QUANTIFICATION OF MILK LOSSES DUE TO SPOILAGE MICROORGANISMS AND ANTIBIOTIC RESIDUES IN RURAL AND PERI-URBAN DAIRY SUB-VALUE CHAINS IN NAKURU, KENYA

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A thesis submitted to the graduate school in partial fulfilment of the requirements for the degree of Master of Science in Food Science and Technology of Egerton University.

EGERTON UNIVERSITY

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DECLARATION AND RECCOMENDATION

DECLARATION

I declare that this is my original work and to the best of my knowledge has not been presented elsewhere for an award.

Signature _____ Date _____

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RECOMMENDATION

This research thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

I dedicate this thesis to my parents, the late Rev Solomon Orwa and Mrs Rebecca Orwa, my Brothers Jeremy Brian, Jude Okoth and James Otieno, my sister Jemima Siphrah, my Husband Fredrick Gudda and my daughter Marie-Becka Awuor.

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ABSTRACT

Dairy losses due to spoilage microorganisms have remained high from previous studies in Kenya. Poor hygiene has been reported to be the main cause of milk contamination with these microorganisms. Antibiotic residues are also continually reported in Kenyan milk due to misuse of veterinary drugs in treating animals by unskilled personnel or farmers themselves. The aim of this study was to quantify milk losses due spoilage microorganisms and determine levels of antibiotic residues in rural and peri-urban dairy sub value chains in Nakuru County. Risk factors were identified by use of questionnaires and observation checklist. Sampling was done following a nested design .Microbiological analysis was based on standard procedures (ISO). Antibiotic residues were screened using Charm II Blue-Yellow and confirmation by High performance liquid Chromatography (HPLC-UV). Data was analysed using statistical package for social scientists (SPSS) and statistical analysis software (SAS). Survey showed that lack of hand and udder drying was done by 11% in rural and 50% in peri urban. There was an increase of 0.5 log cycle in TVC between udder and farm gate in rural location. Regression of risk factors versus microbiological quality of milk revealed that udder swabs were the highest contributor (r=2.73) to milk contamination with spoilage microorganisms. The incidence of occurrence of *Staphylococcuss, Streptococcuss, Bacillus,* and *E.coli* was 26%, 13%, 26% and 23 % in rural and 82%, 64%, 87% and 76% in peri urban respectively. From antibiotic screening, 31.45% (72/229) samples in rural and 28.8% (23/80) in peri urban were positive. None of the positive samples showed presence of tetracyclines while the highest percentage of sulphonamides were detected at cooling centres of rural (23%) and peri urban (100%). Losses of milk as a result of TVC were 8.6% in rural and 10.2% in peri urban. Antibiotic residues contributed to 23% losses in rural and 83.5% in peri-urban. Losses due to spoilage microorganisms are as a result of poor hygiene of hands, udder and water in both locations which recorded counts ranging between 1.8 CFU/ml to 5.5 CFU/ml. Losses due to spoilage microorganisms can be prevented by observing hygiene during milking. Antibiotic residues in milk can be prevented by training farmers on observation of withdrawal periods and use of qualified veterinary personnel.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA – Analysis of Variance

BCR-Bulking container rinse

CAC – Codex Alimentarious Commission

CC-coliform counts

CFU/ ml – Colony Forming Units per millilitre

DMS – Degree Minutes Seconds

EC-European commission

EU-European Union

FAO – Food and Agricultural Organisation of the United Nations

FG-Farm gate

GDP – Gross Domestic Product

GLM –General linear model

IDF – International Dairy Federation

ILRI-International livestock research institute

KEBS-Kenya bureau of standards

LAB – Lactic Acid Bacteria

MCR-Milking container rinse

MRL – Maximum Residual Limit

NMC – National Mastitis Council

PBC – Psychotropic bacterial count

PCA- Plate count agar

SAS – Statistics analysis software

SE- Standard error

SPSS-Statistical package for social scientists

ThBC- Thermophillic bacterial count

TVC – Total viable counts

UHT- Ultra heat treatment

WS-Water source

CHAPTER ONE

INTRODUCTION

1.1 Background information

Livestock contributes about 50% to the agricultural Gross Domestic Product (GDP) in Kenya with dairy production contributing up to 33% of this (National Bureau of Statistics, 2016). Smallholder dairy farmers dominate the dairy industry by accounting for over 75% at the production level (FAO, 2011). Milk production in Kenya is approximately 9 billion litres per year. Out of this about 10% is lost due to spoilage (FAO, 2014). Spoilage microorganisms gain entry into milk initially due to poor pre-milking and post milking handling hygiene (Bonfoh *et al*., 2003, Kumar *et al.*, 2012, Paola *et al.*, 2013).

Pre-milking practices such as hand and udder washing, hands and udder drying, pre dipping of the udder become risk factors if they are not carried out hygienically. Post milking practices such as udder rinsing, post dipping and drying become risk factors if they are not carried out in the right procedure and conditions (Walstra *et al.*, 1999; Kornacki and Johnson, 2001; Petrovick *et al*., 2006; Visser *et al.*, 2007; Coorevitis *et al.*, 2008; Kumar *et al.*, 2012; Al-Hubaety *et al.*, 2013). Use of non-portable water in cleaning milking equipment, hands and udder is a predisposing factor to milk spoilage. Lack of water treatment such as boiling or chlorination also exposes milk to contamination (Matofari *et al.*, 2013). External risk factors include dirty milking parlours with cow faeces and dusts all round and milking in an open non controlled environment (Al-Hubeaty *et al.*, 2013). Plastic containers (Non-food grade) are also risk factors because they develop micropores and hide biofilms during cleaning acting as milk contamination sources. Metal containers if not cleaned following proper sanitation regimes become sources of milk contamination with spoilage microorganisms (Wafula *et al.*, 2016).

Mastitis is a disease of the udder and is common among smallholder farmers due to inadequate access to proper veterinary services. Common clinical practice and research has recommended antibiotic drugs as the best remedy to mastitis. These farmers however tend to turn to non-licenced veterinary personnel. Coupled with lack of observation of withdrawal period and intentional addition to extend milk shelf life, antibiotic residues have been detected in farm milk for over a long period of time (Aboge *et al.*, 2000; Shitandi and Sternesjo 2004; Kangethe *et al*., 2005;Omore *et al.*, 2005; Ekuttan *et al*., 2007; Ahlberg *et al*., 2016).

Apart from the farm, antibiotic residues have been isolated in milk during transportation(Aboge *et al.,* 2000). This shows that milk vendors and transporters add antibiotics in milk to extend its shelf life (Kangethe *et al.,* 2005). This occurrence is also prevalent due to lack of proper monitoring and implementation by the regulating institutions. Therefore the milk bypasses official quality assurance channels posing a public health risk (Ekuttan *et al.,* 2007). Other causes of antibiotic residues in milk include; contamination of feeds with faeces of treated animals and use of un-licensed veterinary drugs (Nisha, 2008). Antibiotic residues in milk along the value chain have been reported to be above the maximum residual levels (MRL) in Kenya (Aboge *et al.,* 2000; Shitandi and Sternesjö 2004).The increase in antibiotic residues in milk along the chain could also be due to pooling of milk from different farms. However since 2001 there has been a rise in the reported levels of antibiotic residues in Kenyan milk (Ahlberg *et al*., 2016).

In Kenya, the prevalence of tetracyclines was recorded to be highest at 55%, followed by sulphonamides at 21% and beta lactams at 6% (Mitema *et al.* 2001; Shitandi and Sternesjö 2004). According to Aboge *et al.* (2000), the most common antibiotics used in the treatment of livestock are; sulfonamides, tetracyclines, beta lactams and aminoglycosides. When milk and other animal products with high levels of antibiotic residues are ingested by humans, there is occurrence of numerous adverse health effects like permanent gene mutation and liver poisoning (Nisha, 2008).

The aim of this study was to quantify milk losses due to spoilage microorganisms and determine levels of antibiotic residues in rural and peri urban dairy sub-value chains in Nakuru County.

1.2 Statement of the problem

Milk production in Kenya is mainly from smallholder farmers who face challenges of milk quality due to unhygienic production practices and lack of preservation chain systems. Most milk is spoiled by contaminating microorganisms during and post-harvest handling. Additionally, antibiotic residues have been detected in farm milk, in milk during transportation, in animal feeds contaminated with faeces of treated animals and use of un-licensed veterinary drugs. Milk bulking has also contributed to increase in antibiotic residue accumulation in milk as some actors in the chain use them for preservation of the milk. Therefore, there has been a steady rise in levels of antibiotic residues in Kenyan milk. These challenges have been investigated but have not been measured as to what extend they contribute to milk loss along the chain, especially the sub-value chains at rural and peri-urban

where most of the milk is produced and handled in Kenya. This study aimed at quantifying milk losses due to microbial spoilage and antibiotic residue contamination in rural and peri-urban sub-value chains in Nakuru county.

1.3 Objectives

1.3.1 General Objective

To contribute to the reduction of dairy value chain losses by characterizing the risk factors associated with microbial milk spoilage and determine levels of antibiotic residues in rural and peri urban dairy value chains.

1.3.2 Specific Objectives

1. To identify the risk factors associated with post-harvest contamination ofmilk by spoilage microorganisms in rural and peri urban dairy sub value chains.
2. To determine the microbial load of milk along the rural and peri urban dairy sub value chain
3. To quantify the levels of antibiotic residues in rural and peri- urban dairy sub value chains.
4. To quantify milk losses associated with spoilage microorganisms and antibiotic residues along the rural and peri-urban dairy sub value chains.

1.4 Hypotheses

1. Practices along the dairy value chain in rural and peri urban are not associated with milk contamination by spoilage microorganisms.
2. The microbial quality of milk in the rural and peri urban sub value chainsdo not fall below the set standards
3. There are no quantitiesof antibiotic residues in milk along rural and periurban sub value chains
4. There are no losses associated with spoilage microorganisms and antibiotic residues in rural and peri urban sub-value chains.

1.5 Justification

The dairy sector contributes significantly to the Kenya's economy. Losses from spoilage microorganisms are estimated at 10%. This affects the farmers, milk vendors and other dairy chain actors economically. This then translates into overall effect to the economy (FAO, 2014). Antibiotic residues have been reported to be in the rise in Kenyan milk in the past few years (Shitandi and Sternesjo 2004; Kangethe *et al.*, 2005; Ekuttan *et al.*, 2007; Omore *et al.*, 2007; Ahlberg *et al.*, 2016). The health effects of the antibiotic residues are numerous and fatal, especially when consumed by young children and the old (Nisha, 2008). To reduce milk spoilage and antibiotic levels, there is need for information on the point at which the losses and antibiotic residues are highest along the value chain. This will aid in developing mitigation measures to curb these problems.

1.6Operational definition of terms

Dairy value chain- the stages at which milk moves from the time of harvesting(farm) to the point of consumption

Rural- Non urbanized settlement

Peri -urban- less urbanized but not rural

Risk factors- practices or factors predisposing milk to microbial spoilage also critical control points (CCP's) for milk

Udder milk- Milk drawn directly from the udder into a sterile container without coming into contact with any other material or milking container but imitates the actual field conditions.

Farm gate- in this context has been used to refer to a value chain node where farm milk is collected. This is usually a product of several cows within the farm and has been transferred from the milking container to the bulking container.

Transporters – in this study refers to a node along the value chain where milk collected from different farms is pooled for transport to the next node

Cooling centre- this is a node along the value chain which receives milk from different transporters where milk is pooled and cooled awaiting collection to theprocessing firms.

CHAPTER TWO

LITERATURE REVIEW

2.1 Forms of milk losses

On farm dairy losses occur in three main forms; spillage spoilage and forced consumption. Spillage is caused by poor roads during transportation; spoilage is caused by spoilage microorganisms which produce lactic acid increasing the milk acidity above the accepted levels (Lore *et al.,* 2005). When the milk fails the alcohol test it is rejected there after it is returned to the farm. At the farm the milk is forcefully consumed, thrown away, or sold at throw away prices leading to economic loss (Muriuki, 2003; FAO, 2011). Losses at the farm have been reported by World Bank to cost the farmers $2 each month in developing countries (Bonfoh *et al.,* 2003; Paola *et al.,* 2013). Losses have also been attributed to by lack of adequate animal health control, inadequate training among farmers and farm employees on milk hygiene (Chye *et al.,* 2004; Chizari *et al.*, 2008; Paola *et al.,* 2013).

Research has showed that most post- harvest milk losses are experienced in small scale dairy farms and at the farm level (Muriuki, 2003; Lore *et al.,* 2005; FAO, 2011). Harvesting of milk which basically takes place at the farm faces many sources of contamination. The animal itself is a risk factor. If the cow is not healthy, then the milk is likely to be contaminated with microorganisms such as *Staphylococcus, Streptococcus* and enteric bacteria in cases of subclinical mastitis at the udder (Paola *et al.,* 2013).

2.2 Sources of milk contamination

Milk produced from the mammary glands of healthy animals is initially sterile (Coorevitis *et al.,* 2008). Post-harvest handling of milk like milking containers and personnel remain as the main sources of milk contamination with microorganisms (Ahmad *et al.,* 2015; Reta *et al.,* 2016). Other significant sources of microbial contamination have been reported to be the animals udder, traditional pre milking and post milking procedures, milking environment and water (Matofari *et al.,* 2013; Pangoli *et al.,* 2008; Al-Hubaety *et al.,* 2013).

Environmental microbial contaminants represent a significant percentage of spoilage microflora. Bacterial count may be high due to growth of bacteria on unsanitary milking equipment, contamination from soiled udder, inadequately cooled milk and milking of mastitis cows (Chye *et al.,* 2004; Chizari *et al.,* 2008; Wafula *et al.,* 2016). Pooling of milk from different suppliers without prior testing has resulted in occurances of *Streptococcus*

agalactiae and *Staphylococcus aureus* in milk (Younan *et al.,* 2001; Susan and Andreas, 2010).

2.2.1 The animal's udder

The external surface of the udder is a prime source of microbial contamination of milk. Bedding materials, mud, faces, soil and other matter all readily stick to skin and are a rich source of microorganisms (Dey and Karim, 2013). Even after washing with water, the microbial count on teat surfaces can be high (Gibson *et al.,* 2008) and the count in milk from washed udders may only be about 1 log cycle lower than from those that were unwashed (Al-Hubaety *et al.,* 2013). Similar low-level reductions in total microbial count and coliform counts on both the udder surface and in milk have been reported even after the use of disinfectants to treat teats (Al-Hubaety *et al.,* 2013, Gibson *et al.,* 2008).

Pangoli*et al.,* (2008) showed that poor cleanliness of cows, and incorrect disinfection of towels used to dry the udder significantly increased the likelihood of contamination. A reduction has been reported in bacterial levels on teats when cows are on pasture and this is reflected in lower bacterial counts in milk during this period (Dey and Karim 2013). Udder surface has been reported to be an important source of coliform bacterial (Islam *et al.,* 2009).*Staphlococcus aureus, Streptococcus faecalis* and coliforms have been isolated from udders. However application of proper cleaning and sanitization has reported significant reduction in these microorganisms (Gleeson *et al.,* 2009; Islam et al., 2009; Al- Hubaety *et al.,* 2013)

2.2.2 Milking Environment

Inadequate lighting of milking parlours and barns is an indication of neglect of milking hygiene (Chizari *et al.,* 2008). It has been suggested that bedding affords the greatest contribution to external udder contamination (Islam *et al.,* 2009; Paola *et al.,* 2013). Presence of faeces in the milking environment plays a significant role in contamination of both the udder and bedding with spoilage and pathogenic microorganisms.Microorganisms that have been isolated from cows bedding include, *Esherichia coli, Staphylococcus aureus, Streptococcus faecalis, Micrococcus spp, Listeria monocytogenes, Bacillus cereus, Salmonella and Campylobacterspp*(Chye et al., 2004; Gleeson eta l., 2009; Paola et al., 2013)

Several potential human pathogens are naturally present in the intestinal tract of cattle and these animals do not show signs of infection, these are usually shed through the faeces (Al-Hubaety *et al.,* 2013).When the animal lies down, the udder is likely to get in touch with the faeces. The microorganisms shed in through the faeces then contaminate the udder which

when not cleaned and sanitized before milking becomes a risk to milk contamination (Gran *et al.,* 2002).

Air is also thought to be an insignificant contributor to microbial contamination of raw milk. It has been calculated that airborne bacteria account for <5 CFU/ml of the bacterial load of milk; of these *Bacillus spp* (spores) constituted <1 CFU/ml (Chye *et al.,* 2004). However *Salmonella* has recently been isolated from air at the milking parlour (Pangoli *et al.,* 2008). Clean, dry and comfortable bedding condition is important to minimize the growth of spoilage microorganisms.

2.2.4 Water

Water used in the production of milk may be one of the problems and arise when untreated water supplies are used to rinse and wash equipment. Such water may contain a diverse array of microorganisms including *Pseudomonas spp., coliforms, Bacillus spp, and Salmonella spp*and *Escherichia coli*(Gran *et al.,* 2002; Matofari *et al.,* 2007; Islam *et al.,* 2009). Bacteria contaminated water can also increase milk bacterial counts in raw milk (Visser *et al.* 2007). When the microbiological quality of the water is low it is used to clean animal udder, milking equipment or even hands of milking personnel, the microorganisms will definitely be found in milk. Being that milk has direct contact with teat opening and milking equipment, milk stands a high risk of contamination with water. Commonly used pre-milking teat preparation method involves washing teats by hand with water and drying teats with a paper towel just before (Al-Hubaety*et al.,* 2013).

Water also plays a primary role in transferring bacteria from the surface of the udder and hands into the milking container if not dried off after cleaning. The drops of water from these surfaces if not disinfected, carry bacteria into the milk which in turn contaminates the milk. There is strong evidence that among all pre-milking procedures, wet cleaning treatment, followed by paper towel manual drying will result in the lowest bacterial counts (Gibson *et al*. 2008; Gleeson *et al.,* 2009). One of the most important aspects of pre-milking udder hygiene is udder dryness at the time of milking (Visser *et al.,* 2007). Drying the udder after washing is more effective than just washing the udder without drying (Islam *et al.,* 2009). Water at the farm can be treated through boiling or use of chemical methods such as chlorination (Yilma, 2012).

2.2.5 Equipment

Milk should be handled in hygienically designed equipment i.e. one that has no dead spaces and crevices, the major control method of surface route of milk contamination, is the type design of container. Failures in the cleaning and disinfection regimes will causes bacterial deposits on the container surfaces thus incubation site for them (Reinemann *et al.* 2003). In particular, dead ends, corners, joints, valves and the hard-to-reach places of milk handling equipment are the most appropriate regions for the existence of microbial contaminants.

Bacteria attach on milk handling equipment surfaces either as single cells or in binary biofilms, which may become difficult to remove (Lindsay *et al.* 2002). Some of the microorganisms isolated from milking equipment and milk handling containers include , *Lactococcus spp, Lactobacillus spp, Leuconostoc spp, Streptococcus spp, Enterobacter spp, Klebsiella spp, Salmonella spp, Staphylococcus spp and E. coli spp* (Chye *et al.*, 2004; Wafua *et al.*, 2016)

The presence of crevices and scratches on equipment surfaces causes accumulation of organic debris that offers good condition for bacterial growth thus high concentration of microbial load whereby some withstand the cleaning and disinfection (Murphy and Boor 2000). Residual bacteria on surfaces that remain after cleaning and disinfection have the potential to proliferate and spoil milk in the dairy value chain (Olivier and Moshoeshoe 2012; Wafula *et al.*, 2016).

2.2.6 Pre milking and post milking practices

Clean cows in dry condition gives better initial bacterial quality of milk compared to washing of udder, milking hands and milking equipment with normal water before milking. Washing and disinfection of udder and milking hands, and sanitary rinse of milking pails just before milking significantly improved initial bacterial quality of milk. Gleeson *et al.*,(2009) attributed higher coliform count to the degree of wetness of udder. The study also showed that udder disinfection (Cl 5 ppm) reduces coliform count in milk by half. Foret *et al.*, (2005) reported a 79.7% and 83.6% reduction ($P<0.01$) in mean bacterial count by washing and disinfection (200 ppm CI) of milker's hands and cows' udder respectively. Bacterial counts can be halved if the udder is washed with a disinfectant as opposed to using a dry cloth for cleaning (Gibson *et al.*, 2008)

Other disinfectants used in pre-milking practice and reduced microbial counts in milk significantly include; iodophor solution, iodine based gel, sodium hypochlorate, dodecyl benzene sulfonic acid (DDBSA), chlorhexidine, phenolics, hibitane, potassium permanganate

and alcohol (McKinnon *et al.*, 1990; Watts *et al.*, 1991; Ingawa *et al.*, 1992; Oliver *et al.*, 1993; Wilson *et al.*, 1997; Oliver *et al.*, 2001; Foret *et al.*, 2005; Gibson *et al*., 2008; Sridar, 2008; Al-Hubaety *et al.*, 2013). Gleeson *et al.* (2008) showed that both iodine based gel and 0.5% iodophor solution significantly reduced milk bacterial count and clinical mastitis occurrence compared to teat washing and drying with paper towels. However, Oliver *et al.* (2001) showed that pre-milking disinfection with 0.25% iodine dip or phenolics reduces the prevalence of microorganisms from the udder and teat. These include microorganisms such as *Streptococcus aureus* and *Streptococcus agalactiaeEscherichia coli spp, Mocrococcus, Lactobacillus*as well as coliforms which are also spoilage microorganisms.

The most recommended post milking udder treatment is the rinsing with portable water, followed by dipping in a disinfectant. This can then be followed by drying of the udder using a clean and dry towel (Gibson *et al.*, 2008; Gleeson *et al.*, 2009). This practice has been reported to reduce the instances of infection with mastitis (Oliver et al. 2001, Foret *et al.*, 2005, Gibson *et al.*, 2008; Al-Hubaety *et al.*, 2013). Post milking teat disinfection helps in prevention of contagious bacteria such as *Staphylococcus aureus* transmission as well as it improves teat condition (Kumar *et al.*, 2012). Proper post milking udder treatment improves on milk production, microbiological quality of milk and therefore, lowers losses.

2.3Mechanism of milk spoilage

Milk is highly nutritious a property that propagates faster growth of microorganisms once it is contaminated. Action of microorganisms can initially be detected through organoleptic tests. Acid formation in the milk is indicated by the sour flavour and coagulation of milk to give a jelly like curd appearance or clear whey nature. Lactic acid fermentation is common in the raw milk at the room temperature (Muir, 1999) because this is the favourable temperature for their metabolism. At temperature from 10^{o} to 37^{o}C souring is mainly due to *Streptococcus lactis*, *Enterococci*, *Lactobacilli* and other coliform bacteria. At temperatures from 37^{o} to 50 ^{o} C the most common contaminants of milk are *S. faecalis* and *S. thermophilus*. Thermophilic bacteria such as *L. thermophilus* can grow in the milk at higher temperatures. Pasteurization is an important process to kill most acid producing microorganisms while permitting the growth of heat resistant microorganisms such as *Streptococcus thermophilus* and *Lactobacilli*.

Other acid producing microorganisms are *Micrococcus* species, *Bacillus* species (mainly lactic acid) and *Clostridium* species (mainly Butyric acid) (De Jonghe *et al.*, 2010). Microorganisms such as *Clostridium spp*. and *Bacillus spp*. can also produce gases such as

hydrogen and carbon dioxide which can be indicated by the formation of foaming at the top of the milk suspension (Rueckert *et al.,* 2004).).

The other way of milk spoilage is the hydrolysis of milk proteins by the growing microorganisms. The release of peptides in the milk leads to a bitter flavor to the milk. Ropiness, sliminess in the milk, is caused by the release of slimy capsular material from the cells. *Enterobacter aerogenes*, *Escherichia coli*, *Micrococcus freudenreichii* are examples of microorganisms that can cause ropiness in the milk. Oxidation of unsaturated fatty acids can also lead to change in odour and taste of milk. Production of alkaline products such as ammonia, urea, and carbonates can also produce off flavours to milk (Rueckert *et al.,* 2004).

2.3.1 Spoilage by psychrotrophic microorganisms

Psychrotrophic microorganisms are a group of microorganisms which can grow at 0^{O}C and have a maximum gowth tempeartures above 20^{O}C. They are widely spread in nature and have been associated with spoilage of frozen foods.They represent a substantial percentage of the bacteria in raw milk, with pseudomonas predominant group. Typically, 65–70% of the psychrotrophs isolated from raw milk are *pseudomonas* species (Griffiths and Phillips, 1990; Muir, 1999). Important characteristics of *pseudomonas* are their abilities to grow at low temperatures (3–7°C) and to hydrolyze and use large molecules of proteins and lipids for growth. Other important psychrotrophs associated with raw milk include members of the genera *Micrococcocus, Aerococcus and LactococcusBacillus.*

An indirect cause of dairy product spoilage is microbial enzymes, such as proteases, phospholipases, and lipases, some of which may remain active in the food after the enzyme-producing microbes have been destroyed. Populations of psychrotrophs ranging from 10^{6} to 10^{7}CFU/ml can produce sufficient amounts of extracellular enzymes to cause defects in milk that are detectable by sensory tests (De Jonghe *et al.,* 2010).

2.3.2 Spoilage by thermophiles

Thermophillic bacteria have the ability to thrive at high temperatures of between 41^{o}C and 122^{O}C. In the food industry they have been associated with spoilage of food which undergo high temperature treatment such a milk. Most prevalent thermophiles in the dairy industry are the spore-formers.Spore-forming microorganisms have a special position among total microflora of milk with regard to their greatest ability to survive thermal treatment of milk and subsequently to propagate in the final products. The thermophilic bacilli for example *Streptococcus thermophilus* are potential contaminants in a variety of industries such as dairy product manufacture where elevated temperatures (40-65°C) prevail during the

manufacturing process or when product is stored (Abo-Elnaga *et al.,* 2002; Janštová *et al.,* 2006; Cempirkova, 2007).

The facultative thermophiles belong to the Bacillus genus and tend to grow at both mesophilic and thermophilic temperatures, depending on the strain. Some examples of species include *Bacillus licheniformis, Bacillus coagulans, Bacillus pumilus Bacillus sporothermodurans* and *Bacillus subtilis* (Scheldeman *et al.,* 2005). Although these contaminants do not constitute a health risk to the consumer but they are used as an index of hygienic measurements. Bacillus spores can cause defect such as sweet curdling, coagulation and diarrhoea and emetic toxin production (Stenfors, *et al.,* 2008).

Traditionally in Kenya, udder preparation and hand washing has implemented the use of water and drying using a cloth (Saran, 1995). Some farmers prefer to let their calves suckle before milking to replace udder washing before milking (Yilma, 2012). These methods however have not proven to be effective in reduction of microbial load on the udder surface or milking hands. Farmers and milk handlers have widely adopted the use of plastic jerry cans to transport milk from one node to the next (Bonfoh *et al.,*2003; Odongo *et al.,* 2016; Wafula *et al.,* 2016) This is due to the fact that this type of container is light and locally available. Research has shown that the most commo methods of cleaning the containers is manly by use of soap and water (either hot or not) (Wafula *et al.,* 2016). Due to lack of sanitizers in the practice the cleaning regimes have not been effective (Arimi, 2005). These practices have however exposed milk further to spoilage and hence contributed to losses along the dairy value chain.

2.4 Antibiotic residues

Antibiotics are antimicrobial substances that are produced either naturally by living organisms or synthetically by laboratory procedures with the ability to inhibit the growth of microorganisms or kill the microorganisms (Wageh *et al.,* 2013). Antibiotics are manufactured for the purpose of the prevention and treatment of animal diseases such as mastitis, arthritis, brucellosis, gastrointestinal diseases, respiratory diseases and many other bacterial infectious diseases (Tollefson and Miller, 2000). In intensified farming antibiotics are also used to improve animal production like increase of growth rate and fattening (Nisha, 2008). When these antibiotics are administered to an animal, they dissolve and distribute rapidly in animal tissues and fluids. Over 90% of these antibiotics bind to plasmic proteins and reach a high concentration between the 3rd and 6th hour of administration (Sulejmani *et al.,* 2012). They are then metabolized in the liver and are excreted through glomerular

filtration. If the right procedure is not used in administration and use of these drugs, they are left in large amounts i.e., residues, in animal products like milk, meat and eggs (Richelle, 2007). Once they are in the milk, there is a carry-over effect along the milk value chains.

Regulatory levels have been established for drug residues in foods in the form of maximum residue limits (MRLs) (Lee *et al*., 2000). MRLs for veterinary drugs refer to the maximum concentration of a residue (resulting from the use of a veterinary drug) that is acceptable in food (CAC, 1997). The quality standards set by the East African Community on quality parameters of milk refers to the Codex Alimentarious Commission (CAC) for veterinary drug and chemical residues (EAS 67: 2000). Table 1 gives some examples of those, which have been set for milk.

Table 1: Residue limits of common veterinary drugs (µg/kg) set for milk

Antimicrobials	Types	EU-MRL (µg/kg)
Sulphonamides	Sulphamethazine	100
	Sulphadiazine	100
	Sulfachloropyradizine	100
	Sulfadimidine	100
	Sulfaquinoxaline	100
	Sulfadimethoxin	100
	Sulfathiazole	100
	Sulfamethoxazole	100
	Sulfadoxin	100
Tetracyclines	Oxytetracycline	100
	Tetracycline	100
	Deotetracycline	100
	Chlortetracycline	100
Macrolides	Erythromycin	40
	Spiramycin	200
	Tylosin	50
	Oleandomycin	50
	Lincomycin	150
Quinolones	Enrofloxaxin	100
	Flumequine	200

Source: Commission Regulation (EU) No. 37/2010

2.5 Contribution of mastitis to antibiotic residues in Kenyan milk

According to Kashongwe *et al.,* (2017), the prevalence of mastitis currently is at 70.8% in rural farms and 53.1% in peri urban dairy cows. Bovine mastitis is the most frequent disease in Kenyan dairy herds (Odongo and Ambani, 1999) and particular problematic in small- scale dairy cattle (Omore *et al.,* 2001). Small sale farmers do not find the practice of sanitizing the udder before and after milking to be economics (Kashongwe *et al.,* 2017). This use of sanitizers in udder washing has been proven to be significant in reduction of microbes at the udderthan the normal use of warm water (Yilma *et al.,* 2012; Wafula *et al.,* 2016).

Mastitis is an inflammation of the mammary glands of dairy cows accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Blood *et al.,* 2003). Over 100 different microorganisms (both Gram-positive and negative) can cause mastitis, and these vary greatly in the route by which they reach the cow and in the nature of the disease they cause (Ma *et al.,* 2000; Murinda *et al.,* 2001). Mastitis is of technological significance in milk processing as valuable components like casein are decreased while undesirable components like ions and enzymes are increased.

Small holder dairy farmers are the mostly affected by mastitis. This is due to the poor hygiene conditions in these farms which expose the animal udder t infection. Lack of proper treatment due to financial constraints have led to these farmers seeking cheap options in treating the animals, these include; self-administration. With the increased frequency of infection of the animal with mastitis so does the frequency of treatment and shedding of antibiotics in milk experienced.

2.6 Common antibiotics in Kenyan animal husbandry

The most common antibiotics used in animal husbandry include sulfonamides, tetracyclines, beta lactams, aminoglycosides, lincosamides, macrolides and pleuromutilins (Lee *et* 2000). The most common sulphonamides are sulfadiazine, sulphadimidine sulfamethoxazole, sulfamerazine, sulfadimethoxine, sulphasalazine, sulfisoxazole and silver sulfadiazine, which have the sulphonamide as the base structure (Chung *et al.,* 2009). In Kenya, the prevalence of tetracycline's was recorded to be highest at 55%, followed by sulphonamides at 21% and beta lactams at 6% (Mitema *et al.,* 2001; Shitandi and Sternesjö 2004). According to Aboge *et al.,* (2000), the most common antibiotics used in the treatment of livestock were beta lactams, sulfonamides, tetracyclines and aminoglycosides.

Antibiotic residues have been quantified in Kenyan milk at levels above the EU MRL/ EAS standards (Aboge et al.,2000; Shitandi and Sternesjo 2004; Kangethe *et al.,* 2005; Ekuttan *et al.,* 2007; Omore *et al.,* 2007; Ahlberg *et al.,* 2016). This indicates that a routine monitoring has not been placed within the chain actors. The main causes of antibiotics residues in milk have been attribute to lack of observing withdrawal period, extra label usage of drugs, contamination of animal feed with faeces of treated animals, or the use of unlicensed antibiotics (Nisha, 2008). Other studies have also attributed the occurrence antibiotics in animal products to lack of educational training in antibiotic use and intentional addition by value chain actors to extend the milk shelf life (Aboge *et al.,* 2000; Shitandi and Sternesjö 2004; Okeke *et al.,* 1995).

2.7Processing challenges associated with antibiotic residues

The starter cultures currently used in the Kenyan dairy industry for the primary acidification of the milk belong mainly to the genera *Lactococcus, Streptococcus, Leuconostoc* and *Lactobacillus*. These starter cultures are mainly lactic acid bacteria used in the production of a range of fermented milk products, including cheese, yoghurt, cultured butter and cultured milks. The primary role of starter cultures in cheese manufacture is the production of lactic acid from lactose at a consistent and controlled rate. The consequent decrease in pH affects a number of aspects of the cheese manufacturing process and ultimately cheese composition and quality (Broome *et al.,* 2002). Antibiotic residues are capable of inhibiting the action of these microorganisms and interfering with the technological process.

2.7 Public health issues of antibiotic residues

When milk and other animal products with high levels of antibiotic residues are ingested by humans, there is occurrence of numerous adverse health effects like permanent gene mutation and liver poisoning (Nisha, 2008). Sulfamethazine has been highly associated with including immunopathological effects, transfer of bacterial resistance to humans, hypersensitivity and carcinogenicity. Mutagenicity and nephropathy have been reported to be caused by gentamycin. Hepatoxicity, reproductive disorders and bone marrow toxicity have been related to the occurrence of some chlorophenical (Wageh *et al.,* 2013). Penicillin however, has been reported to be associated with allergy development. Tetracyclines are also capable of staining teeth in little children.

The non-restrictive usage of antibiotics in animal rearing may lead to problems due to the presence of harmful residues in foods and raw materials of animal origin. Development and spread of antibiotic resistance represents a serious threat with potential public health implications Dissemination of resistance traits could narrow the line of defence against bacterial infections to only a few antibiotic agents and could increase health care costs (Lee *et al*., 2000; Boor, 2001).

2.8 Analytical methods of antibiotic analysis in milk

A control program for antibiotic residues in milk is usually performed in two steps (Screening and confirmation) where a microbial, enzymatic or receptor-based method is used for initial screening (IDF, 1995). The samples found positive are usually confirmed by a chemical method. Since there are no tests that satisfy all requirements with respect to

detection pattern and limits, an integrated control system is the ideal (Heeschen and Suhren, 1996).

2.8.1 Screening methods

In practice, screening is primarily performed using microbiological methods, because of their high cost-effectiveness compared to physical–chemical detection. In general, these assays can be operated: without special training, do not depend upon specialized equipment, and target a broad spectrum of antimicrobial residues within one test (Pikkemaata *et al.,* 2009). The most widely used tests which are commercially available are microbial inhibitor tests with spores of *Bacillus stearothermophilus var. calidolactis* –Delvotest SP, Copan Test, Charm Farm-960 Test,SNAP ß lactam Test Kit, Penzyme milk and Penzyme III, LacTek™ CEF & LacTek™ B-L,Parallux™ ß-Lactam Assay System, Charm II sequential and Charm I Cowside (AOAC, 2003). Microbiological inhibitor tests are generally reliable, have high capacity and are cost-effective. They have a broad detection pattern, which on the other hand makes them unspecific. Their main disadvantage is perhaps the required typically incubation for several hours before the result can be evaluated.

Other types of methods, which can be used for routine screening of residues, include immunoassays, receptor assays and enzymatic assays (Nakazawa *et al.*, 1992; Deshpande and Rocco, 1994; Bremner and Johnston, 1996; Anderson, 1996). These methods can also be applied for a preliminary identification of classes of antibiotics (Sternesjö and Johnson, 1998; Mitchell *et al.,* 1998). The majorities of these tools are quite expensive, and require instrumentation and technical skills but have the advantages of reliability, automation and fast readings of results.

2.8.1.1The principle of Charm II® Blue-Yellow Test

The Charm II® Blue-Yellow Test is a microbial inhibition assay, which detects inhibitors, such as antibiotics, in raw or ultra-pasteurized cow milk. This was the method used in this study. Antibiotics are the most common inhibitors found in raw milk. The test consists of a single service well that contains pre-measured bacterial spores (*Bacillus stearothermophilus var. calidolactis*), media, and a pH indicator. Reagents are unit dosed and compartmentalized to ensure uniformity. This eliminates reagent transfer steps and prevents inadvertent contamination and reagent loss. The Charm II Blue-Yellow II test has superior sensitivity to beta-lactams, sulfonamides, aminoglycosides, and especially tetracyclines. Breakthrough sensitivity to tetracyclines makes it the first inhibition test to closely match EU MRL levels. The starting colour in the well is blue. Milk is added to the microwell and

incubated. Spores germinate and grow, generating acid which is indicated by colour change to yellow. If antibiotics are present in the milk, microbial growth is inhibited so that no acid is generated. Thus antibiotic positive samples remain blue. The positive samples are boiled at 80^{o}c for 10 minutes then screened again to eliminate the presence of other microbial inhibitors(Charm II Blue-Yellow test Manual).

Figure 1: Interpretation chart of Charm II Blue Yellow test

2.8.2 Confirmation methods

A confirmatory method has to be able to identify the molecule present in the sample and to quantitate it. High-pressure liquid chromatography coupled with UV detector (HPLC-UV) is the technique often adopted as a confirmatory method for antibiotic residues (Riediker and Stadler, 2001; Marchetti *et al.,* 2002). This technique has some limitations in a low sensitivity and selectivity; therefore, many purification steps are needed (Ito, *et al.,* 2001; Marchetti *et al.,* 2002). Other techniques used for confirmation of residues include, spectrophotometric, thin-layer chromatographic and bioautographic, gas chromatographic, mass spectrometric, and immunochemical methods (Kennedy *et al.,* 1995; Elliott *et al.,* 1998).

2.8.2.1Principle of HPLC-UV in quantifying the antibiotics

The HPLC comprise of four main stages: Protein precipitation and purification by centrifugation and trichloroacetic acid and McIIvaine-EDTA buffer, sample extraction with Oasis HLB (200mg) cartridge, sample evaporation and, quantification by HPLC with gradient mode on C18 column and UV-detection.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study areas were Olenguruone, Bahati and Dundori located in Nakuru County, Kenya (Fig 1). Nakuru County is rated as a high milk production center in the country. It is estimated to produce over 40 million liters of milk per annum (MoLF, 2012). The divisions within the county where the study was carried out were; Olenguruone, Bahati and Wanyororo. Olenguruone division represented a rural dairy system which lies at 35° 40′60″E and 0° 34′60″S DMS (degree minute seconds). Wanyororo and Bahati divisions represented the peri- urban locations at 36° 16′ 12″ E and 0° 12′ 0″ S. Samples were collected between January 2014 and November 2015.

3.2 Survey of risk factors associated with contamination of milk with spoilage microorganisms

3.2.1 Sampling

A cross-sectional study design was used in administration of the questionnaires. A semi structured questionnaire (Appendix 1) was used to identify risk factors exposing milk to contamination with spoilage microorganisms. An observation checklist (Appendix 2) was used to compliment the information at milking time. Observation checklist, however was used during sample collection but the questionnaire was used majorly during the survey. A total of 250 were filled from both study areas farms. The sample size was guided by the formula of Krejcie and Morgan (1970) described below was used to arrive at the minimum number of respondents for all the dairy systems. Where *ME* (10%) is the desired margin of error, *s* is the standard deviation (1.96 for 95% confidence interval), while *t* is the t-score (from statistical tables) used to calculate the Confidence interval (CI) and n is the sample size to be determined.

$$n = (st/ME)^2$$

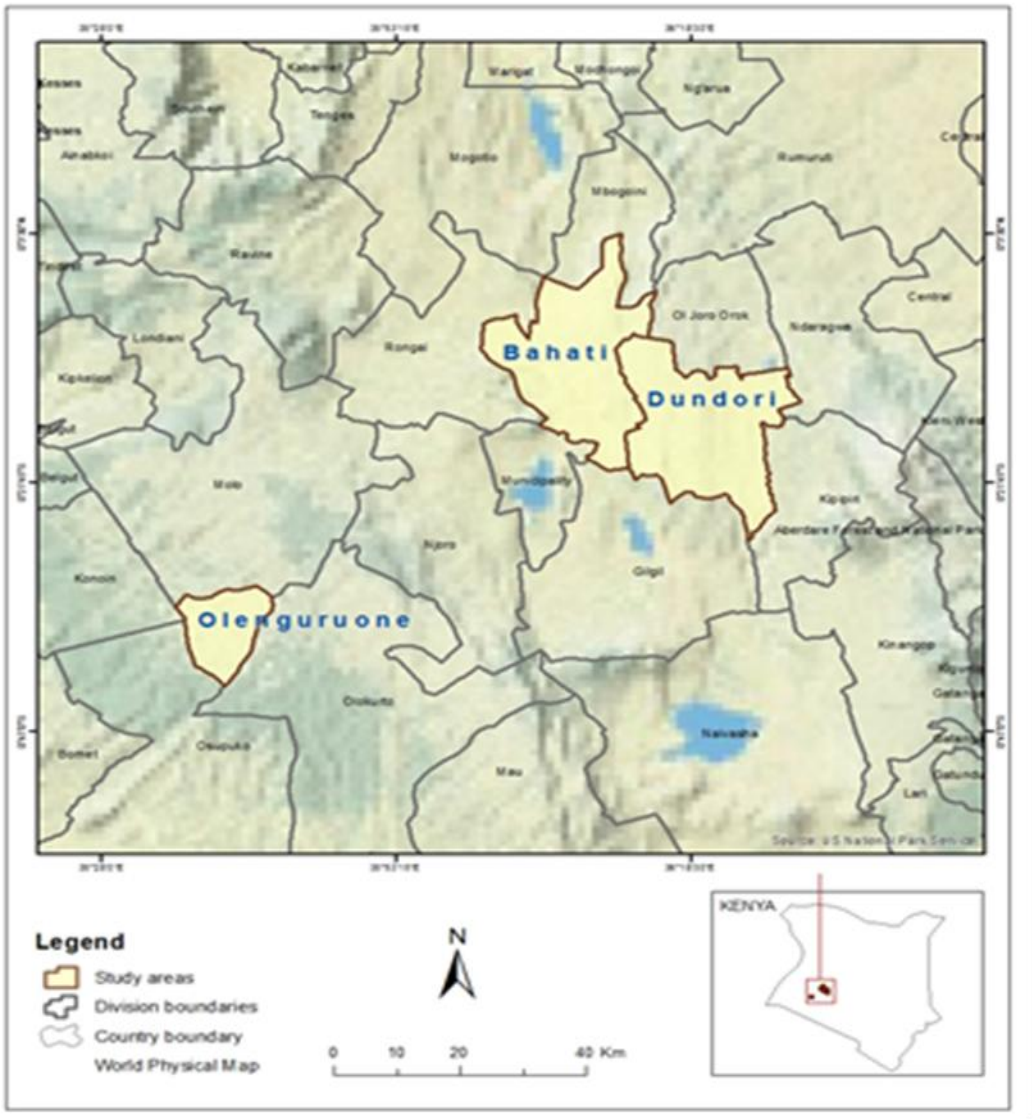

Figure 2 : Rural and Peri urbal locations

Source: Observation Satelitefrom Forestry department of Central Africa (OSFAC), 2014

3.2.2Data analysis

Using SPSS (Statistical package for social scientists) version 20 data was analysed and descriptive statistics was used in representing the data. These were in terms of means and standard deviation. T test was used in mean separation in significant differences.

3.3 Microbiological quality of contamination sources of milk at the farm

Apart from practices identified as risks from the survey, the study evaluated the microbiological quality of potential sources of milk contamination with spoilage microorganisms. The identified sources of milk microbiological contamination included;

water used at the farm, hands of milking personnel, teat udders of the lactating cows, rinses from milking containers and rinses from bulking containers.

3.3.1 Sampling

Stratified sampling was applied and samples were only collected from farms with lactating cows where milk samples were also collected. Samples were collected early in the morning during milking time which ranged between 4.00am to 7.00am in the rural and between 6.00am to 8.00am in the peri-urban areas. Sample collection begun after the milking personnel had prepared the udder and milking containers and ready to start milking. Sterile cotton swabs wrapped in splint wood sticks were used in swabbing hands and udder (Pre-milking practices). The palm of the hands was swabbed using the sterile cotton swab sticks. The swab was then immediately transferred into sterile2% bacteriological peptone water (Oxoid) in a screw cap Bijou bottle. The handle stick was broken while the swab remained in the transport medium. The cap of the bottle was then put back and transferred to the cool box. The teats of the udder were swabbed from the attachment of the teat to the udder downwards while avoiding contact with hair on the udder. Water source used at the farm was sampled in a 500ml sterile sampling bottle, and together with other samples packed in a cool box with sufficient ice and transported to the laboratory within four hours for analysis.

Milking containers and bulking containers at the farm gate were rinsed with 100ml sterile water. Milk from all the quarters of the udder was collected in a sterile sampling bottle (100ml). Milk from all the cows within the farm that had been pooled in a different container at the farm gate for collection to bulking centre was also sampled. The milk samples were used to evaluate the effects of risks factors (udder, hands of milking personnel, milking container, bulking container and water used) on microbiological quality of milk between the udder and the farm gate. Microbial counts from the milk drawn directly from the udder and at the farm gate were regressed against the counts of the risk factors after microbial analysis. A farm with a minimum of one lactating cow provided seven samples for microbiological analysis. In the peri urban 30 farms were visited for sample collection which provided a total of 210 samples. In the rural area 50 farms were visited this provided 350 samples a total of 560 for this objective.

Samples were examined for total viable counts (TVC), coliform counts (CC), Thermophillic bacterial counts (ThBC) and psychrophilic bacterial counts (PBC) by standard procedures (ISO 4833-1: 2013; ISO 4832: 2006; KEBS/EAS, 2006) as explained below.

3.3.2Microbiological analysis using serial dilution method

One millilitre (1ml) of the milk sample was serially diluted six-fold (10^{-6}) using 9ml buffered peptone water. One millilitre of homogenate sample was transferred using a sterile pipette into sterile labelled petri dishes. Approximately 20ml of plate count agar (PCA) which had been autoclaved at 121^{o}C for 15min, cooled and tempered in a water bath at 45^{o}C, was poured into the petri dish. The media and sample were mixed gently by swirling in a figure eight manner.

The petri dish was left to solidify at room temperature and incubated at 37^{o}C for 48h in an inverted manner for Total Viable Counts (TVC) which is an indication of initial bacterial load (ISO 4833-2003). After 48hrs the counts were done using a colony counter and recorded n CFU/m.To count for coliforms which are indicators of hygiene along the value chain from production, a selective media, Mconkey agar was used. The incubation temperature and time were followed as in TVC above.For thermophillic and psychrotrophic bacterial counts (ThBC and PBC), PCA was used but the plates were incubated at 42^{o}C for 24hrs and 10^{o}C for 10 days respectively (ISO 4832: 2006; KEBS/EAS 2006 4.2.1).

Hand swabs, udder swabs, water sources and container rinses were serially diluted in the same manner and then evaluated for TVC, CC, ThBC and PBC following the procedure mentioned above in milk samples. Plates containing 30-300 colonies were selected and counted. An average count of the duplicate plates was calculated and converted into logarithm for recording and analysis. Discrete colonies grown on plate count agar were selected randomly and purified by repeated plating on the same agar. The colonies were subjected to gram stain and their morphology was studied under a microscope (× 100 lens immersion oil).

3.3.3Data analysis

Microbial count data was first transformed to logarithmic values ($\log_{10}$) before subjection to statistical analysis. Milk samples were collected and analysed using a completely randomized design in a nested arrangement experimental plan.The general linear model of SAS version 9.1.3 (SAS PROC GLM) was used to analyse milk microbial quality and the microbial quality of contamination sources. Mean comparison was done by the Fisher's least significant difference (LSD) when analysis of variance showed significant difference in means. Statistical difference was determined at 95% confidence level. Pearsons correlation was determined between contamination sources and farm gate milkwere done in total viable counts of the contamination source and raw milk

3.4 To determine the spoilage microbiological load of milk of milk along the rural and peri-urban sub value chains

Anested design in completely randomised block design(RCBD)was implemented in sampling milk along the value chain. Sampling nodes were established as per the recommendation of Bonfoh *et al.,* (2003) defined as a point of harvest and/or pooling of milk. California Mastitis test (CMT) was also done before sampling, cows testing positive were not sampled. Samples directly from the udder and at the farm gate were sampled as explained in the previous objective.Transporters carrying milk to the cooling centre were met there and samples were collected from them. This was done just before milk was delivered to the bulking centre. This was done randomly targeting transporters with different types of transporting containers and transporting means.

Milk at the cooling centre was also collected in sterile 100ml bottle. The tap of the cooling tank was opened and allowed to run for a few minutes before the samples were picked. The samples were collected at an interval of 30minutes until the time the last transporter arrived at the cooling centre. All samples were kept in a cool box with sufficient coolants and transported to Egerton University labs for analysis within four hours.Microbial groups sought were; total viable counts (TVC), coliform counts(CC), thermophilic bacterial count (ThBC) and psychrophilic bacterial count (PBC).

Spoilage microorganisms sought from plates of TVC after colony purification on the same media were; *Staphylococcus aureus*, *Streptococcus feacalis*, *Escherichia. Coli*, *Bacillus*spp and *Enterobacteriaceae*. Discrete colonies grown on plate count agar were selected randomly and purified by repeated plating on the same agar. The colonies were subjected to morphological (cell shape, cell grouping and endospores), biochemical (catalase, oxidase) and physiological tests and identified to genus level

3.4.1Biochemical tests for selected Genera

E. coli

Colonies from MacKonkey were streaked onto Eosin Methylene Blue agar (EMB) and incubated for 24 hours at 37^{o}C to observe for a green metallic sheen formation (*Escherichia coli*). Colonies with a green shiny metallic sheen were confirmed as *E.coli*(Bargeys Manual, 2003).

Streptococcus faecalis

For the isolation and enumeration of faecal streptococci KF Streptococcal agar with Triphenyltetrazolium Chloride (TTC) supplement was used. A loop full of gram positive cocci, catalase positive, coagulase negative colonies were transferred to the media and incubated at 37^{o}C for 48hours. Red centred colonies were a positive result for faecal streptococcus (Bargeys Manual, 2003)

Staphlococcus aureus

For detection and enumeration of *Staphylococcus aureus*, Baird Parker Agar with egg yolk tellurite supplement was used. A loop full of pure gram positive cocci which are coagulase positive were inoculated onto the agar and incubated at 37^{o}C for 24hrs. The growth of black, shiny, convex colonies with entire margins and clear zones is positive for S*taphylococcus aureus*(ISO 6888-1:2003).

Catalase test

Three to four colonies of the pure culture were picked using a sterile loop and put on a clean glass slide. A drop of 3% hydrogen peroxide (H_2O_2) was added to the organism on a glass slide using a Pasteur pipette at room temperature. Bubbling indicated that the organism was catalase positive

pH

The pH of the milk was done by a calibrated pH meter at each node during sample collection

3.4.2Data analysis

Log transformation was done on data from counts before any analysis was done. General descriptive statistics was done (means). Means and standard error were done by SAS proc glm, mean comparison was done by Fisher's test. Counts between the nodes were done by linear contrasts. Probability of occurrence of indicator microorganisms was according to Matofari *et. al.*, (2007).

$$Incidence\ (\%) = \frac{Number\ \ of\ \ positive\ \ samples}{Total\ \ samples\ \ \ collected}\ X\ 100$$

3.5Determination of antibiotic residues

3.5.1 Sampling

A nested design (in RCBD) was applied in sample collection where the nodes were nested within the locations. Sampling was done in three visits to the dairy system. The first visit 40 samples were collected from the farm, 35 samples from transporters and the three bulking centres were sampled from. This provided a total of 79 samples per visit in the rural dairy system. In the peri urban dairy system, 17 farms were visited with one cow per farm being sampled from, 7 milk transporters were sampled and the two cooling centres in that diary system were also sampled from. This provided a total of 26 samples per visit. Dairy farming is not a priority source of income for the population in peri urban and hence less than fifty per cent of the population carried out dairy farming, hence a relatively smaller sample size was collected from the peri urban dairy system. Sample volumes were 100 ml per sampling point, the samples were then stored at temperatures not higher than 4 °C before analysis.

3.5.2 Equipment Calibration and method validation

3.5.2.1 Calibration

Calibration graphs were first determined by preparation of different concentration of the standard solutions. From a stock solution of 1 mg/ml of each standard the following concentrations were prepared; 2,000 ng/ml, 1,000 ng/ml, 500 ng/ml and 50 ng/ml using mobile phase A. The calibration graphs produced by the standards were used to determine the concentration of drugs in the samples. The calibration curves were used to provide information on recovery, retention factor and the standard deviation. The Limits of detection were also provided, but these were equipment and procedure specific and were provided by the manufacturer of the HPLC-UV.

3.5.2.2 Method validation

The method was validated by the use of blank samples (n =7) spiked with a concentration of 200 ng/ml of all the sulphonamides (Sulfachloropyradizine (SCL) ,Sulfadiazine (SDZ), Sulfadimidine (SMTZ), Sulfaquinoxaline (SQ), Sulfamerazine (SMR), Sulfathiazole (STZ), Sulfamethoxazole (SMX), Sulfadoxin (SDOX), Sulfadimethoxin (SDM), Oxytetracycline (OTC), Doxycycline hyclate (DC), Chlortetracycline hydrochloride (CTC) and Tetracycline hydrochloride (TC)) and tetracycline's (Oxytetracycline (OTC), Doxycycline hyclate (DC), Chlortetracycline hydrochloride (CTC) and Tetracycline hydrochloride (TC).). The spiked and blank samples passed through sample preparation as

other milk samples described above. In the validation procedure Sulfachloropyradizine (SCL) was not eluded in any of the7 spiked samples. However, Sulfadiazine was only eluded from one of the spiked sample hence it was not possible to calculate a standard deviation.

3.5.2Screening

Screening was done using the Charm II Blue-Yellow test.The charm test is done using a kit which is provided by the manufacturer (ALDRICH). The kit has wells containing a media, pre-measured bacterial spores (*Bacillus stearothermophilus var. calidolactis*) and a pH indicator. Milk samples were initially centrifuged and the supernatant (not fat) was used in screening. 50 microliter of the whey was measures and transferred into a well. The procedure was repeated until all samples were transferred to individual wells in duplicate. A positive control containing Penicillin G (4ppb), and a negative control were included before proceeding. Wells with added samples and the controls were then incubated at 64^{o}C in a humidified incubator for three hours. At the end of the three hours, the wells were removed and observed for colour changes which were read using interpretation chart provided by the manufacturer (Figure 1, Charm II Blue Yellow test manual).

Suspect positive samples were further heated to 80^{o}c for 10minutes to eliminate the presence of other antibiotic inhibitors, lysozyme and lactoferrin. The boiled samples were then taken through the charm screening test once again. Samples that tested negative were eliminated but those which remained positive and caution samples were preceded to the HPLC for confirmation and quantification.

3.5.3Confirmation and quantification

Positive suspects were confirmed using HPLC where exact antibiotic quantities were determined. The following antibiotics were sought out based on the high prevalence of Mastitis in both locations. These antibiotics included; Sulfachloropyradizine (SCL), Sulfadiazine (SDZ), Sulfadimidine (SMTZ), Sulfaquinoxaline (SQ), Sulfamerazine (SMR), Sulfathiazole (STZ), Sulfamethoxazole (SMX), Sulfadoxin (SDOX), Sulfadimethoxin (SDM), Oxytetracycline (OTC), Doxycycline hyclate (DC), Chlortetracycline hydrochloride (CTC) and Tetracycline hydrochloride (TC). The antibiotics selected are also wide in spectrum in terms of their activity and are usually used in treating other bacterial diseases among cattle.

The HPLC procedure used had four main steps as described by Mamani and Reyes (2009) and Koesukwiwat *et al.,* (2007). These were; (a) protein precipitation and purification by centrifugation and trichloroacetic acid and McIIvaine-EDTA buffer, (b) Sample extraction

with Oasis HLB (200mg) cartridge, (c) sample evaporation and (d) Quantification by HPLC with gradient mode on C_{18} column and UV-detection.

3.5.4Chemicals and materials

3.5.4.1Standards

The standards used were; Sulfachloropyradizine (SCL) (Sigma-Adrich 46778), Sulfadiazine (SDZ)(Sigma-Adrich 35033), Sulfadimidine (SMTZ) (Sigma-Adrich 46802), Sulfaquinoxaline (SQ) (Sigma-Adrich 45662), Sulfamerazine (SMR) (Sigma-Adrich 46826), Sulfathiazole (STZ) (Sigma-Adrich 46902), Sulfamethoxazole (SMX) (Sigma-Adrich 46850), Sulfadoxin (SDOX) (Sigma-Adrich 46810), Sulfadimethoxin (SDM) (Sigma-Adrich 46794), Oxytetracycline (OTC) (Sigma-Adrich 46598), Doxycycline hyclate (DC) (Sigma-Adrich 33429), Chlortetracycline hydrochloride (CTC) (Sigma-Adrich 26430), Tetracycline hydrochloride (TC) (Sigma-Adrich 87130).

3.5.4.2 Reagents

The reagents included; Acetonitrile (J.T Baker 9017), Methanol (J.T Baker 8402), Trichloroacetic acid (J.T Baker 0344), Disodium hydrogen phosphate (J.T Baker0326), Citric acid (Acros 124912500), Sodium-EDTA (J.T Baker 1073), Calcium Chloride (Merck 1.2378.0500), Sodium Cetate (J.T Baker 0258), Ammonium acetate (J.T Baker 0011).**Mobile Phase A** was prepared by mixing Na acetate (0.075 M) and Calcium Chloride (0.035 M) to Sodium EDTA (0.025 M) and the pH adjusted to 7.0. **Mobile Phase B** was prepared by mixing 75% methanol to 25% Acetonitrite.

3.5.4.3 Equipment

The equipment used in the study was Shimadzu HPLCJapan. Equipped with a UV-vis detector, SID 20A, Column Oven-CTO-10ASVP, X-TerraR MS C18 (3.5 µm, 2.1 × 150 mm column Waters made in Ireland), an XTerra Guard column C18 (3.5 µm, 2.1 × 10 mm) solvent delivery module, LC 20AT, degassing unit DGU-20A3, an auto sampler SIL-20AHT and system controller CBM-20A connected to a HP intergrator with LC Solution Version 3.5 Shimadzu Corp-Japan.

3.5.5Sample preparation

3.5.5.1 Protein Precipitation and purification

5ml of presumptive positive sample was measured into a 25ml centrifuge tube, 2.5ml of 25% TCA in water was added and mixed for 10 seconds by vortexing. 10ml of Mcllavine-EDTA buffer was added to the mixture, vortexed for 10 seconds and then mixed in a

sonicator for 10 minutes. This mixture is then centrifuged at 400 rpm at 10^{O}C. The clear supernatant was poured out to a new 25ml centrifuge tube with the fat remaining in the tube walls. To the old centrifuge tube containing the sub-natant, 10ml Mcllavine-EDTA was added and mixed by vortexing for 10seconds. This was the sonicated for another 10minutes. This was then centrifuged at 4000 rpm. The resulting supernatant was mixed with old supernatant initially collected from the same sample.

3.5.5.2 Solid phase Extraction

The C_{18} cartridges were marked and fixed on the solid extraction vacuum. Additional funnels (20ml) were fixed on the cartridges. The C18 column cartridge was activated by 5ml methanol, followed by 10ml acetonitrile then 5ml Mcllavine-EDTA without letting the cartridge run dry. The clear super-nanatant was then poured to the cartridge funnels so that it trickles through in approximately 20minutes. This was then washed with 5ml methanol in Mcllavine. The cartridge was then dried by using the vacuum drier. After the vacuum was relieved the washes under the cartridge were discarded marked glass tube for sample collection was placed under the cartridges. 5ml of methanol was added to the dry cartridge and allowed to absorb for 5minutes after which the samples were eluded out of the cartridge at aflow rate of 1ml/minute.

3.5.5.3 Evaporation

Glass tubes containing methanol eluent were placed in a sand bath at 50^{O}C to evaporate the methanol and leaving a thick fluid at the bottom of the glass tube.

3.5.6 Quantification by HPLC

Sulphonamides were detected at 265nm while tetracyclines were detected at 385nm. The column temperature was set at 40^{o}C while the flow rate was at 0.2 ml/min. Used gradient was A:B 90:10 at 0-35 min, 65:35 at 35-36 min, 90:10 at 36-45 min and 90:10 at 45-55 min. sample run time was 45 minutes while the injection volume was 10µl. The retention times of Sulphonamides (SDZ,SMX, SMR, SCL, SDOX, SMTZ, SDM, SQ) was 4min, 7min, 7min, 8min, 8min, 14min, 16min and 17min respectively. The retention times for tetracyclines (OTC, DC, TC and CTC) were 11min, 24min, 33min and 36min respectively. In the first run SMX and SCL standards were eliminated to allow for SMR and SDOX to be detected. This is because SMX and SMR shared a retention time (7 min) just as SCL and SDOX (8 min).

The remaining fluid in the glass tube (from sample preparation) was added to 200µl of mobile phase B (75% methanol +Acetonitrite 25%). This was the mixed by vortexing for 15 seconds. 0.3µl of mobile phase A was added to the mixture and vortexed vigorously for

15 seconds. The sample was then filtered through 0.2 µm syringe filter to HPLC vials and put inside the HPLC and results were generated after the run time was completed.

3.6 Determination of milk losses due to milk spoilage

3.6.1 Percentage losses

Determination of probable losses was based on Kenya Bureau of standards grading of raw milk. Samples with Total Viable Counts falling above grade III of milk was considered as lost (Table 2).

Table 2: KEBS Raw milk Grading system

Grade	Counts (per ml)
I	< 200,000
II	>200,000 – 1,000,000
III	>1,000,000 – 2,000,000

Source: KEBS/ EAS 67.2007

Maximum allowable standard counts for thermophiles psychrophiles and TVC for processing milk which were used in arriving at losses from these microbial groups are as outlined below. High levels of these microorganisms indicate poor conditions in production, storage and processing of milk and also the presence of pathogens (Freitas *et al.*, 2009).

Table 3: Standards of microbial counts in raw milk by different regulating bodies

Microbial type	**Maximum Standard count**	**Regulating Body**
TVC	Log $_{10}$6.3 CFU/ml	KEBS/EAS 2007
CC	Log_{10} 4.7 CFU/ml	KEBS/EAS 2007
PBC	Log $_{10}$5.0 CFU/ml	European Council reg No 853/2004

TVC: Total viable counts, CC: coliform counts, PBC: Psychrotrophic bacterial counts, KEBS:Kenya Bureau of Standards, EAS: East African Standards

The percentage exceeding thresholds were calculated based on the formula provided.

$$\%E_{threshold} = \frac{n\ exceeding\ threshold}{N\ sample\ at\ node} \times 100$$

Where:

$\%E_{threshold}$ = Percentage of samples exceeding threshold

$n\ exceeding\ threshold$= Number of samples exceeding threshold

$N\ sample\ at\ node$ = Number of samples collected at a particular node

3.6.2 Losses in Volume

Percentage losses in volume were calculated based on the volume that were sampled which exceeded the maximum threshold as indicated below.

$$L_{volume} = \%E_{threshold} \times V_{sampled}$$
$$V_{sampled} = \pi_{node} \times N_{sample}$$

Where

L_{volume} = Volume lost in liters

$\%E_{threshold}$ = Percentage of samples exceeding the maximum threshold

$V_{sampled}$ = Volume of milk sampled at a particular node in liters

π_{node} = Mean of milk produced at that particular node

N_{sample} = Number of samples at a particular node

3.6.3 National annual dairy losses

Percentage losses throughout the value chain was calculated based on the volume of milk at value chain nodes that did not meet the standard threshold against the total volume of milk in the value chain as explained below.

$$\%Losses = \frac{L_{volume}}{total\ volume\ of\ milk\ in\ value\ chain} \times 100$$

3.7Determination of losses due to antibiotic residues

3.7.1 Percentage of samples with antibiotic residues exceeding EU MRL

$$\%\ \text{Samples with antibiotic residues exceeding EU MRL} = \frac{\text{Number of samples with conc exceeding EU MRL}}{tatal\ samples\ collected} \times 100$$

3.7.2 Volume lost

Volume lost= Number of samples exceeding EU MRL x Mean volume at a node x Sample size

Volume lost total= Volume lost at farm + volume lost transporters + volume lost at bulking centre

Results of calibration are as shown in Table 16. The standard calibration curves used in the generation of results were generated based on a formula by (Sulejmani *et al.* 2012). The absorbance read became a percentage (%) of optical density relative to zero standards B_0 and it is based on the calibration line assigned to each series of standard solutions and has the following formula:

$y = a + b*\ln X$

Y-read signal expressed in% of optical density,

X-concentration of the substance and *a* and *b* coefficients.

In every batch of samples analyzed for values of (Rr^2) recovery ratio, tetracycline's should be at least 0.8278, while the sulphonamides' Rr2 > 0.98. The calibration curve provides information on recovery, retention factor and the standard deviation. The Limits of detections are also provided in the calibration results but these are equipment and procedure specific and were provided by the manufacturer of the HPLC-UV. One such calibration curve has been provided in the appendix.

CHAPTER FOUR

RESULTS

4.1.Risk factors associated with contamination of milk with spoilage microorganisms

From the survey questionnaire and observation checklist, none of the farms visited in both rural and peri urban practiced machine milking. Hand washing was practiced by all farmers in peri urban. Drying of hands and udder was practiced by 11% of farmers in rural and 50% in peri urban. Plastic milking containers were 60% in peri urban and 84% in rural location (Figure 3). Cross tabulation of risk factors practices between location showed that lack of hand drying was significantly different (P=0.007). Plastic milking and bulking containers were significantly different (P =0.04 and P =0.03 respectively) between locations. Lack of water treatment was practiced by 60% in rural and 80% in peri urban this was significantly different (p=0.008).

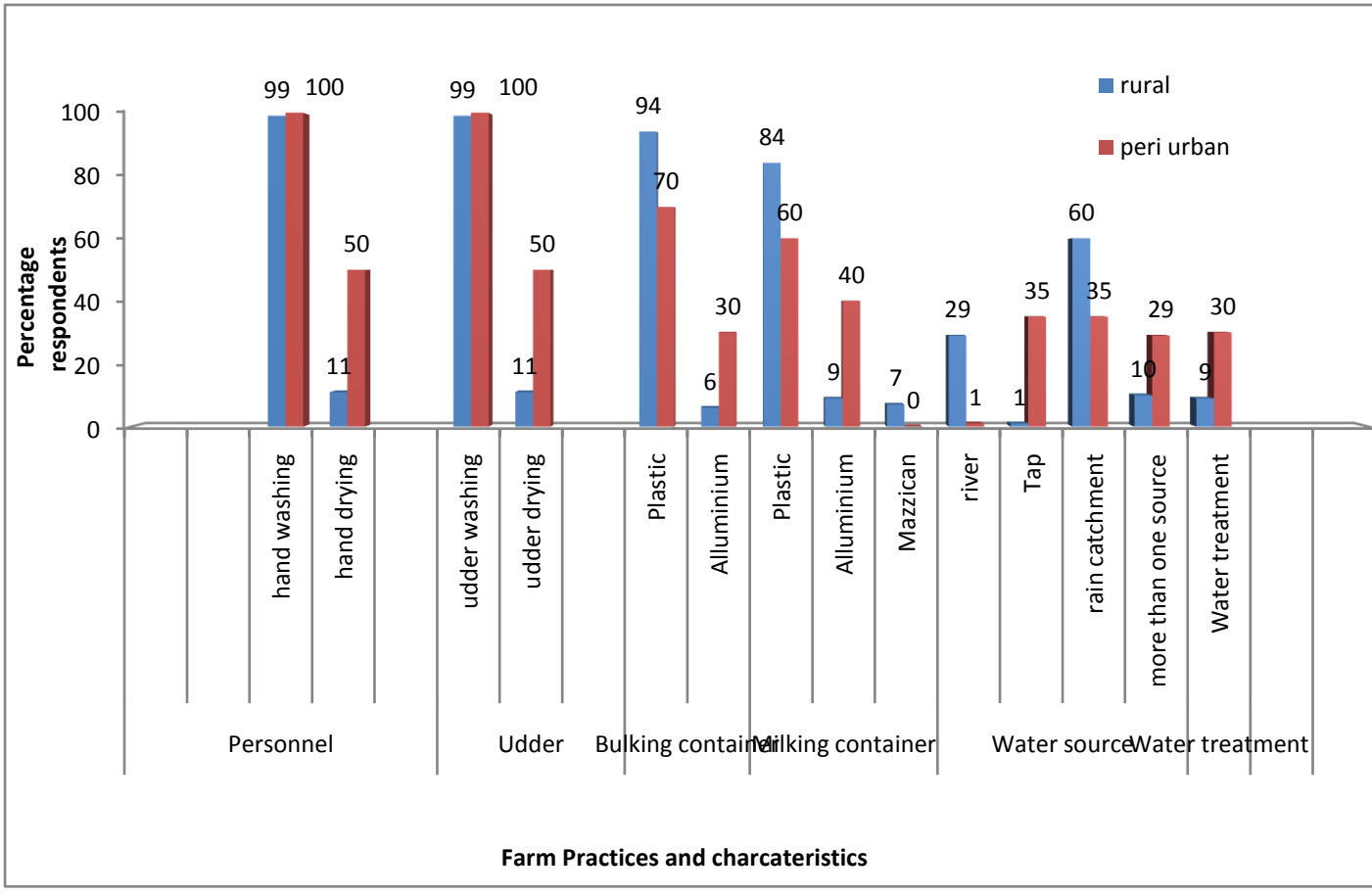

Figure 3 : Graph of farm practices and characteristics of farms in the rural and peri urban locations.

Table 4: Cross tabulation between risk factors and dairy system

Location	Risk factors (%)				
	Lack of washing hands and udder	Lack of drying hands and udder	Plastic milking container	Plastic bulking containers	Lack of farm water treatment
Rural	20[a]	60[a]	94[a]	84[a]	89[a]
Peri urban	3[b]	80[a]	70[b]	60[b]	50[b]

Values followed by different letters in a column are significantly different at ($P < 0.05$).

4.1.1Microbiological quality of contamination sources

From the survey the risk sources of contamination to milk contamination with spoilage microorganisms included udder, hands, milking containers, bulking containers and water sources. Udder swabs recorded the highest counts in TVC($\log_{10}$3.4 CFU/ml) in rural while milking container recorded the highest ($\log_{10}$4.4 CFU /ml) in peri urban. Hands and udder recorded the highest counts in coliform for both locations. Water source recorded the highest in PBC in peri urban location while milking container rinses recorded a significantly ($p<0.05$) lower value for ThBC in peri urban. Lack of water treatment was significantly different between rural and peri urban dairy systems (Table 4). There was a steady rise in microbial counts between the udders to the farm gate in all microbial counts evaluated (Table 5).Increase in TVC from the udder to the farm gate was 0.5log cycle in rural. A significant increase in coliform count was recorded between the udder and farm gate in rural and peri urban milk (Figure 5).

Table 5: Table of microbial counts (Means ± SE) of risk factors and milk drawn directly from the udder and at the farm gate

Microbial count	Location	Risk factors						
		Milk directly from udder	US	HS	MCR	BCR	WS	Milk at the farm gate
TVC	Rural	5.2±0.3[a]	3.4 ±0.2[a]	3.0±0.3[a]	3.3±0.2[a]	2.4±0.5[a]	2.6±0.2[a]	5.7±0.5[a]
	Peri urban	4.8±0.5[a]	3.9±0.2[a]	3.7±0.2[b]	4.4±0.3[b]	2.1±0.1[a]	2.5±0.6[a]	5.2±0.4[a]
CC	Rural	3.2±0.8[a]	2.7±0.3[a]	3.3±0.4[a]	1.3±0.4[a]	1.6±0.7[a]	1.5±0.3[a]	3.9±0.8[a]
	Peri urban	4.2±0.4[b]	3.5±0.3[b]	3.6±0.2[b]	1.1±0.5[a]	1.1±0.2[b]	1.8±0.4[a]	4.7±0.3[b]
ThBC	Rural	5.0±0.4[a]	2.8±0.3[a]	2.8±0.3[a]	2.5±0.3[a]	2.1±0.6[a]	2.2±0.3[a]	5.2±0.5[a]
	Peri urban	2.7±0.5[b]	3.2±0.3[a]	3.4±0.2[b]	1.1±0.3[b]	2.1±0.4[a]	1.4±0.3[b]	2.8±0.6[b]
PBC	Rural	5.5±0.3[a]	3.3±0.3[a]	1.8±0.5[a]	3.3±0.2[a]	1.9±0.9[a]	2.6±0.4[a]	6.1±0.3[a]
	Peri urban	3.7±0.4[b]	3.2±0.3[a]	3.7±0.2[b]	2.5±0.4[b]	2.4±0.4[b]	2.9±0.2[a]	4.7±0.8[b]

US; udder swabs, HS; hand swabs, MCR; Milking container rinse, BCR; bulking container rinse, WS; water source. TVC; Total viable counts, CC; coliform counts, ThBC; Thermophilic bacterial counts, PBC; Psychrophillic bacterial count. Means followed by the same letter in a column within a row are not significantly different at (P > 0.05).

Table 6: The mean square analysis of variance of the microbial types in the two dairy systems and the risk factors within the dairy systems

S.O.E	DF	Mean squares of microbial types			
		TVC	ThBC	PBC	CC
SYSTEM	1	0.679[ns]	0.784[ns]	13.090[***]	9.779[***]
RISK	4	4.511[***]	5.403[***]	2.978[*]	12.904[***]
RISK(SYSTEM)	4	2.064[***]	2.611[*]	2.776[**]	2.436[*]
ERROR		0.384	0.837	1.315	1.138
CV		2.219	4.059	4.943	4.523
R^2		0.940	0.934	0. 732	0.761

SOE; Source of Error, DF; Degree of Freedom, TVC; Total viable counts, CC; coliform counts, ThBC; Thermophilic bacterial counts, PBC; Psychrophilic bacterial count, ns; not significant, *** is significant at P<0.001, ** is significant at P<0.01 and * is significant at P<0.05

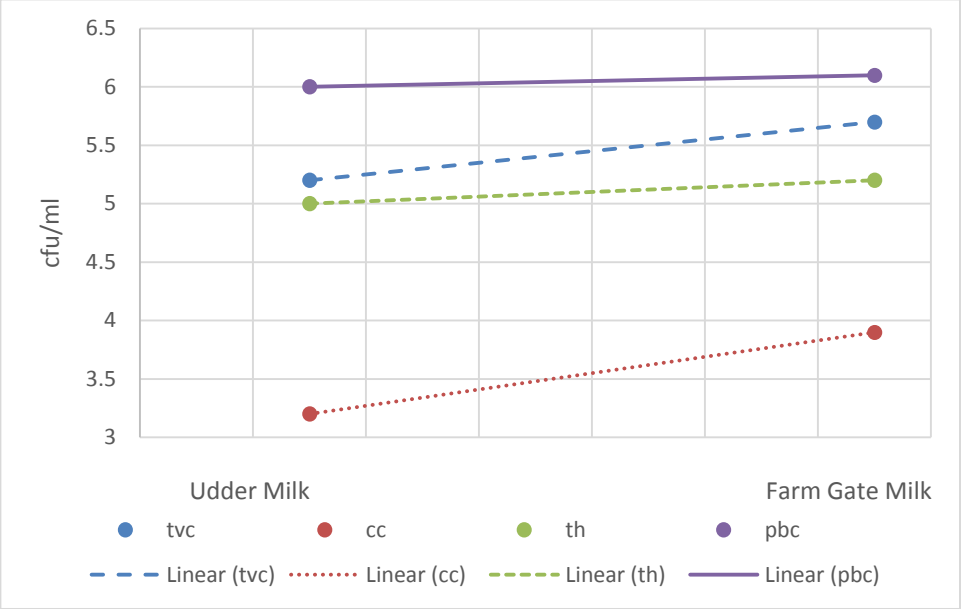

Figure 4 : Comparison of bacterial counts in raw milk drawn directly from the udder and milk at the farm gate in the rural.

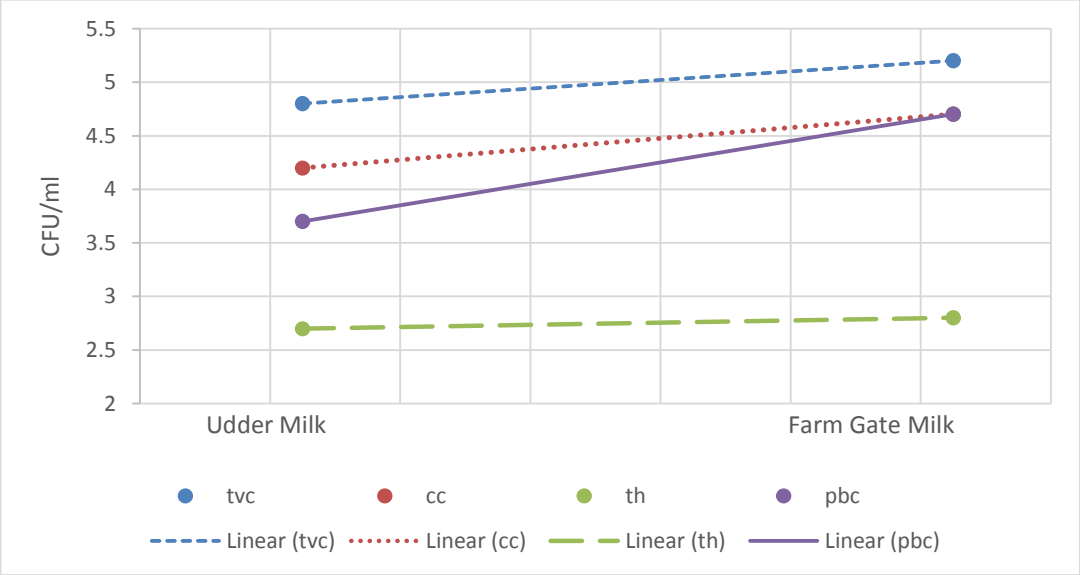

Figure 5 : Comparison of bacterial counts in raw milk drawn directly from the udder and milk at the farm gate in the peri urban.

Regression coefficients were derived from the formula below to determine the most responsible sources of different microbial types in milk at the farm gate. Where *Y* represented each microbial type in farm gate milk (TVC/CC/ThBC /PBC) and was regressed against the microbial type of each risk factor (US, HS, MCR, BCR, and WS) evaluated and X_1 to X_5 are the regression coefficients of the respective risk factors.

$$Y = \beta o + X_1 US + X_2 HS + X_3 MCR + X_4 BCR + X_5 WS + E_i$$

Hands, udder and water source were the highest contributors to coliform counts in rural farm gate milk. In peri urban udders were the highest contributors to coliform counts in farm gate milk (Table 7).

Table 7: Regression coefficients of risk factors versus farm gate milk

Microbial type	**Location**	**Risk factors**					
		US	HS	MCR	BCR	WS	Constant
TVC	Rural	2.73*	2.63*	0.18	0.05	0.12	4.84
	Peri urban	0.87	1.15	1.19	1.51*	0.60	3.29
CC	Rural	0.83	1.46*	0.74	0.16	0.88*	5.34
	Peri Urban	0.58	0.36	0.04	0.31	0.16	4.46
ThBC	Rural	0.22	0.93*	0.02	0.83	0.59	1.85
	Peri urban	0.22	0.31	0.37	0.17	0.08	4.78
PBC	Rural	0.14	0.24	0.48*	0.02	0.01	4.42
	Peri urban	0.46	0.62*	0.55	0.36	0.35	4.39

US; udder swabs, HS; hand swabs, MCR; Milking container rinse, BCR; bulking container rinse, WS; water source. TVC; Total viable counts, CC; coliform counts, ThBC; Thermophilic bacterial counts, PBC; Psychrophillic bacterial count. * Regression coefficient significant at ($P < 0.05$).

4.2Microbiological quality of milk along the dairy value chains

Table 8: Range of microorganisms in milk along the sub value chain % (N)

NODE/VARIABLE	RURAL (%)					PERI URBAN (%)				
	≤30	$\leq 10^5$	$>10^5 \leq 10^6$	$>10^6 \leq 2 \times 10^6$	$>2 \times 10^6$	≤30	$\leq 10^5$	$>10^5 \leq 10^6$	$>10^6$	$>2 \times 10^6$
Farm gate										
TVC	0	33.3	33.3	15.3	15	19	8	33	20	20
CC	41	25	17	8	9	28	11	44	1	7
ThBC	8	25	50	10	7	52	14	24	5	5
PBC	0	8	42	25	25	42	0	11	27	20
Transporters										
TVC	4	44	20	16	16	4	5	52	9	30
CC	44	8	16	16	16	26	22	30	12	10
ThBC	4	64	20	8	4	14	27	27	30	2
PBC	4	24	32	20	20	9	9	18	50	14
BULKING										
TVC	0	50	12	30	8	0	20	33	40	7
CC	25	12	13	40	10	0	12	0	80	8
ThBC	0	12	63	20	5	0	0	25	60	15
PBC	0	12	38	30	20	0	0	12	70	18

TVC; Total viable counts, CC; coliform counts, ThBC; Thermophilic bacterial counts, PBC; Psychrophilic bacterial count.

A steady increase was observed in microbial range between 10^5 and 10^6 CFU/ml along the chain (Table 8). There was a steady increase in TVC, CC, and PBC with lower counts for ThBC at the end of the sub value chain (Table 8). Bulking centres recorded the highest mean counts for CC, ThBC and PBC (Table 8). Total viable counts were highest at transporters node in the rural location. There was no significant difference in TVC between the two dairy systems. A significant difference however, was observed in CC, ThBC and PBC between the dairy systems.

Table 9: The analysis of variance (ANOVA) for milk microbial counts for the rural and peri-urban dairy systems and for the dairy value chain nodes within the two systems

		Mean squares of the microbial load (log_{10} cfu/ml)			
S.O.V	DF	TVC	TCC	THERMO	PSYCH
System	1	0.080^{ns}	30.234^{*}	75.699^{***}	83.480^{***}
Node(System)	6	8.704^{*}	11.463^{ns}	26.763^{***}	14.128^{**}
Error	457	3.685	7.096	4.924	4.892
C.V		3.745	6.396	5.466	4.477
R^2		96.78	87.70	72.940	91.03

S.O.V; Source of variation, DF; Degree of freedom, C.V; Coefficient of variation, R^2; Coefficient of determination, TVC; Total viable counts, TCC; Total coliform count, ThBC; Thermophilic bacterial counts, PSYCH; Psychrophilic bacterial counts, ns; not significant at P<0.05, *significant at P<0.05, **significant at P<0.01 and ***significant at P<0.001.

Table 10: Means ± SE comparison of milk microbial loads for the rural and peri-urban dairy systems and for the dairy value chains nodes.

			Mean milk microbial loads (log_{10}CFU/ml)			
Factor	DS/Nodes	N	TVC	CC	ThBC	PBC
System	Rural	342	5.79 ± 0.22^{a}	3.66 ± 0.35^{b}	4.87 ± 0.21^{a}	5.10 ± 0.15^{a}
	Peri-urban	119	5.14 ± 0.20^{a}	4.51 ± 0.25^{a}	3.51 ± 0.28^{b}	4.36 ± 0.28^{b}
Node (Rural)	Cow	167	6.12 ± 0.61^{b}	3.05 ± 0.65^{b}	4.41 ± 0.53^{b}	4.46 ± 0.35^{a}
	Farm gate	51	6.04 ± 0.26^{ab}	3.18 ± 0.83^{b}	4.73 ± 0.51^{b}	5.09 ± 0.22^{a}
	Transporters	120	5.72 ± 0.30^{a}	3.39 ± 0.63^{b}	4.98 ± 0.24^{ab}	5.47 ± 0.27^{b}
	Cooling centres	4	5.66 ± 0.26^{a}	5.19 ± 0.71^{a}	5.81 ± 0.20^{a}	5.58 ± 0.21^{ab}
Node (peri-	Cow	57	4.63 ± 0.33^{b}	3.93 ± 0.38^{c}	2.65 ± 0.43^{b}	4.21 ± 0.42^{c}
urban)	Farm gate	30	4.84 ± 0.53^{ab}	4.33 ± 0.57^{bc}	2.68 ± 0.61^{b}	4.61 ± 0.65^{c}
	Transporters	30	5.60 ± 0.26^{b}	4.78 ± 0.45^{b}	4.98 ± 0.41^{ab}	5.30 ± 0.49^{b}
	Cooling centres	2	6.91 ± 0.19^{a}	6.03 ± 0.68^{a}	5.63 ± 0.65^{a}	6.69 ± 0.23^{a}

DS is dairy system, TVC is total viable counts, CC is coliform counts, ThBC is Thermophilic bacterial counts, PBC is Psychrotrophic bacterial counts. Means followed by the same letter in a column within a raw are not significantly (p> 0.05) different.

Thermophillic bacterial counts were highly significantly (P<0.001) different within the nodes between the two dairy systems. Coliform counts were significantly (0.05) different between the two systems while TVC where not significantly (0.05) different (Table 9). Total viable counts in milk directly drawn from the cow's udder and at the farm gate did not show any significant difference in both dairy systems. Coliform counts, thermophillic bacterial counts and psychrophilic bacterial counts were significantly different between the two dairy

systems (Table 10). Between the udder and the farm gate no significant difference was observed in CC, ThBC and PBC in rural and peri urban dairy value chains.

4.2.1Per cent microbial groups

In bacterial morphology, gram positive cocci were highest at the farm gate, slightly higher than that of milk drawn directly from the udderin the rural location. The number was significantly lower ($p < 0.05$) at the transporters node (Figure 6). Gram negative rods recorded the highest at the transporters node for both dairy systems while gram positive rods were most at the bulking centres. Gram negative rods were high in transporters node in peri urban location, with gram positive cocci recording the lowest at the cooling centre.

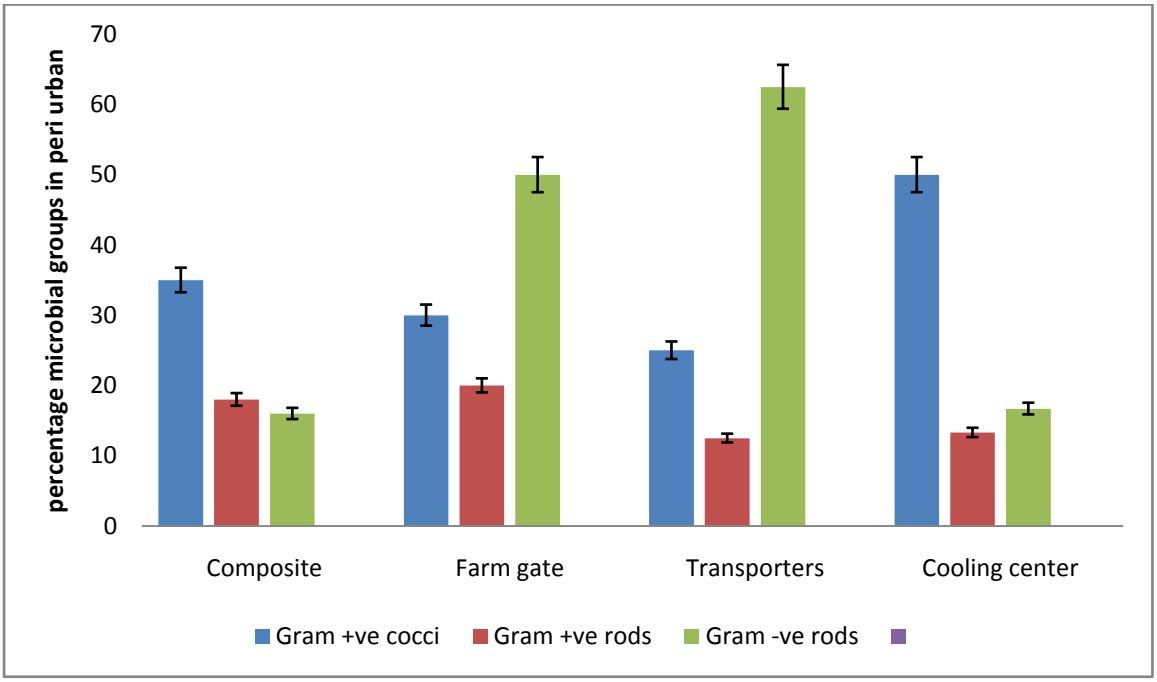

Figure 6 : Comparison of microbial groups in along the value chain in the peri-urban dairy value chain.

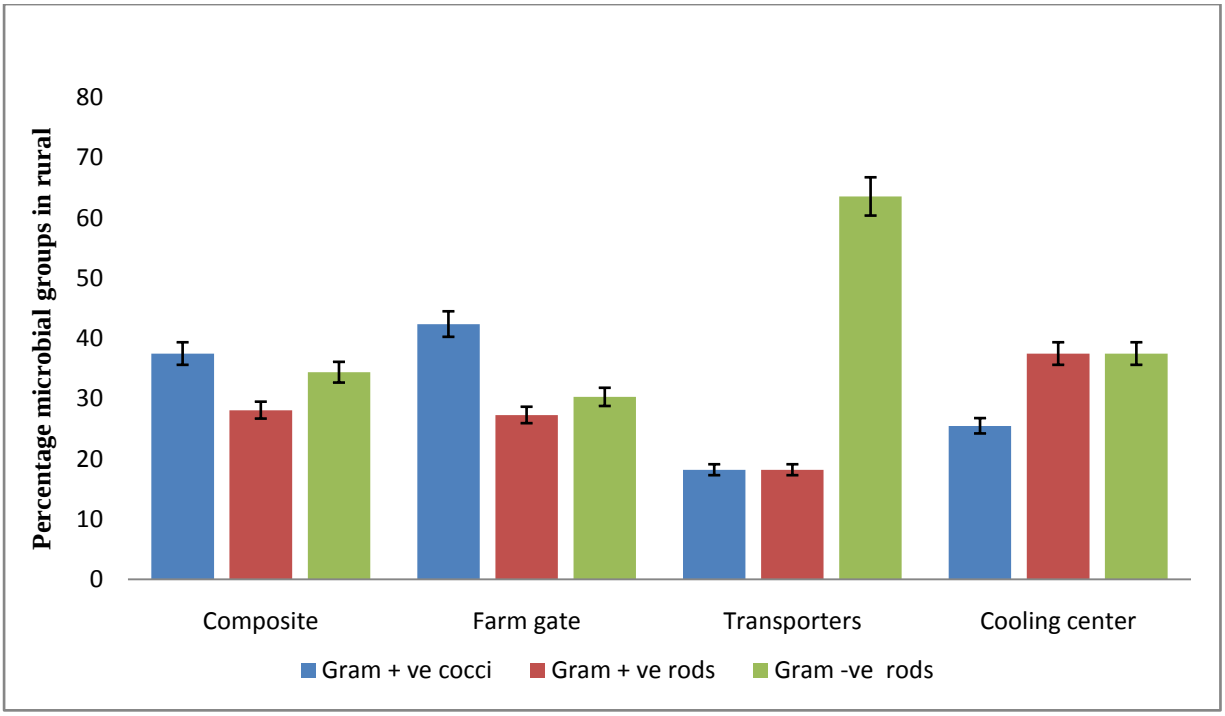

Figure 7 : Comparison of microbial groups in the rural dairy value chain

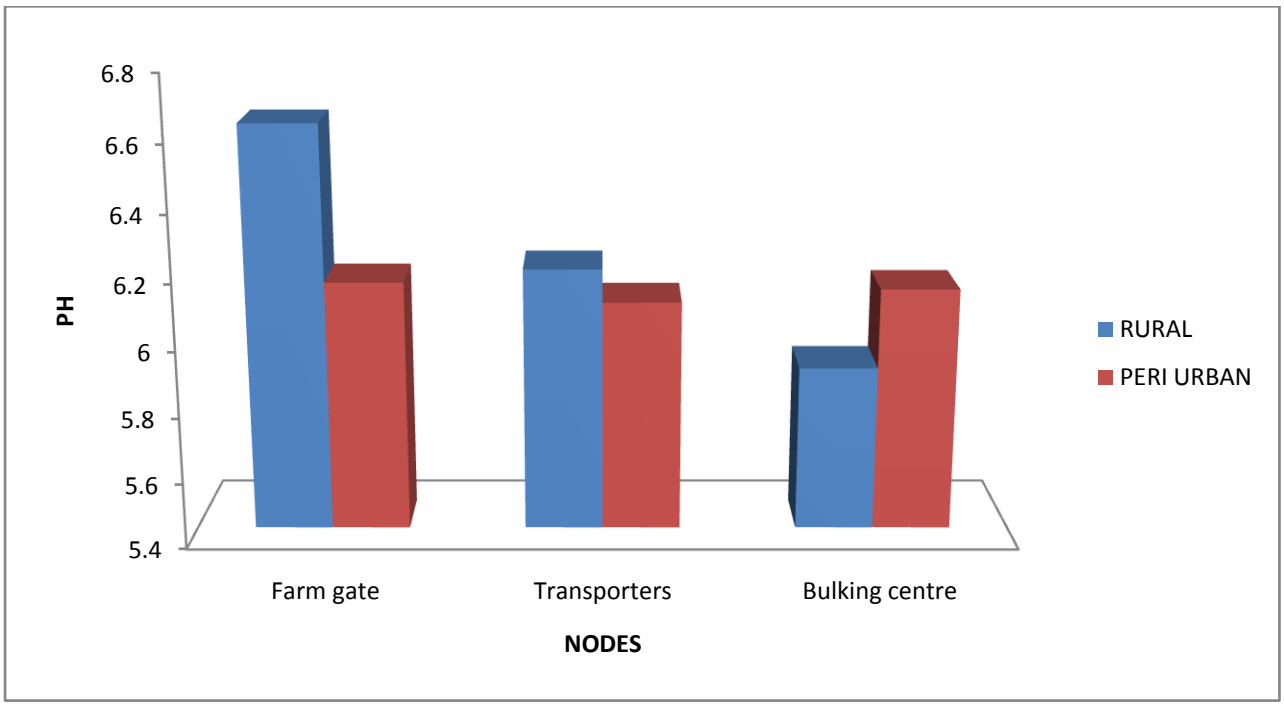

Figure 8 : Change in pH along the value chain nodes in rural and peri- urban

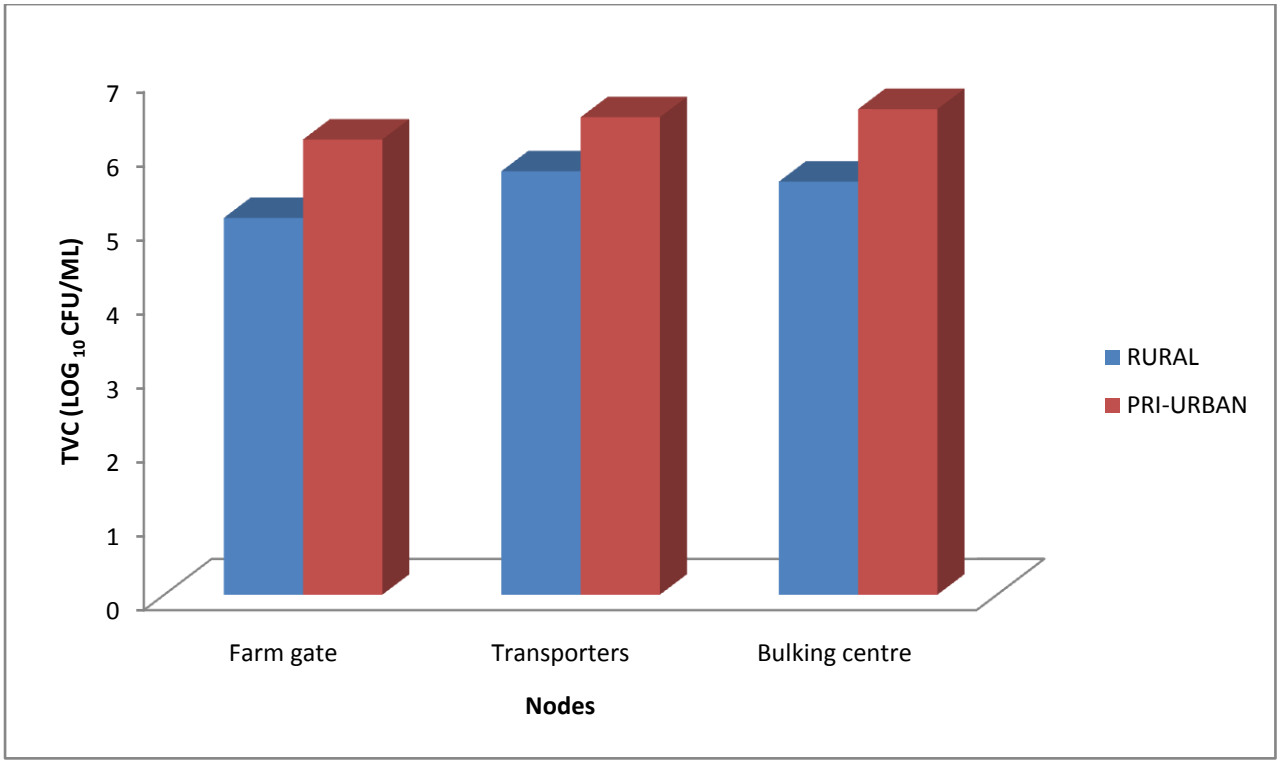

Figure 9: comparison of TVC along the values chain in rural and peri urban nodes

There was a fall in pH as milk moves along the value chain, but a significant increase in TVC was observed in both dairy systems. The incidence of occurrence of *staphylococcus* and *streptococcus* was highest at the farm. *Bacillus spp* had a higher incidence of occurrence at the transporters node. At the cooling centre there was a fall in incidence of occurrence for all indicator microorganisms investigated (Table 11) in the rural location.

Table 11: Incidence of indicator spoilage microorganisms along the value chain in Rural

Node	**N**	***Staphs***	***Streps***	***Bacillus***	***E.coli***
Udder milk	167	37	18	58	55
Farm Gate	51	7	15	7	15
Transporters	120	43	8	17	9
Cooling centre	4	1	7	5	1
Total	341	88	42	83	80
Incidence (%)		26	13	26	23

Staphs-Staphylococcus aureus, Streps-Streptococcus faecalis; E.Coli-Escherichia coli

Table 12:Incidence of indicator spoilage microorganisms in Peri urban

Node	N	*Staphs*	*Streps*	*Bacillus*	*E.coli*
Composite	57	28	13	16	10
Farm Gate	30	30	20	20	10
Transporters	30	20	30	20	28
Cooling centre	2	20	14	4	43
Total	120	98	77	104	91
Incidence (%)		82	64	87	76

Staphs-Staphylococcus aureus, Streps-Streptococcus faecalis; E.Coli-Escherichia coli

There was 82% incidence of *staphs* occurring in peri urban milk. *E.coli* had an incidence of 76% incidence of occurrence. Farm gate milk recorded relatively high positive samples for almost all groups of microorganisms examined during the study (Table 12). Between the farm gate and the cooling centre*E.coli* recorded an increase in incidence while there was a fall in *Bacillus*. For *staphylococcus and streptococcus* the incidence remained relatively the same.

4.3Antibiotic residues (Sulphonamides and tetracyclines) in rural and peri urban dairy systems

4.3.1 Screening results

Out of 229 samples in the rural dairy system, 72 (31.4%) samples tested positive from Charm test out of which 4 (1.7%) samples were treated as caution because the colour change was not distinct. Samples that lacked a distinct colour were treated as positive and were taken to HPLC for confirmation. Out of the positive samples from charm test, 59(56 confirmed positive and 3 caution) were from farm level, 12 (1 caution) samples at the transporters and 1 sample was from the bulking centre. In the peri urban dairy system, out of the 80 samples collected 23 samples were positive while 5 were treated as cautious due to lack of a distinct colour (3 at the farm, 1 transporter and 1 at cooling centre). Out of the distinctly positive samples, 11 of them were recorded at the farm level, 6 at the transporters node and 1 sample was at the bulking centre.

Table 13: Screening results from Charm Blue-Yellow II Test

Dairy system	Nodes	N	Positive	Caution	Total
Rural	Cows	120	56 (45.8%)	3(2.5%)	
	Transporters	105	11 (9.5%)	1 (0.95%)	
	Bulking	4	1(25%)	0	
Total		**229**	**68**	**4**	**72 (31.4%)**
Peri urban	Cows	57	11 (19.5%)	3 (5.3%)	
	Transporters	21	6(28.6%)	1 (4.8%)	
	Bulking	2	1(50%)	1 (50%)	
Total		**80**	**18**	**5**	**23 (28.8%)**

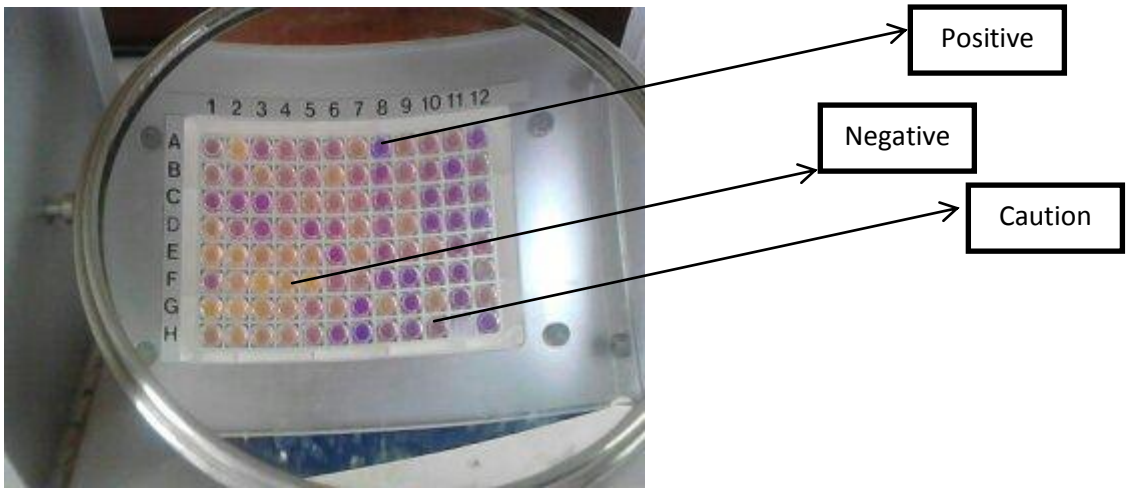

Figure 10 : Charm II Blue-Yellow Kit screening results

4.3.2 Quantification results

Out of the thirteen antibiotics evaluated, results from the HPLC showed that none of the samples contained any of the four tetracyclines tested (OT, CTC, DOC, TC) while three sulphonamides (SMR, SCL and SQ) were also negative. All of the samples that tested positive for sulphonamides at the rural dairy system had values above the EUMRL levels. Positive samples for sulphonamides at the farm node and transporters node also recorded values higher than the EU MRL levels. At the cooling centre in the peri urban dairy system, SDZ and SDM however recorded values less than 100ug/kg in the positive samples. Only 2 samples recorded presence of sulphonamides in the rural farms, 1 sample at the transporters node and 1 sample at the bulking node. Samples positive for sulphonamides contained SDOX (148.78µg/kg,), SDZ(90.03 µg/kg), SDM(66.14 µg/kg) and SMX(8,979.59 µg/kg, 8,979.51

µg/kg) (Table 18).Results for quantity of antibiotics are recorded in Table 4. The mean concentrations of the antibiotic contaminants were significantly different between locations. The highest mean concentration was recorded at peri urban bulking centre, which was highest for all the nodes (Fig. 10).

The mean concentration of antibiotic residues in the rural farm was lower than concentrations at the transporters node. A slight decrease at the cooling centre was observed in third dairy system. A different scenario, however, was observed at the peri urban dairy system. The highest concentration of antibiotic residues was recorded at bulking centres followed by farm gate nodes while transporters recorded the least concentration in the peri urban dairy system.

Table 14: Quantity of Sulphonamides and Tetracyclines

	RURAL (Concentrations in µg/kg)			**PERI (Concentrations in µg/kg)**		
Residue	**Farm**	**Transport**	**Bulking**	**Farm**	**Transport**	**Bulking**
SMR	0	0	0	0	0	0
SDOX	0	0	0	0	0	148.78
SCL	0	0	0	0	0	0
STZ	0	0	0	1923	0	0
SMTZ	389.176	0	0	0	0	0
SQ	0	0	0	0	0	0
SDZ	0	0	0	0	0	90.03
SDM	0	0	2305	0	674.83	66.14
SMX	179.026	2389.844	0	0	0	8979.59 8979.51
Percentage	1.7%	0.95%	25%	1.8%	4.8%	100%
OTC	0	0	0	0	0	0
DC	0	0	0	0	0	0
CTC	0	0	0	0	0	0
TC	0	0	0	0	0	0

Sulfachloropyradizine (SCL), Sulfadiazine (SDZ), Sulfadimidine (SMTZ), Sulfaquinoxaline (SQ), Sulfamerazine (SMR), Sulfathiazole (STZ), Sulfamethoxazole (SMX), Sulfadoxin (SDOX), Sulfadimethoxin (SDM), Oxytetracycline (OTC), Doxycycline hyclate (DC), Chlortetracycline hydrochloride (CTC) and Tetracycline hydrochloride (TC).

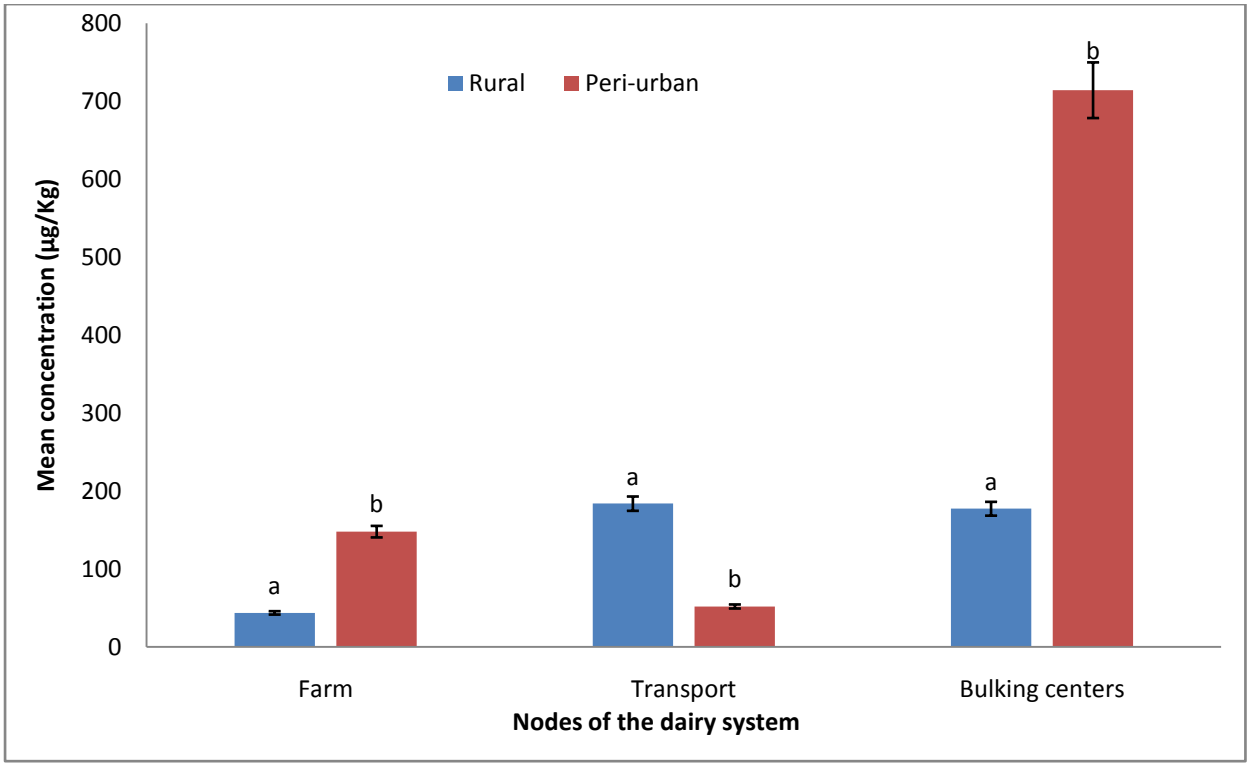

Figure 11 : Comparison of mean concentration of antibiotic residues in milk along the dairy value chains of rural and peri-urban dairy systems

Table 15: Results from method validation using spiked milk samples

Samples	SDZ	SMX	SCL	SMR	SDOX	SMTZ	SDM	SQ	OTC	DC	TC	CTC
Recovery (mean %)	144	92	-	71	71	112	56	42	99	92	70	64
SD	-	6.2	-	5.2	3.9	7.0	3.2	2.7	3.7	5.7	3.2	4.5

Sulfachloropyradizine (SCL), Sulfadiazine (SDZ), Sulfadimidine (SMTZ), Sulfaquinoxaline (SQ), Sulfamerazine (SMR), Sulfathiazole (STZ), Sulfamethoxazole (SMX), Sulfadoxin (SDOX), Sulfadimethoxin (SDM), Oxytetracycline (OTC), Doxycycline hyclate (DC), Chlortetracycline hydrochloride (CTC) and Tetracycline hydrochloride (TC).

Table 16: Calibration curve results for standards

STANDARS	Regression equation from calibration curves	Rr^2	LOD HPLC-UV	RF	SD(%)
SMR	f(x) = 662.48x + 74645.3	1.0	50	737.1	0.08
SDOX	F(x) = 376.583x + 1846.29	1.0	100	378.4	0.004
SCL-rt30	F(x) = 17.72x – 7393.03	1.0	100	10.3	0.62
SDZ	F(x) = 340.51x + 2232.01	1.0	50	342.7	0.005
SMTZ	F(x) = 321.58x + 4987.23	1.0	50	326.6	0.013
SQ	F (x) = 259.997x + 1480.45	1.0	50	258.5	0.005
SDM	F(x) = 292.90x – 8808.51	1.0	80	284.1	0.027
SMX	F(x) = 386.15x – 297.48	1.0	50	385.9	0.001
STZ-32	F(x) = 65.84x + 18977	1.0	50	84.8	0.19
OTC	F(x) = 43.30x + 48278.9	1.0	40	91.6	0.46
DC	F(x) = 55.08x + 29908.4	1.0	40	85	0.3
CTC	F(x) = 127.8x + 18909.9	1.0	40	146.7	0.11
TC	F(x) = 63.85x – 2396.01	1.0	40	61.5	0.034

Rr^2 recovery ratio , RF: retention factor, LOD:limit of detection, SD:standard deviation, Sulfachloropyradizine (SCL), Sulfadiazine (SDZ), Sulfadimidine (SMTZ), Sulfaquinoxaline (SQ), Sulfamerazine (SMR), Sulfathiazole (STZ), Sulfamethoxazole (SMX), Sulfadoxin (SDOX), Sulfadimethoxin (SDM), Oxytetracycline (OTC), Doxycycline hyclate (DC), Chlortetracycline hydrochloride (CTC) and Tetracycline hydrochloride (TC).

4.4Milk losses along the rural and peri urban value chains

Based on the KEBS standard for counts in raw milk, numbers of samples exceeding 2 x 10^6CFU/ml were recorded as probable losses using total viable counts (Table 17). Other standards were used in determining losses caused by Coliform bacteria and psychrotrophic microorganisms. Based on TVC highest losses were recorded at the transporters node for both Rural (16%) and peri-urban (30%) dairy systems. Cooling centres however, recorded the highest losses by coliforms exceeding the maximum threshold. This was observed on both dairy systems. Losses caused by psychrotrophic bacteria were recorded highest at peri-urban cooling centre (100%) and rural farms (92%) (Table20).

Table 17: Percentages of sample size exceeding maximum threshold set for TVC, CC and PBC in both dairy systems and within the nodes

Microbial type	DS	Node	Mean ± SE	Maximum threshold	Citation	Percentage exceeding threshold
TVC				**Log $_{10}$6.3 CFU/ml**	KEBS EAS 2007	
	Rural	Farm	5.09±0.3			15
		Transporters	5.72±0.3			16*
		Cooling centre	5.58±0.3			8
	Peri-Urban	Farm	4.84±0.5			20
		Transporters	5.60±0.3			30*
		Cooling centre	6.61±0.2			7
CC				**Log$_{10}$ 4.7 CFU/ml**	KEBS EAS 2007	
	Rural	Farm	3.18±0.8			34
		Transporters	3.39±0.6			48
		Cooling centre	5.19±0.7			63*
	Peri-Urban	Farm	4.33±0.5			52
		Transporters	4.78±0.5			52
		Cooling centre	6.03±0.7			88*
PBC				**Log $_{10}$5.0 CFU/ml**	European Council reg No 853/2004	
	Rural	Farm	6.04±0.2			92*
		Transporters	5.47±0.3			54
		Cooling centre	5.66±0.2			58
	Peri-Urban	Farm	4.61±0.7			58
		Transporters	5.30±0.5			82
		Cooling centre	6.91±0.2			100*

DS; Dairy system, TVC; Total viable counts, CC; Coliform counts and PBC; Psychrotrophic bacterial counts, *Highest recorded percentage losses within a value chain in a Dairy system.

Table 18: Daily raw milk losses (volume) with reference to total viable counts (TVC) in rural and peri urban dairy systems in volume

Microbial type	Dairy System	Node	N	Mean produced Daily (Litres)	Daily loss in volume (litres)
TVC	Rural	Farm	51	4	30.6
		Transporters	120	7.5	144
		Cooling centre	4	3,550	1,136
					Total: 1,310.6
	Peri- Urban	Farm	30	4.7	28.2
		Transporters	30	6.3	56.7
		Cooling centres	2	800	112
					Total: 196.9

Table 19: Daily raw milk losses (volume) with reference to coliform counts (CC) in rural and peri urban dairy systems

Microbial type	Dairy System	Node	N	Mean produced (Litres)	Daily loss in volume (litres)
Coliforms	Rural	Farm	51	4	69.4
		Transporters	120	7.5	432
		Cooling centre	4	3,550	8,946
					Total:9,447.4
	Peri- Urban	Farm	30	4.7	73.3
		Transporters	30	6.3	98.3
		Cooling centres	2	800	1,408
					Total: 1,579.6

N is the sample size

Table 20: Daily raw milk losses with reference to psychrotrophic bacterial counts (PBC) in rural and peri urban dairy systems

Microbial type	Dairy System	Node	N	Mean produced Daily (Litres)	Daily volume loss (litres)
PBC	Rural	Farm	51	4	187.7
		Transporters	120	7.5	486
		Cooling centre	4	3,550	8,236
					Total: 8,909.7
	Peri- Urban	Farm	30	4.7	81.8
		Transporters	30	6.3	155
		Cooling centres	2	800	1,600
					Total: 1,836.8

N is the sample size

Milk losses from the two value chains, gives a total of **1507.5** litres daily (Rural 1,310.6, peri urban 196.9) based on KEBS TVC standards for maximum microbial count for milk to be processed (Table 18). Losses recorded from volume of milk exceeding standards

of coliform counts were 9,447.44 in rural and 1,579.6 liters in the peri urban daily (Table 19). Physchrophilic bacteria was responsible for higher volume losses in rural (8,909.7 litres) than in peri-urban(1,836.8 litres) dairy system (Table 20).

Percentage losses throughout the value chain was calculated based on the volume of milk at value chain nodes that did not meet the standard threshold (KEBS TVC) against the total volume of milk in the value chain. This is explained below. The average percentage was then extrapolated to estimate possible losses from annual milk production in the country.

$$\%Losses_{Rural} = \frac{1310.6}{15304} \times 100$$

$$= \mathbf{8.6\%}$$

$$\%Losses_{Rural} = \frac{196.9}{1930} \times 100$$

$$= \mathbf{10.2\%}$$

Average % loss = **9.4%** daily

4.4.2 Losses due to antibiotic residues

Losses due to antibiotic residue levels are higher in rural (3,567.1 liters daily) that in peri urban (1,612.3 litres daily). Total daily losses from antibiotic residues are higher that losses due to spoilage microorganisms (1,507.5 liters) from both locations.

Table 21: Table deriving losses in volume from antibiotic residues

Rural	Mean milk per day(litres)	N	n exceeding EU MRL	% exceeding EU MRL	Volume lost (Litres)
Cow	4.5	120	2	1.7	9.2
Transporters	7.5	105	1	1	7.9
Cooling centre	3550	4	1	25	3550
Rural total					**3,567.1**
Percentage					**23.3%**
Peri urban					
Cow	6.0	51	1	1.9	5.8
Transporters	6.3	21	1	4.8	6.4
Cooling centre	800	2	2	100	1600
Peri urban total					**1,612.2**
Percentage					**83.5%**
Total volume lost					**5,179.3**
Percentage					**53.4%**

N is the sample size; n is the number of samples positive for antibiotic residues; EU MRL- European Union Maximum Residual levels.

CHAPTER FIVE

DISCUSSION

5.1 Risk factors associated with contamination of milk with spoilage microorganisms

The study established that farm practices which predisposed milk to microbial contamination included; lack of hand and udder washing, or washing without drying (Figure 3). A similar trend was observed in the peri-urban where 50% practice hand and udder drying (Table 4). Without drying of hands and udder after washing becomes a risk because the water used in washing the udder and hands will drip in the milking container, mixing with the milk. The excess water from hands and udder if not dried off caries microorganisms from hands and udder in unhygienic conditions contributing to high microbial count in milk (Hogan *et al.,* 1979; Gulton *et al.,* 1984; Islam *et al.,* 2009). It was reported that milking in a dry environment provides a significant reduction in microbial load in milk. Thus just washing hands and udder is not as effective as following the procedure with drying of the surfaces with a material like a towel (Islam *et al.,* 2009).

Udder swabs in peri urban recorded high counts in TVC compared to their rural counterparts (Table 5). Due to the small pieces of land in peri-urban compared to the rural areas, most farmers opt to practice zero grazing. Zero grazed animals stay in one place the whole day and are likely to have dirty udders due to defecation in the same spot they feed and spend the night. The proximity of the udder and the rectum of the cow cause easy cross contamination from faecal coliform and other bacteria (Islam *et al.,* 2009). With these factors, compared to the rural where free range grazing was mostly practiced due to availability of land, the hygiene of the udder was better than in peri urban.

Water treatment by either boiling or chlorination was more common in the peri urban than in the rural location. The microbial load in milk from rural areas where minimal water treatment was done, had the highest cumulative microbial counts. The microbiological quality of water used during milking, udder preparation, and equipment cleaning in the farm play an important part in microbial load of raw milk. This study showed that water hygiene is an important aspect of microbiological quality of milk. Previous studies have reported the same findings (Ingawa *et al.,* 1992; Visser *et al.,* 2007; Matofari *et al.,* 2013).

Plastic milking containers were 94% in rural and 84% in peri urban (Figure 3). Rinses from milking containers in the rural recorded the highest in total viable counts. This could be due to the highest percentage of plastic milking containers in the rural location. Plastic containers have been proven to contain micro -pores which facilitate the formation of

biofilms and are therefore difficult to clean and become sources of contamination especially for Psychrotrophic and Thermophillic bacteria (Bereda *et a.l.,* 2012; Mesfine *et al.,* 2015; Wafula *et al.,* 2016).

Bulking containers had a significant regression coefficient value in total viable counts in peri urban unlike milking containers in both locations (Table 9). Milking containers in both locations had a wide opening to reduce spillage during milking from the udder. This property also helps in reducing biofilms due to ease of cleaning. The cleaning material can easily reach all parts of the container effectively. However, the wide opening of the milking container poses a risk of contamination from the milking environment which is always contaminated with cow dung. Bulking containers are however placed away from the milking area and do not get contamination from this kind of environment. Bulking containers are characterized with small openings to reduce spillage during transportation;however, this property makes them difficult to clean since not all areas are easily reached by cleaning material. This characteristic is a risk factor since the containers become hard to clean and promote the development of biofilms (Kaindi *et al.,* 2011; Bereda *et al.,* 2012; Wafula *et al.,* 2016)

The micro-flora of milk at the farm gate is as a result of the contamination it acquires the moment it leaves the udder. Milk drawn directly from the udder had lower readings of TVC compared to the farm gate translating to an increase 8.3%in the rural which is a 0.5 log cycle. Coliform counts increased by 0.7 log cycle, Thermophiles 0.2 log cycle and Psychrophiles 0.1 log cycle (Figure 5). Hygiene in the peri-urban area was generally high compared to rural hence the high increase in microbial load in milk between the udder and the farm gate. This is because hand washing, udder washing and drying of the same was mostly practiced in the peri-urban compared to rural. Water treatment by boiling and chlorination was also practiced more in peri-urban compared to the rural counterparts. Other studies have reported lower counts in milk where proper pre milking and post milking practices were carried out targeting hands and udder (Hogan *et al.,* 1979; Gulton *et al.,* 1984; Gran *et al.,* 2002; Islam *et al.,* 2009; Odongo *et al.,* 2016)

From the regression, the highest source of contaminant was the personnel followed by udder and bulking containers (Table 9). Lack of hand drying, zero grazing and proximity of the udder to the rectum are reasons for the high correlation between hands, udder and the microbiological quality of milk. Hands and udder hygiene are majorly affected by pre-milking procedures and water quality which have shown in this study as being substandard. Mitigation measures in reducing microbial load and improving farm hygiene should target the

practices associated with personnel activities, pre and post milking practices udder washing and drying before milking and using boiled or treated water or detergents to wash hands udder and containers. The microbiological quality and safety of milk is determined by handling and hygiene practices of the farm and the milk. Hygiene milking and post milking practices will ensure a low microbial count in milk with a longer shelf life (Kornacki and Johnson, 2001; Gran *et al*, 2002; Petrovick *et al.,* 2006).

5.2 Microbiological quality of milk along the dairy value chains

Milk from the cow udder directly had counts higher than 30 CFU/ml in rural (Table 8). Contamination sources for this milk are; the udder and hands of milking personnel and also the environment (Kaindi *et. al.,* 2011), since all farmers visited never practiced simple pre milking procedures such as pre dipping. Cleaning of the udder and hands prior to milking was done by farm water whose microbiological quality was unknown. Water used in farm has been reported to be a major contaminant to milk (Ingawa et al., 1992; Visser *et, al.,* 2007; Matofari *et al.,* 2013,). Between the udder and the farm gate, TVC increased by 0.5 log cycle (rural) and 0.4 log cycle (peri urban). Contamination sources for this milk from the udder are the milking container, the sieve and the bulking container as well (Islam *et. al.,* 2009; Kaindi *et. al.,* 2011,). Containers play a significant role in milk contamination with both spoilage and pathogenic microorganisms (Lore *et al.,* 2005; Wafula *et al.,* 2016). About one third of samples collected in the peri urban had counts exceeding 10^5cfu/ml in total viable counts. This indicates that milk production at the farm is a critical control point.

Increase in total viable counts from the time the transporter picks milk from the farm gate to the time it is delivered to the collection centre was the highest for both value chains(Table 10). The time that milk takes to move from the farm gate to the cooling centre is the longest in the sub value chains. This is because the transporters not only pick milk from one farm, but they move from one farm gate to the next before proceeding to the cooling centre. The transporter may take almost thirty minutes moving from one farm to the next before he makes the journey to the cooling centre. The modes of transport used at this node vary from use of donkey, bicycle, motor cycles, vehicles and foot. This hurdle coupled with poor roads allows microorganisms to multiply faster since no cooling is applied at this node (Kaindi *et. al.,* 2011; Bareda *et. al.,* 2012). Due to poor roads which increase the chances of milk spillage during transportation, most transporters prefer to use containers with narrow openings to reduce spillage. These types of containers are majorly plastic and are also light in weight compared to their metal counterparts. However, the narrow opening of the container

makes it difficult to clean (Mesfine *et. al.,* 2015). Plastic materials have macropores which hide biofilms and act as contamination sources (Bareda *et. al.,* 2012; Mesfine *et. al.,* 2015).

The difference in total bacterial counts between the transporter and the cooling centre was not significant (Table 10). Psychrophilic bacterial counts were recorded the highest in both locations at the cooling center. The psychrophilic bacterial count standard has been set at 100,000 cfu/m (Regulation No. 853/2004) by the European (EC) parliament of the Council. High processed dairy products need the limits before milk processing due to the stability of proteins and lipids (Cempirkova, 2007). Milk is cooled to 4^{o}C in the rural and to 7^{o}C in the peri urban. These temperatures slow down the growth of mesophiles and thermophiles andreduce their metabolic rate. Psychrophilic bacteria would probably continue germinating at these temperatures producing thermoresistant extracellular proteolytic and lipolytic enzymes causing qualitative risk at processing and spoilage of final product (Herrera, 2001; Burdova *et. al,* 2002; Chan *et. al.,* 2003; Arslan *et.al.,* 2011). These enzymes are capable of spoiling milk products such as UHT, cheese, ghee, butter, skim milk powder among others (Bhunia, 2008; Ray 2004; Arslan, *et. al.,* 2011). Different species of Pseudomonas (Psychrophillic) species have been isolated from dairy products including, *Pseudomonas fragi, Pseudomonas pseudoalcaligenes, Pseudomonas aeruginosa, Pseudomonas flurescens* (Arslan *et. al.,* 2011) This is because they are heat stable and are not destroyed by heat treatments such as pasteurization and ultra-high temperature heat treatment.

For both locations it was observed that gram negative bacteria increased at the transporters node (Figure 6).*E. coli* and other *enterobacteriacea* might have entered milk through faecal sources like udder which is in close proximity with the cow's anus and is likely to be contaminated with coliforms and related microbes (Islam *et al.,* 2009; Kumar *et. al.,* 2012). Other isolated bacteria in milk with faecal source were *Streptococcus fecalis*(Table 11 and Table 12). Initially these counts were lower in milk drawn directly from the udder and at the farm gate. At the transport node there was more time for proliferation and lack of cooling facilitating their growth. Milk leaving the animal is approximately 37^{o}C but arrives at the collection centre at 34^{o}C-29^{o}C; these temperatures range are still suitable for the growth and proliferation of coliforms and most spoilage microorganisms.

Gram positive cocci was falling steadily along the value chain in peri urban (Figure 4). Microorganisms such as *Staphylococcus* contaminated the milk most at the farm level but as they moved through the value chain, the gram negative coliform, *E .coli* and other *Enterobacteriaceae* competed favourably for the substrates hence affecting the rate of growth for the gram positive cocci. Gram negative bacteria were represented by *E.coli and*

enterobacteriaceae for this study. The sum of the two groups was significantly different between the milk drawn directly from the udder and at the transporters node. Coliforms are used as indicator organisms for hygiene practice. They are capable of breaking down lactose to lactic acid and Carbon II oxide (Kornacki and Johnson, 2001). This property explains the increase in acidity and fall in pH at this node (Figure 7). At the cooling centre the rate of growth of the gram negative cocci fell due to the fall in temperature. However, the mean count for TVC was high due to the growth of Psychrotrophic bacteria.

5.3Levels of Sulphonamides and Tetracyclines in Rural and peri urban dairy systems

Charm Blue-Yellow kit tests for the presence of a wide spectrum of antibiotics including betalactams, sulphonamides, tetracyclines and most of the antibiotics used in animal husbandry. The lack of detection of sulphonamides or tetracyclines at HPLC in some of the samples would indicate the presence of other antibiotics as well in these samples. The charm Blue-yellow II kit eliminates the possibility of analysing samples with inherent (natural antibiotics) through the second stage of screening. In this stage samples were exposed to heat (80^{o}C at 10 minutes) treatment to breakdown natural inhibitors. This temperature time combination also emulates high temperature short time pasteurisation of milk. At this temperature antibiotic (Synthetic) residues are not broken down (Mullan, 2003; Kellnerov *et al.,* 2015; Layada *et al.,* 2016).

Presence of antibiotic residues in milk sampled from individual farms indicates that farmers are not observing withdrawal periods in lactating animals. A study done in Kosovo (Sulejman *et al.,* 2012), sulphonamide residue levels were compared based on time and delivery level. It showed that during the first days of delivery (1-4 days), sulphonamide levels remain high up to mid time after which the drug levels reduce significantly to incalculable levels towards the last days (day 5). This is an indication that since sulphonamides were detected in farm milk, the farmer milked the cow within five days of drug administration (Table 14). Some farmers have attributed lack of observing withdrawal periods to harsh economic times (Shitandi and Sternesjo, 2004). During treatment, the farmer however has to milk the cow to facilitate letdown but is expected to throw away the milk. Most farmers tend to find this practice difficult since the physical appearance of the milk is similar to that from a cow that is not undergoing any form of treatment. A part from lack of withdrawal, animal feed can be contaminated with antibiotics through faeces or poor disposal of treatment kits containing antibiotics (Aboge *et al.,* 2000; Kang'ethe *et al.,* 2005)

The number of positive farm samples in the rural was slightly higher than positive samples in the rural dairy system (Table 13). This shows that consumers in rural setting are more likely to consume milk contaminated with antibiotic residues than those in peri urban. This would be possible if these consumers buy milk directly from the farmers, which is a common practice in the rural area. These findings are similar to those of Aboge *et al.*,(2000) and Kange'the *et al.*,(2005) they reported that rural farmers are three times more likely to consume milk contaminated with antibiotic residues compared to their counterparts in the peri urban farms.

Antibiotic residues in transporters milk, shows that the antibiotics may have been intentionally added to milk to extend their shelf life (Table 14). Transporters collect milk from farms and deliver them to the next value chain node. These include cooling centres which are collection points for dairy processing factories. Transporters face a challenge of milk spoilage since they transport the milk without any cooling facilities. Milk at this node is at a high risk of spoilage due to time taken moving from one farm to the other before reporting to the cooling centre. Most transporters however, have been reported to add antibiotics to milk to prevent milk spoilage (Aboge *et al.,* 2000). Occurrence of antibiotic residues at the transporters node in peri urban is four times higher than in rural location. Consumers of dairy products and milk in the peri urban are more likely to consume milk with added antibiotics to extend shelf life. The number of positive samples for antibiotic contaminants washighest at rural farms (Table 14) while the concentration at this node was significantly lower than at bulking centres in peri urban (Figure 9).

The percentage positive samples from screening results in this study was 30%, these results are slightly higher than those identified in the recent studies in Kenya. In 2004 Shitandi and sternesjo recorded 14%, Ekutta *et al.,* (2007) 4% and Kang'ethe *et al.,* (2005) 14%. The proportion of antibiotic groups in milk samples differs sparingly in this study compared to other studies done in Kenya. These results show that sulphonamide occurred at 4.1% while tetracycline was at 0%. A study by Ahlberg *et al.,* (2016) recorded sulphonamides at 0.4% and tetracyclines at 2.5%. Mitema *et al.,* (2001) recorded sulphonamides at 24% and tetracyclines at 61%.

Different parts of Africa have reported different results. In Tanzania over 36% of milk supply chain was reported to contain antibiotic residues (Kurwijila *et al.,* 2006). Other studies in Africa have also recorded the presence of antibiotic residues in milk like in Egypt (Goudah *et al.,* 2007), Ghana-35% (Addo *et a.,* 2011), South Africa 15% (Bester and Lombard, 1979), Ethiopia 36% (Myllniemi *et al.,* 2000), Sudan 23% (El-tayeb *et al.,* 2012)

and Nigeria 44% (Olufemi and Agboola, 2009). In other nations of the world antibiotic residues have been reported in raw milk. In 2007Kress *et al.*,reported 1.6% of samples containing sulphonamides in Germany. These are slightly lower than sulphonamides identified in raw milk in Netherlands at 16% by Abjean (2000). Sulphonamides in milk have also been reported in Mexico at 51.3% (Tolentino *et al.*, 2005), Turkey 12%(Alkan, 2007) and Korea 23%(Chung *et al.*,2009). In the most recent studies, sulphonamides, tetracyclines and other antibiotic residues in milk have been recorded at levels above EU MRL at different value chain nodes (Olatoye *et al.*, 2016; Chowdhury *et al.*, 2016; Layada *et al.*, 2016).

All sulphonamides detected read values above the EU MRL value except sulfadiazine and sulfadimethoxine which were below 100µg/kg (Table 14). The rest of the samples were detected above the Limit of detection values of each antibiotic residue. This indicates that even higher levels than the read value might have been present. Sulfadiazine is a common antibiotic used in the veterinary practice in several countries and was recorded at the bulking centre in the peri urban dairy system only.

Mean concentration of antibiotic residues along the value chain nodes varied significantly ($P<0.05$) (Figure 9). The mean concentration of Sulphonamides at the farm was higher than the recommended EU MRL value while at the transporters node the level falls below 100ug/kg. It is possible that farmers contribute the highest concentration than transporters in the peri urban dairy system. Since high concentrations are found in milk during the first four days of treatment, it is possible that the farmers milk cows without completely observing even a day of withdrawal (Sulejman *et al.*, 2012). Being that the percentage of transporters who tested positive for antibiotics is high, there is a possibility of a dilution factor or addition of antibiotics at lower levels compared to farm levels. There is however a significant increase at the cooling centres in antibiotic concentration. This may be due to the fact that cooling centres in the peri urban are adding sulphur based antibiotic residues in the milk or there might be a cumulative effect of antibiotics added to milk through the previous nodes since this milk does not leave the value chain.

In the rural setting the scenario in mean concentration of antibiotics along the value chain is quite different. Sulphonamide concentrations at the farms are lower than concentrations at transporters node. These concentrations also fall below the EU MRL values (Figure 9). Since the level of antibiotics shed in milk during animal treatment starts to fall on the fifth day of treatment, the results could mean that the farmers withdraw from the animal for the first 1-4 days of treatment. Milking for sale and distribution into the value chain however begins between the fourth and fifth day. This would explain the presence of

antibiotics in milk but at a lower level at the farm compared to the transporters node. Transporters node had a higher concentration of antibiotics residues. This could be due to the fact that these transporters intentionally add antibiotics in raw milk to extend its shelf life before delivery to the cooling centre. Between the bulking centre and transporters node in rural dairy system there is a non-significant fall in antibiotic residue concentration. This would mean that cooling centres in the rural area do not add antibiotics in milk.

The positive samples from Charm Blue-Yellow II test does not differentiate whether the result is due to antibiotic inhibitor or other growth inhibitors. Growth inhibitors used in the treatment of worm infections such as anthelmintics are possible sources of error for the Charm II Blue- Yellow Kit. According to this study, The Charm II Blue-Yellow was 97.1% efficient in distinguishing between positive and negative samples since only 9 out of 309 samples were not clearly differentiated. These were labelled as caution samples and preceded to HPLC UV for confirmation. None of the caution samples however, recorded the presence of sulphonamides or tetracyclines under investigation. There is a high possibility that some sulphonamides were not detected due to presence of impurities in the sample. Sulphonamides are detected at a lower UV range of 268nm where many impurities of biological origin can interfere with the analysis.

When milk is stored at ambient temperature, antibiotics degrade (Marth and Steele, 2001). When milk is slightly spoiled, the beta lactamase enzyme is produced and this would breakdown beta lactam antibiotics. The same is likely to occur to other antibiotics (Guay*et al.,* 1997).

5.4 Raw milk losses along the dairy sub value chains

Kenya Bureau of Standards (KS EAS, 163: 2007) has graded milk in three classes based on TVC (Table 2). Grade one milk has counts less than 200,000 CFU/ml, grade two has counts falling between the range of 200,000 – 10^6cfu/ml, while grade III milk has counts ranging between 10^6-2 ×10^6 CFU/ml. Milk with counts beyond 2× 10^6 CFU/ml was used as a benchmark for losses determination because beyond this point the milk spoilage effects would be visible to the eyes with curd formation and strong off flavours, this type of raw milk would have unstable proteins and would not withstand pasteurization. Losses were recorded to be highest at the transporters node (16% rural and 30% peri urban). Farms recorded 15% loss in rural and 20% in peri urban.

This report is quite different from the one done in 2005 by Lore, *et al.,* (2005) where loss hot spot was identified to be the farm. The farm losses were estimated to be 4.5% of total

milk produced at the farm with the main causes being spillage and spoilage. The study however did not include transporters as a node. This is because milk which did not meet standards at the market were returned back to the farmer. FAO 2011 reported the loss to be higher than 6% and also maximum at the farm. These losses have been attributed to, poor post-harvest handling hygiene of milk, long distance to the market, use of substandard plastic containers (Lore *et. al.,* 2005; FAO, 2011). Poor hygiene has been incriminated to be the main contributor in dairy losses as by spoilage microorganisms by this study. Other recent studies have reported the same (Odongo *et. al.,* 2016).

High percentage losses at the transporters node are also due to the time taken for the milk to be transported between the farm and the cooling centre. Most transporters Start picking milk from farms during milking time. However, the transporter will visit other farms as well before transporting milk to the cooling centre. This practice increases time at which milk is out of the cold chain. Many sources of milk contamination occur at the farm (Orwa *et al.,* 2017). These include, the udder, hands of milking personnel, milking container and bulking container. The factors which contribute to milk spoilage at the transporters node are the equipment used to transport milk and time taken to reach the cooling centre (Bonfoh *et al.,* 2003). Therefore, microorganisms which contaminated milk at the farm have a lot of time to multiply. If time taken between farm gates and cooling centre is shortened then these losses would be minimised.

The latest study in dairy has recorded 10% losses in Kenya (FAO, 2014) with an annual milk production of 5billion. When these losses are compared by those of 2005 (Lore *et al.,* 2005) which were 54million litres annually, there has been a ten times increase in about 10 years. This study however has given a percentage loss of 9.4%. If the study were to determine losses at the processors and consumers level, then the losses would be higher. This result shows that there is possibility of even higher losses in the present Kenyan dairy value chain.

Between the farm gate and cooling centre, losses due to coliform counts are increasing. Losses at the cooling centre were 1,947.4 litres and 1,579.6 litres daily (Table 19). There is a cumulative effect of coliforms as milk moves along the value chain hence high losses are observed towards the end of the value chain. Psychrophilic bacterial counts accounted for high losses (8,909.7 litres daily) in rural farms (Table 20). This is due to the low temperature (upto less than 10^{o}C) at the time of milking in the rural dairy system. Poor hygiene also facilitates contamination of milk and multiplication of these microorganisms in milk.

The farm has the highest number of sources of milk contamination with spoilage microorganisms and practices contributing to milk spoilage (Figure 3, Table 4). Milk leaving the farm in rural location recorded mean counts of 5.09 ±0.26 SE in TVC (Table 10). Regression of this value with contamination sources showed that hands and udder are the highest contributors to losses at this node (Table 7). A cross tabulation of farm practices also reveal that lack of water treatment (89%) is a major contributor to milk spoilage (table 4). This is because the same water is used in cleaning hands and udder. If drying is not done before milking then the risk of contamination of milk with spoilage microorganisms from water, hands and udder increases. Farmers in the rural areas use plastic containers for milking (94%) and bulking (84%) compared to their counterparts in peri urban (Table 4). The practices and low microbial quality of possible sources of milk contamination in both dairy farms are identified as risk factors to milk contamination with spoilage microorganisms. These have led to on farm milk losses of 30.6 litres daily in the rural farms and 28.2 litres in the peri urban.

Losses due to antibiotic residues are higher in volume compared to losses due spoilage microorganisms (Table 18 and Table 19). This indicates that misuse of drugs by value chain actors is a common practice in both locations. The risk of consuming milk with antibiotic residues in therefore higher than the risk of purchasing milk with high microbial count.High levels of antibiotic residues may be responsible to lower losses from spoilage microorganisms. Antibiotic residues have the ability to inhibit growth of microorganism (Nisha, 2008) especially at levels recorded by this study (Figure 9).

Milk lost to antibiotic residues exceeding the set standards (EU MRL) is higher (23.2%) in rural dairy value chain compared to peri urban dairy value chain (83.3%). This is due to lack of information on effect of antibiotics among value chain actors or access to high quality veterinary services. Rural locations in Kenya are characterised by poor infrastructure and this may contribute to lack of information in these dairy systems (Lore *et al*.,2005) . Regulation bodies such as Kenya Dairy Board (KDB) have most of their agents in peri urban than in rural locations.

Mean concentrations of antibiotic residues are relatively high inperi urban location compared to rural dairy value chain (Figure 9). This indicates that as much as misuse of antibiotics is not rampant in the peri urban location, those involved in negligence in handling antibiotic residues allow high levels of antibiotics to persist in the milk. Farmers for example are suspected to not observe withdrawal period while transporters may be adding high levels of antimicrobials to milk. Hence, the peri urban population is likely to develop health

complications associated with consumption of milk with high levels of antibiotic residues. The maximum residual limit of sulphonamides in milk is set at 100 parts per billion by European Union. The mean concentration for instance, in milk at the cooling centre is 714.19 ppb in the peri urban; this figure is 7times high above the set limit.

This study focused on two main antibiotic drugs commonly use in animal husbandry in Kenya. Tetracyclines were not detected in any sample while sulphonamides being present were used as a benchmark to derive these losses. It is suspected that other antibiotics may be present in the milk apart from sulphonamides alone. In this case, losses from antibiotic residues are expected to be higher than what this study has reported.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. Udder swabs were the highest source of contamination of milk (r =2.73) in the peri urban. In the rural dairy system, hands of milking personnel recorded the highest for TVC (log10 3.7 CFU/ml). It is evident that effective udder cleaning and observation of high personal hygiene by the hand milker's may reduce the risk of microbial contamination of milk with spoilage microorganisms in both systems.
2. High counts were recorded along all value chain nodes (up to 10^ 7 CFU/ML). Hygiene needs to be high from the farms, transporters to the cooling centers. Cooling points should be introduced along the value chain while use of food grade milk handling equipment should be introduced and implemented.
3. Presence of antibiotics in the farm is more common in the rural farms that the peri urban farms. The level of antibiotics in the peri-urban increased through the transporters to the collection center. Lack of observation of withdrawal period might be a common practice given the high level of antibiotics in the farm milk. Addition of antibiotics for shelf life extension may be practiced more in the peri-urban by milk transporters.
4. Milk losses due to spoilage microorganisms in the rural were 9.4% while in the peri urban it was 10.2%. Losses due to antibiotic residues were 23% in rural and 83.5% in peri urban. Losses due to spoilage microorganisms are due to poor hygiene during milking in both dairy systems. Antibiotic dairy losses are as a result to lack of withdrawal period, self administration by farmers and intentional addition by transporters as a milk preservative.

RECOMMENDATIONS

1. Control of milk losses should target the practices identified to expose milk to contamination with spoilage microorganisms in the study
2. Increase in microbial loads along the value chains can be reduced by introducing cooling points and reducing the time between the farm gate and cooling centres.
3. Value chain actors should be trained on the effects of antibiotics in milk and how to minimise its presence in the milk.
4. Proper hygiene, education of milk handlers and quality veterinary services should be emphasised to milk handlers to reduce these types of losses.

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APPENDICES

Appendix 1: SURVEY QUESTIONNAIRE

FORM NUMBER……

Consent statement

Dear Respondent,

I am an Egerton university student pursuing a Msc in Food Science. I am currently carrying out a study to evaluate milk Quality in relation to milking practices as part of the programme. As a stakeholder in this value chain, I kindly request your participation to provide vital information that would aid the study.

If you accept to volunteer to participate, please sign below

Consent granted…….

Starting time……. End time……..

Please answer the following questions (will be translated in Swahili where necessary).

SECTION A: BIO DATA (of household head (HHD)

a. Sex

   Male { } Female { }

b. Level of education

   Informal { } Primary { } Secondary { } Post secondary { }

Size of household.....

SECTION 1: FARM HYGIENE PRACTICES (pre-milking practices)

1. Who does the milking?

   Family member { } Hired personnel { }

2. Does the milking person have any formal training?

   Yes { } No { }

3. Are there medical checkups for milking personnel?

   Yes { } No { }

4. If yes what is the interval of these checkups?

   Yes { } No { }

5. Do you have a milking shade?

   Yes { } No { }

6. How do you wash your hands before milking?

.....

7. Do you dry your hands after washing

   Yes { } No { }

8. If Yes what do you use to dry your hands

   Towel { } Cloth { } Paper towel { }

9. Do you wear gloves

   Yes { } No { }

10. Do you wash the teat and the udder before milking?

    Yes { } No { }

11. If yes what do you use

    ---

12. Do you dry / wipe the udder before milking?

    Yes { } No{ }

13. If yes what do you use?

    Cloth {} Towels { } Paper tissue { }

14. Do you use one drying material for all the animals?

    Yes { } No { }

15. Do you practice fore-stripping?

    Yes { } No { }

16. Do you do pre-dipping?

    Yes { } No { }

17. If yes, what chemical and at what concentration do you use?

SECTION II: POST HARVESTING MILK HYGIENE PRACTICES

1. Do you practice post dipping?

   Yes { } No{ }

2. If yes, what do you use

   .....

3. Where do you store you milk for transportation?

Metal can { } Plastic Container { }

4. Where do you majorly sell your milk?

Directly to consumers { } To brokers { } To collection centres { }

Directly to the processing factory { }

5. How is your milk transported to its point of sale?

On foot { } Bicycle { } Motorcycle { } Motor vehicle { }

6. When you milk in the evening how do you preserve the milk?

Cooling { } Boiling { } None { } Others Specify.....

7. How do you use the evening milk?

Sell immediately { } Keep for sale next morning { } Ferment for home use { }

Other home use (Specify).....

SECTION III : WASTE DISPOSAL

1. Do you have a toilet

Yes { } No { }

2. Where do you dump your kitchen and other household wastes

Dug pit { } Open ground { }

3. What do you do with accumulated waste

Burn { } Use as manure { } Throw away { } Feed cattle { }

SECTION IV:VETERINARY SERVICES

1. In the last year what how many times did your animal (s) become sick?

   Yes { } No{ }

2. Do you know what the animal was suffering from?

   Yes { } No { }

3. If yes specify the disease.

   .....

4. Who treats you animals when they get sick?

   Veterinary doctor { } Prescriptions at the Agro vet { } Self knowledge { }

5. If self then which medicine do you use
   (Record the commercial name and the components if availed)

.....
.....
.....

6. Do you observe withdrawal periods?
   Yes { } No { }
7. How many days do you withdraw you lactating animal after treatment?
   ---
8. During withdrawal period, what do you use the milk for?
   Pour away { } Feed dogs { } Fermentation { }
   Others specify { }
9. How many times does your cow suffer from mastitis in one lactating cycle?
   ---
10. Do you keep records for treatment of animals in the farm?
    ---
11. Are you aware that medicine used to treat animals can be shed in milk.
    Yes { } No { }
12. If yes where did you get the information from
    Cooperative society { } Extension officers { } Media { } Friends { }

Appendix 2: OBSERVATION CHECKLIST

Observe animal housing if present, cleanliness of the cow shed, cow's udder, milking container, type of container, cleaning procedure, milking practices and report appropriately.

…….......

……..

……..

…….

……

……

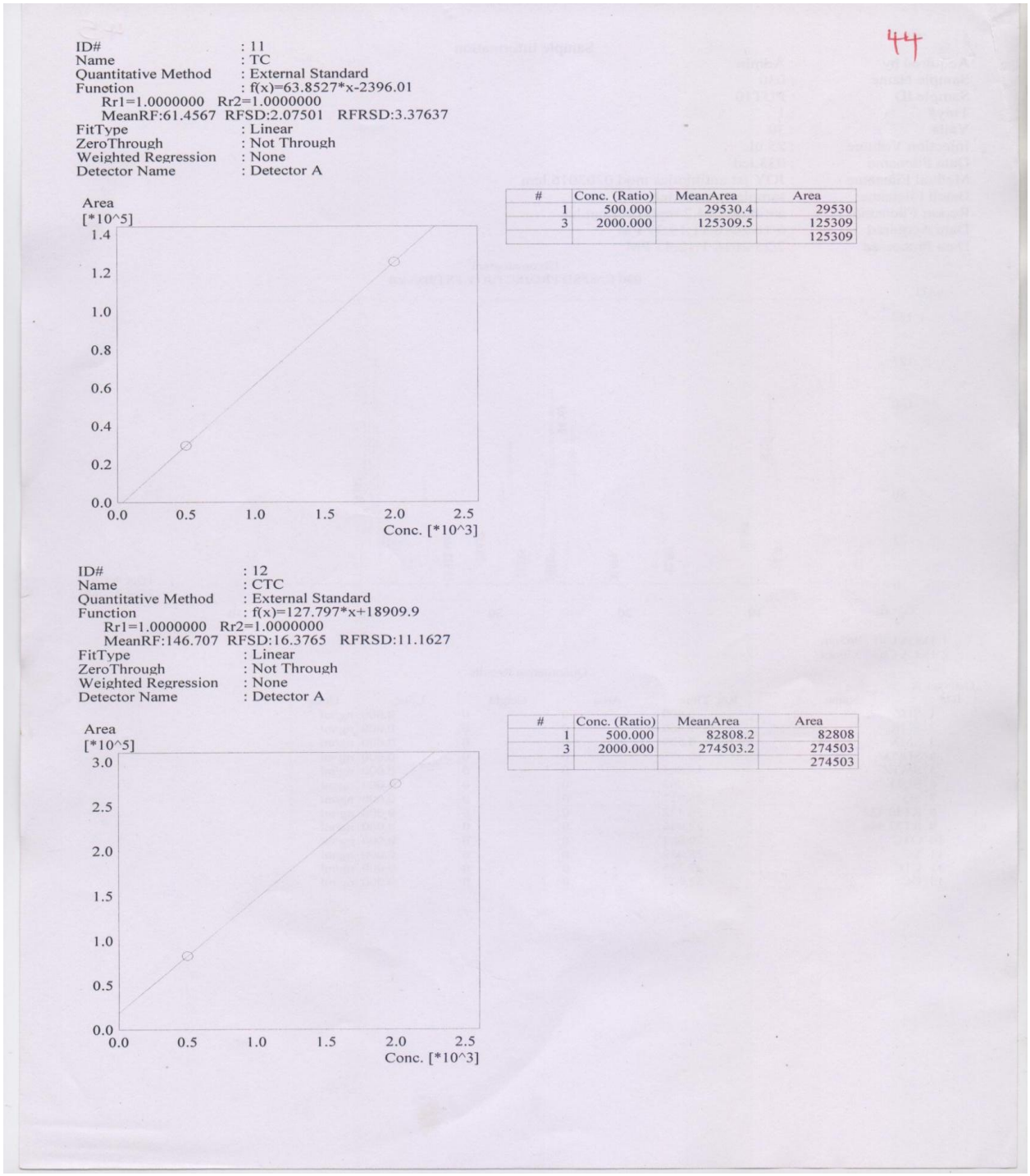
……

…….

……..

……..

Appendix 3: HPLC –UV Graphs generated from calibration and quantification



ID# : 11
Name : TC
Quantitative Method : External Standard
Function : f(x)=63.8527*x-2396.01
Rr1=1.0000000 Rr2=1.0000000
MeanRF:61.4567 RFSD:2.07501 RFRSD:3.37637
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : None
Detector Name : Detector A

#	Conc. (Ratio)	MeanArea	Area
1	500.000	29530.4	29530
3	2000.000	125309.5	125309
			125309

ID# : 12
Name : CTC
Quantitative Method : External Standard
Function : f(x)=127.797*x+18909.9
Rr1=1.0000000 Rr2=1.0000000
MeanRF:146.707 RFSD:16.3765 RFRSD:11.1627
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : None
Detector Name : Detector A

#	Conc. (Ratio)	MeanArea	Area
1	500.000	82808.2	82808
3	2000.000	274503.2	274503
			274503

Sample of a Calibration Graph

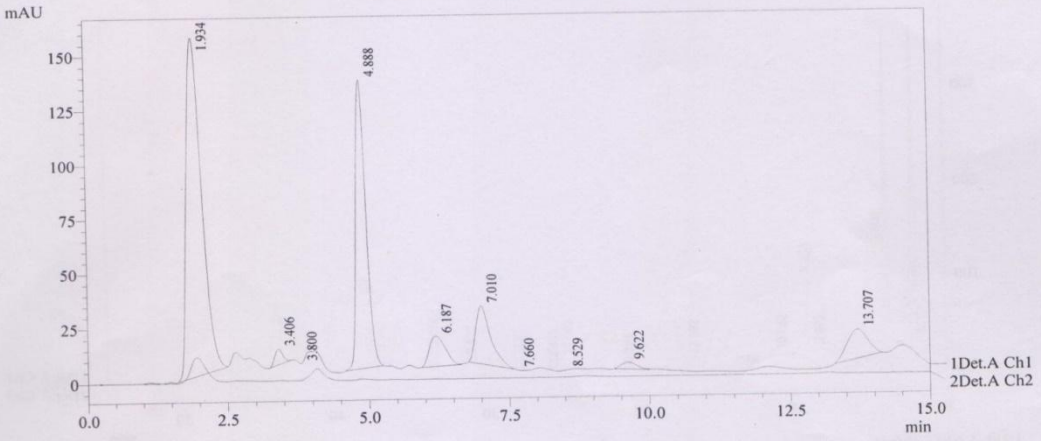
3

Sample Information

Acquired by	: Admin
Sample Name	: 001
Sample ID	: PCC1
Tray#	: 1
Vail#	: 1
Injection Volume	: 25 uL
Data Filename	: 001.lcd
Method Filename	: JOY fst antibiotics mod 0702016.lcm
Batch Filename	: samples procsd.lcb
Report Filename	: antibiotic 14.2 report format.lcr
Date Acquired	: 6/14/2016 12:45:12 PM
Data Processed	: 7/25/2016 1:18:12 PM

Chromatogram

001 C:\SFSD PROJECT\JOY FST\001.lcd



1 Det.A Ch1 / 265nm
2 Det.A Ch2 / 370nm

Quantitative Results

Detector A

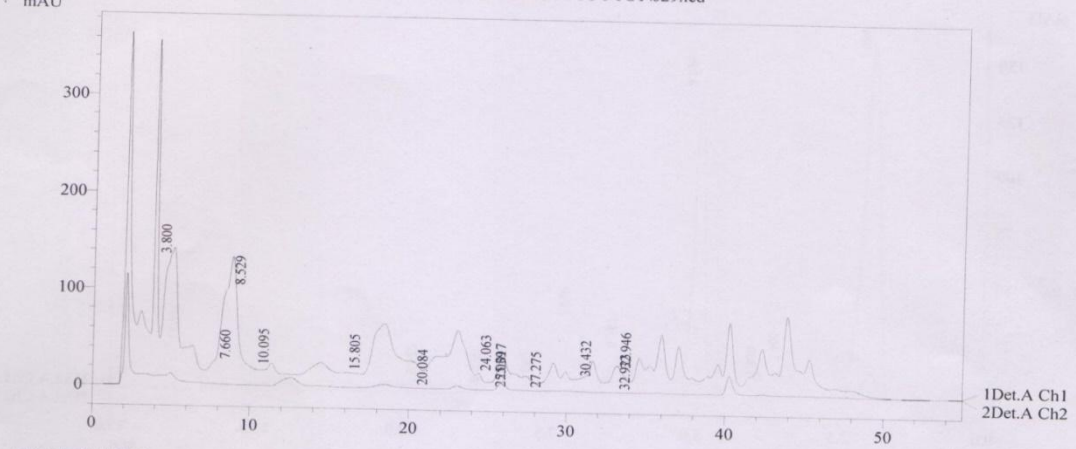
ID#	Name	Ret. Time	Area	Height	Conc.	Units
1	SDZ	3.800	0	0	0.000	ng/ml
2	SMR	7.660	0	0	0.000	ng/ml
3	SMX	8.529	0	0	0.000	ng/ml
4	SDOX	9.622	56577	3014	148.780	ng/ml
5	SMTZ	15.805	0	0	0.000	ng/ml
6	SDM	24.063	0	0	0.000	ng/ml
7	SQ	25.017	0	0	0.000	ng/ml
8	RT30.432	30.432	0	0	0.000	ng/ml
9	RT32.946	32.946	0	0	0.000	ng/ml
10	OTC	20.084	0	0	0.000	ng/ml
11	TC	25.039	0	0	0.000	ng/ml
12	CTC	27.275	0	0	0.000	ng/ml
13	DC	32.923	0	0	0.000	ng/ml

Graph of a positive sample for SDOX

Sample Information

Acquired by	: Admin
Sample Name	: 026
Sample ID	: DUF13
Tray#	: 1
Vail#	: 26
Injection Volume	: 25 uL
Data Filename	: 029.lcd
Method Filename	: JOY fst antibiotics mod 0702016.lcm
Batch Filename	: samples procsd.lcb
Report Filename	: antibiotic 14.2 report format.lcr
Date Acquired	: 6/16/2016 7:37:25 PM
Data Processed	: 7/25/2016 1:12:59 PM

Chromatogram
026 C:\SFSD PROJECT\JOY FST\029.lcd



1 Det.A Ch1 / 265nm
2 Det.A Ch2 / 370nm

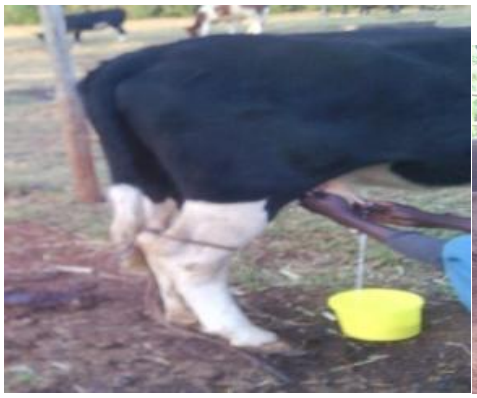
Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Height	Conc.	Units
1	SDZ	3.800	0	0	0.000	ng/ml
2	SMR	7.660	0	0	0.000	ng/ml
3	SMX	8.529	0	0	0.000	ng/ml
4	SDOX	10.095	0	0	0.000	ng/ml
5	SMTZ	15.805	0	0	0.000	ng/ml
6	SDM	24.063	0	0	0.000	ng/ml
7	SQ	25.017	0	0	0.000	ng/ml
8	RT30.432	30.432	0	0	0.000	ng/ml
9	RT32.946	32.946	0	0	0.000	ng/ml
10	OTC	20.084	0	0	0.000	ng/ml
11	TC	25.039	0	0	0.000	ng/ml
12	CTC	27.275	0	0	0.000	ng/ml
13	DC	32.923	0	0	0.000	ng/ml

Graph of a negative sample for tetracycline and sulphonamide

Appendix 4: Pictures from the dairy value chain nodes



Rural farm

Peri-urban Farm

Transporters

Collection centers

Appendix 5: PUBLICATIONS

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International Journal of LivestockProduction

Handling practices and microbial contamination Sources of raw milk in rural and peri urban small holder Farms in Nakuru County, Kenya

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ABSTRACT

The cow, the milking and milk handling procedures at the farm level expose the milk to potential risk of contamination with spoilage microorganisms. Milk contamination if not prevented will lead to milk losses along the dairy value chain. The objective of this study was therefore to identify the risk factors associated with contamination of milk with spoilage microorganisms at the farms in rural and peri urban in Nakuru County Kenya. A survey was conducted using a pre-tested semi structured questionnaires (250) and an observation checklist to identify the risk factors. A total of 560 samples obtained from the following identified contamination sources; the udder, hands, milking and bulking containers and water sources were analyzed for total viable counts (TVC), Coliform counts (CC), thermophillic bacteria counts (ThBC) and psychrophilic bacteria counts (PBC). The results from the survey showed that only 11% of rural farmers practiced hand and udder drying compared to 50% in peri-urban. Water treatment by either chlorination or boiling was done by 11% in rural and 30% in peri-urban respectively. Regression of risk factors versus farm gate milk from viable colony counts, showed that udder swabs were the highest source of contamination of milk (r =2.73). In the rural, hands of milking personnel recorded the highest for TVC (log10 3.7 CFU/ml). It is evident from the results that effective udder cleaning and observation of high personal hygiene by the hand milker's may reduce the risk of microbial contamination in both systems of milk production.

Key words: Risks, handling practices, contamination, rural, peri urban.

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DOI 10.1186/s40550-017-0050-1

Assessment of sulphonamides and tetracyclines antibiotic residue contaminants in rural and peri urban dairy value chains in Kenya

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Abstract

Background: Antibiotic residues are drug substances found in food from plants or animals initially exposed to antibiotics. In animal husbandry antibiotics have widely been used for the treatment of animal diseases. These residues have the ability to expose the public to serious health hazards. In Kenya drug residues have not only been related to lack of withdrawal periods but also to intentional addition to extend milk's shelf life.

Results: The aim of this study was to investigate the occurrence of 13 veterinary drugs of tetracyclines and sulphonamides along the dairy sub value chain. The study was carried out in Nakuru County which is the leading milk producer in the country. A total of 229 samples were analysed from rural and 80 samples from peri-urban. These were collected from different nodes of the value chain; the farm, milk transporters and at the bulking centers between January 2014 and November 2015. Screening of samples was done by Charm II Blue -Yellow-test while confirmation was done by HPLC-UV for sulfachloropyradizine (SCL), sulfadiazine (SDZ), sulfadimidine (SMTZ), sulfaquinoxaline (SQ), sulfamerazine (SMR), sulfathiazole (STZ), sulfamethoxazole (SMX), sulfadoxin (SDOX), sulfadimethoxin (SDM), oxytetracycline (OTC), doxycycline hyclate (DC), chlortetracycline hydrochloride (CTC) and tetracycline hydrochloride (TC). In the rural 72 out of 229 (31.4%) samples were positive after screening while none of the samples confirmed the presence of tetracyclines after analysis with HPLC-UV. Sulphonamides confirmed after analysis with HPLC-UV were all above the EU MRL limits. In the peri urban 28.8% (23/80) of the samples were positive for antibiotic residues. Tetracyclines were not detected in confirmation while 60% of the positive samples were positive for sulphonamides out of which 71% were above the regulatory limits. Highest percentage of antibiotics was detected in rural farms (46.7%) and at peri urban bulking centers (50%).

Conclusion: The study concluded that antibiotic residues along the dairy value chain are majorly from the farm due to lack of withdrawal periods followed by intentional addition along the value chain. Value chain actors should also be trained on ways of avoiding antibiotic residues from entering the dairy value chain to protect the public from health effects related to antibiotic residues.

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Full Length Research Paper

Microbiological quality of raw milk along the rural and peri urban dairy systems of Nakuru county- Kenya

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Abstract

The study aimed at profiling the microbiological quality of raw milk from the udder to the cooling centres in rural and peri urban. Samples were collected directly from the udder, at the farm gate, from transporters delivering to cooling centres and from the bulking centres. A total of 461 raw milk samples were collected. Microbiological analysis were done following standard procedures of ISO and American Public Health Association, these included Total Viable Count (TVC), Coliform Counts (CC), Thermophilic bacterial counts (ThBC) and Psychrophillic bacteria counts (PBC). Indicator microorganisms enumerated were *Streptococcus*, *Staphylococcus, Enterobacteriaceae E. coli* and *Bacillusspp*. For both nodes the collection centers recorded the highest in TVC(Rural 10^5cfu/ml, Peri urban 10^6cfu/ml) with transporters at both nodes recording the highest percentage for gram negative rods (rural 63.3%, peri urban 62.5%). ThBC was significantly different at the farm and bulking centre in both dairy systems. PBC recorded highest counts at cooling centres in both dairy systems. Given the high counts recorded at all nodes (up to 10^7 CFU/ml), hygiene need to be high from milk production (farms) throughout the value chain. Cooling points along the value chains need to be introduced and use of food grade equipment to handle and transport milk would help in reducing microbial load in raw milk.

Keywords: Microbiological quality, raw milk, value chain, rural, peri-urban.