity of the *Staphylococcus* species was confirmed and revealed by coagulase test. The incidence of CPS isolates was lower in the present study compared with that reported by Addis *et al*. (2011), who obtained CPS prevalence of 23.5% in raw

milk in Debre Zeit, Ethiopia. In Dodoma and Morogoro regions, Tanzania Karimuribo *et al*. (2005) reported high levels of enterotoxigenic *Staphylococcus* species at a prevalence of 35.3% in milk from pastoral herds. In Dar es Salaam, Kilango (2011) established a prevalence of 23.19% of *S. aureus* in milk from farmers in Temeke Municipality. Also, Smith *et al*. (2007) obtained the value of 54% in bovine mastitis milk isolates and Salandra *et al.* (2008) established the value of 55.9% from dairy products in Italy. In other studies, Tsegmed (2006) obtained Staphylococcal enterotoxin in 19% of the 26 investigated *S. aureus* strains. Although the CPS prevalence obtained in the present study was low, raw milk may carry a potential risk of poisoning along the value chain, if the milk is subject to conditions and storage temperatures conducive to the multiplication of CPS, with subsequent production of enterotoxins (Nádia *et al*., 2012). Inappropriate handling of raw milk could result in bacterial growth and substantially increase the potential risk to consumers of raw milk and raw milk products. Thus vigilance in maintaining hygienic conditions in milking and along the milk value chain is of crucial importance (Van Kessel *et al*., 2004).

Three strains of *Listeria spp*. namely *L. innocua*, *L. ivanovii* and *L. monocytogenes* were confirmed in the present study. *Listeria spp*. forms the largest prevalence of all species identified in this study. *Listeria* cases have been reported by previous authors Worldwide. In a study in Nigeria (Yakubu *et al*., 2012), *L. innocua* was detected in 51.3% of 39.58% isolated *Listeria spp.*, which is a higher value than what was obtained in the present study. In another recent study in Syria by Al-Mariri *et al*. (2013), the bacterium was detected in 17.8% of 10.96% isolates of *Listeria spp.*, which is lower than what is reported in the present study. *L. innocua* is important because it is very similar to the food-borne pathogen *L. monocytogenes* but non-pathogenic in character due to the fact that it lacks the 10-kb virulence locus that is needed for pathogenicity (Abee *et al*., 2004). Although,

the bacterium is non-pathogenic its presence in milk could influence the microbial load resulting to milk of poor microbiological quality. Milk contamination by this bacterial organism should be avoided through maintaining the standard hygiene at all steps of the value chain.

Concerning *L. ivanovii*, its prevalence in bulk milk at the collection centre may be due to contamination from the environment during milking, transportation and storage along with udder infection (Sarangi *et al*., 2009). Al-Mariri *et al*. (2013) reported isolation of 10.96 % of *Listeria s*pp. from milk in Syria, which contained 14.3% *L. ivanovii* similar to the results obtained in extensive farming system but lower than that obtained in semi-intensive/intensive farming system, in the present study. However, other investigators from Nigeria found a higher incidence of *L. ivanovii*, 18.4% of 39.58% isolates of *Listeria spp.* in raw milk from cattle herds (Yakubu *et al*., 2012). *L. ivanovii* is circumscribed to causing disease in ruminants, which is associated with eating spoiled silage or hay suggesting foodborne origin (Gaya *et al*., 1996). It shares certain characteristics with *L. monocytogenes* (e.g., hemolysis) and is occasionally associated with abortion in ruminants (Czuprynski *et al*., 2010). However, *L. ivanovii*, has been previously isolated from infected humans indicating pathogenic potential (Nyenje *et al*., 2012). Guillet *et al*. (2010) reported that *L. ivanovii* can also cause bacteremia in immunocompromised, debilitated patients, but the associated infection by this microorganism is extremely rare in humans. Therefore the isolation of *L. ivanovii* in the present study might reflect a health risk to the public.

In this study *L. monocytogenes* showed the greatest prevalence led by the EFS on one hand and household samples on the other hand. Similar studies on the prevalence of *Listeria* in raw milk from different parts of the world have provided results ranging from

higher to lower than the findings in the present study. Jackson *et al*., (2012) reported isolation of *L. monocytogenes* in 99 (49%) of 202 raw milk samples. In Spain Vardar-ünlü *et al*. (1998) reported higher levels of *L. monocytogenes* in the milk ranging from 44.7% to 45.3%. Moreover, Mugampoza *et al*. (2011) reported a low prevalence of 13% in raw milk in Uganda. Also, isolation of *L. monocytogenes* was reported by Yakubu *et al*. (2012), Al-Mariri *et al*. (2013) and Jamali *et al.* (2013) in other places. Moreover, Warke *et al*. (2007), Kalorey *et al.* (2007), Aurora *et al.* (2006) and Varder-Unlu *et al.* (1998) have reported as low as 4.7%, 5.1%, 1.69%, and 4% of *Listeria* isolates in raw milk respectively. Literature suggest that varying environmental condition between, sampling season, sampling occasion as well as method of detection may alter prevalence (Sarangi *et al*., 2009). Among all species of *Listeria, L. monocytogenes* has been reported as the leading cause of human listeriosis.

Even though high rates of contamination of milk with *L. monocytogenes* have been reported, listeriosis is a relatively rare disease as compared with other common foodborne infections. However, because of its high case fatality rate of approximately 20 – 30%, listeriosis has been ranked second, after Salmonellosis as the most recurrent cause of foodborne infection-related deaths in Europe (Nyenje *et al*., 2012). *L. monoctogenes* infection in ruminants is associated with eating spoiled silage or hay, as happens with *L. ivanovii*, suggesting foodborne origin. The incidence of *Listeria* species in animal feed might be a risk factor for presence of *Listeria* in the farm environment, cow infections, their presence in milk and thus also in human body causing infections (Czuprynski *et al*., 2010). It is advised that feeding ruminants with spoiled silage or hay should be avoided as it may expose the animals to infection by *Listeria* species. Surprisingly, bacterial organisms were isolated in boiled milk.

The presence of microorganisms in boiled milk could be due to insufficient boiling, poor personal hygiene handling, dirty utensils among others (Kitagwa *et al.,* 2006).

4.9.1 Other microorganisms

Based on colony morphology and gram stain other bacteria include *Klebsiella spp*., *Proteus spp., Staphylococcus spp*., *Enterococcus faecalis*, *Bacillus cereus* and *Pseudomonas spp.* The possible causes of these bacterial contaminations in milk could be due to either unhygienic handling practices or mastitis cows. Coagulase Negative Staphylococci (CNS) was the most common microorganism found in majority of milk samples. The high (50%) proportion of CNS isolated in the current study may be due to the fact that they are part of the normal teat skin flora and mucosa of humans and animals and some of the species are found free living in the environment. In addition, unpasteurized raw milk might have CNS if the cow suffers from mastitis of CNS. An overall CNS prevalence of 16% in raw bovine milk has been reported in Ethiopia (Addis *et al*., 2011), which was lower than the investigation in the current study. Also, *Staphlylococcus spp., Bacillus spp. Proteus spp.* and *Coliforms* such as *Pseudomonas aeruginosa, Klebsiella spp*. *Enterobacter spp*. and *E. coli* also have been reported by Karimuribo *et al*. (2005) and Knutson *et al*. (2010). Common bacterial species isolated in this study are shown on Plate 11.

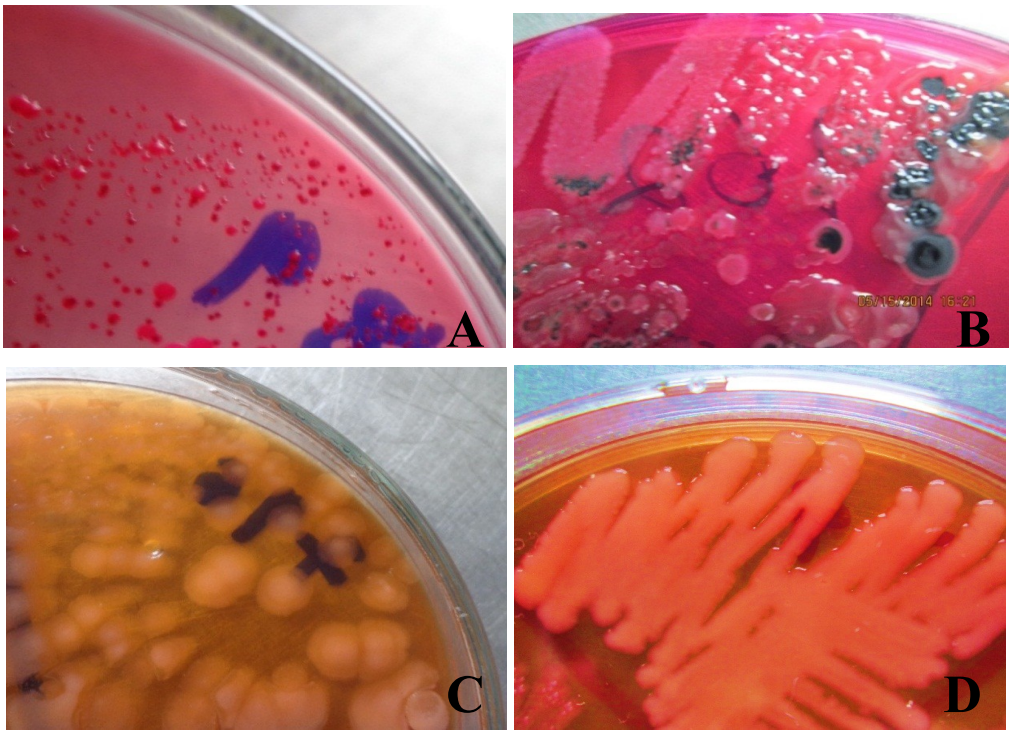

Plate 11: Common bacterial isolates (A) *Enterococcus faecalis* on MA plate (B) *Proteus spp.* on XLD agar plate (C) *Pseudomonas spp.* on MA plate and (D) *Klebsiella spp*. on MA plate

4.10 Possible Sources of Milk Microbial Hazards in the Milk Value Chain

The results of the current study have indicated that conditions for bacterial contaminations of milk at the selected critical points were diseased animals due to poor husbandry practices, less hygienic pre and post milking practices, poor sanitation practices associated with milking and storage containers, source of water used in sanitary activities, pooling of milk, storage conditions and time, cleanliness of the selling points and sub-optimal hygiene of milk handlers. Similar findings have been reported in recent studies in Tanzania (Swai and Schoonman, 2011; Bukuku, 2013 and Shija, 2013) and elsewhere (Omore *et al*., 2005; Oliver *et al*., 2009 and Mosalagae *et al*., 2011). According to Knutson *et al*. (2010), the origins of potentially harmful microorganisms in raw milk lie in both the milk as it is excreted and in subsequent contamination during the time of

collection, processing, distribution and storage. The milking procedure, subsequent pooling and the storage of milk carry the risks of further contamination from man or the environment or growth of inherent pathogens. Thus, hygienic handling of milk throughout the value chain is essential to ensure the safety and suitability of milk for consumption.

Also, the study found that most of the people at nodes of the value chain had no formal training on safe food handling, which suggests that they may have limited knowledge on food hygiene. Education of food handlers in the principles of safe food handling is an essential step towards reducing the incidence of food-borne diseases resulting from cross-contaminations during handling of foods (WHO, 2005). Inadequate hygiene training and/or instruction of all people involved in food related activities pose a potential threat to the safety of food and its suitability for consumption (Kitagwa *et al*., 2006). It is therefore important that all personnel will be aware of their role and responsibility in protecting food from contamination or deterioration (Kilango, 2011).

The results on milk handling practices indicated that most of the farmers were either skipping or not following at all some of the important steps during milking. The steps such as fore-stripping and teat-dipping were not followed at all which was influencing microbial contaminations in milk. Also, the type of milk containers used by most of the respondents for milk storage, handling and transportation was of poor quality as per Tanzania Bureau of Standards (TBS) and international (Codex) standards for milk handling. These non-food grade plastic containers were influencing high microbial load in milk (Kivaria *et al*., 2006). The plastic containers can thus be a source of *B. cereus* endospores and other similar kinds of bacteria in milk (Shija, 2013). Moreover, the use of plastic bags in fitting lids of milk buckets and scooping of milk were among the causal factors of microbial contaminations in milk.

Water is known to be a potential source of milk microbial contamination. Water supplies is not a problem if an approved piped supply is available. Otherwise it must be assumed that water is contaminated and therefore it should be boiled or hypochlorite should be added at the rate of 50 parts per million (ppm) to the cleaning water (FAO, 1989). When water from non-tap sources is used for cleaning purpose, it is important that the handlers should at least filter and heat treat it before use (Yilma, 2012).

Pooling of milk from different sources without quality checks was causing bacterial pathogens in milk from one of the sources to grow and multiply in bulked milk, hence spreading the risk to many people upon consumption of the milk. Also, in the present study, it was found that milk was kept at room temperature for a long time during storage and delivery. Equally, Kivaria *et al*. (2006) reported that lack of cold chain, long time for delivery, poor milk handling and transportation, account for the high microbial load in milk. The transport and collection of the surplus milk to the point of sale or processing should not take very long to minimize post-harvest spoilage (Pandey and Voskuil, 2011).

Moreover, identified dirty restaurant premises might be causing microbial contaminations in milk received at those points. Restaurants provide chances for outbreaks of foodborne disease since large quantities of various foods are handled in the same kitchen. Also, failure to wash hands, utensils or countertops could lead to contamination of foods including milk. Furthermore, due to poor hygienic conditions of all handlers brought from unwashed hands and dirty clothes, they might unknowingly introduce pathogenic microorganisms into the milk. Thus, unhygienic handling of milk may have contributed a lot of its contamination. However, in this study, there was no statistical significant association between the high bacterial load in milk and most of the unhygienic practices that were observed ($P > 0.05$). Only, the statistical significant association ($P < 0.05$) was

observed between dirty milk containers and high bacterial load in milk from the suppliers, street vendors and restaurants (Appendix 6).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the findings of this study it can be concluded that:

i. Milk produced and handled along the milk value chain in Handeni and Lushoto districts is of poor quality and hazardous for human consumption and can be a potential source of milk-borne infections.

ii. There is high prevalence of *L. monocytogenes* in milk produced and supplied along the value chain, which raises a public health concern about its safety to consumers.

iii. Farm management practices that would affect the occurrence of zoonotic pathogens in milk are poor and managerial factors such as unhygienic housing systems, contaminated feeds and animal diseases are fundamental conditions affecting the quality of milk.

iv. Handling practices towards pre and post milking, sanitation associated with milking and storage containers, storage conditions and time and hygiene of people are poor and has greater influence on the microbial contamination of raw milk.

v. Most of the sources of water used in sanitary activities along the milk value chain are poor, which contributes to microbial contamination of milk.

vi. Majority of people mainly in the extensive farming system consume raw and/or raw fermented milk, which can result into health problems. This is supported by evidence of milk-borne zoonotic pathogens isolated in this study, which provides an insight into the magnitude on health risks associated with consumption of raw milk.

vii. The organization of milk supply chains, dysfunctional state of the regulatory agencies and quality control structures are compromizing the hygienic quality of milk along the value chain, which predispose the public to risk of contracting milk-borne infections.

5.2 Recommendations

In view of the conclusions above, it is therefore recommended that:

i. Animal husbandry practices should be improved to control microorganisms from lactating animals, environment and equipment by adhering to general hygiene practices and environmental cleanliness.

ii. All actors along the milk value chain should be organized and educated to increase their awareness on management of animals, general milk handling, milk hygiene and commercialization of milk.

iii. Limited awareness on health risks associated with consumption of raw and/or raw fermented milk needs to be spoken by responsible authorities such as Tanzania Food and Drugs Authority (TFDA) and Prime Minister Office – Regional Authority and Local Government (PMO-RALG) health sector and the existing regulations must be instituted in order to safeguard community health.

iv. Sector policies, organizational structures and support services for farmers and other actors must be properly concentrated in order to stimulate dairy sector development particularly by strengthening the dominant informal sector.

v. Further studies should be conducted to investigate presence of other *verocytotoxigenic E. coli* serotypes such as the EHEC serogroups O26, O111 and O103 and *L. monocytogenes* in milk, which are pathogenic for both humans and animals and confirm their prevalence.

vi. The research into public health risks in milk should continue and in particular consideration should be focused at the cultural and milk handling practices likely to predispose humans to infectious diseases through consumption of various traditional milk and milk products which are so common in various parts of the country.

REFERENCES

Abee, T., van Schaik, W. and Siezen, R. J. (2004). Impact of genomics on microbial food safety. Review article. *Trends in Biotechnology* 22 (12): 653 – 660.

Addis, M., Pal, M. and Kyule, M. N. (2011). Isolation and Identification of *Staphylococcus* species from Raw Bovine Milk in Debre Zeit, Ethiopia. *Medwell Journals. Veterinary Research* 4(2): 45 – 49.

Addo, K. K., Mensah, G. I., Anning, K. G., Nartey, N., Nipah, G. K., Bonsu, C., Akye, M. L. and Smit, H. L. (2011). Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana. *Tropical Medicine and International Health* 16(2): 227 – 232.

Al-Mariri, A., Younes, A. A. and Ramadan, L. (2013). Prevalence of *Listeria* spp. in raw milk in Syria. *Bulgarian Journal Veterinary Medicine* 16(2): 112−122.

AENOR (Spanish Association for Standardization and Certification). (2005). UNE-EN ISO 11290-1:1997/A1:2005 – Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method – Amendment 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data (ISO 11290-1:1996/AM1:2004). 1 – 18pp.

Ahlstrom, C. (2011). Molecular ecology of *Listeria spp*., *Salmonella, Escherichia coli* O157:H7 and non-O157 SHIGA toxin producing *E. coli* in Northern Colorado wilderness areas. Dissertation submitted in partial fulfillment of the requirements for the Degree of Master of Science at Colorado State University, Fort Collins, Colorado, USA. 1 – 63pp.

Ahmad, N., Ubaid-ur-Rahman, Ali, L., Malik, A. P., Safeer, M. and Ubaidullah (2011). Contamination of raw milk with *Escherichia coli* sold in Peshawar University Campus and adjacent area, Pakistan. 3(2): 72 – 75pp.

Allen, M. E. (2005). MacConkey agar plates protocol. MicrobeLibrary Atlas Protocol Project [*http://www.microbelibrary.org/edzine/details.asp?id=1977*]. Site visited on 19/7/2014.

Anand, S. K. and Griffiths, M. W. (2011). Pathogens in milk/*Enterobacteriaceae*. Encyclopedia of Dairy Sciences, 2nd Edition. Elsevier B.V. 67 – 71pp.

Aurora, A., Prakash, A. and Prakash, S. (2006). Occurrence of pathogenic *Listeria monocytogenes* in raw milk and ready to eat milk products in Agra city, India. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* 27(2): 87 – 93.

Bauman, D. E. (2004). The enhancement of milk fat with fatty acids of importance in human health maintenances and diseases prevention. [*http//vivo.cornell.edu/ entity?home=&id=5502*]. Site visited on 25/8/2013.

Baylis, C. (2009). Raw milk and raw milk cheeses as vehicles for infection by verocytotoxin-producing *Escherichia coli*. *International Journal of Dairy Technology* 62: 293 – 307pp.

Betts, R. and Blackburn, C. W. (2009). Detecting pathogens in food. In: Foodborne pathogens: hazards, risk analysis and control, 2nd Edn. Edited by: Blackburn, C. W. and McClure P. J. WoodheadPublishing, Oxford, UK. 17 – 65pp.

Bisi-Johnson, M. A., Obi, C. L., Vasaikar, S. D., Baba, K. A. and Hattori, T. (2011). Molecular basis of virulence in clinical isolates of *Escherichia coli* and *Salmonella* Species from a tertiary hospital in the Eastern Cape, South Africa Gut Pathogens 3(1): 9 – 12.

Blodgett, R. (2010). Most probable number from serial dilutions. Bacteriological Analytical Manual Appendix2. [*http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm109656.htm*]. Site visited on 1/9/2013.

Bonfoh, B., Roth, C., Traoré, A. N., Fané, A., Simbé, C. F., Alfaroukh, I. O., Nicolet, J., Farah, Z. and Zinsstag, J. (2006). Effect of washing and disinfecting containers on the microbiological quality of fresh milk sold in Bamako (Mali). *Journal of Food Control* 17: 153 – 161.

Bonfoh, B., Wasem, A., Traoré, A. N, Fané, A., Spillmann, H., Simbé, C. F., Alfaroukh, I. O., Nicolet, J., Farah, Z. and Zinsstag, J. (2003). Microbiological quality of cow's milk taken at different intervals from the udder to the selling point in Bamako (Mali). *Journal of Food Control* 14: 495 – 500.

Boor, K. J., Brown, D. P., Murphy, S. C., Kozlowski, S. M. and Bandler, D. K. (1998). Microbiological and chemical quality of raw milk in New York State. *Journal of Dairy Science* 81: 1743 – 1748.

Brenner, Don J., Krieg, N. R. and Stanley, J. T. (2005). Bergey's Manual of Systematic Bacteriology 2B (2nd Ed.). New York: Springer. 1108pp.

BSI (British Standards Institute). (2002). BS EN ISO 6579: incorporating Corrigendum No. 1. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella spp*. London: BSI. 1 – 27pp.

BSI (British Standards Institute). (2003). BS EN ISO 6888-1: Incorporating Amendment No. 1 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). 2003 Edition. London: BSI. 1 – 13pp.

BSI (British Standards Institute). (2007). BS EN ISO 6785. Milk and milk products. Detection *of Salmonella spp.* 2007 Edition. London: BSI. 1 – 34pp.

Bukuku, J. N. (2013). Awareness of Health risks as a result of consumption of raw milk in Arusha city and Meru district, Tanzania. Dissertation for Award of MSc. Degree at Sokoine University of Agriculture. Morogoro, Tanzania. 1 – 54pp.

Cappuccino, J. G. and Sherman, N. (2002). *Microbiology*. A laboratory manual. 6th edition. Pearson education inc. San Francisco, California. 215 – 224pp.

Centinkaya, F., Cibik, G., Soyuteniz, E., Ozkin, C., Kayali, R. and Levent, B. (2008). *Shigella* and *Salmonella* contamination in various foodstuffs in Turkey. *Journal of Food Control* 19: 1059 – 1063.

Chang'a, J. S., Mdegela, R., Ryoba, R., Løken, T. and Reksen, O. (2010). Calf Health and Management in Smallholder Dairy Farms in Tanzania. *Journal of Tropical Animal Health and Production* 42: 1669-1676.

Chatterjee, S. N., Bhattacharjee, I., Chatterjee, S. K. and Chandra, G. (2006). Microbial examination of milk in Tarakeswar, India with special reference to coliforms. *African Journal of Biotechnology* 5(15): 1383–1385.

Claeys, W. L., Cardoen, S., Daube, G., De Block, J., Dewettinck, K., Dierick, K., De Zutter, L., Huyghebaert, A., Imberechts, H., Thiange, P., Vandenplas, Y. and Herman, L. (2012). Raw or heated cow milk consumption: Review of risks and benefits. *Journal of Food Control* 31: 251 – 262.

Clark, C. G., Farber, J., Pagotto, F., Ciampa, N., Dore, K., Nadon, C., Bernard, K. and Ng, L. K. (2010). Surveillance for *Listeria monocytogenes* and listeriosis in Canada, 1995–2004. *American Journal of Epidemiology and Infectious Disease* 138: 559 – 572pp.

Czuprynski, C. J., Kathariou, S. and Poulsen, K. (2010). "Listeria," in *Pathogenesis of Bacterial Infections in Animals*, Wiley-Blackwell, 4th edition. 167 – 187pp.

Dagmar, S., Andreas, M., Benedict, L. and John, L. (2013). Microbiological Quality of Milk in Tanzania: From Maasai Stable to African Consumer Table. *Journal of Food Protection* 11: 1824 – 1993.

Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S. and Courbois, C. (1999). *Livestock to 2020 the Next Food Revolution*. Food, Agriculture, and the Environment Discussion Paper 28. International Food Policy Research Institute. 79pp.

Dinges, M. M., Orwin, P. M. and Schlievert, P. M. (2000). Exotoxins of Staphylococcus aureus. *Clinical Microbiology Reviews* 13: 16 - 34.

EAS (East African Community Standard). (2006). EAS 67: 2000 Raw cow milk — Specification [*http://www.dairyafrica.com/documents/EAS%2067%20raw%20milk%20cleaned.doc*]. Site visited on 24/8/2013.

EAS (East African Community Standard). (2007). EAS 67:2007 Raw Cow Milk — Specification. 1 – 19pp.

FAO (Food and Agriculture Organization). (1989). Milking, milk production hygiene and udder health. FAO animal production and health paper 78. Edited by Akam, D. N., Dodd, F. H. and Quick, A. J. Rome, Italy. 119pp.

FAO (Food and Agriculture Organization). (1995). Milk supply to urban centres in Tanzania with particular reference to the city of Dar es Salaam – General overview by L.R. Kurwijila, and Henriksen, J. Rome, Italy. [*http*://*www. fao.org/docrep/x5661e/x5661 e07.htm*]. Site visited on 23/8/20124.

FAO (Food and Agriculture Organization). (2005). Developing Countries and the Global Dairy Sector. Part I – Global Overview, by V. Knips. Rome, Italy. 2pp.

FAO (Food and Agriculture Organization). (2006). Considerations of Food Safety and Consumer Protection. [*http://www.fao.org/docrep/006/y8705e/y8705e09.htm*] Site visited on 25/8/2013.

FAO (Food and Agriculture Organization). (2010a). Status of and Prospects for Smallholder Milk Production – A Global Perspective, by T. Hemme and J. Otte. Rome. 1 – 5pp.

FAO (Food and Agriculture Organization). (2010b). Recommendations for Smallholder Dairy Development. [*http://www.thedairysite.com/articles/2527/recommendations-for-smallholder-airy*-development]. Site visited on 29/7/2013.

Forshell, L. P. and Wierup, M. (2006). Salmonella contamination: a significant challenge to the global marketing of animal food products. *Scientific and Technical Review of the Office International des Epizooties*. 25(2): 541 – 554pp.

Frey, R. J. and Sherk, S. D. (2006). Enterobacterial Infections. Gale Encyclopedia of Children's Health: Infancy through Adolescence. [*http://www.encyclopedia.com/doc/1G2-3447200215.html*]. Site visited on 29/7/2013.

Fsihi, H., Steffen, P. and Cossart, P. (2001). Listeria monocytogenes. In E. A. Groisman (ed.), Principles of Bacterial Pathogenesis. Academic Press, San Diego. CA: Academic Press. 751–803pp.

Gaya, P., Saralegui, C., Medina, M. and Nuñez, M. (1996). Occurrence of *Listeria monocytogenes* and other *Listeria spp*. in raw caprine milk. *Journal of Dairy Science* 79: 1936 – 1941.

Goff, H. D. and Hill, A. R. (1993). Chemistry and physics. In Y. H. Hui (Ed.), Dairy Science and Technology Handbook. Principles and properties, Vol. 1. VCH Publishers, New York. NY: John Wiley and Sons. 1 – 82pp.

Gopalakurup, P. T. (2002). Clean milk production and marketing of milk from the farmers' perspective. *Indian dairyman: journal of the Indian Dairy Science Association* 54: 92 – 94.

Guillet, C., Join-Lambert, O., Le Monnier, A., Leclercq, A., Mechaï, F., Mamzer-Bruneel, M. F., Bielecka, M. K., Scortti, M., Disson, O., Berche, P., Vazquez-Boland, J., Lortholary, O. and Lecuit, M. (2010). Human listeriosis caused by *Listeria ivanovii*. *Journal of Emerging Infectious Diseases* 16(1): 136 – 138.

Häsler, B., Fornace, K., El Tolth, M. and Rushton, J. (2013). Rapid assessment of nutrition and health risks in informal dairy value chains in Tanzania. Tanzania report. 17 – 18pp.

Hayes, M. C., Ralyea, R. D., Murphy, S. C., Carey, N. R., Scarlett, J. M. and Boor, K. J. (2001). Identification and characterization of elevated microbial counts in bulk tank raw milk. *Journal of Dairy Science*. 84: 292 – 298.

Hudson, J. A. (2010). Evaluation of methods for detection of Coagulase Positive Staphylococcus and Staphylococcal toxin in Milk and Cheese. New Zealand Food Safety Authority. Report. 1 – 30pp.

ISO (International Organization for Standardization). (1999). ISO 6887-1. Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions. 1st ed., 1999. International Organization for Standardization, Geneva, Switzerland. 1 – 5pp.

ISO (International Organization for Standardization). (2004). Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony count method. 1st ed., 2004. International Organization for Standardization, Geneva, Switzerland. 1 – 9pp.

ISO (International Organization for Standardization). (2013). Microbiology of the food chain — Horizontal method for the enumeration of microorganisms – Part 2: Colony count at 30 °C by the surface plating technique. 1st ed., 2013. ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*. International Organization for Standardization, Geneva, Switzerland. 1 – 20pp.

Jackson, E. E., Erten, E. S., Maddi, N., Graham, T. E., Larkin, J. W., Blodgett, R. J., Schlesser, Joseph, J. E. and Reddy, R. M. (2012). Detection and Enumeration of Four Foodborne Pathogens in Raw Commingled Silo Milk in the United States. *Journal of Food Protection*. 75(8): 1382 – 1393pp.

Jamali, H., Radmehr, B. and Thong, K. L. (2013). Prevalence, characterisation, and antimicrobial resistance of *Listeria* species and *Listeria monocytogenes* isolates from raw milk in farm bulk tanks. *Journal of Food Control* 34(1): 121 – 125.

Joseph, E. (2014). Asssessment of Microbiological Hazards along the Milk Value Chain in Kilosa and Mvomero Districts - Tanzania. Dissertation for Award of MSc. Degree at Sokoine University of Agriculture, Morogoro, Tanzania. 30 – 51pp.

Kalorey, D. R., Kurkure, N. V., Warke, S. R. and Barbuddhe, S. B. (2007). Evaluation of indirect and avidin-biotin enzyme linked immunosorbent assays for detection of anti-listeriolysin O antibodies in bovine milk samples. *Journal of Zoonoses and Public* Health 54(8): 301 – 306.

Kang'ethe E. K., Arimi, S. M., Omore, A. O., McDermott, J. J., Nduhiu, J. G., Macharia, J. K. and Githua, A. (2000). The prevalence of antibodies to *Brucella abortus* in marketed milk in Kenya and its public health implications. *In. Proceeding of the 3rd All Africa Conference on Animal Agriculture*, 6 – 9 November 2000, Alexandria, Egypt. 25 – 30pp.

Kang'ethe, E. K., Onono, J. O., MacDermott, B. and Arimi, S. M. (2007). Isolation of *E. coli* O157:H7 from milk and cattle faeces from urban dairy farming and non-dairy farming neighbour households in Dagoretti Division, Nairobi, Kenya: prevalence and risk factors. *East African Medical Journal* 84: 65 – 75.

Kaplinsky, R. and Morris, M. (2000). 'A Handbook for Value Chain Research', prepared for the IDRC, Institute of Development Studies: Sussex. 4pp.

Karimuribo, E. D., Kusiluka, L. J., Mdegela, R. H., Kapaga, A. M., Sindato, C. and Kambarage, D. M. (2005). Studies on mastitis, milk quality and health risks associated with consumption of milk from pastoral herds in Dodoma and Morogoro regions, Tanzania. *Journal of Veterinary Science* 6(3): 213 – 221.

Karns, J. S., Van Kessel, J. S., McCluskey, B. J. and Perdue, M. L. (2005). Prevalence of Salmonella enterica in bulk tank milk from US dairies as determined by polymerase chain reaction. *Journal of Dairy Science* 88(10): 3475 – 3479.

Keller, S. E. and Miller, A. J. (2006). Microbiological safety of fresh citrus and apple juices. In: Sapers, G. M., Gorny, J. R. and Yousef, A. E. (Eds.) (2006). Microbiology of fruit and vegetables. Boca Raton, FL: CRC Press Taylor and Francis Group. 211 – 224pp.

Khan, A. A., Massod, F. A. and Bhat, B. A. (2011). Bacteriological quality and safety of raw milk in Kashmir valley. *Wayamba Journal of Animal Science* 3: 2102 – 5789.

Kilango, K. G. (2011). Food Safety in Milk Markets of Smallholder Farmers in Tanzania: A Case of Peri Urban Wards in Temeke Municipality. Dissertation for Award of MSc. Degree at Sokoine University of Agriculture, Morogoro, Tanzania. 6 – 58pp.

Kitagwa, W. G. I., Bekker, J. L. and Onyango, R. O. (2006). The influence of knowledge, attitudes and practices of food handlers on food kiosk hygiene. Eldoret, Kenya. *Environment and Health International* 8(2): 19 – 29.

Kivaria, F. M., Noordhuizen, J. P. T. M. and Kapanga, A. M. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region, Tanzania. *Journal of Tropical Animal Health Production*, 38: 185 – 194.

Knutson, D. R., Currier, W. R., Ribera, L. and Goeringer, P. (2010). Asymmetry in raw milk safety perceptions and information: Implications for risk in fresh produce marketing and policy. In: *The Economics of Food, Food Choice and Health.* (Edited by Knutson, D. R. *et al*.), 15 – 17 September 2010, Freising, Germany. 1 – 22pp.

Kostić, T. and Sessitsch, A. (2012). Microbial Diagnostic Microarrays for the Detection and Typing of Food- and Water-Borne (Bacterial) Pathogens. Review 1: 3 –24pp.

Kurwijila, L. R. (1998). Dairy Processing marketing in Tanzania: Lessons and Future Challenges *Tanzania Veterinary journal* 18(4): 192 – 208.

Kurwijila, L. R., Joseph, W. E., Omore, A., Mdoe, N. S. Y., Mnenwa, K. R., Staal, S., Kazwala, R. R. and Burton, D. (2003). Assessment of Public Health Hazards Associated with Informal Milk Marketing in Tanzania. SUA – ILRI Milk Marketing and Public Health Project Proc. Final Stakeholder Workshop, VETA Chang'ombe, 5 March, 2002. 1 – 26pp.

Latimer Jr., G. W. (Ed.) (2012). Official Methods of Analysis of the Association of AOAC International. 19th Ed. Association of Official Analytical Chemists (AOAC). Washington DC. USA 17: 1 – 279pp.

López-Campos, G., Martínez-Suárez, J. V., Aguado-Urda, M. and López-Alonso, V. (2012). Detection, Identification, and Analysis of Foodborne Pathogens; In. *Microarray Detection and Characterization of Bacterial Foodborne Pathogens* (Edited by López-Campos, G., Martínez-Suárez, J. V., Aguado-Urda, M., López-Alonso, V.), 2012, Springer Briefs in Food, Health and Nutrition. 23pp.

Lubote, R., Shahada, F. and Matemu, A. (2014). Prevalence of Salmonella spp. and Escherichia coli in raw milk value chain in Arusha, Tanzania. *American Journal of Research Communication* 2(9): 1 – 13.

Mangwayana, E., Giller, K. E. and Baggs, E. M. (2000). Food Safety in Horticultural Markets. [*http://www.dfid.gov.uk/r4d/PDF/Outputs/R7519c.pdf*]. Site visited 25/8/2013.

Matofari, J. W., Shalo, P. L., Younan, M., Nanua, J. N., Adongo, A., Qabale, G. and Misiko, B. N. (2013). Analysis of microbial quality and safety of camel (*Camelus dromedarius*) milk chain and implications in Kenya. *Journal of Agricultural Extension and Rural Development* 5(3): 50 – 54.

Mattos, M. R., Beloti, V., Tamanini, R., Magnani, D. F., Nero, L. A., Barros, M. A. F., Pires, E. M. F. and Paquereau, B.P.D. (2010). Quality of raw milk produced in agresteregion of Pernambuco, Brazil. *Semina: Ciências Agrárias* 31 (1): 173 – 182.

Mhone, T. A., Matope, G. and Saidi, P. T. (2012). Detection of *Salmonella spp*., *Candida albicans*, *Aspergillus spp.,* and antimicrobial residues in raw and processed cow milk from selected small holder farms of Zimbabwe. *Veterinary Medicine International* 2: 1 – 5.

Minja, M.G. and Latonga, P.M. (Unpublished, 2003) Improving Processing, Marketing Efficiency and Reducing Public Health Risks in Hai District, Northern Tanzania. Final Technical Report. Tarp II Project. pp 10 – 14.

MoAC (Ministry of Agriculture and Co-operatives)/SUA (Sokoine University of Agriculture)/ ILRI (International Livestock Research Institute). (1998). Rapid appraisal of the Tanzania dairy subsector: *Executive summary.* ILRI, Nairobi, Kenya. 1 : 224pp.

Mosalagae, D., Pfukenyi, D. M. and Matope, G. (2011). Milk producer's awareness of milk-bornezoonoses in selected smallholder and commercial dairy farms of Zimbabwe. *Tropical Animal Health and Production* 43: 733 – 739.

Mosu, S., Megersa, M., Muhie Y., Gebremedin, D. and Keskes, S. (2013). Bacteriological quality of bovine raw milk at selected dairy farms in Debre Zeit town, Ethiopia. *Journal of Food Science and Technology Research* 1(1): 1 – 8.

Mugampoza, D., Muyanja, C. M. B. K., Ogwok, P., Serunjogi, M. L. and Nasinyama, G.W. (2011). Occurrence of *Listeria monocytogenes* in bulked raw milk and traditionally fermented dairy products in Uganda. *African Journal* of *Food*, *Agriculture*, *Nutrition* and *Development* 11(2): 4610 – 4622.

Mwangi, A., Arimi, S. M., Mbugua, S., Kangethe, E. K. and Omore, A. O. (2000). Assurance of marketed milk quality in Kenya. Paper presented at the Faculty of Veterinary Medicine Biennial Scientific Conference, 30 – 31 August 2000, University of Nairobi, Kenya. 1 – 4pp.

Nádia, M., Diane, S., Débora, O. and Mirlei, R. E. (2012). Evaluation of Microbiological quality of Raw Milk produced at two properties in the Far West of Santa Catarina, Brasil. *Journal of Food and Public Health* 2(3): 79 – 84.

NADIS (National Animal Disease Information Service). (2013). Mastitis Control and Management. Mastitis part 7 – Teat Disinfection, by R. Laven. UK. from [*http://www.nadis.org.uk/bulletins/mastitis-control-and-management/mastitis-part-7-teat-disinfection.aspx?altTemplate =PDF]* Site visited on 23/08/2013.

Nangwala, S. W. (1996). Effect of mastitis on milk yield response to improved feeding. Dissertation for Award of M. Sc. Degree at Sokoine University of Agriculture. Morogoro, Tanzania. 5pp.

Ndambi, O. A., Hemme, T. and Latacz-Lohmann, U. (2007). Dairying in Africa – Status and recent developments. *Livestock Research for Rural Development.* [*http://www.lrrd.org/lrrd19/8/ ndam 19111.htm*]. Site visited on 29/7/ 2013.

Nene, V., Morzaria, S., Baker, L., Odono, A., Rege, E., Zerbini, E. and Bishop, R. (1999). Genomics Research: Prospects for Improving Livestock Productivity. I*n Agricultural Biotechnology and the Poor. CGIAR Secretariat Publication*. pp. 86 –195.

Nhachi, C. F. B. and Kasilo, O. M. J. (1996). Pesticides in Zimbabwe: Toxicity and Health Implications. University of Zimbabwe Publications. 110pp.

Njombe, A. P., Msanga, Y., Mbwambo, N. and Makembe, N. (2011). The Tanzania Dairy Industry: Status, Opportunities and Prospects. Department of Animal Production, Livestock Products and Marketing Infrastructure. Ministry of Livestock and Fisheries Development. Paper Presented to the 7th African Dairy Conference and Exhibition held at MovenPick Palm Hotel, Dar es Salaam, 25 – 27 May 2011. 1 – 19pp.

Nyenje, M. E, Odjadjare, C. E, Nicoline F. Tanih, N. F., Green, E. and Ndip, R. N. (2012). Foodborne Pathogens Recovered from Ready-to-Eat Foods from Roadside Cafeterias and Retail Outlets in Alice, Eastern Cape Province, South Africa: Public Health Implications *International Journal of Environmental Research and Public Health* 9: 2608 – 2619.

O'Connor, C. B. (1995). Rural Dairy Technology. ILRI Training Manual 1. ILRI (International Livestock Research Institute), Nairobi, Kenya. 119pp.

OECD (Organisation for Economic Co-operation and Development)/FAO (Food and Agriculture Organization). (2011). Agricultural Outlook 2011 – 2020. OECD and FAO Secretariats Publication. pp.162.

OIE (World Organization for Animal Health). (2008). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals – Chapter 2.9.7 *Listeria monocytogenes.* 6th Edition. 12 rue de Prony, 75017 Paris, France. 2: 1238 – 1254.

OIE (World Organization for Animal Health). (2008). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals – Chapter 2.9.11 Verocytotoxigenic *Escherichia coli*. 6th Edition. 12 rue de Prony, 75017 Paris, France. 2: 1294 – 1304pp.

Oliver, S. P., Boor, J. K., Murphy, C. S. and Murinda, E. S. (2009). Food safety hazards Associated with consumption of raw milk. *Journal of Food-borne Pathogens and Disease* 6: 793 – 806.

Omore, A., Lore, T., Staal, S., Kutwa, J., Ouma, R., Arimi, S., and Kang'ethe, E. (2005). Addressing the public health and quality concerns towards marketed milk in Kenya SDP Research and Development Report No.3 Smallholder Dairy (R& D) Project. 1– 45pp.

Omore, A., Staal, S., Kurwijila, L. R., Aning, G., Mdoe, N. and Nurah, G. (2001). Indigenous market for dairy products in Africa:trade-offs between food safety and economics. Proceedings of Symposiums on Dairy Development in the Tropics, Utrecht University, Utrech Netherlands, 19 – 24pp.

Pandey, G. S. and Voskuil, G.C.S. (2011). Manual on Milk safety, quality and hygiene. Golden Valley agricultural Research Trust, Zambia. 52pp.

Pantoja, J. C. F., Reinemann, D. J. and Ruegg, P. L. (2011). Factors associated with coliform count in unpasteurized bulk milk. American Dairy Science Association. *Journal of Dairy Science*. 94: 2680 – 2691.

Parekh, T. S. and Subhash, R. (2008). Molecular and Bacteriological examination of milk from different milch animals with special reference to Coliforms. *Journal of Current Research in Bacteriology* 1(2): 56 – 63.

Plotter, H. M. (2002). Raw milk and milk products for human consumption. Dairy Division, Indiana State Board of Animal Health, Indianapolis, IN. [*http://ohioline.osu.edu/ fse-fact/0003.html*]. Site visited 25/08/2013.

RLDC (Rural Livelihood Development Company). (2009). Dairy sub sector development strategy 4 – 7pp.

Robert, J. H. (1996). Controlling Contagious Mastitis: National Mastitis Council Regional Meeting Proceedings. 11pp.

Ruegg, P. L. (2003). The role of hygiene in efficient milking. Babcock Institute Dairy Updates. Review. Milking and milk quality No. 406. [*http://babcock.wisc.edu/ sites/ default/files/ documents/ productDownload/du_406.en_.pdf*]. Site visited on 23/8/2014.

Ruijter de Wildt, M., Elliott, D. and Hitchins, R. (2006). *Making Markets Work for the Poor: Comparative Approaches to Private Sector Development*, Berne: Swill Agency for Development and Cooperation. 4pp.

Rwehumbiza, J. M., Ryoba, R. and Karimuribo, E. D. (2013). Assessment of microbiological status and presence of antibiotic residues in cow milk from mallholder production systems in Bagamoyo and Kisarawe districts, Tanzania. *Tanzania Veterinary Journal* 28: 60 – 69.

Ryan, K. J. and Ray, C. G. (Eds.) (2004). *Sherris Medical Microbiology* McGraw Hill, UK. 733pp.

Salandra, G., Goffredo, E. Pedarra, C., Nardella, M. and Parisi, A. et al. (2008). Occurrence, Characterization and antimicrobial resistence pattern of *Staphylococcu*s species isolated from dairy products in Southern Italy. *International Journal of Food Microbiology* 9: 327 – 360.

Sarangi, L. N., Panda, H. K., Priyadarshini, A. Sahoo, S., Palai1, T. K., Ranabijuli, S. Senapati, S. and. Mohanty, D. N. (2009). Prevalence of *listeria* species in milk sample of cattle of Odisha. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* 30(2): 135 – 136.

Sharma, M. (2009). Management Techniques for Clean Milk Production in Dairy Animals. 1 – 2pp. [*http://www.scribd. com/doc/15779660/Management-for-Clean-Milk-Production*]. Site visited on 9/08/ 2014.

Shija, F. (2013). Assessment of Milk handling practices and bacterial contaminations along the dairy value chain in Lushoto and Handeni districts, Tanzania. Dissertation for Award of M. Sc. Degree at Sokoine University of Agriculture, Morogoro, Tanzania. 36 – 45pp.

Sikira, A. and Ndanu, H. (2012). Draft report on participatory rural appraisal to inform the next step of the three project of Moremilkit, Safe Food Fair Food and Milkit projects in Morogoro and Tanga regions, Tanzania. Sokoine University of Agriculture. Morogoro, Tanzania. 1 – 104pp.

Simforian, E. (2013). Assessment of bacterial quality and associated handling practices of unpasteurised fruit juices vended in Dar es Salaam city, Tanzania. Dissertation for Award of MSc. Degree at Sokoine University of Agriculture. Morogoro, Tanzania. 4pp.

Smith, K., Peter, K., Daniela, H. and Melchior, S. (2007). Food borne pathogenic microorganisms and natural toxins. Food drug Administration center food safety, Applied Nutrition 10: 119 – 150.

Sivapalasingams, S., Friedman, C. R., Cohen, L. and Tauxe, R. V. (2004). Fresh produce: a growing cause of outbreaks of foodborne illness in the United States. *Journal of Food Protection* 67(10): 2342 – 2353.

Staal, S. J., Delgado, C., Baltenweck, I. and Krusha, R. (2000). Smallholder dairy (research and development) project research report. Spatial aspects of producer milk price formation in Kenya: a joint household-GIS approach. Berlin: International Association of Agricultural Economists Meeting; 2000. 68pp.

Streeter, R. N., Hoffsis, G. F., Bech-Nielsen, S., Shulaw, W. P. and Rings, D. M. (1995). Isolation of Mycobacterium paratuberculosis from colostrum and milk of subclinically infected cows. *American Journal of Veterinary Research*. 56: 1322–1324.

Swai, E.S. and Schoonman, L. (2011). Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania, *Asian Pacific Journal of Tropical Biomedicine* 1(3): 217 – 222pp.

Swaminathan, B. (2001). *Listeria monocytogenes*. *In:* Food Microbiology: Fundamentals and Frontiers, Second Edition, Doyle M.P., Beuchat L.R. and Montville T. J., eds. ASM Press, Washington, DC, USA 383 – 409 pp.

Tassew, A. and Seifu, E. (2011). Microbial quality of raw cow milk collected from farmers and dairy cooperatives in Bahir Dar Zuria and Mecha district, Ethiopia. *Agriculture and Biology Journal of North America* 2(1): 29 – 33.

Tebaldi, V. M. R., Oliveira, T. L. C., Boari, C. A. and Piccoli, R. H. (2008). Isolation of coliforms, staphylococci, and enterococci in raw milk from communitarian expansion refrigeration tanks: identification, lipolytic and proteolytic action. *Ciência* e *Tecnologia de Alimentos* 28: 753 – 760.

Tesha, Y. C. (2010). Effect of feeding different concentrates to Ayrshire and Friesian cows on milk yield and Fatty Acid composition of Butter Fat. Dissertation for Award of MSc. Degree at Sokoine University of Agriculture. Morogoro, Tanzania. 5pp.

Thaker, H. C., Brahmbhatt, M. N. and Nayak, J. B. (2012). Study on occurrence and antibiogram pattern of *Escherichia coli* from raw milk samples in Anand, Gujarat, India. *Journal of Veterinary World.* 5(9): 556 – 559.

Tsegmed, U. (2006). Staphylococci isolated from raw milk of yak and cattle in Mongolia. Studies on the occurrence, characterization, detection of enterotoxin and antimicrobial susceptibility profile of the isolates. Master's thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden. 25 – 31pp.

Unnevehr, L. and Hirschhorn, N. (2000). *Food safety issues in the developing world*. World Bank Technical Paper No. 469. The World Bank, Washington DC. 72pp.

Van Kessel, J. S., Karns, J. S. Gorski, L. McCluskey, B. J. and Perdue, M. L. (2004). Prevalence of Salmonellae, *Listeria monocytogenes* and Fecal Coliforms in Bulk Tank Milk on US Dairies, *Journal of Dairy Science* 87(9): 2822 – 2830.

Vardar-ünlü, G., Ünlü, M. and Bakici, M. Z. (1998). Incidence of *Listeria spp*. from raw milk in sivas. *Turkish Journal of Medical Science* 28: 389 – 392.

Vazquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Dominguez-Bernal, G., Goebel, W., Gonzales-Zorn, B., Wehland, J. and Kreft, J. (2001). "Listeria Pathogenesis and Molecular Virulence Determinants". *Clinical Microbiology Reviews* 14: 584 – 640.

Vigano, A., Pellisier, N., Hamad, H. J., Ame, S. A. and Pontello, M. (2007). Prevalence of *E. coli,* thermotolerant coliforms, *Salmonella spp*. and *Vibrio spp.* in ready-to-eat foods: Pemba Island, United Republic of Tanzania. - *In: Annali di igiene, medicina preventiva e di comunità* 19(5): 395 – 403pp.

Vojdani, J. D., Beuchat, L. R. and Tauxe, R. V. (2008). Juice-associated outbreaks of human illness in the United States, 1995 through 2005. *Journal of Food Protection* 71: 356 – 364.

Warke, S. R., Kalorey, D. R., Kurkure, N. V. and Barbuddhe, S. B. (2007). Prevalence and genotypic characterization of *Listeria monocytogenes* of bovine intramam mary origin. *Royal Veterinary Journal of India 3*: 113 – 117.

Wehr, H. M. and Frank, J. H. (Eds.) (2004). Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C. 1–570pp.

WHO (World Health Organization). (2005). Drug-resistant Salmonella REF 1 – 6 Fact sheet No. 139. Geneva: Food Safety Department WHO; Revised April, 2005. [*http://www.who.int/mediacentre/factss/fs139/en/*]. Site visited on 27/08/ 2014.

Winn, Jr., W. C., Allen, S. D., Janda, W. M., Koneman, E. W., Procop, G. W., Schreckenberger, P. C. and Woods, G. L. (2006). Koneman's Color Atlas and Textbook of Diagnostic Microbiology— Chapter 6. *The Enterobacteriaceae*, 6th edition. Lippincott Williams and Wilkins, Philadelphia, PA. 212 – 300pp.

Wilson, D. J., Das, H. H., Gonzalez, R. N. and Seas, P. M. (1997). Association between management practices, dairy herd characteristics and somatic cell count of bulk tank milk. *Journal of the American Veterinary Medical Association* 210: 1499 – 1505.

Yakubu, Y., Salihu, M. D., Faleke, O. O., Abubakar, M. B., Junaidu, A. U., Magaji, A. A., Gulumbe, M. L. and Aliyu, R. M. (2012). Prevalence and antibiotic susceptibility of *Listeria monocytogenes* in raw milk from cattle herds within Sokoto Metropolis, Nigeria. *Sokoto Journal of Veterinary Sciences*, Volume 10(2): 13 – 17.

Yilma, Z. (2012). Microbial Properties of Ethiopian Marketed Milk and Milk Products and Associated Critical Points of Contamination: An Epidemiological Perspective, Epidemiology Insights, (Edited by Dr. Maria De Lourdes Ribeiro De Souza, Da Cunha). In Tech., [*http://www.intechopen.com/books/epidemiology-insights/microbial-properties-of-marketedand-ethiopianfermentdproducts–and-associated-critica*]. Site visited on 28/8/2014.

Zadernowska, A. and Chajęcka, W. (2012). Detection of *Salmonella spp*. Presence in Food, Salmonella - A Dangerous Foodborne Pathogen, (Edited by Barakat, S.M.). In Tech. [*http://www.intechopen. com/books/salmonella-a-dangerous-foodborne-pathogen/detectionofsalmonella -spp-resence-in-food*]. Site visited on 3/9/2014.

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 – Nairobi). 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:		Signature (or other mark)
Address:		Date
Phone #		
Witness (staff) Name		
Witness Position Title		
Witness Signature		

Appendix 2: Questionnaires survey for respondents in the study area

Potential health risks in the milk value chain

This questionnaire is designed to collect information related to people's knowledge and awareness of milk safety along the milk value chain. It will take less than thirty minutes to complete. Please note that your answer is absolutely confidential and your name will not be discussed in any report. Also, your individual answer will not be shared with anyone.

I. Questionnaire survey for farmers

1. Questionnaire number……..
2. GPS co-ordinates…….
3. Sample number……
4. Form of the sample……..
5. District…….
6. Division……
7. Ward…….
8. Village……..

Part A: Personal particulars

1. Name of respondent:……
2. Age (years)….....

3. Gender:

   ☐ Male ☐ Female

4. Highest level of education of the respondent

   ☐ No school

   ☐ Primary school

   ☐ Secondary school

   ☐ College education

   ☐ University

   ☐ Other (specify):……

5. Position of the respondent in the household:

   ☐ Head of the household (Father) ☐ Spouse ☐ Son

   ☐ Daughter ☐ Employee ☐ Others (Specify)…….

Part B– 1: Farm management and general zoonoses exposure practices

1. Type of cattle raised:

   ☐ Indigenous cattle ☐ Exotic (Specify)……

   ☐ Hybrid (Specify)……..

2. Number of animals:……

3. How many lactating cows do you have in this herd…….

4. What farming system are you practicing?

   ☐ Semi-intensive/intensive system

   ☐ Extensive system

   ☐ Others (Specify)……

5. Where do you commonly graze your cattle? (single choice)

   ☐ Open space - communal grazing fields

   Open space - private grazing fields

☐ Dumping sites

☐ Zero grazing

☐ Others (specify)……

6. Does this herd come into contact with other herds (e.g. during watering or in communal grazing land)? (Mandatory)

☐ Yes ☐ No

7. If yes how often do they come into contact with other herds?(single choice)

☐ Everyday

☐ At least once a week

☐ At least once a month

☐ Less often

8. Are your animals housed?

☐ Yes ☐ No

9. If yes, what type of floor/bedding are they in?

☐ Natural earth

☐ Concrete

☐ Others (Specify)……

10. What is the water source for your animals?

☐ Tap water

☐ Water pans/flood water

☐ Local River/streams

☐ Local wells/boreholes

☐ Other (Specify)…….

11. Which breeding method do you use in the farm?

☐ Artificial Insemination

Bull

☐ Both

12. If you use bull for breeding, where do you obtain the bull?

☐ Neighbours

☐ Special breeders

☐ Others (Specify)…….

13. Do you keep other animals apart from cattle?

☐ Yes ☐ No

14. If yes, which and how many?

☐ Camels…….

☐ Donkey……

☐ Chicken……

☐ Goats……

☐ Sheep……

☐ Dogs……..

☐ Cat……

☐ Others (Specify)……..

15. Do you receive any Veterinary services?

☐ Yes ☐ No

16. Who normally administer treatments to your cattle?

☐ Self

☐ Veterinarian

☐ Animal health worker

☐ Other (Specify)……..

17. Is it to get veterinary assistance?

☐ Easy

Difficult

☐ Not sure

18. How do you rate the cost of veterinary drugs?

☐ Expensive

☐ Cheap

☐ Reasonable

☐ Not sure

19. What is the general health status of lactating animals in your herd

Good ☐ Sick ☐ Don't know ☐

If sick, what is the problem? (Specify)……...

20. Is there any routine screening and prevention of diseases?

☐ Yes ☐ No

21. If Yes, for what diseases?

☐ Anthrax

☐ Brucellosis

☐ Helminthiosis

☐ Tuberculosis

☐ Other (Specify)…….

22. What do you do with milk from your animals?

☐ For family consumption

☐ Sale to milk vendors/traders

☐ Sale to milk collection centres

☐ Sale to neighbours and members of the community

☐ Other (Specify)…….

23. If selling milk, for how long do you keep the milk before reaching the market?

Mention: …….

24. Which form of milk are you selling?

☐ Raw milk ☐ Boiled milk

25. What is the practice when cow is sick?

☐ Milking ☐ Not milking

26. If you milk sick cow, what do you do with its milk?

☐ Family consumption

☐ Sale the milk

☐ Leave for calves

☐ Discard

☐ Other (Specify)…….

27. Do you consume raw milk? (Mandatory)

☐ Yes ☐ No

28. If yes above, how often?

☐ Always

☐ Sometimes

☐ Don't know

29. Do you consume raw fermented milk?

☐ Yes ☐ No

30. Do you believe or know that raw milk can be a potential source of transmission of infectious diseases to humans?

☐ Yes ☐ No

31. Do you know any source(s) of microbial contaminations in milk?

☐ Yes ☐ No

If Yes, mention:…….……..……...

32. Are there any cases of occurrence of mastitis?

☐ Yes ☐ No

If Yes above, what actions do you take?……

Part B – 2: Milk handling practices at farm level

1. How do you milk?

   ☐ Hand milking ☐ Machine milking

2. What are the sanitary measures that you are taking during milking, including milkers?

   ☐ Clean the shed before milking and dispose the dung away from the shed

   ☐ Wash the milking vessels with clean water and dry them

   ☐ Wash the udder with clean water before milking

   ☐ Fore-strip each quarter and observe for signs of mastitis

   ☐ Wipe and dry the udder after washing using clean dry towel

   ☐ Wash hands with soap and dry the hands with towel

   ☐ Apply milking jelly/lubricant

   ☐ Milk the animal

   ☐ Disinfect the teats by teat dip

3. What is the source and status of water that you are using for sanitary measures, including washing of hands, utensils and/or equipment? Mention:……...

4. Are there any routine check-ups or screening of health status of those people who are handling milk, including milkers and sellers?

   ☐ Yes ☐ No

5. What kind of utensils and/or equipment that are used during milking and handling?

   ☐ Plastic containers

   ☐ Aluminium/Stainless steel containers

   Wooden containers

☐ Traditional pots

☐ Other (Specify)…….

6. How frequently do you wash the utensils/equipment used for milk activities?

Daily ☐ Weekly ☐ Monthly ☐ Others (Specify)……..

7. How do you handle the milk at household?

Always covered soon after milking ☐ Not covered at all ☐

8. Did you get any kind of/or formal training on milk handling and marketing?

☐ Yes ☐ No

9. How do you store your milk, including storage conditions?

☐ Refrigerator ☐ Chiller ☐ In bucket/can at room temperature

☐ Others (Specify)……

10. Do you sell milk to the neighbouring households/milk processing plant?

☐ Yes ☐ No (For household consumption) ☐ Both

If No, skip question 11.

11. What means of transportation are you using to reach the customers, including handling facilities and storage conditions? Mention:…… ……

12. Which form of milk do your customers prefer most?

☐ Raw milk ☐ Boiled milk ☐ Other (Specify)……..

13. Did you ever encounter any rejection of your milk by customers?

☐ Yes ☐ No

If Yes, what was the reason (s):…… …….....

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

II. Questionnaire survey for vendors/traders and restaurants/kiosks

1. Questionnaire number……..
2. GPS co-ordinates…….
3. Sample number……
4. Form of the sample……...
5. District……..
6. Division……
7. Ward…….
8. Village……..

Part A: Personal particulars

1. Name of respondent:…….……
2. Age (years)….....
3. Gender:

   ☐ Male ☐ Female

4. Highest level of education of the respondent

   ☐ No school

   ☐ Primary school

   ☐ Secondary school

   ☐ College education

   ☐ University

   ☐ Other (specify):……..

5. Position of the respondent:

   ☐ Head of the household (Father) ☐ Spouse ☐ Son

   ☐ Daughter ☐ Employee ☐ Others (Specify)…….

6. What type of business do you run?

   ☐ Supplier ☐ Street vendor ☐ Milk kiosk ☐ Restaurant

   ☐ Others (Specify)…….

Part B: Milk handling at the vendor/trader and restaurants/kiosks level

1. From whom do you purchase your milk from?

   ☐ Own farm

   ☐ Other farm

   ☐ Milk bulker

   ☐ Market

   ☐ Other (specify):……

2. Where do you purchase milk from? Location:.....

3. Do you complete any checks for milk quality before buying?

   ☐ Yes ☐ No

   If yes above, what checks do you perform…….

   ……

4. How do you transport the milk? (Use transporters)

   ☐ Refrigerated vehicle

   ☐ Other vehicle

   ☐ Bicycle/cart

   ☐ Motorcycle

   ☐ Hire transporter

   ☐ Other (specify):……

5. Do you mix milk from different farms or sources?

   Yes No

6. How long does it usually take to transport the milk from source to final destination? .....
7. How long do you Keep the milk from transport until sale?.....
8. Did you get any kind of/or formal training on milk handling and marketing?

   ☐ Yes ☐ No

9. Which equipment do you use to store the milk?

   ☐ Plastic containers

   ☐ Glass bottles

   ☐ Aluminium/Stainless steel containers

   ☐ Other (specify):……

10. How do you keep the milk until sale?

    ☐ At room temperature

    ☐ Refrigerator

    ☐ Other (Specify)……..

11. How frequently do you clean the milk containers?

    ☐ Never

    ☐ Infrequently

    ☐ Monthly

    ☐ Weekly

    ☐ Daily

    ☐ Other (specify):……

12. What do you use to clean milk containers?

    ☐ Cold water only

    ☐ Hot water only

    ☐ Hot water with detergent/soap

    ☐ Other (Specify)……..

13. What cleaning agent do you use?

☐ None

☐ Bar soap

☐ Bleach

☐ Detergent

☐ Other (Specify)……..

14. What is your source of water for cleaning?

☐ Tap water

☐ Water tank

☐ Local River/streams

☐ Local wells/bore holes

☐ Other (specify):……

15. Do you use disinfectant?

☐ Yes ☐ No

16. What do you use for washing your hands?

☐ Cold water only

☐ Warm water only

☐ Cold water and soap

☐ Warm water and soap

☐ Other (specify):……

17. If you don't sell all the milk in 24 hours, what do you do with the remaining milk?

☐ Sell

☐ Discard

☐ Consume

☐ Other (specify):……

18. Which form of milk do your customers prefer most?

☐ Raw milk ☐ Boiled milk ☐ Other (Specify)……...

19. Do you process milk?

☐ Yes ☐ No

If yes above, mention the products that you process milk for:……...

If No, skip to direct observation

20. Is there a hand-washing area with soap in the processing location?

☐ Yes ☐ No

21. When do you wash your hands?

☐ After using the toilet

☐ Before handling milk

☐ Regularly during day

22. What do you use to clean surfaces and utensils?

☐ Tap water

☐ Hot water

☐ Soap

☐ Detergent

☐ Bleach

☐ Other (specify):……

Direct observation

23. Cleanliness of the vendor/server

☐ Well-clean

☐ Dirty

24. Storage equipment status

☐ Clean ☐ Dirty

25. Is the storage equipment covered?

☐ Yes ☐ No

26. Type of container used to fetch milk from the large container

☐ A cup with handle

☐ A cup without handle

☐ Other (specify):……..

27. How is the milk served?

☐ From a large container/thermal flask and pour into a cup

☐ By immersing a cup in the large container/cooking pan (Scooping)

☐ Cold from the fridge

☐ Other(s) specify……..

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

III. Questionnaire survey for the collection centres

1. Questionnaire number……..
2. GPS co-ordinates…….
3. Sample number…….
4. Form of the sample……
5. District……...
6. Division…….
7. Ward……..
8. Village……
9. Name of the collection centre……Owner…….....
10. Name of respondent:…….…….

Milk handling practices

1. How much litres of milk do you collect per day?……
2. Which equipment do you use to get milk to the collection centre?

   ☐ Plastic containers

   ☐ Aluminium/Stainless steel containers

   ☐ Other (specify):……

3. Do you complete any checks for milk quality before accepting/rejecting?

   ☐ Yes ☐ No

   If yes above, what checks do you perform…….

4. What are the acceptance/rejection standards?.....
5. Is there a chilling/cooling facility? (Observe)

   ☐ Yes ☐ No

6. How long does the milk stay until transport?.....
7. How long does it usually take to transport the milk to final destination? .....

8. How is the milk transported? (Use transporters)

   ☐ Refrigerated vehicle

   ☐ Other vehicle

   ☐ Other (specify):……

Note: Record any other relevant information that is not asked from the list of questions

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

Appendix 3: Results of SPSS analysis

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Number of cattle * Type of animal house	54	100.0%	0	.0%	54	100.0%

Number of cattle * Type of animal house Crosstabulation					
Variable		Type of animal house			Total
		Boma	Null	Shed	
Number of cattle	10 – 20	6	2	0	8
	> 20	17	11	0	28
	< 10	0	1	17	18
Total		23	14	17	54

Floor design					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Beddings	1	1.9	1.9	1.9
	Concrete	2	3.7	3.7	5.6
	Deep litter	10	18.5	18.5	24.1
	Natural health	27	50.0	50.0	74.1
	Null	14	25.9	25.9	100.0
	Total	54	100.0	100.0	

Sources of water for animals and sanitary activities at households					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Local River	16	29.6	29.6	29.6
	Local River/Wells	1	1.9	1.9	31.5
	Wells/boreholes	13	24.1	24.1	55.6
	Tap water	7	13.0	13.0	68.5
	Tap water/dam	2	3.7	3.7	72.2
	Dam/flood water	9	16.7	16.7	88.9
	Dam/well	3	5.6	5.6	94.4
	others	3	5.6	5.6	100.0
	Total	54	100.0	100.0	

Containers used for milk storage at households					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Don't know	1	1.9	1.9	1.9
	Metal/Aluminium	7	13.0	13.0	14.8
	Null	4	7.4	7.4	22.2
	Plastic	35	64.8	64.8	87.0
	Calabash	7	13.0	13.0	100.0
	Total	54	100.0	100.0	

Means of washing hands					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Water only	19	35.2	35.2	35.2
	Water with soap	18	33.3	33.3	68.5
	null	17	31.5	31.5	100.0
	Total	54	100.0	100.0	

Means of washing cow teats					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Cold water	9	16.7	16.7	16.7
	Warm water only	12	22.2	22.2	38.9
	null	33	61.1	61.1	100.0
	Total	54	100.0	100.0	

Hand milking technique used					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Squeezing action	2	3.7	3.7	3.7
	Stripping (Pulling the teat)	49	90.7	90.7	94.4
	null	3	5.6	5.6	100.0
	Total	54	100.0	100.0	

Means of milk quality assurance among milk suppliers, street vendors and restaurants					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Clot on boil	2	3.6	3.6	3.6
	Lactometer	11	19.6	19.6	23.2
	None	32	57.1	57.1	80.4
	Pour on ground	4	7.1	7.1	87.5
	Visual and smell	7	12.5	12.5	100.0
	Total	56	100.0	100.0	

Means of milk transport/delivery among milk suppliers, street vendors and restaurants					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Bicycle	22	39.3	39.3	39.3
	Motorcycle	7	12.5	12.5	51.8
	Onfoot	17	30.4	30.4	82.1
	Supplied	9	16.1	16.1	98.2
	Vehicle	1	1.8	1.8	100.0
	Total	56	100.0	100.0	

Pooling of milk					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid		4	6.7	6.7	6.7
	No	17	28.3	28.3	35.0
	Yes	39	65.0	65.0	100.0
	Total	60	100.0	100.0	

Containers used for milk storage among milk suppliers, street vendors and restaurants					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Glass bottle	1	1.8	1.8	1.8
	Plstic container	53	94.6	94.6	96.4
	Thermos	2	3.6	3.6	100.0
	Total	56	100.0	100.0	

Source of water used in sanitary activities among milk suppliers, street vendors and restaurants					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Dams	6	10.7	10.7	10.7
	Local river/streams	5	8.9	8.9	19.6
	Rainwater & dam	9	16.1	16.1	35.8
	Tapwater	25	44.6	44.6	80.4
	Wells	11	19.6	19.6	100.0
	Total	56	100.0	100.0	

Means of cleaning milk containers					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Cold water and soap	6	10.7	10.7	10.7
	Hot water and soap	50	89.3	89.3	100.0
	Total	56	100.0	100.0	

Types of soap/detergents used for cleaning milk containers among milk suppliers, street vendors and restaurants					
Variable		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Barsoap	23	41.1	41.1	41.1
	Wshngpowder	33	58.9	58.9	100.0
	Total	56	100.0	100.0	

Appendix 4: SAS results on mean TPC and Enterobacteriaceae count

------------------------------Overall mean TPC and Entcount ---------------------------------

Variable	N	Mean	Std Dev	Minimum	Maximum	Coeff of Variation
TPC	80	17389625.06	78031991.78	33636.00	636363636	448.7272814
EntCount	43	1876268.51	6219543.48	1363.00	32272727.00	331.4847230

-- FS=Extensive --

Variable	N	Mean	Std Dev	Minimum	Maximum	Coeff of Variation
Entcount	25	1979072.76	6634582.94	1363.00	32272727.00	335.2369387
TPC	40	6373454.60	13266057.83	33636.00	70000000.00	208.1454825

-- FS=Semi-intensive/intensive -------------------------

Variable	N	Mean	Std Dev	Minimum	Maximum	Coeff of Variation
Entcount	18	1733484.83	5778349.66	1818.00	24545454.00	333.3371915
TPC	40	28405795.53	109129225	36364.00	636363636	384.1794363

--- SM=Households -------------------------------------

Variable	N	Mean	Std Dev	Minimum	Maximum	Coeff of Variation
Entcount	17	4340400.94	9478028.61	7727.00	32272727.00	218.3675827
TPC	30	42460212.07	124600925	33636.00	636363636	293.4533732

--- SM=Milk suppliers ---------------------------------

Variable	N	Mean	Std Dev	Minimum	Maximum	Coeff of Variation
Entcount	10	519591.10	1309359.17	1364.00	4238182.00	251.9979982
TPC	24	2621969.83	4928739.59	36364.00	24772727.00	187.9785011

--- SM=Restaurants ------------------------------------

Variable	N	Mean	Std Dev	Minimum	Maximum	Coeff of Variation
Entcount	12	134318.33	98959.94	1364.00	254091.00	73.6756759
TPC	16	2367045.63	1496826.34	354546.00	4945455.00	63.2360576

--- SM=Street vendors ---------------------------------

Variable	N	Mean	Std Dev	Minimum	Maximum	Coeff of Variation
Entcount	4	21249.75	28404.35	1363.00	61818.00	133.6690805
TPC	10	1656363.70	1681692.77	122727.00	5590909.00	101.5291972

-- FM=Boiled --

Variable	N	Mean	Std Dev	Minimum	Maximum	Coeff of Variation
Entcount	8	129318.38	107595.51	1364.00	251364.00	83.2020265
TPC	9	2206565.78	1467907.52	354546.00	4500000.00	66.5245303

---------- FM=Raw ----------

Variable	N	Mean	Std Dev	Minimum	Maximum	Coeff of Variation
Entcount	35	2275571.40	6848353.33	1363.00	32272727.00	300.9509316
TPC	71	19314238.21	82693529.57	33636.00	636363636	428.1480257

Appendix 5: SAS GLM procedure for the effect of farming system, source and form of milk on TPC and *Enterobacteriaceae* count

Dependent Variable: TPC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Farming system	1	1.080097E15	1.080097E15	0.18	0.6739
Source of milk	3	2.7785637E16	9.2618789E15	1.53	0.2141
Form of milk	1	837818840171	837818840171	0.00	0.9906
FS*SM	3	1.6453359E16	5.4844529E15	0.91	0.4425
FS*FM	1	6264169202.9	6264169202.9	0.00	0.9992
Error	70	4.2350929E17	6.0501327E15		
Corrected Total	79	4.8103035E17			

R-Square	Coeff Var	Root MSE	TPC Mean
0.119579	447.2931	77782599	17389625

Least Squares Means

FST	TPC LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1=LSMean2 Pr > |t|
EFS	4896242.5	24597017.5	0.8428	0.6739
SIFS	19323428.0	23683279.3	0.4173	

SM	TPC LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
Households	42227598.4	24339450.0	0.0872	1
Milk suppliers	2389356.1	25354025.3	0.9252	2
Restaurants	2398636.6	19767087.2	0.9038	3
Street vendors	1423750.0	31555522.6	0.9641	4

Least Squares Means for effect SM
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: TPC

i/j	1	2	3	4
1		0.0656	0.2305	0.1553
2	0.0656		0.9998	0.9738
3	0.2305	0.9998		0.9799
4	0.1553	0.9738	0.9799	

FM	TPC LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1=LSMean2 Pr > |t|
Boiled	11877221.6	35257810.5	0.7372	0.9906
Raw	12342449.0	11014434.4	0.2663	

Dependent Variable: *Enterobacteriaceae* count

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Farming system	1	52418567876	52418567876	0.00	0.9726
Source of milk	3	1.3922155E14	4.6407184E13	1.06	0.3804
Form of milk	1	599990000.04	599990000.04	0.00	0.9971
FS*SM	3	4.37148E12	1.45716E12	0.03	0.9917
FS*FM	1	44595139876	44595139876	0.00	0.9748
Error	33	1.4481985E15	4.3884802E13		
Corrected Total	42	1.6246743E15			

R-Square	Coeff Var	Root MSE	Entcount Mean
0.108622	353.0710	6624561	1876269

Least Squares Means

FS	Entcount LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
EFS	1216284.18	2413787.97	0.6177	0.9726
SIFS	1344155.28	2804049.51	0.6348	

SM	Entcount LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
Households	4464696.89	2634409.88	0.0995	1
Milk suppliers	512091.16	2915935.59	0.8617	2
Restaurants	136818.31	2028349.12	0.9466	3
Street vendors	7272.56	4329257.15	0.9987	4

Least Squares Means for effect SM
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Enterobacteriaceae count

i/j	1	2	3	4
1		0.1506	0.2523	0.2937
2	0.1506		0.9243	0.9085
3	0.2523	0.9243		0.9797
4	0.2937	0.9085	0.9797	

FM	EntCount LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
Boiled	1272719.79	3608610.11	0.7266	0.9971
Raw	1287719.67	1432084.65	0.3751	

Appendix 6: SAS General Linear Model procedure for the effect of milk handling practices on TPC and *Enterobacteriaceae* count among the suppliers, street vendors and restaurants

Dependent Variable: TPC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Quality checks	1	1.1403632E13	1.1403632E13	0.80	0.3776
Pooling of milk	1	9.5451656E12	9.5451656E12	0.67	0.4191
Transport length	5	1.3808394E13	2.7616787E12	0.19	0.9631
Training	1	3.3533824E12	3.3533824E12	0.23	0.6310
Means to clean containers	1	429058951293	429058951293	0.03	0.8634
Cleanness of business agent	1	3.6512148E13	3.6512148E13	2.56	0.1188
Storage equipment appearance	1	8.5049802E13	8.5049802E13	5.96	0.0199
Milk serving	1	9652452916.8	9652452916.8	0.00	0.9794
Error	35	4.9982653E14	1.4280758E13		
Corrected Total	47	6.2332709E14			

R-Square	Coeff Var	Root MSE	TPC Mean
0.198131	162.9619	3778989	2318940

Least Squares Means

Qltychecks	TPC LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
No	1779728.89	1423898.79	0.2196	0.3776
Yes	567049.07	1494562.36	0.7067	

Milkpooling	TPC LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
No	450762.54	1465737.59	0.7603	0.4191
Yes	1896015.42	1659676.67	0.2610	

Transportlth	TPC LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1hr	967119.23	2034424.18	0.6375	1
2hrs	1662296.47	1957135.71	0.4015	2
30min	1972423.32	2065945.84	0.3463	3
Lessthan30in	-25320.59	2024866.30	0.9901	4
Morethan1hr	1134470.07	1954412.02	0.5653	5
Nil	1329345.39	1752987.31	0.4533	6

Least Squares Means for effect Trsptlth
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: TPC

i/j	1	2	3	4	5	6
1		0.7962	0.6598	0.6312	0.9292	0.8668
2	0.7962		0.9139	0.5427	0.8412	0.9001
3	0.6598	0.9139		0.3656	0.6964	0.7595
4	0.6312	0.5427	0.3656		0.5512	0.5081
5	0.9292	0.8412	0.6964	0.5512		0.9213
6	0.8668	0.9001	0.7595	0.5081	0.9213	

Least Squares Means

Training	TPC LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
No	1620230.29	1069301.38	0.1387	0.6310
Yes	726547.67	1974233.67	0.7151	

Meansequipcl	TPC LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
Coldwtrs	981160.01	2249365.55	0.6654	0.8634
Htwtrsoa	1365617.95	860558.28	0.1215	

Agentcleanness	TPC LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
Clean	-592139.19	1924725.70	0.7602	0.1188
Dirty	2938917.15	1440193.93	0.0489	

Strgeqappear	TPC LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
Clean	3463031.50	1572138.14	0.0343	0.0199
Dirty	-1116253.53	1621513.14	0.4957	

Milkserving	TPC LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
Pouring	1190951.01	1381373.47	0.3945	0.9794
Scooping	1155826.95	1531282.81	0.4554	

Dependent Variable: *Enterobacteriaceae* count

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Quality checks	1	21604779967	21604779967	0.02	0.8948
Pooling of milk	1	16128584751	16128584751	0.01	0.9090
Transport length	5	714758979875	142951795975	0.12	0.9852
Training	1	11242216145	11242216145	0.01	0.9240
Means to clean containers	1	7243789704	7243789704	0.01	0.9390
Cleanness of business agent	1	7706154257.4	7706154257.4	0.01	0.9371
Storage equipment appearance	1	79143676026	79143676026	0.07	0.8004
Milk serving	1	439641568007	439641568007	0.37	0.5538
Error	12	1.4217459E13	1.1847882E12		
Corrected Total	24	1.6558351E13			

R-Square	Coeff Var	Root MSE	Entcount Mean
0.141372	394.8708	1088480	275654.6

Least Squares Means

Qlty checks	Entcount LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
No	200398.635	551256.466	0.7225	0.8948
Yes	118942.834	644504.189	0.8567	

Milkpooling	Entcount LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
No	203087.940	598113.443	0.7401	0.9090
Yes	116253.529	675682.894	0.8663	

Transportlth	Entcount LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1hr	68372.31	868355.64	0.9385	1
2hrs	26687.40	1316051.45	0.9842	2
30min	250332.85	1401791.87	0.8612	3
Lessthan	71164.94	752680.96	0.9262	4
Morethan	539392.66	1020193.97	0.6066	5
Nil	2074.24	655057.73	0.9975	6

Least Squares Means for effect Trsptlth
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Entcount

i/j	1	2	3	4	5	6
1		0.9783	0.9198	0.9976	0.5486	0.9439
2	0.9783		0.9115	0.9773	0.7548	0.9873
3	0.9198	0.9115		0.9092	0.8791	0.8789
4	0.9976	0.9773	0.9092		0.6076	0.9221
5	0.5486	0.7548	0.8791	0.6076		0.5697
6	0.9439	0.9873	0.8789	0.9221	0.5697	

Least Squares Means

Training	Entcount LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
No	200761.532	461633.920	0.6714	0.9240
Yes	118579.937	824691.931	0.8881	

Meansequipcl	Entcount LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
Coldwtrs	120617.014	918204.927	0.8977	0.9390
Htwtrsoa	198724.455	439496.801	0.6592	

Agentcleanness	Entcount LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
Clean	210928.214	938593.161	0.8260	0.9371
Dirty	108413.255	681368.617	0.8762	

Strgeqappear	Entcount LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
Clean	33855.109	664950.502	0.9602	0.8004
Dirty	285486.360	754400.591	0.7117	

Milkserving	Entcount LSMEAN	Standard Error	H0 :LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
Pouring	345280.465	629912.378	0.5936	0.5538
Scooping	-25938.996	571168.143	0.9645	