HYGIENIC AND COMPOSITIONAL QUALITY OF RAW MILK PRODUCED BY SMALLHOLDER DAIRY FARMERS IN LUSAKA PROVINCE OF ZAMBIA

BY

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UNIVERSITY OF ZAMBIA

DECLARATION

I, **KUNDA BONAVENTURE** do hereby declare that this dissertation represents my own work and that it has never been submitted before for the award of a degree or any other qualification at this university or any other university.

Signature……..

Date……..

DEDICATION

This work is dedicated to my daughters Zoey and Zena, and my wife Bwalya. I love you all.

ABSTRACT

This study was carried out to evaluate the hygienic and compositional quality of raw milk produced by smallholder dairy farmers (SHDFs) in Lusaka province of Zambia. It was conducted during the months of January and February 2014. The hygienic and compositional quality of raw milk was determined by assessing its Somatic Cell Count (SCC), Total Bacteria Count (TBC), Total Coliforms Count (TCC), Antibiotic Residues (ARs), added water and milk components. Altogether, 83 samples of raw milk were collected and analyzed at the University of Zambia, School of Veterinary Medicine Public Health Laboratory. Somatic Cell Count (SCC) of milk was measured using a DeLaval Cell Counter (DeLaval International AB, Sweden). Total Bacteria Count (TBC) and TCC were determined by culturing the samples of raw milk on Standard Plate Count Agar (SPC) and Violet Red Bile Glucose Agar (VRB) respectively, followed by colony counting after 48 hours of incubation at 32°C. Milk composition and added water were determined using a LactiCheck Ultrasonic Milk Analyzer (Page & Pedersen International Ltd, USA) while testing for presence of ARs was done using the Copan Milk Test 100 (Copan Diagnostics Inc., Denmark).

On composition, it was found that Butter Fat (BF) for raw milk from 23 out of 83 farms (27.7%) was below recommended standards and minimum legal limit of 3.2% fat. Solid Not Fat (SNF) for raw milk from 26 out of 83 farms (31.3%) was below recommended standards and minimum legal limit of 8.3%. Density for raw milk from 28 out of 83 farms (33.7%) was below recommended standards of 1.028 g/cm^3. On adulteration of milk with water, it was found that 26 out of 83 farmers (31.3%) had added some quantity of water varying from 9.46 – 34.3 % to their raw milk. 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. It was therefore concluded that water adulteration was the probable cause of low density, low BF and low SNF in milk.

Total Bacteria Count (TBC) ranged from 445 to 2.6 x 10^6 cfu/ml of raw milk. Milk from 5 out of 83 farms (6.02%) had TBC above the recommended standards and maximum legal required limit of 200,000 cfu/ml of raw milk. Total Coliforms Count (TCC) ranged from 100 to 100,000 cfu/ml of raw milk. Milk from 4 out of 83 farmers (4.82%) had TCC above the maximum recommended

limit of 50,000 cfu/ml of raw milk. The study concluded that sanitary quality of milk produced by smallholder dairy farmers in Lusaka province of Zambia, as far as bacterial contamination was concerned in totality was within acceptable standards opposite to the findings in many other countries in the region. This might be attributed to the fact that recently there has been a lot of emphasis and support towards clean hygienic production of milk and good price paid by milk processing companies for raw milk which has low bacteria content.

On SCC, milk from 51 out of 83 farmers (61.45%) did not conform to recommended standards. It had somatic cells more than the maximum recommended number of 300,000 cells / ml of raw milk. Somatic Cell Count (SCC) ranged from 263 to 22.3 x 10^6 cells/ml of raw milk. This was due to subclinical mastitis not noticeable on clinical observation.

Raw milk from 25 out of 83 farms (30.1%), tested positive for ARs while the other 58 farms (69.90%) were found negative for ARs. This study has produced the first ever report on ARs in milk in Zambia. A higher percentage of ARs in raw milk was found in the study than what has been reported in many other countries in the region.

The high SCC, ARs and added water found in the milk is a matter of serious concern. It indicates that there is need for further education and training of the farmers in aspects of good milk production which should include correct usage of antibiotics and observance of withdrawal period after antibiotic treatment. There is also need to extend this study to wider locations of milk producing areas in Zambia.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ARs	Antibiotic Residues
BF	Butter Fat
cfu/ml	Colony Forming Unit per mililitre
DAZ	Dairy Association of Zambia
DMCC	Direct Microscopic Cell Count
DPB	Dairy Produce Board
EU	European Union
FP	Freezing Point
GART	Golden Valley Agricultural Research Trust
GHP	Good Hygienic Practices
MCC	Milk Collection Centre
MRL	Maximum Residue Limit
RT	Room Temperature
SCC	Somatic Cell Count
SD	Standard Deviation
SHDFs	Smallholder Dairy Farmers
SNF	Solids Not Fat
SPA	Standard Plate Count Agar
SPC	Standard Plate Count
TBC	Total Bacteria Count
TCC	Total Coliform Count
USA	United States of America
VRBGA	Violet Red Bile Glucose Agar
ZABS	Zambia Bureau of Standards

CHAPTER 1

1.0 INTRODUCTION

Milk is highly nutritious and it is the primary source of nutrition for young mammals before they are able to digest other types of food (Bankole *et al*., 2011). It is composed of approximately 87.2 % water, 3.7 % fats, 3.5% protein, 4.9% lactose 0.7% ash and has a pH 6.8 (Olatunji, 2012). Due to its high moisture content, pH which is close to neutral and the diversity of nutrients, milk is a good growth medium for several types of microorganisms (Acuri *et al*., 2006). Microorganisms can enter into milk during milking stage, storage or transportation to the market (Garedew *et al*., 2012). These microorganisms can come from the environment, animals being milked, milkers or from equipment used in the milking parlour (Gran *et al*., 2002). Once they enter into milk, microorganisms can multiply and cause changes to its quality. If pathogenic microorganisms are involved, they can cause harm to consumers by causing human illnesses and diseases (Barros *et al*., 2011).

It is well established that consumers want clean, wholesome and nutritious milk that is produced and processed in a sound, sanitary manner and is free from pathogens (Farhan and Salk, 2007). To fulfill this demand by consumers, it is necessary that farmers produce milk of good quality. Good quality milk is that which is free from pathogenic bacteria and harmful toxic substances, free from sediment and extraneous substances, of good flavor, with normal composition, adequate in keeping quality and low in bacterial counts (White, 1993). Milk processing companies pay for raw milk produced and supplied by farmers in accordance with its quality (Yambayamba and Zulu, 2011). Therefore, in order to maximize revenue, it is important that farmers produce raw milk of good hygienic and compositional quality (Pandey and Voskuil, 2011). According to Khan *et al*. (2008) raw milk of poor hygienic quality poses a public health risk, has reduced processing properties and its products have got a reduced shelf life.

The smallholder dairy farming industry in many African countries, including Zambia, is important because it plays a significant role in ensuring food security and alleviation of poverty. It provides households with the much required employment, income generation and nutritious food (Pandey, 2014). However, the farmers are often unaware of the quality of milk that they are

producing. It is therefore important that studies of this nature are conducted to assess and monitor the hygienic and compositional quality of raw milk being produced by farmers. This will assist to generate evidence-based information that can be used to educate and train farmers on improved dairy production. With improved dairy production, farmers will help prevent public health risks associated with raw milk of poor quality and Zambia's milk will become more competitive in meeting regional and international standards.

1.1 General objective of the study

The aim of the study was to evaluate the hygienic and compositional quality of raw milk produced by SHDFs in Lusaka Province of Zambia.

1.2 Specific objectives of the study

i. To determine the Somatic Cell Count (SCC) of raw milk produced by SHDFs in Lusaka province of Zambia.
ii. To determine the Total Bacteria Count (TBC) and Total Coliforms Count (TCC) in raw milk produced by SHDFs in Lusaka province of Zambia.
iii. To qualitatively test for presence of Antibiotic Residues (ARs) in raw milk produced by SHDFs in Lusaka province of Zambia.
iv. To determine the compositional quality of raw milk produced by SHDFs in Lusaka province of Zambia.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Nutritional importance of milk

Milk is a major component in human diet all over the world (Gran *et al*., 2002). It is an important source of nutrients required for growth in infants and for maintenance of health in adults. It is a sole natural food for infants (Boor *et al*., 1998). Milk is a valuable source of protein, fat, carbohydrates, vitamins and minerals (Faraz *et al*., 2013). Milk protein contains all the nine essential amino acids required by humans, especially young ones, for growth and development. The nine essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Huth *et al*., 2006). Milk proteins are needed in the body to build and repair tissues and to produce antibodies which circulate in blood and help to resist infection (Javaid *et al*., 2009). Milk lactose and fat are good sources of energy whereas milk vitamins play many roles in the body which include being cofactors of metabolism, hormone precursors and antioxidants (Boor *et al*., 1998). Vitamins help the body use carbohydrates, proteins and fats. Some of the minerals found in milk are calcium, magnesium, phosphorus, potassium, selenium, zinc, copper, iron, manganese and sodium. Milk minerals have many uses in the body that include enzyme functions, bone formation, water balance and maintenance of the body and oxygen transport (Khanik, 2007).

2.2 Somatic Cell Count (SCC) in raw milk

Milk SCC is an indicator of udder health and prevalence of clinical and subclinical mastitis in dairy herds (Hamann, 1996). High SCC is associated with an increased risk of clinical mastitis, decreased milk yield and shorter shelf life of dairy products (Hutton *et al*., 1990). Mastitis in both clinical and subclinical form affects milk production. Apart from lowered productivity, mastitis reduces milk quality as a result of changes in milk composition and also by contamination of milk by drugs used for treatment of the disease (Karimuribo *et al*., 2005). Somatic cells found in milk are primarily leukocytes (white blood cells) and some epithelial cells shed from the lining of the mammary glands (Eberhart *et al*., 1982). The leukocytes are derived from blood and consist of macrophages, lymphocytes and neutrophils (Harmon, 1994). The macrophages are involved in immune recognition and are the predominant cell type present in milk from uninfected mammary glands. Lymphocytes are responsible for immune memory. The neutrophils are involved in defense against an invasion of the mammary gland by microorganisms and are the predominant cell type in milk from infected glands (Karimuribo *et al*., 2005).

Somatic cell count (SCC) of milk directly represents the inflammatory status (mastitis) of the mammary gland (udder) from where milk was collected (Salman and Elnasri, 2011). Somatic Cell Count (SCC) from normal mammary glands should be lower than 200,000 cells/ml of milk. Somatic Cell Count (SCC) between 200,000 and 300,000 cells/ml of milk is indicative of a degree of infection or initial stages of infection and that the cow is infected with a form of mastitis (Politis and Ng-Kwai-Hang, 1988).

In Zambia, the maximum limit of the number of somatic cells per ml of milk that is acceptable by law is not stated. The Food and Drug Act of 2001 only states that milk sold in Zambia for manufacture into dairy products should not contain any inflammatory products and somatic cells are the main inflammatory product of mastitis. However, the Zambia Bureau of Standards (ZABS) has set 300,000 cells/ml in raw milk as the maximum limit. This maximum limit for somatic cells is low compared with standards set in many other countries. In EU and USA, maximum limit is 400,000 and 750,000 cells/ml of milk respectively (Smith and Hogan, 1999). In South Africa, according to section 15(1) of the Foodstuffs, Cosmetics and Disinfectant Act

(Act No. 54 of 1972), the maximum accepted limit for somatic cells is 500,000 cells/ml of milk. The reason for higher maximum accepted limit for somatic cells in these other countries, especially the developed countries, might possibly be due to the fact that high producing exotic breeds of cattle (breeds found in developed countries) are more prone to mastitis (Rauberts and Shook, 1982).

In the dairy industry, especially in the developed countries, SCC has become one of the most reliable indicators for determining milk quality and the price of raw milk (Jayarao *et al*., 2006). In Zambia, however, there is lack of information on quality of raw milk with regards to SCC. The only available research based published literature on SCC of raw milk is by Pandey *et al*. (1996) who attempted to study the sanitary quality of raw milk from dairy farms supplying milk to the now defunct Dairy Produce Board (DPB) in Lusaka, Zambia. The study by Pandey *et al*. (1996) focused on commercial dairy farmers who had high yielding exotic breeds while the current study focused on SHDFs who have average yielding local and cross breeds. It was therefore envisaged that this study would generate updated information on the quality of raw milk produced by SHDFs in Lusaka province of Zambia.

2.3 Total Bacteria Count (TBC) in raw milk

Microbial load of milk is a major factor in determining its quality (Khan *et al*., 2008). It indicates udder infection and the level of hygiene exercised during milking, that is, cleanliness of udders for the cows, milkers and milking equipment. It also indicates the condition of storage and the manner of transportation of milk after milking (Karikari *et al*., 1998). Milk must be cooled to temperatures below 5^{o}C soon after milking (within 2 hours) to avoid proliferation of bacteria (Mubarack *et al*., 2010).

Milk is synthesized in specialized cells of the mammary glands and it is virtually sterile when secreted into the alveoli of the udder. Beyond this stage, microbial contamination of milk can occur (Mdegela *et al*., 2004). From the time milk leaves the udder, until it is dispensed into containers, everything with which it comes into contact is a potential source of microorganisms (Mutukumira *et al*., 1996). Improper cleaning and sanitizing of dairy equipment, dirty udders, dirty milkers and inadequate cooling of the raw milk or keeping raw milk at room temperature for a long time can all lead to an increase in milk TBC (Grimaud *et al*., 2007). Good quality raw milk, which is produced under good farm hygiene conditions and cooled adequately (cooled immediately after milking to temperature less than 5°C) usually have TBC which is less than 5,000 cfu/ml of milk (Salman and Elnasri, 2011). Raw milk with high microbial load has poor keeping quality and products manufactured from it are of inferior quality and have a reduced self life (Hayes *et al*., 2011). It must be appreciated that nearly all changes which take place in taste, odour or appearance of the milk after milking are as a result of microorganisms, especially bacteria (Yambayamba and Zulu, 2011).

In Zambia, the law states 200,000 cfu/ml of raw milk as the maximum legally recommended limit of bacterial count in raw milk but it does not state the grades of milk according to the bacterial load (The Food and Drugs Act, 2011). However, milk processing companies have a grading system and pay farmers according to the grade of their milk. They grade raw milk with ≤50,000 cfu/ml is Grade A, 50,001 to 200,000 cfu/ml Grade B while >200,000 is Grade C (Yambayamba and Zulu, 2011). According to Yambayamba and Zulu (2011), when a farmer produces milk which is not Grade A, he incurs a loss in his income. This loss can be calculated by taking income from milk per litre according to the milk grade and comparing with the income

that would have been obtained if the farmer was producing Grade A milk. The difference of these income levels is the income the farmer is losing.

2.4 Total Coliforms Count (TCC) in raw milk

The genera *Escherichia, Klebsiella, Enterobacter, Serratia,* and *Citrobacter* are collectively called Coliforms (Boor *et al*., 1998*).* Coliforms are almost always present in raw milk but with good methods of production their number can be kept low (Bae and Seung, 1992). The presence of these organisms in milk and milk products is an indication of unsanitary production and improper handling of either milk or milking utensils (Boor *et al*., 1998). Coliforms are mainly part of the normal intestinal flora of mammals. In immune compromised individuals, for example HIV patients in humans, some coliforms can cause a wide range of infections as opportunistic pathogens (Boor *et al*., 1998).

Presence of coliforms in milk is generally associated with fecal contamination (Chandan and Hedrick, 1979). Kagkli *et al*. (2006) showed that in addition to faecal contamination, other factors such as milking wet udders, inadequate cooling of milk and udder infection are the main sources of coliforms in milk. *Escherichia coli* (*E. coli*) is the most commonly isolated coliform from milk in the clinical laboratory (Ahmed and Salam, 1991). Coliforms count of less than 100 cell/ml is considered acceptable, but count of less than 10 cell/ml is achievable and desirable (Boor *et al*., 1998). Coliforms count above 500 cell/ml indicates poor hygiene either during equipment cleaning or between milking with common contaminants such as bedding, manure, soil or water (Boor *et al*., 1998). Coliforms are used as an indicator of hygienic condition during handling and processing of milk and milk products (Cousin, 1982).

In Zambia, the law does not state the acceptable maximum limit of coliforms count in raw milk. The Zambia Bureau of Standards (2009) however has set 50,000 cfu/ml of raw milk as the maximum limit acceptable number of coliforms in raw milk.

2.5 Antibiotic Residues (ARs) in raw milk

Antibiotics are usually used for the prevention and treatment of animal diseases and to improve the efficacy of animal production (Sana *et al*., 2005). They have been used in cows for many years to treat infections such as mastitis (Seymour *et al*., 1988). The past several years have seen increased pressure on milk producers to increase milk production from each and every cow. This pressure often results in more infections, and the increased use of antibiotics to counter these infections (Shitandi and Kihumbu, 2004).

After treating with antibiotics, if farmers milk their cows without adhering to the withdrawal period recommended by the manufacturer of the antibiotics used, ARs can be detected in milk (Khanik, 2007). Antibiotic Residues (ARs) are important for three major reasons. First, microorganisms can develop antibiotic resistance when exposed to milk ARs at sub lethal doses (under dosage). This renders antibiotic treatment against these microorganisms ineffective (Mitchell *et al*., 1998). Second, some antibiotics have side effects and therefore consumption of milk containing ARs can cause similar complications in consumers. Examples of such complications are allergies such as urticaria, dermatitis, asthma and rhinitis (Nero *et al*., 2007). Some antibiotics, including nitrofuranes and chloramphenicol have a carcinogenic effect in laboratory animals. This represents a potential risk to consumers of milk which contain ARs (Movassagh and Karami, 2010). Third, the bactericidal and bacteriostatic activity of ARs can adversely affect the fermentation process of milk into cheese and cultured products, such as mabisi (sour milk) and yoghurt by inhibiting the starter cultures. Starter cultures contain bacteria (Kang'ethe *et al*., 2005).

The Maximum Residue Limits (MRLs) for ARs in milk have been established in many countries and MRLs for European Union (EU) are shown in Appendix 2. These MRLs are set at levels which are not likely to be exceeded if the veterinary drugs are used in accordance with approved label instructions (Nouws *et al*., 1998). In Zambia, MRLs have not been established. However, the law only states that milk sold for manufacture into dairy products is required to have no antibiotics or other antimicrobial substances (The Food and Drugs Act, 2001).

2.6 Added water

Water is the most common adulterant in milk which is often added to milk by unscrupulous milk dealers who want to increase the volume in order to earn easy money (Ombui *et al*., 1995). Addition of water to milk reduces its nutritive value and if the water added is contaminated, there is a health risk posed to consumers (Van Kessel, 2004). Water used to adulterate milk might be from an unsafe source of water supply which can be contaminated with pesticides, fecal material, heavy metals and micro-organisms. Such contaminated milk can be harmful to consumers if consumed raw and if it is processed, the products have reduced shelf life (Kandpal *et al*., 2012).

It is important that SHDFs do not add water to their raw milk because milk processing companies have introduced stiff penalties to farmers and dairy cooperatives who do not adhere to their acceptable standards of milk (Pandey and Voskuil, 2011). For example if a farmer or cooperative is discovered to have added water (adulterated) in milk once, as a penalty, that farmer or cooperative will be considered to have added water to the whole supply of milk during that month. At Milk Collection Centres (MCCs), officials test milk delivered by farmers and milk which does not meet the processors' standards is rejected (Kenny and Mather, 2008).

2.7 Smallholder Dairy Farmers (SHDFS) in Zambia

Zambia's dairy sector is made up of three groups of farmers. These are commercial, smallholder and traditional farmers (Pandey, 2014). A smallholder dairy farm is a farm where the number of animals per farm or per herd usually does not exceed 10 heads of cattle, milking machines are generally not used and milk is not chilled but is generally transported to the market in unrefrigerated cans (Kusiluka *et al*., 2006). In Zambia, SHDFs are generally found in urban areas, peri urban areas and in resettlement scheme areas (Valeta, 2004). According to Pandey (2014) SHDFs mostly use crossbred cows (Friesian crossed with indigenous breeds in most cases) which are largely supplied by Golden Valley Agricultural Research Trust (GART) and other commercial dairy farms. These cows produce on average 10 - 20 litres of milk per cow per day (Valeta, 2004). The SHDFs have got a market oriented approach towards milk production and they are comparatively resilient to rising prices of stock feed because they usually only use a small quantity of purchased stock feed during the dry period of the year (Pandey, 2014). Zambia's domestic annual milk consumption is approximately 3.0×10^8 litres per annum and it is estimated that half of it is produced by SHDFs, while 23% of it is produced by commercial dairy farmers and the remainder (27%) is imported as milk and milk products (Valeta, 2004).

2.8 The public health importance of milk

Although milk is an important component of a healthy diet for humans, it can present a health risk due to possible contamination with hazards such as pathogenic bacteria, chemical and antibiotic residues (White *et al*., 2009). Bacteria can originate from udders, milkers or milking equipment if they are dirty (Oliver *et al*., 2005)**.** If pathogenic microorganisms are involved, they can cause diseases in milk consumers such as tuberculosis which is caused by *Mycobacterium spp*, brucellosis caused by *Brucella abortus* and Q fever caused by *Coxiella burnetii*. Hepatitis A is an example of a milkborne disease that can result from viruses coming from personnel and environment contaminating milk (Jayarao and Henning, 2001).

The occurrence of chemical residues in milk is also a matter of public health concern. Most of the chemical contaminants in milk are veterinary drugs such as antibiotics, anthelminthitic drugs, hormones and pesticides (Khaniki, 2007). Pesticides can enter the cow as residues of herbicides on forage while antibiotics, anthelminthitic drugs and hormones can enter when given to cows orally, by injection, or as intra mammary infusions for the treatment of mastitis. Chemical contaminants can also enter milk from equipment after milking.

2.9 Milk quality studies

In Uganda, Grimaud *et al.* (2007) found a high bacterial load of 2 x 10^6 cfu/ml of raw milk in a survey on milk quality that was carried out in Mbarara major milk producing region in Uganda, between June and August 2004. The high bacterial3 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 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 7.8x10^4 - 1.3x10^6 cfu/ml and coliforms from 2.4x10^2 - 2.3x10^3 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 3.4x10^7 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, which 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 1,490 x 10^6 cfu/ml of raw milk (range 0.25 x 10^6 - 25,100 x 10^6 cfu/ml of raw milk) and coliforms count 149 x 10^3 cfu/ml of raw milk (range 0.10 x 10^3- 1,540 x 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. Kenyan national standards (maximum bacteria counts/ml) for 'good' milk are 2,000,000 cfu/ml and 50,000 cfu/ml for total and coliforms count, respectively, for raw milk while 50,000 cfu/ml and 10 cfu/ml for total and coliforms count, respectively, for pasteurised milk.

In Swaziland in a study done from October, 1999 to April, 2000, TBC in raw milk was high (greater than 1 x 10^7 cfu/ml of raw milk) and coliforms count were high (greater than 7 x 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 cross-sectional study that was done 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 x 10^6 cfu/ml of raw milk (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 study on milk quality in Tanzania found 22% of raw milk had specific gravity below 1.026 suggesting adulteration with water.

Lues *et al.* (2010) in a study done in South Africa found total viable micro-organisms ranging from 1×10^4 to 1×10^7 cfu/ml of raw milk with the highest recorded count at 6.08×10^7 cfu/ml of raw milk. Only 6.1% of the samples complied with the 2×10^5 cfu/ml of raw milk guideline set by the regulations. South African regulations (R.489 of 2001) states that standard plate counts may not exceed 5×10^4 cfu/ml (raw milk intended for consumption) and 2×10^5 cfu/ml (raw milk for further processing). The mentioned legislation further states that, for both the purpose of direct consumption and further processing, coliforms must be below 20 cfu/ml of raw milk Lues *et al.* (2010). In Zimbabwe, Mutukumira *et al*. (1996) studied only 10 raw milk samples from smallholder farmers and found TPC ranging from 6.2×10^3 - 7.8×10^7 cfu / ml of raw milk. Coliforms count ranged from 3.2×10^2 - 2.3×10^5 cfu/ml of raw milk, protein 3.19%, BF 3.52%, total sold 11.76%, SNF 8.25 % and pH 6.15 - 6.65 indicating poor quality of milk.

In Nigeria, Olatunji *et al.* (2012) reported a total viable bacteria count ranging from 1×10^6 - 5.6×10^7 cfu/ml in raw milk in Abuja. Laba and Udonsek (2013) studied bacteriological quality and safety of raw milk in North Central Nigeria and found total viable count in raw milk ranging from 1.16×10^6- 2.60×10^6. Authors also isolated *Listeria monocytogenes, Staphylococcus aureus, E. coli, Klebsiella spp, Pseudomonas spp, Proteus spp* and *Bacillus cereus.* The results indicated the potential health risk of consuming raw cow milk under the current production and collection conditions. Recently, Fasae and Olusesan (2015) also from Nigeria reported mean value of viable bacteria count of 5.97×10^6 cfu/ml of raw milk in Bunagi breed of local cows which is five times higher than required standard of 1×10^6 cfu/ml of raw milk. On composition they further found 3.43% protein, 4.70% BF, 12.5% total solid. Authors concluded that the high TBC might have been due to lapses in milk sanitation, conducive ambient temperature and relative humidity for the growth of bacteria accompanied by lack of refrigeration in the situation of long distance milk transportation.

In a study of 120 samples of raw quality in Morocco, Hadrya *et al*. (2012) found total mesophilic aerobic bacteria count of 6.9×10^8 cfu/ml of raw milk (range 2.7×10^5 - 7.0×10^9 cfu/mi of raw

milk) and faecal coliforms count 4.2 x 10^7cfu/ml of raw milk (range 0 - 1.7 x 10^9cfu/ml of raw milk) suggesting bad sanitary quality and need for improved hygienic standards. In Nepal, Lekhraj *et al.* (2010) attempted to study the microbiological quality of raw milk supplied by smallholder dairy farmers and found TBC ranging from 2.78 x 10^6 - 13.29 x 10^6 cfu/ml of raw milk with mean value of 9.03 x 10^5 cfu/ml of raw milk which clearly indicated very poor quality of milk from microbiological point of view. Rezaei *et al.* (2014) reported in a quality study of raw milk samples in Iran an average TBC of 3.8 x 10^7 cfu/ml of raw milk with range of 2.56 x 10^6 - 7.3 x 10^7cfu/ml of raw milk.

Rutaro (2015) in Uganda studied SCC in raw milk to compare Southwestern Uganda's milk quality against international standards. The milk's SCC was analyzed using a DeLaval DCC. The study found that the 100 farms had an average SCC of 507,000 cells/ml of raw milk and about 34% of farms in the study had SCC under 200,000 cells/ml of raw milk, an indication of high quality milk while 7% of the farms with SCC over 1,000,000 cells/ml of raw, the remaining 93% had an average SCC of 276,000 cells/ml of raw milk, a level comparable to international standards, well below the EU threshold of 400,000 cells/ml of raw milk.

In Zambia, Pandey *et al*. (1996) studied the sanitary quality and cell count of raw milk from 95 dairy farms supplying milk to the dairy produce board in Lusaka. They found cell count of milk varying from 0 - 122 x 10^5 cells/ml of raw milk and standard plate count ranging from log 7.66 - log 9.15 per ml of raw milk suggesting a high bacterial load in milk. Coliforms count ranged from log 6.06 - log 7.20 per ml of raw milk. The authors concluded that the sanitary quality of raw milk of about 45% dairy farms around Lusaka was not good and originated from inflamed udders. Another study was done in Zambia by Yambayamba and Zulu (2011) involving 16 smallholder farmers in Magoye (Southern Province) to determine the level of bacterial contamination of milk across different types of environment based on housing types. 1.13 x 10^5 cfu/ml of raw milk was found as mean TBC across the different types of housing.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in Lusaka Province of Zambia during the months of January and February 2014. Lusaka province lies in the south central part of Zambia between 28 and 30 degrees east and between 15 and 16 degrees south (Figure 1). It shares borders with Mozambique and Zimbabwe on the southern and eastern sides respectively. Domestically, it shares borders with Southern province on the south-west, Central province on the north and Eastern province on the east. The province has a human population of 2,191,225 people and it covers a total area of 75,261 square kilometers (CSO, 2012). There are a total of 444,419 households in the province out of whom 65,213 are agriculture households (CSO, 2012). Eighty three (83) SHDFs (30 of whom belonged to Mapepe MCC in Chilanga district, 26 to Palabana MCC in Chongwe district and 27 to Lusaka West farming block in Lusaka district participated in this study.

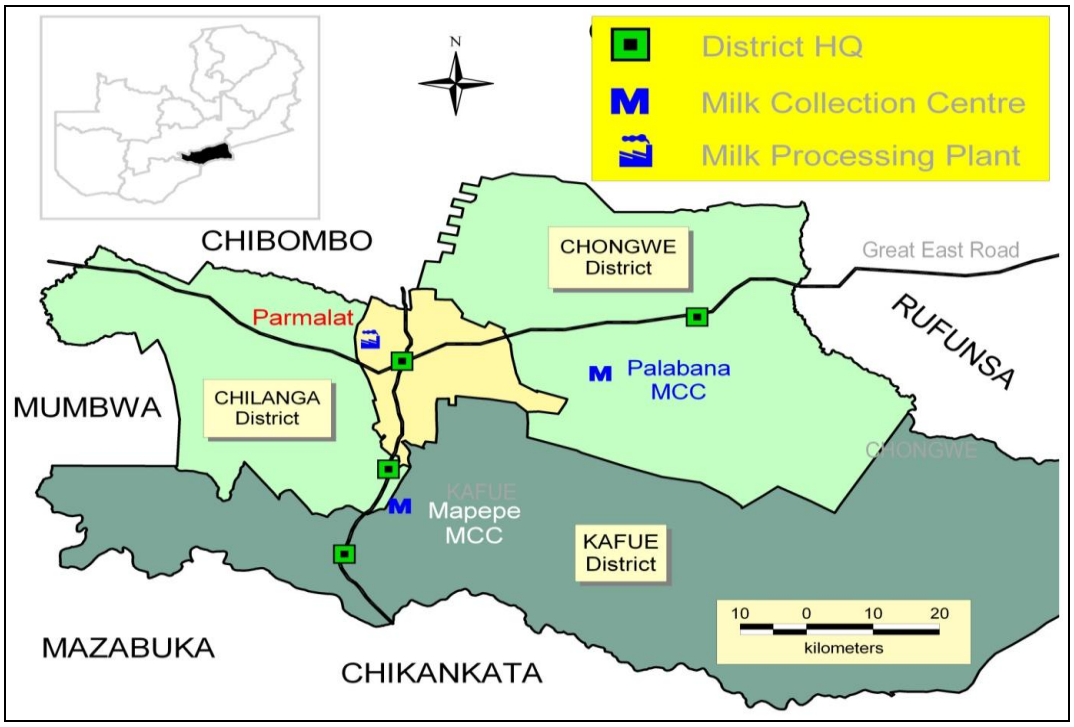

Figure 1: Map of Lusaka Province of Zambia

3.2 Study design and sample size determination

A cross-sectional study design was used in the study implementation. A list of SHDFs belonging to Palabana dairy scheme (Palabana MCC), Mapepe dairy co-operative society (Mapepe MCC) and Lusaka west farming block (Parmalat) was compiled using records at the milk collection centres (MCCs) and the Dairy Association of Zambia (DAZ). This constituted the sampling frame. Sampling units were individual smallholder dairy farms. Smallholder Dairy Farmers (SHDFs) from these three areas were selected for this study because that is where the majority of smallholder dairy farms in Lusaka Province are situated.

There are 106 SHDFs who supply milk to the 3 milk centres Palabana dairy scheme (Palabana MCC), Mapepe dairy co-operative society (Mapepe MCC) and Lusaka west farming block (Parmalat).

The sample size was calculated using the following formula:

$n=pq\ (Z/e)^2$ (Shajahan, 2009)

Where

- n is the minimum sample size required
- p is the proportion belonging to the specified category = 0.5
- q is the proportion not belonging to the specified category = 0.5
- Z is the z value corresponding to the 95% level of confidence required = 1.96
- e is the margin of error required = 0.05

$n=pq(Z/e)^2 = 0.5 \times 0.5 \times (1.96/0.05)^2 = 384$ farmers

Adjusted for a finite population

Since there are 106 SHDFs, the sample population was adjusted to take into account selection from a finite population. This was calculated according to Shajahan (2009) using the following formula:

$n'=n/(1+(n/N))$

Where:

- n' is the adjusted minimum sample size
- n is the minimum sample size = 384 (as calculated above)
- N is the total number of smallholder dairy farmers who will be targeted = 106

$n'=n/(1+(n/N)) = 384/(1+(384/106)) = 83$

Therefore the minimum sample size required for the study was 83 farmers.

3.3 Milk sample collection

From the list of SHDFs (n=106) in Lusaka province of Zambia, 83 were randomly selected from Palabana dairy scheme, Mapepe dairy cooperative and Lusaka West farming block (Figure 1). Chairpersons of the mentioned areas were requested to assist in organizing meetings with the SHDFs. These meetings were used to sensitize the SHDFs about this study. After the farmers accepted to participate in the study, raw milk samples (50 ml from the bulk raw milk of each of the 83 selected farms) were collected aseptically into separate sterile 50 ml sample bottles as milk was being delivered to MCCs. The collected samples were stored in an ice packed cooler box and transported to University of Zambia (UNZA), School of Veterinary Medicine Public Health laboratory for further analysis (Figure 2).


Figure 2: Images of farmers delivering milk to MCC and of the laboratory at UNZA

3.4 Laboratory analysis of milk

Somatic Cell Count (SCC)

A DeLaval Cell Counter (DeLaval International AB, Sweden) was used to count somatic cells in the milk. To measure somatic cells, the cassette of the DeLaval Cell Counter, which contains a reagent (a DNA specific fluorescent probe), is used to collect 1.0 µl of milk sample (Figure 3). Once inside the cassette, milk mixes with the reagent. The reagent then reacts with the nuclei of the somatic cells and when the cassette is inserted in the DeLaval Cell Counter, it is exposed to light emitted by the DeLaval Cell Counter. This gives rise to fluorescence signals which are recorded in an image. The image is used to determine the number of somatic cells in the milk which appears on the screen of the DeLaval Cell Counter (DeLaval, 2005).

Figure 3: Image of DeLaval Cell Counter (left) and its cassette (right).

Total Bacteria Count (TBC)

Total bacteria count (TBC) was determined using the Standard Plate Count (SPC) method where using peptone water each of the 83 milk samples was serially diluted into three dilutions of 1:10. 1:100 and 1:1,000. From each dilution, 1 ml was placed on a sterile petri dish using a sterile pipette and pour plated with15 ml molten (55°C) Standard Plate Count Agar (SPA). The plates were allowed to solidify for 15 minutes after which they were incubated for 48 hours at 32°C. Bacteria (or clusters) that grew and became visible colonies were counted using a colony counter and expressed as number of Colony Forming Units per milliliter (cfu/ml) of milk (Richardson, 1985).

Total Coliforms Count (TCC)

Similar to TBC, milk samples were homogenized and serially diluted into three dilutions of 1:10. 1:100 and 1:1,000. One milliliter of the appropriate dilutions was placed on petri dishes and pour plated with15 ml molten (55°C) Violet Red Bile Glucose Agar (VRBGA). The plates were allowed to solidify for 15 minutes after which they were incubated for 48 hours at 32°C. Finally,

coliforms that grew were counted using a colony counter and expressed as number of colony forming units per milliliter (cfu/ml) of milk (Richardson, 1985).

Antibiotic Residues (ARs)

Copan Milk Test 100 (Copan Diagnostics Inc., USA) which is a qualitative test for detecting the presence of antimicrobials in milk was used. In this test, *Bacillus stearothermophilus var. calidolactis* spores are enclosed within an agar based gel matrix containing nutritive substances and a pH indicator. When milk sample which is free from antimicrobials is added and incubated at 64°C for 3 hours, the bacterial spore germinates, produce acid and that results in a pH drop. The pH drop causes a colour change from purple to yellow. However, if milk sample contain antimicrobials, the spores do not germinate and therefore no acid will be produced and the colour will remain purple or unchanged (Copan innovation, 2013).

Added water and composition of milk

A LactiCheck Ultrasonic Milk Analyzer (Page & Pedersen International Ltd, USA) was used to determine the composition of milk samples (Figure 4). This is an automated machine, which works rapidly and effectively in analyzing the major components of milk (BF, SNF, protein and lactose), milk density, added water and FP. To analyze milk, the sample cup of the LactiCheck Ultrasonic Milk Analyzer, is filled with 20 ml of milk sample which must be at room temperature. The sample cup bottom is then fixed on the sample cup holder so that the aspirator of the LactiCheck Ultrasonic Milk Analyzer is immersed in the milk sample. The start button is then pressed to start the test which takes about 85 seconds depending on the milk temperature and ambient room temperature. When the measurement is completed, the display shows the results (LactiCheck, 2010). The LactiCheck Ultrasonic Milk Analyzer operates using a simple principle of physics: the motion of any wave will be affected by the medium through which it travels. When a milk sample is ready for analysis, the LactiCheck Ultrasonic Milk Analyzer launches a high frequency sound wave by exciting the ultrasonic transducer with a continuous wave impulse. The sound travels through the milk. An amplitude theory implemented in the LactiCheck Ultrasonic Milk Analyzer software predicts the magnitude of each of the scattering and absorption mechanisms. The movement of the particles of, for example BF and SNF, relative to the continuous phase causes visco-inertial losses as the sound wave propagates through the

sample. Drag between the liquid and the particles cause sound energy to be lost as heat. The velocity of the ultrasonic pulse and the temperature change of the sample are precisely measured. As the sound velocity and temperature are directly correlated to the particulates in the sample, the LactiCheck Ultrasonic Milk Analyzer provides reliable analyses of critical components such as BF and SNF by accurately assessing changes in these parameters. Other characters, such as protein, added water and FP, are calculated based upon the percentage of components measured using an exact mathematical formula (LactiCheck, 2010).

Figure 4: Image of LactiCheck Ultrasonic Milk Analyzer.

3.5 Statistical analysis of data

Data that was generated from laboratory tests on the 83 samples of raw milk was first entered into Microsoft excel then transferred to SPSS version 20 for analysis. Means and percentages of milk parameters were calculated and the following statistical tests were conducted:

- ANOVA (Analysis Of Variance) was to establish whether the levels of milk production for the 3 study areas were significantly different from each other.
- Correlation coefficient test to determine association between added water and density and added water and SNF.

CHAPTER 4

4.0 RESULTS

4.1 Location of farmers who participated in the study

The study was conducted in three areas, namely Lusaka west farming block, Mapepe dairy cooperative and Palabana dairy scheme. These areas were selected because that is where majority of the SHDFs in Lusaka Province of Zambia are located. A total of 83 SHDFs selected at random participated in the study. The distribution of the farmers by study area is shown in Table 1.

Table 1: Location of the farmers (n=83)

Variable	Study area (Location)	Number of farms	Percent
Location of farm	Lusaka West	27	32.5
	Mapepe	30	36.2
	Palabana	26	31.3
TOTAL		83	100

4.2 Levels of milk production

For each of the 3 study areas, overall minimum and maximum amount of milk in litres produced per farmer per day and overall mean amount of milk in litres produced per day are shown in Table 2.

Table 2: Farmers' daily milk production levels in Lusaka Province

Study area	No. of farms	Mean litres of milk	Minimum litres	Maximum litres	Std deviation
Lusaka west	27	257.41	20	700	207.85
Mapepe	30	42.10	3	200	41.96
Palabana	26	80.04	20	190	49.12
Total	83	124.02	3	700	154.86

Results of ANOVA test showed a significant difference ($p=0.001$) in the levels of milk production among the 3 study areas. Lusaka west farming block was the highest followed by Palabana dairy scheme and the least was Mapepe dairy cooperative.

4.3 Somatic Cell Count (SCC)

Results for SCC are summarized in Table 3. Somatic Cell Count (SCC) of milk from the 83 farms ranged from 263 to 2.312 x 10^6 cells/ml of raw milk. Milk from 50 out of the 83 farms (60.2%) had SCC above the recommended maximum limit of 300,000 cells/ml of raw milk.

Table 3: Summary of SCC test results

Study area	n	Number not meeting standards	Percent (%)
Lusaka west	27	16	59.3
Mapepe	30	18	60.0
Palabana	26	16	61.5
Total	83	50	60.2

n = number of farms

4.4 Total Bacteria Count (TBC)

Results for TBC are summarized in Table 4. It was found that TBC of raw milk from 5 out of the 83 farms (6.02%) was above the maximum and legally accepted number in Zambia (200,000 cfu/ml of raw milk). The TBC ranged from 445 to 2.6 x 10^6 cfu/ml of raw milk. Figure 5 gives an example of bacteria colonies that grew after culturing.

Table 4: Summary of TBC test results

Study area	n	Number not meeting standards	Percent (%)
Lusaka west	27	0	0
Mapepe	30	4	13.3
Palabana	26	1	3.8
Total	83	5	6.02

n = number of farms

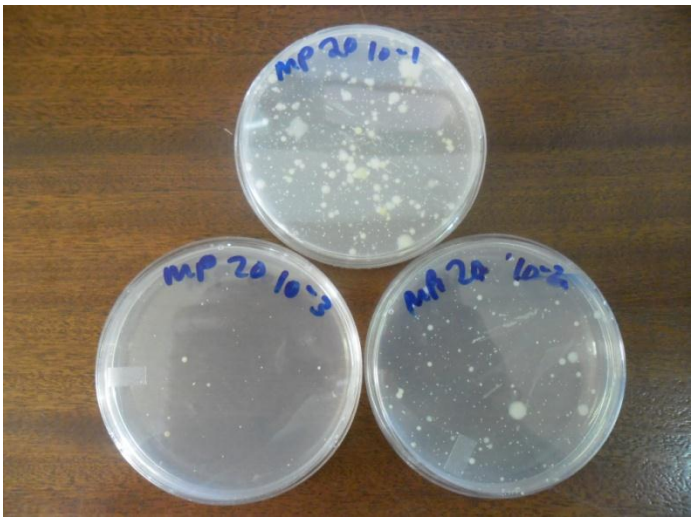

Figure 5: Some of the bacteria colonies that grew after culturing

4.5 Total Coliforms Count (TCC)

Total Coliforms Count (TCC) of milk from 4 out of 83 farms (4.8%) did not conform to recommended standards (50,000 cfu/ ml of raw milk). Total Coliforms Count (TCC) ranged from 100 to 1.0 x 10^5 cfu/ml of raw milk. Overall results on TCC are summarized in Table 5 and Figure 6 gives an example of coliform bacteria colonies that grew after culturing.

Table 5: Summary of TCC test results

Study area	n	Number not meeting standards	Percent (%)
Lusaka west	27	0	0
Mapepe	30	3	10.0
Palabana	26	1	3.8
Total	83	4	4.8

n = number of farmers

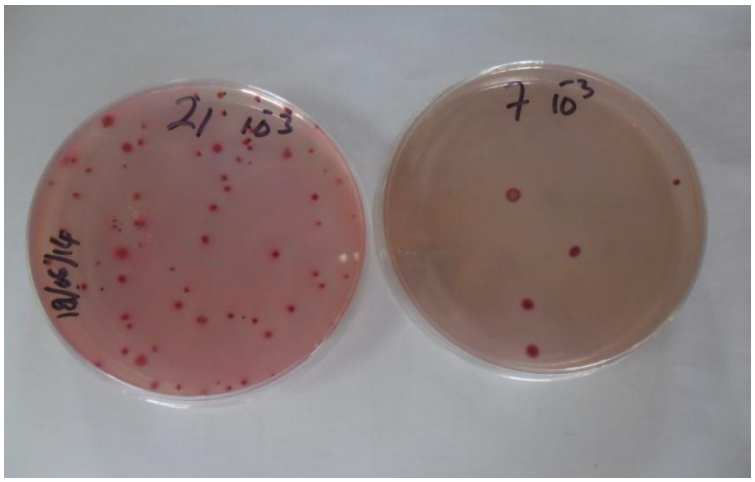

Figure 6: Some of the coliform bacteria colonies that grew after culturing

4.6 Added water

Test results on water adulteration of milk are summarized in Table 6. It was found that milk from 26 out of 83 farms (31.3%) had added water. The quantity of water added ranged from 9.46 to 34.3%.

Table 6: Summary of test results for water adulteration of milk

Study area	n	Positive for water adulteration	Percent (%)
Lusaka west	27	8	29.6
Mapepe	30	7	23.3
Palabana	26	11	42.3
Total	83	26	31.3

`

4.7 Milk Composition

A summary of results on milk composition is presented in Tables 8, 9 and 10. Butter Fat (BF) for milk from 23 out of 83 farms (27.7%) was below recommended standards (3.2% fat) and SNF of milk from 26 out of 83 farms (31.1%) was below recommended standards (8.3%). Density of milk for 28 out of 83 farms (33.7%) was below recommended standards (1.028g/cm^3). Figure 7 gives an example of components of a milk sample reading on the LactiCheck Ultrasonic Milk Analyzer.

Correlation coefficient tests, at a significance level of 0.01, were conducted to establish whether there was an association between added water and density and between added water and SNF in the milk. The results (Table 7 and Figures 8 and 9) showed that there was a strong negative

association (r = -.959; p=0.001) between added water and milk density. There was also a strong negative association between added water and SNF (r = -.916; p=0.001).

Table 7: Correlation coefficient test results between added water and density and between added water and SNF

		SNF	Density (g/ml)
Water	Pearson Correlation	-.916	-.959**
	Sig. (2-tailed)	.001	.001
	N	83	83

**. Correlation is significant at the 0.01 level (2-tailed).

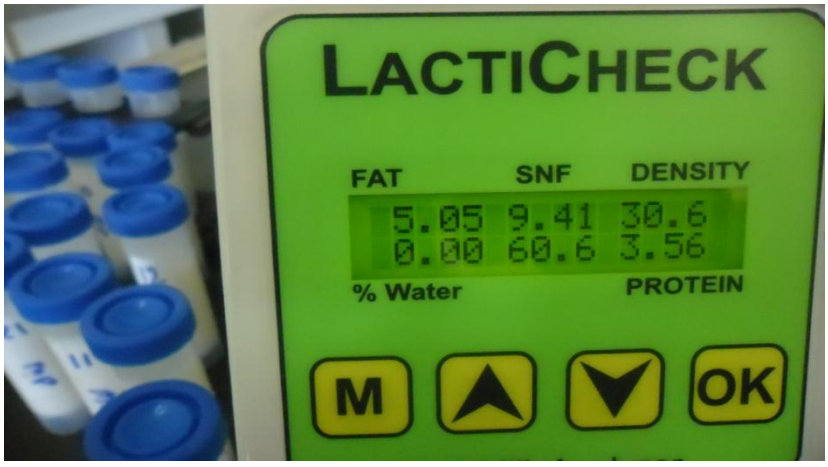

Figure 7: A close up image of LactiCheck Ultrasonic Milk Analyzer showing readings of components of a milk sample.

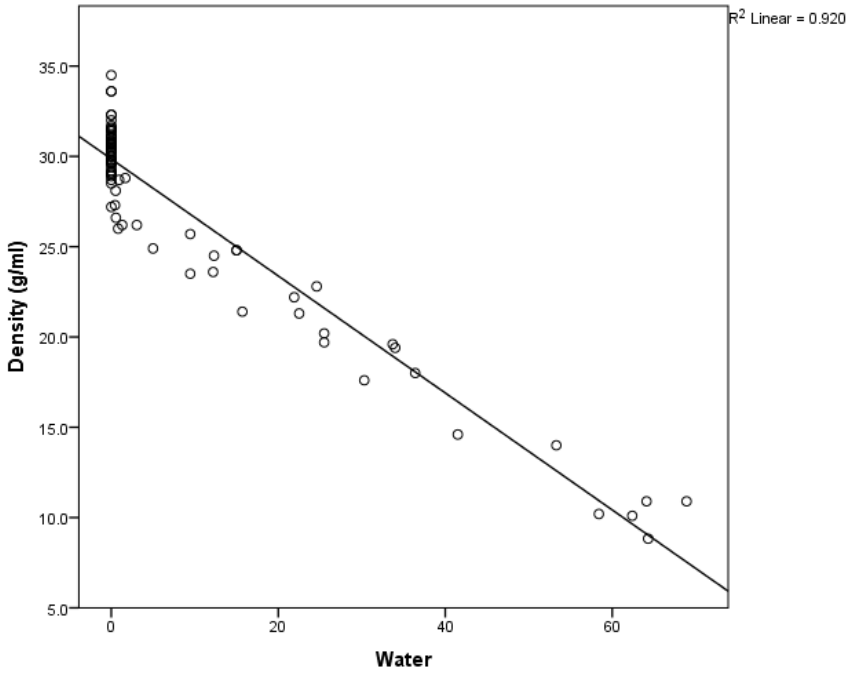

Figure 8: Correlation between added water and density

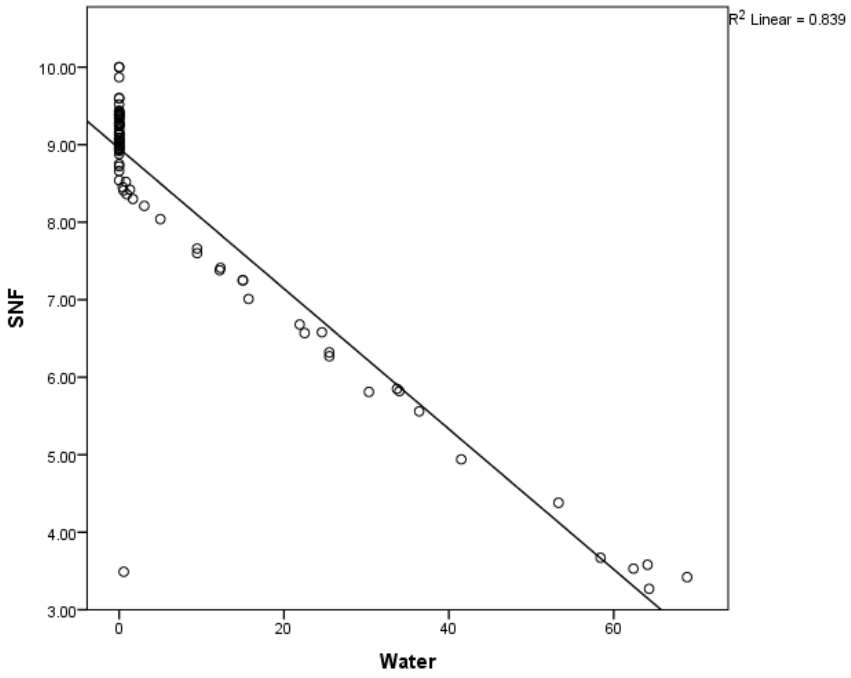

Figure 9: Correlation between added water and SNF

Table 8: Composition of raw milk from Lusaka west farming block (n=27).

S/N	Sample no.	BF	SNF	Added water (%)	Density (g/cm^3)
1	1	**2.79**	8.92	**13.6**	1.031
2	2	**3.02**	**4.94**	**41.5**	**1.015**
3	60	3.30	9.05	0.00	1.031
4	61	3.71	9.52	0.00	1.032
5	62	3.38	9.39	0.00	1.032
6	63	4.16	9.14	0.00	1.030
7	64	4.00	9.42	0.00	1.032
8	65	3.97	9.38	0.00	1.032
9	66	3.77	9.05	0.00	1.030
10	67	3.81	8.94	0.00	1.030
11	68	3.21	**6.27**	**25.5**	**1.020**
12	69	3.59	9.05	0.00	1.031
13	70	3.50	9.27	0.00	1.031
14	71	3.59	9.03	0.00	1.031
15	72	**1.29**	**4.38**	**53.3**	**1.014**
16	73	3.84	9.29	0.00	1.031
17	74	3.54	8.98	0.00	1.030
18	75	3.74	8.72	0.00	1.029
19	76	3.79	9.60	0.00	1.032
20	77	**1.14**	**6.58**	**24.6**	**1.023**
21	78	4.63	**7.01**	**15.7**	**1.021**
22	79	**1.89**	**7.25**	**15.0**	**1.025**
23	80	4.72	9.35	0.00	1.031
24	81	**2.48**	8.36	0.00	1.029
25	82	**2.79**	**6.57**	**22.5**	**1.021**
26	83	4.86	8.88	0.00	1.029
27	84	5.58	9.13	0.00	1.029

NB: Bold figures indicate samples which did not conform to recommended standards.

Table 9: Composition of raw milk from Mapepe dairy cooperative (n=30).

S/N	Sample no.	BF	SNF	Added water (%)	Density (g/cm^3)
1	3	3.92	9.15	0.00	1.031
2	4	**2.19**	**6.68**	**21.9**	**1.022**
3	5	**2.62**	9.6	**37.1**	1.034
4	6	4.22	8.94	0.00	1.029
5	7	4.59	**8.21**	0.00	**1.026**
6	8	3.95	9.08	0.00	1.030
7	9	4.72	9.35	0.00	1.031
8	10	4.65	10.0	0.00	1.034
9	11	**2.24**	**3.27**	**64.3**	**1.009**
10	12	4.00	9.16	0.00	1.030
11	13	3.43	9.28	0.00	1.031
12	14	**1.88**	**3.53**	**62.4**	**1.001**
13	15	3.40	8.41	0.00	1.028
14	16	4.43	9.39	0.00	1.031
15	17	**2.50**	**3.67**	**58.4**	**1.001**
16	18	5.05	9.25	0.00	1.030
17	19	4.08	8.66	0.00	1.028
18	20	**1.89**	**7.25**	**15.0**	**1.025**
19	21	3.81	9.40	0.00	1.032
20	22	3.43	9.13	0.00	1.031
21	23	**2.92**	**7.41**	**12.3**	**1.024**
22	24	3.83	9.25	0.00	1.031
23	25	5.01	8.54	0.00	**1.027**
24	26	3.81	9.24	0.00	1.031
25	28	4.62	9.37	0.00	1.031
26	29	3.33	10.0	0.00	1.034
27	30	4.37	9.02	0.00	1.030
28	31	4.25	9.43	0.00	1.031
29	32	3.94	8.96	0.00	1.029
30	33	5.43	9.20	0.00	1.029

NB: Bold figures indicate samples which did not conform to recommended standards.

Table 10: Composition of raw milk from Palabana dairy scheme (n=26).

S/N	Sample no.	BF (%)	SNF (%)	Added water (%)	Density (g/cm^3)
1	34	5.30	**8.04**	0.00	**1.025**
2	35	4.71	8.96	0.00	1.029
3	36	5.32	9.27	0.00	1.030
4	37	5.07	9.0	0.00	1.029
5	38	4.91	**7.60**	**9.46**	**1.023**
6	39	4.29	9.09	0.00	1.030
7	40	**1.92**	**5.56**	**36.4**	**1.018**
8	41	**2.10**	8.30	**13.7**	1.028
9	42	**2.66**	**7.66**	**9.46**	**1.026**
10	43	6.87	9.87	0.00	1.031
11	44	**1.48**	**5.82**	**34.0**	**1.019**
12	45	6.23	8.52	0.00	1.026
13	46	4.31	8.99	0.00	1.029
14	47	**2.83**	**6.32**	**25.2**	**1.020**
15	48	**1.24**	**3.58**	**64.1**	**1.011**
16	49	4.70	9.08	0.00	1.029
17	50	3.53	**5.81**	**30.3**	**1.018**
18	51	3.76	**7.38**	**12.2**	**1.024**
19	52	6.90	9.44	0.00	1.029
20	53	5.53	8.42	0.00	**1.026**
21	54	**1.43**	**5.85**	**33.7**	**1.020**
22	55	**1.53**	**3.42**	**68.9**	**1.011**
23	56	4.43	8.45	0.00	1.027
24	57	**3.09**	8.75	0.00	1.029
25	58	5.41	**3.49**	0.00	**1.027**
26	59	4.58	9.33	0.00	1.031

NB: Bold figures indicate samples which did not conform to recommended standards.

4.8 Antibiotic Residues (ARs) in milk

A summary of test results for presence of ARs in milk are presented in Table 11. Milk from 25 out of 83 farmers (30.1%) tested positive for presence of ARs.

Table 11: Results for presence of Antibiotic Residues (ARs)

Study area	n	Positive for ARs	Percent (%)
Lusaka west	27	9	33.3
Mapepe	30	11	36.7
Palabana	26	5	19.2
Total	83	25	30.1

n = number of farms

Figure 10 shows an example of Copan Test Milk 100 results which were found in the study where P = a positive control, 5 = a positive milk sample, while 7, 8 and 85 = negative milk samples.

Figure 10: Example of Copan Milk Test 100 results.

CHAPTER 5

5.0 DISCUSSION

In this study, the hygienic and compositional quality of raw milk produced by SHDFs in Lusaka Province of Zambia was established by assessing its SCC, TBC, TCC and ARs, added water and milk components.

Production of raw milk of good hygienic and compositional quality by farmers is important to milk processing companies, milk consumers and the farmers themselves. This is so for milk processing companies because raw milk of poor hygienic and compositional quality has reduced processing properties and processed milk and milk products made from such raw milk have a reduced shelf life (Oliver *et al*., 2005). For consumers, consumption of milk contaminated with pathogenic bacteria, toxins and ARs, can lead to diseases, allergic reactions, toxication and a risk of microorganisms developing resistance. For SHDFs, producing milk of good quality is important because milk processing companies pay farmers in accordance with the hygienic and compositional quality of raw milk delivered to them (Yambayamba and Zulu, 2011).

On composition, the study found that BF for raw milk from 23 out of 83 farms (27.7%) was below recommended standards and minimum legal limit of 3.2% fat. Solid Not Fat (SNF) for raw milk from 26 out of 83 farms (31.3%) was below recommended standards and minimum legal limit of 8.3%. Density for raw milk from 28 out of 83 farms (33.7%) was below recommended standards of 1.028 g/cm^3. On adulteration of milk with water, it was found that 26 out of 83 farmers (31.3%) had added some quantity of water varying from 9.46 – 34.3 % to their raw milk. 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. It was therefore concluded that water adulteration was the probable cause of low density, low BF and low SNF in milk. In similar studies done in other countries, Donkor *et al*. (2007) and Karimuribo *et al*. (2005) found 18% farmers in Ghana and 5% farmers in Kilosa district, Tanzania respectively had added water to their milk. In Kenya, Mwangi *et al*. (2000) found13% of raw milk samples from Kiambu and Nairobi were adulterated with water. In comparison, these findings from other countries were lower than the 31.3% of water adulterated milk in Zambia as found by this study. 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. Addition of water to milk should be avoided because it reduces the nutritive value of milk, and if contaminated, it poses a health risk to consumers (Kandpal *et al.*, 2012).

For TBC and TCC, milk from only a small number of farmers did not conform to recommended standards. Milk from 5 out of 83 farms (6.02%) had TBC above the recommended standards and maximum legal limit of 200,000 cfu/ml of raw milk while milk from 4 out of 83 farmers (4.82%) had TCC above the maximum recommended limit of 50,000 cfu/ml of raw milk. In similar studies conducted in other countries in the region, Shitandi and Kihumbu (2004) in Malawi found a high mean TBC of 3.4×10^7 cfu/ml of raw milk. In Tanzania, Kivaria *et al*. (2006) found a high TBC of 8.2×10^6 cfu/ml of raw milk in the Dar es Salaam region while in Kilosa district Karimuribo *et al*. (2005) found 13.4% of milk had TBC which did not conform to recommended standards of that country. In Mbarara, the major milk producing region in Uganda, Grimaud *et al*. (2007), found a high bacterial load of 2×10^6 cfu/ml of raw in a survey on milk quality done between June and August 2004. Mwangi *et al*. (2000) in Kiambu and Nairobi, Kenya found 82% and 58% of raw milk samples did not meet recommended standards for TBC and coliforms count respectively. Total Bacteria Count (TBC) was 1.49×10^9 cfu/ml of raw milk and coliforms count was 1.49×10^6 cfu/ml of raw milk. In Swaziland in a study done from October, 1999 to April, 2000, Fakudze and Dlamini (2001) found high TBC ($> 1 \times 10^7$ cfu/ml of raw milk) and high coliforms count ($> 7 \times 10^4$ cfu/ml of raw milk). In Zambia, only one study has so far been done on the sanitary quality of raw milk produced by SHDFs. This was done by Yambayamba and Zulu (2011) in Magoye (Southern Province of Zambia) and mean TBC was 1.13×10^5 cfu/ml of raw milk. The current study was done in Lusaka Province of Zambia and only 5 out of 83 milk samples (6.02%) had TBC above the recommended standards. It was therefore concluded that the sanitary quality of milk produced by smallholder dairy farmers in Lusaka province of Zambia, as far as bacterial content was concerned in totality was within acceptable standards opposite to the much higher findings in some other countries in the region (Shitandi and Kihumbu (2004) in Malawi, Kivaria *et al*. (2006) and Karimuribo *et al*. (2005) in Tanzania, Grimaud *et al*. (2007) in Uganda, Fakudze and Dlamini (2001) in Swaziland and Mwangi *et al*. (2000) in Kenya). This might be attributed to the fact that recently there has been a

lot of emphasis and support towards clean hygienic production of milk and good price paid by milk processing companies for raw milk with low bacterial count.

In this study, SCC of raw milk ranged from 263 - 2.312 x 10^6 cells/ml of raw milk which is much lower compared to those reported earlier by Pandey *et al.* (1996) from Zambia. However, keeping in mind the current Zambian standards, 51 out of 83 farmers (61.45%) did not conform to recommended standards of 300,000 cells/ml of raw milk. This was an indication that raw milk came from cows with inflamed udders (mastitis) and that the farmers were making losses in milk production because cows with high SCC (mastitis) have decreased levels of milk production. In addition, when cows are put on mastitis treatment, milk is discarded and not sold during treatment and withdrawal periods. In similar studies done in other countries, Salman and Elnasri (2011) also found high SCC in a study conducted in Khartoum State of Sudan. They found SCC of raw milk produced by 56.70% SHDFs did not conformed to that country's recommended standards of 5.0 x 10^5 cells/ml of raw milk. In a study done in Mbarara and Kiruhura districts, the major cattle corridor in Uganda, Rutaro (2015) found an average SCC of 507,000 cells/ml of raw milk and samples of milk from 66 out of 100 farms had SCC which did not conform to recommended European standards. Zambian standards for SCC seem to be quite high (300,000 cells/ml of raw milk) as compared to those in South Africa 500,000celss/ml of raw milk, European Union 400,000 cells/ml of raw milk, USA 750,000 cells/ml of raw. The higher values for the standards for SCC in these countries is possibly due to their high yielding dairy breeds of cows which are more prone to mastitis.

This study has produced the first ever report on ARs in milk in Zambia and a high percent of milk was positive for ARs. Raw milk from 25 out of 83 farms (30.1%), tested positive for ARs. Farmers were probably not using antibiotics correctly and were not observing recommended withdrawal periods of antibiotics. Similar studies on ARs have been conducted in other countries. Findings in this study were close to those reported in Pakistan, Iran and Brazil. In Pakistan, Khaskheli *et al*. (2006) found ARs level of 36.5%, while in Iran 32.9% of raw milk tested was found positive for ARs (Mokhtari *et al*., 2013). In Triangle Region of the State of Minas Gerais, Brazil Tetzer *et al*. (2005) found 33.3% of raw milk samples positive for ARs. In Zimbabwe, Mhone *et al.* (2012) found 2.5% of raw milk from smallholder dairy farms positive for ARs and Shitandi and Kihumbu (2004) in Kenya found 21% of raw milk positive for ARs.

These findings in Zimbabwe and Kenya are both lower than the 30.1% in Zambia which was found in this study. In Zambia, antibiotics for pharmacological and agricultural use are sold without prescription and used indiscriminately by farmers. This could have contributed to higher number of milk samples being positive for ARs. Processing of milk does not eliminate ARs contained in milk (Moats 1988, Loksuwan 2002, de Oliveira *et al* 2012). There is therefore need for enforcing the withdrawal period of milk after antibiotic treatment and the enforcing by the regulatory authority the sale of antibiotics on prescription by veterinary personnel.

CHAPTER 6

6.1 CONCLUSION

This study found that raw milk produced by SHDFs in Lusaka Province of Zambia conformed to recommended standards and legal requirement on TBC and TCC but not on SCC, ARs, added water and composition.

6.2 RECOMMENDATIONS

- The study recommended that training in good dairy farm management practices should be conducted regularly in order to assist SHDFs adhere to correct usage of antibiotics, avoid adding water to their milk and improve the udder health of their cows.
- It was also recommended that the regulatory authority should establish maximum acceptable limits for SCC, TCC and ARs in raw milk.
- There is also need to extend this study to wider locations of milk producing areas in Zambia.

CHAPTER 7

7.0 REFERENCES

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8.0 APPENDICES

Appendix 1**:** List of antibiotics detected by Copan Milk Test 100

ANTIBIOTIC		COPAN TEST DETECTION LIMIT PPB	MRL (EU) PPB
B-Lactams	Penicillin	2	4
	Ampicillin	2	4
	Amoxcillin	2	4
	Cloxacillin	12	30
	Dicloxacillin	5	30
	Oxacillin	5	30
	Nafcillin	4	30
	Ceftiofur	25	100
	Cefquinom	80	20
	Cefapirin	4	60
	Cefoperazon	30	50
	Cefalexin	>45	100
	Cefalonium	12-15	20
	Cefacetrile	25	125
	Cefazolin	6	50
	Cefuroxime	60	-
Tetracyclines	Chlortetracycline	450	100
	Oxytetracycline	450	100
	Tetracycline	450	100
	Dioxycycline	150	100
Sulonamides	Sulfathiazol	50	100
	Sulfamethazine	150	100
	Sulfadioxine	150	100
	Sulfadimethoxine	50	100
	Sulfadiazin	50	100
	sulfamethoxazole	50	100
	Sulfamerazine	60	100
	Sulfamonometosina	50	100
	Sulfacetamide	100-150	100
Aminoglycosides	DH-Streptomycin	1750	200
	streptomycin	1750	200
	Neomycin	500-2000	500
	Gentamicin	400	100
	Spectinomysin	7500	200
	Kenamycin	4000-5000	200
Macrolides	Erythromycin	600	40
	Spiramycin	5000	200
	Tylosin	100	50
	Tylmicosin	75-100	50
Other Antibiotics	Dapson	2-4	0
	Trimethoprin	135	50
	Tiemfenicol	>100	50
	Chloramphenicol	5000-7500	0
	Flumequine	5000-6000	50
	Lincomycin	500-700	150