# BACTERIAL CONTAMINATION DETERMINATION AND PERFORMANCE OF A SOLAR HEATER IN THE PASTEURISATION OF COW MILK IN WESTERN PROVINCE, ZAMBIA

**BY**

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## DECLARATION

I Mary Chibwe declare that the work presented in this dissertation was done by myself and has not been presented to this or any other university for the award of any degree.

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## APPROVAL

This thesis of Mary Chibwe has been approved as fulfilment for the award of the degree of Master of Science in Public Health and Zoonosis of the University of Zambia.

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## ABSTRACT

Although milk contains many nutrients necessary for growth, raw milk may harbor numerous pathogens. The milk pathogens may be shed in harvested milk from an infected animal and may transmit zoonotic diseases. Consumption of raw milk is common in developing countries. However, milk needs to be pasteurised to render it safe for human consumption. In resource poor communities milk pasteurization is hindered due to lack of access to electricity and heat energy forcing people to consume raw milk. In this study, the presence of pathogens in raw milk was investigated and the identified pathogens were subjected to solar heating in order to determine the possible use of solar energy for inactivation of milk pathogens.

Raw milk from indigenous cattle of Mongu, Nalolo and Limulunga districts of Western province was collected. The collected milk was subjected to bacterial contamination determination and identification of selected bacterial species. *Escherichia coli, Staphylococcus aureus* and *Bacillus* species were identified as the major contaminants. Milk contaminated with these pathogens was subjected to heating using conventional methods and solar heating in the months of April, May, June and July 2017. Furthermore, other organisms that included *Salmonella* and Methicillin resistant *Staphylococcus aureus* were also subjected to solar heating. Further, 63 farmers who supplied milk to the milk collection centres in the study area were interviewed in order to capture bio-data, milk handling and consumption practices. The results indicated that solar heating is not effective in the cooler months of the year. It was also observed that sterilized milk allows rapid bacteria proliferation when contaminated compared to raw milk suggesting the presence of intrinsic antimicrobial factors in unsterilized milk. Pasteurization using electricity is expensive for rural communities and solar energy is a cheaper source of energy which can be used to inactivate pathogens in the months with no cloudy cover. This study also demonstrated that consumption of raw milk is a common traditional practice in western province where knowledge and application of good hygiene practice during milk collection, storage and transportation is inadequate. There is need to discourage consumption of raw milk and promote boiling of milk using affordable and environmentally friendly methods. More milk collection centres need be established and the traditional farmers should be trained in milk handling.

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## DEDICATION

I dedicate this work to my father Mr. Oliver Chibwe and my mother Mrs. Violet Chikonoko Chibwe for all the support you have given me. I also dedicate this work to Mr. Charles Musonda and Mrs Peggy Musonda for being there for me in my academic life.

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| | |
|---|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| ALP | Alkaline Phosphatase |
| CDC | Centers for Disease Control and Prevention |
| CFU | Colony Forming Units |
| CoNS | Coagulase Negative Staphylococcus |
| CSO | Central Statistics Office |
| DNA | Deoxyribonucleic Acid |
| ESBL | Extended Spectrum Beta lactamase |
| FAO | Food and Agriculture Organisation |
| HIV | Human Immunodeficiency Virus |
| LAB | Lactic Acid Bacteria |
| MAP | *Mycobacterium avium paratuberculosis* |
| MCC | Milk Collection Centre |
| MRL | Maximum Residue Limit |
| MRSA | Methicillin Resistant *Staphylococcus aureus* |
| PCR | Polymerase Chain Reaction |
| RDI | Recommended Daily Intake |
| STEC | Shiga Toxin Producing *Escherichia coli* |
| UHT | Ultra High Treatment |
| UNZA | University of Zambia |
| WAPI | Water Pasteurisation Indicator |
| WHO | World Health Organisation |

# CHAPTER ONE

# INTRODUCTION

## 1.1. Background

Milk is a major component of the human diet in many parts of the world. It is an important source of nutrients required for growth in infants. Milk is rich in proteins, fats, carbohydrates, vitamins, minerals and essential amino acids (Muehlohoff *et al*., 2013; Kerstetter *et al.,* 2004). In addition to these rich nutrients, milk also has a near neutral pH and a high water activity making it a favourable habitat for many microorganisms (Quigley *et al*., 2013).

Milk is a major source of dietary energy, protein and fat in humans. Globally it is estimated that milk contributed 134 kcal of energy/capita per day, 8 g of protein/capita per day and 7.3 g of fat/capita per day on average. It is further estimated that cow milk accounted for 83 percent of global milk production in 2010 (Muehlohoff *et al*., 2013). Milk can be obtained from different animal sources and these include cows, goats, sheep and buffalo. Humans also produce milk which is commonly referred to as breast milk and is consumed by babies. Cow milk is widely consumed and is an important source of proteins (Dror and Allen, 2011). Its protein and minerals content is higher than that of human milk (Soliman, 2005). The calcium and phosphorus content for cow milk is 129 mg/100 g and 104 mg/100 g respectively (Lucey, 2015). The protein in cow milk is of high-quality i.e. protein that supports maximal growth and contains a good balance of all the essential amino acids, including lysine. The fat content for cow milk is between 3 and 4 g of fat/100 g and saturated fatty acids about 75 g/100 g total fatty acids. Milk is a good source of the B vitamins (Riboflavin or B2 and B12) (Muehlohoff *et al*., 2013).

Milk is an example of an environment that contains a diverse and complex microbial population and may harbour numerous pathogens. This is because milk has a high protein, fat and carbohydrate content. Milk also contains vitamins, minerals and essential amino acids, and it's near neutral pH and a high water activity makes it a suitable habitat for many microorganisms. The microbial load for fresh milk from a healthy cow is low but storing the milk without cooling can increase the microbial load (Quigley *et al*., 2013). The microorganisms present in milk multiply when the temperature is higher. Cooling of milk reduces the microbial activity

hence reducing the microbial population (Samaržija *et al.*, 2012). Microorganisms in milk originate from a variety of sources and can play a number of niches while in milk. Some microorganism's roles is to facilitate milk fermentation. These microorganisms include *Lactococcus, Lactobacillus, Streptococcus, Propionibacterium* and fungal populations. Microorganisms such as *Pseudomonas, Clostridium, Bacillus* and other spore-forming or thermoduric microorganisms are spoilage organisms. Other microorganisms promote health (e.g. *Lactobacilli* and *Bifidobacteria*) while *Listeria, Salmonella, Escherichia coli, Campylobacter* and mycotoxin-producing fungi are examples of pathogenic microbes (Quigley *et al*., 2013).

The Lactic Acid Bacteria (LAB) in milk include *Lactobacillus casei subsp. paracasei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus curvatus, Lactobacillus brevis, Lactobacillus fermentum; Leuconostoc lactis, Leuconostor cremoris; Enterococcus faecium, Enterococcus faecalis, Enterococcus durans* and *Pediococcus spp.; P. pentosaceus, P. acidilactici*. *Lactobacilli* are inactivated by pasteurisation but some strains may survive the heat treatment and proliferate in dairy during products production and storage. Bluma and Ciprovica (2015) isolated *Lactobacillus paracasei, Lactobacillus brevis, Lactobacillus fermentum Lactobacillus curvatus, Lactococcus lactis, Lactobacillus plantarum* and *Lactobacillus rhamnosus* in raw milk. Studies suggest that some raw milk LAB can contribute to the human health by aiding digestion and decreasing the risk of allergies such as asthma, hay fever and atopic sensitisation (Zastempowska *et al.*, 2016).

*Pseudomonas, Bacillus* and *Acinetobacter* spp are a major component of milk, especially at refrigeration temperatures because they are psychrotrophic in nature (Malek *et al*., 2015). Another type of microorganisms present in raw milk are the thermoduric bacteria. These are microorganisms which are able to withstand high temperature and they include the various species of genus *Bacillus, Microbacterium, Micrococcus, Enterococcus, Lactobacillus* and *Corynebacterium* (Zastempowska *et al*., 2016). Various yeasts and moulds are also present in raw milk. The yeasts that have been isolated from raw milk from healthy animals or those with mastitis include *Kluyveromyces, Rhodotorula, Debaryomyces, Saccharomyces, Geotrichum, Pichia, Candida, Trichosporon* and *Cryptococcus* spp (Delavenne *et al*., 2011). Moulds have also been isolated from milk although they are at a lower level than yeasts. These molds are from the genera *Penicillium, Aspergillus, Mucor, Fusarium* and *Cladosporium.* These are present in milk from both sick and healthy animals (Zastempowska *et al.*, 2016).

Annually, millions of diseases worldwide can be associated with consumption of food contaminated by pathogens. The pathogens may include bacteria, fungi, viruses and parasites (Quigley *et al*., 2013). Among the contaminated food is milk. Milk contains nutrients necessary for growth and can harbour populations of food-borne pathogens. Milk pathogens can be a cause of severe human illness and even death. The presence of *Campylobacter* spp., *Salmonella* spp., *Brucella melitensis, Mycobacterium bovis* and tick-borne encephalitis virus (TBEV) in milk cause diseases in humans. Other pathogens associated with milk include the bacteria *Bacillus cereus, Brucella abortus, Listeria monocytogenes, Staphylococcus aureus, Yersinia enterocolitica, Yersinia pseudotuberculosis, Corynebacterium* spp., *Streptococcus suis subsp. zooepidemicus* and the parasites *Toxoplasma gondii* and *Cryptosporidium parvum* (Quigley *et al.*, 2013).

*Salmonella* spp., *Campylobacter* spp., *E. coli O157:H7, Yersia enterocolitica* and *Listeria monocytogenes* as well as intoxications by *Staphylococcuss aureus* have been associated with human outbreaks as a result of consumption of raw milk or dairy products made from contaminated raw milk (Claey *et al.,* 2013). The most common pathogen associated with raw milk consumption is *Campylobacter* spp followed by *Salmonella* spp. and human pathogenic verocytotoxigenic *E. coli* (*E. coli* O157:H7) and pathogenic *E. coli* (non-O157:H7). *Listeria monocytogenes* and *Staphylococcus aureus* are not frequently associated with outbreaks due to raw cow milk consumption. This is because their growth in raw milk is limited by the commensal microbes present in milk. The other reason is that *Listeria monocytogenes* has a high infectious dose. *Staphylococcus aureus* can only produce enterotoxin that are pathogenic to humans if it is in high numbers (Pouillot *et al*., 2016; Claey *et al.*, 2013).

*Staphylococcus aureus* is part of the human normal flora. It's presence in raw milk can originate from poor hygienic practises such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking. Milk can also be contaminated by *Staphylococcus aureus* when there is infection of the mammary gland. *Staphylococcus* was isolated from the hands and on the uniform of milk handlers and may therefore lead to milk contamination (De Oliveira *et al.*, 2011).

The development of a disease after consuming contaminated raw milk depends on a number of factors. These include the pathogenicity of the micro-organism, the toxicity of the toxin

produced by microorganisms, the number of ingested microorganisms, quantity of toxins, the infective dose in humans, age and the health status of the consumer (Claey *et al.,* 2013). Everyone can be affected by pathogens in raw milk, but, babies, the elderly, pregnant women and immune-compromised persons are mostly at risk. Common symptoms of milk-borne infections include diarrhoea, vomiting, nausea, fever, abdominal cramps, etc. Some individuals can however develop more severe clinical symptoms such as Guillain-Barré syndrome (*Campylobacter* spp) and hemolytic uremic syndrome (HUS) (*E. coli* O157:H7), or long-term and sometimes chronic complications such reactive arthritis, or even death (Claey *et al.,* 2013).

The presence of foodborne pathogens in milk may either be due to direct contact of safe milk with contaminated sources in the environment during milking and processing, or due to excretion from the udder of an infected animal (Swai and Schoonman, 2011). A variety of factors greatly influence the prevalence of food borne pathogens in milk. Among these include; the number of animals on the farm, size of the farm, farm management practices, hygiene practices, climate, seasons and geographical location (Oliver *et al*., 2005). Unhygienic milking equipment is a hidden source of psychrotrophic and thermophilic bacteria, as well as the destructive enzymes. The presence of coliform bacteria may be due to contamination by manure, soil and water during milking and handling. Milk handlers who practice poor personal hygiene may also contaminate milk. Milk may also be contaminated by water containing human discharges. The common cleaning practice on most dairy farms is flushing animal houses with water to remove manure. This practice is effective and quickly removes manure but may distribute faecal flora throughout the farm environment. This can expose large numbers of animals to pathogen organism which are found in faecal matter (Chye *et al.*, 2004).

Milk pathogens may be shed in milk harvested from an infected animal and may transmit zoonotic diseases. Diseases naturally transmitted between animals and humans are referred to as Zoonotic diseases. Zoonotic diseases have serious public health and economic implications (WHO, 2006). Those transmitted through milk consumption include Tuberculosis, transmitted by the *Mycobacterium tuberculosis complex*, Brucellosis through *Brucella* spp, *Staphylococcus* and *Bacillus anthracis*. A great number of people in developing countries, especially the poor, marginalized communities are affected by these diseases since they are associated with poverty, poor farm management practices, and high levels of illiteracy (WHO, 2006). The risk of transmission of zoonotic diseases to humans is greatly tied to the prevalence of these diseases in the animal population (Abbas and Aldeewan, 2009).

Although scientific research has revealed that raw milk may harbour pathogens, consumption of raw milk remains an issue for debate. In order to guarantee its microbial safety and to prolong its shelf life, milk is heat treated. Since the introduction of milk pasteurization in 1938 in the United States of America, a reduction of foodborne diseases associated with milk has been recorded. Recent studies have reported that the majority of milk-borne outbreaks in the US occur in states that permit the sale of raw milk. Similar observations have been made in England and Wales where a significant drop of the incidence of diseases related to milk consumption has been recorded since the introduction of pasteurisation (Claey *et al.,* 2013).

In developed countries, Bovine tuberculosis used to be a serious zoonosis. During that period, it represented a huge public health problem mainly through consumption of contaminated unpasteurized milk. This disease was controlled in cattle through designed test and slaughter schemes (Pandey *et al*., 2013). In rural areas of Africa, consumption of unpasteurized milk is a common practice. Studies have confirmed the presence of zoonotic *M. bovis* in the milk (Pandey *et al*., 2013). The population consuming raw milk is therefore at risk of contracting diseases. Young children, babies and HIV/AIDs affected individuals are at a higher risk of contracting the disease (Pandey *et al.,* 2013).

Raw milk must therefore be pasteurised in order to protect public health. Consumption of raw milk is common in developing countries, including Zambia. Pasteurisation of raw milk reduces microbial load of milk and limits the number of spoilage microorganisms (Hudson *et al*., 2003). The heat treatment of milk typically reduces psychrotrophic and mesophilic populations but the thermoduric microorganisms are able to survive (Quigley et al., 2013). *Bacillus cereus* is a major spoilage organism of pasteurised milk and milk products stored at refrigeration temperature and is also a concern in food safety as it can produce different types of toxins which can cause food poisoning (Quigley *et al.,* 2013).

Pasteurisation of milk is rarely practiced in rural communities. This is because access to electricity or heat energy is limited. Where electricity is available, electricity tariffs are high for traditional cattle holders to afford (Zahira *et al.,* 2009). In the Western province of Zambia for instance there is limited firewood as a result of the vast plains. This may be one of the reasons, most households are unable to boil their milk and hence they consume raw milk.

Western province experiences plenty sunshine during the year. Solar energy can therefore be utilised to pasteurise milk.

This study was therefore meant to investigate the presence of pathogens in milk from Western province, specifically *Staphylococcus*, *Streptococcus*, *Escherichia coli*, *Salmonella*, *Brucella abortus* and *Mycobacterium bovis.* Further the identified pathogens in milk were subjected to solar heating to determine the possible use of solar energy for inactivation of pathogens in milk.

### 1.2 Problem Statement and Justification

The consumption of unpasteurised milk is one of the major means of transmission of zoonotic diseases. For example, pathogens such as *Escherichia coli, Salmonella, Staphylococcus, Brucella abortus* and *Mycobacterium bovis* which are found in milk can cause illnesses in human beings, especially vulnerable individuals. Among these may include; children, pregnant women, the elderly and the immunocompromised population (Tesfaye *et al*, 2013; Lauzardo and Askin, 2000).

Milk must therefore be pasteurised in order to inactivate pathogens in milk. Pasteurisation is very expensive for rural communities in Zambia due to limited access to electricity and inadequate firewood in some parts of Zambia such as Western province. This makes many rural households consume milk without boiling it or heat inactivation of bacterial contaminants in milk. Lack of refrigeration also results in quick spoilage of milk. There are few milk collection centres in Western province. Poor road network and long distances to the few existing milk centres are some of challenges faced by traditional farmers in Western province. Solar pasteurisation is a cheap and simpler method of rendering milk safe for human consumption thus reducing the risk of exposure to zoonotic diseases. Solar cookers can be easily accessed on the Zambian market and they can also be fabricated locally. Use of solar energy can reduce the use of firewood and hence contribute to the reduction of greenhouse gases in the atmosphere (Goswami and Besarati, 2013).

Solar energy is a reliable form of energy. This source of energy is cheap and available throughout the year in Zambia. It is estimated that the amount of solar energy intercepted by the earth per minute is greater than the amount of energy (fossil fuel) that is used globally each year. The total annual solar radiation falling on the earth is more than 7 500 times the world's total annual primary energy consumption (Goswami and Besarati, 2013). Despite the earth

receiving this abundant energy from the sun, solar energy is not the main source of energy in many countries (Zahira *et al.,* 2009). The common source of energy in Zambia is hydropower. Hydropower is not accessible in many rural areas of Zambia. Furthermore, the tariffs charged to consumers have made electricity expensive and unaffordable in Zambia. In the face of climate change, hydropower has become unreliable as there is insufficient water due to drought. Therefore solar energy from the sun may be the only alternative in providing a consistent and steady source of power which is readily available. The use of solar energy reduces the use of charcoal and does not cause air pollution during use (Hamududu and Killingtveit., 2012).

## 1.3. Objectives

### 1.3.1. General Objective

To assess bacterial contamination in milk as well as determine the efficiency of Bacterial inactivation in solar heated milk in Western Province of Zambia.

### 1.3.2. Specific Objectives

1. To assess the health risks associated with raw milk consumption among traditional farmers.
2. To determine the level of bacteria contamination of milk consumed at household level in Western Province, Zambia.
3. To investigate the effect of solar heating on the survival of pathogenic and spoilage bacteria in traditionally consumed bovine milk.

## 1.4. Research Questions

1. Is cow milk consumed at household level free from bacterial pathogens?
2. Is solar heating a suitable way of inactivating milk pathogens for improved milk safety?

## 1.5. Expected Benefits

Understanding the spoilage and pathogenic organisms in milk will help in formulating important public health control measures and other storage strategies at traditional level. The study will help in gathering information on the possibility of using solar power in energy resource constrained areas as a means of pasteurizing milk. This will help rural communities

identify cheaper and eco-friendly sources of energy that can be used to improve milk safety and increase the shelf-life of milk.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1. Milk Production and Consumption

Global estimates by the Food Agriculture Organisation (FAO, 2015) indicate that there are over six billion consumers of milk and milk products with the majority of these living in developing countries. It is also estimated that between 750 and 900 million people live within dairy farming households, corresponding to 12 to 14% of the world's population with the majority of these farmers being poor people with an average herd size of two dairy animals and average milk harvest of 11 litres per day (FAO, 2015).

Milk consumption in developing countries has almost doubled. There has been an increase in per capita consumption of milk and other livestock products in parts of the developing world (Blasko, 2011). However, adverse conditions such as higher ambient temperature and/or humidity affect milk production in these countries. These conditions are harsh for the dairy cattle as they may reduce milk production even when the cows have the genetic potential. Unlike large-scale dairy producers who are well organised traditional small-scale farmers have little or no mechanization or technological innovations. The smallholder sector in developing countries significantly contributes to milk production. In Kenya for example, it accounts for about 85 percent of total milk production (Blackmore *et al*., 2000). Despite having the potential for increasing milk production, small scale dairy farmers in developing countries produce low quantities of milk. This is mainly due to poor animal management, poor quantity and quality of feed (Cheruiyot and Otieno 2017; Muehlohoff *et al*., 2013). Milk yield can be increased by improved feeding and increasing concentrate supplementation. Dairy farmers are faced with the challenges of ensuring that milk is safe and that it does not easily deteriorate. In addressing these challenges, technological advancements have resulted in production of machinery that reduces wastage, optimize production and maximize utilization of milk constituents (Seck *et al*., 2016; Muehlohoff *et al*., 2013).

Over 80% of the milk consumed in developing countries is informally marketed as loose, raw milk (Kilango *et al*., 2012). This informal trade has persisted due to the various social and

economic benefits that it allows for the various participants (Kilango *et al.*, 2012). There is also little or no quality control for milk produced and handled in the informal market channels, hence the risk of contamination by zoonotic pathogens, adulterants, antimicrobial drug residues and others, which constitute a high public health risk to consumers (Kurwijila *et al*., 2006).

In Zambia, it is estimated that 300,000 traditional cattle-owning households produce milk at small scale (Mumba and Pandey, 2013). The majority of these are in the Southern and Western parts of Zambia. A large percentage of the milk produced by these household does not enter the formal markets. The milk is either consumed or sold locally without being processed (Mumba and Pandey, 2013). A study that was conducted in 15 districts of Zambia (including some districts in Western province), estimated that 38 percent of the milk produced by the small-scale farmers is sold informally, 28 percent is consumed domestically and 28 percent is wasted. This is because milk marketing is difficult in some areas. In Mongu for example, about 3,000,000 liters of milk are produced annually but only a small percentage of this milk reaches the co-operatives (Mumba and Pandey, 2013).

### 2.2. Pathogens Associated with Raw Milk

Milk and dairy products are a habitat for many microorganisms. These microorganisms include many zoonotic bacteria and some viruses such as retroviruses and cytomegalovirus (Losnedahl *et al.,* 1998). In healthy animals, milk may contain very few microorganisms (Rawat, 2015). The natural inhibitory systems in milk hinder microbial growth for the first three or four hours even if the milk is kept at ambient temperatures (Losnedahl *et al.,* 1998). Apart from the Lactic Acid Bacteria (LAB) which are part of the normal flora of milk and produce bacteriocins and other substances that inhibit the growth of other microorganisms, milk also contains lactoferrin, lactoperoxidase, lysozyme, and N-acetyl-ß-D-glucosaminidase. These proteins have antimicrobial activities and may prolong the shelf-life of milk (Losnedahl *et al.,* 1998). After secretion, external sources of pathogens can result in contamination of milk. Dairy cattle are known to harbour populations of *Salmonella* spp, *Campylobacter* spp, *Clostridium* spp, Shiga Toxin-producing *Escherichia coli* (*STEC), Cryptosporidium parvum, Brucella* spp. and *Mycobacterium bovis*. Other pathogens in milk may originate from external sources (Gwida and EL-Gohary, 2013). *Listeria* species are naturally common in the environment. *Listeria monocytogenes* has been isolated in raw milk and soft cheeses and can cause infection in pregnant women resulting in spontaneous abortions or stillbirth (Claey *et al.,* 2013). Other

pathogens that may colonise raw milk include, *Staphylococcus aureus*, pathogenic *E. coli* and *Yersinia enterocolitica* (Gwida and EL-Gohary., 2013)

Milk-borne infections may cause diarrhea, vomiting, nausea, fever and abdominal cramps. In some individuals severe clinical symptoms such as Guillain-Barré syndrome (*Campylobacter spp*.), hemolytic uremic syndrome (HUS) (*E. coli* O157:H7), reactive arthritis, or even death have been recorded (Claey *et al.,* 2013).

The presence of commensal lactic acid bacteria in raw milk inhibits the multiplication of bacteria, including those with pathogenic potential. The Lactic Acid Bacteria (LAB) produces bacteriocins. Bacteriocins have antimicrobial activities against bacteria that are related to the LAB (Zacharof *et al*., 2012). Apart from bacteriocins, the LAB also produces lactic acid, hydrogen peroxide and diacyls. All these hinder the growth of microorganisms (Mokoena, 2017). *Lactobacillus lactis* has shown anti-microbial activity against *Staphylococcus aureus* and *E.coli* (Nakhdjavani *et al*., 1996). The LAB also exhibit antimicrobial activity against *Clostridium, Staphylococcus aureus* and *Listeria monocytogenes* (Zacharof *et al*., 2012). Pathogens that have been involved in foodborne outbreaks associated with the consumption of milk include *Listeria monocytogenes*, *Salmonella*, *Campylobacter, Staphylococcus aureus, Bacillus cereus* and *Clostridium botulinum* (Claeys *et al*., 2013).

Other bacterial organisms such as *Staphylococcus*, *Streptococcus, E. coli* and *Salmonella* are also among the biological milk adulterants (Hill *et al*., 2012). Mycobacteria are non-spore forming acid-fast bacilli which exhibit some resistance to environmental influences due to their lipid-rich cell walls (O'Mahony *et al.*, 2009). Bovine tuberculosis, caused by *Mycobacterium bovis,* is predominantly a disease of cattle even though all warm blooded animals, including humans, are susceptible to infection to varying degrees (UK Zoonoses report, 2010). *M. bovis* is closely related to *Mycobacterium tuberculosis* as they both belong to the *Mycobacterium Tuberculosis Complex*, along with *M. africanum* (Mostowy *et al.,* 2005).

In developed countries, Bovine tuberculosis was a serious zoonosis. Bovine brucellosis and bovine Tuberculosis are both classified as neglected zoonoses of interest by the World Health Organisation with potential to affect most notably, public health and secondly, people's livelihoods, as they perpetuate poverty (WHO, 2006). Although these diseases are classified as neglected zoonosis, they have a strong association with poverty and still remain endemic in

most developing countries (FAO, 2009). Bovine Tuberculosis represents a huge public health problem mainly through consumption of contaminated unpasteurized milk. This disease was controlled in cattle through designed test and slaughter schemes. Milk pasteurization and increased public awareness level and education have resulted in reduced incidences. In rural areas of Africa, consumption of unpasteurized milk is a common practice (Pandey *et al*., 2013). Some studies have confirmed the presence of zoonotic *M. bovis* in the milk (Pandey *et al*., 2013). The population consuming raw milk is therefore at risk of contracting diseases. Young children, babies and HIV/AIDs affected individuals are at higher risk of contracting the disease. (Pandey *et al.,* 2013).

A study by Danbirni *et al*., (2010) also found that apparently healthy lactating cows can also shed viable *M. bovis* in milk thereby posing a serious public health problem in areas where unpasteurised milk is consumed and also where improper milk handling and processing is done. In developing countries, both these practices are still common (Danbrirni *et al*., 2010). Fortunately, human to human transmission of *M. bovis* infection is rare in absence of immunosuppression (Grange, 1995). *Brucella* spp, the genus to which *B. abortus* belongs, are small, non-motile aerobic gram-negative bacteria responsible for causing brucellosis. Bovine brucellosis is an infectious and contagious disease of cattle commonly caused by *Brucella abortus*, which induces abortions, and causes still births and weak calves to be born among cattle (Ibrahim *et al.,* 2012).

Another bacterial pathogen commonly found in milk is *Staphylococcus* spp. The vegetative cells of *Staphylococcus aureus* for example do not survive pasteurisation, this bacterium produces a heat-stable enterotoxin which survives pasteurisation (De Oliveira *et al.*, 2011). *Staphylococcus aureus* is one of the causative agents of mastitis in dairy herds. Mastitis involves inflammation of the mammary glands and this can result in the shedding of this bacteria and their toxins in milk (Abebe *et al*., 2016). The presence of large concentrations of *Staphylococcus aureus* may indicate mastitis in a dairy herd. *S. aureus* is an enterotoxin-producing pathogen but its concentration needs to exceed $10^5$ cfu/ml for sufficient toxin to be produced to cause human illness (Hill *et al*., 2012).

*Staphylococcus aureus* in raw milk can also come from poor personal hygiene practices such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking (Tegegne and Shimels., 2017; De Oliveiria *et al*., 2011). Presence of this

pathogen in foods such as milk can cause public health problems. This bacteria produces enterotoxin and is capable of coagulating plasma (De Oliveiria *et al*., 2011). *Staphylococcus aureus* can produce a heat stable enterotoxin that causes food poisoning and was linked to a large-scale outbreak that occurred in June 2000 in Japan. This outbreak was caused by consumption of low-fat milk produced from skimmed-milk powder contaminated with *S. aureus* enterotoxin A (Asao *et al*., 2003).

*Salmonella* is a significant pathogen found in milk as a result of endogenous and exogenous contamination. The presence and the population of *Salmonella* in bulk milk tanks on farms depend on many factors. These factors include geographical location, herd size and subclinical shedding, farm management practices and its presence in the environment (Hill *et al.*, 2012). The geographical location affects the survival of *Salmonella* spp because geographical areas with high temperatures and favourable humidity promote the growth of this microorganism (Akil *et al.*, 2014). *Salmonella* spp in milk is usually as a result of faecal contamination or from animals suffering from asymptomatic mastitis. Although salmonellosis is still a neglected zoonosis in developing countries, it is the most common food-borne bacterial disease worldwide. There has been an increase in the outbreaks of human salmonellosis in most parts of the world and has been isolated from raw milk and milk products (Karshima *et al*., 2013).

*Streptococcus* spp is also another common pathogen in milk. *Streptococcus agalactiae* is a common cause of non-clinical mastitis infections in dairy cows. Lyhs *et al*., (2012) isolated *Streptococcus agalactiae* from milk samples. *Streptococcus* is a microorganism which is commonly found in organic matter. Studies have shown that *Streptococci* was isolated from cattle manure (Weaver *et al*., 2005). Due to the presence of *Streptococci* in cattle manure, this microbe is frequently isolated from bedding. The risk of transmitting it to uninfected cows can be increased by poor udder cleanliness, inadequate stall management, and damaged teat ends (Gorgy *et al*., 2016).

*Streptococcus* species that are known to cause mastitis include *Streptococcus agalactiae, S. dysgalatiae, S. uberis, S. dysgalatiae, S. epidemicus, S. bovis* and *S. equinus*. *Streptococcus uberis* is associated with many cases of clinical and subclinical mastitis in lactating cows. It is also the predominant organism isolated from mammary gland during the non-lactating period (Gorgy *et al*., 2016).

*Escherichia coli* is a normal inhabitant of the intestines of warm blooded animal and is excreted together with faecal matter. It's presence in food or water indicates faecal contamination. Although most strains of *E. coli* are non-pathogenic, it's isolation from food or water may be of public health concern due to the possible presence of enteropathogenic and/or toxigenic strains which lead to severe gastrointestinal disturbance (Zeinham and Abdel-latef, 2014; Hernandes *et al*., 2009). Because this microorganism does not survive pasteurisation, its presence in processed foods is usually as a result of recontamination (Baranceli *et al*, 2014).

Enterohemorrhagic *E. coli* strains such as serotype O157:H7 are pathogenic. *E. coli* O157:H7 causes haemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Mayer, 2012). Outbreaks linked to enterohemorrhagic *E. coli* strains have been as a result of consuming meat products such as underdone steaks and hamburgers, unpasteurized milk and dairy products manufactured from raw milk (Zeinham and Abdel-latef, 2014). *Escherichia coli* is associated with raw milk which has been contaminated by faecal matter. Enteropathogenic *E. coli* can also be isolated from the milk of animals with mastitis (Holsinger *et al.,* 1997). The detection of indicator organisms such as *E. coli* and coliforms implies a risk that other enteric pathogens may be present in the sample. Despite significant reduction in the global importance of *E. coli* as a causative agent for diarrheal illness over the past 50 years, *E. coli* still remains a major cause of illness in under-developed nations. This is mainly due to poor sanitation (Chye *et al*., 2004).

The presence of pathogenic *E. coli*, including STEC and enteroggregative *E. coli* (EAEC) in raw milk, inadequately pasteurised milk and raw milk products has resulted in outbreaks. STEC have been detected in raw milk from healthy animals. Enterohaemorrhagic *E. coli* strains of STEC such as serotype 0157 have a much lower occurrence (Muehlohoff *et al*., 2013).

*Bacillus cereus* is a major spoilage organism of pasteurised milk and milk products stored at refrigeration temperature and it is also a concern for food safety as it can produce different types of toxins and is a potential food-poisoning agent (Quigley *et al.,* 2013). *Bacillus licheniformis* and *Bacillus cereus* are the most commonly isolated species of *Bacilli* present in milk at various processing stages. *B. cereus* is a ubiquitous microorganism that has been isolated from soil, air, dust and water (Bottone, 2010; Tabit, 2010). Processed food products such as rice, dairy products, meat, spices and egg are other sources of this microbe. Even with very low doses, *B. cereus* can cause food spoilage and food poisoning. *B. cereus* poisoning is

by an emetic toxin that causes vomiting, while that which is caused by enterotoxins causes diarrhoea. *B. licheniformis* is associated with septicemia, peritonitis, ophthalmitis and food poisoning in humans. It is a common contaminant of dairy products, cooked meats and vegetables. In animals suffering from mastitis, heat-stable toxin-producing *B. licheniformis* and *B. pumilus* have been isolated from milk. *B. stearothermophilus* has been noted to cause contamination of dairy products, especially milk powder. Some *Bacilli* species (e.g. *B. stearothermophilus*) produce thermophilic spores that can withstand pasteurisation at 73 °C for 15s and can grow at 65 °C. *Bacillus sporothermodurans* and strains of *Paenibacillu*s produce endospores that can withstand industrial sterilisation and Ultra High Treatment (UHT) of milk. (Tabit, 2010).

The microorganism *Cronobacter* spp. have been associated with outbreaks of meningitis and enteritis in infants after the consumption of powdered infant formula (Beuchat *et al*., 2013). *Mycobacterium avium paratuberculosis* (MAP) has also been isolated in milk and is suspected to be possibly linked with Crohn's disease, a chronic inflammatory bowel disease that affects the lining of the digestive tract (McNees *et al*., 2015). It is not inactivated by pasteurisation and viable MAP has been isolated in bovine milk (Muehlohoff *et al*., 2013).

The spread of zoonotic diseases among animals and from animals to milk can be prevented if some measures are put in place. These include controlling infection from feed and fodder, improving shelter and hygiene of animals and the environment, safe waste management and good management of veterinary drug application (Muehlohoff *et al*., 2013).

### 2.3. Raw Milk Consumption and Consequences

Although there is evidence that milk can contain pathogens and chemical substances that may cause illness, consumption of raw milk is still high in developing countries (Popović-Vranjes *et al*., 2015; Muehlohoff *et al*., 2013). Outbreaks implicating milk and dairy products have been documented but information on the health and economic burden attributable to unsafe milk consumption is limited. This lack of information is more pronounced in developing countries and limits the ability to identify which food pathogens or chemical substances present the greatest risk to consumer health (Muehlohoff *et al*., 2013).

Consumption of raw milk was linked to the spread of disease in the early 1800's. It was associated with human diseases such as diphtheria, typhoid, tuberculosis, and brucellosis

(CDC, 2014). In the US, numbers of foodborne illnesses associated with milk consumption have reduced over the years as a result of on-farm programs to control animal diseases such as brucellosis, tuberculosis and mastitis. Enhanced farm sanitation practices, temperature control of milk products from the farm to the consumer and pasteurization of milk have also contributed to this reduction (CDC, 2014). The nature of dairy-borne human illnesses in the US has also changed over the past 20 years (CDC, 2014). Illnesses from dairy product consumption are now usually associated with *Salmonella enterica*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Escherichia coli* O157:H7. These microorganisms are introduced in milk as a result of contamination from external sources. In December 2005, 18 people fell ill and were hospitalised after consuming milk contaminated with *E. coli* O157:H7 at a farm in USA. The five people that were hospitalised were aged 1-13 years old and four of these patients developed Hemolytic Uremic Syndrome (Dhanashekar *et al*., 2012; CDC, 2014). *E. coli* O157:H7 also caused illness in six children from California and two children in Washington in the year 2006 (CDC, 2014). In 2006, *Campylobacter jejuni* infections were diagnosed in two people in Ohio and five people in Colorado. These people consumed milk products made from raw milk. Over 50 people were also diagnosed with *Campylobacter jejuni* after consuming cheese made from raw milk in Wisconsin (CDC, 2002). In 2007, other illnesses linked to consumption of raw dairy products were recorded and these included a *Salmonella* outbreak that affected 29 in Pennsylvania, a *Campylobacter* outbreak in the state of Kansas that affected 67 people, and listeriosis infections among four pregnant women in North Carolina. This listeriosis resulted in three miscarriages and a premature delivery (CDC, 2014).

Many studies have clearly demonstrated that milk can be a major source of foodborne pathogens (Zastempowska *et al*., 2016). Consumption of raw milk contaminated with *Salmonella* has resulted in outbreaks of salmonellosis. For example, in 2002–2003 outbreaks of *Salmonella* in Illinois, Indiana, Ohio, and Tennessee states in the US occurred. Infections were linked to an Ohio dairy farm that legally sold raw milk and milk products (Zastempowska *et al*., 2016; LeJeune *et al*., 2009). Although consumption of raw milk has been recognized as a major cause of foodborne diseases, the true incidence of milkborne disease in the United States is unknown. There is clear scientific evidence which demonstrates that consumption of raw milk is a risk factor of human foodborne disease (Zastempowska *et al*., 2016).

Consumption of raw milk is believed to be low in Australia because raw milk consumption is not permitted and access is very limited. Despite the limited availability, consumers of raw cow

milk purchase milk in the form of "pet milk" or via "cow share" programs (Juffs and Deeth, 2007). Dairy producers who have ready access to raw milk also consume raw milk. A study in the US found 42% of farmers in Pennsylvania consumed raw milk citing taste and convenience the reason why they prefer raw milk. Similar studies revealed that 34.9% of dairy producers consumed raw bulk milk (Juffs and Deeth, 2007).

### 2.4. Antibiotic Resistance

During the animal production, chemicals are used and these can leave residues which may find themselves in milk. Examples of such residues include veterinary drugs and pesticides. Good agricultural and veterinary practices at farm level are very important to assure food safety. Farmers must ensure the safety of milk and animal products as they use veterinary drugs. Antimicrobial drugs are the most important for milk. This is because of the high degree of local treatment, i.e. intermammary infusions (Muehlohoff *et al*., 2013). To safeguard public health, antibiotic residues in milk must not exceed the Maximum Residual Limits (MRL). The MRL for antibiotic residues in milk is the maximum concentration of an antibiotic residue, resulting from the registered use of a legally permitted antibiotic. The MRL is measured in mg/kg, mg/l, parts per million (ppm) or parts per billion (ppb). The MRL differs from antibiotic to another (Hassan and Arooba, 2017). Countries such as the USA and the United Kingdom have their own MRL. Zambia is yet to develop its own MRL but the Food and Drugs Act of 2001 specifies that milk sold for manufacture into dairy products must be free from antibiotics and other antimicrobial substances (Food and Drug Act, 2001). Through good veterinary and husbandry practices, antibiotic residues in milk can be reduced. Farmers must adhere to the recommended withdrawal period before milking animals previously on antimicrobial treatment. Failure to do so is one of the reasons for antimicrobial residues in milk (Muehlohoff *et al*., 2013). Incorrect route of administration and dosage, use of antimicrobials not registered for dairy cows and incorrect use without taking into consideration lactation status have also been cited as the cause of high levels of antimicrobial residues in milk. Misuse or inappropriate use of antimicrobials in treatment and prevention of diseases in food animals may lead to the emergence and spread of micro-organisms resistant to antimicrobials (Muehlohoff *et al*., 2013).

The greatest threat to the use of antibiotics is antibiotic resistance. This resistance can be intrinsic or acquired. Intrinsic resistance is a natural characteristic of a microorganism that allows it to grow in the presence of the corresponding antibiotic, while acquired resistance results either from spontaneous mutation in the bacterial genome or from the acquisition of

genes encoding resistance through transduction, conjugation or transformation (Fair and Tor., 2014; Quigley *et al*., 2013). *Lactococci*, *enterococci* and *lactobacilli* are intrinsically resistant to some antibiotics, the strains of these that are found in foods are typically quite sensitive to clinically important antibiotics such as ampicillin, penicillin, gentamicin and vancomycin. *Leuconostoc* strains are generally sensitive to antibiotics (Quigley *et al.,* 2013).

Resistant microbes from animals can be pathogenic to humans or they may transfer antimicrobial resistant genes to pathogens of public health importance. Use of antibiotics in public health and animal husbandry has increased and so has antibiotic resistance in pathogens (Economou and Gousia, 2015). Apart from spreading resistant zoonotic foodborne pathogens, food-related commensal bacteria or opportunistic pathogens can become potential carriers of resistance genes, and pose health risks to consumers. In milk and milk products resistant mastitis-causing bacteria, such as *Staphylococci* and *Enterococci* are very relevant and their presence in milk or dairy products could be of public-health relevance to consumers (Kumar and Prasad, 2010). Coagulase-negative S*taphylococci* (CoNS) an important microorganism involved in subclinical bovine mastitis and *Staphylococcus aureus* have become resistance to antimicrobial agents (Pyörälä and Taponen, 2009). *Enterococci* species are frequent carriers of resistance genes. Many studies have demonstrated the potential risk of acquired antimicrobial resistance in enterococci, and transfer of mobile genetic material to other bacteria (Zdolec *et al*., 2016; Van Hoek *et al*., 2011). Apart from studying antimicrobial resistance on udder pathogens and milk samples from drug-treated animals, the presence of resistant bacteria in regularly collected raw milk samples from clinically healthy animals should also be conducted. This will help in assessing the potential spread of resistant strains from raw material to dairy products (Zdolec *et al.,* 2016).

Studies demonstrated that *P. fluorescens, P. aeruginosa, Stenotrophomonas maltophilia* and *Burkholderia* species isolated from milk were resistant to several b-lactam and non-beta lactam antibiotics. This trait appears to increase in occurrence through the cold chain transportation of raw milk. Some *Acinetobacter* species isolated from raw milk have also exhibited antibiotic resistance. *Lactococcus lactis* isolated from milk displayed resistance to tetracycline, clindamycin and erythromycin while *L. garvieae* exhibited resistance to tetracycline, streptomycin and quinupristin– dalfopristin (Quigley *et al.,* 2013). Some strains of *S. aureus* have shown resistance to antibiotics such as penicillins, oxytetracycline, streptomycin and/or gentamicin. Consumers of milk and dairy products are therefore exposed to antibiotic residues.

Animals suffering from mastitis should not be milked and a withdrawal period must be observed after antibiotic treatment (Quigley *et al.*, 2013).

Antibiotic resistance has led to reduced effectiveness of antibiotics in treating diseases in humans and animals. Countries that are at greater risk are those with weak, inadequate or non-existent national policies, regulatory, surveillance and monitoring systems for antimicrobial resistance and antimicrobial drug usage. Guidelines for risk analysis of foodborne antimicrobial resistance and a code of practice to minimize and contain antimicrobial resistance have been adopted by the Codex Alimentarius Commission (Bruno and Mackay, 2012).

**2.5. Pasteurisation of Milk**

Historical data has clearly demonstrated that milk pasteurization has led to the reduction of foodborne diseases associated with milk. In the United States of America, it is estimated 25% of all foodborne and waterborne disease outbreaks in the US were associated with milk before 1938 (Claey *et al.*, 2013). Currently the percentage of such outbreaks associated with milk has reduced and is estimated to be below 1% (Claey *et al.*, 2013). The average annual milk-borne outbreaks have reduced from 29 (before pasteurisation was adopted) to 2.4 after pasteurisation was adopted. Recent studies have reported that the majority of milk-borne outbreaks in the US occur in states that permit the sale of raw milk (Claey *et al.*, 2013).

Pasteurisation is a function of time and temperature. Milk is commonly pasteurised by holding at 71.7°C for 15 seconds, or 62.7°C for 30 minutes. Although this process is named after Loius Pasteur the French scientist, pasteurisation was practised even before Louis Pasteur. Bottled milk fed to infants was heated to temperatures above 65°C for 1 hour. The first commercial pasteuriser was made in Germany in 1882. Currently, milk and other foods are pasteurised mainly by commercial pasteurisers. These pasteurisers are electrically powered and expensive to purchase and maintain (Juffs and Deeth, 2007).

In the industrialised dairy sector, pasteurisation technologies are routinely applied and regulated. On the other hand many small-scale dairy farmers in developing countries sell their milk through the informal markets (Kilango *et al*., 2012). Most of the milk in this informal market is unpasteurised, lacks a cold chain and may have little or no regulatory control. In some cultures people prefer consumption of raw milk. However, despite the differences, for both the formal and informal sector, countries should apply relevant food safety and animal

health programmes, regulatory controls and monitoring and compliance systems to protect the health of their citizens. The challenge to all food-safety policy-makers is to ensure that appropriate action are taken to prevent food-borne illnesses associated with milk. These may include implementation and support of hygiene practices and education for dairy farmers, suppliers and consumers (Kilango *et al*., 2012). In addition to this use of cheaper energy sources can be promoted in developing countries. With so much emphasis on use of cleaner technologies, solar energy can be used to heat milk to pasteurisation temperatures in parts of the world with plenty of sunshine. In some regions of the world such as in the tropics sunlight is plenty and this sunlight has been converted to solar energy and has been used to pasteurise food and water (Desail *et al*., 2013).

Although pasteurisation is well known to be very effective at removing pathogens in milk, some schools of thought suggest that raw milk is better than pasteurised milk. Some sectors of society actually encourage consumption of raw milk as opposed to pasteurised milk. There are claims that pasteurisation of milk negatively affects the quality of milk and that raw milk is more nutritious than pasteurised milk (Melini *et al*., 2017; Lucey, 2015). Pasteurisation of milk is believed to destroy beneficial components of milk. These components include proteins, vitamins, minerals and beneficial microbes (Lucey, 2015).

Heating can cause degradation of vitamins, denaturation of whey proteins, maillard reactions between reducing sugars and the epsilon amino groups of lysine residues in proteins and reactions of lactose (Muehlohoff *et al*., 2013). Vitamin C is easily oxidised in the presence of oxygen and cations and it is also easily degraded during heating. Studies show that losses in vitamin C, folate and vitamin B12 increase with severity of heat treatment (Yeh *et al*., 2017). Sterilization caused significant losses of all vitamins shown above except riboflavin. Several studies have looked at the effect of packaging materials and storage conditions on vitamin stability (Manharbhai, 2014). Vitamin C content of raw and heat treated milks decreased significantly even during storage for two weeks in a freezer (Muehlohoff *et al*., 2013). It was also reported that niacin, biotin and pantothenic acid were relatively stable during Ultra High Temperature (UHT) treatment. Most fat soluble vitamins i.e. Vitamins A, D and E are heat stable after pasteurization (72 ℃, 15 seconds) or UHT treatment while protein denaturation in pasteurized milk is 0.4% and 56% denaturation in UHT milk (Muehlohoff *et al*., 2013). The solubility of milk is reduced during heat treatment because β-lactoglobulin and κ-casein aggregate. The antioxidant stability of milk is changed and the milk proteins are modified by

the Maillard reaction. The maillard reaction mainly involves lysine residues in casein reacting with lactose and other sugars leading to browning of milk, as a result of formation of brown melanoidins. Maillard reactions do not occur during pasteurization and UHT treatment. Heat treatment of milk has been reported to cause isomerization of lactose to lactulose (Parekh *et al.,* 2016). The sugar lactulose stimulates the growth of *bifidobacteria (*Ishibashi *et al.*, 1997*).* The more intense the heating, the higher the amount of lactulose formed. Heat treatment also causes isomerization of some fatty acids with pasteurization showing increased trans-isomer content of milk while UHT treatment did not result in significant increases in trans-isomer content. High temperatures and storage temperatures had no effect on the ash content of milk. Heat treatment is essential to ensure total microbiological safety, but it can also reduce various nutrient contents (Muehlohoff *et al*., 2013).

Lucey, (2015) reported that heat is able to modify the protein structure. Although this modification affects the solubility and emulsification properties, it does not affect the digestibility and nutritional properties. A study by Macdonald *et al*., (2011) demonstrated that pasteurisation has no effect on the concentration of Vitamin B6. Lucey, (2015) also reported that pasteurisation has no effect on the concentration of B1 and Vitamin B6 in milk but it significantly reduces the concentrations of folate and Vitamin C. The reduction of the concentrations of these two vitamins has little effect on the contribution to the Recommended Daily Intake (RDI). Pasteurisation has no impact on the mineral and fat content of milk. There is also a claim that pasteurisation destroys probiotics. Probiotics are commonly known as the "good" bacteria as they are associated with a number of health benefits. These effects of probiotics are only manifested when probiotics are present in high concentrations. These high concentrations are achieved in dairy products such as yoghurt but not in milk. Milk does not contain high levels of probiotics because probiotics do not compete well with most types of lactic acid bacteria. Since pathogens can be easily isolated from raw milk, the International Dairy Federation strongly recommends consumption of pasteurised milk and discourages consumption of unpasteurised milk (Lucey, 2015).

### 2.5.1 Methods of Heat Inactivation of Milk Pathogens

#### a. Boiling

Since time in memorial, food preservation by exposure to high temperatures has been practised. Exposure to high temperatures not only helps in preserving food but also kills pathogenic

organisms (Zahira *et al.,* 2009). At household level, milk can be boiled using charcoal, firewood or electricity. WHO has recommended boiling of milk as a way of reducing food spoilage organisms and rendering milk safe (Metwally *et al*., 2011).

**b. Commercial Systems**

At industrial level, there are several methods of inactivating microorganisms in milk. FAO has defined a number of methods of heat treatment of milk. The heat treatment methods are as follows;

1. Thermization: "The application to milk of a heat treatment of a lower intensity than pasteurization that aims at reducing the number of micro-organisms. A general reduction of log 3–4 can be expected. Micro-organisms surviving this process will be heat-stressed and become more vulnerable to subsequent microbiological control measures".

2. Pasteurization: "Pasteurization is a microbiocidal heat treatment aimed at reducing the number of any pathogenic micro-organisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard".

3. Ultra-High Temperature (UHT) treatment: UHT treatment of milk and liquid milk products "is the application of heat to a continuously flowing product using such high temperatures for such time that renders the product commercially sterile at the time of processing. When the UHT treatment is combined with aseptic packaging, it results in a commercially sterile product".

4. Commercial sterilization: "The application of heat at high temperatures for a time sufficient to render milk or milk products commercially sterile, thus resulting in products that are safe and microbiological stable at room temperature" (Metwally *et al*., 2011) .

**2.5. 2. Significance of solar pasteurisation**

Solar energy is renewable energy. Renewable energy is energy which comes from the natural energy flows on earth. This form of energy cannot be exhausted unlike other forms of energy. Because this source of energy is free from pollution it is referred to as "green energy", "clean energy", "sustainable energy" or "alternative energy". It is believed that reflective substances were used to concentrate the sun's rays to light fires as early as the 7th Century B.C. In the 21st

century, solar technology ranges from simple solar cells to solar-powered buildings and even solar powered vehicles (Desail *et al*., 2013).

Studies have demonstrated that exposing water in clear plastic or glass jars to sunshine inactivates bacteria. This effect of sunshine varies from no inactivation to an approximately 3-log decrease in 1.5hrs. The variability is as a result of transparency of the container, water turbidity, water temperature reached, altitude, aerobic or anaerobic conditions, and the amount of solar radiation received. This procedure is limited to clear liquids because direct exposure to sunshine cannot pasteurise raw milk. Solar cooker International (SCI) developed a simple reflective solar cooker in 1995. This solar cooker was introduced as a cooking device to three refugee camps in Kenya and Ethiopia (Safapour, 1999).

The cooker (Figure 2.1) is designed in such a way that the foiled reflective panels of the Cookit direct sunshine onto a dark pot which is enclosed in a clear polypropylene bag. This dark pot converts sunshine into heat, which is trapped within the bag and cooks the food (Safapour, 1999).

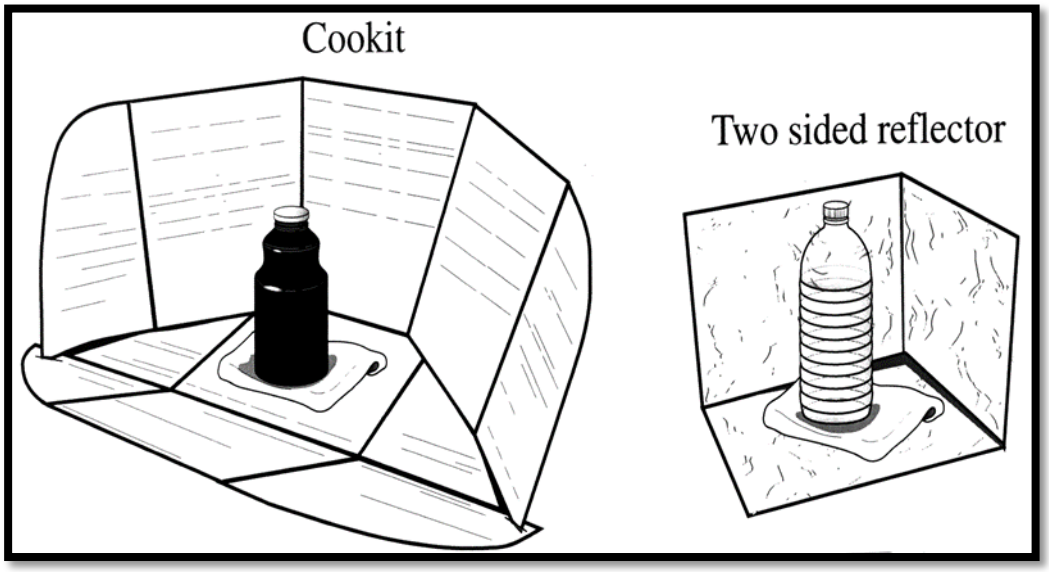


Figure 2.1: Reflective Solar Cooker

Data from experiments conducted on a cloudless day in which Enterobacteria phage T2 and *E.coli* were exposed to solar heating showed that Enterobacteria phage T2 was much more resistant to solar inactivation than *E. coli*. Both *E. coli* and Enterobacteria phage T2 were completely inactivated at 60°C in 1 h and 70°C in 1.5 h respectively (Safapour, 1999). Separate studies demonstrated that 3.7 litres of water was heated to 70°C in 3-4 hours on sunny days

(Safapour, 1999). Many people in developing countries experience fuel wood shortages and expensive modern fuels. Fortunately most of these developing countries receive plenty of sunshine and can use solar energy for inactivating bacteria. The solar cooker does not work on cloudy days but this technology can still be effective on 200 to 300 sunny days/year in many developing countries such as Zambia, Kenya and Ethiopia (Safapour, 1999).

Metcalf and Safapour (1999) conducted experiments and concluded that the temperatures in water which could kill at least 90% of microbes within one minute were as follows; 55°C for worms, and cysts of the protozoa *Giardia, Cryptosporidium*, and *Entamoeb*a; 60°C for the bacteria *Vibrio cholerae*, *Samonella typhi, Shigella spp*, and Enterotoxigenic *Escherichia coli*, and 65°C for *Hepatitis A* and for rotavirus virus. Inactivation time decreases as the temperature increases. This study and other similar studies demonstrated that heating contaminated water to 65°C could pasteurize the water and make it safe to drink. A Cookit solar cooker made from cardboard and aluminium foil and developed by Solar Cookers International was used in these experiments. To pasteurize water a dark covered metal or glass container containing water was placed in the Cookit facing towards the sun. In full sunshine, it took about two (2) hours to pasteurize two (2) litres of water and about three (3) hours to pasteurize four (4) litres (Safapour and Metcalf, 1999).

Solar cookers are relatively cheap and low-tech devices. They do not require fuel and operation costs and this makes them suitable for use by people in rural areas. Use of solar cookers reduces air pollution and slows deforestation and desertification, caused by use of firewood for cooking. Investment in solar energy technology should be encouraged and government should invest in this technology. It is pollution free, renewable, reliable and has low maintenance costs (Desail *et al*., 2013).

Safapour and Metcalf (1999) developed a simple and reliable method for pasteurising water and milk. Solar cookers were used to cook food and pasteurise contaminated water in Kenya. A water pasteurisation indicator (WAPI) was used to indicate if pasteurisation occurred (Safapour and Metcalf, 1999). A low cost solar milk pasteuriser was used for water and milk pasteurisation in Pakistan. The solar milk pasteuriser attained pasteurisation temperature in 1 to 1.5 hours so it can be commercially used (Zahira *et al.,* 2009). Many other scientists have utilised solar energy to destroy bacteria in water. To confirm if pasteurisation has taken place, the alkaline phosphate test is used. Alkaline Phosphatase (ALP) is an enzyme which is naturally

present in all raw milks. Alkaline Phosphatase is widely used as an indicator of proper milk pasteurization. Complete pasteurization inactivates the enzyme to levels which are not detectable by conventional methods. The heat stability of ALP is greater than that of pathogens which may be present in milk; hence the enzyme is used as an indicator of proper pasteurisation (Zahira *et al.,* 2009). The WAPI indicator used (Safapour and Metcalf, 1999) to verify that water temperatures reach 65°C during pasteurisation of water can also be used. The WAPI is a clear polycarbonate tube, partially filled with a wax, and sealed on both ends. This WAPI wax melts at 65°C and is placed at the bottom of the container during solar pasteurisation. The WAPI wax melts and falls to the bottom of the tube, when pasteurization conditions have been achieved. This indicator can also be used for milk pasteurisation (Safapour and Metcalf, 1999).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1. Study Location

The study was conducted in Western province, Zambia. Western province is located between latitude 15° South and lines of longitude 24° East. This province covers an area of 126,386 square kilometres and its human population in 2010 was 902,974 (CSO, 2010). Cattle rearing is a major agricultural activity and it supports about 80% of the population. Cattle is not only a source of income but it is a source of draught power and manure for crop production. The cattle population in Western Province in the year 2000 was 604,000 (CSO, 2010) which was second to Southern province. The cattle are kept in the plains of the Zambezi River, hence grazing taking place in the Zambezi plains. The animals are left to graze freely and are only brought back in the evening enclosures at the homesteads. The people milk their animals on a subsistence level, despite efforts by government for them to commercialise the operations. This study was ethically cleared and the clearance number is L.R.B No 00005948.

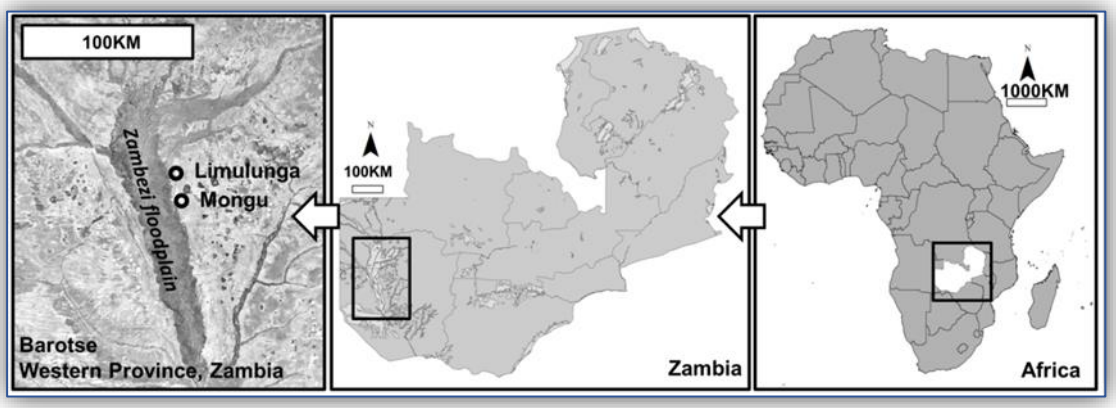


Figure 3.1: Western Province, Zambia. (Source: Knight-Jones et al, 2016)

## 3.2 Study Design

The study was descriptive cross sectional in nature.

### 3.3 Sampling frame

Selecting the correct sample size from a larger population is imperative in order to avoid research and/or inference mistakes/bias (Shajahan, 2009). Shajahan (2009) further stated that a sample is a group selected from the complete population to make the task of surveying less costly and more manageable and that the selected sample should be representative and have the same characteristics and attributes as the overall population. The following is the calculation of the sample size using the formula from Krejcie and Morgan (1970).

$$S = \frac{x^2 NP(1-P)}{d^2(N-1) + x^2 P(1-P)}$$

Where

S is the Required Sample Size

N is the given population

P is the Prevalence

$d^2$ is the degree of accuracy

$X^2$ is 1.96 Confidence level

The number of milk collection centre was too low and hence this formula could not be applied. Milk samples were collected from all the milk collection centres. There were three established milk collection centres namely Mongu, Limulunga and Nalolo. There were also three other centres which Zammilk, a subsidiary of Zambeef PLC, collected milk from and processed it. These three centres are Lealui, Sefula and Nabubela. At these centres, farmers had formed cooperatives and each farmer delivered milk and the milk was pooled and the collection centres proceed to take the milk for processing. A total of 63 farmers who sold their milk to milk collection centres were purposefully identified and interviewed according to the questionnaire indicated in Appendix 1.

### 3.4 Sample Collection

Milk samples were collected at Milk Collection Centres (MCC) in 50ml sterile falcon tubes. The milk samples were collected in triplicate for each sample. The number of milk samples collected depended on the availability of milk which was brought to the Milk Collection Centre. The milk samples were collected between April and September 2015. The tubes were labeled using a permanent marker. Aseptic techniques were observed during the sample collection. The

milk was poured into the falcon tubes and tightly closed. A total of 52 milk samples were collected for analysis as indicated in Table 3.1.

Table 3.1: Total number of samples collected at each Milk Collection Centre

| **Sampling Area** | **Pooled Samples Collected** |
|---|---|
| Limulunga 1 | 6 |
| Limulunga 2 | 3 |
| Limulunga 3 | 2 |
| Mongu Farm 1 | 2 |
| Mongu Cooperative | 8 |
| Mongu Harbour | 3 |
| Nalolo | 3 |
| Lealui 1 | 2 |
| Lealui 2 | 5 |
| Lealui 3 | 6 |
| Lealui 4 | 4 |
| Lealui 5 | 3 |
| Lealui 7 | 5 |
| **Total samples collected** | **52** |

The samples were kept in a cooler box which contained ice and transported to the University of Zambia laboratory in a car refrigerator. The samples were then frozen at -20℃ until microbiological analysis was conducted.

## 3.5 Sample analysis

### 3.5.1 Bacteria contamination determination

Milk was taken from the freezer and brought to room temperature. A milliliter of raw milk was added to 9 ml sterile normal saline. After mixing, 1ml was pipetted and added to 9 ml of sterile normal saline. The mixture was further diluted by pipetting 1 ml of the mixture and adding it to 9ml normal saline. An aliquot of 0.5 ml of each dilution was pipetted and inoculated onto sterile media and incubated at 36.5ºC for 24 hours. The media used was MacConkey Agar (Himedia, Mumbai, India), Nutrient Agar (Himedia, Mumbai, India), and Blood Agar (Himedia, Mumbai, India). The numbers of colony forming units (cfu) were counted using a colony counter after 24 hours of incubation.

### 3.5.2 Bacteria Identification

The bacteria were identified by colony characteristic, Gram's staining, differential and selective media growth and by appropriate conventional biochemical tests using the Analytical Profile Index (API) 20E systems (Biomerieux SA, Marcy-1 Etoile France) and according to Cowan *et al*., (1993).

### 3.5.3 Heat inactivation of milk pathogens

The bacteria suspension was prepared by picking bacteria colonies using a sterile loop. The Mc Farland standard (Mc Farland Standard REF 70900 Biomeriuex Inc) was used to estimate the concentration of bacteria in the suspension. This standard is a series of different opacity which estimates the bacteria concentration. The standard that was used in this study was the 0.5 standard and this corresponds to $1.50 \times 10^7$ CFU/ml. Three 50ml samples of milk were prepared. One of these samples was sterilised and one sample was maintained as the control, while the other one was inoculated with known bacteria. The bacteria suspension (1ml) was added to the sterilized milk sample and 1ml of the bacteria suspension was added to the raw milk sample. No bacteria was added to the control. The bacteria species that were artificially inoculated into the milk are *Staphylococcus MRSA, Salmonella, Bacillus* and *E. coli*. Separate experiments were conducted for each bacteria species. The *Salmonella, Bacillus* and *E. coli* isolates were isolated from this study, while the *Staphylococcus MRSA* was a standard reference strain.

In the first part of the experiment, the samples were subjected to heating using an electrically powered water bath. The water bath was heated to 72 ºC and milk sample containers were placed in a hot water as shown figure 3.2. The milk temperature was measured and when the milk temperature reached 72 ºC (Water temperature increased to slightly above 72 ºC as the milk was being heated). When the milk temperature reached to 72 ºC, the milk samples were maintained in the water bath at 72 ºC for 1 minute. After the one minute had elapsed the samples were removed from the water bath and left on the bench to cool. The milk samples were diluted using sterilized normal saline and inoculated on to MacConkey and Nutrient agar to enumerate bacteria counts. The thermometer used to measure milk temperature was sterilized using alcohol.

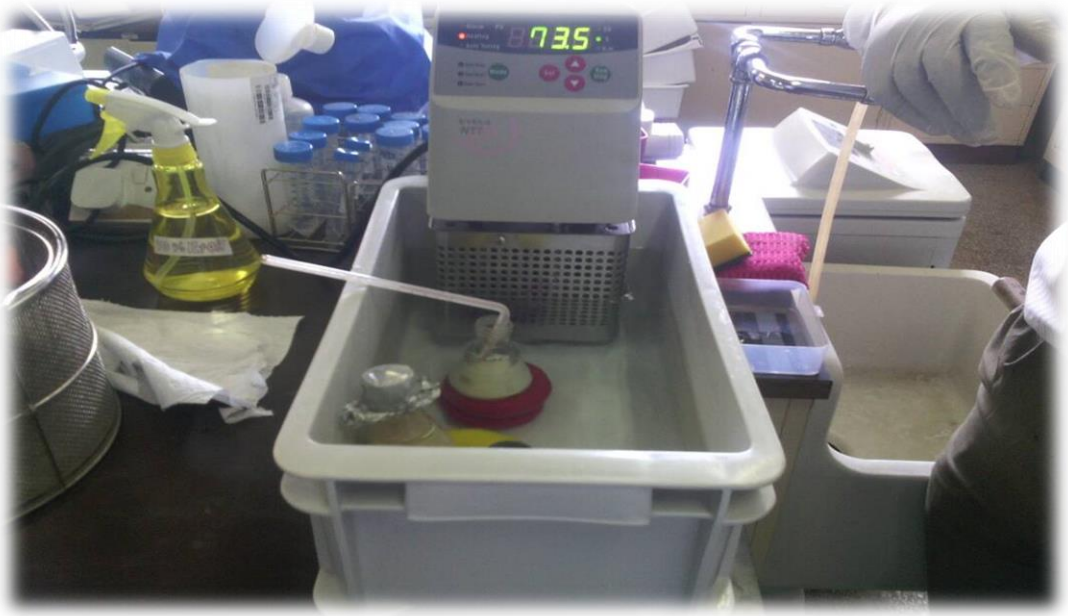

Figure 3.2: Heating of milk using an electrically powered water bath.

For Solar inactivation, a parabolic solar cooker (Manufactured by Sun Fire International, South Africa) was assembled as shown in the picture in figure 3.3.

a. b.

Figure 3.3: A solar cooker before (a) and after assembly (b).

This solar cooker traps sunlight converting it into heat energy by focusing the heat energy at one point (see Figure 3.4). The solar cooker needs sunlight, strong enough to cast a shadow.

Figure 3.4: Heat energy focussed onto a pot.

The performance of the solar cooker was first tested in Mongu, Senanga and Lealui in Western province, Zambia (Figure 3.5). The solar cookers were assembled together with the local people in the villages. The solar cookers were used to trap light energy and convert it to heat energy and used to cook food, boil water and boil milk. The cooker was tested on sunny days, cloudy days and a rainy day in these trial study areas.

Figure 3.5: Women cooking on solar cookers (on a partially cloudy day) in Western province

The solar cooker was also tested at the University of Zambia, School of Veterinary Medicine laboratory and at Evelyn Hone College on separate days (Figure 3.6). On these days the times taken to boil milk and water was taken.

Figure 3.6: Assembling a solar cooker at Evelyn Hone College

For heat inactivation of bacteria in milk, milk was artificially contaminated (spiked) as described in the water bath experiments. One liter of water was poured into a pot and the pot was placed on a Solar Cooker (Figure 3.7). The position of the solar cooker was adjusted in order to receive maximum sunlight. The ambient temperature was recorded and the initial water temperature was measured. Milk samples in 50ml flasks were placed in the pot and the milk temperatures was recorded. The milk samples were exposed to solar heating for 1 hour and the highest milk temperature was recorded. The milk samples were allowed to cool and samples were diluted and inoculated on Nutrient Agar and Mac Conkey agar to isolate and enumerate bacteria.

Figure 3.7: Heating milk samples on a solar cooker at UNZA

### 3.5.4 Antimicrobial susceptibility testing:

The isolate bacteria from the collected milk samples from Western Province were tested for antimicrobial susceptibility using standard procedures. Briefly, the antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar (Becton, Dickinson and Company, MD, USA) based on the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2009), The antibiotic discs (Becton, Dickinson and Company, MD, USA) used included ampicillin (10µg), sulfamethoxazole/trimethoprim (1.25/23.75µg), streptomycin (300µg), ciprofloxacin (5µg), tetracycline (30µg), gentamicin (10µg), nalidixic acid (30µg), chloramphenicol (30µg), ceftazidime (30µg), norfloxacin (10µg) and cefotaxime (30µg). Furthermore, the phenotypic confirmation of Extended spectrum beta lactamase (ESBL) producing isolates was done by the combination of disc approximation method using either ceftazidime (30µg) or cefotaxime (30µg) alone followed by over- night incubation at 37°C for 18 – 24 hrs. Interpretation of susceptibility patterns on other anti-microbial discs was done using guidelines laid down in the CLSI, which provides break points corresponding to zone of inhibition diameter. An increase in antibiotic zone diameter (5 – 12 mm) for either ceftazidime or cefotaxime indicated ESBL production (CLSI, 2009). Quality

control Standard laboratory procedures were strictly adhered to avoid contamination. *Escherichia coli* ATCC 25922 was used as a quality control organism.

### 3.5.5 Extended Beta Lactamase Detection in *Escherichia coli* Isolates

*E. coli* isolates from milk samples collected in Western Province were subjected to ESBL determination. In order to detect Extended Beta Lactamase (ESBL), the *E. coli* isolates were cultured in brain heart-infusion broth at 37 ºC for 24 hours. Phenotypically, the cultured bacteria was grown on the inoculated MacConkey agar (Oxoid, Basingstoke, UK) containing 2mg/L of cefotaxime (Sigma-Aldrich, Munich, Germany) for preliminary screening of ESBL *E. coli* producing bacteria according to Reich *et al.* (2013). The plates were later incubated at 37°C for 24 hours. For genetic detection, *E. coli* isolates were cultured on brain-heart-infusion broth (Nissui, Tokyo, Japan) at 37°C for 24 hours. After incubation, DNA was extracted by boiling. The *E. coli* isolates were subjected to PCR for confirmation of resistance genes TEM (Temoniera), SHV (Sulphydryl Variable) and CTX-M (Cefotaxime – Munich) using primers previously used by other workers (Reich *et al*., 2013 and Batchelor *et al*., 2005). The primers used are listed in Table 3.1.

Table 3.2: Primers used for amplification of targeted genes and significance of the gene in ESBL producers

| Gene | Primer | Gene Significance |
|---|---|---|
| *bla*$_{SHV}$ | 5′-ATG CGT TAT ATT CGC CTG TG-3′<br>5′-TGC TTT GTT ATT CGG GCC AA-3′ | Shares 68% of its amino acids with TEM-1. Commonly found in *K. pneumoniae* and is responsible for up to 20% of the plasmid-mediated ampicillin resistance (Paterson *et al*., 2003). |
| *bla*$_{TEM}$ | 5′-TCG CCG CAT ACA CTA TTC TCA GAA TGA-3<br>5′-ACG CTC ACC GGC TCC AGA TTT AT-3′ | Up to 90% of ampicillin resistance in *E. coli* due to the production of TEM-1. The amino acid substitutions responsible for the ESBL phenotype cluster around the active site of the enzyme and change its configuration, allowing access to oxyimino-beta-lactam substrates. (Boyd *et al*., 2004). |
| *bla*$_{CTX-M}$ | 5′-ATG TGC AGY ACC AGT AAR GTK ATG GC-3′<br>5′- TGG GTR AAR TAR GTS ACC AGA AYCAGC GG-3 | Ability to hydrolyse cefotaxime and are located on highly transmissible plasmids, facilitating fast and efficient spread of resistance (Boyd *et al*., 2004; Bonnet, 2004) |

### 3.5.6 Questionnaire data

A structured pretested questionnaire was administered to the farmers who sold milk to the milk collection centres. The questionnaire captured Bio data, milk production, handling and consumption. A total of 63 farmers from the six (6) MCC's under the study were interviewed. The questionnaire is included in Appendix 1.

### 3.5.7 Data analysis

Laboratory results and questionnaire data was entered into MS-EXCEL and then analysed using STATA version 9.2.

# CHAPTER FOUR

# RESULTS

## 4.1 Milk handling and consumption among traditional farmers

Information on milk handling and consumption was collected from the farmers (n=63) who were interviewed. The information collected included bio-data, information on milk boiling, raw milk consumption, milk preference, cleaniliness and training in milk handling. From this study it has been observed that consumption of raw milk was very high among traditional farmers in Western Province. In this study, 96.8% of the respondents consumed raw milk and only 3.17% didn't (Figure 4.1). The local people prefered raw milk to boiled milk for two main reasons that were cited and these are taste and tradition. Some consumers of milk stated that they consumed raw milk because it tasted better and makes very good sour milk while others stated that consuming raw milk was their tradition.

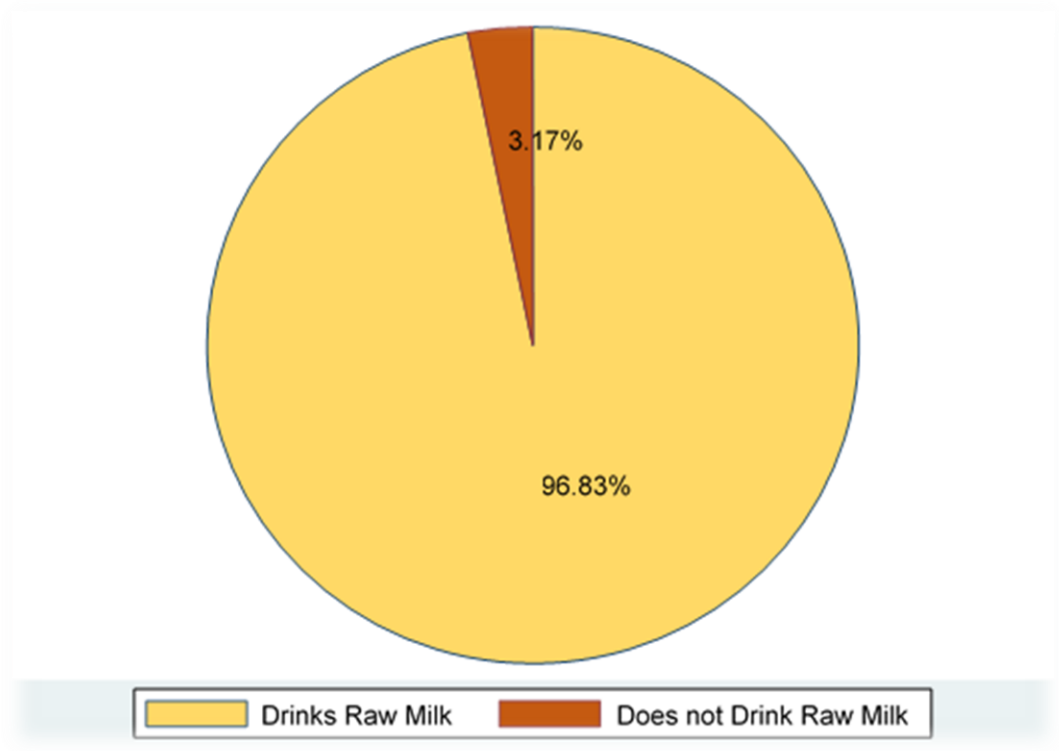


Figure 4.1: Raw milk consumption in Western Province

It was also observed that milking places were characterised by poor hygiene and uncleaniliness was common among the farmers. The commonly used milking place was the animal housing

structure. The animal housing structure was made of wooden poles and grass. Observations indicated that only 1.59% of animals housing structures were very clean i.e the structures were free from cow dug, cleaned daily and disinfected( Figure 4.2). Out of all the animal housing structures that were observed, the majority of the animal housing structures (77.8 %) were classified as average in terms of cleaniliness. These were cleaned at least once a week and the cow dung was swept out of the house. There was no use of disinfectants or cleaning with large volumes of water. The percentage of animal housing structures that were charcteristised by very poor hygiene conditions was 20.6%. Cleaning was rarely done and there was accumulation of cow dung in the animal housing structure. Milking of cows was usually done under very poor hygiene conditions in most cases. This may be the reason for high numbers of pathogens in milk.

Milking utensils were also observed and 25% of the milking utensils were very dirty (i.e. the originally white containers appeared brownish in colour and the interior was slimy) and 75% were categorised as clean. The milking utensils were not cleaned with disifectants.

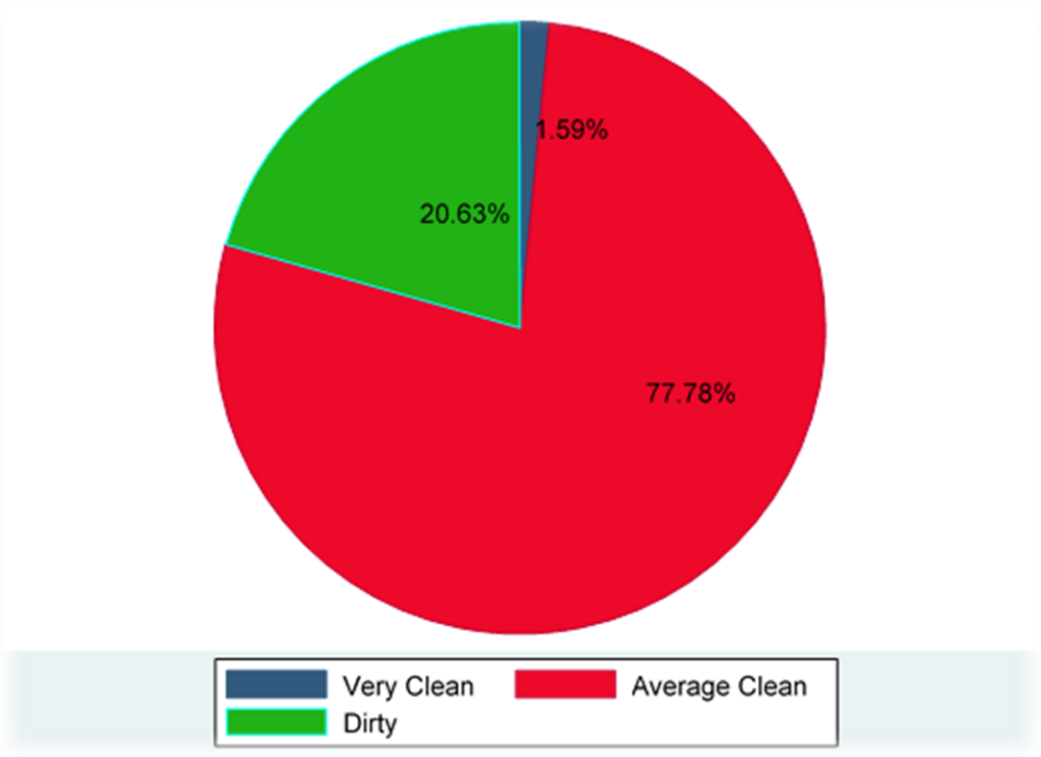


Figure 4.2: Animal Housing Cleanliness

This study demonstrated that 58% of the respondents use plastic containers as their milk storage containers. These plastics are usually containers for cooking oil which are washed and used for this purpose. Table 4.1 Shows the types of milk containers used by the traditional farmers.

Table 4.1: Types of Milk Containers

| Milk Container | Frequency | Percentage | Cumulative Frequency |
|---|---|---|---|
| Plastic | 58 | 92.6 | 92.6 |
| Stainless Steel | 1 | 1.56 | 93.65 |
| Aluminium | 3 | 4.76 | 98.41 |
| Others* | 1 | 1.59 | 100 |
| Total | 63 | 100 | |

*Includes containers made of clay and calabashes

Majority of the farmers (over 60%) that were interviewed had only attained primary education and may lack knowledge on good hygiene practices concerning milk handling. Table 4.2 shows that only 23.8% of the respondents had been trained in milk handing. These farmers may not be aware of the risks associated with consumption of raw milk and hygiene practices when handling milk.

Table 4.2: Milk Handling Training

| Milk Handling Training | Frequency | Percentage | Cumulative Frequency |
|---|---|---|---|
| Trained | 15 | 23.81 | 23.81 |
| Not Trained | 48 | 76.19 | 100 |
| Total | 63 | 100 | |

## 4.2 Bacteria contamination of milk

The bacterial count for pooled milk samples on three kinds of media are shown in Figures 4.3, 4.4 and 4.5. One of the milk samples from Limulunga area recorded the highest bacterial count on Mac Conkey Agar (Limulunga 2). On Blood Agar, high bacterial counts were observed in milk from Limulunga (Limulunga 2), Lealui (Lealui 7) and Mongu Cooperative. On Nutrient Agar, a similar pattern was observed as that obtaining in blood agar results Limulunga (Limulunga 2), Lealui (Lealui 7) and Mongu Cooperative.

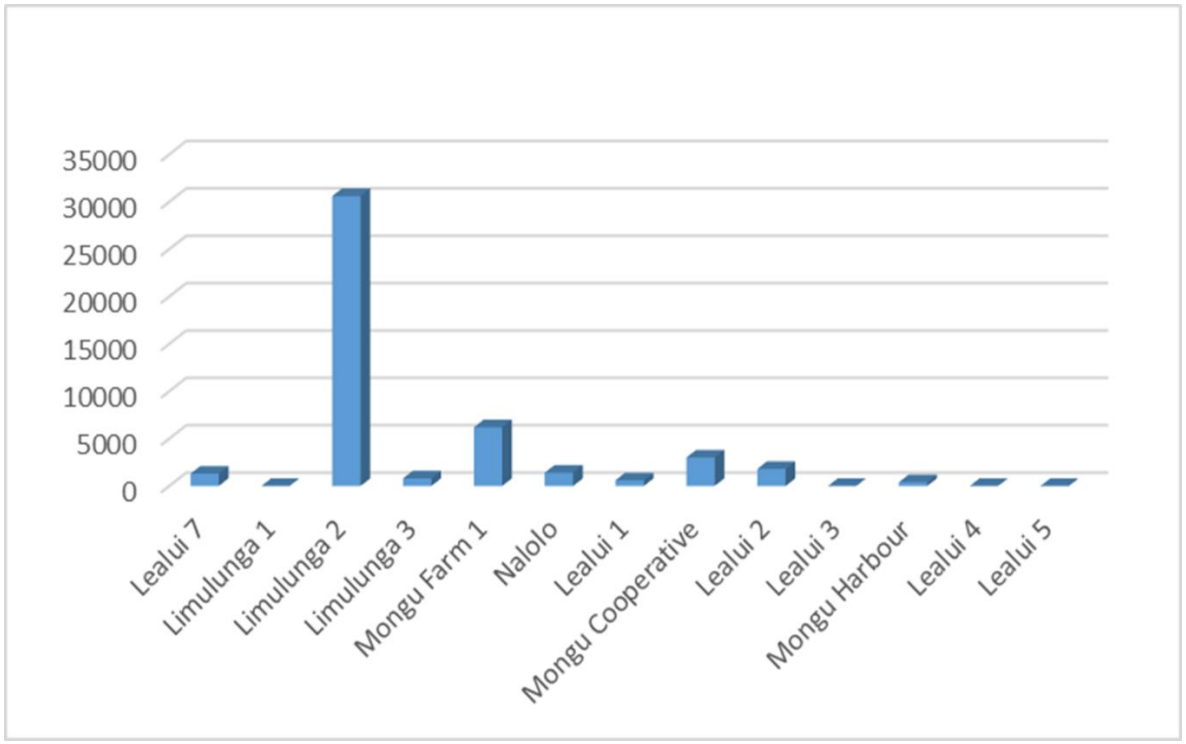


Figure 4.3: Bacterial Count on Mac Conkey Agar (in CFU)

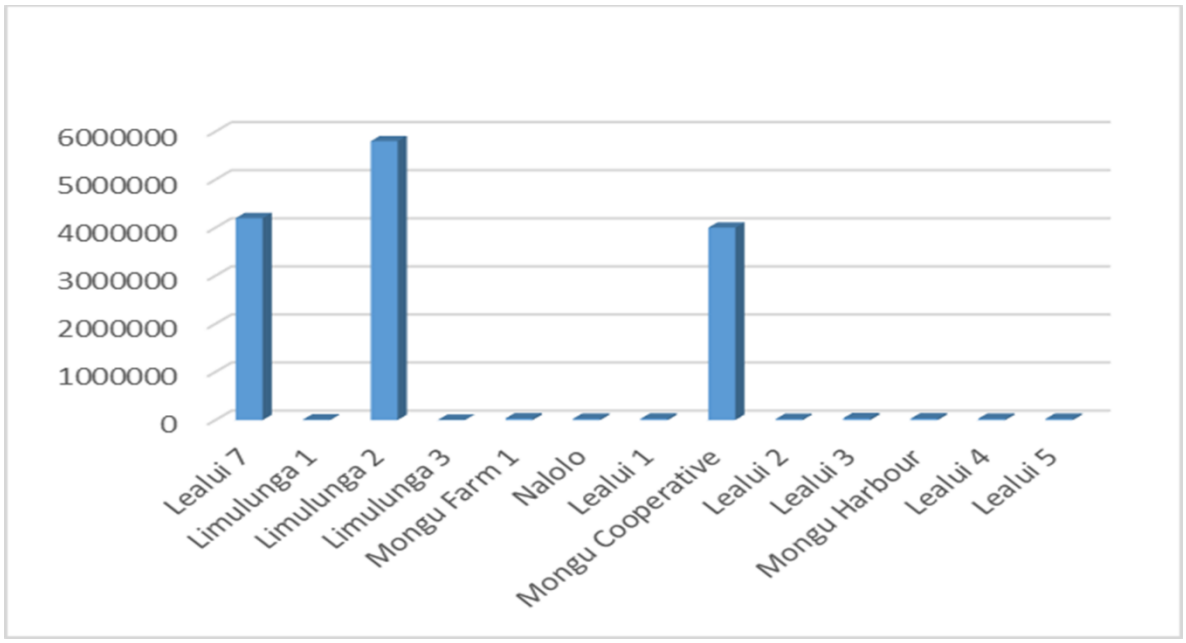


Figure 4.4: Bacterial Count on Blood Agar (in CFU)

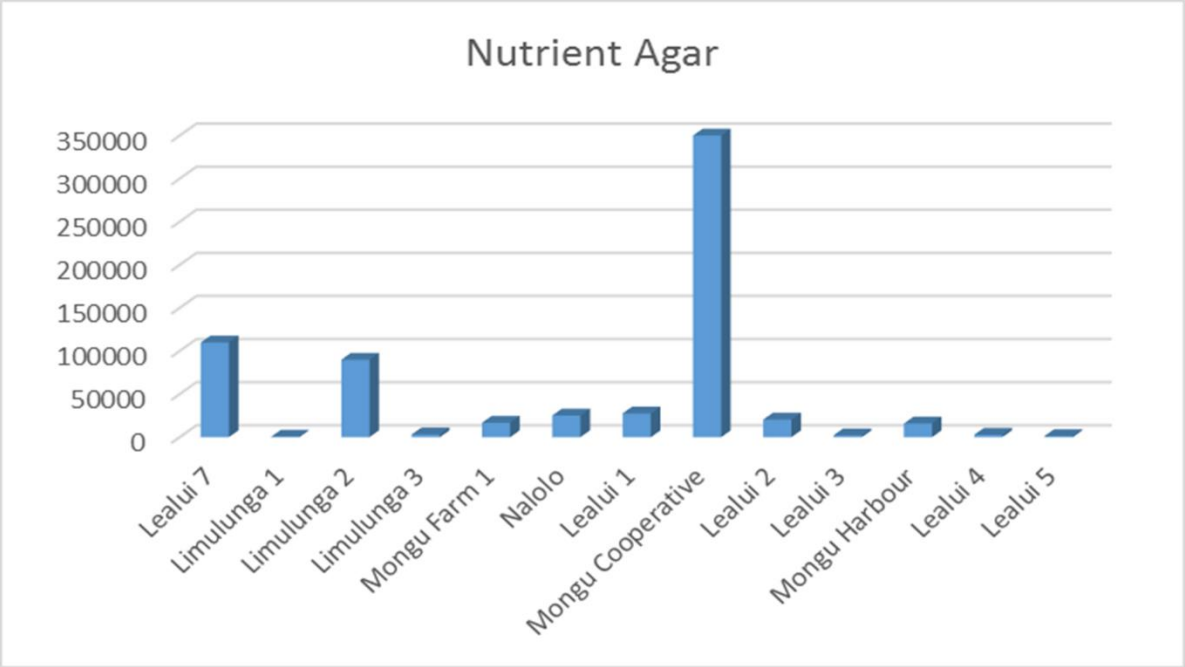


Figure 4.5: Bacterial Count on Nutrient Agar (in CFU)

The bacteria identified from the milk included *Staphylococcus, E coli, Bacillus* and *Streptococcus.* Some other bacteria could not be identified as a result of the restrictive nature of the biochemical reactions. *Staphylococcus* was isolated from all the pooled milk samples. The pooled milk samples from Limulunga and three sampling points in Lealui did not contain *E coli*. The bacteria identified in the pooled milk samples collected from milk collection centres and collection points is shown in Table 4.3.

Table 4.3: Bacteria Identified in Raw Milk

| **Sampling Area** | **Bacteria Identified** |
|---|---|
| Limulunga 1 | *Staphylococcus* |
| Limulunga 2 | *E coli, Staphylococcus, Bacillus,* |
| Limulunga 3 | *E coli, Staphylococcus* |
| Mongu Farm 1 | *E coli, Staphylococcus, Bacillus, Streptococcus* |
| Mongu Cooperative | *E coli, Staphylococcus, Bacillus, Streptococcus* |
| Mongu Harbour | *E coli, Staphylococcus, Bacillus* |
| Nalolo | *E coli, Staphylococcus, Streptococcus* |
| Lealui 1 | *E coli, Staphylococcus, Bacillus,* |
| Lealui 2 | *E coli, Staphylococcus, Bacillus* |
| Lealui 3 | *Staphylococcus* |
| Lealui 4 | *Staphylococcus* |
| Lealui 5 | *Staphylococcus* |
| Lealui 7 | *E coli, Staphylococcus, Bacillus, Streptococcus* |

## 4.3 Antibiotic Sensitivity

Selected isolates of *E coli* (10), *Staphylococcus* (10), *Streptococcus* (10) and *Bacillus* (10) were tested for antibiotic sensitivity. The antibiotics used were Cefotaxime/Ceftazidime, Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Streptomycin, Sulfamethoxazole/Trimethoprime and Tetracycline. The antibiograms are indicated in Table 4.4.

Table 4.4: Results of Antibiotic sensitivity Test

| **Antibiotic** | ***E. coli*** | ***Staphylococcus*** | ***Streptococcus*** | ***Bacillus*** |
|---|---|---|---|---|
| Cefotaxime/Ceftazidime | All[#] | All | All | All |
| Ampicillin | NS | All | All | All |
| Chloramphenicol | All | All | All | All |
| Ciprofloxacin | All | All | All | All |
| Gentamicin | All | ND* | ND | ND |
| Streptomycin | All | ND | ND | ND |
| Sulfamethoxazole/Trimethoprime | All | All | All | All |
| Tetracycline | All | All | All | All |

ND*: Not done

All[#]: All isolates were susceptible to the antibiotic used

NS: Not susceptible

The antibiotic sensitivity tests showed that isolates were susceptible to the antibiotics used in this study except in the case of *E. coli* which showed resistance to ampicillin. Extendend Beta-Lactamase detection results showed that all the isolates did not posess beta lactam properties. Milk samples in this study were collected from traditional farmers. The usage of antibiotics among traditional farmers is very low. The farmers usually use a combination of herbal remedies and antibiotics to treat their sick animals.

## 4.4 Performance of a solar heater in heating

The effectiveness of the solar cooker was tested in Western Province in the month of May. Although the solar cooker only needs sunshine enough to cast a shadow, the weather conditions

in the month of May were unfavourable. Most days were either cloudy or rainy. The solar cooker was however tested on a few days which were partially cloudy (Figure 4.6).

Figure 4.6: A partially cloudy sky in September, 2017 (Lusaka).

One litre of water was heated to temperatures above 50 ºC within 30 minutes. Two liters of milk was boiled in 44 minutes (Figure 4.7).

Figure 4.7: Boiling milk on solar cooker

## 4.5 Effect of solar heating on the survival of Milk Pathogens

*E coli*, *Salmonella*, *Staphylococcus MRSA* and *Bacillus* were subjected to heating using electricity and solar energy. The reduction in bacterial population after exposure to heating using an electrically powered water bath is shown in Table 4.5.

Table 4.5: Effect of Heating on survival of pathogens (Using Electricity)

| **MICROBE** | **PRE-STERILISED MILK** | | **RAW MILK** | | **CONTROL** |
|---|---|---|---|---|---|
| | BEFORE | AFTER | BEFORE | AFTER | |
| *E coli* | 1.5 x $10^7$CFU/ml | 4 x $10^2$ CFU/ml | 1.5 x $10^7$CFU/ml | 2x $10^2$ CFU/ml | 2 CFU/ml |
| *Salmonella* | 1.5 x $10^7$CFU/ml | 7.1 x $10^3$ CFU/ml | 1.5 x $10^7$CFU/ml | 2.6 x $10^3$ CFU/ml | 0 CFU/ml |
| *Staphylococcus MRSA* | 1.5 x $10^7$CFU/ml | 7.4 x $10^3$ CFU/ml | 1.5 x $10^7$CFU/ml | 3.4 x$10^3$ CFU/ml | 1 CFU/ml |
| *Bacillus* | 1.5 x $10^7$CFU/ml | 9.2 x $10^3$ CFU/ml | 1.5 x $10^7$CFU/ml | 8 x $10^2$ CFU/ml | 2 CFU/ml |

The results showing the reduction in bacteria population after solar heating are shown in Table 4.6. Solar heating depends on weather conditions and the results shows the effects of heating under, three different weather conditions. The highest milk temperature recorded was 61℃ and the lowest was 56 ℃.

Table 4.6: Effect of solar heating on survival of pathogens

| MICROBE | AMBIENT TEMP | WEATHER | MAX MILK TEMP | BACTERIA CONC | RAW MILK | PRE-STERILISED MILK | CONTROL |
|---|---|---|---|---|---|---|---|
| *E coli* | 28° C | Sunny day | 61° C | 1.5 x $10^7$CFU/ml | 0CFU/ml | 400 CFU/ml | 6 CFU/ml |
| | 26° C | Partially cloudy | 58° C | 1.5 x $10^7$CFU/ml | 400 CFU/ml | 600 CFU/ml | 15 CFU/ml |
| | 25° C | Partially cloudy | 56° C | 1.5 x $10^7$CFU/ml | 4800CFU/ml | 18400 CFU/ml | 20 CFU/ml |
| *Salmonella* | 28° C | Sunny day | 61° C | 1.5 x $10^7$CFU/ml | 3600CFU/ml | 19200 CFU/ml | 6 CFU/ml |
| | 26° C | Partially cloudy | 58° C | 1.5 x $10^7$CFU/ml | 8400CFU/ml | 39200 CFU/ml | 15 CFU/ml |
| | 25° C | Partially cloudy | 56° C | 1.5 x $10^7$CFU/ml | 9600CFU/ml | 43400CFU/ml | 20 CFU/ml |
| *Staphylococcus* MRSA | 28° C | Sunny day | 61° C | 1.5 x $10^7$CFU/ml | 2000 CFU/ ml | 3200 CFU/ml | 6 CFU/ml |
| | 26° C | Partially cloudy | 58° C | 1.5 x $10^7$CFU/ml | 27800 CFU/ml | 48600 CFU/m/ | 15 CFU/ml |
| | 25° C | Partially cloudy | 56° C | 1.5 x $10^7$CFU/ml | 29600 CFU/ml | 51200 CFU/ml | 20 CFU/ml |
| *Bacillus* | 28° C | Sunny day | 61° C | 1.5 x $10^7$CFU/ml | 98200 CFU/ml | 112000 CFU/ml | 6 CFU/ml |
| | 26° C | Partially cloudy | 58° C | 1.5 x $10^7$CFU/ml | 121000CFU/ml | 142000 CFU/ml | 15 CFU/ml |
| | 25° C | Partially cloudy | 56° C | 1.5 x $10^7$CFU/ml | 153200 CFU/ml | 178200 CFU/ml | 20 CFU/ml |

# CHAPTER FIVE

# DISCUSSION

From this study, it has been observed that raw milk consumption among traditional farmers in Western province was prevalent. From the interaction, it was clear that most respondents lacked basic education and hence they may not know the health risks associated with raw milk consumption. The raw consumption of milk has been documented to be a vehicle of pathogens (Muehlohoff *et al*., 2013). Out of the 63 respondents only two respondents had attained tertiary education and one of these respondents did not drink raw milk and preferred boiled milk. Despite having attained tertiary education, the other of the two respondents consumes raw milk stating that it was traditional to do so. A study by Headrick *et al*. (1997) showed that people with less than a high school education were more likely to consume raw milk than those who had completed high school, suggesting that the level of education may influence a person's choice to consume raw milk. In this study however, attaining secondary education did not affect the choice to consume raw or boiled milk. This may be due to a small number of respondents who had attained secondary education and traditional beliefs. Consumption of raw milk was a traditional belief regardless of the educational status of a person.

Although the traditional farmers in the study area preferred raw milk, raw milk samples are likely to have higher numbers of possible pathogenic bacteria. This high number can be attributed to poor hygienic conditions. Observations that were conducted during interviews and captured in the questionnaire revealed that household surroundings, animal housing and milking utensils were characterised by poor hygiene conditions like piled up cow dung, stagnant water, dirty and stained milk containers. Hill *et al* (2012) and Hutchison *et al*., (2005) also reported that the presence of pathogens in raw milk is believed to be influenced by environmental factors, such as indoor housing of cattle and poor quality feed, such as silage. A study by Millogo *et al.,* (2010) in Burkina Faso also demonstrated that the lack of hygiene in the milk handling chain between cow and consumers subjected the consumer to a high risk of milk borne diseases. They reported that the hygienic quality of the milk became decreased along the dairy chain which was attributed to contamination by manure or dust, milk maids, the environment or dirty storage vessels. Use of disinfectants for cleaning animal houses and

milking utensils was very low. Use of plastic milk containers was observed in this study as reported by Gemechu and Amene (2017). The plastic milk containers which are used were not designed for milk storage and lacked cleanliness. These containers were not usually disinfected and this may lead to formation of biofilms. Biofilms are a thin but robust layer of mucilage adhering to a solid surface and containing a community of bacteria and other microorganisms (Donlan, 2002). Biofilms have great importance for public health because of their role in certain infectious diseases and importance in a variety of device-related infections such as milking utensils used in this case. Several frank bacterial pathogens have been shown to associate with, and in some cases, actually grow in biofilms. Murga *et al*., (2001) demonstrated that the presence of biofilms in potable and healthcare water systems can serve a means of survival of *Legionella pneumophila.* This can cause contamination of water in the distribution system. *Staphylococcus aureus* and other microorganisms were isolated from catheters of patients in intensive care units (Raad *et al*., 1992) while *Listeria monocytogenes* has been also reported to grow on stainless steel (Wirtanen *et al*., 1996). *Salmonella typhimurium* and *Escherichia coli* O157:H7 have been reported to form biolfilms on stainless steel in food systems (Hood and Zottola, 1997). Other microorganisms that are able to form biofilms and have been isolated are *Vibrio cholerae* (Watnick *et al*., 1999), and *Helicobacter pylori* (Stark *et al*., 1998). The biofilms cannot easily be removed by gentle rinsing. Some workers like Millogo *et al*., (2012) recommended that plastic bottles should be replaced with milk containers with a large opening which can be easily cleaned inside. The best method of cleaning is the one that may involve scrubbing inside the container with a cloth (mutton cloth) and a brush.

Further observations, were that the traditional farmers lacked knowledge on zoonoses acquired from milk and through milk handling. The majority of them had received any form of training on milk handling and hygiene. There is need for effective and continuous training on milk handling as recommended by Zeinham and Abdel (2014). In previous studies a number of diseases have been documented from Western province such as anthrax, tuberculosis, brucellosis and other milk spoilage microorganisms (Mumba and Pandey, 2013: Pandey *et al.*, 2013; Siamudaala *et al.*, 2006; Phiri, 2006). These findings highlight that milk from Western province may be contaminated with disease causing agents.

The milk in Western province was found to contain a number of microorganisms of Public Health importance as reported by Knight-Jones *et al*., (2016). The organisms reported in this study were *E. coli*, *Staphylococcus*, *Streptococcus* and *Bacillus*. Therefore, there is need to

promote boiling of milk before consumption as some of these organisms may produce toxins such as *Staphylococcus* and *Bacillus*. Introduction of inexpensive and appropriate technology for rural communities can help protect the public from milk borne infections. Heating milk using solar energy can reduce the bacterial load of milk. The bacterial load in milk can also be reduced through heating using an electrically powered water bath, boiling with firewood or using solar heating, indicating the importance of both measures. The bacteria identified in this study i.e. *E. coli, Staphylococcus, Bacillus* and *Streptococcus* are potential pathogens. Some *Bacillus* species produce an emetic toxin which causes vomiting while others produce enterotoxins like *Staphylococcus* which results in diarrhoea. The presence of *E. coli* indicates the presence of other enteric pathogens and some strains such as *E. coli* O157:H7 causes haemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Claey *et al*., 2014). Gorgy *et al.,* (2016) reported that some *Streptococcus* species are known to cause mastitis. *Staphylococcus aureus* produces an enterotoxin that coagulates plasma (De Oliveiria *et al.,* 2011).

Solar heating has been used in a number of countries such as Kenya, India and Argentina. In Kenya, a solar pasteuriser was used to pasteurise water which had over 180 CFU *E coli*. In this experiment, results showed that there was 100% inactivation of *E. coli* (Safapour and Metcalf, 1999). The solar pasteuriser in this case was made using local materials. Franco *et al.,* (2008) designed a system for pasteurising goat milk used in cheese production in Argentina while solar based systems were introduced at village cooperative level to boil water for processing milk and for cleaning purposes in India by Desai *et al*., (2013).

In this study, bacteria in milk which was sterilized using an autoclave before being artificially contaminated was more resistant to heat than bacteria which was in raw milk as some of the fats or proteins that would hold heating for a much longer time were not present. Studies by Claey and others (2013), indicate that raw cow milk has antimicrobial properties that inhibit the growth of micro-organisms and/or contribute to the immunity of the young calf. Bacteriocins such as nisin may also be present in milk. Bacteriocins are proteins produced by bacteria of one strain and they have antimicrobial effect on closely related strains. Antimicrobial systems responsible for antimicrobial properties are inactivated after sterilization of milk and this could be the reason why milk which was first sterilised before being artificially contaminated had higher number of microorganisms compared to the one which was not sterilised. The presence of commensal lactic acid bacteria in raw milk may have

inhibited the multiplication of bacteria that was inoculated and this could have caused a low number of microbes in this milk.

Weather conditions were unfavourable during this study hence pasteurization temperatures were not reached. The temperatures reached could be described as thermisation temperatures. Thermisation is pre-treatment used only to extend the shelf-life of refrigerated milk by reducing the microbial population of milk (Metwally *et al*., 2011). It is important to note that sunlight has other properties such as UV radiation that may have minimal or no effect on pasteurisation of milk. This is an important matter that may require further investigation in future studies as UV light has been used to destroy pathogens in water placed in clear containers (Reed, 2004). Safapour and Metcalf (1999) however suggested that UV light may not be suitable for opaque liquids such as milk. This school of thought is yet to be expanded as UV light may be absorbed leading to changes in bacterial survival.

The use of solar cookers is limited to days when there is plenty of sunshine. Solar cookers can therefore be used in the months (August to November) of the year when there is plenty of sunshine. This can minimise usage of charcoal and reduction on the cutting down of trees. Solar heating is cheap and clean. In a village setting a point can be selected where the solar heating equipment is placed for ease of use. At the time this study was being conducted the average cost of firewood in the villages was K1.26/kg ($0.13/kg) while the cost of charcoal was K1.16 ($0.12/kg). The weekly charcoal and firewood consumption per household was approximately 31.37kg and 38.66kg, respectively. On average the cost of firewood and charcoal translates to K48. 7 ($5) and K36.4 ($4) per week. The solar cookers were purchased at K1067 ($110) and the solar cookers have a life span of 12 years. This shows that solar energy in rural areas is cheaper than use of firewood or charcoal. Solar cookers are clean, pollution free method that can be used for heating milk, cooking food and boiling drinking water. Embracing this kind of technology will not only promote environmental sustainability but it will also improve public health through promoting of heat inactivation of pathogens in milk and in water. The subject of climate change should also discuss the use of cleaner energy so that such investments are placed in poor resource communities to avoid over indulgency in trees that are important in the maintenance of a clean environment.

# CHAPTER SIX

# CONCLUSION

From this study, it was concluded that;

1. Raw milk from selected parts of Western Province contained pathogens and heating milk inactivated the organisms.

2. The raw milk samples collected in this study had high levels of bacterial contamination. This milk is usually consumed raw and consumption may results in zoonotic diseases.

3. Pathogens in milk can be inactivated by using various heat treatment methods such as solar energy. Solar heating is a simple and cheaper technology which can be employed for heat inactivation of pathogens in milk.

4. Solar heating technology is limited to sunny months or days.

# CHAPTER SEVEN

## RECOMMENDATIONS

This study has revealed information on raw milk safety and milk consumption patterns in western province, Zambia. This study therefore recommends the following;

1. There is need to raise awareness of the dangers of consuming raw milk among traditional farmers. Public health workers in the rural communities must raise consumer awareness on the consumption of boiled milk through health promotion programs.

2. Farmers in Western province need to be trained on good milk handling and hygiene practices.

3. Social change which will encourage consumption of boiled milk should be advocated for in the rural areas of Zambia where consumption of raw milk is traditional.

4. Mandatory heating/boiling of milk before consumption through the use of cheaper methods of heat harnessing such as solar heating should be promoted.

5. Adequate infrastructure in form of milk collection and processing centres must be established in Western Province.

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## APPENDICES

### APPENDIX ONE

### QUESTIONNAIRE

### PART ONE: GENERAL INFORMATION

| | |
|---|---|
| Questionnaire code | |
| Name of interviewer | |
| Date of interview: | |
| Consent given? (1=Yes, 2=No) If consent is denied, continue with this questionnaire at another household that agreed to participate. | |
| If no, why? | |
| Region: | |
| District: | |
| Ward | |
| Village: | |

### PART 2: BIO-DATA AND FARM INFORMATION

| | |
|---|---|
| Head of household's name | |
| Respondent's name | |
| Respondent's age | o 20-35<br>o 35-50<br>o Above 50 |
| Respondent's sex | o Female (1)<br>o Male (2) |
| Respondent's relationship to Household head | o Household head<br>o Spouse<br>o Child<br>o Other family member specify _____<br>_____<br>o Other non-family member specify___<br>_____ |

| | |
|---|---|
| Education level of respondent: | ○ None<br>○ Primary<br>○ Secondary<br>○ Higher education (Diploma level or higher) |
| Who works on the farm? | ○ Family labour<br>○ Hired labour |
| Do you drink raw milk? | ○ Yes (1)<br>○ No (2) |
| Do you boil your milk before drinking? | ○ Yes (1)<br>○ No (2) |
| Which milk do you prefer and why? | ○ Raw milk<br>○ Boiled milk<br>Why? _____ |
| Do you sell your milk? | ○ Yes (1)<br>○ No (2) |
| How do you sell your milk? | ○ Directly from farm<br>○ Collect in bulk with other farmers<br>○ Don't sell |
| To whom do you sell your milk? | ○ Consumers<br>○ Bicycle boys<br>○ Retailers/shop keepers<br>○ Processors<br>○ Others; specify |
| Do you carry out on-farm milk processing? | ○ Yes (1)<br>○ No (2) |
| If yes, what products do you make? | |
| Do you have cooling facilities for milk storage? | ○ Yes (1)<br>○ No (2) |
| Do you have trouble selling all your dairy products? | ○ Yes (1)<br>○ No (2) |

| | |
|---|---|
| If yes, please mention why? | ○ |
| How much money do you charge for your milk? | _____ per litre |
| What breeds do you keep and in what numbers? | ○ Indicate the number adjacent to breed type.<br>○ Local breeds _____<br>○ Cross breeds _____<br>○ Exotic breeds_____ |
| How many dairy cattle do you keep/own | _____ cattle |

**PART 3: GENERAL ANIMAL HEALTH STATUS**

| | |
|---|---|
| Do you tag/identify your animals? | ○ Yes<br>○ No |
| Do you keep only dairy cattle | ○ Yes<br>○ No |
| If no, what other animals do you keep? | ○ Goats<br>○ Sheep<br>○ Pigs<br>○ Poultry<br>○ Others: specify_____ |
| Do these different animals mix up on the farm in any way? | ○ Yes<br>○ No |
| Do you separate the healthy animals from the sick ones and those receiving therapy? | ○ Yes<br>○ No |
| What animal rearing method do you use? | ○ Free grazing<br>○ Zero grazing<br>○ Mixed |
| Is the animal feed easily available all year round? | ○ Yes<br>○ No |

| | |
|---|---|
| If no, which months is it unavailable (write all that apply) | |
| Do you use any mineral supplements and/or concentrates? | ○ Yes<br>○ No |
| If yes, specify | ○ Maize bran<br>○ Legumes<br>○ Roots and tuber peelings<br>○ Mineral blocks |
| Where are they sourced from? | ○ Purchased<br>○ Home made<br>○ Others; specify |
| Do you provide sufficient portable water for your animals? | ○ Yes<br>○ No |
| Where do you get the water from? | ○ Piped water<br>○ Well water<br>○ Borehole<br>○ Rainwater<br>○ Borehole<br>○ Rainwater<br>○ Others; specify _____ |
| What type of housing do you use for your dairy cows? Indicate code<br>Coding<br>1. Brick<br>2. Stone<br>3. Mud/earth<br>4. Concrete/cement<br>5. Stone<br>6. Iron sheets<br>7. Wire mesh<br>8. Grass thatched/dung<br>9. Others; specify _____ | ○ Floors;_____<br>○ Walls;_____<br>○ Iron sheets; _____ |

| | |
|---|---|
| Do you keep health records for your animals? | ○ Yes<br>○ No |
| If not, give your reasons | |
| What is contained in the health records? Tick all that apply | ○ Treatment dates<br>○ Animal identification<br>○ Dosages<br>○ Routes of administration<br>○ Withdrawal time for meat and milk<br>○ Individual who administered the drug<br>○ Drug used<br>○ Duration of treatment therapy |
| Are your cattle vaccinated against tuberculosis? | ○ Yes<br>○ No<br>○ Some of them<br>○ I don't know |
| Are your cattle vaccinated against brucellosis | ○ Yes<br>○ No<br>○ Some of them<br>○ I don't know |
| Who diagnoses, treats and administers the drugs to sick animals? | ○ The farm hands<br>○ Veterinary practitioners<br>○ Animal health and welfare officers<br>○ Others; specify _____<br>○ I don't know |
| What do you use to treat your sick animals? | ○ Nothing<br>○ Veterinary drugs only<br>○ Herbal remedies only<br>○ A mixture of both vet drugs and herbal remedies<br>○ Don't know |
| What signs have your animals suffered from in the last 12 months? | ○ Weight loss<br>○ Malaise |

| | |
|---|---|
| Tick all that apply | ○ Weakness<br>○ Reduced milk production<br>○ Coughing<br>○ Foetal abortions<br>○ Low appetite<br>○ Worms<br>○ Diarrhoea<br>○ Unspecified diseases<br>○ Others: mention _____<br>_____ |

**PART 4: FARM AND HOUSEHOLD SANITARY CONDITIONS (OBSERVE)**

| Item | Rate | Materials used to clean (Indicate all that apply) |
|---|---|---|
| Household | | |
| Animal housing and environment | | |
| Milking shed | | |
| Milking utensils | | |
| | Rating<br>1-Excellent<br>2-Medium<br>3-Poor | Codes<br>1. Soap and warm water<br>2. Warm water only<br>3. Soap and cold water<br>4. Cold water only<br>5. Water and disinfectant<br>6. Soap, water and disinfectant |
| How often do you clean the animal housing facilities? | ○ Daily<br>○ Weekly<br>○ Rarely<br>○ Never | |

**PART 5: MILK HANDLING, STORAGE AND PRODUCTION**

| | |
|---|---|
| Where do you normally do the milking? | ○ In the animal housing structure<br>○ In a milking shed<br>○ Other: specify_____ _____ |
| How often do you milk the cows in the day and at what time/s? (24 hours) | ○ Once<br>○ Twice<br>○ Thrice<br>○ Four times<br>○ It varies |
| How much total milk volume is collected per day? | _____ litres |
| What preliminary preparations do you take before milking? | ○ Milking area cleaned<br>○ Utensils cleaned prior to milking<br>○ Utensils disinfected<br>○ Hands washed<br>○ Disinfect hands<br>○ Udders cleaned<br>○ Udders disinfected |
| How do you stimulate milk let-down and lubricate the teats? | ○ Using the calf<br>○ Using milking salve<br>○ Using cooking oil<br>○ Using petroleum jelly |
| Is the milk filtered after milking? | ○ Yes<br>○ No |
| What do you use to filter? | ○ Clean white cloth?<br>○ Other; specify _____ |
| What materials are your milk storage containers made of? | ○ Plastic<br>○ Stainless steel<br>○ Aluminium<br>○ Other; specify _____ |
| What do you do with the harvested milk? | ○ Drink it |

| | |
|---|---|
| (tick all that apply) | ○ Refrigerate it<br>○ Boil it<br>○ Sell it<br>○ Process it into other products; specify which products _____ _____ |
| Do you boil your milk before selling? | ○ Yes<br>○ No |
| Do you add anything to the milk before selling it? | ○ Yes<br>○ No |
| If yes, what do you add? | ○ Water<br>○ Others: specify |
| How is the milk stored? | ○ Heat treated<br>○ Cold storage; specify temperature _____<br>○ Room temperature |
| How do you transport the milk to customers? | ○ On foot<br>○ By bicycle<br>○ By motorcycle<br>○ By car<br>○ Others; specify _____ |
| Have you received any training on milk handling? | ○ Yes<br>○ No |
| If yes, how many times have you attended such training? | _____ times |
| What organisation conducted the training? | ○ Government organisation<br>○ Civil society organisation<br>○ NGO<br>○ Don't know |
| Was the training helpful to you? Y/N | ○ Yes<br>○ No |

**PART 6: KNOWLEDGE AND ATTITUDES ABOUT ZOONOSIS**

| | |
|---|---|
| Have your animals suffered from these illnesses in the last 12 months? Indicate 1=Yes and 2= No | ○ Tuberculosis _____<br>○ Brucellosis _____ |
| If you answered yes above, what course of action did you take? | ○ Culled them<br>○ Treated them<br>○ Sold them<br>○ Did nothing<br>○ I don't know |
| Mention some signs of brucellosis in cattle | |
| Mention some signs of tuberculosis in cattle | |
| Do you harvest milk from sick animals? | ○ Yes<br>○ No |
| If yes, what reasons do you have for this practice? | |
| If yes, under what circumstances is this done? | ○ Fulltime/any time<br>○ With new born animals<br>○ With sickly animals |
| How can you prevent the dangers you have mentioned above? | |
| Do you consume any other raw animal products? | ○ Yes<br>○ No |
| How do you handle aborted foetuses? (tick all that apply) | ○ Wear gloves<br>○ Bury the foetus<br>○ Burn the foetuses<br>○ Disinfect birthing premises<br>○ Don't wear gloves<br>○ Don't disinfect birthing premises |

| | |
|---|---|
| Do you or your household members share housing with any of your animals? | o Yes<br>o No |
| Have you observed any signs of illnesses amongst the family members? | o Yes<br>o No |
| If yes, mention the signs of illness you have noticed. Tick all that are mentioned | o Weight loss<br>o Recurring fever<br>o Coughing<br>o Back pain<br>o Joint pain<br>o Malaise<br>o Others: specify_____<br>_____ |