MICROBIAL CONTENT AND QUALITY OF RAW BOVINE MILK FROM SELECTED FARMERS IN NAMWALA DISTRICT OF ZAMBIA

BY;

MWEEMBA BOYD

A dissertation submitted to the University of Zambia in partial fulfilment of the requirements for the Degree of Masters of Science in Epidemiology

THE UNIVERSITY OF ZAMBIA
LUSAKA,

2019
COPYRIGHT

No part of this dissertation may be reproduced, stored in any retrieval system or transmitted in any form, or by any means without the prior written permission of the author.

© 2019 by Mweemba Boyd
DECLARATION

I, Mweemba Boyd, do hereby declare to the Senate of University of Zambia that this dissertation is my own original work and has neither been submitted nor been concurrently submitted for degree award in any other Institution.

__________________________
Signature

__________________________
Date
CERTIFICATE OF APPROVAL

The University of Zambia has approved the dissertation by Mweemba Boyd as fulfilling the requirements for the award of the Master of Science in Epidemiology.

Examiner 1: ________________________________

Signature: ___________________________ Date: ________________________________

Examiner 2: ________________________________

Signature: ___________________________ Date: ________________________________

Examiner 3: ________________________________

Signature: ___________________________ Date: ________________________________

Chairperson board of examiners: ________________________________

Signature: ___________________________ Date: ________________________________

Supervisor: Dr. Bwembya A. Phoebe

Signature: ___________________________ Date: ________________________________

Co-Supervisor: Dr. Banda Jeremiah

Signature: ___________________________ Date: ________________________________
ABSTRACT

Contamination of milk by pathogenic microbes may be a hazard to human health. Worldwide, pathogenic microbes contribute to numerous cases of diarrheal diseases and outbreaks. This study set out to determine microbial content, nutrient quality and associated risk factors regarding contamination of raw bovine milk in Namwala district of Zambia.

A cross sectional study was conducted in March, 2017 among small scale dairy farmers. Total enumeration of three milk collection centres (MCCs) was carried out. A simple random sampling method was employed to select study participants. Probability proportion to size was considered in selecting the sample size per MCC. A total of seventy (70) farmers were included in the study. Laboratory forms were used to collect data on the laboratory outcomes (microbial content and milk quality) and questionnaire was used to collect data on the probable risk factors to contamination. Observation checklist was also used to record hygiene practices during milking. The microbial content and quality of raw milk was determined by comparing Total Bacteria Count (TBC) and Total Coliforms Count (TCC) against the requirements by the Zambian Bureau of Standards (ZABS) and the Food and Drugs Act (FDA). Bacterial speciation was determined through isolation of microbes of public health importance such as E. coli and S. aureus while milk compositional quality was determined by comparing the levels of added water in milk, nutrients such as butter fat content, solid nonfat, density and protein. Data were analyzed using STATA version 13.0. Multiple logistic regression was used after which Step wise regression model (machine led) was adopted to establish association between the dependent and independent variables adjusting for confounding factors.

A total of 70 farmers participated in the study out of which 16 (22.8%) were from Namwala central, 24 (34.3%) from Nchole and 30 (42.9%) from Mungaila dairy farmers cooperatives. Total bacterial count (TBC) of raw milk from 32 (45.7%) farms was above the maximum legally accepted limits in Zambia. Total Coliforms Count (TCC) from 19 (27.1%) farms did not conform to recommended Zambian standards. Nchole had the highest contamination of S. aureus (41.8%) with Namwala central having the lowest (19%). The overall prevalence of pathogenic E.coli (0157:H7) was at 21% with Namwala central having the highest (10%) contamination among the MCCs in Namwala district. Milk samples from Namwala did not conform to only Zambian standards but also to regional and international standards. Water adulteration was detected in 50% of the samples. About 56% and 47% samples were below standards for butter fat (BF) and solid nonfat content, respectively. The milk density was below the recommended standards for all samples. Microbial content as defined by Total Bacterial Count (TBC) was found to be an average of $2.8 \times 10^6 \pm 9.8 \times 10^5$ cfu at producer level. Total coliform count (TCC) values for farmers in Namwala district averaged of $3.7 \times 10^5 \pm 6.3 \times 10^5$ cfu. Based on milk composition quality, about 50% of farmers practiced water adulteration. Factors associated with contamination of the milk in Namwala district included use of family members in milking 1.26 ($p=0.50$, 95% CI 0.64-2.50) and age 1.47 ($p=0.31$, 95% CI 0.69-3.09). About 83% (n=58) of the farmers in Namwala district did not follow good hygienic practice of hand washing when milking.
The study found that milk in Namwala district was of poor quality with high water adulteration. This suggests the need for a milk processing facility and active surveillance in the area to improve hygienic practices to safeguard public health.

**Keywords:** Raw bovine milk, quality, microbial content, dairy farmers, risk factors, contamination.
DEDICATION

This work is dedicated to my mother Mrs. Mweemba Vine, my ever loving and supporting wife Mayaba Lumamba Mweemba and my lovely sons Joseph and Boyd Mweemba Jnr.
ACKNOWLEDGEMENTS

I am grateful to God our heavenly Father for the health, knowledge, insight and wisdom in this work that I can now with gladness say “Ebenezer-this far you have brought me Lord”! I am deeply indebted to my able supervisors Dr. Bwembya Phoebe, Dr. Banda Jeremiah and Prof. Michelo Charles for their tireless guidance and patience throughout the study period. Without them this work would not have been possible.

Special thanks go to the various officials of the Bacteriology Department at University of Zambia, school of Veterinary Medicine for allowing me to conduct laboratory analysis at their place. I am highly indebted to Dr Mubita, Prof. Pandey and Mr. Penjani Kapila whose precious advice and supervision made the laboratory analysis part of this work possible. I am also grateful to Ms. Jessy Zygambo and Mr. Given Moonga from Department of Epidemiology and Biostatics, University of Zambia for their guidance in data analysis.

I wish also to thank Dr. Showa the District Veterinary Officer for Namwala district who allowed me to collect milk samples in his catchment area and for his input and encouragement to push on. Last but not least, I am grateful and indebted to my loving mom and other family members for their encouragement during the entire period of this study. A special thanks to my study group friends and all my classmates for standing with me. May the Lord richly reward and bless you.
# TABLE OF CONTENTS

COPYRIGHT........................................................................................................................................i
DECLARATION..................................................................................................................................... ii
ABSTRACT........................................................................................................................................... iv
DEDICATION....................................................................................................................................... vi
ACKNOWLEDGEMENTS ...................................................................................................................... vii
TABLE OF CONTENTS ......................................................................................................................... viii
LIST OF TABLES ................................................................................................................................... x
LIST OF FIGURES ................................................................................................................................ xi
LIST OF APPENDICES ........................................................................................................................ xii
ACRONYMS ......................................................................................................................................... xiii

## CHAPTER ONE: INTRODUCTION........................................................................................................... 1

1.1 Background ..................................................................................................................................... 1
1.2 Problem statement ......................................................................................................................... 3
1.3 Justification .................................................................................................................................. 4
1.4 Research questions ....................................................................................................................... 5
1.5 Objectives .................................................................................................................................... 5
1.5.1 General objective ..................................................................................................................... 5
1.5.2 Specific objectives .................................................................................................................... 5
1.6 Conceptual framework ................................................................................................................ 5

## CHAPTER TWO: LITERATURE REVIEW ................................................................................................. 8

2.1 Hygiene, Handling and Microbial Quality of Raw Milk .................................................................. 9
2.2 Health and economic impact of unsafe foods ............................................................................... 15

## CHAPTER THREE: METHODOLOGY ...................................................................................................... 17

3.1 Study design ............................................................................................................................... 17
3.2 Study population ........................................................................................................................ 17
3.3 Study setting ............................................................................................................................... 17
3.3.1 Inclusion criteria .................................................................................................................... 17
3.3.2 Exclusion criteria ................................................................................................................... 17
3.4 Recruitment procedures ............................................................................................................. 18
3.4.1 Sample size consideration................................................................. 18
3.5 Variables included in the study............................................................. 19
3.6 Data collection ..................................................................................... 20
3.6.2 Laboratory data collection ................................................................. 21
3.7 Laboratory procedures ......................................................................... 22
3.8 Milk composition .................................................................................. 24
3.9. Observed farm level hygiene practices ............................................... 24
3.10 Data management and analysis ............................................................ 25
3.11 Ethical consideration .......................................................................... 25

CHAPTER FOUR: RESULTS ....................................................................... 27
4.1 Microbial content in raw milk............................................................... 27
4.2 Bacterial characterization and speciation............................................... 28
4.3 Milk nutrient content ............................................................................ 29
4.4 Farmer’s characteristics associated with milk contamination.............. 31

CHAPTER FIVE: DISCUSSION .................................................................. 34

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS ......................... 39
6.1 Conclusion .......................................................................................... 39
6.2 Recommendations ............................................................................... 39

REFERENCES ......................................................................................... 41
APPENDICES ............................................................................................ 44
LIST OF TABLES

Table 1: Microbial limits in raw milk ........................................................................................................ 11
Table 2: Bacterial types commonly associated with bovine milk ............................................................. 11
Table 3: Variables considered in the study and scale of measures .......................................................... 20
Tables 4: Social demographic characteristics for the study participants .............................................. 27
Table 5: Total Bacterial Count milk samples ............................................................................................... 28
Table 6: Total Coliform Count milk samples .............................................................................................. 28
Table 7: Prevalence of *S. aureus* and *E. coli* in milk ........................................................................... 29
Table 8: Milk samples below nutrients standards ....................................................................................... 29
Table 9: The observed probable risk factors to contamination ................................................................. 30
Table 10: Final model stepwise regression on farm level characteristics ................................................. 31
LIST OF APPENDICES

Annex 8.1: Questionnaire

Annex 8.2: Participant information sheet

Annex 8.3: Participant informed consent form

Annex 8.4: Translated tools (Chitonga)

Annex 8.5: Raw milk quality from Namwala Central Dairy Corporative

Annex 8.6: Raw milk quality from Nchole Dairy Corporative

Annex 8.7: Raw milk quality from Mungaila Dairy Corporative

Annex 8.8: Checklist for observing contamination risk factors

Annex 8.9: Logistic regression outcome for farm level characteristics on milk contamination
LIST OF FIGURES

Figure 1: Conceptual framework. .................................................................................. 6
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cfu/ml</td>
<td>Colony Forming Unit per Milliliter</td>
</tr>
<tr>
<td>DAZ</td>
<td>Dairy Association of Zambia</td>
</tr>
<tr>
<td>DVO</td>
<td>District Veterinary Office</td>
</tr>
<tr>
<td>GHP</td>
<td>Good Hygiene Practice</td>
</tr>
<tr>
<td>MCC</td>
<td>Milk Collection Centre</td>
</tr>
<tr>
<td>SHDF</td>
<td>Small Holder Dairy Farmers</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SPC</td>
<td>Standard Plate Count</td>
</tr>
<tr>
<td>TBC</td>
<td>Total Bacterial Count</td>
</tr>
<tr>
<td>TCC</td>
<td>Total Coliform Count</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>ZABS</td>
<td>Zambia Bureau of Standards</td>
</tr>
<tr>
<td>DEC</td>
<td>Diarrhoeagenic E. coli</td>
</tr>
<tr>
<td>EPEC</td>
<td>Enteropathogenic E. coli</td>
</tr>
<tr>
<td>ETEC</td>
<td>Enterotoxigenic E. coli</td>
</tr>
<tr>
<td>EIEC</td>
<td>Enteroinvasive E. coli</td>
</tr>
<tr>
<td>EaggEC</td>
<td>Enteroaggregative E. coli</td>
</tr>
<tr>
<td>DAEC</td>
<td>Diffusely adherent E. coli</td>
</tr>
<tr>
<td>MoH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>VTEC</td>
<td>Vero cytotoxin- producing E. coli</td>
</tr>
<tr>
<td>STEC</td>
<td>Shiga Toxin-producing E. coli</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>BF</td>
<td>Butter Fat</td>
</tr>
<tr>
<td>FBD</td>
<td>Food Borne Diseases</td>
</tr>
<tr>
<td>SNF</td>
<td>Solid Non Fat</td>
</tr>
</tbody>
</table>
OIE  World Organisation for Animal Health
CHAPTER ONE: INTRODUCTION

1.1 Background

Milk (raw, heat treated or pasteurized) may harbour a variety of pathogens which can be a source of many human foodborne diseases (Bishop and White, 1986; Sorhaug and Stepaniak, 1997). Milk meant for human consumption must be free from any pathogenic organisms (Bertu et al., 2010). Microbial contamination in milk may cause milk-borne diseases to humans or cause milk spoilage (Kanyeka, 2014). Once microbes enter into milk, they can multiply and if pathogenic, may lead to illnesses among consumers (Barros et al. 2011). Improper processing, handling and management may also lead to contamination of processed milk (Bonfoh et al., 2003). Humans may be infected with milk-borne pathogens through consumption of infected raw or unpasteurized milk and milk products (Bertu et al., 2010).

Despite the existence of milk quality control measures and regulations at different points before processing and consumption, over 75% of milk marketed in many developing regions (including East, Central and Southern Africa) is sold raw or unpasteurized through informal channels, putting consumers at risk of milk borne diseases (Bertu et al., 2010; Oliver and Murinda, 2011). Milk is consumed raw at household and/or village level especially by pastoral and agro-pastoral communities who do not believe that milk could be a potential source of human infections (Bertu et al., 2010). Selling and consumption of raw or untreated milk and milk products poses health risks such as diarrheal diseases to consumers and the general public (Baynes, et al., 1999). Concerns about human health risks from the market pathways need to be addressed in the context of consumer practices. For example, boiling reduces or eliminates potential infection without discouraging the markets through which the majority of smallholder dairies and livestock keepers sell their milk (Kang’ethe et al., 2000).

The quality of milk is determined by its composition and overall hygiene. Many milk-borne epidemics of human diseases are spread through milk contamination. Sources of microbial contamination in milk include primary microbial contamination from the infected or sick lactating animal. The major secondary causes of microbial contamination occurs along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipment and water supplies used in sanitary activities. Other
secondary sources of microbial contamination occur during milk handling, transportation and storage. Tertiary microbial contamination occurs mainly through re-contamination of milk after processing due to unhygienic conditions and/or poor or improper handling and storage of milk for consumption (Parekh and Subhash, 2008). Consumption of contaminated food like milk may lead to food-borne diseases (FBDs).

The World Health Organisation (WHO) has described FBDs as illnesses of an infectious or toxic nature caused by, or thought to have been caused by the consumption of food and water (Adams and Motarjemi, 1999). It is estimated that up to a third of people in developed countries are affected by FBDs (WHO, 2009). FBDs are caused by the consumption of foods exposed to hazards that may be biological or pathogenic (viruses, bacteria, parasites), chemical (heavy metals and toxins), and physical such as glass fragments and bone chips (Schmidt et al., 2003). According to the WHO, about 62% of all human pathogens are zoonotic (Taylor et al., 2001); while World Organisation on animal health (OIE) estimates that 75% of all emerging human diseases originate from animal reservoirs (Vallat, 2007). Consequently, animal product foods have been found to be responsible for the majority of FBDs (De Buyser et al., 2001). Incidences rise with increasing access to such foods especially where there is inadequate hygiene, limited safety inspection or unsatisfactory heating to kill pathogens (McCrindle, 2008).

There are several diarrheal diseases associated with water and poor sanitation which occur in many African countries. In places where dairy farming is practiced, milk can be another source of diarrheal diseases. In Zambia, many rural areas are involved in cattle ranching and produce high volumes of raw milk making it easy for household members to access raw milk. Many milk processing plants are found in urban and not in rural areas. This contribute to selling unprocessed milk to consumers through informal channels (Pandley, 2014). Namwala is among the districts in Zambia where cattle ranching is highly practiced and there is no milk processing plant making it easier for many people to have access to raw milk. However, many farmers may be unaware of the microbial milk content they are producing. This poses a great risk to consumers as the milk may be a source of many diarrheal diseases in the area. In Namwala district, there is limited information about risk factors associated with microbial contamination of milk at farm level. Therefore, there was need to generate evidence with regard to quantifying and identifying bacteria species that are common contaminants of milk from smallholder dairy farmers in
Namwala district. There was also need to determine the concentration of nutrients in milk and the amount of added water to milk. These qualities were compared against the Zambian standards.

1.2 Problem statement

Raw milk is an important vehicle for the transmission of milk-borne pathogens to humans, and milk can easily be contaminated during milking and handling (Eneroth et al., 2001). Milk borne contamination by pathogens is one of the public health concerns worldwide as it contributes to many diarrheal diseases and outbreaks (Baylis, 2009). Being a highly perishable commodity and highly nutritious food, milk serves as an ideal medium for the growth and multiplication of various microorganisms (Galbraith et al., 1982). Poor or improper handling of milk can exert both a public health and economic constraint, thus requiring hygienic vigilance throughout the milk value chain (Swai and Schoonman, 2011). In many parts of the world, milk remains a significant source of microbial infections and other food borne diseases (Pandley et al., 2014). In England and Wales, yearly outbreak of food poisoning from Salmonella and Campylobacter jejuni in milk not receiving heat treatment or imperfectly pasteurized have been reported (Al-Tahiri, 2005). Zucali et al., (2015), indicated that consumption of contaminated milk with pathogens has continued to be a public health challenge in the United States (US).

The World Health Organisation (WHO, 2010) estimated that every year there are about 47.8 million milk borne illnesses in the United States (16,000 cases for 100,000 inhabitants), 2 million in the United Kingdom (3,400 cases for 100,000 inhabitants) and 750,000 in France (1,220 cases for 100,000 inhabitants). The WHO, (2010) also estimated that about 2.1 million people died in the year 2000 due to milk borne diarrheal diseases. In 2010, about 30% foodborne illness outbreaks in the US were connected to raw milk consumption (Riahi-Zanjani and Balali-Mood, 2013). According to a study conducted by Scholder, et al, (2013) in Tanzania, microbial assessment of bovine milk showed the presence of pathogens such as E. coli O157:H7 (78%) and Salmonella (66%).

In Southern Africa, Staphylococcus aureus has been isolated from samples (79%) of raw milk and lightly heated dairy products in Zimbabwe (Al-Tahiri, 2005). In Zambia, contamination of milk with pathogens poses a great danger to the public as many people in the peri urban and rural areas where cattle ranching are practiced drink milk in its raw form (Pandley, 2014). Such milk
may be contaminated with pathogens or milk spoilage microbes putting the public at risk of developing diseases. Over 90% of households in Namwala District have animals and have easy access to raw milk at farm level (Showa, 2016). However, little is known about the quality of milk consumed in Namwala and associated contamination risk factors.

1.3 Justification

Microbial contamination in milk poses a risk to the public as it may lead to milk borne diseases and illnesses. Much of the milk available in rural areas is consumed in the form of unpasteurized, sour milk or milk products. Namwala is among the districts in Zambia where cattle ranching is highly practiced but lacks a milk processing plant. Thus, making it easier for many people to have access to raw milk. This poses a great risk to consumers as the milk may be the source of many diarrheal diseases.

Although it is argued that Zambia has not been recording outbreaks associated to milk born infection, this does not mean absence of milk borne illnesses/diseases. For instance, in Namwala where this research was conducted, there was limited information to ascertain the microbial content and quality of raw milk in relation to the risk factors. Many studies conducted in Zambia have focused on the microbial level content in milk. For instance, Kunda, et. al, (2014) focused their study on microbial levels in milk in Lusaka district without specifying the types of microbes isolated in milk and associated risk factors.

It was anticipated that the study would inform policy and provide a basis for monitoring the quality of milk produced in Namwala District, as well as bring awareness to farmers on the best hygienic practices. The research also intended to inform the general public about the common species of bacteria responsible for microbial contamination in milk at farm level and how to reduce contamination. In addition, evidence-based advice was to be provided to the District Health officials and other stakeholders at health facility level to educate people on dangers of consuming untreated milk and the best milking and handling practices in Namwala district.
1.4 Research questions

What is the microbial content and quality of raw milk produced and what are the possible risk factors associated with microbial contamination of milk from small holder farms in Namwala district?

1.5 Objectives

1.5.1 General objective

To determine the level of microbial content and quality of raw milk produced and associated risk factors in Namwala district.

1.5.2 Specific objectives

i. To determine the microbial content of raw milk produced from selected small holder farmers in Namwala district and its conformity to set Zambian standards.

ii. To characterize bacterial speciation of *S. aureus* and *E. coli* in raw milk from selected farms in Namwala district.

iii. To determine the nutrient content of raw milk produced from selected small holder farmers in Namwala district and its conformity to set Zambian standards.

iv. To identify possible risk factors associated with microbial contamination in raw milk from selected farms in Namwala District.

1.6 Conceptual framework

To realize the objectives of the study, it was important to consider the risk factors associated with *E.coli* and other *S. aureus* in milk at farm level. The concept of food safety of milk from small holder farmers under this study focused on the microbiological composition and risk factors associated with contamination.

The conceptual framework by Kanyeka, (2014) was modified to explain the factors. The framework identifies factors likely to have an effect on milk quality. These include hygiene practices, milk composition quality, microbial contamination in milk and socio-demographic factors (Figure 1).
Figure 1: Conceptual framework (Source: Kanyeka, 2014).

The model considers milk as safe when it is microbial free such that even after being consumed by humans does not cause any disease. The conceptual framework also explains the different pathways through which milk can be contaminated from the producer to the consumer. Milk consumed can be contaminated at different levels. In Namwala, possible points for contamination may occur at producer level, where factors include health of the animal, hygiene of workers and their practices and type of utensils used. Socio demographic factors refer to age, sex and educational level were other factors considered in the study. The milk consumed without
treatment may be a risk factor to the consumers at the house hold level. The vendors may also contaminate the milk through containers used and their hygienic practices which also pose a risk to consumers. The attitude of consumers (boiling the milk) may lead to poor milk quality which can allow microbial infection through handling and affect human health. The milk collection center is another point where milk can be contaminated, particularly if milk containers are not routinely cleaned. These factors can affect the milk quality and ultimately the health of the consumer.
CHAPTER TWO: LITERATURE REVIEW

The quality of milk consumed contributes to safeguarding the health of the consumer. Appearance and chemical composition is critical to ensuring provision of an acceptable product that does not lead to infections in humans. Milk is a yellowish-white non-transparent food for offspring of mammals before they are able to eat and digest other types of food. It contains a balanced form of the necessary and digestible elements for building and maintaining the body (Pandey and Voskuil, 2011). The main composition of milk is water (87 – 88%), while the remaining part is total milk solids. These include carbohydrates, fat, proteins and ash or minerals. Moreover, milk is an excellent source of high quality protein, vitamins, minerals such as calcium and phosphorus. Fresh milk has a pleasant soft and sweet taste and carries hardly any smell (Angulo et al., 2009).

Milk is sterile when it is in the udder of a healthy animal but becomes contaminated with bacteria mainly during or after milking (Karimuribo et al., 2005; Makerere University, 2011). It may be a result of an infected or sick animal, human, environment, water and equipment used for milking, or contamination during storage of milk. Exposure of milk to these sources or conditions may lead to increased microbial contamination and affect its quality. Sometimes re-contamination may occur after processing, mainly due to unhygienic conditions, poor or improper handling of milk during consumption (Parekh and Subhash, 2008). In addition, water used for adulteration by unscrupulous and unfaithful workers/sellers may be contaminated and may cause health problems (Karimuribo et al., 2005; Swai and Schoonman, 2011).

The quality of milk may be contaminated and graded as poor. Consumption of poor quality milk is considered a public health concern (Karimuribo et al., 2005; Syit, 2008; Mdegela et al., 2009). Diseases arising from intake of poor quality milk include bovine tuberculosis, brucellosis, anthrax, listeriosis, salmonellosis, leptospirosis, Q fever, and campylobacteriosis. *E. coli* O157:H7 has also recently been reported as an emerging new milk-borne bacterial pathogen with very serious health effects (Sivapalasingams et al., 2004).

Harmful pathogenic bacteria that may be isolated from milk present serious threat to human health and contribute up to 90% of all dairy related diseases (De Buyser et al., 2001; Sivapalasingams et al., 2004; Donkor et al., 2007).
In promoting safety, proper milking, cleaning and sanitizing procedures of equipment and environments are essential to ensuring quality of milk. Many countries have implemented laws and regulations concerning the composition and hygienic quality of milk and milk products to protect the consumers and promote public health (Pandey and Voskuil, 2011). However, these laws and regulations are not often adhered to in developing countries, making milk-borne diseases a higher health risk to the public. This is exemplified by over 75% of milk marketed in many developing countries which is sold raw/unpasteurized through informal channels (Bertu et al., 2010; Oliver and Murinda, 2011). It is documented that a big percentage of people in Tanzania especially in rural areas consume raw milk (Mullins, 1993; Kurwijila et al., 1995). This predisposes them to the risk of contracting zoonosis, and other milk-borne diseases. In Zambia, the number of people who may be exposed to zoonotic diseases is unsubstantiated.

2.1 Hygiene, Handling and Microbial Quality of Raw Milk

After milking environmental contamination which occurs increases the total bacteria count up to 50,000 per ml. In some cases the bacterial count may reach several millions per milliliter (Pandey and Voskuil, 2011). That count level indicates a very poor hygienic standard of milk during milking and handling or milk from a diseased animal. The presence of coliform bacteria, particularly *E. coli* in raw milk is an indicator of faecal contamination which implies poor hygienic conditions and un-sanitized environment (Pandey and Voskuil, 2011). Of the approximate 600 million tonnes per annum of milk produced in the world today, 85% is bovine milk, 11% is buffalo milk, 2% is caprine milk and 2% is ovine milk (Jayarao et al., 2006). In developing countries, most of the milk produced by small scale farmers is dominated by local herds of cattle (Pandey and Voskuil, 2011).

If the milk is cooled to 4°C immediately after milking, it nearly maintains its original quality and remains safe for processing and consumption. Temperature of storage and time after milking are also important in determining milk quality. These factors influence the rate at which bacteria increases in number (Omore et al., 2005). To prevent quick multiplication of bacteria, the milk has to be produced as hygienic as possible and should be cooled or heated quickly (Pandey and Voskuil, 2011).

Among pathogens causing infections in humans, *E. coli* strains isolated from intestinal diseases have been grouped into at least six different diarrhoeagenic *E. coli* (DEC) groups based on
specific virulence factors and phenotypic traits. These include enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enteroaggregative E. coli (EAggEC), diffusely adherent E. coli (DAEC), and Vero cytotoxin-producing E. coli (VTEC) or Shiga toxin-producing E. coli (STEC) (Oliver et al., 2005). Food-borne outbreaks have been particularly associated with E. coli VTEC, and to a lesser extent EPEC, ETEC and EaggEC strains. Among the VTEC strains, E. coli O157: [H7] (for instance VTEC O157) has become widely recognized as a very important cause of food-borne illness over the last two decades (Oliver et al., 2005).

2.1.1 Udder health and milking hygiene

The bacteria that cause udder infections in a herd mainly come from infected quarters or cows and the environment in which animals are kept (Blood and Radostits, 1989). Spread of contagious bacteria to teats of uninfected quarters or cows, occurs primarily at milking time (NMC, 1987). The rate of new infections is however, greatly reduced if proper milking hygiene practices are followed at all times. Pre-milking udder hygiene for example washing with clean water and drying using hand towels reduces milk contamination by transient bacteria located on the udder. Teats and the lower portion of the udder must be washed with a warm sanitizing solution, which should be changed periodically to prevent accumulation of pathogens in the solution (Robert, 1996). The use of post milking teat disinfectants has proved to be an effective measure in reducing new infections because it reduces the resident teat skin bacterial population, which is the main source of infection for the mammary gland (Kurwijila, 1991).

2.1.2 Personal hygiene and bacterial quality of raw milk

People involved in dairying should maintain cleanliness and it is important for milkmen to be in good health so that they do not become a source of infectious diseases. Organisms may drop from hands, clothing, nose, and mouth and from sneezing and coughing (Kurwijila, 1998).

Coliforms counts indicate the level of hygiene, since coliforms are microorganisms of faecal origin. To improve the quality of food consumed, East African Countries have harmonized standards for some products including milk. The standard plate count per milliliter (or gram) for raw reconstituted (prepared) milk or pasteurized milk (at the plant in the final container) does not exceed 30,000 (EAS, 2007). Table 1 shows the classification for Standard Plate Count/ ml in raw milk in East African countries.
Table 1: Microbial limits in raw milk

<table>
<thead>
<tr>
<th>GRADE</th>
<th>cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>A or I</td>
<td>&lt;200,000</td>
</tr>
<tr>
<td>B or II</td>
<td>&gt;200,000-1,000,000</td>
</tr>
<tr>
<td>C or III</td>
<td>&gt;1,000,000-2,000,000</td>
</tr>
</tbody>
</table>

**Source:** East African Standards (2007)

Table 1 clearly shows the grading of the microbes with the first class of A (I) indicating less than 200,000 standard plate count per milliliter (cfu/ml) and worst quality with the higher microbial content ranging from 1,000,000 to 2,000,000 (cfu/ml).

Ordinary bacteria (Table 2) easily found in milk multiply under favorable temperatures to cause spoilage and/or become a health risks due bacterial infection or production of toxins. Some bacteria such as *Staphylococcus aureus* and *E.coli*, if allowed to multiply may produce heat labile toxins that cause illness. Time elapsed since milking and temperature at which milk is stored are the main factors that influence bacterial counts in milk. The major milk-borne pathogens of concern are zoonoses and environmental coliforms of faecal origin. The latter are commonly introduced in milk due to poor handling at farm and along the market pathway (Pandey and Voskuil, 2011).

Table 2: Bacterial types commonly associated with bovine milk

<table>
<thead>
<tr>
<th>TYPE OF BACTERIA</th>
<th>EFFECT ON MILK AND CONSUMERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus: L. lactis, L. bulgaricua, L. acidophilus, Leuconostoc lactis, Propionibacterium</td>
<td>Acid production/fermentation</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>Pathogenic and Spoilage</td>
</tr>
<tr>
<td>Staphylococci: Staph. Aureus</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>Streptococcus: Strep. Agalactiae</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>Coliforms (mostly introduced through poor hygiene)</td>
<td>Some are Zoonotic and pathogenic (e.g. E. coli-0157:H7)</td>
</tr>
</tbody>
</table>

**Source:** Adapted from Kaiza Kilango G. (2011)

Microbes have an ability to grow in unprocessed food such as milk, in an environment allowing amplification, and long term survival. Thus, promoting opportunities to infect humans through contaminated foods (Newell *et al*., 2010). It is estimated that risk factors account for more than 80% milk contamination (FAO, 1989). The rapid growth of microorganisms, particularly at high
ambient temperature can cause marked deterioration in quality of milk and dairy products (FAO, 1989).

Microbes in milk can lead to ill health. A study conducted in England to assess communicable diseases associated with milk and its products showed 179 (77%) of the outbreaks and 7350 (78%) of the cases were due to unpasteurized or defectively pasteurized cows' milk or due to milk contaminated after pasteurization (Galbraith et al., 1982). It also showed that 133 (74%) of these outbreaks were due to salmonellas (Galbraith et al., 1982).

A study conducted in Zimbabwe by Goulet, (2014) to determine total microbial content of milk produced by small holders farmers found the presence of microbial contamination. The milk had high Total Bacterial Count (TBC), faecal coliforms with E. coli and S. aureus present in raw and processed milk. Similarly, in a Rwandan study 5.2% of the samples contained Salmonella pathogens (Kiyota et al., 2014). Another study by Scholder, et al, (2013) in Tanzania also showed the presence of pathogens (85%) such as E. coli O157:H7 and Salmonella.

2.1.3 Staphylococcus aureus in Milk
Milk may be contaminated with Staphylococcus aureus, a facultative anaerobic, Gram-positive coccus, which appears as grape-like clusters. When viewed through a microscope, it also appears as large, round, golden-yellow colonies. It is often associated with hemolysis, when grown on blood agar plates (Ryan and Ray, 2004) as quoted by Kaiza, (2011). The golden appearance is the etymological root of the bacteria's name; aureus which means "golden" in Latin. S. aureus is catalase positive (meaning that it can produce the enzyme "catalase") and able to convert hydrogen peroxide (H2O2) to water and oxygen. This characteristic makes the catalase test useful to distinguish staphylococci from enterococci and streptococci. A small percentage of S. aureus can be differentiated from most other staphylococci by the coagulase test. S. aureus is primarily coagulase-positive (meaning that it can produce "coagulase", a protein product, which is an enzyme) that causes clot formation while most other Staphylococcus species are coagulase-negative (Ryan and Ray, 2004). However, while the majority of S. aureus are coagulase-positive, some may be atypical in that they do not produce coagulase. The most common organism in patients with nosocomial bacteremia is coagulase-negative staphylococcus (Matthews et al., 1997). Incorrect identification of an isolate can impact implementation of effective treatment and/or control measures (Matthews et al., 1997).
2.1.4 Staphylococcal food poisoning

*Staphylococcus aureus* is an important food-borne pathogen. It is a versatile pathogen of humans and animals and causes a wide variety of diseases ranging in severity from slight skin infection to more severe diseases such as pneumonia and septicemia. Of particular relevance to the food processing industry is the ability of some *S. aureus* strains to produce heat stable enterotoxins that cause staphylococcal food poisoning (SFP), which ranks as one of the most prevalent causes of gastroenteritis worldwide (Dinges *et al*., 2000). The intoxication is characterized by enteric responses like diarrhea, abdominal cramps, and vomiting within 1 to 6 hours after consumption of contaminated food (Leenalitha and Peter, 2007). The toxins are heat stable proteins (Leenalitha and Peter, 2007). The bacterium is heat labile and does not compete well with other microorganisms. Therefore, contamination usually occurs after the food has been processed when there is little competition from other microorganisms.

*Staphylococci aureus* usually gains access to foods from food handlers or other surfaces like the processing equipment. Although Staphylococci are commonly found on animal skins, as well as water and soil, bacteria from food handlers and other human sources are considered as the most important contributing factors to intoxications associated with food (Leenalitha and Peter, 2007). Food poisoning is of great concern to food industries and regulatory agencies as it represents massive health and economic losses. The foods that are commonly contaminated by staphylococcus endotoxins (SEs) are baked dessert items such as cream filled pastries, cream pies, chocolate éclairs, meat and meat products, potatoes, tuna, chicken, turkey, ready-to-eat salads, eggs, poultry, dairy and milk products (Leenalitha and Peter, 2007).

*Staphylococcus aureus* does not form spores. Thus, *S. aureus* contamination can be readily avoided by heat treatment of food. Nevertheless, it remains a major cause of food borne diseases because it can contaminate food products during preparation and processing. *Staphylococcus aureus* is found in the nostrils, and on the skin and hair of warm-blooded animals. Up to 30-50% of the human populations are carriers (Le Loir *et al*., 2003).

*Staphylococcus aureus* is able to grow in a wide range of temperatures ranging from 7°C to 48.5°C with an optimum of 30°C to 37°C (Schmitt *et al*., 1990), pH of 4.2 to 9.3 and an optimum of 7 to 7.5 and Sodium chloride (NaCl) concentrations of up to 15% (Bergdoll, 1989). These characteristics enable *S. aureus* to grow in a wide variety of foods. This, also explains
their ecological and their presence in foodstuffs that require manipulation during processing, and their appearance in fermented food products, such as cheeses.

Grimaud et al. (2007) found a high bacterial load of $2 \times 10^6$ cfu/ml in raw milk in a survey that was carried out in Mbarara a major milk producing region in Uganda, between June and August, 2004. The high bacterial load was attributed by the authors to poor hygiene conditions at production and lack of an efficient preservation system to limit bacteria proliferation during transportation to the market. In a study of raw milk in Namibia, Bille et al. (2009) found total protein 3.2%, BF 3.63 %, total solids 12.33 %, SNF 8.7 % and pH ranging from 6.0 - 6.7. The total aerobic count ranged from $7.8 \times 10^4$ - $1.3 \times 10^6$ cfu/ml and coliforms from $2.4 \times 10^2$ - $2.3 \times 10^3$ cfu/ml. The authors concluded that the high number of bacteria in milk might have been associated with unclean udders, teats and milk storage tanks.

Sindani (2012) in a study in Malawi, found overall bacteria count of raw milk to be high, with a mean of $3.4 \times 10^7$ cfu/ml of raw milk collected from smallholder farmers. The high bacteria count indicated that the quality of milk produced by farmers and subsequently collected by processors was of poor quality. This calls for better hygienic measures during production and handling of milk. In a study of raw milk in Kenya, Mwangi et al. (2000), found TBC at $1,490 \times 10^6$ cfu/ml (range $0.25 \times 10^6$ - $25,100 \times 10^6$ cfu/ml of raw milk) and coliforms count at $149 \times 10^3$ cfu/ml (range $0.10 \times 10^3$ - $1,540 \times 10^3$ cfu/ml of raw milk). Eighty two (82) % and 58 % of raw milk samples did not meet Kenyan national standards for total bacteria and coliforms count respectively. Approximately 13% of samples were adulterated with water. According to Kenyan national standards, maximum bacterial counts per millilitre for raw milk is 2,000,000 cfu/ml and for total and coliforms count is 50,000 cfu/ml. Pasteurized milk standards for total bacterial count is 50,000 cfu/ml and 10 cfu/ml for total coliforms count.

In a Swaziland study conducted from 1999 to April, 2000, TBC was high (greater than $1 \times 10^7$ cfu/ml of raw milk) and coliforms counts were also high (greater than $7 \times 10^4$ cfu/ml of raw milk). Antibiotic residues were present in 35% of the raw milk samples while water adulteration was at least 5% of the milk volume. Fat was greater than 3.5%, protein greater than 3.4% and lactose greater than 4.4% (Fakudze and Dlamini, 2001). In a similar study conducted to determine the quality of raw milk in the Dar es Salaam region of Tanzania, 7.0% of the raw milk was positive for ARs and TBC was $8.2 \times 10^6$ cfu/ml (Kivaria et al., 2006). Authors concluded
that the milk in the Dar es Salaam region was of poor quality. Schooman and Swai (2011) in another Tanzanian study found 22% of raw milk had specific gravity below 1.026 suggesting adulteration with water.

In Zambia, available evidence from a study to assess the composition and microbial quality of pasteurized and Ultra High Temperature treated milk in Lusaka found presence of microbial contamination. The Total Bacterial Count (TBC) was present from pasteurized milk samples in the range of 6,000 to 38,000 cfu/ml. In addition, Total Coliform Count from the samples ranged from 68-188 cfu/ml (Kunda, *et al*, 2014). These figures are higher than the acceptable standards of 5cfu/ml (Kunda, *et al*, 2014). Although pathogenic milk contamination poses a great danger to the public available literature in Zambia is limited on explaining risk factors associated with milk contamination. In Namwala, no study has been carried out to ascertain the microbial contamination in milk. Therefore, it is important to identify the microbes present in raw milk and associated risk factors in order to provide specific interventions to protect the public from harmful microorganisms.

2.2 Health and economic impact of unsafe foods

Food safety is an essential public health concern for all countries. Foodborne diseases due to microbial pathogens, bio toxins, allergens, and chemical contaminants in food represent serious threats to the health of thousands of millions of people. Serious outbreaks of foodborne disease have been documented on every continent in the past decades, illustrating both the public health and social significance of these diseases. Consumers everywhere view foodborne disease outbreaks with ever-increasing concern. Outbreaks are likely, however, to be only the most visible aspect of a much broader, more persistent problem. Foodborne diseases not only significantly affect people's health and well-being, but they also have economic consequences for individuals, families, communities, businesses and countries. These diseases impose a substantial burden on health-care systems and markedly reduce economic productivity. Poor people tend to live from day to day, and loss of income due to foodborne illness perpetuates the cycle of poverty (FAO, 2006).

Food safety issues are a sensitive area in terms of public health management especially from an economic point of view. The subject is made more confusing because the sources of contamination are variable and can take place at any point in the food production and marketing
chain. Currently there is limited scientific information to quantify the magnitude of the problem and to provide baseline data from which informed decisions can be made. More information is needed that will help improved regulatory policy decisions to be made. Scientific information will also help ensure more effective control when outbreaks occur (Mangwayana et al., 2000).

Unnevehr and Hirschhorn (2000) reported that 70% of deaths among children under 5 are linked to biologically contaminated food and water globally. Impacts include fatalities in vulnerable groups, severe and disabling long-term effects such as joint disease, kidney failure, cardiac, retinal and neurological disorder. Evidence is growing that in developing countries, ill health is not only a personal and household tragedy, but a major factor in causing and perpetuating poverty (Lawson, 2004).

The cost of food borne diseases is estimated to exceed $5 billion per year in the United States (Foegeding et al., 1994), and $1.3 billion annually in Canada (Todd, 1989). Economic burden on people in India affected by an outbreak of *Staphylococcus aureus* food poisoning was found to be higher than in case of a similar outbreak in the US (Sudhakar et al., 1988). Unsafe food and food borne illnesses also affect producers because they earn a poor reputation which may take time to overcome. Those who are engaged in marketing unsafe food such as vendors or wholesalers also receive a tarnished reputation. This means that they lose their market and therefore their incomes become eroded (Nhachi and Kasilo, 1996).

In promoting food safety the Zambian government adopted the East African standards on milk safety in order to safeguard health of the public. The Zambian Bureau of Standards (ZABS), the body established to standardize and control the quality of foods and other commodities in the country outlines the standards. The standard plate count per milliliter (or gram) for raw reconstituted (prepared) milk or pasteurized milk (at the plant in the final container) is stated not to exceed 50,000 while Total bacterial count should not exceed 200, 000 per milliliter (ZABS, 2011). The government has legalized these standards through the Food and Drugs Act chapter 303 of the laws of Zambia. The standards further stipulate that no pathogenic microbes (*E.coli, S. aureus* etc.) should be found in all foods produced, imported or sold in Zambia (FDA, 2005). However, it is unclear if milk producers in rural areas do abide by the stipulated standards.
CHAPTER THREE: METHODOLOGY

3.1 Study design

The study employed a one staged cross sectional design to establish the magnitude of microbial contaminants in raw bovine milk and risk factors associated with contamination.

3.2 Study population

The study population was individual small holder farmers in rural areas of Namwala District and each farmer represented a study unit. It was the same farmers with lactating cows from whom raw cow’s milk was collected and questionnaires administered.

3.3 Study setting

The study was conducted in Namwala districts located in Southern Province of Zambia. The district was purposely selected because when compared to other districts in Zambia, Namwala has the highest number of small-scale livestock keepers (CSO, 2010). These small livestock keepers practice different farming systems (pastoralist, agro-pastoralist and smallholder dairying). They also supply milk to the local community and milk collection centres. In Namwala, there are 106 Small holder Dairy Farmers who supply milk to Parmalat collection center; and it has the majority of milk suppliers as compared to other milk collection centres in the province (Parmalat, 2016). In Namwala, more than 90% of the people are dependent on raw milk to supplement their diet. There is also no milk processing company in Namwala (Showa, 2016). Because of this, the population is at a very high risk of contracting milk borne illness and zoonotic diseases.

3.3.1 Inclusion criteria

The study included all small holder dairy farmers. These were men and women with lactating cows. Each study participant produced at least 10 liters of milk per day at the time of the study.

3.3.2 Exclusion criteria

Small holder dairy farmers whose cows had inconsistence milk supply for a period of six months prior to the study were not eligible. In addition, small holder dairy farmers who were unwilling to participate in the study were not considered.
3.4 Recruitment procedures

Three Milk Collection Centres (MCCs) in Namwala district were included in the study out of which the sampling frame was generated. Considering that each milk collection centre had a different number of dairy farmers, probability proportion to size was employed. The probability sampling method (simple random sampling) was employed where each farmer was given equal chances of being selected. From the total population of farmers (106), at least 60 of the farmers were required in order to detect the difference in the study at 80% power. This study recruited a total of 70 farmers increasing the power to above 80%. The study participants were recruited as follows: Namwala central (n=16), Nchole (n=24) and Mungaila (n=30).

The study collected data using laboratory analysis and a survey. Milk used for laboratory analysis was obtained from the 70 farmers. Microbial risk assessment was carried out by assessing milk for microbial load and the presence of Staphylococcus aureus or E. coli from the same farmers (n=70). These farmers from whom milk was collected were also relied upon to collect information using a questionnaire. These two approaches were employed in order to link the laboratory outcome to contamination risk factors.

3.4.1 Sample size consideration

The number of milk samples to be used in the study was determined by using the formula according to Fisher et al., (1991). The sample size was estimated based on an estimated prevalence of 85% (prevalence of S. aureus from smallholder dairy and pastoral cattle herds in the urban and peri-urban areas of the Dodoma municipality in Central Tanzania and from pastoral herds in Dodoma and Morogoro regions, Tanzania) as reported by Mdegela et al. (2005) where:

\[ n = (z)^2 \times p \times (1-p)/d^2 \]

- \( n \) = the minimum sample size required
- \( Z \) = is the estimated standard variation at 95% confidence interval (CI) considered the point of the normal distribution corresponding to the level of significance \( (Z=1.96) \).
- \( P \) = Estimated prevalence of Staphylococcus aureus
- \( 1-P \) = the probability of having no hazards disease;
• \( d \) = precision level (0.09)
• \( e \) = is the estimated margin of error to be considered at 0.05 or 5%.

\[
n = 3.84 \times 0.85(1-0.85)/0.10^2
\]

**n=60 samples (Actual=70 samples)**

The prevalence of *S. aureus* in cow’s milk has been estimated in previous studies as 85%. A total of **70 cow milk samples** (one from each farmer) were required to detect 40% reduction at 80% power for a two sided 5% level of significance using Pearson’s chi squared test. The actual samples collected in the study were 70 which increased the power of the study to above 85%. The milk samples were collected from the same farmers to whom the questionnaires were administered.

**3.5 Variables included in the study**

The dependent variables considered in the study were milk quality, microbial contamination level and microbial characterization and speciation. The independent variables were age, sex, education level, butter fat content, solid nonfat, *E. coli*, *S. aureus*, and water adulteration in milk. Table 3 shows the variables included in the study. The first dependent variable was considering the microbial characterization and speciation specifically looking at the prevalence of *S. aureus* or *E. coli*.

The second dependent variable focused on microbial contamination levels in milk in relation to the Zambian bureau of standards and the Food and Drugs Act, chapter 303 of the laws of Zambia (Total Bacterial Count at 200, 000 cfu/ml and Total Coliform Count at 50, 000 cfu/ml standard limits). The third dependent variable focused on the milk quality which considered the levels density, solid nonfat, water adulteration and fat content in milk. The legal limits for the nutrients in raw milk are that butter fat content should not exceed 3.2%, solid nonfat should not exceed 8.3% while density should not exceed 1.028g/cm³. In keeping with ZABS, no milk should be adulterated with water (ZABS, 2011).
<table>
<thead>
<tr>
<th>Type of variable</th>
<th>Variable</th>
<th>Indicator</th>
<th>Scale of Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent</td>
<td>Microbial characterization-colony count and speciation</td>
<td>% of samples contaminated with <em>S. aureus</em> and <em>E. coli</em></td>
<td>Binary</td>
</tr>
<tr>
<td></td>
<td>Microbial contamination level</td>
<td>% of samples with high TBC and TCC</td>
<td>Binary</td>
</tr>
<tr>
<td></td>
<td>Milk composition quality</td>
<td>% of samples with poor nutrient quality</td>
<td>Binary</td>
</tr>
<tr>
<td>Independent</td>
<td><em>E. coli</em></td>
<td>Proportion of samples tested positive of <em>E.coli</em></td>
<td>Proportion</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>Proportion of samples tested positive of <em>S.aureus</em></td>
<td>Proportion</td>
</tr>
<tr>
<td></td>
<td>Butter fat content</td>
<td>Proportion of samples with high BF content</td>
<td>Proportion</td>
</tr>
<tr>
<td></td>
<td>Solid non fat</td>
<td>Proportion of samples with high SNF content</td>
<td>Proportion</td>
</tr>
<tr>
<td></td>
<td>Density</td>
<td>Proportion of samples with high density</td>
<td>Proportion</td>
</tr>
<tr>
<td></td>
<td>Water adulteration</td>
<td>Proportion of samples tested positive of E.coli</td>
<td>Proportion</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>Years at last birthday</td>
<td>Interval</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>Male/Female</td>
<td>Nominal</td>
</tr>
<tr>
<td></td>
<td>Education level</td>
<td>No education, primary, secondary and tertiary</td>
<td>Nominal</td>
</tr>
</tbody>
</table>

### 3.6 Data collection

The study employed total enumeration of all milk collection centres from which the sampling frame was generated. Simple randomly sampling method was employed to select the required sample size (70) of the farmers. Questionnaires were administered to the same farmers from whom milk samples were collected.
3.6.1 Field data Collection

The structured questionnaire was used to collect information from small scale dairy farmers with lactating cows. The questionnaire was made with pre-coded response choices (closed-ended questions with a few open-ended questions). The questionnaire was also translated into the local language (Tonga) to make it easier to communicate with respondent (Annex 8.5). The questionnaire was administered using face-to-face interview with selected small scale farmers (70) at their households (farms). Information was collected on possible risk factors for microbial contaminations and compositional quality of milk. The information collected included milk production, types and practices of milking and milk handling, sanitary measures taken during milking, utensils used for milking, milk storage and storage conditions, uses of milk (for selling or domestic purposes), consumption of raw milk and milk products as well as health care seeking behavior of farmers (Annex 8.1). After the interview, direct observations on general cleanliness, hygienic conditions and practices with regard to milk were also noted using a checklist (Annex 8.8).

Prior to start of data collection, pre-testing of questionnaire was carried out at one of the villages not included in the study. This was meant to check for clarity, sequence and applicability of the questions and estimating the duration for each questionnaire.

3.6.2 Laboratory data collection

3.6.2.1 Sampling of milk

The Principal researcher with support from two trained Field Research Assistants reached out to households in the selected dairy cooperatives using a list of the selected eligible farmers. Milk samples were obtained from each of the 70 farmers. Milk samples were collected directly from the storage containers used by corresponding farmers in the visited households. Approximately 50 ml of milk was aseptically collected and put into a sterile screw capped falcon tubes. All samples were drawn from pooled containers containing milk that were milked on that particular day. This is the same milk which was either consumed at household level or sold to the public or both.
3.6.2.2 Milk sample handling
All samples were coded with randomly selected numbers for identification and stored in a cool box with ice packs during field work. Thereafter, the samples were stored at -20°C temperature after each day’s field work. Later on samples were transported within 48 hours after collection under controlled temperature to the University of Zambia, School of Veterinary Medicine for bacteriological analysis. The data collected on the microbial status in milk were entered in the laboratory forms in readiness for use at analysis stage.

3.6.2.3 Laboratory Analysis of Milk Samples
Analyses were carried out in the Microbiology Laboratory in the School of Veterinary Medicine, University of Zambia. Analysis for microbial status of raw milk involved establishing the total bacterial counts (TBC) and total coliform count (TCC). Bacterial speciation and characterization involved isolation of some common pathogenic microbes such as *E. coli* and *S. aureus*. The milk composition was determined by the levels of nutrients in milk using the lactiCheck ultrasound machine (Page & Pedersen International Ltd, USA) and compared to Zambian standards. The primary outcome for milk samples was calculated as the total number of cow milk that failed microbial standards and expressed as a percentage.

3.6.2.4 Determination of microbial content and quality of raw bovine milk

3.6.2.4.1 Media preparation and storage
All the media used in this study were prepared according to manufacturer’s instructions. The guidance clearly delineates the requirements of both quality control laboratories and the manufacturers of culture media in maintaining compliance throughout the performance testing process (EN ISO 11133: 2014).

3.7 Laboratory procedures

3.7.1 Preparation of control isolates
About 500 ml of raw cow milk was collected from School of Veterinary Farm University of Zambia (UNZA) as a control sample. The sample was sterilized by boiling, cooled and placed in a sterile bottle. Part of the sample was inoculated with reference strains of *Escherichia coli* ATCC® 2262-79 (DEC9B) and *Staphylococcus aureus* NCTC 6571/ATCC® 9144. A sterile
pipette was used to transfer 25 ml of the milk sample into a sterile conical flask containing 225 ml of Buffered Peptone water (BPW).

3.7.2. Sample preparation, inoculation and incubation

A total of 3 sterile test tubes were dispensed with 9 ml of sterilized Buffered Peptone water (BPW). Samples were removed from the freezer and thawed at room temperature. Using a sterile pipette 1 ml of the milk sample was transferred into a conical flask containing 9 ml of Buffered Peptone water (BPW) and mixed well. Three-fold serial dilution of the inoculums from $10^1$ to $10^3$ into sterile BPW solution using disposable sterile pipettes tips was performed. One milliliter of the prepared inoculum was transferred into test tube containing 9 ml of BPW ($10^1$ dilution). Then using another sterile pipette, 1 ml of the resulting dilution was transferred into a second test tube containing 9 ml of BPW (10-2 dilution). The dilutions were mixed using a vortex mixer for 5 – 10 seconds.

3.7.3 Total Bacterial Count (TBC) and Total Coliform Count (TCC)

Determination of total bacterial count (TBC) and total coliform count (TCC) was carried out using ISO 4833-1:2013 protocols. These protocols take into account all the procedures required to isolate the microbes in question.

3.7.4 Counting of bacterial colonies

After the incubation period, bacterial colonies on the culture plates were counted. Two critical dilutions per each sample were counted. Colony forming units were counted on at least two critical dilution plates by the aid of colony counter. Two consecutive plates with less than 300 colonies were considered for record (ISO 4833-1:2013).

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

Identification of S. aureus in milk samples was done by using ISO 6888-1:1999 protocols. This takes into account all the stages from catalase to coagulase in order to confirm the presence of S. aureus in milk.

3.7.6 Detection of E. coli

As part of the laboratory analysis, all test samples that showed positive bacterial growth during bacterial count and those suspected as E. coli colonies during detection of Staphylococci were
removed from the refrigerator, thawed at room temperature and used for detection of *E. coli*. Petri dishes with MacConkey Sorbitol Agar media were labelled and divided into four equal parts. A sterile loop was dipped into a thawed milk sample and streaked onto MacConkey Sorbitol Agar plates as a differential media for identification of *E. coli*. Then, the plates were inverted and incubated at 37ºC for 24 hours. After incubation period, the plates were examined for typical and atypical colonies. Parallel with the test samples, controls which were used was known *Escherichia coli* (ATCC® 25922).

The presumed well-selected typical and atypical colonies were again sub-cultured in the media (MacConkey Sorbitol agar) and under the same conditions in order to get pure colonies of *E. coli*. After the next 24 hours of incubation, well-isolated colony was selected and sub-cultured further onto Nutrient agar (NA) so as to be used for biochemical confirmation. Tests such as Gram staining and biochemical reactions like oxidase and indole tests were performed to confirm the presence of *E. coli* in the test samples.

### 3.8 Milk composition

#### 3.8.1 Adulteration of milk

To determine adulteration with water and composition of milk, samples were placed in a LactiCheck Ultrasonic Milk Analyzer (Page & Pedersen International Ltd, USA). The analyzer is an automated machine, which works rapidly and effectively in analyzing the major components of milk such as butter fat, solid nonfat, protein, milk density, added water and freezing point. Milk Analyzer provides reliable analyses of critical components such as butter fat and solid nonfat by accurately assessing changes in the parameters (Annex E, F and G). Other characters, such as protein, added water and freezing point, are calculated based upon the percentage of components measured using an exact mathematical formula (LactiCheck, 2010).

### 3.9. Observed farm level hygiene practices

Besides the questionnaire assessment, this study used a check list to observe milk hygiene practices at farm level. Specifically, observations focused on hand washing, cleanliness of the protective clothing for people milking, cleanliness of the milking containers, cleanliness of the water used during milking, the environment under which milking was done and specific assignment of people to milk the animals (Annex 8.8).
3.10 Data management and analysis

Laboratory analyses of 70 milk samples for microbial content and quality of raw milk established the total bacterial count (TBC), Total Coliform Count (TCC) using ISO 4833-1:2013 protocols ([https://www.iso.org/standard/53728.html](https://www.iso.org/standard/53728.html)). *E. coli* and *Staphylococcus aurous*, the milk born bacteria of interest were isolated using [ISO 6888-1:1999] and (ATCC® 25922) protocols ([https://www.iso.org/obp/ui/#iso:std:36145:en](https://www.iso.org/obp/ui/#iso:std:36145:en)). Milk composition was also checked for levels of nutrients and water adulteration using the LactiCheck ultrasound analyzer (Page & Pedersen International Ltd, USA). Results for microbial content, quality, nutrients and water adulteration were compared with the Zambian standards (ZABS, 2011 and FDA, 2005). Laboratory outcome and interviews data were entered in Microsoft excel (Annexes E, F and G). Cleaning of data for any errors was carried out and later exported to STATA V.13 (STATA Corporation, TX, USA) for analysis. Observations were summarised in Annexes 8.8.

3.10.1 Statistical analysis

Means with associated standard deviations were used to summarise and describe continuous variables such as milk quantity produced and levels of nutrients in milk. Colony forming units (cfu) were counted to determine the microbial status in milk, milk quantity and other variables were also determined and compared with Zambian standards. The association between social demographic characteristics and other risk factors on milk contamination was measured and expressed as dichotomous outcome. Multiple logistic regression (Annex 8.9) and machine led stepwise regression was used to identify risk factors for contamination of milk at 95% significance level. In addition, tables were used in presentation of the results.

3.11 Ethical consideration

A research clearance to conduct this study was sought from ERES Converge Institutional Review Board and permission from Ministry of Agriculture and Livestock, Zambia Dairy Farmers Union (ZDFU) and the Local District Veterinary Office (DVO) before commencement of the study. Before going to the field, communication was made with the Livestock Extension Officer (LEO) to conduct the study in their area. Farmers participating in the study were informed about the study and participated on voluntary basis. Prior to commencement of the interviews, the purpose and importance of the study were explained to the selected participants to
be interviewed. Consent was obtained from each of the selected small-holder dairy farmers participating in the interviews. During interviews, confidentiality was maintained by ensuring that each farmer was interviewed individually in a scheduled place.

All the information collected from the participants and laboratory results obtained after milk sample analysis were kept under the custody of the researcher as confidential. Data on the computer were protected with a password. Study participants were anonymized. Questionnaires were identified by coded numbers. Participants were assured that information collected from them was not linked to them and their participation in the interview would not affect services that they were currently receiving. It was empathized to participants that confidentiality and anonymity would be maintained. If data is published in local or international journals, it would not be linked to names of participants.
CHAPTER FOUR: RESULTS

A total of 70 farmers participated in the study. Of these farmers, 16 (22.8%) were from Namwala central, 24 (34.3%) from Nchole and 30 (42.9%) from Mungaila dairy farmers cooperatives. Most 44.3% (n=31) of the participants were between 15 and 29 years of age. Out of the total samples (70), the majority of the participants 93% (n=65) were males (Table 4).

Table 4: Social demographic characteristics for study participants in Namwala district (n=70).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Namwala Central (n=16)</th>
<th>Nchole (n=24)</th>
<th>Mungaila (n=30)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age group (Years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-29</td>
<td>4 (25%)</td>
<td>11 (46%)</td>
<td>16 (53%)</td>
<td>31 (44%)</td>
</tr>
<tr>
<td>30-44</td>
<td>7 (44%)</td>
<td>8 (33%)</td>
<td>10 (33%)</td>
<td>25 (33%)</td>
</tr>
<tr>
<td>45+</td>
<td>5 (31%)</td>
<td>5 (21%)</td>
<td>4 (14%)</td>
<td>14 (23%)</td>
</tr>
<tr>
<td>2. Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (94%)</td>
<td>22 (92%)</td>
<td>28 (93%)</td>
<td>65 (93%)</td>
</tr>
<tr>
<td>Female</td>
<td>1 (6%)</td>
<td>2 (8%)</td>
<td>2 (7%)</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>3. Education level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>1 (6%)</td>
<td>16 (67%)</td>
<td>17 (57%)</td>
<td>34 (49%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>10 (63%)</td>
<td>6 (25%)</td>
<td>13 (43%)</td>
<td>29 (41%)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>3 (19%)</td>
<td>1 (4%)</td>
<td>0</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>No formal education</td>
<td>2 (12%)</td>
<td>1 (4%)</td>
<td>0</td>
<td>3 (4%)</td>
</tr>
</tbody>
</table>

4.1 Microbial content in raw milk

4.1.1 Total Bacterial Count (TBC)

Total bacterial count (TBC) of raw milk from 32 (45.7%) farms was above the maximum legally accepted limits in Zambia (200,000 cfu/ml of raw milk). Total bacterial count ranged from 50 to above $2.6 \times 10^6$ cfu/ml. About 50% (n=12 and n=15) samples from Nchole and Mungaila were contaminated with high bacterial load (Table 5).
Table 5: Total Bacterial Count milk samples (n=70)

<table>
<thead>
<tr>
<th>Location</th>
<th>Within legal limit (&lt;2×10^6 cfu/ml)</th>
<th>Above legal limit (&gt;2×10^6 cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Namwala Central (n=16)</td>
<td>11 (69%)</td>
<td>5 (31%)</td>
</tr>
<tr>
<td>Nchole (n=24)</td>
<td>12 (50%)</td>
<td>12 (50%)</td>
</tr>
<tr>
<td>Mungaila (n=30)</td>
<td>15 (50%)</td>
<td>15 (50%)</td>
</tr>
</tbody>
</table>

*Zambian legal limits: (<2×10^6 cfu/ml)*.

4.1.2 Total Coliforms Count (TCC)

Total Coliform Count (TCC) from 19 farms (27.1%) did not conform to recommended Zambian standards (50,000 cfu/ml of raw milk). Levels of coliforms ranged from 20 cfu/ml to above 5.0 x 10^4 cfu/ml. Mungaila had the highest number 10 (33%) of the samples with high coliform counts. Overall, 30% (n=19) of the samples from Namwala district were contaminated with high total coliform count (Table 6).

Table 6: Total Coliform Count milk samples (n=70)

<table>
<thead>
<tr>
<th>Location</th>
<th>Within legal limit (&lt;5×10^4 cfu/ml)</th>
<th>Above legal limit (&gt;5×10^4 cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Namwala Central (n=16)</td>
<td>12 (75%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Nchole (n=24)</td>
<td>19 (79%)</td>
<td>5 (21%)</td>
</tr>
<tr>
<td>Mungaila (n=30)</td>
<td>20 (67%)</td>
<td>10 (33%)</td>
</tr>
</tbody>
</table>

*Zambian legal limits: (<5×10^4 cfu/ml)*.

4.2 Bacterial characterization and speciation

Table 7 shows that of the samples analyzed (n=70), 23 (32.9%) had isolates of S. aureus. Nchole had the highest number of samples with contamination of S. aureus (n=10, 41.7%) while Namwala Central had the lowest (n=3, 18.8%). Overall, 29 (41.4%) of all milk samples were contaminated with general E. coli. Out of these 6 (21%) had pathogenic E. coli. The highest number of samples contaminated with general E. coli was observed for milk samples from Mungaila (n=18, 60%).
Table 7: Prevalence of *S. aureus* and *E. coli* in raw milk from Namwala district, Zambia (n=70)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Namwala Central (n=16)</th>
<th>Nchole (n=24)</th>
<th>Mungaila (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> in milk</td>
<td>3 (18.8)</td>
<td>10 (41.7)</td>
<td>10 (33.3)</td>
</tr>
<tr>
<td>General <em>E. coli</em> in milk</td>
<td>3 (18.8)</td>
<td>8 (33.3)</td>
<td>18 (60)</td>
</tr>
<tr>
<td><em>E. coli</em> 0157:H7</td>
<td>3 (18.8)</td>
<td>1 (4.2)</td>
<td>2 (6.7)</td>
</tr>
</tbody>
</table>

*Zambian legal limits: 1=0, 2<8.3% and 3=0)*.

4.3 Milk nutrient content

With regard to milk nutrient content, the study considered the butter fat content, solid nonfat content as well as density for milk samples. Butter fat (BF) content for milk from 25 (35.7%) farms was below recommended minimum Zambian standards. Solid nonfat (SNF) of milk from 30 (43%) farms was below Zambian standards. Density of milk for all samples was above recommended Zambian standards. The protein content in all the samples ranged from 1.40 to 4.31 (Table 8). The study found that 43 (61.4%) of the samples were adulterated with water. According to the Food and Drugs Act (CAP 303 of the laws of Zambia), no water should be added to milk. The percentage of water added in milk ranged from about 3% to 68%. Mungaila had the highest number, 19 (63%) of farmers practicing water adulteration.

Table 8: Samples below nutrient standards from Namwala district, Zambia (n=70)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Namwala Central (n=16)</th>
<th>Nchole (n=24)</th>
<th>Mungaila (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water adulteration</td>
<td>8 (50%)</td>
<td>16 (66.7%)</td>
<td>19 (63%)</td>
</tr>
<tr>
<td>Samples below solid nonfat standards</td>
<td>8 (50%)</td>
<td>8 (33%)</td>
<td>14 (46.7%)</td>
</tr>
<tr>
<td>Samples below density standards</td>
<td>16 (100%)</td>
<td>24 (100%)</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>Samples below fat standards</td>
<td>8 (50%)</td>
<td>8 (33%)</td>
<td>9 (30%)</td>
</tr>
</tbody>
</table>

*Zambian legal limits (1=0, 2<8.3%, 3<1.028g/cm³, 4<3.2%)*.
4.3.1 Hygienic practices at farm level in Namwala district

Based on the observations, the study found that 58 (82.9%) of the farmers did not practice the hygiene of washing their hands with soap or using a sanitizer before milking. About 53 (57.7%) wore protective gear during milking. Warm water can be one way of sanitizing the milking containers and the study found that 42 (60%) of participants used warm water to clean their milking containers. It was also observed that about 36 (51.4%) of the farmers had people specifically assigned to do the milking. Among the farmers 45 (64.3%) covered their milk during milking and this is one of the good hygiene practices. The type of water used when cleaning the milking utensil is critical to infection or contamination of milk. Therefore, the study found that 48 (68.6%) of the farmers used water from dirty sources. The majority 47 (67.1%) of the milking parlors had earthed floors while the remaining few had concrete floors (Table 9).

Table 9: Observed risk factors on milk contamination in Namwala district (n=70)

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>FREQUENCY</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand washing with soap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>17.1</td>
</tr>
<tr>
<td>No</td>
<td>58</td>
<td>82.9</td>
</tr>
<tr>
<td>Wearing of clean protective clothing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>24.3</td>
</tr>
<tr>
<td>No</td>
<td>53</td>
<td>57.7</td>
</tr>
<tr>
<td>Washing of milking containers with warm water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>42</td>
<td>60</td>
</tr>
<tr>
<td>No</td>
<td>28</td>
<td>40</td>
</tr>
<tr>
<td>Are there specific people assigned to milk animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36</td>
<td>51.4</td>
</tr>
<tr>
<td>No</td>
<td>34</td>
<td>48.6</td>
</tr>
<tr>
<td>Covering of the milk during milking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>45</td>
<td>64.3</td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td>35.7</td>
</tr>
<tr>
<td>Type of water for use during milking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean</td>
<td>22</td>
<td>31.4</td>
</tr>
<tr>
<td>Dirty</td>
<td>48</td>
<td>68.6</td>
</tr>
<tr>
<td>Type of floors in the parlor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concrete</td>
<td>23</td>
<td>32.9</td>
</tr>
<tr>
<td>Earthed</td>
<td>47</td>
<td>67.1</td>
</tr>
</tbody>
</table>
4.4 Farmer’s characteristics associated with milk contamination

To identify the farm-level characteristics that were influencing the microbial contamination of milk in Namwala district, the study investigated the association between contamination with a particular species of bacteria of public health significance and probable risk factors. These were social demographic characteristics, hygiene related factors and milking environment related factors. Logistic regression was adopted to assess the association between risk factors and contamination outcome in milk.

Bivariate analysis was conducted at a $P$ value < 0.05 and none of the risk factors were associated with milk contamination. In order to adjust for confounding factors, all variables were considered for the multivariate modeling (Annex 8.9). Those who had secondary education were 50% less likely to contaminate milk ($OR = 0.49; 95\% CI = 0.23–1.02; P = 0.05$). Step wise regression model (machine led) was adopted at 0.5 significance level with all variables included (Table 10).

Table 10: The final stepwise regression model on the effect of farm level characteristics on milk contamination

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Bivariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude Odds Ratio</td>
<td>Unadjusted P-value</td>
</tr>
<tr>
<td>1. Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>0.27(0.029-2.58)</td>
<td>0.26</td>
</tr>
<tr>
<td>2. Education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>0.63(0.23-1.71)</td>
<td>0.36</td>
</tr>
<tr>
<td>Tertiary</td>
<td>0.30(0.28-3.14)</td>
<td>0.31</td>
</tr>
<tr>
<td>No formal education</td>
<td>0.44(0.04-5.38)</td>
<td>0.52</td>
</tr>
<tr>
<td>3. Age group (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-29</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>30-44</td>
<td>1.09(0.38-3.15)</td>
<td>0.88</td>
</tr>
<tr>
<td>*45+</td>
<td>1.85(0.52-6.62)</td>
<td>0.35</td>
</tr>
<tr>
<td>4. The people milking the cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Owner</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>*Family member</td>
<td>1.57(0.51-4.83)</td>
<td>0.43</td>
</tr>
<tr>
<td>External employee</td>
<td>1.05(0.14-7.93)</td>
<td>0.96</td>
</tr>
<tr>
<td>Both family and employees</td>
<td>1.05(0.14-7.93)</td>
<td>0.96</td>
</tr>
</tbody>
</table>
After adjusting for the confounding effects of all the hypothesized risk factors such as washing of teats before milking, milking of animals suspected to be infected, milking environment, age, gender, time the infected animal is milked, the persons milking the animal and storage containers, the study found that compared to milk from farms whose owners only had primary school-level education, no formal education and tertiary education, milk from farms whose owners had secondary education were 50% less likely to be contaminated with bacteria [Adjusted Odds Ratio: 0.49 (P=0.05, 95% CI 0.23-1.02)].

After adjusting for the confounding effects of all the hypothesized risk factors, the study found that when compared to storing milk under the shade, farmers who did not store their milk under the shade were 80% less likely to have their milk contaminated with bacteria [Adjusted Odds Ratio: 0.23 (p=0.04, 95% CI 0.05-0.99)]. After adjusting for the confounding effects of all the hypothesized risk factors, the study found that compared to milk from farmers who milked their animals under sanitary conditions, those who milked their animals under unsanitary conditions were 3 times more likely to contaminate the milk with bacteria [Adjusted Odds Ratio: 3.04 (p=0.08, 95% CI 0.88-10.49)]. However, the milking environment was not really considered as a risk factor to contamination (p>0.05).
After adjusting for the confounding effects of all the hypothesized risk factors, the study found that compared to animals milked by the owner, farmers whose milking was done by family members were 1 time more likely to be contaminated with bacteria [Adjusted Odds Ratio: 1.26 ($p=0.50$, 95% CI 0.64-2.50)]. After adjusting for the confounding effects of all the hypothesized risk factors, the study found that farmers whose milking was done by people whose age was older than 45 years were 1.5 times more likely to have their milk contaminated with bacteria [Adjusted Odds Ratio: 1.47 ($p=0.31$, 95% CI 0.69-3.09)].
CHAPTER FIVE: DISCUSSION

This study determined the microbial content and quality of raw bovine milk in Namwala district of Zambia. Contamination with enteric pathogens such as *E. coli* and *S. aureus* is common and presents a public health risk to consumers (Pandey, 2005, Eneroth, *et al.*, 2001 and Jayarao *et al.*, 2014).

5.1 Microbial content in raw milk

This study demonstrated that milk from the three milk collection centres (MCCs) was of poor quality based on the high number of total bacterial count (TBC) and high total coliform count (TCC) in milk samples. The study also found presence of pathogenic microbes and widespread adulteration of milk from Namwala district. High TCC values indicate feacal contamination of milk (of animal or human) and environmental origin (Blowey, 2000). A high bacterial count reduces the shelf-life and enhances the risk of milk-borne bacterial infections if milk is not properly heated or if thermally injured pathogens recover under suitable temperatures (Kayihura *et al.*, 2008). When such milk is consumed may lead consumers to develop milk borne diseases. Using the Zambian standard (ZABS, 2011 and FDA, 2005), East African Community Standard (EACS, 2006) and European standards, 19 (27%) of the samples had above normal coliform counts, demonstrating a poor and hazardous quality of milk consumed in Namwala communities. This implies that local people are exposed to health risks that bring about ill helath. In rural areas such as Namwala with limited health facilities, milk born infections may lead to experiencing ill health for long periods which in turn may lead to diminished productivity and erosion in income.

The proportion of samples with high TCC reported in the current study is lower than the 39%-69% reported by Omore *et al.*, (2001) from the Kenyan highlands. This difference could be due to different levels of hygiene, and possibly the variability in ambient temperatures between the two areas. The total coliform counts from the three MCCs which were higher than acceptable standards (50, 000 cfu/ml), could be attributed to improper hygienic management of the milking utensils and plastic containers used in keeping the milk. Hygienic practices by farmers are an important factor in contamination as it influences the quality of milk consumed or sold to the general public.
This study found that the longer the distance to reach the MCC, the higher the bacterial contamination level in milk. The results of this study were consistent with studies from Kenya, Malawi and Tanzania which revealed higher bacterial counts at farm level, suggesting poor handling during the collection process (Omore et al., 2001, Kivaria et al., 2006 and Shitandi, 2007). Similar findings were noted in Malawi (Shitandi and Kihumbu, 2004); in Tanzania (Kivaria et al. 2006) and in Swaziland (Fakudze and Dlamini, 2001). It has been suggested that use of dirty plastic containers, scooping of milk with dirty utensils, high milk temperature, the long duration and lack of cold chain at milking points may be factors contributing to rapid bacterial multiplication. In the case of Zambia, the distance may reduce frequency of supervisory visits by the relevant authorities. Small scale farmers without proper support may not care about hygiene practices nor adhere to recommended standards of keeping milk free from contamination. Therefore, milk contamination appears to be a general problem in rural areas with poor settings. Considering that poor subsistence farmers produce milk for both home consumption and selling, contaminated milk from such farmers not only presents a health problem but also erodes the financial returns. Local farmers if known to sell contaminated milk may lose clients and subsequently their income.

The current study carried out in Namwala District found that 32 out of 70 milk samples (45.7%) had TBC above the recommended standards. It was therefore concluded that the sanitary quality of milk produced by smallholder dairy farmers in Namwala district of Zambia, as far as bacterial content was concerned was unacceptable as the figure was not within acceptable Zambian standards. It is also clear that the farmers in the study area did not abide by the stipulated Zambian standards. To support farmers improve the quality of their milk may require deliberate efforts that bring the necessary awareness to communities. Relevant government authorities may need to extend their reach by identifying those farmers who need targeted support.

5.1.1 Microbial characterization and speciation in raw cow’s milk

The current study further revealed that 41% of milk samples showed positive E. coli suggesting that almost half of the milk derived from animals is infected with some E. coli species. It must however, be realized that since pooled milk samples were studied, the findings do not directly reflect the status of individual cows or herds. The proportion of E.coli positive samples from Mungaila milk collection centre (60%) was significantly higher than those from other Milk
Collection Centres (MCCs). Mungaila was located farthest among the MCCs from the central administration in Namwala district indicating that milk may be kept longer at temperatures favorable for microbial multiplication at household level. For such communities, there is need to encourage investment in household coolers to ensure milk is kept under conditions that minimize microbial multiplication.

In other African countries, studies reveal much lower *E. coli* contamination in milk, 13.5% in Nigeria (Bertu *et al* 2010) and 3.5% in Kenya (Omore *et al* 2002). The high proportion (41%) of unclassified *E. coli* observed in this study is therefore a source of concern since in the presence of a verocytotoxigenic *E. coli*, toxins may be produced to cause illness to consumers. Despite the fact that most people claim to boil the milk that may destroys verocytotoxins, the possibility of inadequate treatment cannot be ruled out. The fact that the majority of Namwala residents consume raw milk increases the risk of milk-borne *E. coli* poisoning. This study suggests that contaminated milk (due to both *E. coli* and *S. aureus*) could be a contributing factor to diarrheal and food borne diseases diagnosed in Namwala district.

Consumption of milk contaminated with pathogenic bacteria and toxins can lead to diseases, allergic reactions, toxication and a risk of microorganisms developing resistance. For instance, the pathogenic *E. coli* (0157:H7) is a risk factor to diarrhea especially to children under the age of five years and people who are immune compromised such as those with HIV/AIDS. This study found 8.6% of the samples were contaminated with the pathogenic *E. coli* (0157:H7) risking the consumers of that milk. *S. aureus* is a risk factor to diarrhea, food poisoning, allergic reaction and toxication to consumers.

### 5.1.2 Compositional quality of raw milk

Overall, 50% of samples from the three MCCs had density below 1.028g/cm³, suggesting water adulteration (intentional or accidental). Adulteration of milk by addition of water may introduce chemical or microbial health hazards as well as reduce the nutritional and processing quality, palatability and marketing value of the milk. The practice of water adulteration in milk is more common during the dry season when milk is scarce and market demand is high (Pandey, 2005). However, verification of this seasonal observation could not be ascertained in the present study because sampling was carried out only during the rainy season (March, 2017).
Mungaila showed higher water adulteration practice than the other milk collection centres (Annex 8.6). By location, this centre was located farthest to Namwala district administration centre. The community may be under-supervised by veterinary extension workers and may also have more people without formal education. These factors may have contributed much more to the prevalence of water adulteration. Generally, Mungaila seems to have challenges in maintaining milk quality and may require attention to minimize health problems associated with consumption of adulterated milk. This suggests the need for active surveillance to support farmers to adhere to acceptable practices.

It is worth to mention that all samples which were found to be adulterated with water had low density, low BF and low SNF content (Annexes 8.5, 8.6 and 8.7). This was so because as water was added to milk, the nutrients concentration reduced. It is also worth mentioning that most of the samples with added water had high TBC and TCC as well as having confirmed microbes such as *S. aureus* and *E. coli*. This might have implied that the water used either for cleaning the utensils or added to the milk for profit maximization was contaminated with bacteria hence, high TBC, TCC and the presence of pathogenic microbes. When there is high demand for milk, unscrupulous milk dealers sometimes add water to milk in order to increase its volume so that they can earn easy money. For Namwala which is a remote district, small holder dairy farmers may also not access safe water, or have the necessary capacity to acquire appropriate equipment or adhere to acceptable milking practices.

It was found that when family members milked the animals contamination increased. A family member is likely not to be supervised and may likely take things for granted forgoing the good milking practices. In case of an employee milking the animals, the owner would take interest to know what is happening especially in terms of theft. This in turn will make those milking observe everything including good hygiene practices. As farmers grew older, the frequencies of milking animals reduce as they left such responsibilities to younger ones who may not observe good hygiene practices.

### 5.1.3 Risk factors associated with milk contamination

It was observed that more farmers 58 (82.9%) did not practice hand hygiene and few 17 (24.3%) farmers wore clean protective clothing during milking. This implies that one of the ports of entry
for contamination was poor hand hygiene and lack of specific protective clothing. Unacceptable milking practices such as not milking animals from concrete parlors which are easy to clean also contributed to increased contamination risks and compromised milk quality. Similarly, in Tanzania, 76% of the milk farmers had poor hygienic practices during milking Kanyeka, (2014). Contaminated milk with pathogens increases opportunities for new human health problems in dairy farming communities and where cattle ranching is practiced, animals (including products) may be reservoirs for of zoonotic bacteria which may cause ill health to humans.

5.1.4 Strengths and limitations of the study

The study was conducted among small scale farmers based in Namwala and therefore generalizations of the study are limited to Namwala district. However, the study provides evidence on selected pathogenic microbes isolated and quality of milk in relation to risk factors associated to contamination. Verification of water adulteration was limited to the period when sampling was carried out (during the rainy season in March, 2017).
CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion
Findings of this study show that milk produced by small scale dairy farmers in Namwala district of Zambia is of poor microbial and nutrient quality. Contributing factors to this problem include the high prevalence of water adulteration, poor handling and poor hygienic practices and the use of unsterile milk storage equipment. In addition, high education levels of farmers has greater influence on the microbial contamination of raw milk.

The presence of pathogens in milk such as *E. coli* and *S. aureus*, the high counts of coliforms and the high levels of bacteria are indicative of a potentially hazardous product. The available milk poses a serious public health risk to consumers, particularly if the milk is not pasteurized or adequately boiled at household level. It is concluded that milk produced by small holder dairy farmers from Namwala district is of poor quality, hazardous for human consumption and can be a potential source of milk-borne infections to the general population in the area.

6.2 Recommendations

i. To improve the availability of quality milk to prevent consumers from milk borne illnesses in Namwala district there is need to bring to the general public information about the importance of consuming milk that is free from contamination. This requires policy makers to take deliberate efforts to ensure that milk consumed, particularly in rural communities conform to acceptable standards. This suggests that interventions be directed at routine surveillance to monitor hygiene practices by farmers at farm level. This effort will prevent milk contamination especially by enteric organisms that may become opportunistic pathogens to human health.

ii. The Ministry of Health has a major role to play in creating awareness in the community about possible health problems (including the type of diarrheal diseases) that may result from contaminated milk. Inspection of all milk and milk products in the area should be encouraged to ensure no milk of poor quality is sold to the public. The general public should be encouraged to avoid taking raw milk as it may harbor pathogenic microbes which may later cause disease or death. The Ministry of Health can conduct training for farmers to sensitize them on hygienic practices during milking and the dangers of
contaminated milk at household level and the general public. The producers of milk should also be taught on the legal implications of food that does not meet the legal standards but intended to be sold to the general public. For Namwala, the Ministry of Health is encouraged to disseminate health information at all levels including the health facility and within catchment areas.

iii. The Ministry of Livestock at district level should take a leading role in ensuring animal health is given a priority and animals are kept free from diseases. This will in turn prevent the public from many zoonotic diseases. Awareness in good milking practices for all farmers in the cooperatives should be encouraged by the all extension officers.

iv. The Ministry of Local Government and Housing is recommended to ensure creation of designated places for selling milk rather than selling through informal channels. This will facilitate routine checks for all the milk sold to the public. The local authority is also encouraged to work with the business community to create awareness in the communities on the importance of hygiene and reducing consumption of raw milk to avoid ill health.

v. Namwala is an area where people depend on milk and milk products. In order to safeguard the health of the public (those based in Namwala and everyone who can have access to milk produced from Namwala), it is recommended that policy makers consider installation of a milk processing facility. Raw milk safety information in all cattle ranching areas in Zambia and abroad should be disseminated so as to protect the human health.

vi. Further research is to quantify the magnitude of *E. coli* (0157:H57), *S. aureus*, other pathogens in milk and water adulteration throughout Zambia. It is important to substantiate the reasons for adulteration in order to prevent possible occurrence of toxins produced by *E. coli* and *S. aureus* and other pathogenic spore forming microbes in milk. The linkage between diarrheal diseases in Namwala and milk contamination should be studied to determine the exact contribution of milk to the burden of disease.
REFERENCES


APPENDICES

Appendix 8.1: Questionnaire

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

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

1. Questionnaire number:………………………………

2. Farm code:……………………………………

3. Date of interview:……/……../20………

4. GPS co-ordinates:…………………………

5. Province:…………………………………

6. District:…………………………………..

7. Village:……………………………………

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

1. How old were you on your last birth day?
   Mention (years)…………………………

2. Sex of the respondent:
   1= Male [     ]
   2= Female [    ]

3. Level of education of the respondent (tick)
   Mention (grade)……………………………

4. Do you own all the cattle in this herd?
   1= Yes [      ]
   2= No [       ]
   If no, how many different owners own this herd? Mention……………………

5. For how long have you kept cattle?
   Mention (years)…………………………
6. For how long have you been in dairy farming?
Mention (years)……………………………………………………………

FARM MANAGEMENT PRACTICES (herd associated factors)
(I will now ask you some questions on how you manage your herd)

7. Do you raise beef cattle in the same herd?(Mandatory)
1= Yes [ ]
2= No [ ]

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

9. Do you practice transhumance/nomadism? (Move with the herd in search of pasture/water)
1= Yes [ ]
2= No [ ]

10. Do you own other animals apart from cattle?
1= Yes [ ]
2= No [ ]
If yes, mention them ……………………………………………………………

11. Where do you COMMONLY graze your cattle?(Multiple choice)
1= Open space - communal grazing fields [ ]
2= Open space - private grazing fields [ ]
3= Dumping sites [ ]
4= Zero grazing [ ]
5=others (specify)……………………………………………………………

12. How often does your herd graze in outside pastures DURING THE YEAR? (Multiple choice)
1= All year round [ ]
2= Few months per year (seasonal) [ ]

13. Does this herd come into contact WITH OTHER HERDS (e.g. during watering or in communal pasture)? (Mandatory)
1= Yes [ ]
2= No [ ]

14. If yes how often do they come into contact with other herds?(Single choice)
1= Everyday [ ]
2= Atleast once a week [ ]
3= Atleast once a month [ ]
4= Less often [ ]
15. Does this herd come into contact with wild game animals? (Mandatory)
1= Yes [   ]
2= No [   ]

16. If yes, mention the wildlife/wild animals species that your herd come into contact with most frequently:.................................................................

17. How often does your herd come into contact with wild animals? (Single choice)
1=Less often [   ]
2=Everyday [   ]
3=Atleast once a week [   ]
4=Atleast once a month [   ]

18. Are your animals enclosed?
1=Yes [   ]
2=No [   ]
3=Both [   ]

19. What type of animal house floor is in? (Mandatory) (Single choice)
1= Covered with manure [   ]
2= Concrete [   ]
3= Earthed floor [   ]
4= others (specify).................................................................

20. What is the water source for your cattle herd?
Mention.................................................................
HYGIENE RELATED QUESTIONS
21. How do you most commonly feed milk to your calves? (Multiple choice)
   1= Bucket feeding [   ]
   2= Suckling from the dam [   ]
   3= Others (specify)………………………………………………………………………………………………

22. Who primarily milks the lactating cows? (Multiple choice)
   1= Myself [   ]
   2= Family member only [   ]
   3= External employees [   ]
   4= Both family member and employee[   ]
   5= Others (specify)………………………………………………………………………………………………

23. Is it primarily males who milk cows?
   1= Male [   ]
   2= Female [   ]
   3= Both [   ]

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

25. For how long have you been producing the milk at your farm? (Mandatory)
   Mention (years)………………………………………………………………………………………………

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

27. Do you wash your hands before milking?
   Yes[   ]
   No[   ]

28. What do you use to wash your hands?
   1= Water only [   ]
   2= Water with soap [   ]
   3= Water with ash[   ]
   4= Water with any other disinfectant[   ]

29. Do you wash the teats of the animal before milking?
   Yes[   ]
   No[   ]

30. What do you use to wash teats? (Multiple choice)
   1= Warm water only [   ]
   2= Cold water only[   ]
   3= Water with a disinfectant [   ]
31. Which milking technique do you use? (Multiple choice)
1= Hand milking [ ]
2= Machine milking [ ]
= Both [ ]

32. Can you recognize if your cow has an infection/ a problem in the udder?
1= Yes [ ]
2= No [ ]

33. If yes, how do you detect? (multiple choice) (Tick)
1= Change of color of the udder/teats [ ]
2= Udder feels warm than usual [ ]
3= Changed consistency of the udder [ ]
4= Changed size of the udder[ ]
5= Presence of visible lesion on the udder [ ]
6= Udder veins are engrossed [ ]
7= Changed milk consistency and color [ ]
8= Others (specify)………………………………………………………

34. If yes, do you milk animals with udder problem?
1= Yes [ ]
2= No [ ]
3= Sometimes [ ]

35. If yes, when do you milk the animal with udder problem(s)?(Options)
1=Before the health animals [ ]
2= After the healthy animals [ ]
3=Just mix the milking exercise depending on which one comes first[ ]
4= Others (specify)………………………………………………………

36. What do you do with the milk obtained from cows with udder infection?
1= Discard [ ]
2= Consume in the your household [ ]
3= Sale to the market [ ]
4= Feed to calves [ ]
5= Others (specify)………………………………………………………

GENERAL CONTAMINATION EXPOSURE PRACTICES
(I will now ask you some general questions on your behavior towards disease in animals and human)
37. What do you do with milk from YOUR cattle herd?(Multiple choice- mandatory)
1= Consume within the family[ ]
2= Sell to milk vendors [ ]
3= Sell to local businesses (restaurant, hotels schools, Parmalat) [ ]
49

4= Sell to milk processing company [ ]
5= Sell to neighbors and members of the community [ ]
6= Other (specify)……………………………………………………

38. Do you cover the milk after milking at household level?
1= Yes [ ]
2= No [ ]
3= Don’t know [ ]

39. Do you boil the milk consumed at household level?
1= Yes [ ]
2= No [ ]

40. What type of milk storage containers do you use during milking?
1= Calabash (Wooden) [ ]
2= Plastic container [ ]
3= Glass bottle [ ]
4= Metal can [ ]
5= Don’t know[ ]

41. Do you store the milk under shade during milking?
1= Yes [ ]
2= No [ ]

42. How long do you keep the milk before consuming it?
Mention…………………………………………

43. How long do you keep the milk before selling it?
Mention…………………………………………

44. When the animal is sick, what do you do with the milk?
1= Don’t milk the animal [ ]
2= Sell the milk [ ]
3= Consume it in the family [ ]
4= Use it for the calves [ ]
5= Other (specify)……………………………………………………

45. And if the sick animal is treated with a medicine, what do you do with the milk?
1= Don’t milk the animal [ ]
2= Sell the milk [ ]
3= Milk the animal and discard the milk [ ]
4= Consume it in the family [ ]
5= Use it for the calves [ ]
6= Other (specify)……………………………………………………
46. Do you consume raw milk? (Mandatory-single choice) (Note: Raw being unprocessed milk, NOT BOILED, not pasteurized or homogenized)
   1= Always (regarded as yes) [    ]
   2= Sometimes (regarded as yes) [    ]
   3= No [    ]

47. Do you consume milk products made from raw milk? (Mandatory)
   1= Yes [    ]
   2= No [    ]

48. If yes, which ones? (Multiple choice)
   1= Yogurt [    ]
   2= Sour milk [    ]
   3= Ghee [    ]
   4= All milk products [    ]
   5= Others (specify) ..............................................................................

49. How do you handle an animal that is close to die or dies (dead one) on the farm?
   1= Bury (after exitus) [    ]
   2= Burn (after exitus) [    ]
   3= Slaughter and sale and/or eat the meat [    ]
   4= Others (specify) ..............................................................................

MEDICAL SERVICES
50. When you seek healthcare where will you go first?
   1= Private clinic [    ]
   2= Religious prayers [    ]
   3= Government clinic or hospital [    ]
   4= Traditional healer [    ]
   5= A clinic owned by a non-governmental or faith based organization [    ]
   6= Stay home [    ]
   7= Other (specify) .................................................................

51. In the last year how many times have you gone to a clinic or hospital TO GET TREATED YOURSELF? (Single choice)
Mention.................................................................

52. What is the distance covered to the nearest clinic or hospital?
Mention.................................................................

Thank you very much for devoting your time to participate in this study
Appendix 8.2: Participant information sheet

Study Title: Microbial quality of raw bovine milk from selected farmers in Namwala district of Zambia.

IRB NAME: ERES Converge No.: 00005948

Introduction

Hello, my name is Mweemba Boyd and my assistants’ names are __________ I am from The University of Zambia, School/Department of Public Health. I would like to discuss the reason for coming here and invite you to participate in the study. During the discussion, I and my colleagues will ask questions. Please feel free to stop us and to ask us questions at any time.

Purpose of research project

This study is part of my practicum for my training in Master of Epidemiology and Biostatics, which I am doing with the University of Zambia. The purpose of the practicum is to find out the different types of microorganisms that may be found in milk. Also the levels of microorganisms the milk could contain in this area. To do so, I first want to find out what your challenges or the challenges of your colleagues are, in terms of milk handling and purification. I also want to learn if there are any other training programs in terms of milk handling and purification previously conducted in this area by the ministry or any organization accessible to small scale farmers in this area.

As part of my study, I will also collect milk samples to check whether the milk is contaminated or not. I will provide feedback on the status of the milk found in the area.

Why you are being asked to participate?

Potential participants for the study are people living in Namwala. Others are small scale farmers who have been producing a minimum of 10 liters of milk in the past 6 months and live in Namwala District. You have been asked to participate because you live in this area. To get information, I would like to talk to at least sixty small scale farmers to participate in this study interview.

Procedures

If you agree to participate in this study:

- I would like to invite you to take part in this interview. This interview will take about 20 minutes. It will be carried out in a private place. If you permit me, I will tape record the
discussion to help pick all you will say. If not, I will ask you if it will be ok for me to write notes. The information from tape or notes will not linked to your name and will not be included in the tape recording or the typed documents.

- I will also collect milk samples from your animals. I will take samples to the University of Zambia laboratory to check the presence of microorganisms which may be present in the milk.
- At a later stage, I will come back to let you know about the quality of your milk.

**Risks/discomforts**

There are no physical risks to participants in this study. However, I recognize some information you may tell me may be personal or maybe sensitive to other stakeholders. In that case, I would like to assure you the information that I will get from you will not be shared with anyone outside this study. The information you will be in confidence and will not be shared.

**Benefits**

I have arranged with the Agriculture Extension officers to provide training on milk management at community level. If you are participating in the training program, you will benefit knowledge regarding the good practices to avoid milk contamination. You will also know the type of microorganisms found in milk produced in the area. This may improve your milking and storage practices of milk. Overall, there are no direct benefits to you. Instead, you will contribute to the generation of new knowledge regarding the good practices attitude and beliefs to improve the milk quality. It is my hope that effort will benefit the current and future small scale farmers and the community at large in Namwala District.

**Payment**

I want to mention that there is no payment for participating in this study.

**Protecting data confidentiality**

I have put up steps to protect the information I will get from you. First, only my assistants and I will have access to the information. The collected data will be locked in a secured place. The information I will type will be protected using a password. I will destroy all data within 3 years after typing the information. I will keep copies of typed information on CDs in case we have a problem with the computer.

**What happens if you do not want to participate in this?**

You are free to decide whether you want to take part in this study. This will not bring any problem to you. If you decide not to participate in the study you will not be disadvantaged in any way. You are free also to withdraw at any time if you decide to participate in this study. If you decide to participate in the study, you are free not to answer questions that you may feel to be so personal.

**Who do I call if I have questions or problems?**
In case you have any questions, you call:

- Call me, <<Mr. Boyd Mweemba>>, at <<+260-978-160357>> if you have questions, concerns and complaints about this study.
- Call <<Dr. Bwembya Phoebe>> at <<0966171175>>
- Call <<Prof. Michelo Charles>> at <<0979232403>>
- Or you can also write or call to the Ethics Committee, the ERES Converge IRB office at the following physical address: 33 Joseph Mwilwa Road, Rhodes Park, Lusaka, Zambia; phoning the office on +260 955 155633 or +260 955 155634; sending an email to eresconverge@yahoo.co.uk

Appendix 8.3: Participant informed consent form

Study Title: Microbial quality of raw bovine milk from selected farmers in Namwala district of Zambia.

What does your signature (or thumbprint/mark) on this consent form mean?

Your signature (or thumbprint/mark) on this form means:

- You have been informed about the study’s purpose, procedures, possible benefits and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to be in this program

________________________   _____________________________   __________
Print name of Participant              Signature of Participant                          Date

________________________   _____________________________   __________
Print name of Person Obtaining Consent  Signature Consent Date

Ask the participant to mark a “left thumb impression” in this box if the participant (or participant’s parent) is unable to provide a signature above.
Appendix 8.4: Translated tools (Chitonga)

Mibuzyo kubalimi bakama bazulilwa ku namwala

Nzila tuzunda nzyotukonzya kunjila mumukupa ciindi noucili ku mpulasi
Mundando wa mibuzyo oyu uyanda kuziba nzila zikonzya kuletela kuti tuzunda twaandeene
andeene kuti tunjile mumukupa ciindi nouli a mpulasi. Tacico kutola ciindi cilamfu pe kuvwiila
mibuzyo eeyi. Amuzibe kuti bwiinguzi bwenu tabukozibizigwa kubantu bamwi pe elyo aboobo
izina lyenu talikalembwi ali oonse.
1. mundando wa mibuzyo nambala:..............................
2. Mpulasi :.............................................
3. buzubaba mibuzyo:...../....../20........
4. busena:............................
5. Cooko:.................................
6. Cilikiti:........................................
7. Mumunzi mwa:.............................

CIBEELA A: BAINGUZI (Ndilasaaguna kumubuzya mibuzyo ijatikizya ndinywe)

1. Muli amyaka yongaye?
   Myaka........................................

2. Bainguzi sena:
   1= Mbalombwana [] naa
   2= Mbakaintu [    ]

3. sena mwakasakana buti mulwiiyo lwenu?
   Giledi.................................

4. Sena nywebo mulijisi ngombe mucimpati oomu?
   1= inzya [    ]
   2= peepe [    ]
   Kuti naa peepe, sena bali bongaye bavubile ngombe ezi? Mweelwe......................

5. Catola ciindi cilamfu buti kamuli bavwubi ba ngombe?
   Myaka.................................

6. Calampa ciindi cilamfu buti kamuli balimi bakama mukupa ku ngombe eez?
BUBAMBE BWAAMPULASI (kujatikizya bubambe bwa ngombe)
(ndiyakubuzya mibuzyo ijatikizya mbomubamba ngombe)

8. Mujisi ngombe zyongaye zikamwa?
Mweelwe…………………………

9. Sena mulavwuba banyama bambi kunze a ngombe?
…………………………………………

11. Sena nkukuli nkomucezyela ngombe?
……………………………………………………………………

15. Sena ngombe noziya kumacelelo zila swaangana abanyama bamusokwe?
…………………………

24. Kuti naa inzya, sena mbanyama nzi mbozi swaangana
limwi:……………………………………………………………………

18. Sena mucimpati muyalilwe nzi ansi?
……………………………………………………………………

19. Sena nkukuli nkozinywa meenda ngombe?
……………………………………………………………………
MIBUZOYI IJATIKIZYA BULONDO

20. Sena boombe mubelesya nzi ciindi nomawisya mukupa?

21. Sena mbabani bavula kukama mukupa lyoonse?

22. Sena aba bantu bakama mbalombwana lyoonse?

23. Sena mukupa munji buti ngomukama ku ngombe abuzuba?

25. Kuti musike mpomwiikama ngombe nintaamu nzi nzyomucita:

26. Sena inga mulasamba kumaanza kamutana kama?

27. Sena mubelesya nzi kusamba kumaanza?

28. Sena inga mulasanzya nkolo zya ngombe kamutana kama?

29. Ino inga mubelesya nzi?

30. Ni nzila nzi njomubelesya kukama?

31. Sena mulakonzya kuziba kuti ngombe yenu ijisi butongo kucibele?

32. kuti naa inzya, inga mwaziba buti?

33. Kuti naa mwajana butongo kucibele ca ngombe, mulazumanana kwiikama?

34. kuti naa pe, mucita buti ku ngombe eeyi?

35. Mukupa ngomukama kuzwa ku ngombe ilaa butongo kucibele inga mucita buti?
NZILA TUZUNDA MBUTIKONZYA KUNJILA MU MUKUPA
(Ndilamubuzya mibuzyo ijatikizya malwazi akonzya kuba kubanyama abantu)
36. Sena mukupa ngomukama ku ngmbe inga mucita nzi anguwe?
)........................................................................

37. Sena inga mulavunika mukupa wenu mwamana kukama ku maanda?
.................................................................

38. Ku mukupa ngomulya amunzi inga mulaujika?
.................................................................

39. Ciindi mwamana kukama ziyobwedo nzi zyomubelesya?
.................................................................

40. Sena nomukama mukupa mulaubikka acinvule?
.................................................................

41. Kwiinda ciindi cilamfu buti kuzwa nomwamana kukama a no mulya mukupa wenu amunzi?
.................................................................

42. Kwiinda ciindi cilamfu buti kuzwa nomwamana kukama a no sambala?
.................................................................

45. Sena mulanywa mukupa? (Mukupa: nkokuti uutajikilwe, nokuba kubambwa kufumbwa munzila imbi kunze ambuwakamwa ku ngombe) .................................................................

46. Sena mulalya ku bambwa ku mukupa?
.................................................................

47. Naa inzya, sena ninzi zyomuvula kulya?
.................................................................

48. Ino kuti naa ngombe iyanda kufwa naa yalifwida inga mucita buti anjiyo?.........................

BUSILISI
49. kuti ciindi nomwayanda kusilikwa inga mugama kuli? .................................................................

50. Kumwaka wainda ayuuno mwainda ziindi zyongaye kamuya kucibadela kuya kukusilikwa? Myeelwe.................................................................

51. Mpalamfu buti mpomusilikilwa? .................................................................

Twamulumba muciindi cibotu kutola lubazu mumubandi ooyu.
Twaambo twakuzibya basikutola lubazu

Mutwe wa ciiyo: misyobo ya tuzunda tukonzya kujanwa mumukupa kuzwa kubalimi basyoonto bazulilwa ku namwala mucisi ca zambia.

INKAMU YEENDELEZYA: ERES Converge          No.: 00005948

Busanduluzi

Mwabonwa, mebo ndime Mweemba Boyd alimwi ndabagwasyi bangu bali azyina lya………………………………………………. Ndizwa kucikolo cipati ca University of Zambia cizulilwa mu dolopo lya Lusaka. Lwiiyo lwangu lulanganya inseba zya bantu elyo aboobo ndalombozya kuti tubandike kumuzeezo ngundalonda okuno nkeakaambo kaako mwatambwa kutilo tule lubazu mumubandi ooyu. Mukwiizya kwses, tuli kubuzya mibuzyo yaandeene andeene elyo mwalombwa kutilo mulinwve kwaanguluka kutubuzya mibuzyo aswebo kufwumbwa ciindi.

Muzeezo waciyo eeci.


Kucita kutilo tuzyiba tuzunda tukonzya kujanwa mumukupa, ndaya kubweza mukupa musyoonto ku mulimi amulimi. Mukupa ooyu uyooyandika kupimwa a mincini kucita kutilo tuzyibe kujatikizye kulanga na tuzunda tulajanwa mu mukupa. Twaakumana mulimo ooyu, tuyaakuzyiba bube mbounoobede mukupa.

Nkaambo nzi ncomuyandika kutola libazu?


Mayale aamalailile

• Kunze akooku, ndiyooyandika kubweza mukupa ngomukama kuzwa ku ngombe kyenu kucita kuti ukalangwe langwe amincini okuya kucikolo cipati ca University of Zambia. Kucita kuti tukazibe tuzunda tukonziza kujanika kutola lubazu mumubandi ooyu. Eeci tacizubulului kuti mazina enu pe.

Butongo bukonzya kujanika


Bulumbu bukonzya kujanika

mulaangulukile kuzwa kufumbwa ciindi. Kumibuzyo njomutakonzyi kuvwiila, mulaangulukile kutaiwvilia.

**Sena Nguni ngomukonzya kubuzya ciindi nomwaba aamubuzyo naa penzi?**

Muciindi nomwaba amubuzyo inga mwatuma kumanambala aya:

- Inga mwanditumina mebo ndeba **Boyd Mweemba** aluwaile lwa +260-978-160357.
- Inga mwabaita aba Dr. Bwembya Phoebe aluwaile lwa 0966171175.
- inga mwabaita ba Prof. Michelo Charles aluwaile lwa 0979232403.
- Naa pe inga mwalembela kunkamu ilanganya bweendiyo coonse ya **ERES Converge IRB ku opesi lipati lijanwa mumugwagwa wa 33 Joseph Mwilwa Road, Rhodes Park, mudolopo lya Lusaka, Zambia; inga mwatuma aluwaile oolu +260 955 155633 naa +260 955 155634; inga mwabatumina muluwo ku eresconverge@yahoo.co.uk**

**Kuzumina kutola lubazu**

**Mutwe wa ciiyo:** misyobo ya tuzunda tukonzya kujanwa mumukupa kuzwa kubalimi basyoonto bazulilwa ku namwala mucisi ca zambia.

**Ino kusimba busimbo bwenu ansi aawa caamba nzi?**

Busimbo bwenu bwaamba kuti:

- Mwazibizigwa muzeeko, malailile, bubotu naa bubi bukonzya kujanika kwiinda mukutola lubazu mu ciiyo eeci.
- Mwalipelwe ciindi cakubuzya kamutana simba busimbo bwenu.
- Mwalyaaba kutola lubazu mu ciiyo eeci.

________________________   _____________________________        _______

Mazina eenu  Busimbo  Mubuzuba bwa

Inga mwasimba cigumo waciwensyi muka bbokesi aaka kuti naa tamucikonzyi kulemba atala.
Appendix 8.5: Raw milk quality from Namwala Central Dairy Corporative

<table>
<thead>
<tr>
<th>S#</th>
<th>FAT</th>
<th>SNF</th>
<th>DENSITY</th>
<th>% WATER</th>
<th>PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3.41</td>
<td>8.61</td>
<td>28.9</td>
<td>0*</td>
<td>3.25</td>
</tr>
<tr>
<td>2.</td>
<td>1.24*</td>
<td>7.38*</td>
<td>25.9</td>
<td>13.9</td>
<td>2.77</td>
</tr>
<tr>
<td>3.</td>
<td>3.27</td>
<td>9.98</td>
<td>34.5</td>
<td>0*</td>
<td>3.75</td>
</tr>
<tr>
<td>4.</td>
<td>1.24*</td>
<td>7.38*</td>
<td>25.9</td>
<td>13.9</td>
<td>2.77</td>
</tr>
<tr>
<td>5.</td>
<td>3.27</td>
<td>9.98</td>
<td>34.5</td>
<td>0*</td>
<td>3.75</td>
</tr>
<tr>
<td>6.</td>
<td>1.16*</td>
<td>4.05*</td>
<td>12.8</td>
<td>58.1</td>
<td>1.57</td>
</tr>
<tr>
<td>7.</td>
<td>7.96</td>
<td>11.4</td>
<td>35.9</td>
<td>0*</td>
<td>4.31</td>
</tr>
<tr>
<td>8.</td>
<td>1.16*</td>
<td>4.05*</td>
<td>12.8</td>
<td>58.1</td>
<td>1.57</td>
</tr>
<tr>
<td>9.</td>
<td>2.65*</td>
<td>6.66*</td>
<td>21.7</td>
<td>21.6</td>
<td>2.53</td>
</tr>
<tr>
<td>10.</td>
<td>4.37</td>
<td>9.91</td>
<td>33.2</td>
<td>0*</td>
<td>3.74</td>
</tr>
<tr>
<td>11.</td>
<td>4.82</td>
<td>10.1</td>
<td>33.8</td>
<td>0*</td>
<td>3.83</td>
</tr>
<tr>
<td>12.</td>
<td>1.98*</td>
<td>4.62*</td>
<td>14.2</td>
<td>48.2</td>
<td>1.78</td>
</tr>
<tr>
<td>13.</td>
<td>2.19*</td>
<td>9.68</td>
<td>34.3</td>
<td>0*</td>
<td>3.63</td>
</tr>
<tr>
<td>14.</td>
<td>5.37</td>
<td>10.7</td>
<td>35.4</td>
<td>0*</td>
<td>4.03</td>
</tr>
<tr>
<td>15.</td>
<td>3.33</td>
<td>6.4*</td>
<td>20.1</td>
<td>23.8</td>
<td>2.44</td>
</tr>
<tr>
<td>16.</td>
<td>1.43*</td>
<td>4.49*</td>
<td>14.3</td>
<td>51.5</td>
<td>1.73</td>
</tr>
</tbody>
</table>

*Samples falling within acceptable legal limits*

Appendix 8.6: Raw milk quality from Nchole Dairy Corporative

<table>
<thead>
<tr>
<th>S#</th>
<th>FAT</th>
<th>SNF</th>
<th>DENSITY</th>
<th>% WATER</th>
<th>PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.73*</td>
<td>4.1*</td>
<td>13.4</td>
<td>58.9</td>
<td>1.58</td>
</tr>
<tr>
<td>2.</td>
<td>0*</td>
<td>3.65*</td>
<td>12.2</td>
<td>67.7</td>
<td>1.41</td>
</tr>
<tr>
<td>3.</td>
<td>1.67*</td>
<td>5.89*</td>
<td>19.6</td>
<td>32.7</td>
<td>2.24</td>
</tr>
<tr>
<td>4.</td>
<td>3.32</td>
<td>9.89</td>
<td>34.1</td>
<td>0*</td>
<td>3.72</td>
</tr>
</tbody>
</table>
### Annex 8.7: Raw milk quality from Mungaila Dairy Corporative

<table>
<thead>
<tr>
<th></th>
<th>FAT</th>
<th>SNF</th>
<th>DENSITY</th>
<th>% WATER</th>
<th>PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.34*</td>
<td>5.32*</td>
<td>18.5</td>
<td>43.2</td>
<td>2.02</td>
</tr>
<tr>
<td>2.</td>
<td>1.19*</td>
<td>6.2*</td>
<td>21.2</td>
<td>29.5</td>
<td>2.34</td>
</tr>
<tr>
<td>3.</td>
<td>1.84*</td>
<td>5.2*</td>
<td>16.7</td>
<td>41.2</td>
<td>1.99</td>
</tr>
<tr>
<td>4.</td>
<td>3.6</td>
<td>3.49*</td>
<td>8.52</td>
<td>56.6</td>
<td>1.4</td>
</tr>
<tr>
<td>5.</td>
<td>3.05*</td>
<td>5.94*</td>
<td>18.5</td>
<td>29.6</td>
<td>2.27</td>
</tr>
</tbody>
</table>

*Samples falling within acceptable legal limits*
<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td>0.88*</td>
<td>8.8</td>
<td>31.9</td>
<td>0*</td>
<td>3.29</td>
</tr>
<tr>
<td>7.</td>
<td>4.09</td>
<td>9.97</td>
<td>33.7</td>
<td>0*</td>
<td>3.76</td>
</tr>
<tr>
<td>8.</td>
<td>3.14*</td>
<td>6.46*</td>
<td>20.5</td>
<td>23.4</td>
<td>2.46</td>
</tr>
<tr>
<td>9.</td>
<td>2.11*</td>
<td>4.56*</td>
<td>14</td>
<td>48.6</td>
<td>1.76</td>
</tr>
<tr>
<td>10.</td>
<td>1.22*</td>
<td>4.47*</td>
<td>14.4</td>
<td>52.4</td>
<td>1.72</td>
</tr>
<tr>
<td>11.</td>
<td>1*</td>
<td>6.76*</td>
<td>23.6</td>
<td>22.3</td>
<td>2.54</td>
</tr>
<tr>
<td>12.</td>
<td>4.36</td>
<td>9.69</td>
<td>32.4</td>
<td>0*</td>
<td>3.66</td>
</tr>
<tr>
<td>13.</td>
<td>3.06*</td>
<td>6.74*</td>
<td>21.7</td>
<td>20.2</td>
<td>2.56</td>
</tr>
<tr>
<td>14.</td>
<td>3.63</td>
<td>5.55*</td>
<td>20.3</td>
<td>30.21</td>
<td>1.56</td>
</tr>
<tr>
<td>15.</td>
<td>2.41*</td>
<td>4.28*</td>
<td>24.16</td>
<td>36.85</td>
<td>2.37</td>
</tr>
<tr>
<td>16.</td>
<td>2*</td>
<td>8.64</td>
<td>22</td>
<td>24.59</td>
<td>1.82</td>
</tr>
<tr>
<td>17.</td>
<td>4.25</td>
<td>9.01</td>
<td>23.3</td>
<td>40.98</td>
<td>1.73</td>
</tr>
<tr>
<td>18.</td>
<td>1.89*</td>
<td>7.41*</td>
<td>15.8</td>
<td>0*</td>
<td>3.39</td>
</tr>
<tr>
<td>19.</td>
<td>2.68*</td>
<td>6.72*</td>
<td>12.63</td>
<td>18.37</td>
<td>2.64</td>
</tr>
<tr>
<td>20.</td>
<td>3.32</td>
<td>9.95</td>
<td>18.96</td>
<td>25.51</td>
<td>3.992</td>
</tr>
<tr>
<td>21.</td>
<td>2.43*</td>
<td>8.61</td>
<td>29.7</td>
<td>0*</td>
<td>3.24</td>
</tr>
<tr>
<td>22.</td>
<td>3.89</td>
<td>9.89</td>
<td>33.6</td>
<td>0*</td>
<td>3.73</td>
</tr>
<tr>
<td>23.</td>
<td>5.87</td>
<td>10.3</td>
<td>33.7</td>
<td>0*</td>
<td>3.91</td>
</tr>
<tr>
<td>24.</td>
<td>2.1*</td>
<td>7.89*</td>
<td>27.1</td>
<td>6.81</td>
<td>2.97</td>
</tr>
<tr>
<td>25.</td>
<td>3.16*</td>
<td>8.85</td>
<td>30</td>
<td>0*</td>
<td>3.33</td>
</tr>
<tr>
<td>26.</td>
<td>1.86*</td>
<td>3.94*</td>
<td>11.8</td>
<td>57.2</td>
<td>1.54</td>
</tr>
<tr>
<td>27.</td>
<td>3.11*</td>
<td>9.62</td>
<td>33.2</td>
<td>0*</td>
<td>3.62</td>
</tr>
<tr>
<td>28.</td>
<td>2.96*</td>
<td>8.42</td>
<td>31.9</td>
<td>26.3</td>
<td>3.02</td>
</tr>
<tr>
<td>29.</td>
<td>2.98*</td>
<td>8.63</td>
<td>29.3</td>
<td>0*</td>
<td>3.25</td>
</tr>
<tr>
<td>30.</td>
<td>3.58</td>
<td>9.46</td>
<td>32.1</td>
<td>0*</td>
<td>3.56</td>
</tr>
</tbody>
</table>

*Samples falling within acceptable legal limits*
Annex 8.8: Checklist for observing contamination risk factors

1. Hand washing with soap
2. Wearing of clean protective clothing
3. Washing of milking containers with warm water
4. Are there specific people assigned to milk animals
5. Covering of the milk during milking
6. Type of water for use during milking
7. Type of floors in the parlor

Annex 8.9: Logistic regression outcome for farm level characteristics on milk contamination

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Bivariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude Odds Ratio (95%CI)</td>
<td>Unadjusted P-value</td>
</tr>
<tr>
<td>1. Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>0.27(0.029-2.58)</td>
<td>0.26</td>
</tr>
<tr>
<td>2. Education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>*Secondary</td>
<td>0.63(0.23-1.71)</td>
<td>0.36</td>
</tr>
<tr>
<td>Tertiary</td>
<td>0.30(0.28-3.14)</td>
<td>0.31</td>
</tr>
<tr>
<td>No formal education</td>
<td>0.44(0.04-5.38)</td>
<td>0.52</td>
</tr>
<tr>
<td>3. Age group (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-29</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>30-44</td>
<td>1.09(0.38-3.15)</td>
<td>0.88</td>
</tr>
<tr>
<td>*45+</td>
<td>1.85(0.52-6.62)</td>
<td>0.35</td>
</tr>
<tr>
<td>4. The people milking the cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Owner</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>*Family member</td>
<td>1.57(0.51-4.83)</td>
<td>0.43</td>
</tr>
<tr>
<td>External employee</td>
<td>1.05(0.14-7.93)</td>
<td>0.96</td>
</tr>
<tr>
<td>Both family and employees</td>
<td>1.05(0.14-7.93)</td>
<td>0.96</td>
</tr>
<tr>
<td>5. Storing milk under the shade during milking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>*No</td>
<td>0.62(0.24-1.59)</td>
<td>0.32</td>
</tr>
<tr>
<td>6. When the infected animal is milked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t milk the animal</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Before health animals</td>
<td>1.50(0.14-16.54)</td>
<td>0.74</td>
</tr>
<tr>
<td>After health animals</td>
<td>0.4(0.06-2.57)</td>
<td>0.33</td>
</tr>
<tr>
<td>Question</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Just mixing</td>
<td>1.11 (0.19-6.22)</td>
<td>0.91</td>
</tr>
<tr>
<td>7. Milking environment*</td>
<td>Sanitary</td>
<td>1</td>
</tr>
<tr>
<td>*Unsanitary</td>
<td>1.6 (0.59-4.29)</td>
<td>0.35</td>
</tr>
<tr>
<td>8. Do they milk animals they suspect to be infected</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2.5 (0.68-9.56)</td>
</tr>
<tr>
<td></td>
<td>Sometimes</td>
<td>0.4 (0.08-2.31)</td>
</tr>
<tr>
<td>9. Washing of teats before milking</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1.4 (0.52-3.50)</td>
</tr>
</tbody>
</table>

**Abbreviation; CI=Confidence Interval**