EVALUATION OF MICROBIAL CONTAMINATION IN MILK OF HEALTHY AND MASTITIC COWS IN SELECTED DISTRICTS IN TANZANIA

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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.

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ABSTRACT

The production of milk from cows in Tanzania has not reached the optimum levels due to several constraints including poor genetic potential of animals, lack of feeds especially good quantity and good quality during the dry seasons, prevalence of diseaseases, generally poor management and lack of good husbandry practices. Mastitis is one of the important disease that lowers the milk production and quality in local and dairy cattle in Tanzania. This cross sectional study was conducted between November 2013 and February 2014 in Kilosa and Mvomero districts in Morogoro Region; Lushoto and Handeni districts in Tanga Region. The objectives of the study were to establish the prevalence of mastitis in lactating cows, assess the types of mastitis and establish the bacterial contamination in raw milk. A total of 78 cows were examined for clinical and subclinical mastitis using udder palpation and California mastitis test respectively. Subsequently, milk samples were collected for bacteriological analysis using standard protocols. It was found that the prevalence of mastitis was 75.6% of the total samples examined; 12.8% of the examined cows had clinical mastitis while 62.8% had subclinical mastitis. Breed wise, the prevalence of mastitis was 82.1% and 72% in dairy and local cows respectively. Handeni district had the highest prevalence of mastitis (88%) while Kilosa district had the lowest prevalence (60%) within districts samples analyzed. Lushoto and Mvomero had prevalence of 70% and 83% respectively. Bacteria isolated were *Staphylococcus aureus* (34.6%), *Streptococcus epidermidis* (51.3%) and *Escherichia coli* (12.8%). For the first time in Tanzania, this study isolated *Listeria* spp. in particular *Listeria monocytogene* (32.1%), *Listeria ivanovii* (15.4%) and *Listeria inoccua* (30.8%) from raw cow milk. This study concludes that the magnitude of mastitis in lactating cows in the study areas was high. It is recommended that livestock experts in the districts should apply different methods to prevent and control mastitis in cattle and further studies are

needed to establish the virulence and behaviour of different pathogenic microorganisms that cause mastitis in dairy cattle in Tanzania.

DECLARATION

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

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LIST OF ABBREVIATIONS AND SYMBOLS

ATCC	American Type Culture Collection
BHI	Brain Hart Infusion
BPA	Baird Parker Agar
CAMP	Christine Atkinson Munch Peterson
cfu/ml	Colony forming unit per millitre
CI	Confidence interval
DF	Degree of freedom
EAC	East African Community
EAS	East African Community standard
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
ISO	International Organization for Standardization
LFO	Livestock Field Officer
MDR	Multi-drug resistance
Ml	milliliter
MPEE	Ministry of Planning, Economy and Empowerment
PHCT	Population and Housing Census, Tanzania
SAS	Statistical Analysis System
SCC	Somatic cell count
SFFF-II	Safe food ,fare food project phase two
SUA	Sokoine University of Agriculture
TSHZ	Tanzania short-horned Zebu
USA	United States of America

WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

The production of milk from cows in Tanzania has not reached the optimum levels due to several constraints including poor genetic potential of animals, lack of feeds especially good quantity and good quality during the dry seasons, prevalence of diseases and generally poor management and lack of good husbandry practices (Njombe et.al.,2011) The optimal milk productivity of milking cows in Tanzania has not yet been realized due to several constraints that include poor animal management, poor feeding particularly during the dry season when feeds for ruminants become scarce, poor genetic potential and the high prevalence of diseases as a result of poor disease control. The major diseases affecting the dairy sector of Tanzania include tick borne diseases, bacterial diseases viral, worms and nutritional disorders (Mdegela *et al*., 2009). Of all bacterial diseases Mastitis is the most important disease in the dairy sector worldwide and is more prevalent in dairy cattle (Robello *et al*., 2005). Until recently, these two types of mastitis known to exist worldwide. Sub-clinical mastitis, is the first form of mastitis and is the most prevalent in cattle which cause reduction in milk yields (Karimuribo *et al.,* 2005, Mdegela *et al*., 2009). Furthermore, subclinical mastitis is associated with reduction in milk production of up to 70% and significant increase in somatic cell count (SCC) (Mdegela *et al*., 2009; Mbeho, 2012). Clinical mastitis is associated with swollen, painful and discoloration of the udder, blood in milk, pus and flakes (Shekimweri, 1992; Mdegela *et al*., 2004). Economic consequences of clinical or sub-clinical mastitis include poor quality milk, increased culling rate and increased cost of veterinary services and medicine (Rahman *et al*., 2009). Mastitis could only be minimized if adequate data on health risks are gathered

and risk assessments carried out in order to help to devise effective udder infections mitigation strategies (Mdegela *et al*., 2009).

Subclinical mastitis does not manifest visible changes in the udder or milk. It is not easily recognized and it is more prevalent than clinical mastitis. Also subclinical mastitis has been shown to cause losses in milk production in the normal lactating animals (Mdegela *et al*., 2009). Clinical or sub-clinical mastitis in cattle are caused by microorganisms that may be isolated from milk and milk products. Microorganisms that are commonly found in milk include *Staphylococcus aureus*, *Streptococcus epidermidis, Listeria monocytogenes, L. ivanovii, Listeria innocua, Escherichia coli, Salmonella* spp*., Brucella abortus, Mycobacterium* spp*., Campylobacter* spp*., Leptospira* spp*., Clostridium* spp., *Pseudomonas aeruginosa* and *Proteus* spp. (Piepers *et al*., 2007; Abdel-Rady and Sayed, 2009; Kasalica *et al*., 2011; Al-mariri *et al*., 2013; Hosseinzadeh and Saei, 2014; Kanyeka, 2014). Milk is known to be a major vehicle of transmission of these milk-borne pathogens some of which may pose a serious threat to humans. Occasionally, mastitis is associated with milk borne zoonotic infections which may be transmitted to humans such as tuberculosis and brucellosis. The study carried out in the Southern Highlands of Tanzania isolated Mycobacterium spp from raw milk of indigenous cattle (Shirima *et al.,* 2003).

Presence of microbial contamination in milk is of public health importance. There are many sources of microbial contamination in milk. These sources include, infected or sick lactating animal (Abdel-Rady and Sayed, 2009), microbial contamination along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipments (Jones and Bailey, 2009; Sohrabi *et al*., 2013). Water sources that are used in sanitary activities in washing milk containers, milking machines, and udders contribute to the microbial load in milk. Other sources of

microbial contamination occur during milk handling, transportation and storage. Contamination may also occur due to re-contamination of milk after being processed due to unhygienic conditions, improper handling and storage of milk during consumption (Parekh and Subhash, 2008; Kasalica *et al*., 2011; Kanyeka, 2014; Ngasala *et al*., 2015). Normally milk serves as an ideal medium for growth and multiplication of various microorganisms due to its nutritional value, therefore, milk contamination may cause spoilage and milk-borne diseases to consumers (Ngasala *et al*., 2015).

Prevalence of clinical and sub-clinical mastitis has been observed in different breeds in Tanzania Karimuribo *et al*. (2006) reported that the prevalence of mastitis in Iringa was 61.2% in dairy cows, and 26% in local cows Mbeho (2012) reported variation of prevalence of mastitis in local Tanzania breeds. He found Maasai zebu to have high prevalence of mastitis of 52% followed by Iringa red (48.2%) and Gogo strain with only 22.2%. It can be concluded that breeds have a significant contribution to the prevalence of mastitis in dairy cows (Gwandu, 2013; Kanyeka, 2014). Abdel-Rady and Sayed (2009), also noted that in Egypt the differences in prevalence of mastitis in Friesian breed was 20.4% at the cow level and 6% at the quarter level while in native breed it was 16.7% at the cow level and 5% at the quarter level.

Likely factors of mastitis in cattle herd include confinement in which the infections rate can be as high as 25%. High yielding cows are associated with high levels of mastitis levels compared to average yielders (Gröhn *et al.,* 1990). Environmental factors such as unhygienic housing, warm and humid weather, and the general lack of farm cleanliness and sanitation, may account for the observed high prevalence of environmental pathogens. On the other hand, deficient milking procedures, poor hygiene, lack of awareness and mindset on milk handling and consumption as well as treatment practices could contribute

to a high prevalence of contagious pathogens (Radostits *et al.,* 2000; Shem *et al*., 2001; Kivaria *et al.,* 2004; Kanyeka, 2014). The general lack of knowledge on dairy cattle husbandry by the smallholder producers, and the paucity of livestock extension services, may be important factor for mastitis (Kivaria *et al*., 2004). Other factors include the stage and number of lactations and parity of the animals as well as breed of individual cow. In developing countries milk is still a source of zoonotic diseases and *Mycobacterium* spp have been frequently isolated from milk (Kanyeka, 2014). Outbreak of food poisoning from *Salmonella* spp, *Campylobacter* spp and *Staphylococcus* spp in milk not receiving heat treatment or imperfectly pasteurized have been frequently reported in different countries (Kanyeka, 2014). Therefore, microbiological assessment of milk is essential to establish the degree of contamination and recommend means of interventions.

1.2 Problem Statement and Justification

The production of milk from cows in Tanzania has not reached the optimum levels due to several constraints including poor genetic potential of animals, lack of feeds especially good quantity and good quality during the dry seasons, prevalence of diseases and generally poor management and lack of good husbandry practices. (Njombe *et al.,* 2011) Clinical and subclinical mastitis is one of the important disease that lower the milk production and quality in local and dairy cattle in Tanzania (Kivaria *et al*., 2004).

Raw milk is a good media for the transmission of milk-borne pathogens to humans, as can be easily contaminated during milking and handling. Since milk is highly perishable and highly nutritious food, it serves as an ideal medium for the growth and multiplication of various microorganisms (Kanyeka, 2014). There is little knowledge about dairying among farmers in study area, due to lack of technical supervision and poor veterinary services and education in the dairy sector. General herd management and bovine udder health, on the

other hand, deficient milking procedures, poor hygiene due to lack of water supply and treatments could contribute to mastitis (Kivaria *et al*., 2004; Mdegela *et al*., 2004).

Therefore this study was conducted to establish the prevalence of mastitis in the study area. This information will be useful to educate the public on the importance of knowing the microbial quality of milk they consume and to safeguard the public from zoonosis that may be transmitted through milk.

1.3 Objectives

1.3.1 General objective

To find out the association between bacteria in milk and mastitis disease in cattle from smallholder dairy farmers and agro-pastoral communities in Kilosa, Mvomero, Handeni, and Lushoto districts.

1.3.2 Specific objectives

i) To identify the types of mastitis in local and dairy cattle.

ii) To establish the prevalence of mastitis in cattle from selected cows.

iii) To isolate and identify milk bacteria which are associated with mastitis disease from mastitis and non – mastitis cows.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background Information

The dairy industry is a very important component of the livestock sector of Tanzania. The industry has greater potential for improving the living standards of people through improved nutrition and increased income from sales of milk and milk products (Njombe *et al*., 2011). The industry contributes one-third of the 4.7% livestock industry's contribution to the Gross Domestic Product (GDP) (Njombe *et al*., 2011). Out of the 21 million cattle in Tanzania, about 680 000 are dairy cattle mainly cross of Friesian, Jersey, and Ayrshire breeds with the Tanzania Shorthorn Zebu (TSHZ). Total annual milk production is currently estimated at 1.65 billion litres (NBS, 2012; NLR, 2012). About 70% of the milk produced comes from the traditional sector (indigenous cattle) kept in rural areas, while the remaining 30% comes from improved cattle mainly kept by smallholder producers.

In 2011, the total milk processing capacity was about 384 100 litres per day; however the actual processed amount is about 105 000 litres per day. Per capita milk consumption in the country is estimated at 43 litres (Njombe *et al*., 2011). This consumption level is relatively low compared to the recommended milk per capita milk consumption of 200 litres by FAO (Njombe *et al*., 2011). Dairy production is a biologically efficient system that converts feeds and roughages into milk, and is very nutritious food that is rich in carbohydrates, protein, fats, vitamins and minerals (Mekibib *et al.*, 2010). However, due to increase of human population milk production often does not satisfy the need and demand of milk requirements due to a multitude of associated factors that include mastitis (Mekibib *et al*., 2010).

2.2 Mastitis

Mastitis is a condition accompanied by inflammation of the udder which affects cattle, sheep, goats, camels and buffaloes (Karimuribo, 2002). It is the inflammation of the mammary gland and udder tissue, and is a major endemic disease of dairy cattle. It usually occurs as an immune response to bacterial invasion of the teat canal by variety of bacterial sources present on the farm, and can also occur as a result of chemical, mechanical, or thermal injury to the cow's udder (Arnold, 2011; Hosseinzadeh and Saei, 2014; Erskine, 2014).

According to Radositits *et al.* (2000), mastitis is subdivided into two forms namely clinical and subclinical mastitis. Usually clinical mastitis is associated with obvious signs of changes in the milk and udder. The affected udder produce milk with poor consistence, due to presence of flakes or clots, and also the colour is abnormal with red, yellow and sometimes blue, watery, or blood stains. Also the udder becomes hard and swelling of the affected quarter, which sometimes results in an asymmetrical appearance (Kalorey *et al*., 2001; Mbeho, 2012).

Subclinical form of mastitis does not show clinical manifestation, it is associated with increased somatic cell counts due to inflammatory change and tissue damage caused by bacterial colonization. This is the most prevalent form of mastitis reported in Tanzania. It is accompanied with reduced milk production of the cow (Mdegela *et al*., 2004; Mbeho, 2012). It is the form of mastitis which takes a very long duration to be recognized and difficult to detect. It can be confirmed by laboratory means by detecting the milk cellular contents by use of somatic cells and bacteriology examination (Mbeho, 2012). Prevalence of subclinical mastitis varies with the season of the year. Abdel-Rady and Sayed (2009), reported that mastitis decreases as weather changes from summer with high incidences

through spring with moderate infections to low infection in cold weather as during winter and autumn.

2.3 Causes of Mastitis

Mastitis is caused by a number of microorganisms which are in different groups such as bacteria, protozoa, yeast/fungi and algae. In Tanzania, the causative agents of mastitis isolated are *Staphylococcus* spp, *Streptococcus* spp*, Klebsiella* spp, and *P. aeruginosa* (Kivaria *et al*., 2007, Mdegela *et al*., 2005, Karimuribo *et al*., 2005). In Zanzibar (Unguja and Pemba) the pathogens of mastitis isolated were *E. coli, Klebsiella* spp*, Serratia* spp*, Alcanobactor pyogens and Bacillus* spp (Suleiman, 2013). Mastitis pathogens that can be routinely monitored in subclinical form are contagious ones such as *S. aureus* and *S. agalactiae* (Mona *et al*., 2008). Sources of microbial contamination in milk include primary microbial contamination from the infected or sick lactating cows, while the secondary causes occur along the milk value chains from milk handlers, unsanitary utensils and milking equipments as well as water supply used for cleaning (Ngasala *et al*., 2015). A cow with mastitis has the potential to shed large number of microorganisms into the milk supply. The influence of mastitis on the total bacterial count of milk depends on the strain of infected microorganisms, stage of infection and percentage of the herd infected (Donkor *et al*., 2007).

The most common mastitis pathogens are found in the udder tissues, spreading from cow-to-cow (contagious pathogens) or in the herd's surroundings (environmental pathogens), such as bedding materials, manure and soil. This distinction may be important when assessing the challenges present in a herd and the measures which may be taken to reduce or treat mastitis (Erskine, 2014). Contagious pathogens that cause mastitis tend to live on the cow's udder and teat skin and transfer from affected cow (or quarter) to unaffected cow

(or quarter) during milking. They adhere easily to the skin, colonizing the teat end and then 'grow' into the teat canal, where infection occurs; because of this, post-milking teat disinfection and dry cow therapy play an important role in controlling contagious mastitis. Farms with a high level of contagious mastitis often have high Somatic Cell Counts (SCCs) (Abdel-Rady and Sayed, 2009; Erskine, 2014). Environmental mastitis pathogens - present in the housing and bedding - can transfer during milking or between milkings, when the cow is loafing, eating or lying down. The pathogen can enter the teat canal by force during milking, for example, when liner slippage occurs (Piepers *et al*., 2007). These environmental pathogens do not generally possess the same ability as contagious pathogens to adhere to and colonise the teat. High levels of environmental pathogens in a herd may cause normal SCCs (Abdel-Rady and Sayed, 2009). Mastitis pathogens can infect cows both during the dry period and when cows are lactating, and it is important to identify and recognize the source of these infections, as approaches to control, prevention and treatment of the pathogen's effects can differ according to whether the infection occurs when the cow is dry, or in lactation (Erskine, 2014).

2.4 Causative Agents of Mastitis

Bacteria which cause mastitis can be classified into two groups namely major and minor pathogens. *S .aureus, S. agalactiae, Streptococcus uberis* and *E. coli* are the most common etiological agents involved in subclinical and clinical cases of mastitis in dairy cows. *Pseudomonas aeroginosa, Corynebacterium pyogenes* and some *Aerobacter spp*. are less common (Gonzalo *et al*., 2002; Abdel-Rady and Sayed, 2009). With exception of *Mycoplasma* spp, which may spread from cow to cow through aerosol transmission and invade the udder subsequent to bacteremia, contagious pathogens are spread during milking by milkers' hands or the liners of the milking unit. Additionally, contagious transmission infrequently occurs for pathogens typically associated with environmental

reservoirs, e g, through the development of host-adapted virulence factors (*Escherichia coli*) or by shedding of overwhelming numbers of bacteria from infected udders (Erskine, 2014). Raw milk is one of the most common paths for transmission of *L .monocytogenes,* mainly due to sick animals on the farm. Healthy animals are often carriers of *L .monocytogenes* and as such can be source of contamination of the environment, or milk (Kasalica *et al.,* 2011). It is particularly dangerous to individuals who have weakened immune systems, including pregnant women, AIDS patients, and the very young and very old (Erskine, 2014).

2.5 The Predisposing Factors of Mastitis

The occurrence of mastitis has been considered to be contributed by factors such as poor farm management, improper milking procedures and susceptible breed of cows (Byarugaba *et al.,* 2008). Also age of the cow, lactation stage, and presence of teat lesion are contributing factors that predispose a cow to mastitis infection (Madut *et al.,* 2009).

Biffa *et al*. (2005), reported that number of parity, location, season of the year and grazing system had an effect on occurrence of mastitis in Ethiopia. The season of the year is among the potential risk factor as in wet season the prevalence of mastitis is higher compared to dry season (Peeler *et al.,* 1994; Rahman *et al*., 2009). One general factor that contributes to mastitis infection is shortcomings in dairy husbandry. Superficial washing of the udder, in some cases without any disinfectants, the use of same udder cloths for several cows and poor hygiene of the milkers are contributory factors to mastitis in milking cows (Shem *et al*., 2001) Moreover, unhygienic milking procedures, wet and muddy barn-floors, persistent teat lesions, the absence of pre-and-post milking teat dips, and the failure to use dry period therapy, lack of proper treatment for clinical cases, and the absence of culling may be risk factors for clinical mastitis throughout the lactation

2.6 Detection of Mastitis

2.6.1 Physical methods for detection of mastitis

Usually clinical mastitis does not require laboratory assistance, it shows all cardinal signs or changes in colour, discoloration, presence of clots/or blood stains in milk as well as the physical changes of the udder such as swelling, hot and painful udder all these signs can be easily detected during clinical examination of infected cows (Shearer and Harris, 2003). The most common way to detect clinical mastitis is stripping a few squirts of milk into a strip cup at the beginning of milking to check for abnormalities such as clots and flakes. Visual observation and palpating the udder for signs of inflammation can also help to identify clinical mastitis but should not be the substitute for stripping.

2.6.2 California Mastitis Test (CMT)

California Mastitis Test (CMT) is a detection method for subclinical mastitis in dairy cattle (Radositits *et al*., 2000). In the epidemiological studies, CMT is important for diagnosis of subclinical mastitis as a reliable, easy, rapid and cheap tool for diagnosis of mastitis since it detects prevalence of SCC (Abdel-Rady and Sayed, 2009; Erskine, 2014). Furthermore, CMT is reliable and easy method to use at village level and it provides results with high sensitivity (Mbeho, 2012). It is an indirect method to detect the increased number of somatic cell content in milk. The principle behind the test is the reaction that takes place between the reagent and the nucleic acid of the cell leading to gel formation as the number of cell increases beyond a certain threshold (Abdel-Rady and Sayed, 2009, Erskine, 2014). The CMT was chosen in several investigations because it is more perfect, efficient and reliable than other field and chemical tests for diagnosis of subclinical mastitis. Furthermore the results of CMT also are in a good agreement with bacteriological results (Erskine, 2014). Apart from CMT, several other diagnostic methods can be

included like Modified White Side test (MWT), pH, chlorine and catalase tests (Abdel-Rady and Sayed, 2009).

2.6.3 Bacterial isolation and identification

This is the reliable method for detection of udder infections through bacteriological examination of collected milk samples (Shearer and Harris, 2003, Petrovisk, 2006). Also fresh or incubated milk smears can be used for microscopic examination. Culture is made on special media such as McConkey agar and blood agar and incubated at a specified temperature and time. The samples have to be collected carefully and aseptically to avoid contamination from the environment which may lead to incorrect laboratory results (Shearer and Harris, 2003).

After culturing the isolated bacteria may be identified phenotypically by macro and micro morphology examination. Different biochemical methods are used for confirmation. Molecular methods are also available for definitive identification of the bacteria. Sensitivity test to commonly used antibiotics in treatment of mastitis may be used to ascertain the type of antibiotics which the bacteria are sensitive to.

2.7 Control Measures for Mastitis

Control of mastitis should focus on the type of mastitis, i.e. environment to teat of the cow, within cow udder quarter infection and cow to cow infection. The prevalence of mastitis can be reduced through early mastitis detection which requires examination of milk from all quarters; any cow that shows clinical mastitis should be examined and treated. Dry cow therapy (DCT) is used when a cow is at the end of lactation period by introducing intramammary infusions in the teats. Also proper milking procedure and hygiene reduces potential for traumatic injury to the teats and udder (Mbeho, 2012).

Generally milking procedures prevent occurrence of mastitis by washing the teats, clean and dry teats before attaching the milking machine teat cups, milking wet udders increases chances of mastitis.

Mastitis can be prevented by focusing on management efforts on milking technique and hygiene. Clean and dry bedding, clean and dry udders at the time of milking, and lack of teat-end lesions all have a positive effect on control. The single most important management practice to prevent transmission of new infections is the use of an effective germicide as a postmilking teat dip (Abdel-Rady and Sayed, 2009; Erskine, 2014). Other practices that augment teat dipping include use of individual towels for drying teats, use of a premilking germicide, attachment of units at the proper time after teat stimulation (60–120 sec), cleaning milking units after an infected cow has been milked, or segregation of infected cows into a separate milk group. Intramammary infusions of β-lactam antibacterial drugs 7–14 days before expected calving dates have been reported to reduce the rate of intramammary infections at calving (Erskine, 2014). Control practices also include identification and treatment or culling of infected animals, maintaining skin and streak canal health and proper use of an effective teat dip. In some situations, a back-flush system may be warranted (Petersson and Mullarky, 2012). Milk cows with mastitis last in the milking order to minimize the chances of cross-contamination (Arnold and Bewley, 2011). If milking is by milking machines, it should be well managed and maintained to avoid frequent liner slips and teat end impacts which may increase environmental mastitis.

2.8 Food-borne Pathogens in Milk

Milk and milk products derived from milk of dairy cows can harbour a variety of microorganisms and can be important sources of food borne pathogens. The presence of food borne pathogens in milk is due to direct contact with contaminated sources in the

dairy farm environment and to excretion from the udder of an infected animal (Olive *et al*., 2005; Karimuribo *et al*., 2005). Entry of food-borne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in biofilms. Subsequent contaminations of processed milk products can exposure consumers to pathogenic bacteria (Olive *et al*., 2005). Pasteurization may not destroy all foodborne pathogens in milk. Furthermore, pathogens such as *L. monocytogenes* can survive and thrive in post-pasteurization processing environments, thus leading to recontamination of dairy products (Kasalica *et al*., 2011). These pathways pose a risk to the consumer from direct exposure to foodborne pathogens present in unpasteurized dairy products as well as dairy products that become re-contaminated after pasteurization. Majority of livestock keepers in Tanzania consume raw milk that predispose them to the risk of contracting zoonoses, and other milk-borne diseases like tuberculosis, brucellosis, listeriosis, salmonellosis and leptospirosis (Kanyeka, 2014). Also milk is one of the main components of the diet of the rural society as well as urban and pre-urban areas. If it is consumed in the raw state by village communities may be a problem. The presence and consumption of such milk constitute a public health hazard (Kanyeka, 2014; Demme and Abegaz, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Selection of the Study Districts, Villages and Households

The selection of study districts was based on the availability of livestock keepers in both production systems. These were agro pastoral community and smallholder dairy farms. Also the understanding and mutual participation of the pastoralists in villages were considered in the SFFF II project in these areas.

The four districts were selected purposely because they have many smallholder dairy farmers and agro-pastoralist who supply milk to the local community and nearby milk processing plants. Sixteen villages in all four districts were purposely selected by SFFF II project mostly based on the number of livestock keepers and accessibility. With the help of Livestock field Officers (LFO), households with cattle in the selected villages were identified to be used for study based on inclusion and exclusion criteria.

3.1.1 Inclusion and exclusion criteria

The study inclusion criteria for the households were: smallholder farmers with lactating cows during the study, willing to participate in the study and availability of milk at the time of data collection. The exclusion criteria were: unwilling to participate in the study and absence of milk at the time of data collection.

3.1.2 Description of Kilosa and Mvomero districts

Kilosa and Mvomero are located in Morogoro region (Fig. 1). This region is located in between latitude 5^0 58" and 10^0 0" to the South of the Equator and between longitude 35^0 25" and 35^0 30" to the East of Greenwich. The region is bordered with Dodoma and Iringa

regions on the Western part, on the Northern part it is bordered with Tanga and Manyara regions. On the Easten side it is bordered with Costal belt regions of Pwani and Lindi, and Ruvuma region on the Southern part. According to the 2012 National census, Kilosa district has a population of 438 175 people (PHCT, 2012) and land area of 14 245 km^2 , with cattle population of 215 040 cattle (MPEE, 2007; Ndanu *et al.*, 2012).

Mvomero district has a population of 312 109 people (PHCT, 2012) and land area of 7 325 km^2 . The population of cattle in Mvomero district is estimated to be 172 827 (MPEE, 2007), Both districts have insignificant water areas (MPEE, 2007). Kilosa and Mvomero districts were selected to be included in the study because they are characterised by agropastoral and smallholder dairy production systems, also the SFFF II project conducts research activities for livestock development in these districts within Morogoro region.

3.1.3 Description of Handeni and Lushoto districts

Handeni and Lushoto districts are located in Tanga region. (Figure 2) Lushoto is a mountainous area 1200–1900 m above sea level (Google Earth), with a mean annual rainfall of 800–1400 mm, (ILRI 2007). It has a relatively high human population and intensive farming practices. Most of the district's land is steeply sloping, characterized by a mixed farming system. Rainfed agriculture is the most important land use followed by irrigated agriculture, livestock keeping in semi intensive system (Msita *et al*., 2010). Handeni, on the other hand, is in the lowlands (500–900 m above sea level) and much drier, with a mean annual rainfall of 600–800 mm. It has low human population and extensive farming activities (Morris *et al*., 2014). It is the largest district covering an area of 7,366.41 square kilometres and its human population is about 355, 702 (PHCT, 2012). Morris *et al.* (2014); did a comprehensive livestock environmental assessment on the

production status of these small holders in Lushoto and their constraints including diseases in their livestock.

3.2 Study Animals, Population and Husbandry Practices

In this study, the animals involved in the study were lactating cows found in the areas of production system of agro-pastoralists and small scale dairy farms in the four districts. In these systems there are traditional breeds of cattle especially TSHZ, and their crosses of Friesian, Ayrshire, and Jersey crossed with other indigenous breeds of cattle. The model of production of indigenous cattle was free grazing of large herds of cattle in grazing lands especially in Kilosa and Handeni districts, while in Lushoto and Mvomero districts dairy cattle are managed with semi intensive system whereby cattle are kept in feeding stalls for some-time and fed with cut and carry pasture

3.3 Sample Size

A formula by Kothari (2004), $n = Z^2SD^2/e^2$ was used to calculate the sample size for this study. Where Z, is the estimated standard variation at 95% confidence interval (CI) which was considered the point of the normal distribution corresponding to the level of significance (Z=1.96). Prevalence rate was estimated at 12.8% and e, is the estimated error and was considered at 0.05 or 5%.

Therefore, the sample size 'n' was calculated as:

$$n = \frac{(1.96)^2 \times (0.128)^2}{(0.05)^2} = 25.17$$ approximately n = 25 samples per each district.

Based on the above formula, in each district 25 lactating cows were supposed to be involved in sampling making a total of 100 milk samples for all four districts. But during the time of sample collection it was a dry season, therefore many cows in the selected villages were not lactating despite of using inclusion and exclusion criteria thus, 78 milk samples were collected (Table 2).

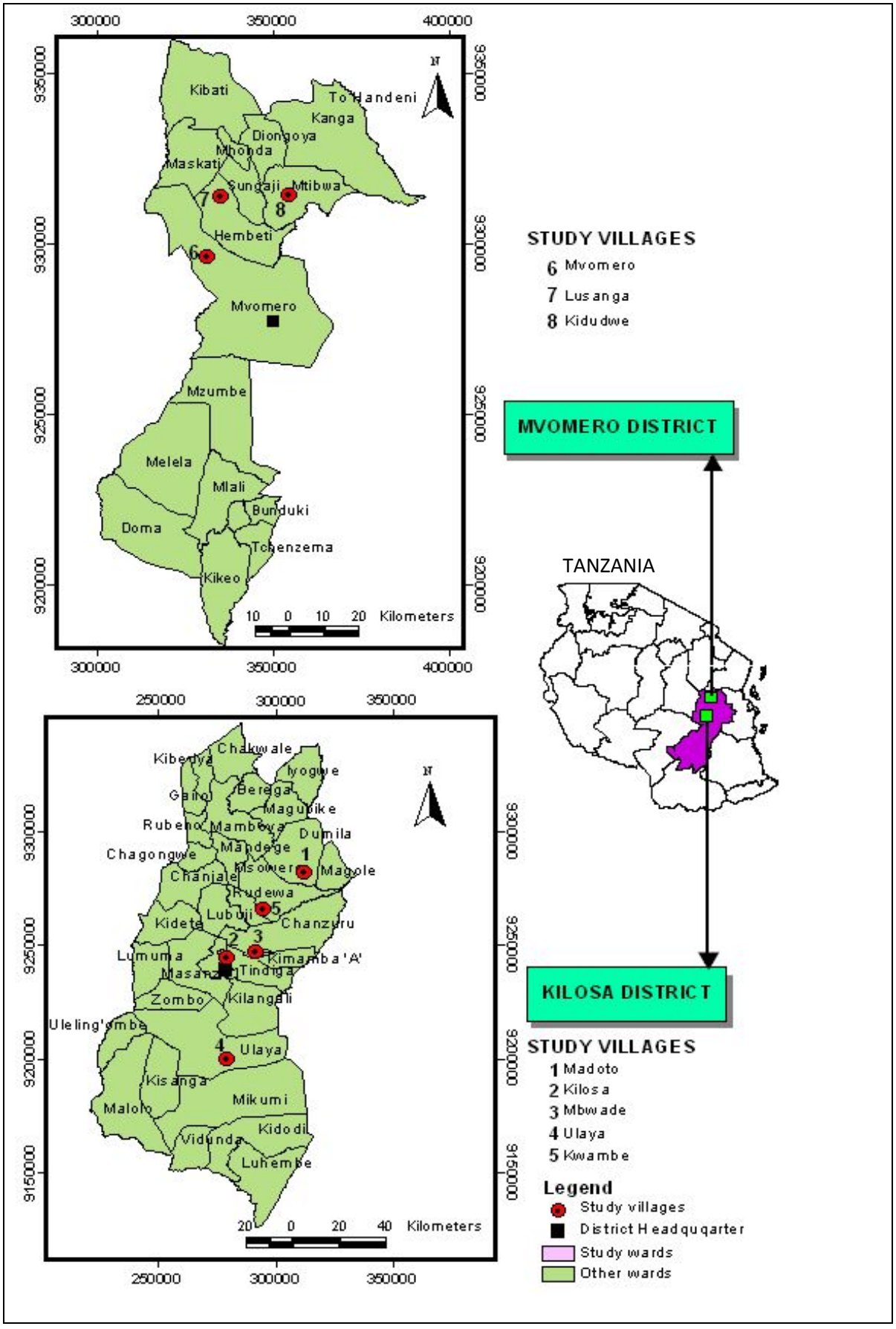

Figure 1: Map of Kilosa and Mvomero districts study areas in Morogoro Region

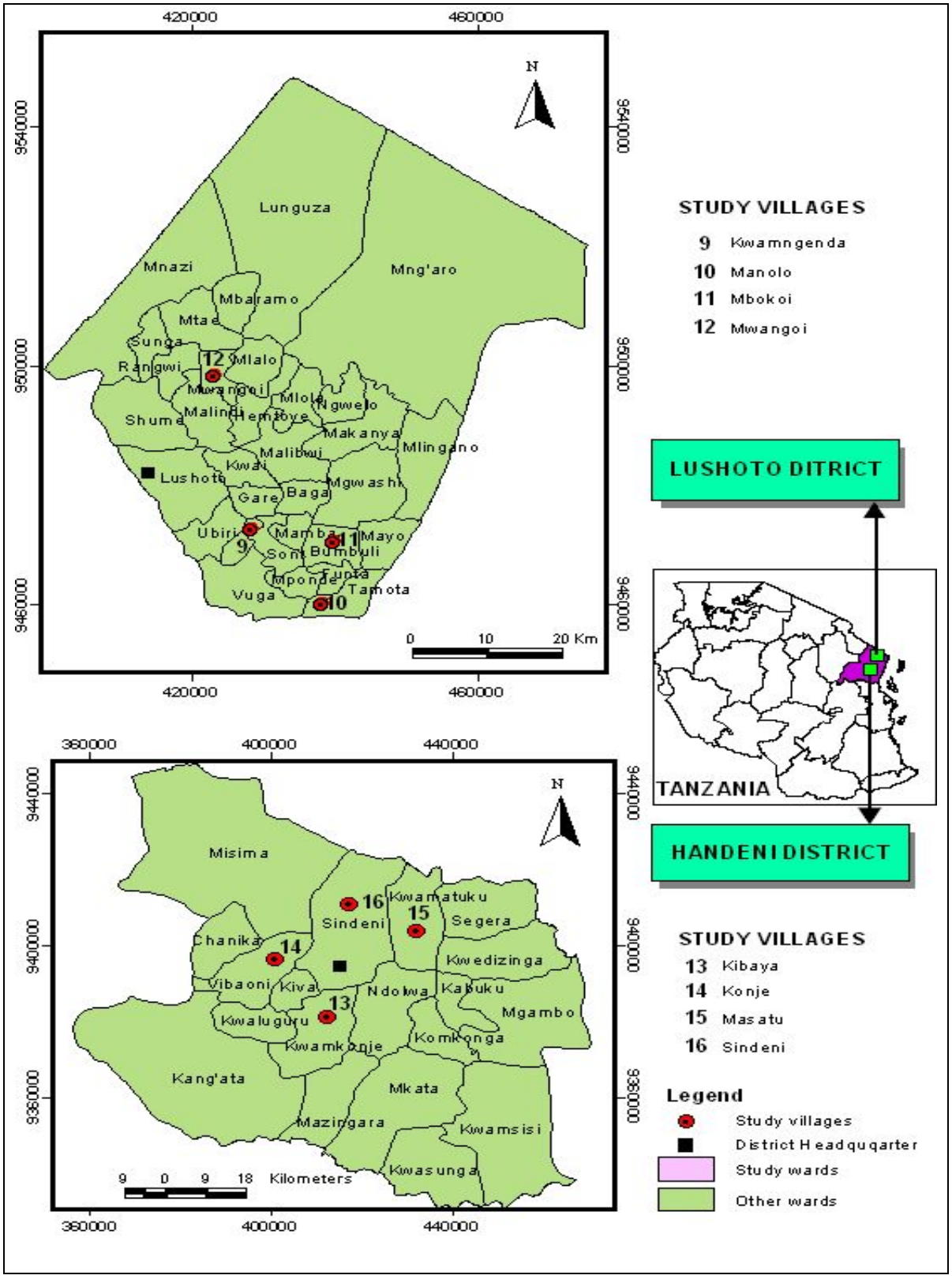

Figure 2: Map of Handeni and Lushoto districts in Tanga Region

3.6 Study Design

A cross sectional study was conducted to smallholder farmers and agro-pastoral dairy production systems in Lushoto, Handeni, Kilosa and Mvomero districts. The selected cows were clinically examined for mastitis concentrating on the size of the udder, colour of the milk and other signs for clinical mastitis. There after subclinical mastitis was examined by the use of CMT (Table 1). This study was carried out in the period between November 2013 and August 2014, under the coordination of SFFF II Project.

3.7 Examination of Clinical Mastitis

Before collection of milk samples cows were examined for clinical mastitis. The signs of emphasis included udder swelling, hotness of the udder as well as lesions on the teats. Stripping a few squirts of milk into a strip cup at the beginning of collection of milk samples to check for abnormalities such as clotted/blood stained milk, changes in colour, clots and flakes was also used as a test for mastitis. Visual observation and palpating the udder for signs of inflammation was also carried out for observation of clinical mastitis.

3.8 Subclinical Mastitis Test by CMT

All 78 lactating cows from the selected household herds were screened for subclinical mastitis. Milk was drawn from each quarter into corresponding CMT white plastic paddle, approximately 2 mls in each receptacle (Table 1). The CMT reagent (Shalma test reagent R) was squirted without frothing from a CMT reagent dispenser until an amount equal to the volume of milk in each of the test cup had been added. Mixing was done by gentle circular motion of the paddle in a horizontal plane. The reaction developed its peak colour within 10 seconds. Each sample tested was graded, interpreted and scored in four categories as in Table 1.

Table 1: Califonia Mastitis test scores and equivalent somatic cell counts

CMT test score	**Reaction observed**	**Equivalent milk SCC/ml**	**Health quarter**
Negative	Homogenous mixture, no thickening	0-200 000, 0-25% Neutrophils	Health quarter
Trace	Slight (a faint cloud like) thickening that disapper about 10 minutes	150 000-500 000, 30-40% Neutrophils	Possible infection if all quarters read trace there is infection. If one or two quarter read trace, infection are possible
1	Distinct slime formation occurs immediately after mixing but no tendency to form gel, this slime does not disappear over time	400 000-500 000, 30-60% Neutrophils	Subclinical mastitis
2	Distinct slime formation occurs immediately after mixing with a slight gel formation	500 000-800 000, 60-70% Neutrophils	Subclinical mastitis
3	Distinct slime formation occurs immediately after mixing, the distinct gel is formed and the surface of the mixture becomes elevated like fried egg,	>800 000, 70-80% Neutrophils	Subclinical mastitis

CMT=Califonia Mastitis Test, SCC= Somatic Cell Counts, Kivaria (2007)

3.9 Collection of Milk Samples in the Study Districts

A total of 78 lactating cows with a combination of dairy (n=28) and local cows (n=50) were screened for subclinical mastitis (Table 2). Milk samples were collected directly from the individual cows in the visited households. Forty five milliters of milk samples were collected aseptically from each quarter and mixed in one sterile screw capped falcon tube. All samples were collected from lactating cows after being tested for mastitis using CMT. All collected samples were coded with numbers for identification and packed in a cool box with ice packs. Thereafter, the samples were transported to SUA laboratories and were stored at -20 ºC pending further analysis.

Table 2: Lactating cows and their breeds from the districts

District	Number of Samples collected	Number of cattle Breeds	
		Dairy	Local
Handeni	25	2	23
Kilosa	25	0	25
Lushoto	10	10	0
Mvomero	18	16	2
Total	**78**	**28**	**50**

3.10 Laboratory Analysis of Milk Samples

Laboratory analysis of milk samples was done in the Microbiology laboratory of the Department of Veterinary Medicine and Public Health, SUA. Specific bacterial pathogens namely *E. coli*, *S. aureus, Strepotococcus* spp and *Listeria* spp. were targeted by using selective media.

3.11 Media Preparation

3.11.1 Buffered peptone water

The medium (BPW powder, Oxoid® Ltd., Basingstoke, Hampshire, England, CM0509, Lot 1 442 805) is composed of 10 g/l Peptone, 5 g/l Sodium chloride, 3.5 g/l Di-sodium phosphate and 1.5 g/l Potassium di-hydrogen phosphate. The medium was prepared according to manufacturer's instructions whereby 20 g of the powdered medium was dissolved in 1 litre of distilled water. The culture medium was mixed well and 10 ml were dispensed into capped test tubes. The test tubes were sterilized by autoclaving at 121 ºC for 15 minutes and cooled to 25 ºC before use. All the unused prepared media were stored in a refrigerator at -20 ºC.

3.11.2 Nutrient agar

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

3.11.3 MacConkey agar

The medium (Oxoid® Ltd., Basingstoke, Hampshire, England, CM0007, Lot 1367351) is composed of 20 g/l Peptone, 10 g/l Lactose, 5 g/l Bile salts, 5 g/l Sodium chloride, 0.075

g/l Neutral red, 12 g/l Agar and final of pH 7.4 ± 0.2 at 25 ºC. The medium was prepared according to the manufacturer's instructions whereby 52 g of the powdered medium was suspended into 1 litre of distilled water. The medium was boiled to dissolve completely followed by sterilization by autoclaving at 121 ºC for 15 minutes and cooled to below 45 ºC and poured onto sterile Petri dishes. The plates were left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37 ºC to check for sterility and to dry the condensed vapour on the plate cover.

3.11.4 Glucose agar

Prepared media tubes composed of 10 g/l enzymatic digest of casein, 1.5 g/l yeast extract, 10 g/l glucose, 5 g/l sodium chloride, 15 mg/l bromocresol purple, 9 g agar and final pH 7.0±0.2 at 25 ºC were received from the supplier and stored in sterile condition at 2 – 8 ºC.

3.11.5 Baird-Parker agar

Baird-Parker agar was prepared plated media (OXOID® Ltd., Basingstoke, U.K.) containing 10 g/l pancreatic digest of casein, 5 g/l meat extract, 10 g/l sodium pyruvate, 12 g/l L-Glycine, 5 g/l lithium chloride, 12 g/l agar and final pH 7.0 ± 0.2 at 25 ºC were received from the supplier and stored in sterile condition at 2 – 8 ºC.

3.11.6 Fraser broth base

The medium (Oxoid® Ltd., Basingstoke, Hampshire, England, CM0895 Lot 1431937) is composed of 5 g/l Proteose peptone, 5 g/l Tryptone, 5 g/l 'Lab-Lemco' powder, 5 g/l Yeast extract, 20 g/l Sodium chloride, 12 g/l Di-sodium hydrogen phosphate, 1.35 g/l Potassium di-hydrogen phosphate, 1 g/l Aesculin, 3 g/l Lithium chloride and final pH of 7.2 ± 0.2 at 25 ºC. To make a full Fraser broth, the medium was prepared according to the manufacturer's instructions by adding 28.7 g of the powdered medium into 500 ml of

distilled water and mixed well to dissolve completely. The medium was then sterilized by autoclaving at 121 ºC for 15 minutes and cooled to below 50 ºC. The contents of 1 vial of SR0156E (Fraser Selective Supplement) was reconstituted as directed in the product insert and aseptically added. The medium was well mixed and each 10 ml were aseptically dispensed into sterile capped test tubes and stored under refrigeration.

3.11.7 Oxford and colorex chromogenic Listeria agar

Prepared plated Oxford agar containing 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 final pH 7.0 ± 0.2 at 25 ºC added with *Listeria* selective supplement were received from the supplier and stored in sterile condition at 2 – 8 ºC. Readymade Colorex chromogenic *Listeria* agar containing 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 and final pH 7.2 ± 0.2 at 25 ºC was also prepared and *Listeria* selective supplement and *Listeria* differential supplement were added. The media was stored in sterile condition at 2 – 8 ºC.

3.11.8 Sheep blood agar base

Prepared plated media (TSA with 5% sheep blood) containing 14 g/l tryptone, 4.5 peptone neutralized, 4.5 yeast extract, 5 g/l sodium chloride, 12 g/l agar and Final pH 7.3 ± 0.2 at 25 ºC added with 5% sheep blood were received from the supplier and stored in sterile condition at 2 – 8 ºC (OXOID® Ltd., Basingstoke, U.K.).

3.11.9 Preparation of initial suspension and decimal dilutions

Thawing of samples was done at room temperature. Using a fresh sterile pipette, 25 ml (or 10 ml) of the sample was transferred into a conical flask containing 225 ml (or 90 ml) of peptone water (PW) and mixed well. Then, using a fresh sterile pipette 1 ml of the initial

inoculum was transferred into test tube containing 9 ml of PW (10^{-1} dilution). Using another fresh sterile pipette 1 ml of the resulting dilution was transferred into a second tube. The procedure continued up to 10^{-7} (or 10^{-5}) dilution and in the last dilution 1 ml was discarded. The dilutions were mixed using a vortex mixer for 5 – 10 seconds. All samples passed through this preparatory step prior to microbiological tests. The remaining samples were used in the initial preparation for isolation and presumptive identification of *Listeria* spp.

3.11.10 Positive control sample

About 500 ml of milk was collected from Magadu farm at SUA as a control sample. The sample was sterilized by boiling, cooled and placed in a clean sterile bottle. Part of the sample was inoculated with strains of *E. coli, S. aureus* and *L. monocytogenes*. Using a fresh sterile pipette 10 ml of the inoculum sample was transferred into a sterile beaker containing 90 ml half Fraser broth for cultivation of *L. monocytogenes.* Also, another 25 ml of the inoculum sample was transferred into a sterile conical flask containing 225 ml of PW. The initial suspension was mixed well and serial dilutions were prepared. Detection and enumeration of the desired microorganisms were done. The resulting colony morphology and colour was used to compare against test samples. The reference strains were cultured and stored in sterile condition at 2 – 8 ºC for use in the entire microbial analysis of milk samples.

3.12 Laboratory Procedures

3.12.1 Detection of E. coli

Paired sterile petri dishes were labeled and by using a sterile pipette, 1 ml of the test sample was transferred from the last dilution to each of two petri dishes. 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 theViolet Red Bile Glucose Agar (VRBG) was added to prevent spreading growth and to achieve semi-anaerobic conditions and allowed to solidify. The procedure was repeated with further dilutions up to the last dilution. The prepared plates were inverted and placed in the incubator set at 37 ºC for 24 hours. The procedure was repeated with the remaining test samples where only consecutive critical dilution steps (from 10^{-3} to 10^{-5} dilutions) were chosen for the inoculation of petri dishes. *E. coli* was analysed in accordance to ISO 21528-2:2004 protocols (ISO, 2004) and it involve the procedures described under sections 3.12.11 to 3.12.1.4.

3.12.1.1 Sub-culturing

Typical colonies are pink to red, with or without precipitation haloes or colourless mucoid colonies, with a diameter of 0.5 mm or more. Five suspect colonies were selected at random from each plate and streaked onto nutrient agar (NA) plates for biochemical confirmation. Also, colonies were streaked onto MacConkey agar (MA) for differentiation of *E. coli*. The plates were incubated at 37 ºC for 24 hours.

3.12.1.2 Confirmation of E. coli

Well isolated colonies were selected from each NA plate for biochemical confirmation. An oxidase test and a glucose fermentation test were performed on each selected colony. Colonies that were oxidase negative and glucose positive were confirmed as *E. coli*.

3.12.1.3 Oxidase test: The test was performed as described by Oxoid® Ltd., Basingstoke, Hampshire, England, Ref MB0266A, Lot 1284539.The presumed and well-isolated colonies were stickled and streaked onto the moistened oxidase detection strips using a

sterile plastic loops, and then the strips were observed for colour change within 10 seconds. If the deep blue or purple colour appeared, was confirmed as a positive reaction.

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

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

3.12.2 Inoculation and incubation of Coagulase Positive Staphylococci (CPS) (*S. aureus*)

Prepared Baird-Parker Agar (BPA) plates were removed from the refrigerator, kept at 25 ºC and labelled prior to inoculation. By means of a sterile 1 ml pipette, 0.1 ml of the test sample was transferred from the last dilution to each of prepared media plates. Using fresh sterile swab the sample was spread on the media surface. The plates were allowed to dry with their lids on for about 15 minutes. The procedure was repeated with the further

dilutions up to the first dilution. The prepared plates were inverted and placed in the incubator set at 37 ºC for 24 hours. Thereafter, all typical and atypical colonies present were counted and the plates were re-incubated at 37 ºC for a further 24 hours. Again, all typical and atypical colonies present were counted. Typical colonies of *S. aureus* grown on BPA are black or grey, shining due to reduced action of telluride; 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. The procedure was repeated with the remaining test samples where only consecutive critical dilution steps were chosen for the inoculation of petri dishes.

Coagulase test

Identification of *S. aureus* in milk samples was done by using ISO 6888-1:1999 protocols (ISO, 1999) through the following stages. Using a sterile wire loop an inoculum was removed from the surface of each selected colony from the plates with *Staphylococcus* spp 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 37ºC. After 4 – 6 hours of incubation, 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 *S. aureus* was added to 0.2 ml of rabbit plasma and incubated.

3.12.3 Identification *Streptococcus* spp

All procedures for culturing of milk samples and obtaining the colonies were the same as described in *Staphylococcus* spp.

Test procedure for identification

All components should were kept at room temperature prior to use. Test latex reagents were re-suspend by the gently inverting the dropper bottle several times and the dropper bottles were examined to ensure that the latex particles are properly suspended before use. Labels were inserted to test tube for each isolate to be tested. One drop of extraction reagent 1 was added to each tube. 1-4 beta-haemolytic colonies were selected using a disposable loop/needle and suspended them in the extraction reagent 1 such that the extraction reagent 1 solution becomes turbid. One drop of extraction reagent 2 was added to each tube. The reaction mixed gently by tapping the tube with a finger for 5-10 seconds. Five drops of extraction reagent 3 were added to each tube and mixed by gently tapping the tube with a finger for 5-10 seconds. One drop of each group latex reagent was dispensed onto separate circles on separate test cards labelled for each isolate being tested. Using a Pasteur pipette, for each test one drop of extract was placed beside each drop of latex reagent. The latex and the extract mixed with the sticks provided, using the complete area of the circles. Gently the cards rocked allowing the mixture to flow slowly over the entire test ring area. Finally agglutination was observed for up to one minute.

Interpretation of results: Positive result was seen rapid strong agglutination of the blue latex particles within one minute with one of the latex reagents indicated the specific identification of the streptococcal isolate. A weak reaction with a single latex reagent was repeated using a heavier inoculum. The repeat test was considered positive if agglutination

occurs with only one of the latex reagents illustrates a suggested scheme for the grouping of streptococci. Negative result: No agglutination of the latex particles.

On Gram staining, both staphylococci and streptococci have round, spherical cell shape, but the arrangement of cells is different due to a different binary fission. Streptococci form a chain of round cells, because their division occurs in one linear direction, whereas staphylococci divide in various directions forming grape-like clusters.

Furthermore differentiation between *Staphylococcus* and *Streptococcus genera* was carried on the catalase test. Staphylococci are catalase positive whereas Streptococci are catalase negative. Catalase is an enzyme used by bacteria to induce the reaction of reduction of hydrogen peroxide into water and oxygen.

3.12.4 Detection of Listeria spp.

Identification of *L. monocytogenes* in milk samples was done by using ISO 11290-1:1996 protocols (ISO, 1996) through the stages (stage 1-stage 4) as indicated below

Stage 1: Primary enrichment in a selective liquid enrichment medium

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

Stage 2: Secondary enrichment in a selective liquid enrichment medium

Test samples were again enriched in a selective liquid enrichment medium with full concentration of selective agents (Fraser Broth). After 24 hours, using a fresh sterile

pipette 0.1 ml of the culture obtained in stage 1 was transferred to a sterile test tube containing 10 ml of Fraser broth. The inoculated test tube was incubated at 37 ºC for 48 hours (sub-culturing).

Stage 3: Plating out and identification

The cultures obtained in stage 1 and 2 above were further inoculated onto two different selective solid media which were Oxford and Colorex *Listeria* agars. Using a sterile loop the culture obtained in stage 1 (primary enriched culture incubated for 24 hrs) was inoculated onto the surfaces of readymade Oxford and Colorex *Listeria* agar plates. The plates were inverted and incubated at 37 ºC for 24 hours and for an additional 24 hours (48 hours). The same procedure was repeated for the culture obtained in stage 2 (secondary enriched culture in the test tube incubated for 48 hrs). After incubation period the plates were examined for the presence of colonies presumed to be *Listeria* spp. Typical colonies of *Listeria* spp. grown on Oxford agar are small, brown-green to dark-brown surrounded by black halos due to hydrolysis of aesculin present in the medium. While for *L. monocytogenes* and *L. ivanovii* grown on Colorex *Listeria* agar are blue-green colonies with well-defined edges surrounded by opaque, white halos, as the medium contains lecithin substrate, which differentiates these bacteria from other *Listeria* spp.

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

Stage 4: Confirmation of Listeria

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

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

2. CAMP Test: This term (CAMP test) describes the synergistic reaction of diffusible substances produced by microorganisms growing adjacent to each other on Sheep Blood agar medium, which results in an enhanced zone of haemolysis in the medium (Fig. 4). Using fresh and separate sterile loops, known cultures of *Staphylococcus aureus (S. aureus)* and *Rhodococcus equi* (*R. equi*) were streaked in single lines onto Sheep blood agar plate parallel and completely opposite to each other. Then, using other fresh and separate sterile loops, several presumed isolated test colonies or cultures were streaked in single lines on the same plate but at right angles to the two known cultures, so that the test cultures and known cultures (*S. aureus* and *R. equi* cultures) were about 1 – 2 mm apart. Parallel with the test samples, control cultures were streaked as well. The plates were inverted and incubated at 37 ºC for 24 hours. After incubation period, the plates were examined for haemolysis. An enhanced zone of β-haemolysis at the intersection between the test culture and each of the known cultures was considered as a positive reaction.

Figure 4: The CAMP test with haemolysis of *Listeria sp*

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

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

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

3.15 Data Management and Analysis

The collected data that included information on clinical mastitis, subclinical mastitis and bactetria identification were entered in Microsoft Excel sheet and analyzed by using SAS (2009). The Chi-square test was used to compare proportions and probability of $P<0.05$ was considered to be statistically significant.

3.16 Limitation of this study

1. This study was carried under the SFFF phase II project. There was no option of changing the study sites including villages. This had effect on the number of samples collected from some of villages and the time frame was fixed. There was variation in the number of samples collected from the districts though the target plan was to collect 25 samples in each district (Table 1). This difference in sample size affected the results in both tests of CMT and bacterial isolation and typing. This is due to the fact that small sample reflected high percentage of positive or negative results as in case of Lushoto District. Apart from dry season it was also observed that in Kilosa and Handeni that livestock keepers are keeping local breeds in large number (Kanyeka, 2014) and it was possible to select the desired sample size compared to Loshoto where they are keeping few animals due to zero grazing and there is also the shortage of pastures and free grazing landbe compared well as they have the same number of samples (25) compared to Handeni (18

samples) and Lushoto (10 samples). Collection was carried out during dry seasons that affected much on the desired sample size because a large number of cows were not lactating; therefore number of samples was not the same in all four districts.

CHAPTER FOUR

4.0 RESULTS

4.1 General Results

A total of 78 milk samples from individual cows were collected in all four districts. Lushoto had lowest number of lactating cows followed by Mvomero while Handeni and Kilosa had equal number of targeted samples (Table 3). Due to variation in number of samples in every district there was significant difference between the districts. Breeds of cows found in all districts are as indicated in Table 3 and were significantly different. The large numbers of cows sampled were local breeds (64.1%, 50/78) compared to dairy cows (35.9%, 28/78). More of local cows were found in Handeni and Kilosa districts while dairy cows were mostly found in Lushoto and Mvomero districts. Regarding the villages involved, Kilosa had more villages (5) followed by Lushoto and Handeni each with four villages while Mvomero had lowest number of villages (3) involved in the study. Apart from variations on the number of villages, there was also a variation on the number of cows sampled in each village. More samples were collected from Sindeni village (9) in Handeni followed by Mbwande village (8) in Kilosa while Konje, Madoto, Kidudwe and Mvomero had equal number of samples (7) while Kambwe and Manolo had only one sample each.

Table 3: Samples collected and cattle breeds from the four studied districts

District	**Samples collected**		**Cattle Breeds (%)**		**P value**
	No	**%**	**Dairy**	**Local**	
Handeni	25	32.1	2 (2.6)	23 (29.5)	0.0001
Kilosa	25	32.1	0 (0)	25 (32.1)	
Lushoto	10	2.8	10 (12.9)	0 (0)	
Mvomero	18	23.0	16 (20.5)	2 (2.6)	
Total	**78**	**100**	**28 (35.9)**	**50 (64.1)**	

4.2 Clinical and Sub Clinical Mastitis in the Studied Animals

The status of mastitis in individual cows in the four studied districts is shown in Table 4. Of all lactating cows examined 59 (75.6%) had mastitis. Subclinical mastitis accounted for 62.7% of the cows examined. Dairy cows were more affected by mastitis (82.1%, 23/28) but the subclinical cases of mastitis were slight higher in local cows (66%). More of mastitis cases were encountered in Handeni District (28.2%, 22/78). For the samples collected in individual districts, Handeni had the highest prevalence (88%, 22/25), followed by Mvomero (83%, 15/18), Lushoto (70%, 7/10) and Kilosa (60%, 15/25). In dairy cows clinical mastitis was found in Mvomero while suclinical mastitis was equally found in Mvomero and Lushoto followed by Handeni. For local breeds, both clinical and subclinical were found more in Handeni followed by Kilosa (Table 4).

Table 4: Status of mastitis in milk from different districts

Parameter	Category	Number (%) of mastitis in different districts				Total	P value
		Handeni	Kilosa	Lushoto	Mvomero		
Types of Mastitis	Sub clinical	20 (25.6)	14 (17.9)	7 (8.9)	8 (10.3)	49 (62.7)	0.00
	Clinical	2 (2.6)	1(1.3)	0 (0)	7 (8.9)	10 (12.9)	29
Mastitis in breeds	Local (n=50)	20 (40)	15 (30)	0 (0)	1 (2)	36 (72)	0.00
	Dairy (n=28)	2 (7.1)	0 (0)	7 (27)	14 (50)	23 (82.1)	01
Mastitis in dairy	Subclinical	2 (2.6)	0 (0)	7 (8.9)	7 (8.9)	16 (57.1)	
cows	Clinical	0 (0)	0 (0)	0 (0)	7 (8.9)	7 (25)	
Mastitis in local	Subclinical	18 (23.1)	14 (17.9)	0 (0)	1 (1.3)	33 (66)	
cows	Clinical	2 (2.6)	1 (1.3)	0 (0)	0 (0)	3 (6)	

4.3 Bacteria Isolates from the Milk Sample

All samples collected were contaminated with bacteria of different species (Table 5). In the current study 74.4% of mastitis milk samples had bacterial contamination while non-mastitis milk samples had only 24.4% bacterial contamination. The isolated species included; *S. epidermidis, S. aureus, L. monocytogenes, L. inoccua, L. ivanovii* and *E. coli*.

In all isolates, the significance difference was observed in *S. epidermidis* from all districts while other isolates were not significant. Over 51% of the milk samples had *S. epidermidis*, followed by *S.* aureus (34.6%), *L. monocytogenes* (32.1%); *L. inoccua* (30.8%), *L. ivanovii* (15.4%) and the lowest one was *E. coli* (12.8%). More of S. *epidermidis, S. aureus,* and *L. monocytogenes* bacteria isolates were found in dairy cows compared to local cows. In local cows, *S. epidermidis, S. aureus, L. monocytogenes* and *L. inoccua* were found more than other bacteria isolates. In district wise Lushoto and Mvomero had more isolates of *S. epidermidis*, *S. aureus*, *L. monocytogenes* in dairy cows while *S. epidermidis*, *S. aureus*, *L. monocytogenes* and *L. inoccua* were found in local cows from Handeni and Kilosa. However, more of *S. epidermidis* isolates were found in Handeni District. Both *L. ivanovii* and *E. coli* were not found in milk samples collected from Lushoto.

Table 5: Bacteria isolates and their distribution in breeds in the districts

Bacteria spp	Total N = 78	Breed	Number (%) of mastitis in different districts					
			Handeni N=25	Kilosa N=25	Lushoto N=10	Mvomero N=18	χ2	P value
S. epidermidis	28	Local	19 (24.4)	8 (10.3)	0 (0)	1 (1.3)		
	12	Dairy	0 (0)	0 (0)	5 (6.4)	7 (9)	6.9	0.0087
S. aureus	14	Local	2 (2.6)	12 (15.4)	0 (0)	0 (0)		
	13	Dairy	1 (1.3)	0 (0)	5 (6.4)	7 (9)	2.97	0.0847
E. coli	8	Local	2 (2.6)	5 (6.4)	0 (0)	1 (1.3)		
	2	Dairy	1 (1.3)	0 (0)	0 (0)	1 (1.3)	2.9	0.0847
L. monocytogenes	14	Local	6 (7.7)	6 (7.7)	0 (0)	2 (2.6)		
	11	Dairy	1 (1.3)	0 (0)	4 (5.1)	6 (7.7)	0.5	0.47
L. ivanovii	8	Local	3 (3.9)	5 (6.4)	0 (0)	0 (0)		
	4	Dairy	1 (1.3)	0 (0)	0 (0)	3 (3.9)	1.9	0.1715
L. inoccua	18	Local	10 (12.8)	8 (10.3)	0 (0)	0 (0)		
	6	Dairy	0 (0)	0 (0)	2 (2.6)	4 (5.1)	1.5	0.22

χ2 = Chi-Square P= Probability level of Chi-Square

4.4 Bacteria Isolates and Their Distribution in Breeds in the Districts

Bacteria isolates from all districts were grouped depending on whether they were from non-mastitis and mastitis with both clinical and subclinical mastitis (Table 6). Regarding to isolates from non-mastitis cows, more samples had *S. aureus* followed by *L. inoccua* and *S. epidermidis*. There was no *E. coli* and *L. ivanovii* isolated from non-mastitis samples collected from Lushoto and Mvomero districts. In case of mastitis more isolates were found in subclinical mastitis compared to clinical mastitis. Despite of few isolates of clinical mastitis found in all districts there was no *E. coli* isolates in clinical mastitis in all the districts.

Table 6: Bacteria isolates found in mastitis and non-mastitis cows in the districts

Bacteria spp	Udder status	Number (%) bacteria isolated in milk from different districts					
		Handeni	Kilosa	Lushoto	Mvomero	χ2	P value
S. aureus	Non						
	mastitis	1 (1.3)	6 (7.7)	3 (3.9)	1 (1.3)	6.67	0.082
	Clinical	0	0	0	2 (2.6)		
	Subclinical	3 (3.9)	6 (7.7)	2 (2.6)	4 (5.1)	4.71	0.194
S. epidermidis	Non						
	mastitis	2 (2.6)	2 (2.6)	0	2 (2.6)	5.42	0.143
	Clinical	2 (2.6)	1 (1.3)	0	4		
	Subclinical	2 (2.6)	5 (6.4)	5 (6.4)	2 (2.6)	9.01	0.291
E. coli	Non						
	mastitis	1 (1.3)	2 (2.6)	0	0	1.96	0.58
	Clinical	0	0	0	0		
	Subclinical	2 (2.6)	3 (3.9)	0	2 (2.6)	2.8	0.424
L. monocytogenes	Non						
	mastitis	1 (1.3)	1 (1.3)	1 (1.3)	1 (1.3)	1.56	0.67
	Clinical	0	0	0	5 (6.4)		
	Subclinical	6 (7.7)	5 (6.4)	3	2 (2.6)	0.67	0.881
L. ivanovii	Non						
	mastitis	1 (1.3)	3	0	0	3.21	0.361
	Clinical	0	1 (1.3)	0	0		
	Subclinical	4 (5.1)	1 (1.3)	0	3 (3.9)	5.05	0.168
L. inoccua	Non						
	mastitis	2 (2.6)	4 (5.1)	1 (1.3)	2 (2.6)	1.33	0.718
	Clinical	0	0	0	1 (1.3)		
	Subclinical	8 (10.3)	4 (5.1)	1 (1.3)	1 (1.3)	2.99	0.393

χ2 = Chi-Square P= Probability level of Chi-Square

CHATER FIVE

5.0 DICUSSION

The current study was carried out to establish the magnitude of mastitis in lactating cows and the microbial quality of milk from mastitis and non-mastitis cows. The results generally indicated that the prevalence of mastitis was high in particular the subclinical form of the disease. This is a serious finding since mastitis is a disease of great economic importance as it may lower the milk production by 75% (Mdegela *et al*., 2009). Therefore efforts need to be taken to control the problem of mastitis otherwise the un-quantified losses encountered by farmers are quite high. In addition most of the milk marketed and consumed in the four study districts was contaminated with different bacteria species. This downgrades the quality of the milk and some bacteria potentially cause mastitis and other diseased in cattle. Nevertheless, majority of bacteria isolated are also known to cause milk-borne diseases in humans. This suggests that the milk produced in the study area is potentially dangerous to the consumers and milk pasteurization is hereby stressed.

The current study has established high prevalence (75.6%) of mastitis in the lactating cows in the study area. These findings could be related with animal husbandry and milk handling practices that are undertaken in the districts. There are several practices at farm level such as type of animal house floor, not washing hands and udder/teats before milking (Kanyeka, 2014). Another factor is milking sick animals and those with udder problems before milking non mastitis cows (Shem, 2001; Gwandu, 2013; Kanyeka, 2014). Water used for cleanliness in washing hands and milk equipments are not clean and may be potential sources of infection. Previous studies in Tanzania had similar observations (Swai and Schoonman, 2011; Kanyeka, 2014).

Treatment of livestock has been left in hands of private sector and paraprofessionals are the main actors in the treatment of sick animals (Karimuribo *et al*., 2005; Morris *et al.*, 2014). This kind of practice could also contribute to improper management of diseases and complicate the situations in the dairy industry. Intensification of experts in livestock sector that will be giving education to livestock keepers is of paramount important in rural areas in order to rescue the dairy industry in Tanzania.

It was further found that dairy cattle were more affected by mastitis at the rate of 82.1% compared to local cattle at 72%. Dairy cattle found in the study area were crosses of pure breeds (Ayrshire, Jersey and Friesian) and local breeds. The local breeds included Tanzania short horn zebu, Boran and Ankole. From the results it is obvious that mastitis had a relationship with the breeds of individual cow as it has been indicated that dairy cattle had more mastitis than local cattle. Karimuribo *et al*. (2006) reported that the prevalence of mastitis in Iringa was 61.2% in dairy cows, while in local cows was only 26%. Mbeho (2012) reported also variation of prevalence of mastitis in local Tanzania breeds. Maasai zebu had high prevalence of mastitis of 52% followed by Iringa red (48.2%) and Gogo strain with 22.2%. Study by Abdel-Rady and Sayed (2009) in Egypt found Friesian breed to be more affected by mastitis (20.4%) than the native breed (16.7%)

It can be concluded that the husbandry practices associated with the breeds have a significant contribution to the prevalence of mastitis in dairy cows. From this observation the breed factor could be more important than the hygienic factors (Rajala and Grohn, 1998; Karimuribo *et al*., 2006; Abdel-Rady and Sayed, 2009). Studies have been carried out to introduce mastitis resistance traits into dairy cow breeding schemes but this was affected by the negative genetic correlation with increased milk yield. It was observed that

heritability of milk yield is markedly higher than that of mastitis resistance (Strandberg and Shook, 1989). Normally in the pastoral community there is extensive system of cattle production and the breeds of cattle found are the indigenous breed (Mbeho, 2012). The local breeds are tolerant to harsh environment and diseases. In smallholder dairy farms/semi-intensive system, the breeds found are mainly crossbreds of indigenous breeds of TSHZ and Boran with exotic breeds of dairy cattle mainly Friesian, these are good producers of milk but are not tolerant to diseases including mastitis compared to pure breeds of Ayrshire, Friesian and Jersey (Laswai *et al*., 2014).

Subclinical mastitis was more prevalent (62.8%) compared to clinical cases. Our finding was supported by that of Abdel-Rady and Sayed (2009). The subclinical form of mastitis is considered 15-40 times more prevalent than clinical form and accounts for greater losses in terms of milk production and it represents a reservoir of infectious organisms. This is the most prevalent form of mastitis reported in Tanzania (Mdegela *et al*., 2009; Mbeho, 2012). It is the form of mastitis which takes a very long duration to be recognized and difficult to detect. Prevalence of subclinical mastitis is associated with different factors including breeding distribution, different seasons and different ages. In relation to age susceptibility, older cows (5-8 year) are more prone to subclinical mastitis than younger cows (2-4 years). When considering different season, Abdel-Rady and Sayed (2009), reported that mastitis decreases as weather changes from summer with high incidences through spring with moderate infections to low infection in cold weather as during winter and autumn. The current study was conducted during dry season which may support the high incidences of mastitis in milking cows as reported by Abdel-Rady and Sayed (2009). Subclinical mastitis pathogens that can be routinely monitored are contagious ones such as *S. aureus* (Mona *et al*., 2008).

More cases of mastitis were found in dairy cows (82.14%, 23/28) compared to local cows (72%, 36/50). All dairy cows found in the study area were crosses of exotic with local breeds. Type of management used was zero grazing to dairy cows where cows were confined in houses all the time (Morris *et al*., 2014). There is substantial evidence that high yields are linked to high mastitis levels and cattle held in confined areas, with high fly populations, can increase the incidence of mastitis. Infections rates can be as high as 25% (Grohn *et al.*, 1990; Rajala and Grohn, 1998). From these findings it can be concluded that lactating cattle in the study area had high levels of mastitis due to breed predisposition and confinement. Morris *et al.* (2014); did a comprehensive livestock environmental assessment in Lushoto on the production status of these smallholders and their constraints including diseases in their livestock.

For the first time, *Listeria* species have been isolated in Tanzania. The isolation rate was 78.2%. (61/78). *L. monocytogene* was the commonest as it accounted for 32.1%. Other *Listeria* species isolates were *Listeria ivanovii* (15.4%) and *Listeria inoccua* (30.8%). The reasons for this high prevalence of *Listeria* spp. in the study area could be due to unhygienic practices during milking and poor milk handling. Also it could also be due to environmental contamination with feacal as in the study areas animal house floor and milking procedures was done in unhygienic manner (Kanyeka, 2014). It is known that the source of *L. monocytogenes* in raw milk is mostly the gastrointestinal tract of animals and the environment, skin of the teats, in particular (Sanaa *et al.,* 1996). Therefore, the presence of *Listeria* spp in raw milk is a public health concern because of the ability of *Listeria* spp survive in different stages of milk processing methods. Over and above, *Listeria* spp grow at low temperatures and survive osmotic stress. Mild preservation treatment is potential risk of milk-borne infection to consumers from *Listeria* spp (Kanyeka, 2014).

The findings from this study are similar to previous studies that reported presence of *Listeria* spp. in raw milk elsewhere. Molla *et al*. (2004), studied *Listeria* species in retail meat and milk products in Addis Ababa, Ethiopia. They reported ice cream samples to be highly contaminated with *L. monocytogenes* (19.6%) followed by *L. innocua* (13%). Furthermore Al-Miriri *et al*. (2013) and Kasalica *et al*. (2011) reported *L. monocytogenes* to be the main cause of listeriosis in humans and animals while *L. ivanovii*, causes abortion in animals' particularly in ruminants and hens. Al-Mariri *et al*. (2013) using conventional bacteriologic methods reported *L. monocytogenes* (41.6%), *L. innocua* (17.8%) and *L. ivanovii* (14.2%) in raw milk. In Mexico City, Carlos *et al.* (2001); studied the incidences of *Listeria* spp. in 1300 raw milk samples, and found that 13% were positive for *L. monocytogenes* (6%), *L. ivanovii*, (4%) *and L. innocua* (1%). Sohrabi *et al*. (2013) reported *L. monocytogenes* (80%) in Esfahan Province, Iran. Their findings were commonly related to special foods and season of the year. Since the milk samples in the current study were collected during the dry season could be a contributory factor for high isolation rate of *Listeria* spp. Al-Mariri *et al*. (2013) reported different species of *Listeria* that included *L. welshimeri* and *L. gravi*. Futher more in Mexico City, Carlos *et al.* (2001) reported *L. seeligeri*. From these studies conducted in different countries it can be concluded that in Tanzania there is still a lot to be done in order to explore different species of bacteria that may be found in milk and milk products.

Other bacteria that was isolated in this study was *S. aureus* that had a prevalence of 34.6%. This bacteria is the main source of contagious type of mastitis both in clinical and subclinical mastitis in milking cows. In the current study, species of *Staphylococcus* were isolated, *S. aureus* and *S. epidermidis* In Addis Ababa, Ethiopia Demme and Abegaz (2015), reported *S. intermidus* and *S. hyicus*. Hosseinzadeh and Saei (2014), reported isolation of *S. haemolyticus, S. chromogenes, S. warneri, S. cohnii, S. simulans, S.*

hominis, S. capitis, *S. xylosus S. haemolyticus,* and *S. chromogenes* from milk samples. The prevalence of *S. aureus* obtained were within the ranges obtained in other studies. Hosseinzadeh and Saei (2014) did a study in North West Iran and reported *S. aureus* of 4.4%. In another study Mbeho (2012), established a prevalence of 65 % of indigenous cattle in Tanzania. Abdel-Rady and Sayed (2009) had the same study in Assiut Governorate and reported a prevalence of 52.5% for *S. aureus*. In Addis Ababa, Ethiopia**;** Demme and Abegaz (2015), found major bacterial pathogen from clinical mastitis cow raw milk to be *S. aureus* (56.5%). This variation within different studies can be associated with hygiene and conditions as well as management practices since *Staphylococcus* species cause contagious mastitis.

Consumption of milk contaminated with *S. aureus* can be a health hazard because about 10% of mastitis staphylococci are known to be producers of enterotoxins which are heat stable toxins (Kivaria *et al*., 2006a). The staphylococcal enterotoxin includes types C, A D and type E (Vasil', 2007). Some studies have associated *S. aureus* with gastroenteritis through these entorotoxins (Bukuku, 2013; Kanyeka, 2014). Since *S. aureus* are contagious and common colonizer of teat end and teat canal, the use of therapy such as dry cow and post-milking teat disinfectants can be of great value in controlling the mastitis disease in lactating cows that is caused by *S. aureus* (Mdegela *et al*., 2009). These measures were not practiced in the study districts and this could be the reason of high incidences of mastitis.

Staphylococcus. epidermidis was isolated with prevalence of 51.3%. Several studies focused on *Streptococcus dysgalactiae* and *Streptococcus uberis*. These bacteria have host-parasite relationship cause contagious mastitis and are transmitted from cow to cow through milking channel.

Escherichia coli were isolated in this study at the rate of 12.8% of the samples. *E. coli* is an indicator bacterium of faecal contamination and is the causative urgent of environmental mastitis, these organisms are commonly found in organic matter, including bedding and manure thus, exposure of the teat end occurs through dirty bedding (Erskine, 2014). This finding cannot be ignored since there are several strains known to be highly pathogenic with a potential of causing illness to consumers and these isolates were not further typed for verocytotoxigenicity. Some strains of *E. coli* are verocytotoxigenic like enterohaemorrhagic type of *E. coli* O157:H7 that are known to cause haemorrhagic colitis (Kanyeka, 2014). Verotoxin producing *E. coli* generally results in a bloody diarrhoea but can cause the haemolytic uraemic syndrome, characterized by thrombocytopaenia, haemolytic anaemia and acute kidney failure, particularly in children (WHO, 1999; Kivaria *et al*., 2006a). A study by Schoder *et al.* (2013) reported occurrence of *E. coli* O157:H7 in raw milk from traditional cattle farms in Tanzania. A similar study by Lupindu (2014) isolated a highly pathogenic *E. coli* O157: H7 in cattle manure in Morogoro urban and peri-urban areas. The results obtained from this study are within the range that has been reported in other studies. In Tanzania Kivaria *et al.* (2006b) reported (4.1%) and 6.3% prevalence was reported by Karimuribo *et al*. (2005). Other studies carried out in Tanzania by Mdegela *et al*., 2009; Ngasala, 2013 also reported higher prevalence of *E. coli* in milk samples. Abdel-Rady and Sayed (2009), reported prevalence of 16.3%.in Egypt. Demme and Abegaz (2015) in Addis Ababa, Ethiopia, reported *E. coli* (18.6%) from clinical mastitis raw cow milk.

These bacteria can be controlled by proper milking procedures, maintaining a clean and dry housing environment and appropriate bedding materials, and vaccinating animals (Petersson-Wolfe and Currin, 2015). *Escherichia coli* O157:H7 is known to cause a deadly

diarrhea in humans and consumption of contaminated raw milk is reported to be among important routes of transmission of these pathogenic bacteria (Kanyeka, 2014).

It was observed in this study that other bacteria were isolated and obtained from non-mastitis milk samples. The most isolated bacteria were *S. aureus* followed by *L. inoccua* and *S. epidermidis*. This finding could be associated with hand milking techniques and hygiene of cows in milk, udder and clothes that are used to clean the udder prior to milking of the cow. Gwandu (2013) also Kivaria *et al*. (2007), found unhygienic condition during milking time, free-running suckling calves clothes that are used to wash the udder, containers that are used to store and transport milk, unclean animal houses and removal of cow dung from the premises to be the bacteria sources that are contaminating the milk (Mulei, 1999). Generally finding such a high number of bacteria species in raw milk samples is of public health significance since majority of the people in Tanzania consume raw milk. This call for extensive education to livestock keepers on proper animal management, better methods of milking to avoid contamination and use of pasteurized milk.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the findings of this study it is concluded that:

i. The prevalence of clinical and subclinical mastitis was high in the study district.

ii. Handeni District had the highest prevalence of mastitis compared to the rest of the districts.

iii. Milk samples collected from lactating cows in Kilosa, Mvomero, Handeni, and Lushoto districts were highly contaminated by potentially pathogenic bacteria. Six types of bacteria with high prevalence from *S. epidermidis, S. aureus, L. monocytogenes, L. inoccua, L. ivanovii* and *E. coli* were isolated from milk samples collected in all four study districts.

6.2 Recommendations

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

i. Livestock keepers in these districts should be educated on the presence of mastitis in their animals and on the ways of how to manage and control the disease. This is the role of extension officers that are found in these studied districts.

ii. Livestock keepers need to be educated on proper animal management, better methods of milking to avoid contamination and the importance consuming heat treated milk (pasteurized or boiled).

iii. Regular studies should be conducted in these districts in order to monitor the extent of the disease and report the findings so that policy makers will be in a position to put forward the regulatory mechanisms through enforcing the laws and by laws governing milk production to safe guard the consumers and the public health.

iv. Further studies should be carried out to identify more microbials that are found in the milk industry as it has been reported in other countries.

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