ASSESSMENT OF MILK HANDLING PRACTICES AND BACTERIAL CONTAMINATIONS ALONG THE DAIRY VALUE CHAIN IN LUSHOTO AND HANDENI DISTRICTS, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PUBLIC HEALTH AND FOOD SAFETY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

Contaminated milk is responsible for up to 90% of all dairy-related diseases of humans. A cross sectional study was carried out in Lushoto and Handeni districts of Tanga, Tanzania to determine the milk handling practices, bacterial contamination and selected milk-borne zoonotic pathogens along the dairy value chain. A total of 93 respondents were interviewed and 184 milk and milk product samples were collected. Laboratory analysis of total and coliform plate counts, detection of *Escherichia coli* O157:H7 and *Brucella abortus* using polymerase chain reaction (PCR) were done. Results showed that, most farmers (57 %) milked their cows under unhygienic conditions. More than 60% of farmers did not clean their hands, wash cow teats and clean animal houses before milking. The majority (92.1%) of farmers were not trained on livestock keeping and milk handling. Although the mean TPC was within the East African Community (EAC) standards, general counts ranged between 3.3 to 5.8 log10. Eighty seven and 93% of milk from farmers and vendors, respectively, did not meet the TPC EAC standards. All the collected milk did not meet the CPC EAC standards, indicating contamination of milk with coliforms. PCR analyses did not detect *E. coli* O157:H7 in all the tested samples while *B. abortus* was detected in 37 out of 87 samples tested. It was concluded that unhygienic practices of milking and post-harvest handling along the dairy value chain possibly contributed to microbial contamination of milk. Detection of *B. abortus* in milk is of public health significance due to its zoonotic potential. It is recommended that veterinary/extension services be provided to livestock farmers on proper animal husbandry and control of zoonotic animal diseases. Public education should be given to all stakeholders in dairy industry on milking and post harvest handling of milk to curtail the likely losses due to rejection of spoiled milk and milk-borne pathogens resulting from contamination of milk.

DECLARATION

I, FORTUNATE SHIJA do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and it has neither been, nor concurrently being submitted for higher degree awards in any other institution.

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I pass my sincere gratitude to the districts' councils of Lushoto and Handeni through their animal health departments for providing me with the support needed especially field assistants during my data collection. I also would like to thank the village leaders in the two districts for helping me with identification of households and restaurants from which the milk samples were collected.

DEDICATION

I dedicate this work to my mother Ritha Shija, my sisters Lilian and Teckla Shija and my brother Kizito Shija. Their love and support during my study time gave me strength and wisdom to accomplish my goal.

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ABBREVIATIONS AND SYMBOLS

µl	microlitre
DNA	deoxyribonucleic acid
MRT	milk ring test
NDB	National Dairy Board
PCR	polymerase chain reaction
SHDF	small holder dairy farmers
STEC	Shiga toxin producing *E. coli*
SUA	Sokoine University of Agriculture
TAMPA	Tanzania Milk Processors Association
TAMPRODA	Tanzania Milk Producers Association
TBS	Tanzania Bureau of Standards
TCC	total coliform count
TDL	Tanzania Dairy Limited
TFDA	Tanzania Food and Drugs Authority
TFL	Tanga Fresh Limited
TPC	total plate count
URT	United Republic of Tanzania

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Food-borne diseases are a serious threat to people in Africa, responsible for 33-90% cases of mortality in children (Flint *et al*., 2005). Although foods of animal origin are a minor constituent in most diets, they are responsible for the majority of incidents of food-borne illnesses; dairy products being implicated (de Buyser *et al.*, 2001). Despite being a nutritional-balanced foodstuff, milk is well known as a medium that favours growth of several microorganisms. Up to 90% of all dairy related diseases are due to pathogenic bacteria found in milk (Ryser, 1998). Weinhaupt *et al*. (2000) and Shirima *et al*. (2003) documented several pathogens known to cause milk-borne zoonotic diseases in humans including brucellosis, tuberculosis, leptospirosis, Q fever and campylobacteriosis. In recent years, there has been emergence of new pathogenic bacteria along the food chain. For example emergence of new milk-borne bacterial pathogens with very serious health effects such as *Eschericia coli* 0157:H7 has been reported (Sivapalasingams *et al*., 2004).

Unlike in developed countries, the dairy industry in most African countries is underdeveloped, dominated by unpasteurized milk and informal markets (Regional Dairy Trade Policy Paper, 2004). The challenges for developing countries remain on how to identify and alleviate technological constraints in order to improve the dairy value chain. Therefore efforts are needed to ascertain the possible solutions towards the improvement of the milk and milk products. Several studies need to be carried out in order to provide reliable information for the improvement of the industry.

The development of new molecular technologies has offered the possibility of testing for a number of pathogens at several points of the value chain and hence gaining a better understanding of the associated pathogens. Polymerase chain reaction (PCR) represents a rapid procedure for the immediate detection and identification of specific pathogenic bacteria from different food materials (Lanzt *et al*., 1994). Therefore it is important to make use of this technology to explore microbial quality of milk and its products so as to be able to institute some control measures.

1.2 Problem statement and justification of the study

Raw milk is known to be a major means for transmission of milk-borne pathogens to humans and the prevalence of zoonotic diseases in animals in Tanzania is high yet many people still prefer to consume raw unpasteurized milk. Milk produced in Tanzania is mostly for the domestic market which prefers raw milk and little amount of processed products (Njombe *et al*., 2011). The population in Tanzania is currently estimated to be 45 million, such an increase has led to the increased demand for good quality dairy products (NBS, 2012)) yet the production remains stagnant. This is reflected by the cattle population in Tanzania which was reported to be 18 million almost 10 years ago (Swai *et al*., 2005) and up to now the population is almost the same. The milk market in Tanzania is mainly informal and most of it is operated by individual smallholder producers. The market faces a number of constraints among them being high risks of microbial contamination due to lack of knowledge on microbial risks related to milk handling and consumption. The presence of microbial pathogens and other hazards in informal market in Tanzania is high, yet the risk to human health is mostly unknown and current food safety management is ineffective and inequitable. Information on the milk handling, quality assessment and marketing linkages along the dairy value chain is inadequate. Karimuribo *et al*. (2005) and Mosalagae *et al*. (2011) reported that the information on the

risks posed by informal milk markets expressing incidences of zoonoses, chemical and drug residues is limited in most of African countries.

Tanga region has one of the biggest milk processors in Tanzania, the Tanga Fresh Limited (TFL). Owing to the presence of TFL, smallholder dairy farmers in Handeni and Lushoto districts that previously used to produce milk at a subsistence basis are now producing for market basis. Still most of the market is informal and hence increased risk of microbial contamination. On the other hand, most of the milk consumed in rural areas is un-hygienically handled and preference is given to raw milk compared to pasteurized and boiled milk. Meanwhile, information on milk handling, risks associated with informal market and unhygienic handling of milk from producer level to consumer.

Therefore, this study was carried out to explore the possible sources of microbial contamination and the risks associated with it along the dairy value chains in Lushoto and Handeni districts. Specifically, the study explored the presence of microbial contamination in the milk through the bacteriological plating and diagnostic PCR. It is anticipated that the findings of this study will be used to provide insight to the public on the health hazards associated with milk and possibly institute some practical measures aimed to mitigate the problems.

1.3 Objectives of the study

1.3.1 Main objective

To assess the milk handling practices, bacterial contaminations and determine the presence of selected milk-borne zoonotic pathogens along the dairy value chain in Lushoto and Handeni districts in Tanga, Tanzania.

1.3.2 Specific objectives

1. To assess the possible sources of microbial contamination of milk from farm to consumer
2. To establish total plate count of bacteria and coliforms in milk from Lushoto and Handeni districts in Tanga region, and
3. To establish the prevalence of *Escherichia coli* 0157:H7 and *Brucella abortus* in milk by using PCR

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Milk production system in Tanzania

Milk production system in Tanzania is mainly characterized by smallholder system with very few large scale farmers and traditional system dominated by indigenous cattle keepers who are either pastoralists or agro-pastoralists. Though still underdeveloped, the dairy industry in Tanzania is dynamic with so many efforts that have been put forward to develop it. The efforts started as early as before independence where milk production was only practiced in areas conducive to dairy cattle rearing, to the present efforts which are geared towards modernization, commercialization and competitiveness of the dairy industry (URT, 2011).

The efforts included the establishment of Zonal Dairy Boards in areas with surplus milk to regulate and develop the industry. The regulation of the industry was done by replacing the Dairy industry ordinance No. 61 (Cap 456) with Dairy Act of 1973 which established a government controlled National Dairy Board (NDB). In addition, the efforts also included the establishment of the programmes to boost dairy development with the efforts mainly geared towards increasing milk production. Most importantly, the efforts were concentrated towards improvement of the indigenous cattle through crossbreeding, diseases control, animal production and the establishment of medium and large scale dairy farms, livestock multiplication units, milk processing plants and milk marketing infrastructures. This resulted in establishment of (i) eight dairy farms under Dairy Farming Company (DAFCO), (ii) seven milk processing plants under the Tanzania Dairies Limited producing reconstituted milk using powered skimmed milk and butter oil which were supplied by the World Food Programme and, (iii) establishment of the

medium and large scale farms to small holder dairy development. These initiatives led to many individuals and agencies to join into the industry as milk producers, processors, marketing agents and facilitating agencies and perform various functions such as promotion of improved dairy breed, milk processing and marketing.

To further improve the industry, the government endorsed a Dairy Industry Act No. 8 of 2004 which resulted to the establishment of the Tanzania Dairy Board with a mandate to develop and regulate the industry. Consequently, all these efforts led to improved and increased milk production in Tanzania. Through the established Tanzania Dairy Board, the efforts of moving the industry from subsistence production to commercialized production are seen with more efforts put towards sustainable production system.

2.1.1 Milk production in Tanzania

It has been reported that total milk production in Tanzania is estimated to be 1.65 billion litres per year, with about 70% of the milk produced by the traditional sectors (indigenous cattle) and 30% from the improved cattle mainly kept by smallholder producers (URT, 2011). Reports have shown that milk production in Tanzania has been increasing over time, (Table 1). However, it should be noted that the increase is due to increased number of cattle and not the production per herd. Still this increase does not go in line with the human population growth. Small proportion of the milk that is produced in the rural areas penetrates the urban markets and the milk processing plants though it is associated with poor infrastructure, such as collection centres, power supply, road network and transport facilities (Njombe *et al*., 2011).

Table1. Production of milk 2000/01-2009/10 in Tanzania (litres)

	Year									
Type of cattle	2000/01	2001/02	2002/03	2003/04	2004/05	2005/06	2006/07	2007/08	2008/09	2009/10
Indigenous	514,000	578,000	620,700	813,700	920,000	941,815	945,524	980,000	1,012,436	997,261
Improved	300,000	322,000	359,800	366,300	466,400	470,971	475,681	520,000	591,690	652,596
Total	814,000	900,500	980,500	1,180,000	1,386,400	1,412,786	1,421,205	1,500,000	1,604,126	1,649,857

Source: Ministry of Livestock and Fisheries development (2011)

2.2 Milk quality and control systems in Tanzania

The Tanzania dairy sector has an elaborate institutional framework been guided by the Livestock Policy, the Tanzania Dairy Industry Development Policy (2002) and the Dairy Industry Act (2004). Its supportive structures include, The Tanzania Dairy Council (TDC), the Tanzania Dairy Board (TDB), the Tanzania Food and Drugs Authority (TFDA) and the Tanzania Bureau of Standards (TBS). Moreover the sector has also strong associations of producers, the Tanzania Milk Producers Association (TAMPRODA) and processors, the Tanzania Milk Processors Association (TAMPA). The current law that governs the dairy products is the Dairy Industry Act, 2004. The act provides for the production, regulation and promotion of the dairy industry, establishment of the Tanzania Dairy Industry Board and repeal of the Dairy Industry Act, 1965.

A big challenge on food safety that has been highlighted by the TFDA is that, Tanzania does not have a defined published policy regarding food safety and quality. On the other hand TAMPA/BEST-AC conducted a study in 2007/08 on the diary sector competitiveness and one of the findings was that the Dairy Industry Act I is the only law that exclusively addresses the dairy industry while other laws and regulations that also address milk quality create overlap of activities which occurs when other regulators undertake functions that are not addressed by the Dairy Industry Act. Based on the outcomes of the study, TAMPA proposed a new framework that should take into account harmonization of the overlapping regulations in the dairy industry. Therefore, following the proposed framework by TAMPA together with other suggestions, the TDB has been working on developing the sector and hence different developments that have been seen and are still being implemented.

2.3 Concepts of food safety and risk analysis

Food-borne disease is still an alarming problem in developing and developed countries leading to severe human suffering and major economic loss. WHO and FAO (2005) estimated that up to 2.2 million deaths each year in developing countries are due to food and water-borne diarrhea diseases. This entails the importance of having the knowledge of the hazards that cause food-borne diseases and the risks associated with the hazards which will enable nations through their agencies to significantly reduce the problem.

Risk analysis is a science based approach to improve food safety decision-making process hence contributing to reduction in the incidence of food-borne diseases and continuous improvement in food safety. This approach makes it possible for the information on hazards in food to be linked directly to the available data on the risk to human health. Risk analysis has proved ability to improve food safety decision making process and improve public health. Risk analysis comprises; risk assessment, risk management and risk communication. Therefore this study employed the risk assessment component of the risk analysis to identify risk factors associated with milk production and handling, and possible bacterial hazards that were present in milk from Lushoto and Handeni districts.

2.4 Factors influencing food safety in dairy value chain

Food safety is the responsibility of everyone involved in the food chain, which include regulators, producers and consumers. Milk is one among the well nutritionally balanced foods and hence its safety is vital. However, de Buyser *et al*. (2001) reported that milk and milk-based food products are highly susceptible to microbial contamination because of their rich composition, which provides a favourable medium for growth of spoilage agents. The

pathogens in milk are derived from several sources including dairy animal, the handler and the environment while the most common external source is contaminated water supply (Kaplan *et al*., 1990). Nonetheless, it should be known that humans can be the most critical source of infection along the milk value chain. For example, following pasteurization milk contamination may occur during packaging and dispensing at shops and/or restaurant.

Much has been documented on how much most consumers in Tanzania prefer raw milk to processed milk. Kurwijila *et al*. (1995) did a survey in Dar es Salaam and found out that 80% of the consumers consumed raw milk while Mullins (1993) found that 51% of households in Dar es Salaam consume raw milk. These practices are contrary to the regulations which are put to protect the public against the milk-borne diseases. As far as food safety is concerned, this is an alarming situation brought up through the dairy industry. Bearing all these in mind several factors have been associated with the safety of milk and milk products along the dairy value chain including animal health factors, hygienic practices and environmental factors.

2.4.1 Animal health factors

Several publications have shown how milk from unhealthy cattle is not safe for consumption unless processed accordingly. Studies by Zvizdic *et al*. (2006) and Makita *et al*. (2008) concluded that human brucellosis occurs through ingestion of milk and milk products or by direct contact with tissues and fluids of infected animal. On the other hand, other zoonotic diseases such as tuberculosis, campylobacteriosis, Q fever and salmonellosis are acquired through drinking milk from infected animals (Charles *et al*., 1999; Weinhaupl *et al*., 2000; Shirima *et al*., 2003). The quality of milk depends very much on the health of the animal.

The health of an animal is assured by combined efforts of the farmer and the veterinarians. The farmer should be keen enough in reporting all the unhealthy conditions to the veterinarians and take up the advice.

2.4.2 Hygienic practices

The hygienic handling of milk from milking to the time it reaches a consumer has a greater influence on safety and quality of the milk and its products. When unhygienically handled, milk can easily be contaminated along the value chain. Possible practices that can lead to milk contamination include, milking, transportation and delivery of milk. Infected personnel involved in milking are also a potential source of milk contamination. Moreover, containers that are used to put milk during milking, storage and delivery may be possible sources of milk contamination. Under poor sanitary conditions, milk can easily be contaminated by several bacteria (Prajapat, 1995; Chatterjee *et al*., 2006). To avoid cross contamination along the milk value chain, proper separation of the activities, thorough cleaning and disinfection of containers is important (Kivaria *et al*., 2006). The hygienic measures taken by milk handlers before, during and after milking play a vital role on milk hygiene and safety.

2.4.3 Environmental factors

A number of environmental factors are associated with the hygiene of milk along the dairy value chain for example water sources, and soil. Bacteria are ubiquitous in air and can easily be introduced into milk. Torkar and Tegar, (2008) have reported that bacterial contamination of milk can originate from different sources such as air, feeds, milking equipment, soil, faeces and grass. Furthermore, Kivaria *et al*. (2006) found that elevated levels of enteric organisms in milk reflect a probable source of contamination via water or soil where faecal-

oral transmission appears to be of epidemiological significance. On the other hand, cow housing system and the environment from which milking activities are done have a greater impact on the safety of milk. Dirty environment with cow dung is one of the many factors that can lead to microbial contamination of the milk during milking. Donkor *et al.* (2007) found that important source of microbial contamination of milk is faecal pollution most probably coming from the cow dung.

2.5 Brucella and *Escherichia coli* infection in relation to dairy milk and its products

2.5.1 *Brucella infection*

Brucella is a wide spread zoonotic pathogen transmitted mainly from cattle, sheep, goats and camels to human through direct contact with blood, placenta, fetus or uterine secretions. Moreover, studies show that transmission of pathogenic *Brucella* strains in humans also occurs as a result of consumptions of contaminated milk and milk products (Young, 1995). The existing literature associates the particular species and biovar with brucellosis in different animals hosts i.e. *B. ovis* with sheep, *B. melitensis* with goats, *B. abortus* with cows, *B. suis* with pigs and *B. neotomae* with desert wood rats (Nielsen, 2002). Worse enough, all these *Brucella* species have a potential of causing brucellosis in humans.

For diagnosis of brucellosis in dairy cattle, bacteriological, serological and molecular methods are used (Neilsen, 1996; Nielsen, 2002). Despite the fact that the isolation of the bacteria leads to the definitive analysis of the disease, serological methods are essentially common. Milk ring test (MRT) has been used as the main screening test for *Brucella* test although its specificity is low (Rolfe and Sykes, 1987). In recent years, the detection of *B. abortus* has been made simple, quick and reliable through use of diagnostic PCR. This is a

very useful method since it can detect bacteria even in samples contaminated with different types of microorganisms (Cortez *et al*., 2001).

2.5.2 *Escherichia coli* infection

There are several types of *E. coli* strains known to contaminate milk but for the purpose of this study, *E. coli* O157:H7 has been investigated. While most of the strains are harmless, *E. coli* O157:H7 is harmful as it can produce toxin leading to severe illness. The toxin is called Shiga toxin (STEC) which is known to cause severe hemorrhagic conditions in humans (Karmali *et al*., 2010). Infections in humans may occur though consumptions of infected raw unpasteurized milk and milk products. Baylis (2009) reported that although the transmission of STEC is associated with consumption of undercooked meat, raw milk and dairy products also significantly contribute to the reported cases of STEC in humans.

For diagnosis and identification of *E. coli* infection in milk traditional microbiological assays have become difficult due to lack of biochemical features distinguishing between pathogenic *E.coli* to non-pathogenic ones. Several molecular assays have been developed and tested for the screening of food products contaminated by pathogenic *E. coli* (Oswald *et al*., 2000; Tarr and Whittam, 2002). Therefore owing to the development of molecular techniques, the study employed the use of PCR to identify the presence of *E. coli* O157:H7 in milk.

2.6 Summary of key observations in literature review

From the literature several things have been highlighted

(a) There is dearth of information on milk handling practices and safety among rural livestock keepers in Tanzania

(b) There is dearth of information on milk-borne infections related to milk handling practices in rural Tanzania

(c) There is need for studies to determine milk handling practices and relations with milk-borne infection among rural farmers, vendors and consumers in Tanzania

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study area

The present study was conducted in Tanga region of the north eastern coastal part of Tanzania, which has a total area of 26,870 km^2 with 75% of arable land and 18% cultivated land. The area is located between longitudes 37º and 39º East and latitudes 4º and 6º South and is characterized by hot and humid tropical climate with rain seasons in March, April, November and December. The mean annual rainfall varies from 500 to 1400 mm with relative humidity ranging from 60% to 90% for most of the year.

Tanga region was chosen for the study due to its long history of livestock keeping and dairy marketing owing to support by several Non- Governmental Organizations of Small Holder Dairy Development programmes which resulted to Tanzania Dairy Limited, producing up to 40,000 litres of milk per day. Two districts of Tanga region: Lushoto and Handeni were selected for the study. Lushoto is bordered by Kenya to the north, Muheza district to the east, Same district to the northwestern and Korogwe district to the south. On the other hand, Handeni is bordered by Kilindi district to the west, Korogwe to the north, Pangani to the east and Bagamoyo to the south (Figure 1).

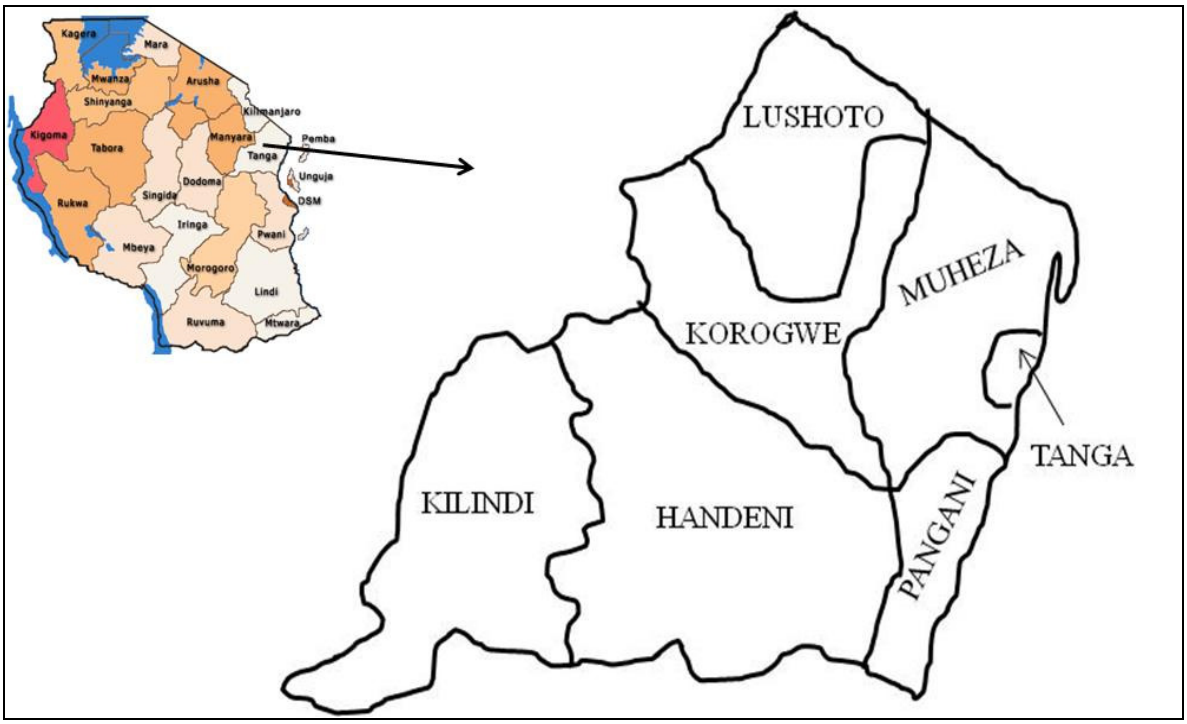

Figure 1. A map of Tanga region showing its districts including Lushoto and Handeni districts that were selected in this study: *Insert* is a map of Tanzania that shows different regions.

3.2 Sample size determination

The sample size was estimated as described by Fisher *et al.* (1991) using the following formula:

$$N = \frac{Z^2P(1-p)}{d^2}$$

Where N = estimated sample size, Z = Confidence interval, P = Estimated prevalence, 1-p = Probability of having no antimicrobial contamination in the sample and d = precision level (acceptable error). Calculating using the following values; Z = 1.96, d = 0.05, p = 0.8, N was equal to 245.9 and hence it was estimated that up to 250 milk samples were to be collected and analyzed.

3.3 Study design and population

A cross sectional study design was employed. The study involved different actors and nodes along the dairy value chain who were farmers, milk collection centres, milk vendors, milk retailers and milk consumers from Lushoto and Handeni districts**.** Two types of farmers were involved in the study including smallholder dairy farmers (SHDF) and traditional farmers keeping Tanzania short horned zebu. The inclusion criteria of the study participants included, availability of milk during the time of sample collection and willingness to participate in the research.

3.4 Selection of study villages and households

Prior to selection of the villages for this study, a survey was done in both districts to identify villages with livestock keepers. A total of 25 villages which had many household with livestock keepers were identified. From the 25 villages chosen, five villages from each district were randomly selected for sample collection. With the help of village leaders, all the households with livestock keepers in the selected villages were identified and each listed on a piece of paper. The papers were mixed and seven households were randomly selected from each village. A total of 35 households which included SHDF and traditional farmers in each village were selected and included in the study

3.5 Milk vendors, restaurants/kiosks and consumers selection

Purposive sampling was done for milk restaurants/kiosks, vendors and consumers. Prior to sampling all the villages from both districts with milk restaurants/kiosks and vendors were identified. Milk samples were taken and questions which generally focused on the type of milk sold, source of milk and hours taken for the milk to finish were administered to all

vendors and restaurants which had milk during the time of sample collection. Consumers were picked from restaurants/kiosks and from a few households.

3.6 Data collection

Two types of data were collected including sociological data and laboratory based data.

3.6.1 Sociological data collection

Structured questionnaires were used which focused on all selected farmers with lactating cattle to obtain information regarding animal management, common animal diseases, milk production, milking and milk handling, marketing/selling, transportation and common problems associated with milk (Appendix 1). In addition, milk vendors and processors and owners of milk restaurants were interviewed on the quality of milk they handle, possible sources of microbial contamination and problems associated with trading milk (Appendices 2 and 3). Lastly a checklist of questions was administered to workers at the milk collection centres (Appendix 4). The questionnaires were made of pre-coded closed ended questions with very few open ended questions.

3.6.2 Pretesting of questionnaires

Prior to starting of data collection, the questionnaires were tested for clarity and time. After testing they were revised and re-written in a better order. The revised questionnaires were translated into Kiswahili which is the language known by the respondents.

3.6.3 Administration of questionnaires

The questionnaires were administered through face to face conversation. While administering questionnaires, direct observation on general cleanliness and hygienic practices with regard to milk was also done and noted. Upon finishing of the administration of questionnaires, milk and milk product samples were collected for laboratory analysis.

3.7 Sampling and handling of milk samples

Milk samples were collected from all the actors along the dairy value chain. In that aspect, milk samples were collected from farmers, restaurants /milk kiosks/milk selling points, milk vendors, milk collection centres and milk consumers. At farm level, milk samples were obtained directly from the containers used during milking, distribution and storage. About 50 ml of milk sample was collected and put in a sterile falcon tubes and placed in a cool box with ice packs. Within four to six hours samples were transported from the field and temporarily stored at -20^{o}C for up to around one week before transporting to the laboratory. Thereafter samples were transported to the Genome Science Laboratory at Sokoine University of Agriculture and stored at -80^{o}C until analysis. Types of milk samples collected are summarized in Table 2.

Table 2. Types of milk samples collected for laboratory analysis

Type of milk	Source	No. of samples
Raw milk	SHDF, traditional famers	108
	Vendors	9
Boiled milk	Restaurant/kiosks, consumers	38
Fermented milk	Vendors	8
Milk products (cheese, quark, butter)	Farmer (processor)	3

3.8 Laboratory analysis

3.8.1 Microbiological analysis

3.8.1.1 Media preparation

3.8.1.1.1 Nutrient Agar

Nutrient agar (OXOID® Ltd., Basingstoke, U.K.) containing 1 g/l of '*lab-lecmo'* powder, 2 g/l of yeast extract, 5 g/l of peptone, 5 g/l of sodium chloride and 15 g/l of agar was prepared according to the manufacturer's instructions. Briefly, 28 g of the powder was dissolved in 1 litre of distilled water. The solution was boiled to dissolve completely and sterilized by autoclaving at 121 ºC for 15 minutes. Before use, the media was cooled up to 45 ºC.

3.8.1.2 MacConkey agar

MacConkey agar (HiMedia laboratories Pvt® Ltd., Mumbai, India) composed of 20 g/l of peptic digest animal tissue, 10 g/l of lactose, 5 ng/l of sodium taurocholate, 0.04 g/l of neutral red and 20 g/l of agar was prepared according to the manufacturer's instructions

where 55 g of the powder was dissolved in 1000 ml of distilled water. The solution was heated to dissolve and sterilized by autoclaving at 121 ºC for 15 minutes. Before use the media was cooled to 45 ºC

3.8.1.2 Total plate count (TPC)

A total of 50 (25 Lushoto, 25 Handeni) milk samples were randomly selected for serial dilution to identify total plate count. Of the 25 samples from each district, 15 were boiled milk samples from the consumers, restaurants and vendors, and 10 were un-boiled milk samples from farmers, vendors and collection centre.

3.8.1.2.1 Sample preparation and incubation

A total of 10 tubes were dispensed with 9 ml of sterilized normal saline. Tenfold serial dilution of the sample from 10^{-1} to 10^{-10} in sterile normal saline solution was done. Then, 1 ml of the milk sample was added into the 9 ml normal saline (10^{-1} dilution). Then, 1 ml of the resulting solution was transferred into a second tube containing 9 ml normal saline (10^{-2} dilution). The procedure was repeated for more dilutions as shown in Figure 2.

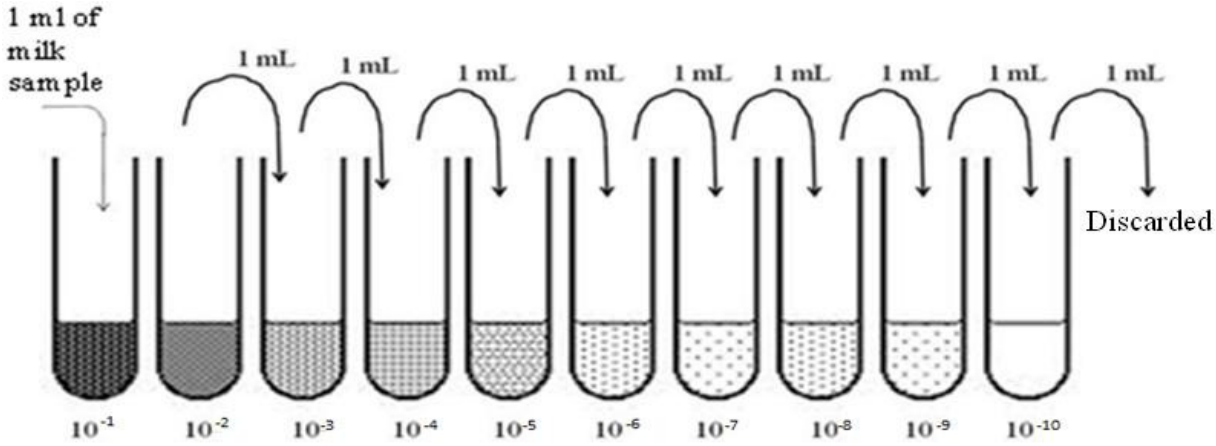

Figure 2. Serial dilutions of milk samples in sterile normal saline before inoculation

After the serial dilutions, 1 ml of the diluted milk sample was added into a sterile Petri dish. Then approximately 22 ml of molten agar (45 ºC) was poured into inoculated Petri dish. The inoculum and the medium were carefully mixed by gentle shaking of the Petri dishes and allowed to solidify by leaving the Petri dishes standing on the horizontal surface of the biological safety cabinet. After complete solidification, all the Petri dishes were inverted and placed in the incubator at 37 ^{o}C ± 1 ºC for 24 hours to allow for bacterial growth.

By using a bacterial colony counter, the number of colony forming units was counted. Two consecutive plates with countable colonies were considered for record.

The number of counted bacteria was expressed in colony forming units per ml using the following formula:

$$\text{Number of bacteria} = \frac{\text{Number of colony forming unit (CFU)}}{\text{Volume plated (ml)} \times \text{total dilution factor}}$$

3.8.1.3 Coliform count

The dilutions and inoculation was done as for the total bacteria count (section 3.8.1.2) except that this used Mac Conkey agar.

3.8.2 Molecular analysis of milk bacterial contaminants

Conventional PCR was used to identify *B. arbotus* and pathogenic *E. coli* 0157:H7 in milk samples using their specific primers.

3.8.2.1 Milk sample preparation and DNA extraction

A total of 2 ml of milk was boiled for 30 minutes and centrifuged at 17,000 g for 5 minutes**.** The pellet was discarded and supernatant used for DNA exttraction. DNA was then extracted from the supernatant using the QIAamp® Viral Mini Kit-Qiagen (QIAGEN Sciences, Maryland, USA) following the manufacturer's instructions.

A known isolate for *E. coli* O157:H7 was kindly provided by Athuman Lipindu of the Department of Veterinary Medicine and Public Health of the Faculty of Veterinary Medicine, Sokoine University of Agriculture. DNA was extracted by boiling the *E. coli* O157:H7 isolate at 80 ºC for 30 minutes in a thermo-cycler followed by centrifugation at 17,000 g for 5 minutes. The pellet was discarded and supernatant taken.

A *B. abortus*, positive DNA sample was obtained from the Microbiology laboratory of the Faculty of Veterinary medicine, Sokoine University of Agriculture. The positive control DNA samples of *E. coli* O157:H7 and *B. abortus* were used in optimization of PCR before performing PCR on milk samples.

3.8.2.2 Polymerase chain reaction (PCR) primers

PCR primers BRU-P5 and BRU-P8 were used for the PCR amplification of bp fragment of the rDNA of *B.abortus* while primers O157-3 and O157-4 were used for the PCR amplification of bp fragment of the hyl A gene of *E.coli* O157:H7. The primer sequences as indicated in Table 3.

Table 3. Primer sequences used for *B. abortus and E.coli* O157:H7

Organism	Primer name: primer sequence (5'-3')	Target gene	Reference
B. abortus	BRU-P5: TCGAGAATTGGAAAGAGGTC	16S-23S	Nancy *et al*., 1996
	BRU-P8: GCATAATGCGGCTTTAAGA	16S-23S	
E .coli	0157-3: GTAGGGAAGCGAACAGAG	*hly* A	Wang *et al*., 1997
	0157-4: AAGCTCCGTGTGCCTGAA	*hyl* A	

3.8.2.3 PCR amplification

The *B. abortus* 16S-23S sequence was amplified as previously described by Nancy *et al.* (1996) with some modifications in the total master mix and annealing temperature. Briefly PCR was performed in a total volume of 25 µl containing 0.5 µl of *Taq* DNA polymerase, (Invitrogen Carls bad, CA), 12.5 µl of 2X reaction buffer, 7 µl of RNase free water, 10 pmol of each primer and 3 µl of DNA template. The mixture was then subjected to 40 cycles of amplification in a thermal cycler (StepOne PCR systems, Applied BioAsystems). The initial denaturation was for 10 minutes at 95 ºC.The cycle consisted of denaturation for 30 seconds at 95 ºC, annealing for 30 seconds at 55 ºC and extension at 72 ºC for 30 seconds. The final extension step was performed at 72 ºC for 10 minutes.

The *E. coli hyl*A gene was amplified as previously described by Wang *et al*. (1997) with some modifications. Briefly, the PCR mixture consisted of 25 µl containing 0.5 µl of *Taq* DNA polymerase (Invitrogen Carls bad, CA), 12.5 µl of 2X reaction buffer, 8 µl of RNase free water, 10 pmol of each primer and 2 µl of DNA template. The PCR reaction was performed in a thermo cycler at a denaturation temperature of 72 °C for 10 minutes. A total of 35 cycles at 95 ºC, 55 ºC and 72 ºC each for 30 seconds followed denaturation. The final extension was performed at 72 ºC for 10 minutes. After DNA amplification, PCR products were analyzed using 1.5% agarose gel at 100 Volts for 30 minutes and afterwards visualized and imaged using a BioDoc-IT imaging system (UVP, Upland, CA).

3.8.2.4 Preparation of agarose gel

Agarose gel was prepared by mixing 1.5 g of agarose powder in 100 ml of 0.5X (Tris-Acetate-EDTA) buffer to obtain a 1.5% concentration of the gel. The mixture was completely dissolved by boiling on a hot plate while stirring using a magnetic stirrer. Agarose transferred to a 50 ml disposable plastic falcon tube and a 2 µl of GelRed nucleic acid stain (Phenix Research Products, Candler, N) was added into the 50 ml of the molten agarose and mixed gently. The agarose was then poured in the horizontal electrophoresis casting equipment (Mupid One, Japan) in the presence of a comb and left to set for about 15 minutes.

3.8.2.5 Loading of PCR products in agarose gel and electrophoresis

A volume of 5 µl of the PCR products was mixed thoroughly with 1 µl of blue/orange 6X loading dye (Promega, Madison, USA) on a laboratory parafilm. The PCR products were loaded in the wells of the agarose gel and 10 µl of a 1 kb molecular weight marker mix

(Promega, Madison, USA) was loaded in a parallel track. The horizontal gel electrophoresis was carried out at a constant voltage of 100V for 30 minutes.

3.9 Ethical consideration

Research permit was provided by the Vice Chancellor Sokoine University of Agriculture and permission letters were obtained from Executive Directors of Lushoto and Handeni districts. Verbal consent was obtained from each respondent after explaining the purpose and importance of the study prior to commencement of interviews and sampling. Participation in the study was on voluntary basis. All the information collected from the participants and the laboratory results obtained after milk samples analysis were kept under the custody of the researcher as confidential.

3.10 Data analysis

The collected data was entered into Microsoft office excel worksheet for cleaning and preliminary analysis. The cleaned data was then copied into STATA I/C 11 statistical package for further analysis. Descriptive statistics like mean, frequencies and percentages were extracted and data presented accordingly. Relationship between different practices as risk factors for microbial contamination in milk was computed against TPC and TCC and statistical significance was established at 95% confidence and critical p value of 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic characteristics of the respondents

A total of 93 (65 farmers, 28 retailers) were interviewed. Of the interviewed farmers 39 (60 %) were traditional catlle keepers and 26 (40 %) were SHDF. The results showed that there were more male vendors compared to females while there were more females involved in restaurant/kiosks business than males (Table 4). The households in Lushoto and Handeni had an average of 6 and 8 family members, respectively. A total of 14 villages were included in the study (Table 4).

Table 4. Demographic characteristics of respondents

Demographic information	Category	Number of Respondents (%)		
		Farmers n =65	Vendors n =16	Restaurants n =12
Sex	Male	49 (75.4)	11 (68.8)	5 (41.7)
	Female	16 (24.6)	5 (31.3)	7 (58.3)
Districts	Lushoto	36 (55.3)	0 (0.0)	8 (66.7)
	Handeni	29 (44.6)	16 (100.0)	4 (33.3)
Number of respondents from each village	Ubiri	10 (15.8)	0 (0.0)	2 (16.7)
	Magamba	10 (15.3)	0 (0.0)	1 (8.3)
	Chakechake	6 (9.2)	0 (0.0)	2 (16.7)
	Irente	2 (3.1)	0 (0.0)	0 (0.0)
	Hamboyo/viti	8 (12.3)	0 (0.0)	3 (25.0)
	Konji	9 (13.8)	4 (25.0)	1 (8.3)
	Kwediyambu	7 (10.7)	1 (6.3)	1 (8.3)
	Sindeni	4 (6.1)	5 (31.3)	1 (8.3)
	Chanika	6 (9.2)	0 (0.0)	0 (0.0)
	Kibaya	3 (4.6)	0 (0.0)	0 (0.0)
	Kilimila	0 (0.0)	1 (6.2)	0 (0.0)
	Kolanda	0 (0.0)	2 (12.5)	0 (0.0)
	Kwemsiha	0 (0.0)	1 (6.2)	0 (0.0)
	Malezi	0 (0.0)	2 (12.5)	0 (0.0)

4.2 Animal management systems

Most of the cow sheds were built of trees and a few of them made of blocks, and iron sheets (Figure 4). There was no significant relationship (p= 0.881 at 95%) between the number of cows and the type of cow shed. Floor materials were generally of mud or earthen followed by stones and a few cemented floors. The feeding systems differed mostly due to the type of cattle kept; however majority of the farmers were not using feed supplements (Table 5).

Table 5. Animal housing and feeding system as reported by farmer respondents (n = 65)

Variable	Category	No. (%) respondents
Types of animal house	Trees/logs "boma"	59 (90.8)
	Block house/mud	3 (4.6)
	Grass	1 (1.5)
	No house	1 (1.5)
Animal house floor material	Under a tree	2 (3.1)
	Mud/earthen	58 (89.2)
	Concrete/cement	3 (4.6)
	Others (timber floor)	2 (3.1)
Animal house floor cleaning	Yes	36 (55.4)
	No	29 (44.6)
Routine cleaning of animal house floor	Once a day	16 (24.6)
	Twice a day	7 (10.8)
	Once a week	12 (18.5)
	Others	30 (46.1)
Feeding system	Cattle & household moved	4 (6.3)
	Only livestock move	25 (39.1)
	Grazing with "boma" feeding or tethered grazing	3 (4.7)
	Stall feeding (zero grazing)	32 (50)
	Others	1 (1.5)
Use of supplementary feed	Maize bran	6 (9.5)
	Mineral supplement	6 (9.5)
	Others	7 (11.1)
	Not using	46 (70.8)

4.3 Hygienic practices during milking, storage and distribution of milk

The main source of water for sanitary activities associated with livestock in both districts was tap water (40%) and was always used during milking in untreated form. The most common type of containers used during milking, storage and distribution were the wide and narrow necked plastic containers (Figure 3). There were no cold storage facilities as all the milk transactions from milking; storage and transportation were being done under room temperature. More than 60% of farmers did not clean their hands, wash cow teats and clean animal sheds before milking. All the farmers reported to do hand milking. The most common means of transport used by farmers in delivering milk was on foot (Table 6).

Table 6. General practices during milking, storage and delivery of milk

Variable	Category	No. (%) farmers respondents
Sources of water	Tap	26 (40.0)
	Wells	21 (32.3)
	Dams and/or streams	19 (29.3)
Milking practices	Cleaning animal shed before milking	28 (43.1)
	Wash hands before milking	46 (70.7)
	Wash cow's teats before milking	41 (63.1)
	Wash hands after milking	47 (72.3)
Containers used for milk storage	Wide necked aluminium vessel	2 (3.1)
	Wide necked plastic vessel	56 (86.1)
	Used water and oil bottles	6 (9.2)
	Cooking pan "sufuria"	1 (1.5)
Containers used for delivery/transportation	Wide necked aluminum vessel	0 (0.0)
	Wide necked plastic vessel	38 (58.5)
	Used water and oil bottles	8 (12.3)
	Cooking pan "sufuria"	3 (4.6)
	Others e.g traditional pots	16 (24.6)
Means of delivery	On foot	37 (56.9)
	By bicycle	9 (13.8)
	By motorcycle	3 (4.6)

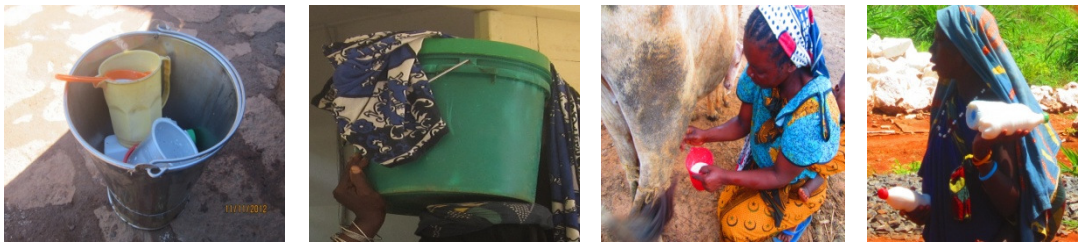

Figure 3.Type of containers used during milking and milk delivery by farmers

4.4 Milk Production and usage by farmers

Lushoto had an average of one lactating cow per household in contrast to Handeni which had an average of seven lactating cows per household been milked. All farmers in Lushoto are smallholder dairy farmers keeping improved cattle and practicing zero grazing while farmers in Handeni keep big herds of traditional cattle. In both districts, it was difficult for to estimate the average production of milk since the calves were left to suck milk from the cows before milking. Most of the milk produced was sold and just little amount was consumed by farmers themselves. Figure 4 shows in detail actors involved in the dairy chain and usage of milk.

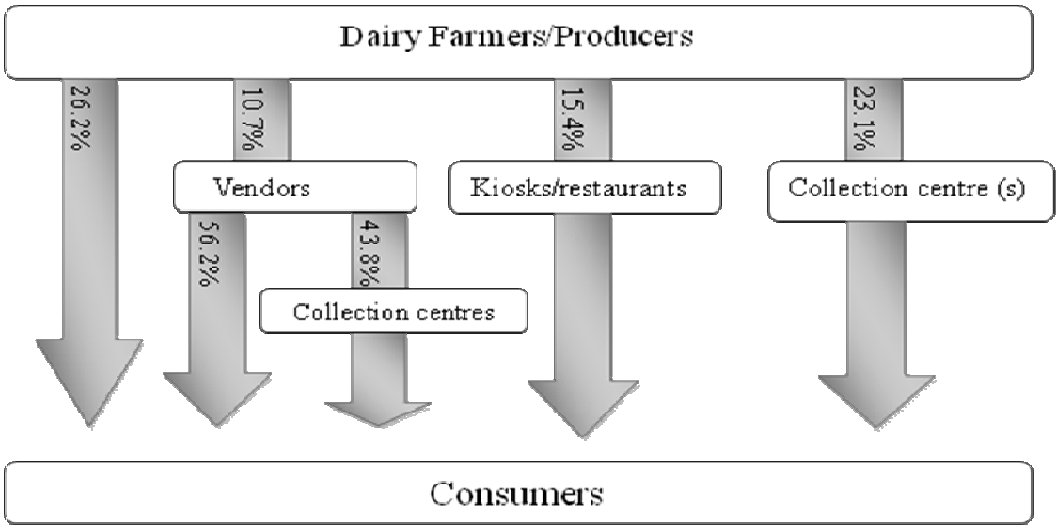

Figure 4. Milk marketing channels in Lushoto and Handeni districts

4.5 Animal health and management

Most famers' respondents (80%) reported that trypanosomiasis, bovine tuberculosis and tick-borne diseases to be the main diseases affecting their animals. Only one farmer in Handeni reported incidence of anthrax in his animals. It was observed that there was no routine screening of cattle for diseases like tuberculosis and brucellosis since the availability of veterinary/extension services were limited. It was further reported that 52.3% of farmers used herbs to treat sick animals. However, 46.2% used veterinary drugs bought from agroshops and 60% of them treated their animals themselves. Moreover, it was reported that a larger populationr (92.1%) of livestock keepers had no training in relation to livestock keeping and general issues related to milk and dairy products.

4.6 Practices by milk retailers in sale and storage of milk

Most of the milk retailers reported to buy milk from different farmers in their villages. The retailers' customers of milk included individual people, milk selling point and collection centres. Containers used for selling and/or distributing milk differed according to the type of retailer (Table 7) and (Figure 5). The milk retailers were also not trained on milk quality and good handling practices.

Table 7. Source, sale and storage of milk by milk retailers

Variable	Category	Number of respondents (%)	
		Vendors n=16	Restaurants/viosks n=12
Source of milk	A farmer in the same village	3 (18.7)	0 (0.0)
	More than one farmer in the same village	11 (68.7)	5 (41.7)
	Farmers in the nearby village	2 (12.5)	3 (25)
	Vendor from the same village	0 (0.0)	3 (25)
	Collection centres	0 (0.0)	1 (8.3)
Type of milk sold	Raw milk	11 (68.7)	1 (8.3)
	Boiled milk	2 (12.5)	10 (83.3)
	Fermented milk	3 (18.7)	1 (8.3)
Customers	Neighbouring households	6 (37.5)	-
	Collection centres	7 (43.7)	-
	Passersby	3 (18.7)	-
Containers used for milk delivery/selling	Wide necked plastic vessels	10 (62.5)	10 (83.3)
	Narrow necked plastic vessel	4 (25.0)	2 (16.7)
	Traditional pots	2 (12.5)	0 (0.0)
How milk is served	Cup	2 (40.0)	-
	Soda/water bottles	3 (60.0)	-
	Hot from a thermal flask in a cup	0 (0.0)	9 (75.0)
	Hot from a cooking pan in a cup	0 (0.0)	3 (25.0)
Handling/storage of excess milk	Consume	-	8 (66.7)
	In a fridge	-	1 (8.3)
	Re-boil next day for sale	-	3 (25.0)

Figure 5. Containers used by retailers for selling milk

4.7 Microbiological quality

A total of 166 milk samples were assessed for microbial contamination by using total plate count (TPC), coliform plate count (CPC), and use of polymerase chain reaction (PCR) for detection of *E. coli* O157:H7 and *B. abortus* in milk.

4.7.1 Total plate count and coliform plate count

The results of TPC for milk from farmers, from vendors and from restaurants are summarized in Table 8. The results showed a mean TPC of 5.3 log10 cfu/ml with more counts reported in milk from vendors ranging from 4.6 to 6.1 log10 cfu/ml. According to the East African community standards of raw cow milk (EAC 67:2007), a good quality raw cow milk should have TPC of less than 5.3 log10 cfu/ml. The results showed that, 87% of milk from famers and 93% of milk from vendors had TPC above the EAC recommended level of 2.0 $x10^5$ cfu/ml. This implied that, most milk from farmers and vendors had poor microbiological quality.

The mean CPC was found to be 4.3 (log10 cfu/ml) with more counts recorded in vendors which ranged from 3.3 to 5.4 (log10 cfu/ml) as indicated in Table 8. Meanwhile according to East African community standards for CPC of raw milk (EAS 67:200), good quality raw cow milk should not exceed CPC of 3 (log10 cfu/ml). This implied that all the milk samples analysed for CPC were above the recommended EAC levels for CPC.

In reference to this limit, indicates unhygienic handling of milk. The average levels of contamination in raw and pasteurized milk (5.3 log10 cfu/ml and 4.9 log10 cfu/ml respectively, for TPC, and 4.3 log10 cfu/ml and 3.6 log10 cfu/ml, respectively, for CPC) were not significantly different ($p > 0.05$).

Table 8. Total plate counts and coliform plate for milk samples from the actors in the value chain

Variable	Observations	Mean (log10 cfu/ml)	Std. Dev (log10 cfu/ml)	Min (log10 cfu/ml)	Max (log10 cfu/ml)
Total Plate Count					
Farmers	21	5.3	5.4	3.3	5.8
Vendors	5	5.8	5.7	4.6	6.1
Restaurants	7	4.9	4.9	0	5.3
Coliform plate count					
Farmers	22	4.8	4.9	2.5	5.5
Vendors	4	4.8	5.1	3.3	5.4
Restaurants	7	3.6	3.9	0	4.3

4.7.2 Risk factors for microbial contamination of milk

4.7.2.1 Risk factors at farmers' level

Several factors related to hygienic practices of the farmers during milking, handling and storage of milk were considered to be possible risk factors for microbial contamination as reflected by TPC and CPC in this study. The factors included; not washing hands, cow teats and not cleaning of animal house before milking and types of milking/storage containers and their cleaning. Statistically, all the factors were found to be not significant ($p > 0.05$) causes of high TPC and CPC (Table 9).

Table 9. Possible risk factors associated with microbial contamination of milk at farmers' level, p-value at 95% CI

	Risk factors		p-value	Mean TPC	Mean CPC	p-value
Milking practices	WHBM	81.8	0.47	2×10^5	5.9×10^4	0.48
	WCTBM	63.6	0.52			0.40
	CAHBM	36.4	0.26			0.31
	WNAC	13.6				
Types of containers	WNPC	72.7	0.35	2×10^5	5.9×10^4	0.39
	Cooking pan "sufuria"	13.6				

Key: TPC= Total pale count, CPC= Coliform plate count, WHBC= Wash hands before milking, WCTC= Wash cow teats before milking, CAHBM= Clean animal house before milking, WNAC= wide necked aluminum container, WNPC= wide necked plastic container.

4.7.2.2 Risk factors at milk vendors and restaurants' level

Risk factors that were considered to be associated with the microbial contamination of milk from vendors and restaurants included source of milk, type of containers used for delivery and serving and/or storage of milk, means of transport during delivery and preparation of milk for selling. However, all these factors when analysed against TPC and CPC were found to be not statistically significant ($p > 0.05$) (Table 10).

Table 10. Risk factors associated with milk containers for milk vendors and restaurants

Factors		Vendors	Restaurants	p-value (TPC)	p-value (CPC)
Plate count	Mean TPC	626100	71175.6		
	Mean CPC	88787.5	4188.4		
Where milk obtained from	1 farmer		20.0 %		
	> 1 farmer		80.0 %	0.28	
Type of milk	Raw	60.0 %			
	Fermented	20.0 %		0.28	0.26
Containers for selling	WNAC		57.1	0.32	0.42
	WNPC		42.9		
How milk is delivered	SSP		85.7 %	0.32	0.71
	MR		14.0 %		
Container used for selling	NNPC	80.0 %		0.28	0.26
	WNPC	20.0 %			
	By bicycle	60.0 %		0.27	0.23
	By motorcycle	20.0 %			
How customers get milk	SSP	20.0 %			

Key: TPC= Total plate count, CPC= coliform plate count,

WNAC= Wide necked aluminum container,

WNPC= Wide necked plastic container, SSP= special selling point,

MR= moving restaurant, NNPC= Narrow necked plastic container

WNPC= Wide necked plastic containers

4.8 PCR determination of *E. coli* O157:H7 and *B. abortus*

In this study a total of 166 and 87 milk samples were tested for *E. coli* O157:H7 and *B. abortus*, respectively. All the tested samples were negative for *E. coli* O157:H7. A total of 37 (42.5%) samples showed positive results for *B. abortus* with highest percentage observed in milk from Handeni and Lushoto farmers (Table 11).

Table 11. *B.abortus* PCR results for milk samples from Handeni and Lushoto

Source of milk samples	Lushoto (%) n = 45	Handeni (%) n = 42	Both districts (%) n =87
Consumers	1 (2.2)	4 (9.5)	5 (5.7)
Restaurant	2 (4.4)	2 (4.8)	4 (4.6)
Farmers	14 (31.1)	11 (26.2)	25 (28.7)
Vendors	-	3 (7.1)	4 (4.6)
Total	17 (37.8)	20 (47.6)	37 (42.5)

Figures 6 and 7 show the bands produced following gel electrophoresis for *B. abortus* and *E. coli*, respectively. The targeted gene for *B. abortus* was 16S-23S gene while for *E. coli* O157:H7 was *hyla* A gene.

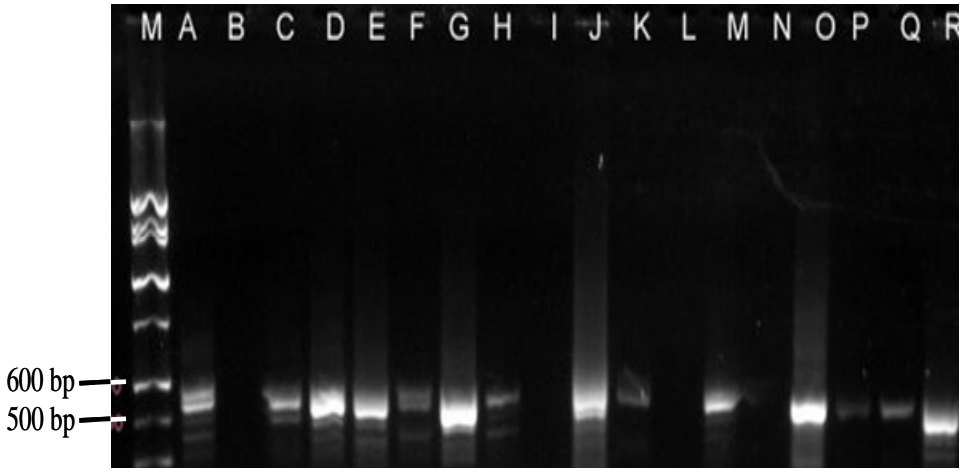

Figure 6. Detection of *B. abortus* by PCR using BRU-P5 and BRU-P8 primer pairs targeting 16S-23S gene producing an expected bp DNA fragment. Note that lane M is a molecular weight marker while lanes A, C, D, E, F, G, H, J, K, M, O, P and Q are positive amplicons whereas lane B, I, L and N are negative amplicons. R is a positive control.

Figure 7. Detection of *E.coli* using O157-3 and O157-4 primer pairs targeting *hyla A* between at 356 bp. Note that lane M is a molecular weight marker, lane A to K are negative amplicons while lane L is a positive control.

CHAPTER FIVE

5.0 DISCUSSION

This study aimed at assessing the milk handling practices and bacterial contaminations and, determining presence of selected milk-borne zoonotic pathogens along the dairy value chain in Lushoto and Handeni districts of Tanga region. Possible risk factors for microbial contaminations along the dairy value chain were explored and the involvement of *B. abortus* and *E. coli* O157:H7 as important milk-borne pathogens was elucidated by using polymerase chain reaction. This was due to the fact that milk produced in Tanzania by the informal sector is not regulated by any agency and such milk may pose a health hazard due to contamination with pathogens. Generally, it was found that, animal housing and feeding, animal health and management, practices of milk harvesting, storage, transportation and retailing predisposed the milk to microbial contamination. Bacteriologically, high TPC and CPC were encountered in most of the samples which were above the recommended East Africa Community standards (EACs, 2007). Interestingly, high prevalence of *B. abortus* was recorded in milk which endangers the health of the milk consumers. Fortunately, *E. coli* 0157:H7 was not detected in all the milk samples analysed.

5.1 Possible factors for microbial contamination

The general hygiene at milking is known to affect the numbers of microorganisms in the milk. It is recommended that before milking, the animal house should be cleaned; the udder should be washed and dried before milking. After milking, teat dipping in suitable disinfectant is necessary to control entry of microorganisms through the teat canal. The personnel and the equipment should be clean. During this study, more than 60% of farmers did not clean their hands, wash cow teats and clean animal houses before

milking. Indeed, the hand milking using unwashed hand practiced by famers may indicate that microorganisms on hands could result in contamination of the milk. In addition, it was observed that milking was done either in the cowsheds or in a kraal with very dirty floor for traditional cattle keepers.This could be another risk practice that contributed to high microbial contamination of milk from farmers. Worse enough, storage and handling of milk under room temperature increases bacteria multiplication. These practices could have contributed to the observed high microbial load in the milk. Previous study by Swai and Schooman (2011) in Tanga reported similar observations. Furthermore, other studies in Zimbabwe, Tanzania and Ghana reported that unhygienic practices along milk value chain predisposed milk to high bacterial load (Gran *et al.,* 2002; Omore *et al.,* 2009). However, in this study, there was no correlation between the high bacterial load in milk and the unhygienic practices that was observed.

On the other hand, the general microbial contamination in milk from vendors and restaurants/kiosks could be associated with the source of milk, bulking, cleanliness of the selling points and storage conditions. Dirty selling environment, lack of cold storage facilities and bulking were all together regarded as main risk factors that contributed to the high bacterial contamination of the milk from restaurants and some vendors. These findings are inline with the study done in Dar es Salaam city by Kivaria *et al*. (2006).

It was realized that the containers used during milking, storage and distribution were the wide and narrow necked plastic containers which sometimes are difficult to wash. Narrow necked plastic containers are not easly washed especially in the inner corners and this lead to sticking of milk residues. In such a situation, microorganisms can rapidly build up in milk residues in milk storage containers, and may contaminate the milk on subsiquent uses. Similar observations were also reported by Kivaria *et al.* (2006) and Bukuku (2013) who reported that plastic containers increased microbial count in milk.

Furthermore, it has been found that the spores of *Bacillus cereus* adhere to surfaces better than do vegetative cells (Peng *et al*., 2001). The plastic containers can thus be a source of *B. cereus* endospores and other similar kinds of bacteria in milk. It is therefore not surprising that the milk storage containers played a significant role in the contamination of milk.

Furthermore, it was noted that over 90% of farmers that were interviewed had no any training on livestock handling and milking hygienic practices. This attributed to most unhygienic practices during milking and generally poor livestock handling reported in the study. Furthermore; most farmers did not see the importance of consulting a veterinary doctor when their animals were sick, some did not know the importance of using feed supplements and others did not know the importance of regular check up on animal health. When asked, most farmers were eager and ready to get knowledge relevant to general animal husbandry and zoonotic diseases. This showed that the extension services in the two districts were limited and hence programs to educate farmers on different matters concerning animal keeping and zoonotic diseases and risks associated to them need to be introduced.

5.2 Microbiological quality of milk

Bacterial load in milk indicates the degree level of hygiene practiced in the whole milk production process. A total bacterial count is an indicator for prolonged storage of milk especially when stored at room temperature. According to international regulations milk should be delivered and refrigerated within 3 hours after milking (IDF, 1990). The results of the present study showed a mean TPC of 5.3 log10 cfu/ml with more counts reported in milk from vendors ranging from 4.6 to 6.1 log10 cfu/ml. According to the East African Community standards of raw cow milk (EAC 67:2007), the mean TPC of milk from

farmers and vendors were above the required standard implying poor microbiological quality. The presence of such high bacterial load in milk may not be surprising since the untreated raw milk harvested from dirty animals; dirty animal houses, the unhygienic environment and general milk handling may have contaminated the raw milk. The results of this study are inline with other done elsewhere in Tanzania by Kweka (2002), Kivaria *et al.* (2006) and Rwehumbiza *et al.* (2013) in which most of the samples tested had higher bacterial count above standards. These findings also compare with studies done in Ghana (Addo *et al.,* 2011), Ethiopia (Tassew and Seifu, 2011) but differ from the study done in Sudan (Adil *et al.,* 2011).

The mean CPC was found to be 4.3 (log10 cfu/ml) with more counts recorded in vendors which ranged from 3.3 to 5.4 (log10 cfu/ml). Meanwhile according to East African Community standards for CPC of raw milk (EAC 67:200), good quality raw cow milk should not exceed CPC of 3 (log10 cfu/ml). In reference to this limit, indicates unhygienic handling of milk. Coliforms are used as indicator microorganisms and the presence of them implies a risk that other enteric pathogens may be present in the milk and implies poor hygiene. The presence of coliforms therefore indicates a safety risk, and the numbers should therefore be of the minimum recommended levels in milk products. Studies by Slaghuish (1996) and Oliver *et al.* (2005) reported that poor housing conditions can be source of contamination of coliforms for housed cows, mainly from bedding material which are mixed with cow dung and urine. Such environment contaminates teats, tail and other body surfaces from which microorganisms gain access into milk during milking. Contamination of bedding materials can be very high due to absorption of urine and faeces. Also as it was observed during this study that water used during milking originated from the tap, wells, dams and/or streams and worse enough it was being used while not treated. Therefore, use of this water for cleaning milking cans

and other associated activities prior to milking subsequently may contaminates the milk with coliforms and other bacteria contaminants.

Surprisingly, the average levels of contamination in raw and pasteurized milk (5.3 log10 cfu/ml and 4.9 log10 cfu/ml respectively, for TPC, and 4.3 log10 cfu/ml and 3.6 log10 cfu/ml, respectively, for CPC) were not significantly different ($p > 0.05$). This finding concurs with that from other studies in pasteurization centers in Gambia, Senegal, and Guinea (Hempen *et al.,* 2004), and also in Brazil (Silva *et al.,* 2009), suggesting that pasteurization is not the only critical step for improving the microbiological quality of milk products. The unsatisfactory quality of pasteurized milk is the consequence of the poor quality of raw milk used and/or a high level of recontamination after pasteurization. Poor handling and storage of pateurized milk in restaurants/viosks observed during this study gave high possibilities for postpasteurization contaminations. These findings highlight the fact that pasteurized milk of such poor microbiological quality poses a threat to consumers.

5.3 Milk-borne zoonotic pathogens: *B. abortus* and *E. coli* O157:H7

The prevalence of *B. abortus* was 42.5% suggesting that there is high contamination rate. More of the samples had come from farmers meaning that the infection had originated from animals. However, it should be noted that the milk was pooled from a bulk collection hence the findings could not reflect the status of individual cow. With such a high prevalence of brucellosis in milk poses a threat to milk consumers. Furthermore, the results revealed a few samples from household consumers especially from Handeni districts to be *B. abortus* positive. This could be associated with the habit of some people in Handeni preferring to drink raw milk to boiled milk as they traditionally believed that raw milk is healthier compared to boiled milk. On the other hand other consumers

consumed fermented milk that had been made from raw milk and hence the chances of contracting *B. abortus* increased. These findings could be related to findings in Tanga by Schooman and Swai (2005) where it was found 56% of the milk marketed in Tanga region (Handeni and Lushoto districts inclusively) were *brucella* positive.

A recent study by Wankyo (2013) established the sero-prevalence of human brucellosis in Morogoro municipality to be 27.3%. In that study, among the risk factors for infection established was consumption of raw unpasteurized milk. Therefore the milk consumers in the Handeni and Lushoto are in dangers of being infected with brucellosis. Indeed, it was reported by the farmer respondents that animals succumbed different diseases and there was no routine screening of cattle for diseases. This was correlated with limited availability of veterinary/extension services. In presence of good animal husbandry coupled with routine screening of diseases to cattle would otherwise have detected and culled all the brucellosis reactor animals. Similar findings were reported by Lyimo (2013) in Morogoro where the prevalence of brucellosis in milk from smallholder dairy farmers was up to 62%. Other studies by Temba (2012) in Morogoro reported a brucellosis seroprevalence of 14.9% in cattle.

All the tested samples for *E. coli* O157:H7 showed negative results. This may show that the bacterium is not present in the cows in the study areas or the milk which was sampled was not contaminated with *E. coli* O157:H7. Previous study by Swai and Schoonman (2011) also did not isolate *E. coli* O157:H7 in milk. In the study by Ndalama *et al.* (2013) reported negative results of *E. coli* O157:H7 in all tested samples from cattle slaughtered at Vingunguti in Dar es Salaam. Elsewhere in Ghana, Addo *et al.* (2011) reported negative results in all 250 milk samples tested. However, Omore *et al.* (2001) isolated *E. coli* O157:H7 in 1% of the samples in milk marketing survey in the Kenyan highlands.

Similarly, Kang'ethe *et al*. (2007) isolated *E. coli* O157:H7 from cattle faeces in urban and peri-urban settings of Nairobi, Kenya.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

From the findings of this study, it is therefore concluded that:

1. Milk produced by farmers and supplied to collection centres and milk vendors in Handeni and Lushoto districts contains unacceptable levels of hygiene indicators and indicates a potential source of milk-borne infections. This raises a public health concern about its safety to consumers
2. Since raw milk is an important vehicle for transmission of zoonoses and other pathogens, this microbial status implies that milk consumers in the study area are at health risk. Indeed, this is supported by detection of *B. abortus* at higher prevalence.

It is therefore recommended that:

1. Veterinary/extension services should be provided to livestock farmers on proper animal husbandry and control of diseases.
2. It is suggested that routine assessment of milk quality produced and consumed by the public be mandetory in order to safeguard the public from milk-borne zoonotic diseases which may emanate through consumption of unsafe milk and milk products.
3. There should be implementation of good hygiene practices throughout the milk chain by training of all stakeholders involved in milking, milk collection and processing, including pasteurization, transport, and delivery, to ensure the safety and quality of milk.
4. Responsible authorities like Tanzania Food and Drug Authority must ensure that existing regulations are instituted and where possible there should be a mandatory

screening of milk before sales to the public. This should also include adequate inspection of milk production facilities with microbiological controls of milk.

5. Farmers should also be educated on good animal husbandry, farm/animal house hygiene, hygienic milking and handling of milk including facilitating them with adequate equipment and facilities for milk storage to minimize unnecessary microbial contaminations.
6. Consumer practices, such as milk boiling, to reduce or eliminate potential infection by milk-borne zoonoses should be further encouraged.

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APPENDICES

Appendix 1: Questioners for milk farmers and milk producers

Questionnaire for farmers and milk producers

1. General information

Date of Survey (DD/MM/YYYY) :	/ /						
Enumerator Name :							
Head of Household Name :							
Did the household consent to the interview? (1= Yes; 2=No)							
If no, why? (code a)							
If no, request a replacement household from supervisor (and continue with this questionnaire)							
Time interview started :	HH:		MM:			Common currency unit:	
Time interview ended :	HH:		MM:				
Site/State/Region/District Name :						Site Code:	
Village/Settlement/Hamlet Name :						Village Code:	
Head of Household Name : (replacement name if original Head above refused)							
Name of survey Respondent :							
Relationship of survey respondent to Household Head (code b) :							
Contact/phone number of the respondent							
Latitudes N/S	Longitude E/W						

No Consent	**Respondent relationship**
1 = Respondent refuses to participate 2 = Respondent does not have the time 3 = Household head (or other knowledgeable member) is not present at the house Other: (specify in cell)	1 = household head 2 = spouse 3 = other family member 4 = other non-family member

The respondent must be the person responsible for most/ all activities related to cattle. It may be the household head, the spouse or another adult household member.

2. Household information

- ***Start with the household head, followed by his wife or wives, children (ranked from old to young) and lastly other household members – include only members who live there at least 3 months per year***

ID	Name	Relationship to HH head (code a)	Gender (1 = Male 2 = Female)	Age (years)	Highest Level of Education (code b)	Primary activity (code c)
1						
2						
3						
4						
5						
6						
7						
8						

Relationship To Head	Highest Level Of Education	Primary Activity
1 = Head 2 = Spouse 3 = Child 4 = Sibling (sister or brother) 5 = Parent 6 = Grandchild 7 = Other relative 8 = Non-relative (including employees who live in house) 9 = Other (specify)	0=No formal and illiterate 1=No formal but literate 2= Kindergarten/pre-school 3= Primary school 4= High / secondary school 5= College 6= University 7= Other (specify)	1 = Crop farming 2 = Livestock & poultry keeping (incl. sales) 3 = Trading in livestock and livestock products (not own) 4 = Trading in agricultural products (excluding livestock!) (not own produce) 5 = Formal Salaried employee (e.g. civil servant, domestic work) 6 = Business – trade / services (non-agric.) 7 = Not working / unemployed 8 = Old/Retired 10 = Infant (<6 years) 11 = Student/ pupil 12 = Disabled 13 = Other (specify)

3. Cattle housing information

3.1 How many cows do you keep?

3.2 Do you keep young and old animals together? Y/N

3.3 How is the housing system? (Observe if possible)

Wall/Roof material	Floor material
Codes **Wall/ Roof material** 1. Thatched 2. Block house 3. Boma/Trees **4.** Other(s) specify	**Floor material** 1. Concrete/cement 2. Mud/earthen 3. Stones 4. Other(s) specify

3.4 Do you clean the floor?

Yes=1 No=2	With what do you use to clean?	How often do you clean?	
Codes **With what do you clean?** 1. Water 2. Water with soap 3. Water with disinfectant 4. Other(s) specify		**How often** Once a day Twice a day Other(s) specify	

4. Feeding

4.1 Feeding practices

Feeding system	Source of fodder(if zero grazing)	Feeding regime(if zero grazing)	Type of fodder

Codes		
Feeding system 1. Pastoral transhumance system (cattle + households moved) 2. Pastoral transhumance system (Only livestock move	**Source of fodder** 1. Public land 2. Planted fodder 3. Purchased fodder 4. Road side fodder 5. Other(s) specify	**Feeding regime** 1. Morning 2. Evening 3. Morning and evening 4. Adlib 5. Other (s) specify

3. Agro pastoral system (mainly grazing with "boma" feeding or tethered grazing 4. Agro pastoral system (only stall feeding (zero grazing)) 5. Other (s) specify	**Type of fodder** 1. Napier grass 2. Guatemala grass 3. Grass legume mixture 4. Fodder trees (e.g. lucaenia etc) 5. Other(s) specify	

4.2 Do you use any feed, mineral supplements and/or concentrates? Y/N

If yes ,type used	If yes, source
Codes: **Types** 1. Maize bran 2. Legumes (beans, soya etc) 3. Roots and tuber peelings 4. Mineral blocks	5. Other(s) specify **Source** 1. Home grounded 2. Purchased 3. Other(s) specify

4.3.1. Is animal feed available throughout the year? Y/N

4.3.1.1 If No, mention times(s) of the year with scarcity of feed._____

5. Information on animal health

5.1 Have your cattle experienced any diseases/conditions in this year Y/N

Diseases/conditions	Control of the diseases	Any mastitis? Y/N	Treatment/control of mastitis

Codes **Causes of death(calves and cows)** 1. Tick-borne diseases 2. Bovine Tuberculosis 3. Trypanasomosis 4. Worms 5. Brucellosis 6. Anthrax	7. Foot and mouth diseases 8. East cost fever 9. Diarrhoea 10. Anaemia 11. Rabies 12. Abortion 13. Q.fever 0. Don't know	14. Others(s)_____ **Treatment Mastitis** 15. Not treated 16. Give antibiotics 17. Other(s) specify

6. Public health

6.1 Knowledge on diseases resulting from milk/milk products consumption

	Y/N	Mention diseases/conditions	Steps taken in case of unhealthy condition	Ways to remove pathogens from milk
Do you know of any diseases that can be caused by drinking raw milk?				
Have you ever experienced any of unhealthy conditions after consumption of milk/milk products				

What do you normally do to remove/reduce pathogens from milk?				

Codes **Diseases** 1. Diarrheoea 2. Tuberculosis 3. Brucellosis 4. Fever 5. Malaria 6. Typhoid Fever 7. Other(s) specify	**Unhealthy conditions** 1. Fever 2. Anorexia (not eating) 3. Diarrheoea (3 or more loose stools in 24hrs) 4. Muscle pain 5. Vomiting 6. Headache 7. Malaise 8. Loss of appetite 9. Yellow fever 10. Amoebic dysentery 11. Coughing 12. Other (specify	**Steps taken** 1. Rest for some hours 2. Visit the health facility for check up 3. Take traditional herbs 4. Take pain killers and rest 5. Take malaria medications (suspecting it is malaria) 6. Other(s) specify	**Ways to remove/reduce pathogens from milk** 1. Sieving/filtering 2. Boiling 3. Letting it to settle-down 4. Fermenting it 5. Other(s)specify

6.2 Information on practices that may lead to acquiring of zoonoses

	Y/N	If yes, Mention the fluid(s)	In what condition is the milk given to children?
Do you drink any other raw fluid(s) from cattle apart from milk?			
Do you prefer giving to young children			

Codes **Fluids** 1. Fresh blood 2. Ruminal fluid 3. Other (s) specify	**Milk condition** 1. Milk that comes straight from the cow 2. Filtered/sieved milk 3. Filtered/sieved +Boiled milk 4. Fermented milk "mtindi" 5. Boiled milk 6. Other(specify)

7. Milk production and practices

7.1 Milk production per day

Number of cows milked	*Estimated amount per day*	*Amount given to cow*	*Amount sold*	*Amount consumed*

7.2 Hygiene in relation to milking practices.

7.2.1 What time(s) of the day do you do the milking? [ ] (codes below)

7.2.2 Do you do any of the following while milking?

	Y/N	If yes, with what?	Source of water used
Clean animal shed before milking			
Tie the cow with rope			
Wash hands before milking			
Dry hands			
Wash the cow's tits before milking			
Wash hands after milking			

Codes

Clean/wash with what?
5. Water
6. Water with soap
7. Water with disinfectant
8. Other(s) specify

Source of water
1. Tap water
2. Well water
3. River
4. Other(s) specify

Time the milking is done
1. Very early in the morning (5am-8am)
2. In the morning hours (9am-noon)
3. Afternoon hours
4. Evening hours
5. Late night

7.2.3 Milk collection, storage and distribution/delivery/sale

	Containers used	How often are containers cleaned	With what are containers cleaned	Means of transportation	Where is the milk sold/delivered	Time the milk delivery is done	How is excess milk stored?
While milking							
For storage							
Milk delivery/sale/distribution							

Codes

Container used
1. Wide necked-aluminum vessels
2. Wide necked-plastic vessels
3. Cooking pan "sufuria"
3. Water with disinfectant
4. Other(s) specify

Means of transportation
1. On foot
2. By bicycle
3. By daladala
4. By motorcycle
5. Other(s) specify

Time for delivery
1. Immediately after milking
2. One hour after milking
3. Two hours after milking
4. Three hours after milking

4. Other (specify__ **How often cleaned** 1. Just before putting in milk 2. Just after delivery of milk **3.** Other (specify **Clean with what** 1. Water 2. Water with soap	**Where is the milk delivered/sold/distributed** 1. Local sales to neighbours 2. To milk vendors 3. Selling points/restaurants 4. Collection centre 5. Other(s) specify	5. Six hours after milking 6. The following day 7. Other (specify)

9. Training

Have u received any training on milk handling? Y/N	When was the training?	Who offered the training?	Was it helpful? Y/N

10. Farmers' organization groups

10.1 A re you a member of any farmers' organization group? Y/N

10.1.1 If yes, do you benefit by being a member? Y/N

10.1.1.1 What benefits do you get?

ASANTE SANA

Appendix 2: Questionnaires for milk vendors

QUESTIONNAIRE FOR MILK VENDORS

A. DEMOGRAPHIC INFORMATION

1. Date _____ 2. Name of the vendor_____

3. Sex Male [ ] 4. Age (Optional) _____

Female [ ]

5. Phone # (interviewee) _____ 6. Ward) _____

7. Name of the village chairperson_____ 8. Phone # (village chairperson) _____

8. District_____9. Village_____

B. MILK COLLECTION AND DELIVERY/SALE

1. What type of milk do you sell
   1) Raw milk
   2) Boiled milk
   3) Fermented milk
   4) Other(s)specify_____
2. Where do you get your milk from?
   1) A farmer in the same village [ ]
   2) More than 2 famers in the same village [ ]
   3) A farmer from neighboring village [ ]
   4) More than 2 farmers in the neighboring village [ ]
   5) Other(s) specify_____
3. How do you get milk from the farmer(s)
   1) Farmer(s) delivers the milk [ ]
   2) Using my own transport(Mention the means of transport) [ ]_____
   3) Go on foot to the farmers place [ ]
   4) Other(s) specify_____
4. What type of container(s) do you use for selling milk?(observe if applicable)
   1) Wide necked-aluminum vessels [ ]
   2) Wide necked-plastic vessels [ ]
   3) Narrow necked plastic containers [ ]
   4) Used plastic water bottles [ ]
   5) Other(s) specify_____
5. What type of container(s) do you use for selling milk?(observe if applicable)
   6) Wide necked-aluminum vessels [ ]
   7) Wide necked-plastic vessels [ ]
   8) Narrow necked plastic containers [ ]
   9) Used water bottles [ ]
   10) Other(s) specify_____

6. Who are your customers?
    1) Neighboring households [ ]
    2) Restaurants/kiosks [ ]
    3) Other(s) specify_____
7. How do your customers get the milk
    1) Deliver to their places (specify the means of transport) [ ]_____
    2) At a special selling point [ ]
    3) Other(s) Specify_____
8. Approximately how many litres of milk do you sell per day_____

9. Approximately how long does it for the milk to finish
    1) 3 hrs after collection
    2) 6hrs after collection
    3) 9 hrs after collection
    4) 12 hrs after collection
    5) Other(s) specify_____

10. How is your cleaning routine for the milk containers?
    1) Cleaning just before putting in milk [ ]
    2) Cleaning after delivery of milk [ ]
    3) Twice a day (before putting in milk and after delivery of milk [ ]
    4) Other(s) specify_____

NB: if there is a special selling place for the vendor, OBSERVE the following

- The cleanliness of the environment
    1. Very clean [ ]
    2. Clean [ ]
    3. Dirty [ ]
    4. Other (s) specify_____
- Delivery of milk to the customers
    1. Hygienically [ ]
    2. Un-hygienically [ ]
    3. Other (s) specify_____
    - Type of container used to fetch milk from the larger container
        1. A cup with a handle [ ]
        2. A cup without a handle [ ]
        3. Other(s) specify_____
    - Type of small containers used to deliver milk to the customers
        1. Narrow necked used bottles with stoppers [ ]
        2. Other(s) Specify_____.
- Observe the general cleanliness of the vendor
    1. Very clean [ ]
    2. Clean [ ]
    3. Dirty [ ]
    4. Other(s) specify_____

Appendix 3: Questionnaire for milk restaurants/kiosk

QUESTIONNAIRE FOR THE RESTAURANTS/MILK KIOSKS

Demographic Information

2. Date _____ 2. Name of the interviewee_____

4. Sex Male [ ] 4. Age (Optional) _____

Female [ ]

6. Name of the kiosk/restaurant_____

7. Phone # (interviewee) _____ 6. Ward) _____7. District _____8.village_____

8. Name of the village chairperson_____ 9. Phone # (village chairperson) _____

Assessment of delivery and sale of milk

1. What type of product(s) do you sell?
    1) Raw milk [ ]
    2) Boiled milk [ ]
    3) Fermented milk [ ]
    4) Other (s) specify_____
2. What time do you get milk/milk products from the producer(s)?
    1) Morning hours [ ]
    2) Afternoon hours [ ]
    3) Evening hours [ ]
    4) Other (s) specify_____
3. Where do you get raw milk from?
    1) A recognized vendor(s) in the area (If more than 1 mention # of vendors) [ ]___
    2) Famer(s) in the neighboring village(If more than 1 mentions #) [ ]_____
    3) Farmer(s) from the same village(If more that 1 mention #) [ ]
    4) Other(s) specify_____
4. Apart from raw milk, do you get other milk products elsewhere? YES [ ] NO [ ]
5. If YES what products do you get?
    1) Fermented milk [ ]
    2) Yoghurt [ ]
    3) Pasteurized milk [ ]
    4) Other(s) specify_____
6. How do you get the raw milk/milk products at the restaurants?
    1) Delivered by the producer
    2) Collect form the producer on foot
    3) Use own transport to get from the producer
    4) Other(s) specify_____

7. What type of container(s) is used to deliver milk to the restaurant?
    1) Wide necked-aluminum vessels [ ]
    2) Wide necked-plastic vessels [ ]
    3) Narrow necked plastic containers [ ]
    4) Used plastic water bottles [ ]
    5) Other(s) specify_____

Preparation and serving of milk for consumption

1. How do you prepare raw milk for consumption?
    1) Sieve and boil [ ]
    2) Boil [ ]
    3) Add water sieve and boil [ ]
    4) Add water and boil [ ]
    5) Other(s) specify_____
2. How do you serve milk?
    1) Hot from a thermal flask and put in a cup [ ]
    2) Hot from the cooking pan and put in a cup [ ]
    3) Customer self-serve from the cooking pan [ ]
    4) Cold from the fridge [ ]
    5) Other(s) specify_____
3. Do you put sugar in the milk? YES [ ] NO [ ]

4. Approximately how many litres of fresh milk do you sell per day_____

5. Approximately how long (hours) does it take for fresh milk to finish? _____

Milk storage

1. What do you do with left-over milk?
    1) Discard [ ]
    2) Given to restaurants' workers to consume [ ]
    3) Stored in the fridge [ ]
    4) Let open in a pan and re-boil the next day for selling [ ]
    5) Put in thermal flasks and sell the next day [ ]
    6) Other(s) specify_____

Public Health

Health of the workers

1. How many workers are there in total? _____
2. Do workers have a regular health check up? YES[ ] NO[ ]
3. When was the last time the workers had their health checked –up?

Customers

1. Have you ever got any complaints from the customer after consumption of milk on the following conditions?
    1) Vomiting
    2) Diarrhoea
    3) Amoebic dysenry
    4) Malaise
    5) Other (s) specify_____

Assessment of the environment

1. a) Do you do fumigation? YES [ ] NO [ ]
   b) If YES how often?

   c) When was the last time fumigation was done?_____

OBSERVE the following

1. How is the premise
   1) Open area under roof
   2) Closed area
   3) Under a shadow of a big tree
   4) Other(specify)_____
2. Cleanliness of the tables (if any)
   1) Very clean
   2) Clean
   3) Dirty
   4) Very dirty
   5) Other (specify)_____
3. Cleanliness of the floor/ground

   1) Very clean
   2) Clean
   3) Very dirty
   4) Dirty
   5) Other(s) specify_____
4. General cleanliness of the kitchen
   1) Very clean
   2) Clean
   3) Very dirty
   4) Dirty
   5) Other(s) specify_____
5. How clean are the restaurant/kiosk servers?
   1) Very clean
   2) Clean
   3) Very dirty
   4) Dirty
   5) Other(s)_____

Appendix 4: Checklist of questions for collection centres

Checklist of question for collection centres

Date_____ Name of the collection centre_____ Owner of the CC_____

Name of the respondent_____ District _____Village_____

Mil collection

How many villages/sub villages/wards does the milk come from?

How much litters of milk do you take_____

What types of containers are used to get milk to the collection centres?

What parameters are checked in order to accept/reject milk?

How is the acceptance/rejection percentage in general?

Is there a chilling/cooling machine? (Observe)

How long does the milk stay before it is transported to the factory/plant?

What type of cars is used to transport milk? (Observe if possible)

What time is the milk transported?

How long does it take to reach the factory/plant?

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

Asante sana