COLIFORM CONTAMINATION OF MILK IN SMALLHOLDER AND PASTORAL DAIRY VALUE CHAINS IN KENYA AND ITS IMPLICATION ON POST-HARVEST LOSSES AND CONSUMER SAFETY

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

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Recommendation

This thesis is has been submitted with our approval as University supervisors.

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DEDICATION

This thesis is dedicated to my parents, Mr. and Mrs. Moses Nato

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ABSTRACT

Coliforms are a marker of hygiene and indicators for pathogenic microbes in milk. Some strains are pathogenic and cause human and animal infections. Coliforms also contribute to milk spoilage by hydrolysing lactose. Their occurrence is mostly due to practices in handling milk along the value chain. The aim of this study was to assess the risk factors for occurrence of coliforms in milk along the dairy value chain in Kenya, their biochemical profiles, phylogenetic distribution, pathogenicity, and antibiotic sensitivity. Nakuru and Isiolo Counties represented Smallholder cow milk value chain and Pastoral camel milk value chain respectively. A cross sectional study was designed for data collection, and data was collected by observation, interviews, and focussed group discussions. In addition, laboratory analyses of milk samples, udder swabs, hand swabs, and water was also done. Quantitative data was analysed using SAS 9.4; and a phylogenetic tree was generated using MEGA version 7. The proportion of milk not meeting quality standards based on coliform counts was 50% for Smallholder rural, 66% for Smallholder peri-urban, and 40% for Pastoral value chains. Risk to high coliform counts was lack of training of milking persons on milk hygiene, use of unhygienic plastic containers and the length of time taken to deliver milk, at high ambient temperature, to secondary collection centres. Additionally, excessive human handling of milk at the pastoral secondary collection centres, and transporting the milk at ambient temperature for a long time was a risk to increased coliform counts for milk delivered to Nairobi market. The dominant coliforms constituting 58% of enterobacteriaceae were *E. coli*, *E. cloacae*, *K. pneumoniae*, and *K. oxytoca*. All *E. coli* isolates were positive for indole which is important in bacterial toxicity, drug defence, and biofilm adhesion. None of the isolates was positive for inositol, ruling out soil as source of the microbes. For *K. pneumoniae*, tests for Lysine decarboxylase in both camel milk isolates (80% +ve) and cow milk isolates (87.5% +ve) was similar to clinical isolates. All *K. pneumoniae* isolates from cow milk tested positive for citrate and urease indicating their role in pathogenicity. Phylogenetic distribution of the coliforms was random with regard to source and value chain. *E. coli* β-haemolytic isolates had a prevalence of 25% in cow milk and 33% in camel milk, and none isolated from the environment. Of the isolates, 12.5% and 19.0% for cow and camel milk respectively were multi-drug resistant to ampicillin, cefotaxime, and cefepime. To reduce Post-harvest losses and enhance food safety, the study recommends addressing the identified risks as well as enhancing antibiotic stewardship in animal disease management.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADH	Arginine dihydrolase
AK	Amikacin
AMP	Ampicillin
AMY	Amygdalin fermentation/oxidation
ATCC	American Type Culture Collection
CC	Coliform Counts
CDC	Centre for Disease Control of the United States
CFU/ml	Colony Forming Units per ml
CIP	Ciprofloxacin
CIT	Citrate utilization
CLSI	Clinical and Laboratory Standards Institute
CTX	Cefotaxim
DVC	Dairy Value Chain
EADD	East Africa Dairy Development
EAS	East Africa Standards
ESBL	Extended Spectrum Beta Lactamases
FAO	Food and Agriculture Organisation of the United Nations
FEP	Cefepime
GARP	Global Antibiotic Resistance Working Partnership
GEL	Gelatinase
GLU	Glucose fermentation/oxidation
GOK	Government of Kenya
H_2S	Hydrogen sulphide
HTST	High Temperature Short Time
IMI	Imipenem
IND	Indole production
INO	Inositol fermentation/oxidation
KDB	Kenya Dairy Board
KEBS	Kenya Bureau of Standards
KNBS	Kenya National Bureau of Statistics
KSh	Kenya Shilling, equivalent to USD 0.1
LDC	Lysine decarboxylase
MAN	Mannitol fermentation/oxidation

MB	Milk Bars
MEL	Melibiose fermentation/oxidation
New-KCC	New Kenya Co-Operative Creameries
ODC	Ornithine decarboxylase
ONPG	O-Nitrophenyl-β-D-galactopyranoside
PPCC	Pastoral Primary Collection Centre
PRSTN	Pastoral Road Side Traders in Nairobi
PSCC	Pastoral Secondary Collection Centre
PUCC	Peri Urban Collection Centre
PUM	Pastoral Udder Milk
PUUM	Peri Urban Udder Milk
RCC	Milk from Rural Collection Centres
RELOAD	Reducing Loses Adding Value
RHA	Rhamnose fermentation/oxidation
RUM	Rural Udder Milk
SAC	Sucrose fermentation/oxidation
SAS	Statistical Analysis System
SD	Standard Deviation
SHUM	Smallholder udder milk
SHUS	Small holder udder swab
SOR	Sorbitol fermentation /oxidation
T3SS	type III secretion system
TDA	Tryptophan Deaminase
TR	Milk from Traders
TVC	Total Viable Counts
TZP	Piperacillin/Tozobactam
UHT	Ultra High Temperature
URE	Urea hydrolysis
USD	United States Dollar, equivalent to KSh.100
VP	Voges Poskauer

CHAPTER ONE
INTRODUCTION

1.1 Background to the study

Kenya's *per capita* milk consumption is projected to reach 220 litres in the year 2030 from 110 litres in the year 2010 (GOK, 2010a). Meeting this demand requires not only increasing animal productivity but also reducing post-harvest losses which are reported to reach 27.2 % of current annual milk production of 4.7 billion litres (FAO, 2011; GOK, 2010a). The losses along the dairy value chain are mainly due to microbial spoilage, spillage and forced consumption, with microbial spoilage being the leading cause of food loss (Ivy *et al*., 2012; Lore *et al*., 2004). Loss due to spillage occurs at the production level due to improper milking techniques, while along the value chain, accidental loss of milk may occur especially during rainy seasons when some roads leading to milk producing areas are hardly passable. Forced consumption essentially is the consumption of milk by the producer household beyond their normal portion because of lack of market for the milk. This is a loss of value to the producer since milk that was meant to be sold is consumed at home (Lore *et al*., 2004). Post-harvest milk loss attributed to microbial spoilage on the other hand is due to microbial infection of the milk. Most often the contamination starts at production and the microbial numbers increase along the value chain (Orwa *et al*., 2017). Milk contamination at production may be attributed to the health of the lactating animal where mastitis cows or camels shed large numbers of microbes into milk, or due to contamination of milk during milking (Wanjohi *et al*., 2013). Contamination of milk during milking would occur due to failure to clean the cows' udder, or improper cleaning of the udder, hygiene of the milking person, cleanliness of the milking containers, cleanliness of the milking parlour, and quality of water used in milking preparation (Fernandes, 2008; Samarzija *et al*., 2012). Along the value chain, the main factors that influence microbial spoilage of the milk are time taken before chilling milk and temperature of storage (Cempirkova, 2007), and type of container for handling milk (Wafula *et al*., 2016).

Microorganisms implicated in spoilage of milk are mostly mesophilic or psychrophilic with the former being important at ambient temperature while the latter being important at refrigeration temperature (Jay *et al*., 2005; Samarzija *et al*., 2012). Psychrophilic microbes are dominant in milk under cold storage and spoil milk by hydrolysing proteins producing undesirable flavour while mesophiles are dominant in milk at ambient temperature. Mesophiles include lactic acid bacteria which breakdown lactose to lactic acid depressing pH of milk from 6.7 to less than 4.6 precipitating proteins leading to curdling of milk (Jay *et al*.,

2005). Equally important are coliforms which cause rapid spoilage in milk because they hydrolyse lactose with the production of acid and gas, and they can also degrade milk proteins (Lu *et al*., 2013). These microorganisms are not a challenge for milk that is cooled immediately after harvesting but pose a huge challenge in dairy value chains that do not have a proper cold chain that is dominant in the informal dairy sector in Kenya (Cempirkova, 2007).

Coliforms are microbes belonging to the family enterobacteriaceae with ability to ferment lactose to form gas and acid within 48h at 32-37^{o}C (Martin *et al*., 2016). These microbes include the genera *Escherichia, Enterobacter, Citrobacter,* and *Klebsiella* which are particularly important in milk because they are enteric and the primary route of contamination of milk is through udders contaminated with cow dung, contaminated water sources, milk receptacles and milking personnel hands (Fernandes, 2008). The occurrence of coliforms in milk is enhanced by various factors which include the type of milk container, cleanliness of the container, general hygiene of milk handler and his environment, and pooling milk from different sources without appropriate quality checks (Younan, 2004). Holding of milk at ambient temperature for a long time either during storage or transport has also been observed as a risk factor (Gleeson *et al*., 2013; Mwangi *et al.*, 2000; Younan, 2004). Mwangi *et al*. (2000) also reported the source of water for cleaning milk containers as a determinant for microbial levels in milk. All these conditions are commonly observed in smallholder and pastoral dairy value chains in Kenya in which informal milk outlets predominate and experience high post-harvest losses (Lore *et al.*, 2004).

In addition to contributing to high post-harvest losses, certain strains within the coliform group are also known pathogens, including some strains of the type species, *E. coli* (Baylis *et al.*, 2011). The pathogens contribute to animal diseases, particularly mastitis and calf pneumonia (Langoni *et al*., 2015; Sharma *et al*., 2015; Younan *et al*., 2013). Coliforms have also been implicated in human intestinal diseases such as infantile diarrhoea, and haemorrhagic colitis (Cohen & Giannella, 1992), and Ochi *et al*. (2017) reported that an outbreak of gastroenteritis in Mandera, Kenya in 2009 was due to *E. coli*. The infections are due to contaminated foods and water. The contaminated foods do not only cause infections in man but also act as reservoirs for *E. coli* causing extra-intestinal infections such as urinary tract infections (Vincent *et al*., 2010). In humans, *K. pneumoniae* colonizes the gastrointestinal tract and less frequently the nasopharynx, whence it gains entry to blood circulation and other tissues causing infection (Piperaki *et al*., 2017). *Klebsiella* accounts for a third of gram negative infections, and also responsible for urinary tract infections, cystitis,

pneumonia, surgical wound infections, endocarditis, and septicaemia (Effah *et al*., 2020). *Enterobacter* spp. on the other hand have recently emerged in community infections other than the hospital settings, causing life-threatening conditions (Anju *et al*., 2020).

Pathogenicity of these strains may be expressed through virulent factors which include β-haemolysins, Extended Spectrum Beta Lactamases (ESBLs) and biofilms which endow the pathogen ability to survive, multiply and cause disease (Kukanur *et al*., 2015). It has also been demonstrated that biochemical profiles of coliforms can be related to pathogenicity. Clinical and environmental sources of microbes can be distinguished based on their Carbon Utilization Profile and Nutrient Utilization Patterns. These profiles and patterns are based on differences among bacterial uses of a wide range of carbon and nitrogen sources for energy and growth (Uzoigwe *et al*., 2007) and this can be used to distinguish between clinical and environmental sources of microbes in milk. For instance, Yaratha *et al*. (2017) demonstrated that lactose non-fermenting *E. coli* are most likely to be pathogenic but less likely to be resistant to later generation cephalosporins such as cefepime and less likely to have ESBL enzymes. Chen *et al*., 2009 found that half the clinical isolates of *K. pneumoniae* have ability to grow anaerobically on citrate. The citrate aids the microbe to express alternative hemophores increasing pathogenesis (Vornhagen *et al*., 2019).

Coliforms shed in the stools of cows contaminate the udder and move up the teat canal not only contaminating the milk but also causing mastitis (Fernandes 2008; Jay *et al*., 2005). The lactating animals suffering from mastitis then shed the microbes with the milk *(*El-Ziney & Al-Turki, 2008; Fernandes 2008*)*. Some of these microbes are pathogenic and also resistant to common antibiotics (Osterblad *et al*., 1999). Antibiotic resistance is one of the biggest threats to global health, food security and development today because it reduces treatment options for common ailments, lengthens hospital stays, and increases mortality (WHO, 2020). Resistance to antibiotics may be acquired by cross gene transfer from resistant to non-resistant microbes or caused by administration of antibiotics to animals without appropriate antibiotic stewardship (Lamuka *et al*., 2017).

The challenges of milk post-harvest loss are more prevalent in Smallholder and extensive Pastoralist dairy systems compared to large scale and intensive dairy systems (Lore *et al*., 2005). This is essentially because smallholder production systems and extensive pastoral systems are less resourced than medium and the large scale systems (Rademaker *et al*., 2015). These loses have continued to be experienced despite efforts by farmers and other stakeholders to reduce the losses.

Post-harvest milk losses as a consequence of microbial contamination of milk as well

as associated food safety risks continue to abound despite many studies on microbial quality of milk (Gitau *et al*., 2013; Matofari *et al*., 2007; Mwangi *et al*., 2000; Omore *et al*., 2005; Orregard, 2013). None of these studies in Kenya has profiled coliforms in milk along the dairy value chain to find their importance as indicators of hygiene as well as expose the risk they pose to public health. There was therefore need for a systematic scientific study and expert knowledge of the risk factors for occurrence of coliforms in milk, their biochemical profiles, genetic distribution, pathogenicity and antibiotic sensitivity along the dairy value chain in Kenya. Addressing these gaps in knowledge was necessary for recommending appropriate interventions to reduce milk spoilage and associated public health risks, thereby increasing food security especially for the poor and vulnerable Smallholder farmers and pastoralists.

1.2 Statement of the problem

Food Post-harvest losses is a global problem but has never been given the desired attention. The estimates of losses vary but generally, it is estimated that 40% of the harvested food is lost post-harvest. Post-harvest losses along Smallholder and Pastoral dairy value chains in Kenya have not been objectively estimated. This is partly due to a lack of a proper methodological framework and the complexity of the value chain. Consistent measurement of food losses is a necessary first step toward reaching the goal of reducing PHLs. Not much progress has been made in this direction due to 'measurement problems'. Currently there is no single definition of food losses, nor are there any agreed upon methodologies for consistent measurement of these losses. Over the past decades, significant focus and resources have been allocated to increase food production. For example, 95% of the research investments during the past 30 years were reported to have focused on increasing productivity and only 5% directed towards reducing losses.

The losses along the dairy value chain can be categorised as either spillage, spoilage or forced consumption. Spoilage of milk is mainly as a result of increased microbial counts as a result of milk contamination. Contamination of milk with microbes contribute to postharvest milk losses in smallholder and pastoral dairy value chains in Kenya with impact on food security, incomes and health of consumers. Among the microbes, coliforms are used as indices for hygiene, while *E.coli* in particular is an index for the presence of pathogenic microorganisms in milk. Coliforms ferment lactose with production of acid and gas which contributes to milk spoilage. The presence of faecal coliforms, particularly pathogenic strains of *E. coli* in milk do not only pose consumer safety concerns but also indicate the presence of

other enteric pathogens like *Salmonella* and *Shigella*. Other coliforms such as *Klebsiella* are not only spoilage agents but are also known to cause about one third of enterobacteriaceae infections, while *Enterobacte*r is listed as an emerging pathogen. In addition, coliforms are some of the microbes that are developing antimicrobial resistance globally thus reducing treatment options for coliform related infections, lengthening hospital stays as well as increasing morbidity.

The value chain concept in Smallholder and Pastoral dairy value chains in Kenya is unstructured with Kenya Dairy Board inadequate in carrying out its regulatory mandate. Currently, there is hardly any concrete information about the risk factors associated with proliferation of microbial counts in milk along the smallholder and pastoral dairy value chains in Kenya and their implication on milk spoilage, consumer safety, and post-harvest losses along the dairy value chain.

1.3 Conceptualization of study objectives

This study was part of a North-South collaborative project titled 'Reducing Losses, Adding Value (RELOAD)' funded by the German Ministry of Education and Research (BMBF) within the framework of GlobE initiative, grant number no. 031A247A-D. Reducing losses and adding value along the Smallholder and Pastoral dairy value chains was one of the sub-projects of the broad project. This sub project was borne out of the realization that high milk losses occur at almost all points along the dairy value chain. These losses may be categorised as either losses in volume (spillage) or value (spoilage) with concomitant health risks due to presence of pathogenic microbes, some of which are resistant to commonly used antibiotics.

1.4 General objective

To contribute to food security as well as reduction of risks associated with microbial flora in milk along the Smallholder and Pastoral dairy chains in Kenya.

1.5 Specific objectives

i) To determine the risk factors associated with the occurrence of coliforms in milk along the dairy value chain in Kenya.

ii) To determine the biochemical profiles and phylogenetic distribution of coliforms in milk along the value chain in Kenya.

iii) To compare the pathogenicity and antibiotic sensitivity of *E. coli* isolated from milk and the environment along the dairy value chain in Kenya.

1.6 Research questions

i) What are the factors associated with occurrence of coliforms in milk along the dairy value chain in Kenya?

ii) What is the biochemical profiles and phylogenetic distribution of coliforms in milk along the dairy value chain in Kenya?

iii) What is the pathogenicity and antibiotic sensitivity of *E. coli* isolated from milk compared to the environment along the dairy value chain in Kenya?

1.7 Justification of the study

Kenya's dairy sector is estimated to be more than USD 1 billion with annual milk production of 4.7 billion litres of which 74.8% is from cows, 18.4% is from camels and the rest from goats (GoK, 2010a; Kenya Dairy Board, 2021). The country's milk production from cows is dominated by Smallholder farmers while camel milk production is dominated by pastoralists (FAO, 2011). Smallholder and pastoral milk producers are faced with many challenges in producing and delivering good quality milk to the market which contribute to milk spoilage.

Milk spoilage is the leading cause of postharvest milk loss worldwide. In Kenya, Smallholder dairy farmers and pastoralists are the most susceptible. There is loss in economic value for producers and actors along the value chain as milk undergoes microbial spoilage as it is transported for long distances without a cold chain to urban markets. Other challenges include lack of proper hygiene during milking due to lack of water in the arid and semi-arid pastoral production areas. Water quality for udder preparation and cleaning of milk containers has been reported to be either scarce or of questionable quality (Noor *et al*., 2013), and contributes to presence of pathogenic microbes in milk, such as *Salmonella, Escherichia coli, and Staphylococcus aureus*. Presence of *E. coli* is an indicator of the presence of other enteric pathogenic microorganisms in the milk (Baylis *et al*., 2011).

Identification of sources of contamination and loss hot spots are important in designing targeted interventions to minimize the loss in quality, nutritional and economic value. In addition, some coliforms are either pathogenic or opportunistic pathogens, with varied resistance to common antibiotics. Further, entry of foodborne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in biofilms, and subsequent contamination of processed milk products and exposure of consumers to pathogenic bacteria. In addition there is need to review, expand, and strengthen the role of regulatory agencies particularly Kenya Dairy Board with a view of

the body working with the operators along the value chain, and not waiting to arrest and prosecute offenders. The principal of quality assurance should be applied along the value chain, right from production at the farm, during transport of the milk to the dairy, during processing until the product reaches the consumers table. Currently, the role of strengthening value chains has essentially been left to development partners with government being a bystander, waiting to arrest and prosecute offenders. This work therefore to a large extent intends to inform policy changes that would increase efficiency in the dairy sector, particularly for the smallholder and pastoral value chains. The work on microbial resistance to antibiotics should stimulate regular research in the study area to establish a trend, and subsequently address the challenges that contribute to the microbial resistance.

Addressing these challenges, particularly through a policy shift, will contribute to the United Nations Sustainable Development Goal number 2 which aims to 'End hunger, achieve food security and improved nutrition and promote sustainable agriculture'.

1.8 Scope and limitations of study

This study focussed on microbial spoilage as one of the causes of post-harvest losses along the Smallholder and Pastoral dairy value chain in Kenya. The study was done in Nakuru, Isiolo and Nairobi counties. Nakuru represented the cow milk Smallholder dairy value chain. The Smallholder value chain was further distinguished into Smallholder Rural value chain, with milk being produced in the rural areas; and the Smallholder Peri-urban value chain with the milk being produced in the Peri-urban zones. Isiolo County represented the Pastoral camel milk value chain. The Isiolo value chain starts with milk being produced and collected in Isiolo. Most the marketed milk ends up in Eastleigh, Nairobi. The value chain is mapped with focus on hygiene and microbial contamination of milk along the chain. The risk factors for high microbial numbers is investigated. Biochemical profiles of the coliforms was also investigated as well as pathogenicity and antibiotic sensitivity of the microbes. The role of Kenya Dairy Board in regulating the dairy industry is also contextualised.

The study was not without limitations. The estimation of post-harvest losses in the dairy industry, particularly along the Smallholder dairy value chain, does not have a globally accepted scientific methodology. The methodology adopted by this research to estimate losses is based on both Kenya and East Africa quality standards. The Post-harvest losses along the chain is defined as the proportion of milk that does not meet the microbial quality requirements. The unstructured form of the Smallholder value chain with about 50% of

operators being unlicensed and therefore operating illegally makes the research more complex. The camel milk value chain is also bereft with its own challenges. The camel *bomas* do not have a permanent location, because the herders are nomadic in search of pasture, water and security. The methodology used in estimating losses along the camel milk value chain is similar to the one used in the Smallholder dairy value chain. However, during the study, it was found that camel milk pricing did not change based on quality of milk. Fresh, slightly fermented and fully fermented milk fetched the same price in Eastleigh, Nairobi. There was therefore no loss in economic value because all the milk sourced from Isiolo is not processed. The camel milk value chain is not regulated by government at all. The macro-level of the value chain is therefore not taken care of. This is due to the limitation of the Dairy Industry Act, 1958 which does not recognise camel milk. These factors make estimation of losses difficult and the information generated may have large variation. The spine of the research is therefore to bring out information about weaknesses of both the Smallholder and the Pastoral dairy value chains that contribute to post harvest milk losses and suggest solutions.

CHAPTER TWO
LITERATURE REVIEW

2.1 Economic importance of milk in Kenya

The dairy sector in Kenya supports a large population of rural poor households. It is the single largest component within the agricultural sector estimated at USD 1 billion with an annual growth rate of 4 % (GOK, 2010a). Annual milk production is estimated to be 4.7 billion litres of which 74.8% is from cows, 18.4% is from camels and the rest from goats (GOK, 2010a; Kenya Dairy Board website, 2021). The sector supports more than 4.8 million households (Bebe, 2003; van der Valk, 2008). Camels are particularly important in North Eastern Kenya and bordering areas, where a large community of Somali and related ethnicity are more familiar with camel milk (FAO, 2011). Milk marketing also offers employment, estimated at more than 365,000 jobs, along the dairy value chain (van der Valk, 2008). Milk consumption in Kenya is 110 litres *per capita*, mainly in form of liquid milk. This level is among the highest globally of any low-income developing country, and Kenyan households spend some 18% of their total income on milk and dairy products (GOK, 2010a; Lore *et al*., 2004).

2.2 The dairy value chain concept

A supply chain is the existing relationship between individual actors, normally found in the market. When this information becomes strategic collaboration between various participating organizations in order to achieve certain objectives in the market over a long time, the relationship is called a value chain. A food value chain in particular is a set of businesses and their interactions that brings farm products from its conception and design to the consumer. That means the producers and consumers form strategic alliances with other supply chain actors such as aggregators, processors, distributors, and retailers to improve financial return (Sidawi *et al*., 2020). Information flow is more extensive in the value chain compared to the supply chain. In addition, the actors in the value chain are more interdependent compared to actors in the supply chain. The expression ‘actor’ refers to a person who performs a certain function within a system or process (Lelea, 2014). This therefore mans that identification of actors should be with reference to a certain human activity system. The term ‘Stakeholder’ on the other hand is a term oftenly used to identify actors with a common interest in a given human activity or process. In addition, the value chain too has an aspect of high value differentiation of products compared to the supply chain. The value chain has various levels. Micro-level – These are the operators for instance

farmers, transporters, and traders. Meso-level –These are Suppliers of services for instance financial services, veterinary services, and feeds. This category includes agrovets, co-operatives and banks. Macro-level- These are actors involved in regulation of the industry for instance Kenya Dairy Board, Kenya Bureau of Standards, and Public Health department. All the actors are stakeholders because they either affect or are affected by particular decisions or actions (Dillmann & Ijumba, 2011).

Identification of stakeholders is an important step in transdisciplinary research. Transdisciplinary research integrates different types off knowledge. Transdisciplinary research searches for answers to complex problems; interconnects areas of specialization as well as between 'science' and the 'real world' (Lelea, 2014). Transdisciplinary research is thus the preferred mode of research for understanding any given value chain. However, there are power differences that need a balance for the success of any study of the value chain. The differences arise from the fact farmers have the local context while the scientist has the scientific context. There are usually different approaches to problem solving. Scientists may be focused on a particular problem while the farmer has complex problems. Scientist usually focus on global or national long-term issues but the farmers/operators are interested in solving his/her own immediate problems. Operators may also have knowledge and practical experience from generations while the scientist may observe issues from expected outputs and theoretical approaches. There are often language differences between the operators and the scientists. The operators use simple language while the scientists more often use scientific jargon. Scientists too may have perceptions of what is 'good' for the operators without a clear understanding of the underlying conditions. In some circumstances, opinion leaders influence the operators (Kaufmann, personal communication, September 22, 2014).

2.3 Perspectives of smallholder dairy value chain in low- and middle-income countries

Globally, a majority of dairy farmers are smallholder producers. Over the years Smallholder land sizes have been diminishing, with increasing livestock numbers. The smallholder dairy farmers lack resilience to and are vulnerable to market shocks. To reduce their vulnerability and improve their value chain position, the farmers usually form co-operatives (Abdulsamad & Gereffi, 2017). The smallholder farmers in these economies are also associated with poor hygiene practices during milking and general milk handling, which contributes to microbial spoilage of the milk (Wafula *et al*., 2018).

Milk in Uganda has been increasing over the last three decades, from an annual production of 58 million litres per year in 1993 on the onset of liberalisation, to 510 million litres in 2014 (Abdulsamad & Gereffi, 2017). Tecnoserve (2008), however estimated that milk production in Uganda in 2008 to be 1 billion litres per year but warns that all dairy data is estimated. The production has been estimated to be increasing at a rate of 7% per year from the year 2000. Like in Kenya, Informal milk traders dominate the market accounting to about 80%-90% of the traded milk (Abdulsamad & Gereffi, 2017). Equally, the contribution of dairy to GDP is almost the same to that of Kenya at 3%, with an estimated 10% loss along the value chain. Primary milk transportation is dominated by use of motorbikes and plastic containers are the dominant form of receptacles for milk (TechnoServe, 2008).

In Tanzania, milk production in the year 2007/2008 was 1.5 billion litres with annual per capita consumption of 43 litres. In Africa, Tanzania is only second to Ethiopia in cattle population. It has a population of about 30.5 million heads of cattle out which 680,000 are dairy cattle. The average production per cow is 3075 litres per cow per annum compared to Kenya with an average of 7083 litres per cow per annum. The proportion of processed milk is only 1.7% while the rest of the milk is traded through informal channels. However, Tanzania, just like Uganda suffers from the problem of reliability of data. Milk production ranges from 0.5 -1 litre per day for local breeds to 10-20 litres per day for intensive production for high breeds. Hawkers play a big role in milk marketing. The hawkers use bicycles, but more recently motorbikes to carry milk. The quantity of milk carried per hawker ranges from 60 litres to 100 litres per day. Processing capacity of milk plants vary from as low as 50 litres to 1000 litres. The processed milk is distributed through different channels, which includes direct sales at factory gates, sales on the streets, and kiosks. Supermarkets are reported to sell selected brands only. The industry is regulated by Tanzania Food and drug Authority and Tanzania Bureau of Standards (Dillmann & Ijumba, 2011).

In Georgia, Sidawi *et al.* (2020) reports that thousands of farmers own 1 ha or less of land which was subdivided after Georgia got independence from the Soviet Union. The rest of the land is owned by municipalities who decide who to use the land, and most of it is used freely for grazing. The country has about 1 million heads of cattle, 50% of being dairy cows. The smallholder dairy has been driven and formalised specifically by the demand for cheese, which is 12.7 kg per capita consumption compared to milk whose consumption is only at 8.3 litres per capita. To ensure food safety standards are maintained for commercial cheese, home-made cheese can only be consumed at home by the household.

Nguyen *et al*. (2017) aptly discusses the dairy value chain in Vietnam. In summary, Smallholder farmers are the dominant milk producers. Growth of smallholder farmers arose from the economic reforms in 1986 that allocated government land to individual owners, liberalised agricultural production, and promoted privatisation. The Dairy herd in Vietnam increased from about 41,000 heads in 2001 to 227,000 in 2014 with an equivalent increase in milk production from 64,000,000 litres to 550,000,000 litres. This surge was supported by amicable policies that focussed on smallholder dairy farming. The dairy value chain in Vietnam has rapidly transformed from being state run before 1990 to being largely privatised in 2008.

2.4 Smallholder dairy value chain in Kenya

Smallholder dairy value chain begins at the farm where milk is produced. The smallholder farm is a dairy production system that is a component of mixed farming systems with integration of dairy and crop production on the same farm, whose average size is less than 10 ha (Bebe, 2003) Smallholder farms contribute about 80% (GOK, 2010a) of the 4.1 billion litres of cow milk produced in Kenya (Kenya Dairy Board website).

The dairy marketing system in Kenya has been one of the most successful success stories in Sub-Saharan Africa in meeting a growing rural and urban milk and milk products demand. It is estimated that 600 million litres (11.3%), out of 5.28 billion litres of milk in Kenya is commercially processed into various value added products, including pasteurised milk, yoghurt, *mala*, cheese, and butter (Kenya Dairy Board website). The processing is carried out mainly by the major dairy processors in Kenya. The processors include Brookside dairy limited, New- Kenya Co-operative Creameries, Githunguri Dairies, Kinangop dairy, and a number of other dairies and mini-dairies scattered across the country (Kenya Dairy Board website). The rest of the milk is sold through licenced milk bars with 55-80% of the marketed milk sold through informal channels (GOK, 2010a; Odero-Waitituh, 2017). A survey conducted by Staal *et al*. (2001) too, found that most of the milk marketed passed through informal channels and was not processed. Sales to neighbouring households comprised the main outlet at 42%. The other outlets were traders (22%), co-operative societies and self-help groups (12%) and processors (12%). The remaining 11% was sold to hotels and shops. Lore *et al*. (2005) found that the informal raw milk sector in Kenya had grown since the late 1980's and represented 86% of the milk sold. In 2010, it was estimated that informal market outlets dominated by hawkers handled between 55 and 70% of domestic marketed milk (GOK, 2010a). The informal milk market comprises producer-sellers, itinerant

traders or “milk hawkers”, wholesalers and retail outlets like shops, kiosks and milk bars (Lore *et al*., 2005). The bulk of the milk handled by the informal sectors stays at ambient temperature for a extended periods of time which contributes to increased microbial levels (GOK, 2010a). Other factors which enhance microbial levels in milk include the hygiene of the udder surface (Orwa *et al*., 2017), the type of milk container, cleanliness of the container, general hygiene of milk handler and his environment, and pooling milk without regard for quality checks (Younan & Abdulrahman, 2004). Mwangi *et al*. (2000) also reported the source of water for cleaning milk containers as a determinant factor for microbial levels in milk. All these conditions are commonly observed in smallholder dairy value chains in Kenya in which informal milk outlets predominate and experience high post-harvest losses (Lore *et al.*, 2005). These losses continue to occur despite many studies on microbial quality of milk (Mwangi *et al*., 2000; Omore *et al*., 2005; Orregard, 2013).

Challenges that have been observed along the Smallholder dairy value chain include a low compliance to safety and quality standards, weak participation of micro-level actors in policy formulation, and non-participation of producers in determination of milk price delivered to the processor bulking centres. Equally, dairy processors face competition from thousands of informal traders who offer farmers better prices and more reliable payments and supply consumers with affordable, convenient milk (USAID, 2015).

2.5 Pastoral camel milk value chain

Camel population (dromedary) in Kenya is the fourth largest in the world at 3.3 million heads in 2010 (KNBS, 2010). Camels are specially regarded by the Somali community who are much familiar with camel milk (FAO, 2011). It is only Chad, Somali and Sudan which have a higher camel population than Kenya (FAO, 2019). In terms of milk production, Kenya is the second largest milk producer in the world at 876,000 tonnes after Somalia with 954,000 tonnes (FAO, 2019). Camel milk production in Kenya has increased tremendously since Kenya attained independence from colonial rule in 1963. The proportion of camel milk to total milk production in Kenya increased from 1% in 1961 to 18% in 2017, valued at USD 876 million (FAO, 2019). Milk supply to Nairobi is dominated by the Isiolo value chain, accounting for 89% of camel milk trade. The rest of the milk came either from Kajiado or Nanyuki (Muloi *et al*., 2018). Nomadic pastoralism is the main camel keeping system in Isiolo and Kajiado. This is characterized by the frequent movement of livestock in search of grazing land and water, and occasionally in search of security. About 80% of pastoralists in this chain were classified as middle-scale with herd sizes between 50–100

camels (Muloi *et al*., 2018)

Camel milk from the pastoral value chain reaches the market through two main channels. The first is an informal channel under which raw milk from the camel herds is transported by informal traders to urban centres, mostly targeting the Somali community residing in Nairobi's Eastleigh estate. Here, most of the milk is bought by households and restaurants. A smaller proportion of the milk taken to other estates in Nairobi and other urban areas, such as Nakuru, Mombasa and Eldoret, and as far as Kampala, Uganda. This channel handles 70% of marketed camel milk from Isiolo district, renamed Isiolo County since the promulgation of the 2010 constitution of Kenya (Muloi *et al*., 2018; Musinga *et al*., 2008). The second channel is a raw milk rural consumer channel where raw milk is supplied directly from the camel herds, usually through informal traders to rural households and restaurants. This is estimated to be 25% of marketed milk from Isiolo County. There is also a minor channel that handles about 5% of milk, which is pasteurised for the high end market (Musinga *et al*., 2008). The challenges along this value chain that are likely to exacerbate the problem of post-harvest losses of camel milk include lack of a policy framework for camel milk since the existing policy only address cow milk, poor road network in the camel milk producing areas, and inappropriate milk transport using public service vehicles from Isiolo to Nairobi. Additionally, there is no consistent cold chain for milk during transport from production to the market (Muloi, *et al*., 2018). A large proportion of the milk remains at ambient temperature for extended periods of time before reaching either the cooling centre or the market which is usually a long distance from the milk production areas (GOK, 2010a). The milk is commonly handled in narrow-necked plastic containers which are not just difficult to clean but also the poor quality of water used to clean them enhances microbial levels in milk (Mwangi *et al*., 2000). Pooling of milk from different sources without carrying out quality tests is also a common practice among milk handlers. This has the potential of contaminating good quality milk with bad quality milk contributing to loss of quality of the bulked milk (Younan, 2004). Milk spoilage was reported by Wayua *et al*. (2012), to be the main problem faced by the traders along this chain. In addition to milk spoilage, studies have also isolated pathogenic microbes from camel milk. Matofari *et al*. (2013) isolated *Salmonella* spp, *Staphylococcus aureus*, and S*treptococcus* spp from camel milk. Odongo *et al*. (2016) and Kashongwe *et al*. (2017) isolated *Staphylococcus aureus*, and S*treptococcus* spp respectively, from camel milk. High prevalence of mastitis has also been noted as a problem in camels. These challenges have contributed to the contamination and increased microbial counts in camel milk along the value chain (Isako & Kimindu, 2019).

2.6 Quantification of PHL along the dairy value chain

Global food availability can be achieved by both increasing food production and reducing post-harvest losses. Over the last three decades, about 95% of resources have been directed towards increasing food production and only 5% towards reducing post-harvest losses.

Post-harvest Food Loss (PHL) is defined as measurable qualitative and quantitative food loss along the supply chain, starting at the time of harvest, processing, marketing, till its consumption or other end uses. Quantitative food loss can be defined as reduction in weight of edible grain or food available for human consumption (Hodges, *et al*., 2011). Food losses can either be quantitative or qualitative. Quantitative food losses can be measured by decrease in weight or volume of food. Qualitative food losses on the other hand are in terms of, for instance, reduced nutrient value and unwanted changes to taste, colour, texture, or cosmetic features of food. Bothe quantitative and qualitative food losses represents loss of significant amount of money and other resources invested in food production (Buzby & Hyman, 2012).

Post-harvest losses along the dairy value chain can be in form of quantity, quality or forced consumption (Lore *et al*., 2004). Spillage is often accidental and would occur especially due to poor roads during transport on a motorbike, and not often quantified. Loss in quality is due to failing to meet quality standards as specified by Kenya Bureau of Standards. The standards specify parameters such as titratable acidity, total microbial counts, coliform counts, presence or absence of *E.coli*, presence of antibiotics, and physico-chemical parameters such as density and proximate properties. The parameters and bench marks are different for different types of milk and also milk products as prescribed by Kenya Bureau of Standards. Forced consumption is also considered as PHL by some researchers (Lore *et al*., 2003). This is the consumption of milk at home as a consequence of not finding the market for the milk. This is estimated to be about 40% (Lore *et al*., 2003) and the volumes are usually higher during the rainy season when pasture is plenty and resulting in a glut milk production. Forced consumption is also associated with evening milk which usually is not marketed.

2.7 Role of regulatory agencies along the dairy value chain in Kenya

2.7.1 Nature and type of regulatory agencies in the Kenyan dairy industry

Regulatory agencies are at the Macro level of the value chain. The major role of the regulatory agencies is to ensure compliance of operators along the value chain to the law and

regulations. The main regulatory agency in the dairy industry is the Kenya Dairy Board established under the Dairy Industry Act, CAP 336, 1958; The Public health department, established under the Public Health Act, Cap 242; and the Kenya Bureau of Standards, established under the Standards Act, Cap 496.

2.7.2 The role of Kenya Dairy Board

The role of Kenya Dairy Board as stipulated in the Dairy Industry Act. The Dairy Industry Act is "an Act of parliament to provide for improvement and control of the dairy industry". The functions of the board are outlined in section 17 of the Act. The functions include organising, regulating and developing an efficient production, marketing, distribution, and supply of dairy produce as well as improve quality of dairy produce. Subsection 17 (1) e states that Kenya Dairy Board is to permit the greatest possible involvement of private sector in the production, processing, and sale of dairy produce, while subsection 17 (1) f mandates Kenya Dairy Board on its own or in association with other government agencies to develop strategies to promote efficiency in the dairy sector. Subsection 17 (1) requires Kenya Dairy Board to promote research in the dairy sector. These are sweeping powers that essentially enable Kenya Dairy Board to effectively carry out its mandate. Section 19 of the Act empowers the board to develop regulations on 22 different items with regard to dairy produce.

2.7.3 The role of the Public Health authorities

The Public Health authorities derive their powers from the Public Health Act Cap 242. The Public Health officers, together with the Kenya Dairy Board inspectors and the Kenya Police enforce regulations concerning milk trade. Section 131 of the Act prohibits sale of unwholesome food. This therefore means that sale of adulterated milk contravenes the Public Health Act. The offenders are prosecuted and the product destroyed. The section also requires operators to take measures to avoid contamination of milk. Section 133 outlines the penalties for a person in possession of unwholesome food. The offender shall be pronounced guilty and shall be fined not more than KSh two hundred thousand (USD two thousand). Or be imprisoned for a maximum period of three years, or both. Section 134 of the Act states the rules for food protection that operators must adhere to. Section 134 (a) specifically requires the Public Health authorities to inspect dairies and milk shops while section 134 (b) requires the authorities to take and examine milk samples and dairy produce. Section 134 (c) requires the Public Health authorities to fix standards for cleanliness of milk, and prescribing warning

for cow keepers, dairy men or purveyors of milk who are found to be operating below the prescribed standard. Section 135 requires the Medical examination of any person in any premises in which any milk or dairy produce is collected, kept or sold, or a person who is engaged in the collection, preparation, keeping, conveyance or preparation of milk. Any milk handler who is a carrier of typhoid, or enteric fever or any infectious disease is prohibited from handling milk. This section also prohibits any person who has been convicted three times of offences under the Public Health Act, to be involved directly in milk trade. Section 135A of the Act requires Municipal councils, with the approval of the minister for Public Health, to make by-laws for regulating, supervising, and licensing milk traders, milk shops, and dairies.

2.7.4 The role of the Kenya Bureau of Standard

Section 3 of the Standards Act, Cap 496 establishes the Kenya Bureau of Standards. Section 9 of the Standards Act give power to Kenya Bureau of Standards to declare any specification, code of practice or standard to be gazetted by the minister in charge of trade. Any person therefore who contravenes the specification, code of practice, or standard is guilty of an offence under the Act. Some of the gazetted standards include Kenya Standards for raw cow milk, Kenya Standards for Pasteurised milk, and Kenya Standards for raw camel milk. It is therefore important that individuals involved in milk trade uphold the standards.

2.8 Microbiology of cow and camel milk

2.8.1 Total viable counts in cow and camel milk

Generally, cows are milked twice a day but high yielders may be milked three times a day. Traditionally, and in many Smallholder farms, hand milking is the only mode of milking. Large scale producers often use milking machines for milk harvesting. For camels, milking is usually done twice in the morning and in the evening too, especially during dry seasons (Ogolla, 2018; Ozer & Yaman, 2014). The ambient temperature for milking may range from 0^{o}C to 30^{o}C or higher for camels domesticated in arid and semi-arid tropical lands. The number of microbes in the milk can vary widely even when the conditions of temperature and period of storage of the milk on the farm is the same. Milk drawn aseptically from the udder is not sterile because of some microbes, especially Streptococci and Micrococci, which naturally inhabit the teat canal are drawn out with the milk. Among other microbes, psychrotrophs, coliforms and thermodurics may also be present in the milk. The most common coliforms include *Escherichia, Enterobacter, Klebsiella, and Citrobacter.*

Some of these gram negative microbes are involved in human ailments. For instance, *Escherichia coli*, including O157:H7 are involved in gastroenteritis, haemolytic uremic syndrome while *Salmonella* is involved in gastroenteritis and typhoid fever. Some of the microbes have been demonstrated to be more resistant to antibiotics than others. For instance, the shiga-toxin producing *E. coli* O157:H7 is more resistant to antibiotics compared to the strain that does not produce shiga toxins. Other microbes that may be found in milk and are pathogenic include *Listeria monocytogenes*, *Staphylococcus aureus* and *Mycobacterium tuberculosis* (Jay *et al*., 2005).

Freshly drawn milk from healthy cows under proper hygienic conditions contains low microbial counts of between 10^2 to 10^3 cfu/ml (Agarwal *et al*., 2012; Jay *et al*., 2005). This count represents the movement up the teat canal of some and the presence of others at the lower ends of teats (Jay *et al*., 2005). These microbial numbers increase along the value chain if milk is not cooled to 4^{o}C within 30 minutes (Gleeson *et al*., 2013) or due to contamination (Orregard, 2013). Microbial counts of less than 200,000 cfu/ml is an indicator of good hygiene while microbial counts greater than 10^5 cfu/ml is indicative of serious problems with maintenance of hygiene. Hygiene during milking is the single most important factor determining microbial load of milk (Orwa *et al*., 2017)

Apart from hygiene during milking, the health of the lactating animal determines the microbial load at milking. Mastitis has been known to contribute to shedding of microbes into milk during milking. The culprit microbes isolated from lactating animals suffering from mastitis are *Streptococcus agalactiae* (Matofari *et al.,* 2013; Younan *et al*., 2013), *Streptococcus dysgalactiae*, *Streptococcus uberis*, coliforms, especially *E. coli*, and *Pseudomonas aeruginosa* (Ozer & Yaman, 2014)

Milk is good source of nutrition for many microbial species. It is a good source of lactose. Some microbes however require amino acids to grow well, which fresh milk does not have. These microbes start thriving in milk only after other microbes have broken the proteins into amino acids for them to utilise.

Lowering the temperature of milk has an enormous effect on growth of all types of microbes, and contributes to enhancing the storage life of milk (Jay *et al*., 2005).

2.8.2 Coliforms in cow and camel milk

Coliforms are members of the enterobacteriaceae family. They are Gram negative straight rods measuring 1.0-.6.0 by 0.3-1.0 um. Endospores or micro cysts are not formed by coliforms. They are chemoorganotrophic, having both a fermentative and respiratory

metabolism and are oxidase and catalase negative. They are heterogeneous in ecology and have pathogenic potential for man, animals, insects and plants. They are also characterized by a rapid generation time (Brenner & Farmer III., 2005). On an agar medium containing bile salts, they can grow aerobically, fermenting lactose, producing both acid and gas within 48h at 37^{o}C. They include organisms belonging to the genera *Escherichia, Enterobacter, Citrobacter, Klebsiella* (Baylis *et al*., 2011; Gilmour & Rowe, 1981; Jay *et al*., 2005). However, coliforms may also include *Hafnia alvei* as well as strains genera such as *Pantoea, Serratia Buttiauxella*, *Yersinia, Leclercia* and others. Currently, there is no scientific agreement on the definition of coliforms as group. The genera or species to be included in this group is not clear (Baylis *et al*., 2011). Nevertheless, the test for coliform organisms are especially important in pasteurized milk (Gilmour & Rowe, 1981).

2.8.3 Importance of coliforms in cow and camel milk

Coliforms are used as indicators for the presence of pathogenic microbes in milk because they are easy and inexpensive to perform (Pantoja *et al*., 2011). Together with positive phosphatase test, presence of coliforms show improper milk pasteurization, whereas the presence of coliforms and a negative phosphatase test shows post pasteurization contamination. Coliforms are found in the colon of most homeotherms, including cows and camels, and contaminate milk through faecal material (Fernandes, 2008). It is then, under suitable conditions, involved in spoilage of milk and milk products. Some encapsulated strains of *E. coli*, as well as *Citrobacter* and *Klebsiella* cause ropiness in milk, even under cold storage contributing to significant economic losses for the agricultural and food industries (Baylis *et al*., 2011; Gilmour & Rowe, 1981). The growth and metabolic activity of these microbes in food can result in organoleptic changes such as colour defects, odours and off-flavours. These changes may arise from enzymatic breakdown of proteins or lipids, production of volatile components and gas production (Baylis *et al*., 2011; Jay *et al*., 2005). In milk and other dairy products, enzymes produced by some coliforms strains of hydrolyse casein. Some of the lipases and proteinases are thermostable and withstand high temperature short time (HTST) and ultra-heat (UHT) treatments (Baylis *et al*., 2011). During extended storage of these products, proteolytic enzymes hydrolyse proteins resulting in bitter flavours and gelation, while lipases break down milk butterfat resulting to development of rancid flavours (Fernandes, 2008). *E. coli* in particular, under suitable conditions can spoil milk and most dairy products, usually with the production of gas and an ‘unclean’ or faecal smell (Gilmour & Rowe, 1981). Some coliforms are regarded as opportunistic pathogens,

especially in clinical settings and these include *Klebsiella* spp, *Serratia* spp. and *Citrobacter* spp. (Baylis *et al*., 2011; Gilmour & Rowe, 1981). Some strains of coliforms produce toxins and/or specific antibiotic proteins (Gilmour & Rowe, 1981). In the same food, *E. coli* serves as both an indicator of faecal contamination as well as an index organism for enteric pathogens such as *Salmonella* (Baylis *et al*., 2011).

2.8.4 Sources of coliforms in cow and camel milk

Sources of coliforms in milk include faeces, water, feed and milking personnel (Fernandes, 2008). The exterior of the cows' udder and teats can contribute both microorganisms that are naturally associated with the skin of the animal as well as reflect the type of microorganisms in the environment in which the cow is housed and milked (Cawe, 2006). Many different microorganisms, including coliforms can be introduced into milk by this means (Fernandes, 2008). Improper udder hygiene during milking results to contamination of milk with microorganisms that are present on the teat skin and udder. It has been demonstrated that udder cleaning without use of a disinfectant does not sufficiently reduce milk contamination (Fernandes, 2008). Microorganisms can enter milk from milking personnel with insufficient personal hygiene (Quigley *et al*., 2013). Milking containers and cleaning water have also been implicated as sources of enterobacteriaceae in milk along the camel value chain (Kaindi *et al*., 2011). In addition, *E.coli, Enterobacter* spp, and *Klebsiella* spp can be shed by cows suffering from mastitis and therefore contaminate milk (El-Ziney & Al-Turki, 2008). Practicing good animal keeping practices and effective cleaning and disinfection of udders surfaces before milking contribute to reduction of microbial contamination of the milk (Fernandes, 2008).

2.9 Factors associated with high microbial load in camel milk along the small holder and pastoral dairy value chains

Milk contamination usually starts at the farm (Orwa *et al*., 2018). Conditions at the farm that contribute to high microbial counts include design and cleanliness of the cow shed and milking parlour, cleanliness of the cow, and milking preparation (Kashongwe *et al*., 2017). Along the value chain, smallholder and pastoral milk producers and transporters hardly observe hygiene requirements in milk handling, storage and transportation. In the informal milk market outlets, use of plastic containers dominate, but being difficult to clean is associated with increased bacteria counts in milk (GOK, 2010a; Lore *et al*., 2005; Younan, 2004). This is compounded by inadequate supply of clean water and lack of waste disposal

system (GOK, 2010a). Microbial spoilage of milk is enhanced by lack of cooling facilities and long distances to collection centres (GOK, 2010a; Lore *et al*., 2005). Milk should be cooled to below 4^{o}C within two hours after milking unless it is heat processed. Long distances between farms, collection centres and dairy plants can result in raw milk being transported for many hours, increasing the probability of bacterial growth, and spoilage (Braunig & Hall, 2005). This is often worsened by poor road infrastructure in the rural milk-producing areas (GOK, 2010a; Lore *et al*., 2005). Inadequate milk collection facilities and inappropriate location of cooling facilities also contribute to the losses (GOK, 2010a). Pooling of evening and morning milk or milk from different producers also increase chance of milk spoilage. In addition, some traders sell milk in open containers in unclean environments which exposes the milk to contamination (Younan, 2004). This is occasioned by the influence of the informal market where 80% of all milk reaching consumers is not subjected to any form of quality checks or control (GOK, 2010a).

2.10 Microbial quality requirement for cow and camel milk

Different countries have different standards for microbial load for cow milk, and some countries such as India have no set standards at all (Wafula *et al*., 2018). The Food and drugs Administration (FDA) of the United States of America requires grade 1 milk to have a Total bacterial count of less than 100,000 cfu/ml while pasteurized milk must have total bacterial count of less than 20,000 cfu/ml (Ozel & Yaman, 2014). Kenya Standards for grade 1 raw milk are less stringent requiring <200,000 cfu/ml while and Kenyan pasteurized milk requirements are more stringent requiring <10,000 cfu/ml. However, only Kenya has standards for camel milk. The quality standards; Total viable counts for raw cow milk (EAS, 2006a), Coliform counts for raw cow milk (EAS, 2006a), Total viable counts for raw camel milk ((KEBS, 2007), and Coliform counts for raw camel milk (KEBS, 2007) is shown in table 1, 2, 3, and 4 respectively. Pasteurised milk require a TVC of less than 10,000 cfu/ml and a coliform count of less than 10 cfu/ml, with Nil *E. coli (*EAS, 2006b).

Table 1: Total plate count/Total viable counts for raw cow milk (East Africa Standards)

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

Table 2: Coliform plate count for raw cow milk (East Africa Standards)

Quality	Counts (per ml)
Very good	0-1,000
Good	1,000-50,000

Table 3: Total plate count/Total viable counts for raw camel milk (Kenya Standards)

Grade	Counts (per ml)
I	<200,000
II	200,001-500,000

Table 4: Coliform plate count for raw camel milk (Kenya Standards)

Grade	Counts (per ml)
I	0-1,000
II	1,001-20,000

2.11 Control of microbial numbers in cow and camel milk

Microbial population in milk and milk products are controlled in four ways. First is to maintain the udder health of the lactating animal against mastitis. Secondly is to reduce contamination of milk at the farm by maintaining good hygiene (Ozel & Yaman, 2014). Third is by chilling milk to a temperature of less than 4^{o}C in less than two hours after milk harvesting (Jay *et al.,* 2005), and lastly, heat treatment, followed by cooling of the milk (Ozel & Yaman, 2014). Wanjala *et al*. (2016) have also demonstrated that use of smoke is effective in reducing microbial numbers in camel milk, with the additional advantage of a smoke flavor preferred by some consumers.

2.12 Biochemical profiles and genetic diversity of coliforms

2.12.1 Biochemical profiles

Coliforms are indicators of hygiene in handling of milk. In addition, they are essentially opportunistic pathogens (Langoni *et al*., 2015). *Escherichia coli* is one of the dominant members of the coliform group (Lues *et al*., 2010). It is naturally commensal but pathogenic types have been isolated (Brenner & Farmer III, 2005). Its main habitat is the gastrointestinal tract from where it is shed out with animal faeces. Contamination of milk

with faecal matter thus is a major source of *E. coli* in milk (Fernandes, 2008). *Enterobacter cloacae* is equally a common member of the coliform group. Terrestrial and aquatic environments (food, water, soil, sewage) are common habitats for *E. cloacae*. The species occurs as commensal microflora in the intestinal tracts of humans and animals. *E. cloacae* is an important opportunistic pathogen (Davin-Regli & Pages, 2015; Paauw *et al*., 2008) and recent studies have pointed to *E. cloacae* developing resistance to multiple antibiotics (Davin-Regli & Pages, 2015). *E. cloacae* has now become the third broad spectrum Enterobacteriaceae species involved in nosocomial infections after *E. coli* and *K. pneumoniae* (Davin-Regli & Pages, 2015). *E. cloacae* has also been associated with Gram negative mastitis in lactating animals. Apart from *E. coli* and *E. cloacae*, two of the most important coliforms contaminating milk are *K. pneumoniae* ssp *pneumoniae* (referred here to as *K. pneumoniae)* and *K. oxytoca*. *K. pneumoniae* is a ubiquitous Gram-negative encapsulated bacterium that resides in the mucosal surfaces of mammals and the environment, including soil and water (Piperaki *et al*., 2017). *K. pneumoniae* is a common environmental agent of both chronic and clinical mastitis affecting dairy herds. If present in the final product, *K. pneumoniae* negatively affects its quality. Mastitis caused by *K. pneumoniae* is more severe due to its poor response to antibiotic treatment, rapid evolution to toxic shock and possible death of the animal (Langoni, *et al*., 2015). The ecological habitats of *Klebsiella* include surface water, sewage, soils and plants, as well as mucosal surfaces of mammals. In humans, *K. pneumoniae* can be present in the nasopharynx and in the intestinal tract (Brisse & Verhoef, 2001). It is from these sources that they find themselves into milk due to poor milk handling practices. *K. oxytoca* is the second most important species of *Klebsiella* after *K. pneumoniae* in terms of probability of isolation in clinical samples. Biochemical difference between *K. oxytoca* and *K. pneumoniae* is fundamentally due to ability to produce indole from tryptophan. *K. pneumoniae* is indole negative while *K. oxytoca* is indole positive. The two species account for the most *Klebsiella* clinical isolates (Alves *et al*., 2006; William *et al*., 2018).

Clinical and environmental sources of microbes can be distinguished based on their Carbon Utilization Profile and Nutrient Utilization Patterns. These profiles and patterns are based on differences among bacterial uses of a wide range of carbon and nitrogen sources for energy and growth (Uzoigwe *et al*., 2007) and this can be used to distinguish between clinical and environmental sources of microbes in milk. Environmental sources of *K pneumoniae* tend to utilize more carbon sources than clinical isolates (Brenner & Farmer III, 2005). Various kits including API20E (bioMereux SA, Lyon France) have been used for

biochemical identification of microbes by profiling their biochemical activities. There is therefore a possibility that biochemical profiles of coliforms from camel and cow milk can be used to indicate the source of these microbes and the chance that the microbes are either pathogenic or non-pathogenic.

Summary of API20E tests is shown in Table 5 below.

Table 5: Summary of API20E tests

Test	Substrate	Reaction tested	Positive	Negative
ONPG*	ONPG	Beta-galactosidase	Yellow	Colourless
ADH	Arginine	Arginine dihydrolase	Red/Orange	Yellow
LDC	Lysine	Lysine decarboxylase	Red/Orange	Yellow
ODC	Ornithine	Ornithine decarboxylase	Red/Orange	Yellow
CIT	Citrate	Citrate utilization	Blue-green/Green	Pale-green/Yellow
H_2S	Na thiosulphate	H_2S production	Black deposit	Colourless/gray
URE	Urea	Urea hydrolysis	Red/Orange	Yellow
TDA	Tryptophan	Deaminase	Brown red	Yellow
IND	Tryptophan	Indole production	Red (2 min)	Yellow
VP	Na pyruvate	Acetoin production	Pink/Red (10 min)	Colourless
GEL	Charcoal gelatin	Gelatinase	Black diffuse	No diffusion
GLU	Glucose	Fermentation/Oxidation	Blue/Blue-green	Yellow
MAN	Mannitol	Fermentation/Oxidation	Blue/Blue-green	Yellow
INO	Inositol	Fermentation/Oxidation	Blue/Blue-green	Yellow
SOR	Sorbitol	Fermentation/Oxidation	Blue/Blue-green	Yellow
RHA	Rhamnose	Fermentation/Oxidation	Blue/Blue-green	Yellow
SAC	Sucrose	Fermentation/Oxidation	Blue/Blue-green	Yellow
MEL	Melibiose	Fermentation/Oxidation	Blue/Blue-green	Yellow
AMY	Amygdalin	Fermentation/Oxidation	Blue/Blue-green	Yellow
ARA	Arabinose	Fermentation/Oxidation	Blue/Blue-green	Yellow

2.12.2 Genetic diversity of coliforms in milk

Coliforms are a ubiquitous group of microbes that ferment lactose with gas production within 24h. They include *E. coli, K. pneumoniae, E. cloacae, and K. oxytoca* (Brenner & Farmer III, 2005). Traditionally, classification of coliforms was based on phenotypic properties. Currently, genotyping of species of enterobacteriaceae, and therefore coliforms, is accepted as a means to separate one species from another. Genetic diversity is a study undertaken to classify an individual or population compared to other individuals or populations. This is a relative measure, as the distance between any pair of entries in the study is greater or lesser depending on all pairwise comparisons that can be made in the study (Abdel-Mawgood, 2012). Milk may get contaminated with *E. coli* from various sources during different stages of production and processing, which could explain the genetic diversity of these bacteria in milk (Oltramari *et al*., 2014). The importance of carrying out genetic diversity of *E. coli* in milk relative to the sources is that, in addition to *E. coli*, human faeces can carry various human enteric pathogens. These enteric pathogens include *Shigella* spp., *Salmonella* spp., and hepatitis A virus. These pathogens do not colonize non-human sources. Determination of whether *E. coli* is of human sources or non-human sources is important in assessing the risk for presence of the pathogens in milk (Parveen *et al*., 1999). Pathogenic *E. coli* too could be identified by phylogenetic analysis which distinguishes isolates into four main phylogenetic groups (A, B1, B2, and D). Phylogenetic groups A/B1 contains strains that are commensal while group B2/D strains are associated with virulence (Gordon *et al*., 2008; Higgins *et al*., 2007; Moser *et al*., 2013).

The use of 16s RNA gene is still useful in separating species although many other genes such as tuf and atpD have been identified in coliforms (Paradis *et al*., 2005). To study bacterial phylogeny and taxonomy, the 16S rRNA gene sequences are very important. This gene is present in most bacteria, often existing as a multigene family and its function has not changed over time. This suggests that random sequence changes are a more accurate measure of time (Suardana, 2014). Studies by Lehner *et al*. (2004) were able to identify *E. sakazakii* using 16S rRNA gene sequence.

2.13 Pathogenicity in coliform bacteria

Enteric *E. coli* infections are divided into 8 pathotypes based on their pathogenicity profiles: Enteropathogenic *E. coli* (EPEC), Enterohaemorhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC), Diffusely Adherent *E. coli* (DAEC), Adherent Invasive *E. coli* (AIEC) and the Shiga

Toxin (Stx) producing Enteroaggregative *E. coli* (STEAEC) (Clements *et al*., 2012). Three pathotypes of *E. coli* (EPEC, EHEC and EIEC) employ a type III secretion system (T3SS) which is assembled within the bacterial cell as well as the plasma membrane of the host cell. This serves to subvert cell processes. The rest do not depend on T3SS and have a comparatively simple and efficient molecular mechanism of virulence requiring effective colonization factors followed by secretion of toxins that subsequently enter the host cell (Clements *et al*., 2012). EHEC is primarily a human pathogen with shiga-toxin and adhesion intimin as the main virulent factors. The role of haemolysin production is low. Ruminants carry this bacterium and are generally asymptomatic (Law, 2001).

In the genus *Klebsiella, K. pneumoniae* and, and to a lesser degree, *K. oxytoca* are considered the most important pathogenic species of Klebsiella although *K. terrigena* and *K. planticola* are also gaining prominence (Podschun & Ullman, 1998). Pathogenicity in *K. pneumoniae* is aided by presence of a thick capsule which helps the organism to evade phagocytosis and serum killing by the host organism, presence of fimbriae which aids the microbe to attach itself to host cells; and expression of hemophores which acquire iron from the host increasing pathogenesis (Li *et al*., 2014; Vornhagen *et al*., 2019).

In *E. cloacae* serum resistance is an important determinant for pathogenicity. It has also been reported that one strain of *E. cloacae* has the ability to produce hemophores. Haemolysis is however absent (Keller *et al*., 1998). It worth noting that that *E. cloacae* is found in 40 to 50% of GIT of human population, and only important as an opportunistic pathogen affecting individuals who are immune compromised (Keller *et al*., 1998).

2.14 Beta-haemolytic *E. coli*

Enteric microbes are associated with the guts of homeotherms and is frequently shed in the faeces of these animals (Fernandes, 2008). In the absence of proper hygiene, these microbes in the faecal matter contaminate the udder and move up the teat canal not only contaminating the milk but also causing mastitis (Jay *et al*., 2005). In this regard, the type of mastitis caused by *E. coli* is not contagious but categorised environmental mastitis (Ahmed & Shimamoto 2011; Iyer *et al*., 2014; Khan & Khan 2006). During milking, the lactating animals suffering from mastitis then shed the mastitis causing microbes with the milk *(*El-Ziney & Al-Turki, 2008; Fernandes 2008*).* Iyer *et al*. (2014) has demonstrated that that cows and camels suffer from udder mastitis in the same way and with involvement of the same type of microbes. *E. coli* that is implicated in mastitis, which is a disease condition, is the

pathogenic types with haemolysin production being one way in which the microbes express pathogenicity (Naveen & Mathai 2005). Expression of haemolysin production in *E. coli* has is associated with strains that cause enteric diseases in many species of animals and extra-intestinal diseases in human populations (Short & Kurtz, 1971).

2.15 Antibiotic sensitivity of *Escherichia coli*

2.15.1 Importance of antibiotic resistance of microbes

In Kenya, just like many other countries, the bacteria that contribute to most severe human infections are those in which there is evidence of antimicrobial resistance, and these include pathogenic *E. coli.* However, like in most of the African continent, there is no formal system for surveillance of antibiotic resistance in agricultural bacterial isolates as opposed to human clinical isolates (GARP, 2011). *E. coli* has been demonstrated to be resistant to a number of antibiotics including ampicillin, ciprofloxacin, Cefepime, amikacin, and imipenem in isolates from human patients (Brinas *et al*., 2005; Chong *et al*., 2009; Neto *et al*., 2003) and those from dairy cows (Adefurin *et al*., 2011; Davis *et al*., 2015). Resistance of *E. coli* to 3rd generation cephalosporins such as cefotaxime is associated with the presence of plasmid mediated Extended Spectrum Beta Lactamase (ESBL) enzymes that are capable of breaking down all beta lactams which are important in the activity of these antibiotics (Ahmed & Shimamoto, 2011). The gene for resistance in these microbes can be acquired by a pathogen via horizontal gene transfer from one organism to another or by mutation. It has also been demonstrated that some organisms are inherently resistant (Vincent *et al*., 2010).

Many studies have reported increasing resistance of *E. coli* to prescribed antimicrobials, especially, beta-lactams. This makes treatment of infections associated with the resistant microbes very difficult (Neto *et al*., 2003; Vincent *et al*., 2010). Many of these studies have been done on patients with urinary tract infections, a few in mastitis animals and even fewer in foods despite the fact that food could be a reservoir for *E. coli* that causes community acquired urinary tract infections (Vincent *et al*., 2010) and also a source of resistant bacteria (CDC, 2013). Presence of pathogenic *E. coli* in milk is a public health concern especially for smallholder and pastoral communities where there is a lot of human contact with milk during milking, transportation and bulking. Pastoral communities too, have a preference for consumption of raw milk that increases risk of infection (Kaindi *et al*., 2011).

The World Health Organization has recognized the importance microbial resistance and the need to devise appropriate strategies for their control. In particular, ESBL-producing *Escherichia coli* are resistant to all penicillins, and cephalosporins (Piccozi *et al*.,

2014). *K. pneumoniae* are generally susceptible to carbapenems such as imipenem or meropenem. However, Podschun and Ullman (1998) isolated ESBL producing carbapenem resistant strains. Similarly, *Klebsiella pneumoniae* with AmpC lactamases, and aminoglycosides-modifying enzymes have been reported. Some *K. oxytoca* are also resistant to imipenem and meropenem (Singh *et al*., 2016). *E. cloacae* has an intrinsic resistance to ampicillin and narrow-spectrum cephalosporins and exhibits a high frequency of mutation to resistance to expanded-spectrum and broad-spectrum cephalosporins (Keller *et al*., 1998).

2.15.2 Causes of antibiotic resistance of microbes

Bacteria may naturally be resistant to certain antibiotics. This may be due to lack of a transport system for the antibiotic, lack of a target of a target for the antibiotic molecule or impermeability of the microbial cell to the antibiotic (Odonkor & Addo, 2011). Resistance of microbes to antibiotics may also be acquired. This may be through transfer of genes for resistance traits from bacteria of different taxonomic and ecological groups using certain mobile genetic elements such as bacteriophages, plasmids, naked DNA or transposons (Levy & Marshal, 2004; Odonkor & Addo, 2011; Sabtu *et al*., 2015). Plasmids and transposons usually mediate high-level resistance. Another way for development of microbial resistance is through sequential mutation in chromosomes. This often is progressive with development of resistance over time. For instance, strains of *E. coli* have developed high level resistance to fluroroquinolones due to gene mutations in the target enzymes and increased expression of membrane proteins that pump the drug out of the microbe (Levy & Marshal, 2004). Long term use of a single antibiotic (usually for more than 10 days) result in resistance to the antibiotic, as well as to other antibiotics that may not even be structurally related.

Growing resistance to antibiotics is generally related to lack of antibiotic stewardship in antibiotic administration (Odonkor & Addo, 2011). For instance, Lamuka *et al*. (2017) reported that that 46% of the pastoralists in Kenya administer veterinary drugs to camels that are sick without consulting veterinary officers. This contributes to either under dosing, overdosing, or administering the wrong drug to the animal and failure to observe withdrawal periods.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

3.1.1 Smallholder dairy value chain

The study was done between 2015 and 2016. Three divisions within Nakuru County were selected for this study (Appendix A). Olenguruone division within Kuresoi Sub County is about 150 km from Nakuru town and represents the smallholder rural value chain while Dundori and Bahati divisions within Nakuru North Sub County are about 10km from Nakuru town and represents the smallholder peri-urban value chain. Nakuru County lies between latitudes 0º 13'N and 1º 10'S, and longitudes 35º 28'E and 35º 36'E at an altitude of between 1520 to 2500 m above sea level. The annual rainfall ranges between 850 to 1660 mm of rainfall. Nakuru County had a population of 286 thousand dairy cattle with a milk production of 291 million litres per year for the year 2014 according to a report by Kenya National Bureau of Statistics (KNBS, 2015).

3.1.2 Pastoral dairy value chain

This study was carried out between February and August 2015 within Isiolo County and along the value chain to Nairobi (Appendix A), Kenya. Isiolo county lies between longitude 36°50' and 39°30' East and Latitudes 0°5' and 2° North and has a total area of 25,605 km^2 (Noor *et al*., 2013). Isiolo County is a typical arid and semi-arid region (Noor *et al*., 2013) with average annual rainfall of 418 mm (Mati *et al.*, 2005), and a camel population of 39,084 (KNBS, 2010). Most camel owners practice pastoralism, with camel milk being an important source of livelihoods (Wayua *et al*., 2012). Most of the marketed milk ends up either in Isiolo town, or Eastleigh market in Nairobi city, a distance of 275 km to the south of Isiolo town (Noor *et al*., 2013).

3.2 Study Design and data collection

3.2.1 Exploratory Study and Identification of stakeholders

Secondary data from published literature on the Dairy Value chain was used to identify stakeholders at the macro level. The stakeholder identified at this level were Kenya Dairy Board, County Government of Nakuru and Isiolo departments of Veterinary services, Kenya Bureau of Standards, and Public Health departments. These were the agencies identified as directly involved with milk trade. However, Kenya Dairy Board had the unique mandate because it dealt only with milk.

Identification of farmer groups and co-operatives was facilitated by the Catholic Diocese of Nakuru. The Catholic Diocese of Nakuru, had an outreach programme that benefitted households through building of water tanks in Dunduri and Bahati, and also supported Olenguruone Farmers' co-operative society to set up a milk cooling facility.

Identification of traders in Nakuru was done with the help of Kenya Dairy Board, Nakuru regional office. Members of the trader groups helped in identification of milk bars that they usually delivered their milk.

Identification of camel herds in Isiolo was done with the aid of Kenya Camel Association. Similarly, the association through its secretary, Mr. Khalif, introduced us to Anolei women group, Tawakal group, and the milk traders at the 'Milk stage'. One trader then introduced us to another trader. The method of identification of stakeholders was largely Snowball in nature (Isaacs, 2014). The stakeholders then voluntarily agreed to be part of the research, with the leaders of the groups being key informants, while members participated in Focus Group Discussions, and provided data through interviews.

3.3.2 Mapping of the smallholder dairy value chain

A cross sectional study was set up to collect data from milk handlers. Primary and secondary data was collected to map the chain and identify the loss hot spots with respect to microbial loads in milk. Primary data was collected using structured interview schedules with respondents being actors along the chain from the farm, collection centres, transporters, traders, and retailers. A total of 63 respondents from the Peri-urban value chain and 82 from the rural value chain were interviewed face to face. The questions were translated into Swahili or the local language of the respondent where appropriate. From each of the divisions, one focussed group discussion was done to corroborate the information collected along the value chain. Expert interviews were held with the Regional manager of Kenya Dairy Board stationed in Nakuru and the Nakuru County Director of Livestock Production while key informant interviews were held with the chairpersons of Olenguruone farmers' co-operative society, Wanyororo farmers' co-operative society in Dundori, and Bahati farmers' co-operative society. Other key informants were the chairman, Mangu Self-help group, Attendant of Olenguruone Farmers co-op Agrovet shop, and the Desk officer, Kenya Dairy commercialization project. All participants in this study voluntarily provided data and information.

3.3.3 Mapping of the pastoral dairy value chain

A cross sectional study was set up to collect data from milk handlers. Primary and secondary data was collected to map the camel milk value chain. Primary data was collected by observation and use of structured interview schedules administered to actors along the chain from producers, collection centre operators, transporters and traders. A total of 90 milk handlers were interviewed face to face. The questions were translated into Swahili or the preferred language of the respondent where appropriate. Key informant interviews were held with the secretary, Camel Owners Association; the chairpersons, Anolei women group and Tawakal group, to validate the findings. All the participants in this study voluntarily provided data/information.

3.3.4 Risk factors for microbial loads along the Smallholder dairy value chain

To determine microbial quality of the milk, a total of 145 raw milk samples, from both peri-urban and rural value chains were taken during milking, and along the raw milk value chain. The milk was sampled from traders/transporters, collection/bulking centres, and milk bars. Another 40 samples of unexpired pasteurized market milk was obtained from supermarkets within Nakuru town. Raw milk was collected in 60 ml sterile sampling bottles and transported in a cool box at 4 to 6^{o}C to the laboratory in Egerton University. The analysis was done within 4 hours after sample collection. The samples were analysed for total viable counts and coliform counts. During sampling, information related to the sample was recorded. This included the sampling point, time of sampling, type of container from which milk is sampled, temperature of the milk sample, whether the person handling the milk is trained on milk quality or not, and mode of transport. Source of water used for milking preparation as well as milk receptacle cleaning was also recorded. For pasteurized market milk, the brand name and expiry date were recorded. Raw milk samples were provided voluntarily by the participants in the study while pasteurized milk was bought from the retail outlets.

Determination of total viable counts (TVC) was done as described by Messer *et al.*, (1985) for the standard plate count. Raw milk sample measuring 1 ml was diluted six-fold while pasteurised milk sample was diluted three-fold (1:10) using buffered peptone water for all the samples. One ml from each dilution was dispensed onto a sterile petri-dish. This was done in duplicate. This was followed by pouring about 20 ml of molten (approx. 45^{o}C) Plate Count Agar (Oxoid, Hampshire, England) prepared according to the manufactures instructions. The inoculum was then gently mixed by alternate clockwise and anticlockwise

movement for 3 minutes, and left for 30 minutes on the bench to set. The plates for TVC were then incubated at 32^{o}C for 48 hours followed by counting of colonies. Plates with colonies between 30 and 300 from the lowest dilution were counted and reported as colony forming units per ml of milk (CFU/ml). Determination of coliforms followed the same procedure except that the media used was MacConkey agar (Oxoid) and the temperature of incubation was 37^{o}C for 24 hours. All the pink/red colonies were counted as coliforms (lactose fermenters).

3.3.5 Risk factors for microbial loads along the pastoral dairy value chain

Milk samples were collected from the camel during milking, and along the value chain from *boma* bulk, primary collection sites near the camel herds, secondary collection centres in Isiolo town, transporters, and traders both in Isiolo and Nairobi. In Isiolo, 76 milk samples were collected from the interviewed actors in Isiolo and 55 milk samples collected from Nairobi traders. The size of the containers from which milk was sampled ranged from 3L, 5L, 10L and 20L. Sampling was done in 60 ml sterile sampling bottles and transported in a cool box at 4^{o}C to the laboratory in Isiolo County referral hospital for samples from Isiolo, and University of Nairobi's Food microbiology laboratory for samples from Nairobi. The analysis was done within 4 hours after sample collection. The samples were analysed for total viable counts and coliform counts. During sampling, information related to the sample was recorded. This included the sampling point, time of sampling, type of container from which milk is sampled, temperature of the milk sample, whether the person handling the milk is trained on milk quality or not, and mode of transport. All the milk samples were voluntarily provided by the participants.

Determination of total viable counts (TVC) was done as described by Messer *et al*. (1985) for the standard plate count. A milk sample measuring 1 ml was diluted six-fold (1:10) using buffered peptone water for all the samples except the samples from Nairobi which were diluted ten-fold (1:10). One ml from each dilution was delivered onto a sterile petridish. This was done in duplicate. It was then followed by pouring about 20 ml of molten (approx. 45^{o}C) Plate Count Agar (Oxoid, Hampshire, England) prepared according to the manufactures instructions. The inoculum were then gently mixed by alternate clockwise and anticlockwise movement for 3 minutes, and left for 30 minutes on the bench to solidify. The plates were then incubated at 32^{o}C for 48h followed by counting of colonies. Plates with colonies between 30 and 300 from the lowest dilution were counted and reported as colony forming units per ml of milk (CFU/ml). Determination of coliforms followed the same procedure

except that the media used was MacConkey agar (Oxoid) and the temperature of incubation was 37ºC for 24h. All the colonies on the plate were counted to represent *Enterobacteriaceae* counts, while pink/red colonies were counted as coliforms (lactose fermenters).

3.3.6 Biochemical profiles coliforms and phylogeny of *E. coli*

A total of 278 milk samples were collected from both camel and cows during milking, and along the value chain. Camel milk was collected from Isiolo pastoralists and along the value chain to Nairobi while cow milk was collected from Smallholder farmers in Nakuru. This was done in 60 ml sterile sampling bottles and transported in a cool box at 4ºC to the laboratory in Isiolo County referral hospital for samples from Isiolo, and University of Nairobi's Food microbiology laboratory for samples from Nairobi. Samples from Nakuru County were analysed at Egerton University department of Dairy, Food Science and Technology. Purification of all isolates and biochemical profiling was done at Egerton University. To select the dominant coliforms, microbes in the Enterobacteriaceae family were selected from MacConkey agar (Oxoid, Hampshire, England) plate incubated at a temperature of 37ºC for 24h. Colonies from each plate were selected based on morphology and growth characteristics. Colonies were purified by streaking, and tested using API20E (bioMereux, SA, Lyon France) according to manufactures instructions. Isolates were identified by the API20E software version 4.1 (bioMereux SA, Lyon France). A total of 244 isolates with significant taxa as 'Good', 'Very good' and 'Excellent' identification were selected for the study. A summary of the API 20E tests is shown in Table 1. In addition, all isolates must test oxidase negative.

Pure bacterial cultures were selected for phylogenetic analysis. DNA extraction was done using the phenol-chloroform method as described by Javadi *et al*. (2014). Bacteria was lysed and protein removed by digestion with proteinase K. Cell wall debris, polysaccharides, and residual proteins were removed by selective precipitation with phenol-chloroform-iso amyl alcohol mixture (24:25:1) . DNA was recovered from the resultant supernatant by iso-propanol precipitation then re-suspended in nuclease-free water, and stored at -20ºC. Quantification of DNA was done by use of Nanodrop spectrophotometry and gel electrophoresis. Amplification of 16s rRNA gene was done with the following bacterial primers.

27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3').

PCR chain reaction was performed in a total volume of 25µL using 13.7 µL distilled

water, 1.0 µL dNTPs (10mM), 2.5 µL PCR buffer, 2 µL $MgCl_2$ (25mM), 1.5 µL forward primer (10mM), 1.5 µL reverse primer (10mM), 0.8 µL Taq polymerase (5U/ µL), and 2.0 µL DNA template. The PCR was set up with the following profile: Initial denaturing step was done at 95ºC for 5 minutes, followed by 45 cycles at 95ºC for 5 minutes (30 seconds) denaturation; 50ºC (30 second) annealing; 72ºC (2 minute) extension; and a final 72ºC (10 minute) extension.

The PCR product was analysed on 1% agarose gel electrophoresis containing 0.5mg/ml ethidium bromide. Amplification patterns were viewed with DNA gel viewer under Ultra Violet light to confirm the presence of a 1,350 bp band. Sanger sequencing of the PCR product was done by Inqaba South Africa. The clean PCR products were sequenced using an ABI sequencing kit (Big Dye Terminator cycle sequencing, Applied Biosystems). Basic Local Alignment Search Tool (BLAST) was used for identification of the sequences. *Escherichia coli* ATCC 25922 was used as a control.

3.3.7 Pathogenicity and antibiotic susceptibility

The study was carried out between February and June 2016. Milk samples were taken directly from the udder during milking, and along the value chain to the market from smallholder producers for cow milk and pastoral producers for camel milk. Udder swabs from the lactating animals and hand swabs from the milking persons were also taken, as well as water for handwashing, udder and milk receptacle cleaning. However, for camel milk, only water for receptacle cleaning was sampled since udder cleaning and handwashing before milking is not practiced. The milk and water samples were collected in 60ml sterile bottles while the swabs were taken using sterile cotton swabs and put in 10ml peptone water in diluent bottles. Samples taken from Northern Kenya (Isiolo) were taken to the Isiolo County referral hospital labs for culturing, while samples taken from Nairobi market were cultured at the University of Nairobi labs. Samples from the Smallholder producers within Nakuru County were cultured at the Egerton University labs where isolation and biochemical identification was done for all isolates. All the samples were transported at 4 to 7 ºC in a cool box, and refrigerated before culturing.

The isolates were cultured on Eosin Methylene Blue agar (Oxoid, Hampshire, England) at 37ºC for 24 hrs. This was repeated until pure *E. coli* colonies were isolated. The isolates were then identified using the API20E system (bioMereux SA, Lyon, France) according to manufacturer's instructions. The isolates were diluted to 0.5 McFarland standards before inoculation onto the ampules. The strips were incubated at 35 ± 2 ºC for 24h

and the colour change in the ampules recorded as positive or negative. The microbial identity was done using an API20E software version 4.1 (bioMereux SA, Lyon, France). The colonies were kept in 1ml of 1 M sucrose solution in 2ml cryo-vials and stored at -18 ^{o}C to await further analysis. When required, the stored culture was re-cultivated in nutrient broth at 37 ^{o}C for 24h.

Test for haemolytic activity was done by plating the isolates on Blood agar (Oxoid, Hampshire, England) with 7% defibrinated sheep blood at 37 ^{o}C for 24h. In summary, the blood was obtained from ewes on the Egerton University farm using sterile hypodermic syringes. The blood was defibrinated and mixed by swirling with the blood agar base at a temperature of 45 ^{o}C then pouring about 20ml onto sterile plates and allowed to set. The isolates were then streaked onto the solid plated and incubated to at 37^{o}C for 24h after which haemolysis was observed.

3.4 Data analysis

3.4.1 Exploratory study and identification of stakeholders

Information generated from the exploratory study separated stakeholders into three categories based on their level and roles. The stakeholders identified at the macro-level were the regulatory agencies, and these were Directorates of livestock at the County level and the Kenya Dairy Board while Operators at the meso-level were institutions that offered services to the operators, for instance, Agrovet shops. At the micro-level were the operators who were actively involved in the milk trade, starting with the producer at the farm level to the milk transporters, traders, milk-bar owners, and processors. The roles and operation of the stakeholders were then described.

3.4.2 Risk factors for high microbial loads in milk

Count data was subjected to statistical analyses using SAS version 9.0 (SAS Institute Inc., Cary, NC, USA). The statistical analyses involved determination of frequencies and arithmetic means; correlation between factors that affect microbial loads; and regression between independent and dependent variables. Shapiro-Wilk test was carried out to determine normality of data when $n<30$.The microbial counts were transformed to $\log_{10}$ and reported as mean ± standard deviation CFU/ml of milk. All the statistical tests were done at a significant level of $p\leq 0.05$. Information from the respondents which was validated by the key informants was used to map the value chain.

3.4.3 Biochemical profiles and phylogeny

Chi square test of equality of proportions for independent samples was used to compare prevalence of *E. coli* isolates and their antibiotic sensitivity, between cow and camel milk, and between udder milk and post-udder milk. The null hypothesis of equal proportions was rejected and that proportions were unequal if $p \leq 0.05$. SAS version 9.4 (SAS institute Inc, Cary, NC, USA) was used for all computations. Data on positive proportions was transformed into percentages and significant difference between proportions for the 20 tests on API20E determined. The Pearson chisq test was used to test the difference between proportions of isolates from camel and cow milk testing positive for given API20E test, as well as between the isolates and expected results by API20E (Manufacturers notice). A summary of API 20E (bioMereux SA, Lyon France) is shown in Table 5

Sequencher version 5.4 (Gene Codes Corp, Ann Arbor. MI) was used for DNA sequence editing, Clustalx version 2.1 for DNA sequence alignment and MEGA version 7 for Neighbour-Joining tree generation with 500 bootstrap repetitions.

3.4.4 Pathogenicity and antibiotic susceptibility

The beta-haemolytic isolates were tested for antibiotic sensitivity based on the principle of the Kirby-Bauer disc diffusion method (Bauer *et al*., 1966). The antibiotics were chosen according to the class they belong to. They were Ampicillin, AMP (Penicillins); Cefotaxime, CTX (3^{rd} generation cephalosporins); Cefepime, FEP (4^{th} generation cephalosporins); Piperacillin/Tozobactam, TZP (Penicillin/ beta-lactamase inhibitor); Ciprofloxacin, CIP (Fluoroquinolone); Amikacin, AK (Aminoglucoside); and Imipenem, IMI (Carbapenem). All the antibiotic susceptibility discs were sourced from Liofilchem, Italy. Briefly, sterile solid plates of Muller-Hinton agar (Oxoid, Hampshire, England) were prepared and a broth of *E. coli* adjusted to 0.5 McFarland turbidity standards spread on its surface. Antibiotic discs were then carefully put on the solid plate, the plate inverted, and incubated at 35±2^{o}C for 24h. The inhibition zone was measured as diameter across the antibiotic disc with a clear zone. The diameter of the clear zone was recorded in mm and interpreted according to the Clinical & Laboratory Standards Institute (CLSI) recommendations (CLSI, 2016). *E. coli* ATCC 25922 was used as a standard.

CHAPER FOUR

RESULTS

4.1 Exploratory study for identification of stakeholders and setting up Stakeholder platforms

4.1.1 Key stakeholders along the dairy value chain in Kenya

The following stakeholders were identified and interviewed with the objective of obtaining information on the dairy value chain, the actors involved, and the their view on the concept of post-harvest losses along the dairy value chain. The key stakeholders interviewed were the chairmen of Olenguruone farmers' co-operative society, Bahati farmers' co-operative, Wanyororo farmers' co-operative society, Mangu self-help group, Anolei Women Co-operative Society, Tawakal co-operative society, Kenya Dairy Board Nakuru Regional Manager, Nakuru County Director of Livestock, Kenya Camel Association chairman, Isiolo County directorate of livestock. Focussed group discussions were also done with members of the farmers groups. The discussions with the farmers groups centred on milk production, collection and transportation. Directorates of livestock provided information on milk production and major challenges faced by farmers, while Kenya Dairy Board provided detailed information on regulatory framework for the dairy industry in Kenya. Secondary data was also collected from the groups and institutions as well as from literature.

4.1.2 General perspectives from key stakeholders along the dairy value chain

Interviews were held with the Chairman, Olenguruone Farmers' Co-operative Society; Agrovet Attendant, Olenguruone Farmers' co-operative society; the Chairman, Wanyororo Farmers' Co-operative Society; the Secretary, Camel Owners Association of Kenya; the Desk Officer, Smallholder Commercialization Project; the County Director, Livestock Department; and the Kenya Dairy Board Nakuru regional officer. The interviews were open with the main aim of finding their roles in the value chain, and find their views on Post-harvest losses of milk along the value chain.

Interview with the Chairman, Olenguruone Farmers' Co-operative Society

Olenguruone Farmers' cooperative was established as a farmers group with the aim of aiding the group members to market their milk at a fair price. Previously, before 1992, KCC was the only institution mandated to carry out milk collection, processing and marketing. After liberalisation of the dairy sector in 1992, many players got involved in dairy product processing. This destabilised the collection and marketing of milk. The establishment of the

co-operative was therefore aimed to help the farmers pool the milk together for cold storage and marketing. The society started with 22 farmers, collecting 800 litres of milk per day. In 2014, the society collected 7,000 litres of milk per day. It sold 4,000 litres to Brookside Kenya Limited and the rest was delivered to Happy Cow limited in Nakuru. The co-operative received milk from the farmers at a price of USD 0.26 and sold to the processor at a price of USD 0.3 per lire, returning USD 0.04 per litre.

The Farmers' co-operative also owned two Agro-Vet stores. One in Olenguruone, and another in Kaplamai. Members of the co-operative were allowed to procure items such as veterinary medicine, dairy supplements, and milk receptacles using their milk delivery as guarantee for payment. This credit facility made it easier for farmers to address challenges of input procurement.

The society also had field extension services. In 2014, it had two field extension officers who worked with farmers in addressing animal disease control, animal feeding, and animal hygiene.

The membership of the co-operative was initially made up of farmers only. However, along the way, Milk traders also registered to deliver milk to the co-operative. Some farmers also pooled milk from none members and delivered to the co-operative. The farmers thus doubled up as farmers, traders and transporters. This describes the complexities of the actors along the value chain.

The co-operative collected milk from the following locations: Amalo, Sinendet, Cheptuech, Silibwet, Kiptagich, Keringet and Kaplamai. Milk collection was within a radius of 12 km. However, some milk was delivered from the neighbouring Narok County. This was usually delivered using donkeys because of the poor terrain.

The cooling plant in Kaplamai was installed in 2013 with the aid of the Danish government. This was done to complement the cooling centre in Olenguruone, and shorten the distance taken to deliver milk to the cooling centre from the farms. The co-operative was tasked with buying land and putting up a building while the Danish government financed procuring of a cooler and setting up an Agrovet.

The co-operative did not collect any information on PHL of milk along the value chain. However, during milk reception, platform tests were done before accepting the milk for bulking. The milk, usually produced in the morning, was received between 6.30am and 12pm at the collection centre, and chilled to a temperature of 2^{o}C. The bulked milk was usually collected in the evening to be transported under the low night temperatures to Nakuru or Nairobi. The milk reaches the destination when it is at a temperature of at most 5^{o}C. It is a

routine practice to test the milk before dispatching it to the processor.

Interview Agrovet Attendant, Olenguruone Farmers' Co-Operative Society

The most important service the Agrovet offers is provision of animal feeds. These include dairy meal and mineral supplements. The price of dairy meal being USD 16 per 70Kg bag, while mineral licks cost between USD 1 and USD 2.5 per kg. Deworming drugs cost between USD 0.8 and USD 2 per dose while acaricides cost between USD 2 and USD 5 per 100ml. Artificial insemination services was priced at between USD 13- and USD 60 per piece. Apart from the Agrovet owned by Olenguruone farmers' co-operative, there were sixteen other Agrovets in Olenguruone. One of the Agrovet was owned by Brookside Dairy limited. The pricing of products and services was the same for both Brookside and the Co-operative Agrovets, and payment was either cash or check-off for those delivering milk to the coolers.

Interview with the Chairman, Wanyororo Farmers' Co-operative Society

Wanyororo Farmers' co-operative was started in 2009 with a membership of 15 members. Most of the farmers owned about 2 to 5 cows and delivering an average of 10 litres of milk each. In March 2014, the co-operative received an average of 400 litres of milk per day. The co-operative had employed two milk transporters who collected milk from the members' homesteads to deliver to the collection centre. The transporters took afferent routes to make sure all the milk was collected. About 50L of milk was delivered straight to a school and the rest of the milk delivered to the collection centre for cooling. All the milk collected for cooling was sold to consumers from the collection facility. The co-operative had employed a dairy technologist from Naivasha Dairy Training Institute to develop milk products, and different flavours of yoghurt were already being sold to consumers. The co-operative was pursuing licensing by the Kenya Dairy Board to be a mini dairy. Similarly, it was working with Kenya Bureau of Standards to be certified to market their products.

The challenges the co-operative faced include inability to get quality artificial insemination services, and animal feeding. Post-harvest losses was not reported because the group sold all the milk that it received. Inco-operation of value addition by processing yoghurt was viewed as a way to preserve and add value to milk especially during the period of glut.

Interview with the Secretary, Camel Owners Association of Kenya

The major regions of milk production in Isiolo were Melbishimi, Mlango, Ngaremare, Chumvi, Nderemara, Attam, Kulamawe, Gotu and Kachuru. The main destination for the milk was Isiolo town. During wet season, most of the milk is sourced from areas close to Isiolo town such as Mlango while during the dry season, milk supply to Isiolo is sourced as far as Kula Mawe which is 70 km from Isiolo town.

The actors along the value chain are usually Individuals who own camels**.** These individuals transport the milk to Isiolo town, where it is cooled overnight and transported to Eastleigh, Nairobi the following morning. This accounts for about 1,000 litres per day, and 3,000 litres per day of milk per day during the dry and rainy season respectively.

Milking of camels is usually done twice in a day. The first milking is done between 4.30am and 6.00am, and the second milking is done between 8.00am and 9.00am. During the rainy season, milking is done at midnight, and early morning. The milk then leaves *boma* at 8.00am, and it reaches Isiolo by 10.00am. In Isiolo town, most of the milk is delivered to Anolei women group collection centre and Tawakal women group, where it is cooled. The rest of the milk is sold at the 'milk stage' in Isiolo town. This milk is not cooled. The milk is sold to households and hotels within Isiolo town. Some of the milk is also sold by the roadside in Isiolo town.

The main co-operative societies in Isiolo were Anolei Women group, Tawakal women group, and Isiolo Camel co-operative. Membership of Anolei women group is only women while Tawakal have a few men as members. Isiolo camel co-operative activities had been diminished by the growing influence of Anolei Women Group. The reason for the dominance of Anolei women group is because of two main reasons. The first is that according to Somali culture, camels belong to the men, who are the heads of the household. The milk, on the other hand is owned by women. Anolei women group also received funding and support from non-governmental organizations such as SNV.

An estimated 99% of the milk collected and chilled in Isiolo is delivered to Eastleigh in Nairobi. The other 1% ends up in Nakuru, Meru and Eldoret. The suppliers to the co-operative are paid on weekly basis. It is the producers who pay the transporters for milk delivery to the co-operative. For some families, a member of the family transport milk to the co-operative bulking centre. During dry season, motorbikes are the common modes of transport while vehicle transport (Land Rovers and Toyota Land Cruisers) are used in wet season. Buses are the means of transport from Isiolo to Eastleigh. Milk is transported in

plastic jerricans from production to the market in Eastleigh. Cleaning of milk containers is usually done in Isiolo using piped water. Disinfection of containers is done from the boma. This is usually done by smoking the containers using *Acacia senegal*. The dry twigs of the tree are lit with fire, and the smouldering part is inserted into clean and dry containers, turning it to reach the most of the interior surface without burning it. This takes a few seconds for each container.

During the wet season, the price of milk at production was USD 0.6 per litre and rose to USD 0.8 per litre in Eastleigh; while during the dry season, the price of milk was USD 0.8 per litre at production and rose to USD 1.5 per litre in Eastleigh, Nairobi.

The challenges experienced along the value chain that contribute to milk spoilage were the terrain and lack of proper roads that made milk collection and transport difficult. The milk thus took a long time under ambient temperature to reach cooling facility in Isiolo town. This was made worse during the dry season. During dry seasons herders moved from one place to another in search of pasture and water. Some of the places they went were almost inaccessible by all modes of transport. In additional, some of the grazing areas were prone to insecurity and animal banditry. Fighting over grazing land and water for animals was common between the Somalis, who kept camels, and Samburus who are cattle and goats keepers. It was not uncommon therefore to find herders armed with AK47 rifles. As a result of the drought, milking of camels was conducted without any form of udder cleaning or hand washing. This exposed the milk to both physical and microbial contamination from both the udder surface and the surface of milking persons' hands. The pastoralists believe that smoking the milk containers extended the shelf life of milk.

Access to market was not a problem along the chain. The supply of milk hardly met the urban demand of camel milk consumers. Most of the milk delivered to Eastleigh in Nairobi was ordered by hotels and households. Some milk was sold at the roadside. The commodity is priced in such a way that 'spoilt' milk fetched the same price as fresh milk.

Unlike in the cow milk value chain, the camel milk value chain were not affected by government regulations. Essentially, Kenya Dairy Board did not cast their net over the camel milk value chain.

Interview with the Desk Officer, Smallholder Commercialization Project

The main objective for the Smallholder commercialization project was to carry out capacity building of actors within the Dairy commercialization areas. The Dairy commercialization areas are defined as areas with a milk volume of more than 90,000 litres of

milk per day. The project targeted Smallholder farmers who were members of co-operatives. The project covered Rongai and Ngata. The main problems faced by Smallholder dairy farmers was breeding due to challenges with Artificial Insemination, accessibility to markets, low prices of milk, low milk animal productivity, and animal diseases.

Interview with the County Director, Livestock Department

The major challenges facing farmers was animal diseases. The common diseases were Foot and Mouth Disease, Lumpy Skin disease and Anthrax/Blackquater. The three are notifiable diseases and controlled by vaccination. Rift Valley Fever is a periodical disease and more prevalent in wet seasons. The tick borne diseases are East coast fever, Anaplasmosis, Tick fever. These are controlled by cattle dipping. Other common diseases include milk fever for high yielding cows, grass tetani which is common during wet weather, and *Brucella arbotus* controlled by vaccination and administration of Tetracycline and Penicillin. Mastitis too is controlled by Tetracycline and Penicillin.

Interview with the Kenya Dairy Board Nakuru regional officer

The Kenya Dairy Board regional office Nakuru covers Nakuru and Baringo Counties. The role of the Kenya Dairy Board in this region is to ensure that dairy actors subscribe to the Code of practice for hygienic milk handling. The emphasis is on handling milk in a hygienic way, transportation and timely handling. And storage using the recommended receptacles. Storage of milk at low temperature is recommended. This code is simplified into modules. There is a module for producers, transporters, and processors. Regulations on bulking milk is simplified into an inspection checklist that is used to inspect the premises before it is licensed. The checklist entails the requirements for the building, equipment, personnel, and services such as water. Kenya Dairy Board advices the premise owners verbally on the requirements in the checklist. The Kenya Dairy Board officers then visit the site to check whether requirements are met, and if not advice further. The owner of the premise then agree with Kenya Dairy Board on a date for inspection. When all the conditions are met, then the premise owner can then apply to be licenced. To ensure full compliance to the regulations, every client is supposed to be inspected once in three months. Kenya Dairy Board inspectors are required to visit the clients. In case the client does not comply fully, the inspector advices accordingly. The visits are usually impromptu. Kenya Dairy Board usually has monthly schedules of premises to visit, so as to visit all the premises within their jurisdiction within three months. For minor violations such as improper record keeping or challenges of

maintaining hygiene, the client is advised on how to improve. This is noted on the inspection check list and a relevant comment for rectification is made. The violation should be found corrected on the next visit. Gross violation of the regulations, for instance intentional adulteration of milk results to charging the client according to the law. Kenya Dairy Board sometimes co-operates with officers from Public Health department to enforce the regulations.

Kenya Dairy Board found that many clients meet the requirements for purpose of licencing, thereafter they relax particularly with regard to maintenance of hygiene. This usually occurs when Kenya Dairy Board breaks for Christmas holidays. The inspections are therefore key to maintenance of milk quality. This applies to the 'formal' sector.

Kenya Dairy Board inspectors also carry out routine random inspection of vendors. Kenya Dairy Board has taken steps to bring together traders who are not licensed (illegal traders) with the aim of formalising them. The aim of Kenya Dairy Board is not to condemn them because they earn a living out of it.

There are milk collection routes that get milk into Nakuru town. These include the Dundori route, Subukia/Bahati route, Kabarak/Ravine route, Ngata/Nakuru route, Mau/Egerton/Njoro route, Lare route. Most of them don't have permits and some transport milk in plastic containers. Some of the traders do not know what the law requires of them. When Kenya Dairy Board inspectors meet them, they advise them on the requirement of the law for instance the type of containers and personal hygiene, milk handling and transportation; and occasionally test the milk. Most of the traders are young and use motorbikes. These traders have been advised to form groups. In most cases they handle small volumes of milk which are not very economical. They are therefore advised to look for a premise in town from where they can sell their milk. So, gradually they can be able to apply and get licensed. Some however are adamant and unwilling to change. They are therefore arrested and charged in court for handling milk under unsanitary conditions and lack of medical certificate. The fines range from USD 50 to USD 500. Kenya Dairy Board identifies routes that have adamant traders and charge them in court. This enhances faster compliance to the law compared to simply asking them to comply.

To be able to accomplish formalisation of the chain, Kenya Dairy Board draws an anti-hawking program every three months and presents it to the head-quarter for funding. The programme needs money because the police, usually in plain clothes, are involved in arrests. Sometimes police manning roadblocks are used for arrests. The operations are done usually once per week, but more frequent during the wet season. Every operations leads into 2 to 5

arrests. It is however not about the number who are arrested but the impact. Usually when an arrest occurs, the message gets across to those who contravene the law, and therefore they reform. This is effective having in mind that the Nakuru office only has 5 staff members, 3 of them being inspectors. In 2013, 60 offenders were apprehended and prosecuted. Usually, after charging offenders, the impact is realised for about a week. Within that period, no milk traders would be seen in town with plastic containers. After about a fortnight, a few traders recant to flouting the regulations.

Kenya Dairy Board discourages the use of ordinary plastic materials because of design and the material of construction. The design is not easy to clean while the material wears out when used for a long time. This becomes a source of contamination. Despite the arrests, some traders got to town using unconventional routes. This was however a small number. Kenya Dairy Board targeted to have almost nil use of plastic containers within the urban areas. Plastic containers however continued to be used in the rural areas because of lack of surveillance. The traders had 'excuses' for use of plastic containers. These include poor terrain leading to milk spillage, mode of transport such as motorbikes and public service vehicles (*matatus*) that have higher risks of milk spillage when using aluminium churns, and that aluminium churns do not carry as much milk as the plastic containers. The traders found the use of plastic containers convenient and cheap, but the rules were to be enforced. After enforcing the rules, the traders devised racks for the motorbikes. This enabled a trader to carry as much as six, 50L Aluminium churns (300L of milk) on a motorbike. Surveillance was more common in urban areas because of the likelihood of adulteration of milk by unscrupulous traders.

Besides the containers, Kenya Dairy Board also tests for milk quality especially adulteration. The common tests done are alcohol test, lactometer test, and clot-on-boiling. Kenya Dairy Board also takes returns of milk volumes within their jurisdiction. For the formal system, it is done from licenced actors. Usually, a record of volumes handled is kept by the licensee and submitted to Kenya Dairy Board.

At the end of the month, Kenya Dairy Board collected some levy from the dairy actors for their sustenance. This amount, also called cess, was USD 0.004 per litre and was paid to Kenya Dairy Board at the end of every month. The amount of milk traded informally could only be estimated. This could be as high as 50% of the total volume of milk handled. It is estimated that the informal sectors handle about 75% of milk countrywide but this is only about 50% in Nakuru. This is because Nakuru is at the centre and most processors compete for milk from the county making prices competitive. Some processors such as Happy Cow ltd

and Guildford dairy (Egerton) are based in Nakuru.

Essentially, we have five major processors in the country. Brookside collects about 300,000litres of milk per day in the county, and the milk processed in Ruiru. New KCC collects about 100,000L per day from the county, the milk is not processed within the county but in the surrounding counties, for instance they have a factory in Sotik (Kericho County), Nyahururu (Laikipia county), Nairobi (Nairobi city county), Eldoret (Uasin Gishu County). Nakuru County which has a New KCC factory, is only a milk collection centre. Sameer (Daima Milk) collected about 50,000L per day in the county and process the milk in Nairobi. Happy cow collects about 20,000L per day, and the milk is processed within the county into fermented milk products such as yoghurt and cheese. Guildford dairy institute (Egerton) is more of a learning institution. We also have mini dairies, cottage industries, and milk bars. Nakuru County has 6 mini-dairies. There are three in Ravine, two in molo and one in Nakuru town. The mini-dairies process a total of 10,000L of milk in a day. The cottage industry are four. These are small farm units of individuals processing cheese on the farm in Ngata, Kabarak area. The milk bars are dominant within the town. In 2013, there were 150 milk bars in Nakuru town and surrounding townships such as Molo, and Egerton. In 2014, the licensing requirements were more stringent and only about 50 milk bars were registered. This was as a result of enforcing the requirement to only sell pasteurised milk by the milk bars.

The National Food Safety committee, which Kenya Dairy Board is a member has been doing surveys for milk quality. The results of the past two years (2012 and 2013) have been alarming because of the parameters for milk quality especially bacterial loads, antibiotic levels, and adulteration. This therefore necessitated enforcing the regulation for the safety of the citizen. This requires that milk in urban centres is pasteurised. This also aimed at growing the industry where the milk bars may start packaging milk before sale.

Kenya Dairy Board also takes samples of pasteurised milk from all processors from the market from all over the country. The samples are taken to registered labs and the results relayed back to the processors. Previously fermented products like cheese had issues with coliforms while pasteurised milk had problems with shelf-life but these issues have been addressed to a great extent. However pasteurised milk still have problems with proximate parameters like butterfat and protein which are below expectation. Antibiotics were also detected in the past but this has been addressed. These surveys are usually done twice per year.

The most alarming quality parameter for raw milk is the bacterial load and presence of antibiotics especially for the smallholder farmers. The large scale farmers such as

Chemusian and Ngogongeri are able to take control of quality because of the level of investment they have. For the small scale farmers, the problem they have is bulking milk from different sources increasing the total viable count especially for the milk which is not chilled.

The terrain in Olenguruone is very rough and the use of plastic containers is very rampant. It is however favoured by the low average temperatures. Dundori is equally colder than Bahati divisions in Nakuru. Dunduri has milk all the year round but has been infested by traders (middle men). Almost half of milk delivered in Nakuru town comes from Dundori. Kenya Dairy Board has made steps in organizing the traders to acquire coolers, pasteurizers and have a premise for selling the milk. This would help transform them into the formal sector. Bahati is equally the same but sometimes it is dry and with no milk.

Kenya Dairy Board, together with the East African Dairy Development (EADD) project, designed a hygienic plastic container (*Mazican*) to be used in areas like Olenguruone but the uptake was very low. This is because of the cost, and the container was only designed for 10L capacity, which was not desirable to traders and transporters. Areas where Kenya Dairy Board has succeeded in reducing use of plastic containers, it has also worked with processors like New KCC and Brookside. The processors in this case complement Kenya Dairy Board in discouraging the use of the plastic containers. However, in Olenguruone, the processors accept milk delivered in plastic containers so long as the quality is good. However, the low ambient temperature is favourable in maintaining good quality milk

Illegal traders include individuals who transport milk commonly on motorbikes with the intention of selling in estates, shops and hotels. The shops which buy milk from the traders and sell to consumers also conduct illegal business. Some butcheries have also been found to participate in illegal sale of milk. The traders on motorbikes who are licenced are about 150 and an equal number are not licenced. The licensed traders are key to providing information to Kenya Dairy Board on which traders are not licensed. This enables creating a level playing field for the traders and enhances quality of milk sold.

Kenya Dairy Board plans to reach out to other government agencies such as county enforcement officers to help in tracking illegal traders. Once this is made possible, it would be possible to eliminate all the illegal traders.

Kenya Dairy Board is encouraging traders to acquire pasteurizers, coolers and milk dispensers. This is hygienic way of handling milk and avoids the cost of packaging which has made pasteurised milk expensive. Kenya Dairy Board advices traders to form groups of about ten people to acquire pasteurizers. This is a gradual process because of the low income levels

of some traders. Kenya Dairy Board often organises discussions with traders to encourage them to raise money to buy pasteurizers and also trains the traders on aspects of milk quality. The sale of raw milk in urban areas is prohibited by the law (Dairy Industry Act), in addition to being a licensing requirement.

Kenya Dairy Board was formed in 1958 under the Dairy Industry Act, and at that time, it was meant to serve the interests of colonial large scale farmers. Their interest was to monopolise the market. Some of the laws passed in the colonial era are not applicable today such as the registration of milk producers. The issue of milk receptacles is contentious too. It is only stated in the code of practice for hygienic milk handling but not in the Dairy Industry Act. Individuals who are prosecuted for using plastic containers as milk receptacles are charged with conveying milk under unsanitary conditions

Though the Dairy Industry Act (1958) only recognises cow milk, Kenya Dairy Board has licenced Vital Camel Milk processor in Nanyuki for processing of camel milk. A goat milk processing plant in Meru has also been licensed by Kenya Dairy Board. Kenya Dairy Board however hopes that the new Dairy Industry bill will be legislated to cater for other sources of milk such as camel and goat milk because the licence for camel and goat milk processing was not anchored on law.

Formal milk sector in Kenya is characterised by milk collection and bulking/cooling. Bulking centres are owned by co-operatives, producer groups or the processors. The formal chain is organized and lower loses are expected. The organization means that the quality is taken care of because the milk collection time and routes are known, the milk is tested for quality. For the informal sector, there is no milk testing. The person collecting the milk has no knowledge of milk hygiene, the milk is transported in plastic containers. The milk may be adulterated. The milk is not cooled despite the high ambient temperatures in Nakuru town. Higher losses in terms of milk spoilage are therefore expected. This has been corroborated by surveys done by Kenya Dairy Board on milk quality. There was however no data taken on post-harvest losses along the dairy value chain. Kenya Dairy Board targeted to increase proportion of pasteurised milk sold to 90% in two years (end of 2015).

4.3 Description of the small-holder dairy value chain in Kenya

4.3.1 Smallholder farm characteristics

Smallholder dairy farmers keep an average of 2 to 3 cows per herd. The dominant breeds are cross-breeds of local zebu with exotic breeds. The annual milk production per cow is about 1800 litres (Migose *et al.*, 2018). The animals are commonly kept on free range

(extensive) on rural farms while zero grazing (intensive) systems was common on peri-urban farms (Kashongwe *et al*., 2017). The common feeds for the cows in smallholder rural value chain was napier grass and pasture while, the smallholder peri-urban, in addition to napier, also fed the cows on concentrates such as dairy meal and mineral supplements (Migose *et al*., 2016). The farmers received veterinary services from private veterinary officers, some employed by farmers' co-operatives and milk processors, while the government plays a role mainly in mass vaccination against notifiable diseases such as foot-and-mouth disease (GOK, 2012) and quarantine of animals in the event of a disease outbreak. Farmers affiliated to Savings and credit co-operative societies such as Olenguruone farmers' co-operative society get support through provision of animal feed, and veterinary services and the expenses reconciled with the milk deliveries. The Sacco also has extension officers who train farmers on aspects of animal husbandry, animal health, and milk hygiene. Farmers who supply milk to processor-owned collection centres such as Brookside dairies also received similar services.

4.1.2 Smallholder milk production and marketing channels

In both smallholder rural and peri-urban value chains, milking is done in the morning and evening producing an average of 4.9 litres and 6 litres per cow per day for peri-urban and rural farms respectively. Morning milk was sold through various channels while evening milk (40% of daily milk produced) was mainly for home consumption and sometimes sold to neighbours. In the rural households, 36.5% of households and 43.8% of peri-urban households boiled the evening milk before storage at room temperature for use the following morning for preparation of breakfast while only 2 % of rural households, and 14.3% of peri-urban households boil, cool, and kept the milk in refrigerators overnight. The rest of the households did not boil the evening milk. The milk is left, usually on the concrete floor, taking advantage of the low night temperatures, until the following day, without undergoing spoilage.

Milk produced in the morning from the smallholder farms has three major outlets (Figure 1). The first outlet is by traders who pool the milk from different farms to deliver it to milk bars, hotels, schools, hospitals, and urban households. This comprised of 76.4% of milk produced from the peri-urban farms compared to only 14.4 % produced from the rural farms. In the second outlet, 31.5 % of the milk produced in the rural value chain was delivered to the processor-owned bulking/chilling centre, while in the third outlet, rural farms delivered 54.1% of the milk to the co-operative owned milk bulking/chilling centre at a price of USD

0.26 per litre during the study period. In the peri-urban value chain, only 23.6% of milk was delivered to the co-operative owned bulking/chilling centre as there was no processor-owned bulking/chilling centre in the study area. The co-operative paid USD 0.3 per litre of milk. On the contrary, farmers who sold to traders received USD 0.4 per litre of milk. The traders sold the milk to the milk bars at a price of between USD 0.45 and USD 0.5. The milk-bars ultimately sold the milk at between USD 0.55 to USD 0.7 depending on the buying price and location.

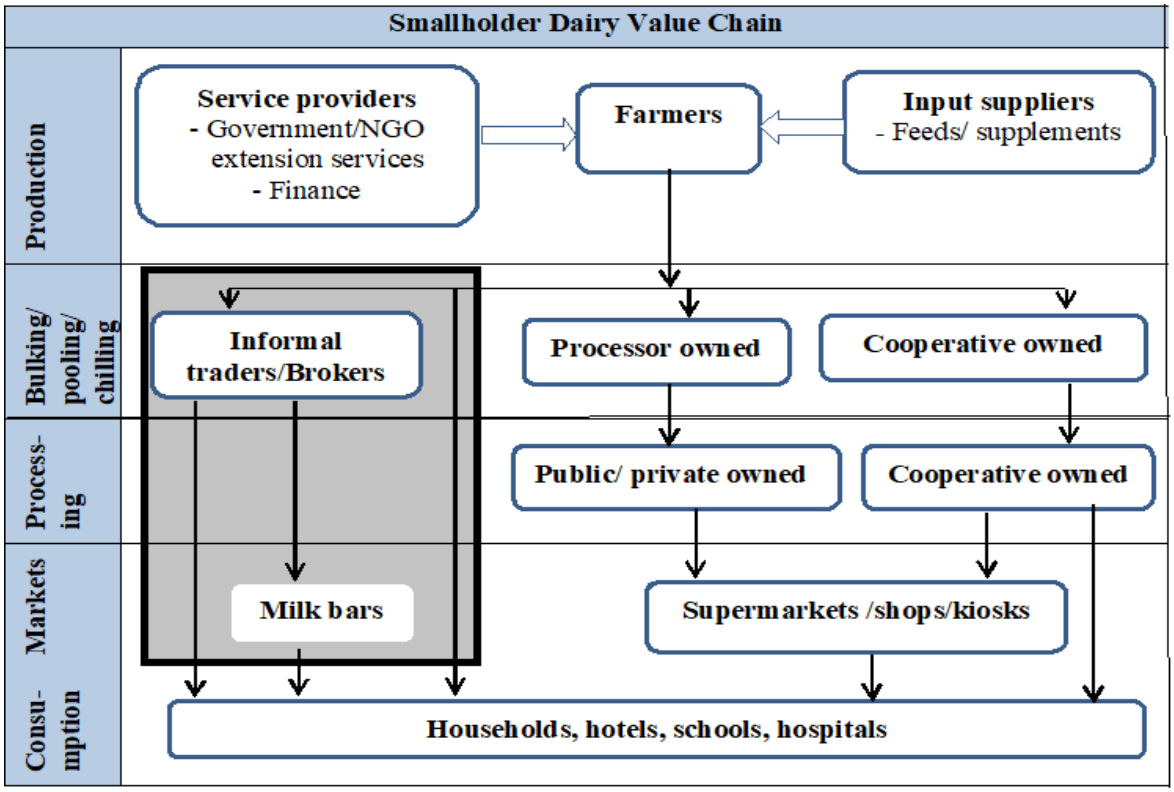

Figure 1: *General map of smallholder dairy value chain in Kenya. Adopted from GOK, 2010a, with modification.*

Informal traders and milk bars (Figure 1, grey shade) were dominant in the peri-urban chain in supplying milk to households, hotels, schools and hospitals, while most of the milk from the rural value chain was bulked for processing either by co-operatives or the processors themselves and sold through supermarkets, shops and kiosks.

Objective quality testing of milk by use of lactometer, alcohol test, or even clot-on-boiling was not carried out after milking in either peri-urban smallholder farms or rural smallholder dairy value chain. All the transporters and traders picking milk from the smallholder farms carried out only visual assessment of the milk. Judgement of milk quality was based on experience.

4.3.3 Milk collection, bulking and chilling along the smallholder dairy value chain

Milk at the processor-owned collection/ bulking/ chilling centre was received between 6.30 am and 12.30pm, and cooled to 4^{o}C in a bulk tank. The milk was then picked in the evening, at about 6.30 pm using a truck with an insulated tank with a capacity of 7,000 litres. The milk was transported at night to the processing plant, a distance of about 300km, where the milk was received at a temperature of 5^{o}C to 6^{o}C. The milk was pumped into silos, and chilled immediately to 4^{o}C awaiting processing. All the milk being delivered to the collection centres in both the smallholder rural and peri-urban value chain was subjected to platform tests before being received, and bulked with the rest of the milk in a chill tank. The temperature of the chill tank was maintained between 2^{o}C and 4^{o}C.

Milk bulked by the co-operative societies in the smallholder rural dairy value chain was sold to private-owned processors under a contract arrangement. For instance, Olenguruone farmers' co-operative society, with a capacity of more than 12,000 litres, sold its milk to Brookside Dairy Limited and Happy Cow Dairy limited. Bulking centres in the peri-urban dairy value chain, such as Wanyororo Farmers' co-operative society, with a capacity of 500 litres sold its milk directly to households, hotels, schools and hospitals. Some of the milk was processed into yoghourt at the bulking centre, and sold to consumers. Such value addition required the farmers' co-operative to register as a mini-dairy with Kenya Dairy Board, and register its product with Kenya Bureau of Standards.

4.3.4 Transportation of milk along the smallholder dairy value chain

Milk transportation from the smallholder rural farms to the bulking centres with regard to number of deliveries is dominated by motorbikes, at 57.3 % followed by delivery on foot at 20.2%, vehicles at 10.1%, donkey at 7.9% and bicycles at 4.5%. In terms of volume of milk transported, motorbikes still dominate with 66.5 % of milk delivered followed by vehicles with 30.4%, donkeys at 2.6%, foot at 0.3% and bicycles at 0.2%. In the peri-urban value chain, milk transport is similarly dominated by motorbikes at 77.2% of volume of milk transported followed by vehicle transport at 22.8% of the milk delivered to the bulking/collection centres. Milk transport to urban consumers, milk bars, hotels, schools and hospitals in the urban centre was dominated by motorbikes at 83.1% of the milk transported, and the rest delivered by vehicles. Some of the motorbikes transporting milk in the peri-urban value chain were customized to carry 50L Aluminium churns as shown in Figure 2.

A customized motorbike can thus carry as much as 200L of milk in Aluminium churns. Since this amount of milk cannot be sourced from one Smallholder farm, it is pooled from different farms. The transporter or trader has a schedule that is informally agreed upon by the farmer on the time of picking milk, and there were no formal contracts. It was observed that the responsibility of cleaning the milk containers was with the trader or transporter, but the farmer cleaned the container again before using it. The milk picking time ranged from 6am in the morning to 10.30am. The milk was either picked from the farm, or at a roadside collection centre. Pooling of milk was practiced by 64% of transporters in the smallholder rural dairy value chain and 83.2% in the smallholder peri-urban dairy value chain. None of the transporters or traders carried out objective quality tests such as alcohol test, or clot-on-boiling before pooling the milk. For some transporters and traders, suspicious milk was carried separately. It was observed that all the transporters in the peri-urban chain had a milk carriage permit from the Kenya Dairy Board while none of the transporters in the rural value chain had the milk carriage permit. From the collection centres in the Smallholder rural value chain, milk was transported to the processor, usually at night taking advantage of the low night temperatures and reduced traffic to reduce time taken on the road. The milk was transported in insulated containers to minimize temperature rise of the milk. The milk temperature on leaving the bulking centre was 2 to 3^{o}C and delivered at a temperature of 6^{o}C. At the reception of the processor, milk was subjected to alcohol test and lactometer test before being cooled again to 4^{o}C to await further processing.

Figure 2: *A customized motorbike carrier for Aluminium churns common in the peri-urban dairy value chain*

4.4 Description of the pastoral camel milk value chain

4.4.1 Pastoral *boma* characteristics

Camel *bomas* are basically temporary homes where the herders pitch their tents. A typical boma is circular with diameter of between 15 m to 20 m (Figure. 3) that may be fenced, usually with acacia twigs. Camels rest in the *bomas* overnight and released in the morning to graze. Camel milking is carried out in *boma*s, usually twice every morning. The first milking is done at about 6am in the morning and the second milking done at 8.00am in the morning. The *boma* would hold between 50 to 100 camels either belonging to one person or a number of people. Several people may put their camels into one herd to take advantage of shared herdsmen, milking persons, and also for security reasons. The *bomas* are generally under-resourced with no permanent dwelling because they always move in search of pasture, water, and security. The *bomas* do not have sanitary facilities for use by the herders. The herders receive basic requirements such as food and water from the milk transporters. The water is hardly enough for cooking and cleaning up. There is therefore no water for either hand cleaning or cleaning the udder before milking. Milk let down from lactating camels is stimulated by calf suckling. Suckling also softens the teats to enable milking. The average

milk production per camel per day as reported by Kashongwe (2017) who carried out a concurrent study in the same study area was 2.1 litres.

Figure 3: Camels resting in a kraal (boma) in Isiolo County

4.4.2 Pastoral camel milk production and marketing channels

The camel milk value chain is summarized in Figure 4

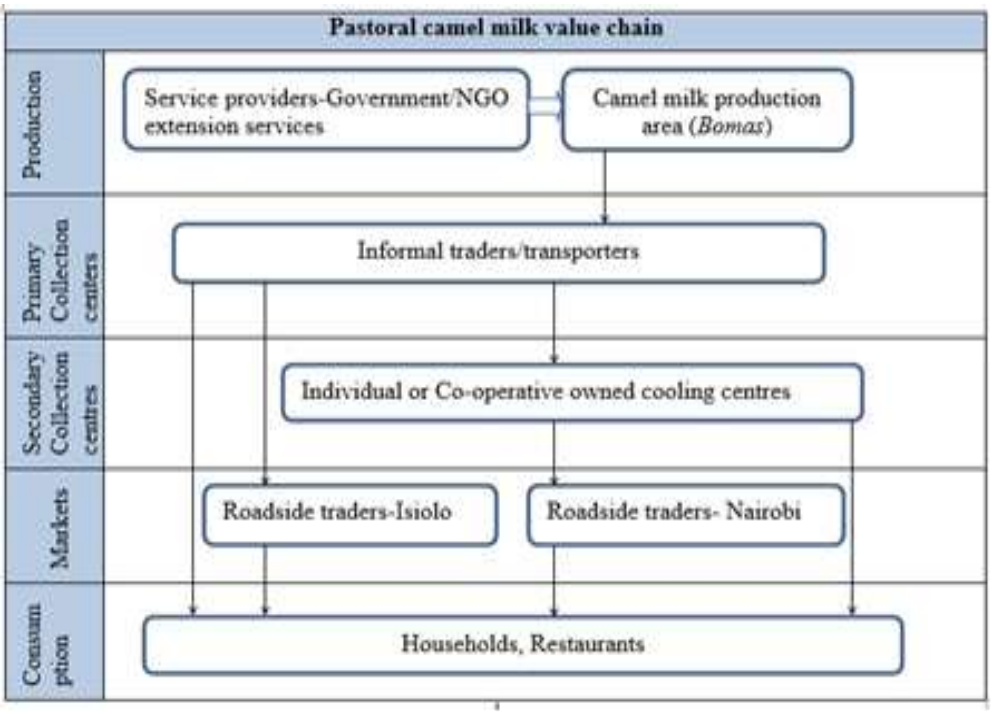

Figure 4: Pastoral Dairy value chain for camel milk from Isiolo County

The major milk producing areas in Isiolo were Burat2, Kulamawe, Shaab, Harakote, Ngaremare, Laglava, Dahayal, Shambani, Mlango, Gotu, Malbi Shilmi, Kobi Fora and Elhat. The main stakeholders involved in the value chain were camel herd owners, cooperative societies, transporters, traders, County veterinary office, and Non-governmental organizations particularly SNV which had supported Anolei Women group to procure milk coolers, and was involved in training the group members on milk quality. The camel herd owners employ herders to look after the camels. For the health of the camels, government extension officers were available from the county offices to provide vaccination and treatment. Non-governmental organizations also offered support in animal health. The herd sizes ranged from about ten camels to hundreds of camels.

The lactating camels were milked twice in the morning, the first milking was usually carried out between 4.30am and 6.00am and the second milking carried out between 8.00am and 9.00am. After milking, the milk was first bulked at the *boma/kraal*, and put under a shade. Milk for the herd owners' home consumption was separated from that to be marketed. The milk was then usually taken to a nearby collection centre, now referred as the primary collection centre, where transporters could easily pick it. This milk was then collected by

transporters either using motorbikes or vehicle transport and taken either to the bulking centres in Isiolo, or to the Isiolo 'milk stage'. At the milk stage, road side traders would buy the milk for resale along the streets of Isiolo town for household use. Some of the milk sold is fresh, while the rest could be *suusa* (sweet sour), *kulel* (bitter sour) or *orawa-kandi* (tastes in between *suusa* and *kulel*).

4.4.3 Milk collection, bulking and chilling

Most of the marketed milk was bulked at the group owned Secondary collection centres run by Anolei women group and Tawakal group. Anolei women group handled up to 2500 litres per day while Tawakal handled 500 litres per day. On arrival at these collection centres, the milk was tested for alcohol stability and bulked in a chill tank at 4^{o}C. Milk that failed the test was not bulked but cooled in its container in a separate cooler. The milk would then be sold as *suusa, kulel* or *orawa-kandi*. The milk bulked in the chill tank would be emptied again into plastic receptacles the following day at 5.00am and loaded on buses for transport at ambient temperature to Nairobi, a journey of about 7 hours. In Nairobi's Eastleigh estate, milk traders received the milk for sale to home consumers, hotels and restaurants within Nairobi. Occasionally, some milk was transported to other towns such as Nakuru. Secondary milk collection centres run by individuals handle between 20L to 100L per day. The operators cool the milk and sale it to traders within Isiolo, hotels, restaurants, and home consumers.

4.4.4 Transportation of camel milk along the pastoral dairy value chain

For the milk sampled in this study, 67% was transported on foot to primary collection centres. Motorbikes were dominant at 42% and 50% in delivering milk to secondary collection centre and to Isiolo roadside traders respectively. Milk was also delivered using vehicles to the 'Milk stage' within Isiolo town where operators picked the milk using wheelbarrows to deliver the milk to the secondary collection centres, a distance of about 1km. This represented 33% of the milk delivered to the secondary collection centres. Only Public service vehicles were available to transport milk to Nairobi, and subsequently to other towns such as Nakuru, Eldoret, Mombasa, and Kampala in Uganda.

4.5 Microbial quality of milk along the Smallholder dairy value chain and associated risk factors

4.5.1 Microbial loads in raw milk along the Smallholder dairy value chain in Kenya

Microbial counts in milk along the value chain is shown in Table 6. There were no milk bars and milk traders along the rural dairy value chain, except a few farmers who were co- operative society members and sourced milk from non-members to supply to the society.

Table 6: *Total Viable Counts and Coliform Counts (Log_{10} cfu/ml) in milk along the Smallholder Rural and Peri-urban (P-urban) value chains*

Microbial type	Value chain	Node along the value chain			
		Udder milk	Collection centre	Milk bar	Traders
TVC	Rural	3.58±0.52	6.22±0.37	…….	.....
	P-Urban	4.70±0.80	6.64±0.31	5.37±1.34	6.38±0.61
CC	Rural	2.85±0.70	6.07±0.33	.....	……..
	P-urban	4.19±0.71	6.42±0.55	4.72±1.73	6.02±1.20

Comparison for the level of differences for microbial counts (TVC and CC) in peri-urban and rural udder milk is shown in Figure 5 while differences in the microbial counts along the chain are shown in Figure 6.

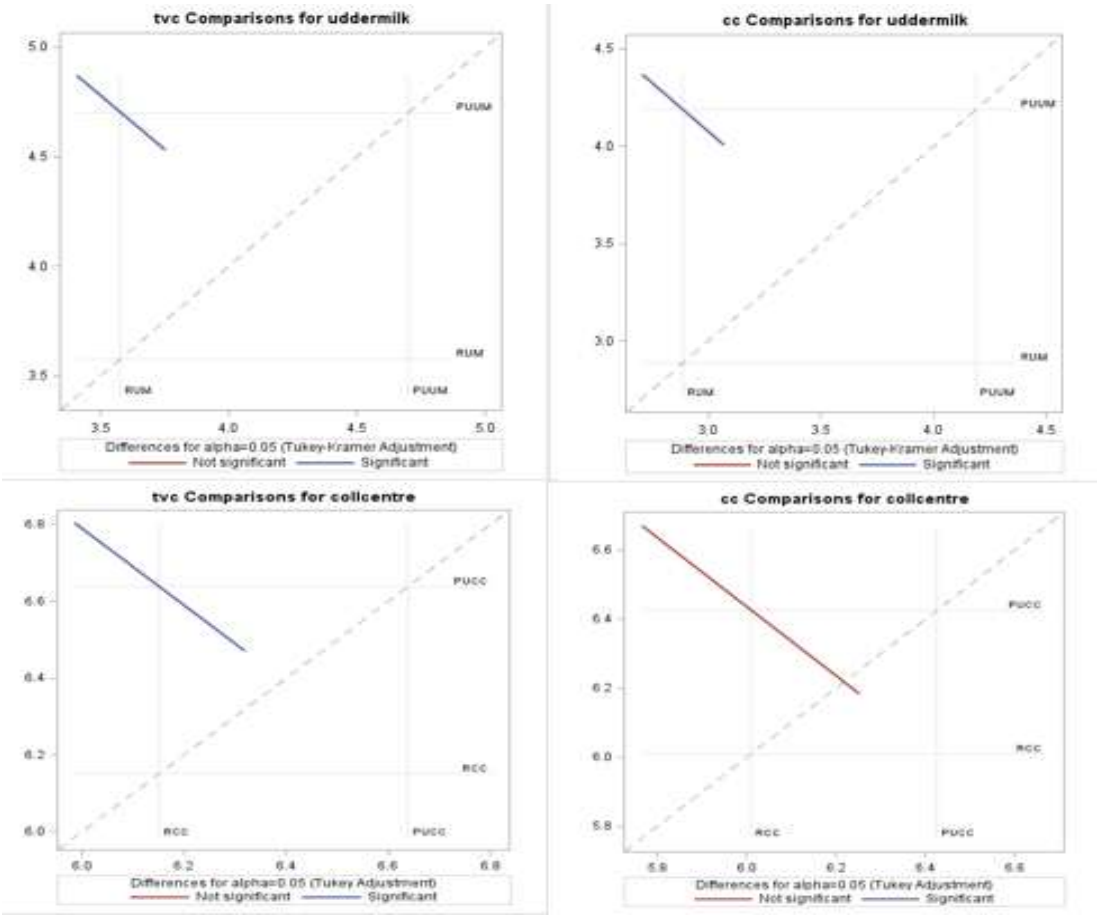

Figure 5: *Least Square mean comparisons (Tukey-Kramer) for TVC and CC in peri-urban udder milk (PUUM) and rural udder milk (RUM) showing differences in microbial counts (Log_{10} cfu/ml)*

TVC and CC in Rural udder milk (RUM) was significantly different from that of Peri-urban udder milk (PUUM). Similarly, TVC in milk in Rural Collection centres (RCC) was significantly different from that in the Peri-urban collection centres (PUCC). However, there was no significant difference between CC in milk in Rural Collection Centres (RCC) and Peri-urban collection centres (PUCC).

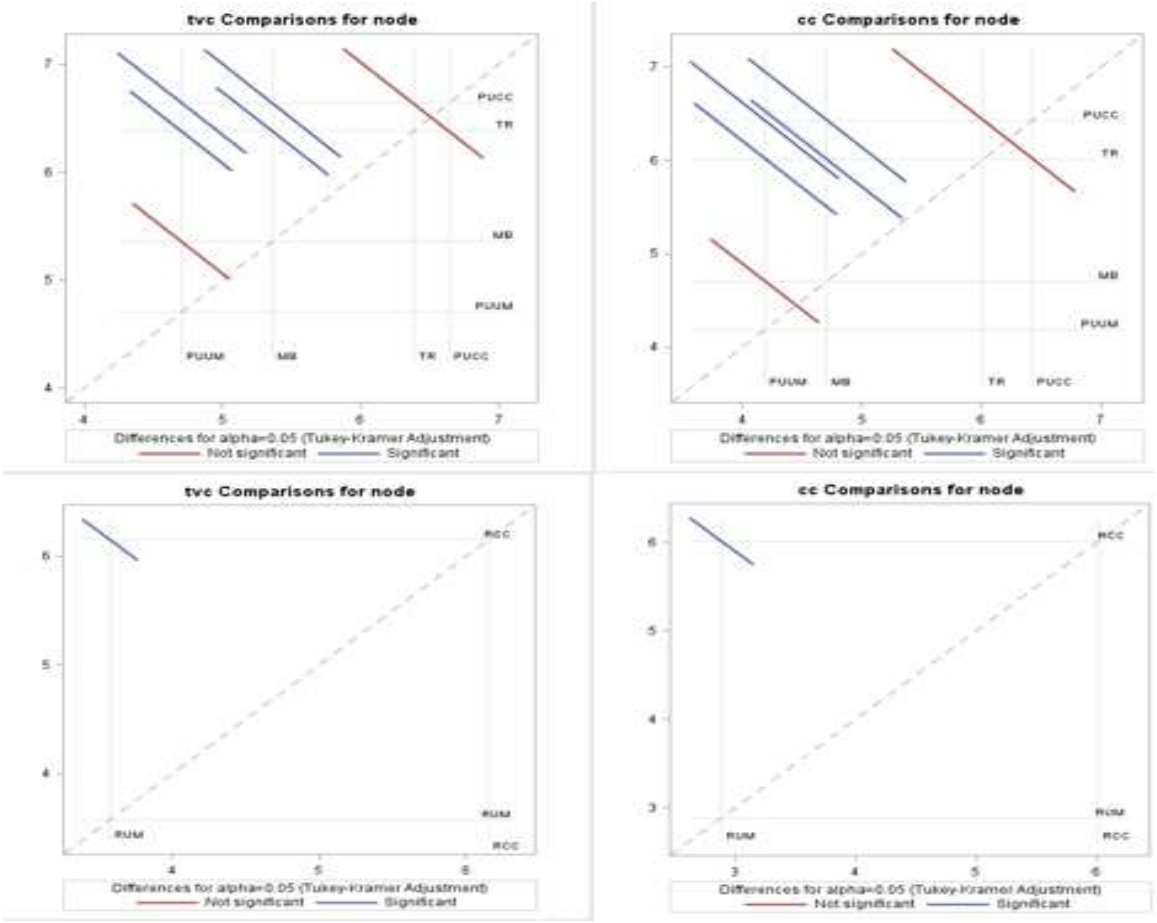

Figure 6: *Least Square mean comparisons (Tukey-Kramer) for TVC and CC along Peri-urban and rural DVC showing differences in microbial counts (log_{10} cfu/ml)*

Key: PUCC- milk from Peri-urban collection centres, MB- milk from milk bars, TR- milk from traders, RUM- udder milk from rural farms, RCC- milk from rural collection centres.

There was no significant difference between counts for both TVC and CC between Peri-urban collection centres and Traders; and Peri-urban udder milk and Milk bars. Counts between the rest of the nodes were significantly different. For the Rural DVC, there was a

significant difference between counts for TVC and CC between Rural udder milk and smallholder rural collection centres.

4.5.2 Compliance of cow milk along the smallholder dairy value chain in Kenya to East African quality specifications

Milk from the smallholder value chain was tested for compliance to East African quality standards (EAS, 2006a) and the results are shown in Table 7 and 8. Milk that had TVC count> 2,000,000 and CC> 50,000 was designated 'poor' and considered as not suitable for processing. It is this proportion of milk that was also considered to have been lost post-harvest, because according to the Dairy Industry Act and the Public Health Act, this milk is supposed to be disposed. Loss hot spots were the nodes along the value chain that experienced highest post-harvest loss. Along the Smallholder rural value chain, based on TVC, the loss hot spots were the collection centres, while along the Smallholder peri-urban value chain the loss hot spots were the traders delivering milk to the urban markets. However, based on coliform counts, there was 100% PHL loss at the collection centre for both the Smallholder rural value chain and the Smallholder Peri-urban value chain. The collection centres were thus the loss hot spots for the two value chains based on coliform counts, which are indicators of hygiene in milk handling. Chi-square test for independence was significant at 0.05 level of significance indicating that quality differences were significantly different along the chain. There was higher compliance of milk along the rural DVC compared to Peri-urban DVC. Milk that was neither in Grade I, II, or III was of poor quality and considered as Post Harvest Loss (PHL) of milk along the value chain. The result shows better compliance to standards for milk in the Rural DVC compared to the Peri-Urban DVC based on CC. Milk designated as 'Poor' represents Post Harvest Loss (PHL) of milk.

Table 7: *Compliance of raw milk (% by volume) along the smallholder peri-urban and rural DVC based on TVC*

Grade	DVC	Nodes along the Smallholder Peri-urban and rural DVC chain				
		Udder milk	Collection centre	Milk-bar	Traders	Mean
I	Peri-urban	77.20	0.00	20.00	0.00	24.30
	Rural	100.00	0.00	-	-	50.00
II	Peri-urban	16.13	0.00	60.00	29.41	26.40
	Rural	0.00	55.56	-	-	27.80
III	Peri-urban	0.00	11.11	20.00	29.41	15.10
	Rural	0.00	22.22	-	-	11.10

Poor PHL)	Peri-urban	6.45	88.89	0.00	41.18	34.10
	Rural	0.00	22.22	-	-	11.10

Chi-square test for independence was significant at 0.05 level of significance.

Table 8: *Compliance of raw milk (% by volume) along the smallholder peri-urban and Rural DVC based on CC according to EAC standards*

Grade	DVC	Nodes along the Smallholder Peri-urban and Rural DVC chain				
		Udder milk	Collection centre	Milk-bar	Traders	Mean
Very good	Peri-urban	3.23	0.00	10.00	0.00	3.30
	Rural	53.13	0.00	-	-	26.60
Good	Peri-urban	80.65	0.00	20.00	22.22	30.70
	Rural	46.88	0.00	-	-	23.40
Poor	Peri-urban	16.13	100.00	70.00	77.78	66.00
	Rural	0.00	100.00	-	-	50.00

Chi-square test for independence was significant at 0.05 level of significance.

Data on pasteurised market milk was taken from four brands representing a large co-operative, a state-owned processor, a small private processor, and a large-scale processor. TVC and CC is shown in figure 7. The mean TVC and CC for pasteurised milk in Kenya was 5.65±2.3 and 1.02±1.5 respectively. The four brands were tested for compliance against East African standards for pasteurised milk (EAS, 2006b) which require pasteurised milk to have TVC and CC of less than 10,000 and 10 cfu/ml respectively. None of the brands fully complied with the EAC standards. The level of compliance is shown in Figure 8.

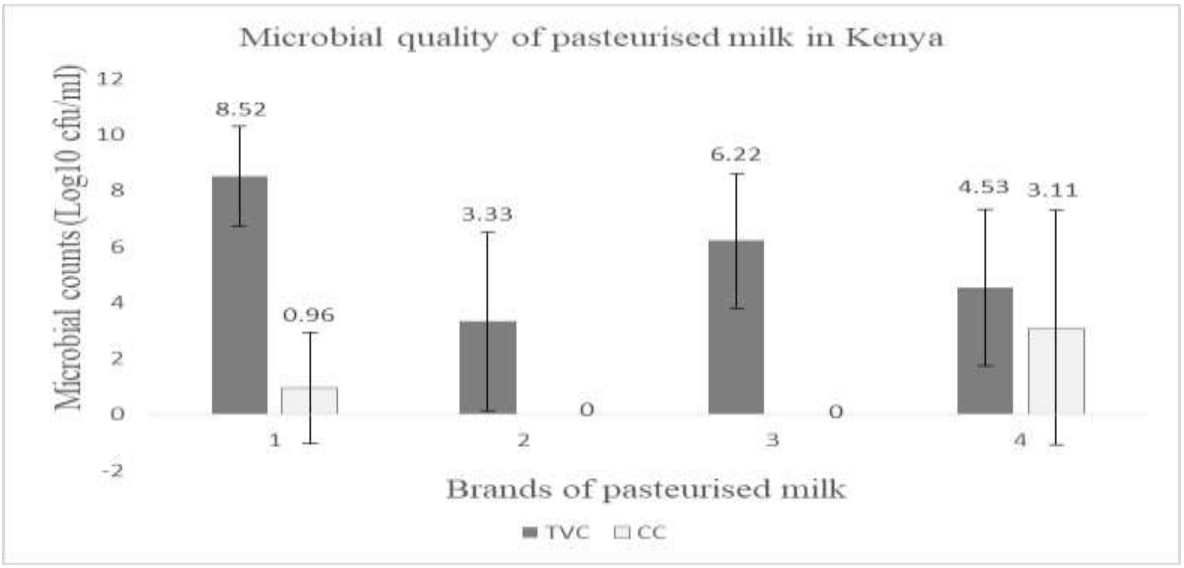

Figure 7: *Microbial quality of different brands of pasteurized milk from different processors in Kenya*

Key: (1) Fresha (Large co-operative), (2) (KCC) State owned processor, (3) Kinangop (Small private processor), and (4) Molo (Large private processor).

The result in Figure 8 shows pasteurised milk for a large co-operative having high TVC while that of a large processor having high CC. The state-owned processor recorded nil CC counts.

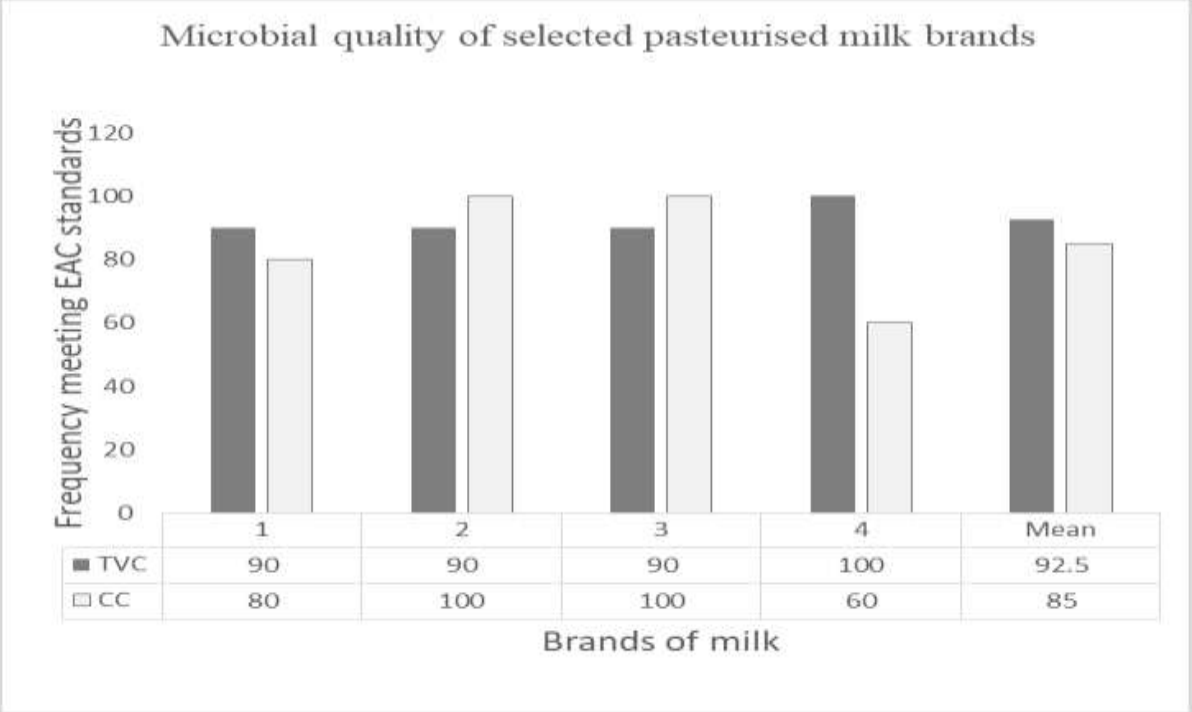

Figure 8: *Quality compliance of selected pasteurized milk brands from different processors to EAC standards based on TVC and CC*

Key: (1) Fresha (Large co-operative), (2) (KCC) State owned processor, (3) Kinangop (Small private processor), and (4) Molo (Large private processor)

The result in Figure 8 shows 100% compliance for State-owned processor and the Small private processor based on CC. The Large private processor had a 100% compliance based on TVC but only 60% compliance based on CC. On average, based on CC, 15% of pasteurised milk does not meet quality standards and is considered lost post-harvest.

4.5.3 Risk factors and their relationship to microbial loads in milk along the Smallholder dairy value chain in Kenya

a) Effect of milk receptacles on microbial loads of milk during transport

The milk receptacles used along the rural dairy value chain were mainly ordinary plastic containers with 88.6% of operators, followed with hygienic plastic containers

(popularly known as *Mazicans)* at 6.8% of operators, and aluminium churns at 4.6% of the operators (Figure 9; Table 9). However, along the peri-urban value chain, only aluminium churns were in use. The microbial counts are shown in Table 9.

Figure 9: *Milk receptacles used along the value chain. From left: Ordinary plastic, Hygienic plastic, and Aluminium churn*

Table 9: *Microbial counts in milk from transporters associated with milk container type along rural and Peri-urban dairy value chains*

Value chain	Container type	% of users	TVC±SD	CC±SD
Rural	Ordinary plastic	88.6	4.80 ± 1.4^{b}	4.30 ± 1.8^{b}
	Hygienic plastic	6.8	4.60 ± 0.8^{c}	4.00 ± 1.1^{c}
	Aluminium churns	4.6	4.50 ± 0.6^{c}	4.00 ± 0.9^{c}
Peri-Urban	Aluminium churns	100.0	6.64 ± 0.3^{a}	6.07 ± 0.3^{a}

Mean ± SD in the same column with the same superscript indicate no significant difference at $p\leq 0.05$

Milk in Aluminium churns had a lower count compared to milk in ordinary plastics in the rural DVC.

b) Effect of water used for milking preparation and cleaning milk receptacles on microbial loads in milk

The microbial counts in milk with regard to source of water for both rural and peri-urban value chains is shown in Table 10. The water sources used in the rural value chain were rain water, river water, water from shallow wells, or a combination of sources of water. In the peri-urban value chain, the water source was mainly rain water and treated tap water. A combination of sources was used by 5.7% and 28.1% in rural and peri-urban dairy households respectively, and these were excluded from the study. Similarly, in the rural small

holder DVC, milk transporters reported use of rain water, water from shallow wells, river water, or a combination of different types of water for cleaning milk containers while in the peri-urban dairy value chain, rain water and treated tap water were the only sources.

Table 10: *Microbial counts (Log_{10} TVC or CC) in milk based on farm water used for cleaning the udder, washing of personnel hands, and washing milk containers in both rural and Peri-urban dairy value chains*

Value Chain	Node along the value chain							
	Farm level				Transporters			
	Water source	% of users	TVC±SD	CC±SD	Water source	% of users	TVC±SD	CC±SD
Rural	Rain	64.7	3.44±1.2	2.98 ±1.5	Rain	61.8	6.13± 0.4	4.03±1.8
	River	30.6	3.59±0.4	3.01 ±1.4	River	6.6	6.31 ±1.4	4.33±1.4
	Well	4.7	3.53±0.5	2.88± 0.5	Well	31.6	6.26± 0.2	4.85± 1.3
Peri-Urban	Tap	52.3	4.72±0.4	4.21 ±1.4	Tap	92.3	6.53± 1.1	3.99± 0.4
	Rain	47.7	4.91±0.7	4.13±0.5	Rain	7.7	6.69 ±0.8	4.04± 1.1

The result in Table 10 shows that water sources had no effect on the microbial counts of milk at farm level and during transport in both the Rural and Peri-urban DVC at 95% confidence level.

c) Effect of time on microbial loads of milk along the Smallholder dairy value chain

In the Small holder rural dairy value chain, the mean duration of time to deliver milk to the collection centre was ranged from 10 minutes for the farms which were close to the collection centre to almost 8 hours for farms that were far from the collection centre. This was also dependent on the terrain. The relationship between time of delivery for milk deliveries and microbial counts is shown in Figure 10. The result shows that there is no significant change of microbial counts with time (p=0.7113).

d) Effect of milk temperature on microbial load along the Smallholder Peri-urban dairy value chain

Along the smallholder peri-urban DVC, the relationship between microbial loads and temperature of milk from traders and milk-bars is shown in Figure 11. The R-square is 0.11 while the mean temperature for the milk during sampling was 20.9±5.5^{o}C, with an environmental temperature of 28.1±3.1 ^{o}C. The milk at the milk-bars and from traders was

sold to urban consumers and hotels the same day.

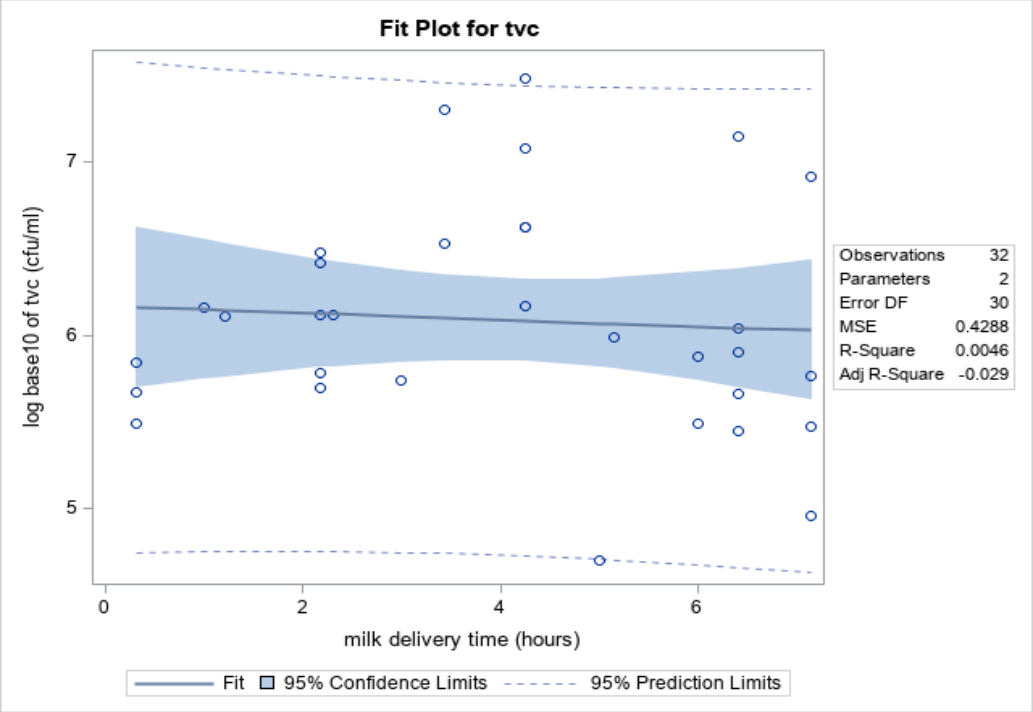

Figure 10: *Fit plot for effect of time of delivery of milk to collection centres in the smallholder rural value chain on TVC of the milk*

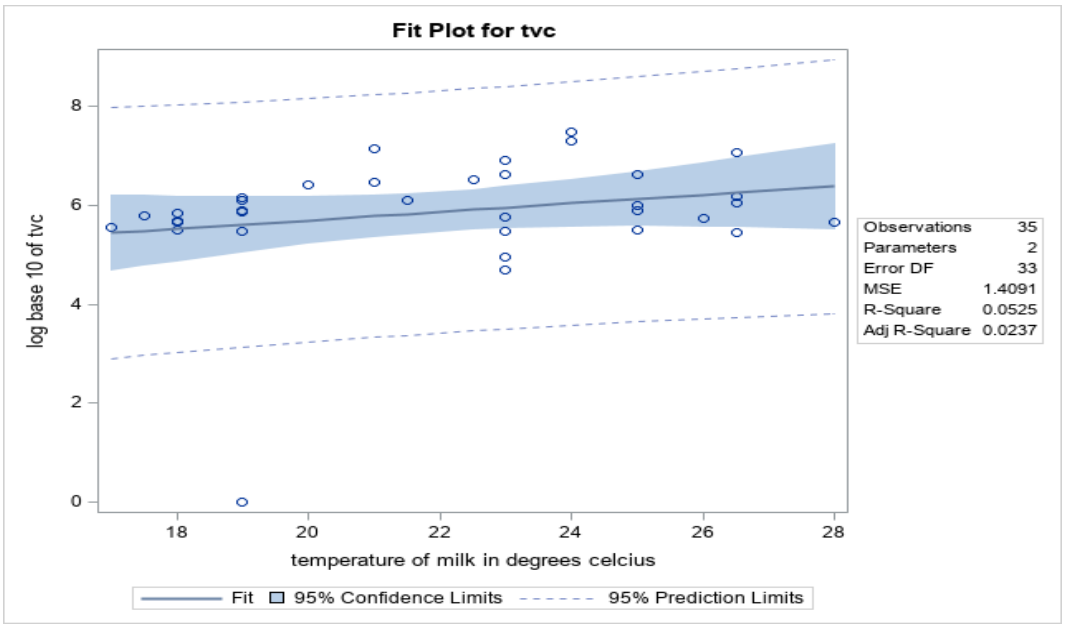

Figure 11: *Fit plot for effect of milk temperature during sampling on microbial count for milk traded in Nakuru town*

The microbial counts increase with temperature of milk delivered to milk-bars by traders though the relationship is not significant, with p= 0.1857.

e) Effect of Volume of milk handled on microbial loads in milk along the dairy value chain

The relationship between milk volume in a receptacle and microbial load of milk from traders in the smallholder peri-urban dairy value chain is shown in fig 12. R-square= 0.0083 at 95% confidence limit, p = 0.6374. The microbial load reduces with increase in milk volume, though the relationship is not significant

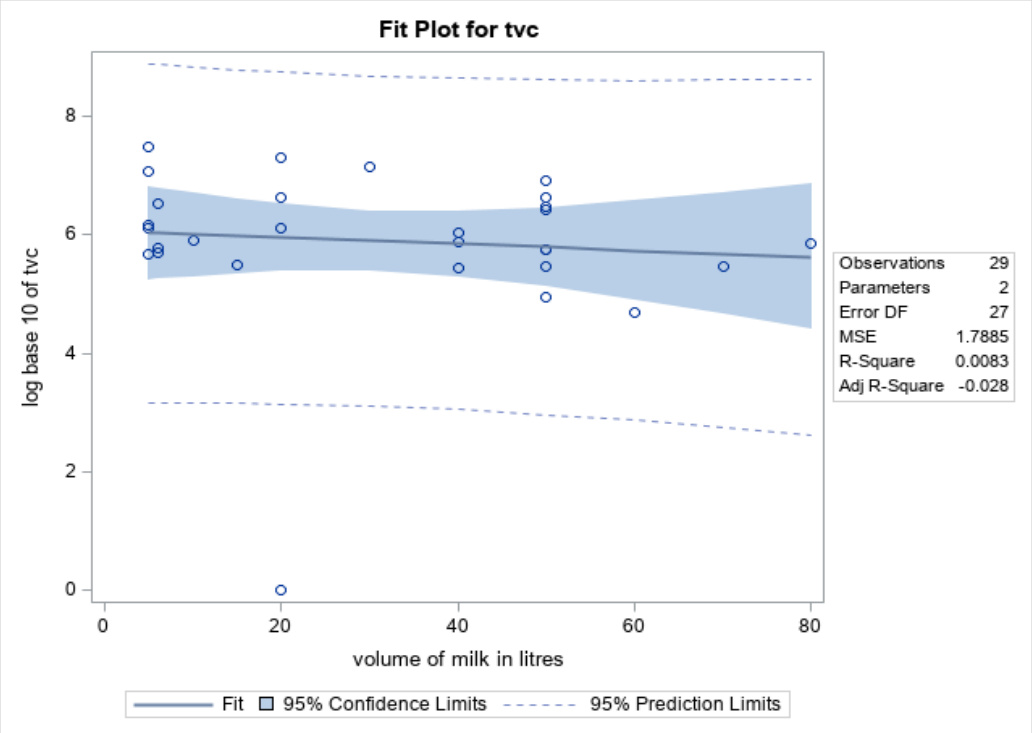

Figure 12: Fit plot for relationship between microbial counts and the volume of milk handled by traders

f) Effect of training of milk handlers on microbial load of milk

In the smallholder rural dairy value chain, none of milking persons had received formal training on milk quality while only 14.3% in the peri-urban household had received formal training on milk quality. In addition, all the transporters in the peri-urban value chain had been trained on milk quality. The effect of training on microbial counts is shown in Table 11. Training of milk handlers had significant impact on microbial counts only at production, but not along the value chain.

Table 11: *Effect of training of milk handlers on microbial counts (Log_{10}) of milk along the smallholder value chain*

DVC	Training	Frequency %	TVC±SD	CC±SD
Peri-Urban (Production)	Trained	14.3	4.42 ± 0.30^{b}	3.9 ± 0.6^{b}
	Not trained	85.7	4.93 ± 0.60^{a}	4.6 ± 1.1^{a}
Rural (Transporters)	Trained	63.8	6.32 ± 0.32^{a}	4.3 ± 0.4^{a}
	Not trained	36.2	6.11 ± 0.41^{a}	4.5 ± 1.2^{a}

4.6 Microbial quality of milk along the pastoral dairy value chain and associated risk factors

4.6.1 Microbial loads in milk along the pastoral dairy value chain

Total viable counts (TVC) of milk from individual camels increased significantly from 4.91±1.04 at production to 6.49±0.77 at secondary collection centre and 7.52±1.32 at the Nairobi market. Coliform count (CC) at production was 3.68±1.28 which also increased significantly to 6.42±1.13 at Nairobi market. Milk bulked at the *boma* had TVC of 5.09±0.60 and CC of 3.44±1.52, both of which increased significantly at the Secondary collection centre and Nairobi market.

At the Primary collection centre TVC and CC were 5.09±0.60 and 3.14±0.93 respectively. These increased significantly during transport of milk to the Secondary collection centre and to Nairobi market. All the counts increased significantly from the secondary collection centre to the Nairobi market. The microbial load along the chain from production to Nairobi market is summarized in Table 12 and significance differences shown clearly in Figure 11. The microbial loads along the pastoral DVC at production and boma bulk were not significant from each other, but were lower than the rest of the nodes. The counts were highest at the Nairobi market.

Table 12: *Microbial loads ($Log_{10,}$ Mean ±SD) along the camel milk value chain*

	Production	*Boma* bulk	Collection centres		Nairobi market
			Primary	Secondary	
TVC	4.91±1.04	5.09±0.60	5.58±1.15	6.49±0.77	7.52±1.32
CC	3.68±1.28	3.44±1.52	3.14±0.93	4.81± 0.51	6.42±1.13

There was no significant difference in both TVC and CC for milk at Production, boma bulk and primary collection centres. There was however a significant increase in TVC from

Primary collection centre to secondary collection centre to the Nairobi market. For coliform counts, there was no significant increase in counts up to the secondary collection centre. The counts increased significantly to the Nairobi market.

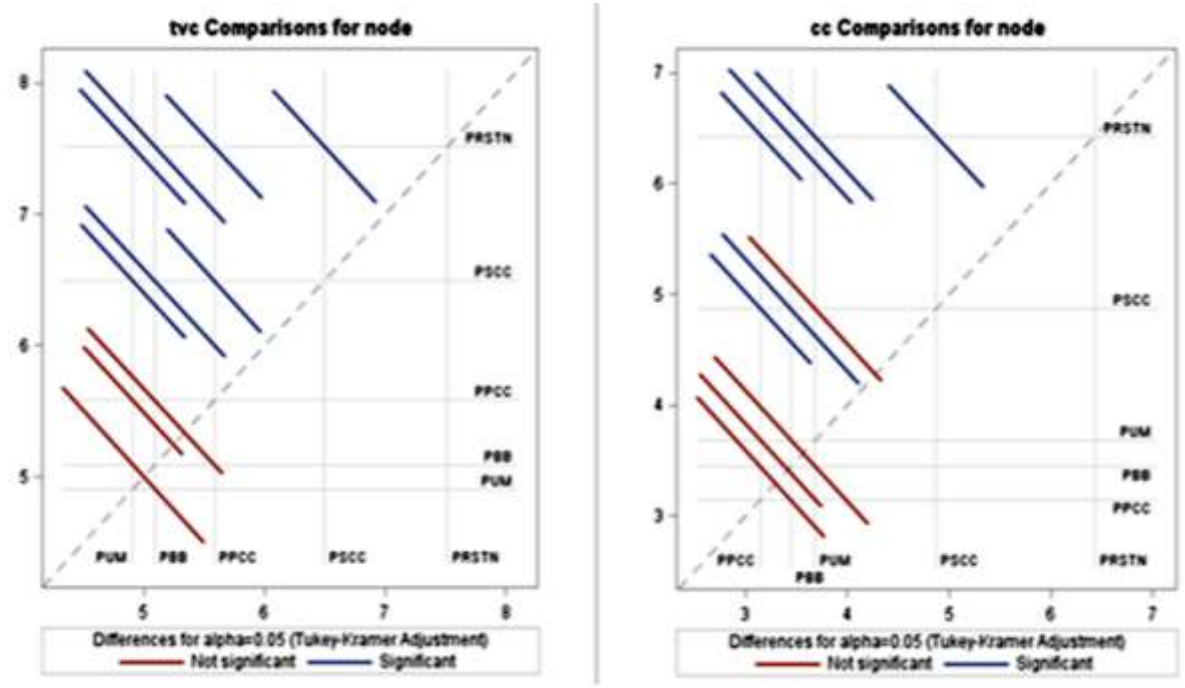

Figure 13. *Least Square mean comparisons (Tukey-Kramer) for TVC and CC in milk along the pastoral DVC at alpha = 0.05*

Key: PUM- pastoral udder milk; PBB- pastoral boma bulk; PPCC- pastoral primary collection centre; PSCC- Pastoral secondary collection centre; PRSTN -Pastoral road side traders in Nairobi. The units for X and Y axes are $\log_{10}$ microbial counts. Intersection of counts on a blue cross-section indicates the difference between the counts not significant but red line shows the differences are significant.

4.6.2 Compliance of camel milk quality to Kenya Standards

Compliance of camel milk along the value chain to Kenya Standards for raw whole camel milk (Kenya Bureau of Standards, 2007) is summarised in Table 13. The proportion of milk that met the standard at production (PUM) based on TVC and CC was the same at 78%. The proportion was equally close at 88.9 % for TVC and 87.5% for CC for *boma* bulked milk. At the Primary collection centre, the proportion that met the standard was 41.4% and 82.3% for TVC and CC respectively. Only 16% and 37.8 % of the milk delivered to secondary collection centres met the standard based on TVC and CC respectively. This further reduced to 8% and 5.4 % at the Nairobi roadside market. On average, 40% of the milk along the chain did not meet the standard based on CC, and categorised as poor. The

proportion that was 'poor' was considered as lost post-harvest. The node where this proportion was highest was considered the loss hot spot. Along the pastoral dairy value chain, the loss hot spot was the Nairobi market where 91.6% and 94.6% was lost post-harvest based on TVC and CC respectively. The average post-harvest loss based on coliform count, as an indicator of hygiene was 39.9%.

Table 13: *Compliance (%) of raw camel milk along the Pastoral DVC based on TVC and CC according to Kenya standards*

Grade	DVC	Nodes along the Pastoral DVC chain					
		PUM	PBB	PPCC	PSCC	PRSTN	Mean
I	TVC	68.9	66.7	29.2	16.0	4.2	37.0
	CC	22.2	37.5	57.7	6.3	0.0	24.7
II	TVC	9.1	22.2	12.2	0.0	4.2	9.5
	CC	55.7	50.0	34.6	31.5	5.4	35.4
Poor (PHL)	TVC	22.0	11.1	58.6	84.0	91.6	53.5
	CC	22.2	12.5	7.7	62.5	94.6	39.9

Key: PUM -udder milk; PBB- *boma* bulk milk; PPCC- Primary collection centre; PSCC- Secondary collection centre; PRSTN- Roadside traders in Nairobi. Chi-square test for independence was significant at 0.05 level of significance.

4.6.3 Predisposing factors for increased microbial counts in milk along the Pastoral dairy value chain

a) General hygiene (udder hygiene, milking personnel hygiene, milking container type and cleanliness, and excessive human handling of milk)

During the study period, there was neither hand washing nor udder cleaning before milking the camels. All the milk containers were made of non-food grade plastic with non-hygienic design that were difficult to clean and sanitize (Appendix G). The containers were previously used for vegetable oil. At the secondary collection centres, group members were involved in all activities from milk reception to record taking, predisposing the milk to further microbial contamination due to excessive human handling. None of the actors handling milk had a medical certificate as required by the Public Health Act, thus predisposing consumers of the milk to risk of infection.

The pastoral milk production area had no water supply, apart from water that was brought by milk traders from Isiolo town. This water was hardly enough for cooking or

drinking, let alone any form of hygiene. All the milk containers were cleaned in either Isiolo town or Nairobi city using running water and soap. The containers were then smoked for disinfection using wood from, especially acacia trees. Although these factors influence microbial loads in milk, they were the same in the study area, and their effect on microbial load in the milk could not be determined from this survey.

b) Effect of holding time and temperature on microbial counts

Milking was done between 6.00am and 8.30am and the milk delivered to the primary collection centre where the milk was picked by transporters or traders to the subsequent nodes along the value chain. The mean pick up time was 10.58am ± 28 minutes. The average delivery time to the secondary collection centre was 11.47am±1h 40 minutes with a range from 10.15am to 6.33pm. On the day of sampling, 200 litres of milk from Kulamawe which is 80 km from Isiolo town had not been delivered by 7.00pm because the vehicle had mechanical problems, which was said not to be unusual. The collected milk is bulked in a chill tank and cooled overnight to 4^{o}C. At 5.00am the following morning, the milk was filled into plastic containers and loaded onto buses for transportation to Nairobi where it is delivered at about 11.30am. However, during the study, 2500 litres of milk from one secondary collection centre (Anolei women group) had not been dispatched to Nairobi because of disagreement on pricing between the owners of milk at the collection centre and the agents in Nairobi. Due to limited capacity of refrigeration, half the volume of milk was not refrigerated but still transported to Nairobi with a delay of 24 hours. Although this is not a common occurrence, it demonstrates the challenges along the value chain that would lead to high microbial count in milk and eventually milk loss through spoilage.

The mean ambient temperature along the chain from production to secondary collection centre was 30.8±1.04 ^{o}C with a minimum of 28^{o}C and a maximum of 32.5^{o}C while the milk temperature had a mean of 30.7±1.4^{o}C with a minimum of 28^{o}C and a maximum of 35^{o}C. The milk at the secondary collection centre is then cooled to 4^{o}C in a bulk tank. It is then returned to the plastic containers and transported to Nairobi where the milk temperature rises again to 17.8±2.7^{o}C. The ambient temperature during milk sampling in Nairobi was 22.5 ^{o}C.

There was a strong correlation between ambient and milk temperature (adj R^2=0.90181, p<0.0001) and therefore, only data on milk temperature was used for modelling in this study. For microbial loads, correlation between TVC and CC was strong with adj. R^2=0.54233 and *p*=0.0004. Only data on TVC was therefore used in further statistical

analysis.

The correlation between time and milk temperature was equally strong (adj. R^2=0.84158, p<0.0001). However, due to their importance in microbial growth, each was subjected to simple regression analysis with TVC. The relationship between TVC and time was found to be significant (Adj. R^2= 0.1473, p=0.0045) (Fig 14); while that between TVC and temperature was found not to be significant (adj. R^2=0.0527, p=0.073).

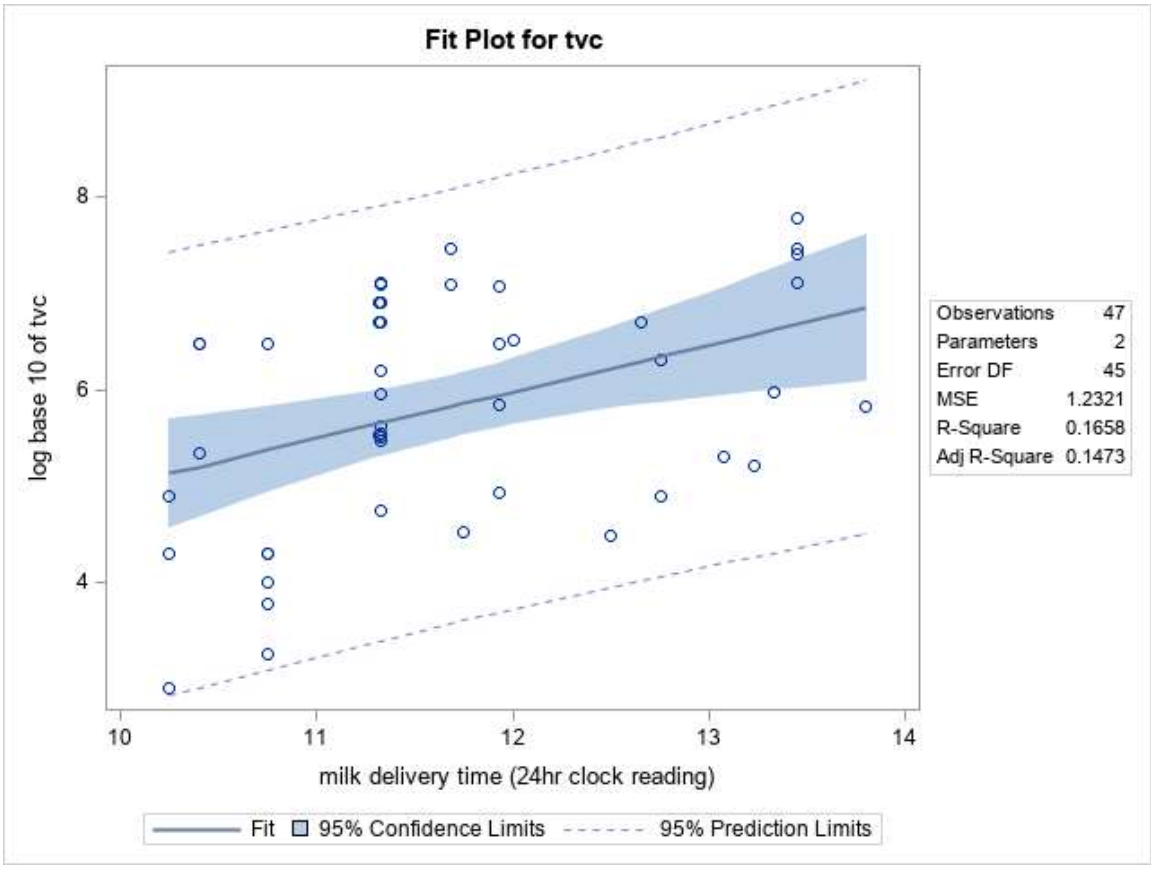

Figure 14: Fit plot for relationship between microbial counts and time of milk delivery to the secondary collection centre in Isiolo town

c) Effect of milk volume on microbial counts

Along the pastoral dairy value chain, from production to the secondary collection centre, the relationship between TVC and the volume of milk contained in a receptacle was not significant with adj.R^2 = 0.015 and p = 0.4117.

d) Effect of level of training of milk handlers on microbial counts

At the production level, none of the operators had any training on milk quality. These were the same operators who transported milk to the primary collection centre. At the secondary collection centre, 44% of the operators had training on milk quality. The mean TVC of the milk handled by those who were trained on milk quality was 6.68 ±0.74 while

those who had not been trained had a mean of 6.18 ±0.71. The counts were however not statistically different with $p = 0.06154$.

4.7 Role of regulatory agencies

The Kenya Dairy Board, established under the Dairy Industry Act, 1958, is the main regulatory body for milk marketing in Kenya. The role of Kenya Dairy Board is outlined in section 17 of the Dairy Industry Act. These functions include organising, regulating and developing an efficient supply chain for milk and dairy products, improving the quality of dairy produce, secure reasonable and stable prices for milk, promote market research with regard to milk, and to promote enterprise in the dairy sector. It may perform these functions on its own or in collaboration with other government agencies (Dairy Industry Act, Cap 336). However, it was observed that Kenya Dairy Board to a large extent carries out policing of the operators, and there was a lot of fear mixed with contempt for Kenya Dairy Board by the operators in the Smallholder peri-urban dairy value chain. The Board had only 3 inspectors for the whole of Nakuru and Baringo counties which was not sufficient of it to carry out its mandate.

4.8 Biochemical profiles and nutrient utilization patterns of coliforms isolated from milk along the dairy value chain in Kenya

4.8.1 Selection of microbes for analysis of biochemical profiles

E. coli, *E. cloacae*, *K. pneumoniae* and *K. oxytoca* made up 58.2% of the 244 culturable isolates of the Enterobacteriaceae family identified by API20E kit (Table 14). *Citrobacter* spp which is part of the coliform group made up only 3.28% with *C. braakii*, *C. freundii*, and *C. youngae* recording 1.64, 0.82 and 0.82% respectively. The biochemical profiles of the four species were thus analysed.

Table 14: *Frequency of coliforms isolated from camel and cow milk as a percentage of culturable Enterobacteriaceae isolates. E. cloacae was the most isolated coliform*

Coliform	Camel milk isolates		Cow milk isolates		Total isolates	
	N	%	*n*	%	*n*	%
E. coli	28	11.48	15	6.15	43	17.6
E. cloacae	26	10.66	22	9.02	48	19.6
K. pneumoniae	15	6.14	8	3.28	23	9.4
K. oxytoca	13	5.33	15	6.14	28	11.5
Total	82	33.6	60	24.6	**142**	**58.2**

4.8.2 Biochemical profile of *Escherichia coli* isolated from camel and cow milk

The results of the biochemical tests showed that four tests for *E. coli* isolates from camel and cow milk were significantly different. These were Arginine dihydrolase (ADH), Lysine decarboxylase (LDC), Ornithine decarboxylase (ODC) and Rhamnose fermentation/oxidation (RHA). Tests for isolates from camel milk that were different from manufactures notice were O-Nitrophenyl-β-D-galactopyranoside (ONPG), Arginine dihydrolase (ADH), Lysine decarboxylase (LDC), Indole production (IND), Sorbitol fermentation /oxidation (SOR), Sucrose fermentation/oxidation (SAC), and Melibiose fermentation/oxidation (MEL) while isolates from cow milk that were different from manufacturers notice were O-Nitrophenyl-β-D-galactopyranoside (ONPG), Arginine dihydrolase (ADH), Indole production (IND), Sorbitol fermentation /oxidation (SOR), Rhamnose fermentation/oxidation (RHA), Sucrose fermentation/oxidation (SAC), and Melibiose fermentation/oxidation (MEL). Ornithine decarboxylase (ODC) was the only test that was significantly different in isolates from camel and cow milk but not from manufacturers notice. Lysine decarboxylase (LDC) was only test that was significantly different between camel and manufacturers notice but not cow milk isolates and manufacturers notice. Rhamnose fermentation/oxidation (RHA) was the only test that was significantly different between cow and manufacturers notice and not camel and manufacturer's notice. This is summarized in Table 15.

4.8.3 Biochemical profile of *Enterobacter cloacae* isolated from camel and cow milk

Results of the biochemical profiles of *Enterobacter cloacae* from milk are shown in Table 16. In this group of microbes, the frequency of positive results for seven tests were different from the manufacturer's notice. Significantly more *Enterobacter cloacae* isolates were positive for Arginine dihydrolase (ADH), and cow milk isolates had high proportion of Lysine decarboxylase (LDC). A significantly higher number of isolates were positive for Sorbitol fermentation /oxidation (SOR), Sucrose fermentation/oxidation (SAC) and Melibiose fermentation/oxidation (MEL). The rest of the test results were not significantly different from what was estimated from the API20E test kit. All isolates from camel and cow milk were negative for Inositol fermentation/oxidation (INO) against the manufactures notice of 12%.

4.8.4 Biochemical profile of *Klebsiella pneumoniae* isolated from camel and cow milk

Results of the biochemical profiles of *Klebsiella pneumoniae* microbes from milk are shown in Table 17. The frequency of positive results for four tests were different from what is estimated by the API20E test kit. Significantly more *Klebsiella pneumoniae* isolates were positive for Arginine dihydrolase (ADH), Ornithine decarboxylase (ODC), and Citrate utilization (CIT). A significantly lower number of isolates fermented/oxidised glucose. The rest of the test results were not significantly different from the manufacturers notice.

Table 15: *Biochemical profiles of E. coli isolates from camel and cow milk*

Biochemical Test	Biochemical activity for isolates from milk		Manufacturers notice	*P value*[a]	*P value*[b]	*P value*[c]
	Camel	Cow				
ONPG	100	100	90	-	0.012*	0.012*
ADH	7.1	20	1	0.0077*	0.0287*	<0.0001*
LDC	96.4	80	74	0.0003*	0.0001*	0.3134
ODC	78.6	60	70	0.0044*	0.164	0.1382
CIT	0	0	0	-	-	-
H_2S	0	0	1	-	0.3161	0.3161
URE	0	0	3	-	0.0810	0.0810
TDA	0	0	0	-	-	-
IND	100	100	89	-	0.0006*	0.0006*
VP	0	0	0	-	-	-
GEL	0	0	0	-	-	-
GLU	100	100	99	-	0.3161	0.3161
MAN	100	100	98	-	0.1552	0.1552
INO	0	0	1	-	0.3161	0.3161
SOR	100	100	91	-	0.0021*	0.0021*
RHA	71.4	93.3	82	<0.0001*	0.0762	0.0152*
SAC	64.3	60	36	0.5347	<0.0001*	0.0007*
MEL	100	100	75	-	<0.0001*	<0.0001*
AMY	0	6.7	3	0.0085	0.0810	0.2233
ARA	100	100	99	-	0.3161	0.3161

P value with * indicates significant difference between obtained and expected populations at p<0.05. *P value*[a] *: P value* for difference between camel and cow milk. *P value*[b]*: P value* for

difference between camel milk and expected proportion. .*P value*[c]*: P value* for difference between cow milk and expected proportion.

Table 16: *Biochemical profiles of Enterobacter cloacae isolates from camel and cow milk*

Biochemical Test	Biochemical activity for isolates from milk		Manufacturers notice	*P value*[a]	*P value*[b]	*P value*[c]
	Camel	Cow				
ONPG	100	100	98	-	0.1552	0.1552
ADH	96.2	95.5	82	0.8040	0.0013*	0.0025*
LDC	3.9	18.2	1	0.0013*	0.1847	<0.001*
ODC	96.2	95.5	92	0.8040	0.2075	0.3066
CIT	96.2	86.4	90	0.0856	0.0837	0.4301
H_2S	0	0	0	-	-	-
URE	0	4.6	1	0.0300*	0.3161	0.1228
TDA	0	0	0	-	-	-
IND	0	0	0	-	-	-
VP	92.3	77.3	85	0.0031*	0.1037	0.1639
GEL	0	0	0	-	-	-
GLU	88.5	95.5	99	0.0681	0.1302	0.0022*
MAN	100	100	99	-	0.3161	0.3161
INO	0	0	12	-	0.0004*	0.0004*
SOR	100	100	90	-	0.0092*	0.0092*
RHA	80.8	86.4	85	0.2849	0.4302	0.7773
SAC	100	100	96	-	0.0434*	0.0434*
MEL	92.3	100	90	0.0047*	0.5669	0.0012*
AMY	100	100	99	-	0.3161	0.3161
ARA	100	100	99	-	0.3161	0.3161

P value with * indicates significant difference between obtained and expected populations at $p<0.05$. *P value*[a]*: P value* for difference between camel and cow milk. *P value*[b]*: P value* for difference between camel milk and expected proportion. *P value*[c]*: P value* for difference between cow milk and expected proportion.

Table 17: *Biochemical profiles of Klebsiella pneumoniae isolates from camel and cow milk*

Biochemical Test	Biochemical activity for isolates from milk		Manufacturers notice	*P* value[a]	*P value*[b]	*P value*[c]
	Camel	Cow				
ONPG	100	100	99	-	0.3161	0.3161
ADH	0	0	0	-	-	-
LDC	80	87.5	73	0.1506	0.2431	0.0100*
ODC	0	0	0	-	-	-
CIT	93.3	100	86	0.0085*	0.0902	0.0001*
H_2S	0	0	0	-	-	-
URE	86.7	100	75	0.0002*	0.0355*	<0.0001*
TDA	0	0	0	-	-	-
IND	0	0	0	-	-	-
VP	80	87.5	90	0.1506	0.0477*	0.5759
GEL	0	0	0	-	-	-
GLU	86.7	100	100	0.0002*	0.0002*	-
MAN	100	100	99	-	0.3161	0.3161
INO	86.7	100	99	0.0002*	0.0007*	0.3161
SOR	100	100	99	-	0.3161	0.3161
RHA	86.7	100	99	0.0002*	0.3161	0.3161
SAC	100	100	99	-	0.3161	0.3161
MEL	100	100	99	-	0.3161	0.3161
AMY	100	100	99	-	0.3161	0.3161
ARA	100	100	99	-	0.3161	0.3161

P value with * indicates significant difference between obtained and expected populations at $p<0.05$. *P value*[a]*: P value* for difference between camel and cow milk. *P value*[b]*: P value* for difference between camel milk and expected proportion. *P value*[c]*: P value* for difference between cow milk and expected proportion.

Table 18: *Biochemical profiles of Klebsiella oxytoca isolates from camel and cow milk*

Biochemical Test	Biochemical activity for isolates from milk		Manufacturer's notice	*P* value[a]	*P value*[b]	*P value*[c]
	Camel	Cow				
ONPG	100	100	100	-	-	-
ADH	0	0	0	-	-	-
LDC	92.3	100	99	0.0047*	0.0202*	0.3161
ODC	0	0	0	-	-	-
CIT	92.3	73.3	89	0.0004*	0.4228	0.0045*
H_2S	0	0	0	-	-	-
URE	76.9	13.3	78	<0.0001*	0.8524	<0.0001*
TDA	0	0	0	-	-	-
IND	100	100	99	-	0.3161	0.3161
VP	92.2	86.7	80	0.2055	0.0186	0.2035
GEL	0	0	0	-	-	-
GLU	100	100	100	-	-	-
MAN	100	100	100	-	-	-
INO	92.3	86.7	99	0.1965	0.0202*	0.0007*
SOR	100	100	99	-	0.3161	0.3161
RHA	100	100	100	-	-	-
SAC	100	100	100	-	-	-
MEL	100	100	99	-	0.3161	0.3161
AMY	100	100	100	-	-	-
ARA	100	100	100	-	-	-

P value with * indicates significant difference between obtained and expected populations at $p<0.05$. *P value*[a]*: P value* for difference between camel and cow milk. *P value*[b]*: P value* for difference between camel milk and expected proportion. *P value*[c]*: P value* for difference between cow milk and expected proportion.

4.8.5 Biochemical profile of *Klebsiella oxytoca* isolated from camel and cow milk

Biochemical results for isolates from camel milk that tested positive for LDC were significantly lower than isolates from cow and manufactures notice. For CIT, camel milk isolates that tested positive were significantly higher than those of cow milk while those from

cow milk were significantly lower than the manufacturers notice. Isolates from camel milk were not significantly different from those of manufacturers notice. For URE, isolates from camel milk were significantly different from those of cow milk but not from manufacturers notice. Isolates from cow milk were significantly lower than those from both camel milk and manufacturers notice. Test for INO had isolates from both camel and cow milk being significantly lower than the manufacturer's notice, but not significantly different from each other. This is summarized in Table 17.

4.9 Phylogenetic distribution of coliforms along the dairy value chain in Kenya

4.9.1 Phylogenetic distribution of *Escherichia coli* along the dairy value chain in Kenya

E. coli isolates was in four clades (Figure 15). The clade with the largest number of isolates also had the type species, *E coli* ATCC 25922 (Appendix W). This isolate was misidentified as *Salmonella arizonae* with API20E code 7344552. The clade had isolates from both camel and cow milk, udder swabs, pasteurized milk and yoghurt. Equally, the rest of the clades had isolates from all sources cultured.

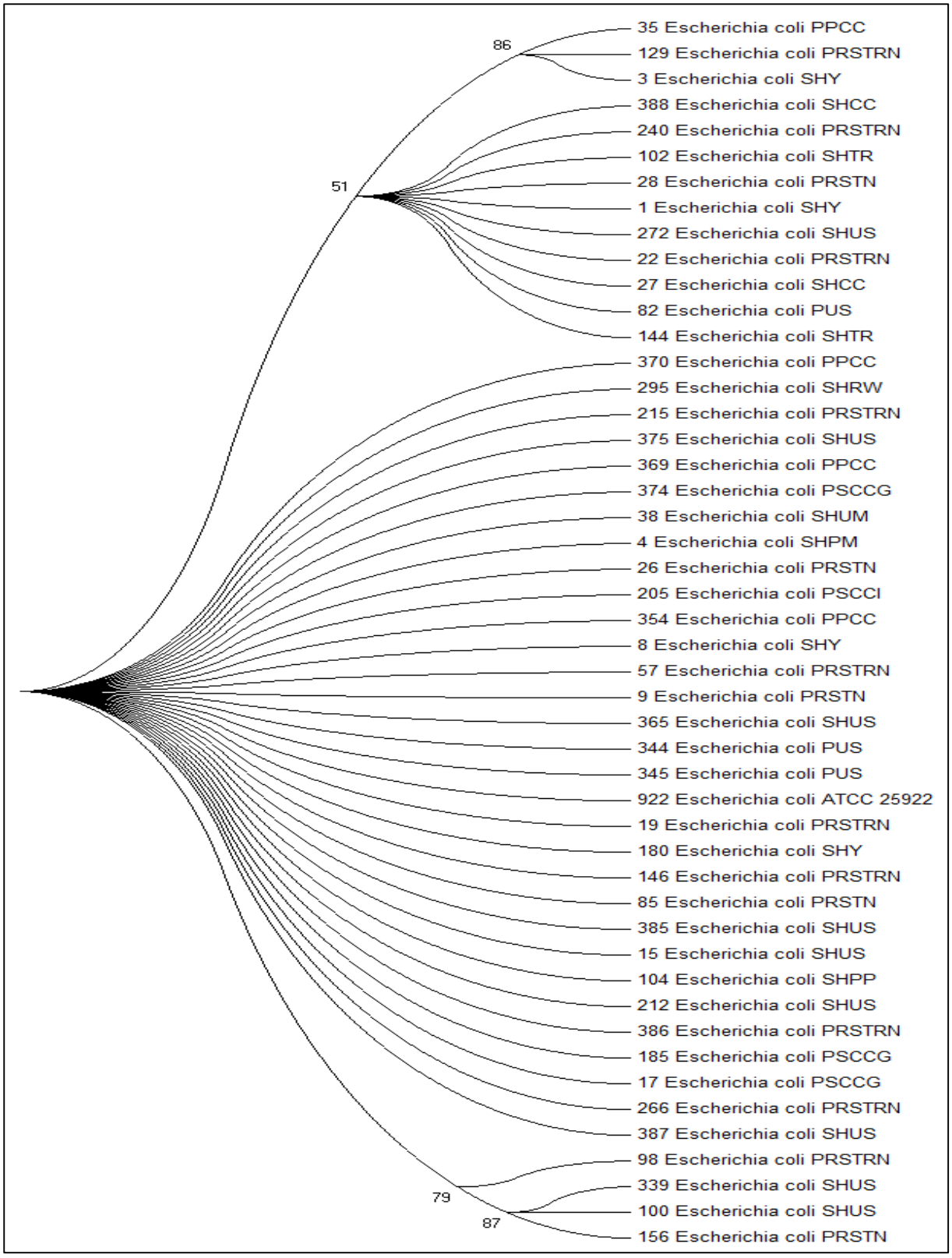

Figure 15: *Escherichia coli phylogeny and distribution along the smallholder and pastoral dairy value chain*

4.9.2 Phylogenetic distribution of *Enterobacter cloacae* along the dairy value chain in Kenya

E. cloacae had five clades (Figure 16). The first clade had only two isolates from the cow udder swab while two other clades had isolates only from the pastoral value chain. Another clade had isolates from all sources. E. hormochei from the smallholder cow milk collection centre was among the isolates in this clade. The clade that follows also had E. hormochei but all the isolates were isoated from camel milk. One clade had Enterobcter cloacae phylogenetic group ony from the Smallhder DVC while another only from the Pastoral DVC.

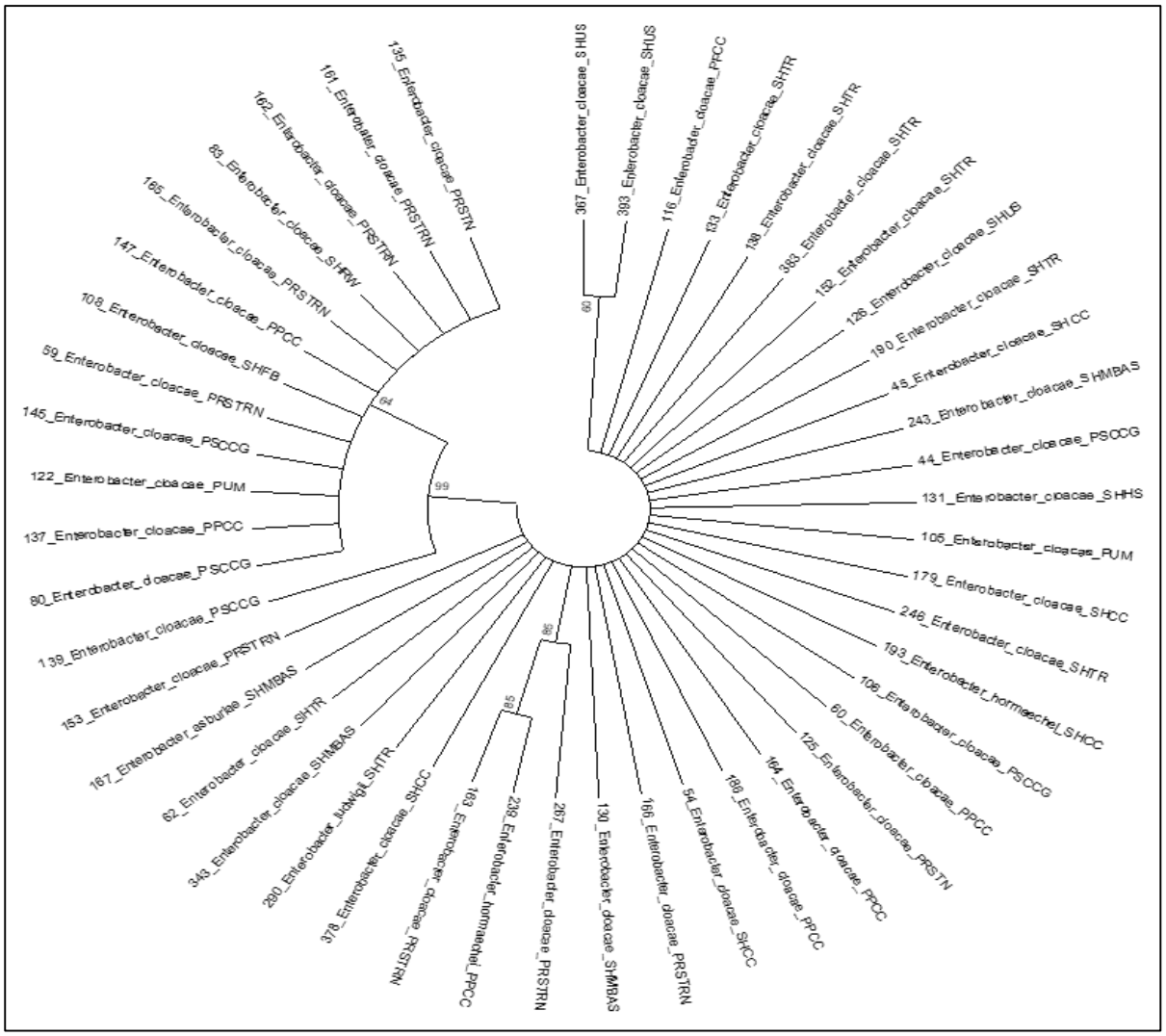

Figure 16: *Enterobcter cloacae phylogeny and distribution along the smallholder and pastoral dairy value chain.*

4.9.3 Phylogenetic distribution of *Klebsiella spp.* along the dairy value chain in Kenya

The microbes in this species were *K. pneumoniae, K. oxytica, K. variicola, and Raoultella* spp. (Figure 17). These were grouped into seven clades. Three clades were occupied by *K. pneumoniae,* two clades by *K. oxytoca*, and one by *Raoultella* spp. The largest clade had *K. pneumoniae* and *K. variicola*. The distribution of isolates based on sources was random. Two clades however had isolates only from camel milk. Also, two clades had *Klebsiella oxytoca*, both found in Smallhoder and Pastroal DVCs. One clade had Klebsiella oxytoca, in which all isolates were distributed in both Smallholder and Pastoral DVCs

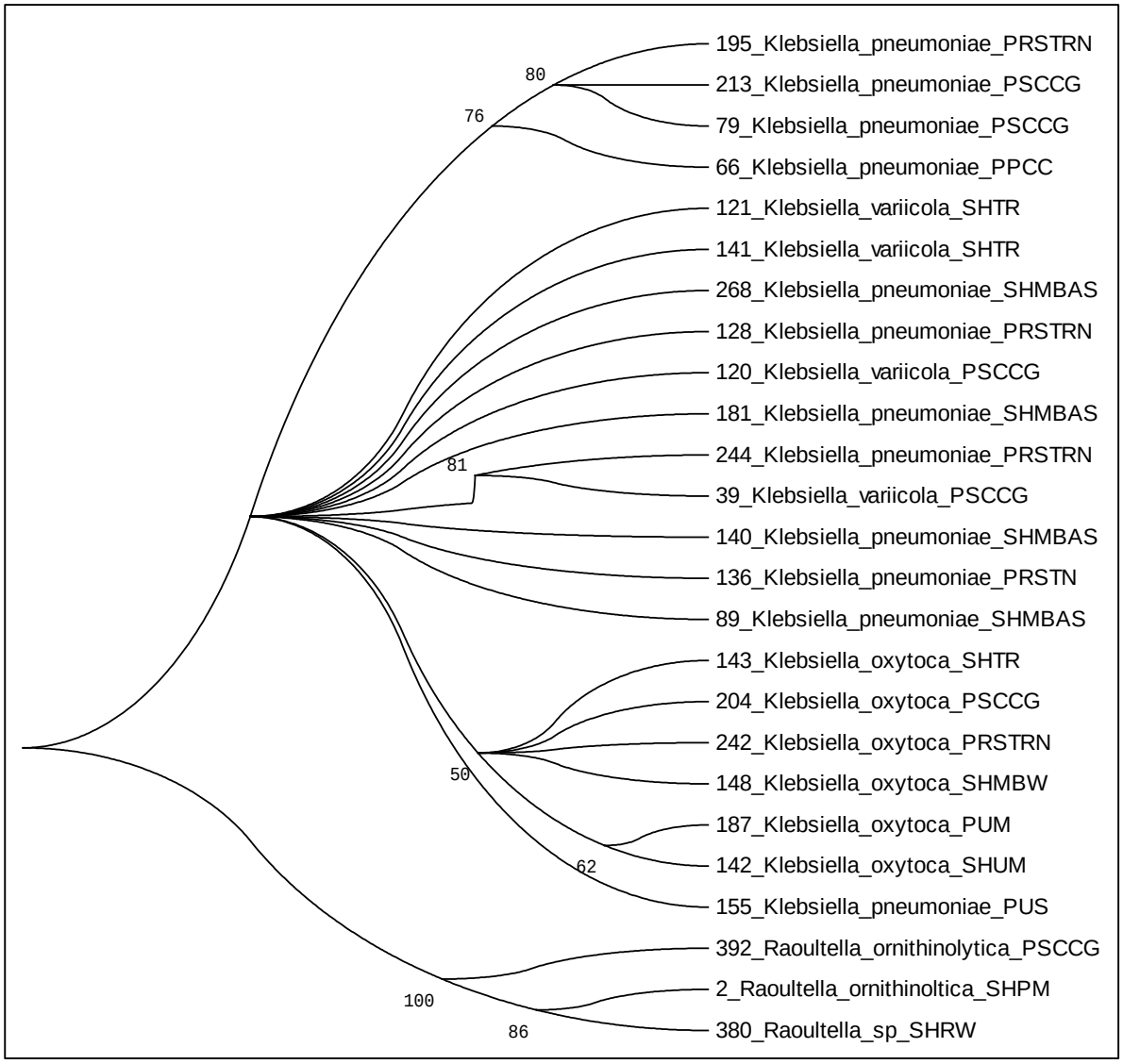

Figure 17: *Klebsiella sp. phylogeny and distribution along the smallholder and pastoral dairy value chain*

4.10 Pathogenicity of *E. coli* isolated along the small holder and pastoral dairy value chains

The sample type, number of samples taken, number of *E. coli* isolates and those tested for pathogenicity (expressed as haemolytic activity) and antibiotic sensitivity are shown in Table 19. Haemolytic activity of the isolate was shown by a clear zone of haemolysed red blood cells on Blood Agar, with 7% defibrinated sheep blood. This is shown in figure 18. Antibiotic sensitivity was observed by a clear zone around an antibiotic disc on a Muller-Hinton agar plate inoculated with a Beta haemolytic *E. coli* isolates, and incubated at 37^{o}C for 24 hours (Figure 19). The diameter of the clear zone was then measured in milli metres and recorded.

Figure 18: *A section of blood agar plate with 7% sheep blood inoculated with beta haemolytic E. coli showing a clear zone*

Figure 19: *A section of a Muller-Hinton agar plate showing susceptibility of β-haemolytic E. coli to Cefepime (clear zone) and Ampicillin*

Table 19: *Types of samples collected along the Smallholder and Pastoral value chain for cow and camel milk respectively*

Dairy value chain	Type of sample	Number of samples	Samples positive for *E. coli*	Number of β-haemolytic isolates
Smallholder dairy value chain for cow milk	Cow milk	142	128	32
	Udder swab	22	16	0
	Milking person hand swab	20	8	0
	Water	10	4	0
Pastoral dairy value chain for camel milk	Camel milk	131	126	42
	Udder swab	16	6	0
	Milking person hand swab	14	4	0
	Water	8	2	0

Plates which had a clear zone around the colony were β-haemolytic, while those without a clear zone were considered non-haemolytic. The proportion of *E. coli* isolates that tested positive for β-haemolysis were 25% and 33.3% for cow milk and camel milk respectively. None of the isolates from udder swab, milking persons hand and water tested positive for β-haemolysis.

Presence of *E. coli* from 142 cow milk samples was 90.1% which was significantly lower than 96.2% in camel milk from 131 milk samples, with $p \leq 0.05$. Prevalence for β-haemolytic *E. coli* as a proportion of *E. coli* isolates was not significantly different between cow and camel milk. The prevalence was 25.0% of 128 *E. coli* isolates for cow milk and 33.3% of 126 *E. coli* isolates for camel milk, with $p = 0.1439$. Along the chain, the prevalence of β-haemolytic *E. coli* in udder milk in both cow and camel milk was not significantly different from the rest of the value chain (post-udder milk) as shown in Table 20.

Similarly, prevalence of β-haemolytic *E. coli* between cow and camel udder milk, and between cow and camel post-udder milk was not significantly different.

In both cow and camel udder milk, the proportion of β-haemolytic *E. coli* was not significantly lower compared to that of the post-udder milk. Similarly, the prevalence of *E.*

coli between cow and camel udder milk as well as between cow and camel post-udder milk was not significantly different.

Table 20: *Prevalence of β-haemolytic E.coli as a proportion of E.coli isolates (n) between cow and camel milk; and between udder and post-udder milk in both cows and camels*

Milk type	Udder milk (%, n)	Post-udder milk (%, n)	*p*-value
Cow milk	20%, 35	26.9%, 93	0.4429
Camel milk	36.7%, 30	30.7%, 101	0.5382
p-value	0.1344	0.5584	

p -values in the rows and columns are for proportions in the rows and columns respectively.

4.11 Haemolytic activity and antibiotic sensitivity of *Escherichia coli* isolated along the small holder and pastoral dairy value chains

The Proportion of β-haemolytic *E.coli* isolates along the chain for both cow and camel milk was significantly different (Table 21). Prevalence of AMP resistant β-haemolytic *E.coli* was 52.4% in camel milk which was significantly higher than 25.0% in cow milk with p = 0.0175. Resistance to Cefotaxime (CTX) was at 37.5% for isolates from cow milk which was not significantly different compared to 23.8% for isolates from camel milk. Similarly, resistance to cefepime (FEP) for isolates from cow milk was at 12.5% and was not significantly different from that of camel milk which was 28.6%. Multi-drug resistance to AMP/CTX/FEP too was not significantly different with 12.5% for isolates from cow milk and 19.0% for those from camel milk (Figure 20). All the isolates were however susceptible to Piperacillin/Tozobactam (TZP), Ciprofloxacin (CIP), Amikacin (AK), and Imipenem (IMI).

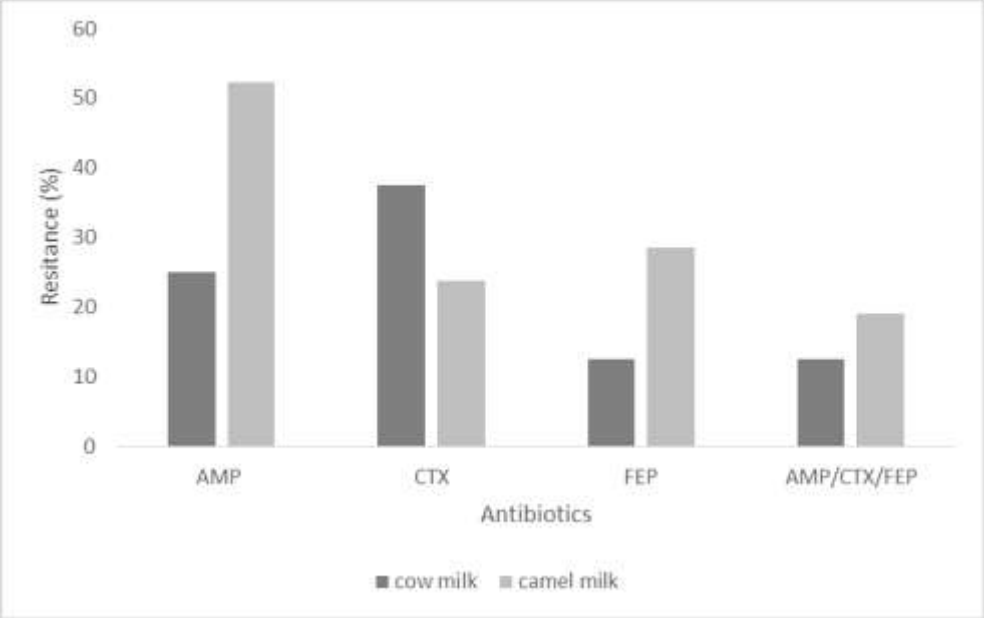

Figure 20: Resistance of β-haemolytic E. coli from cow and camel milk to antibiotics

Table 21: *Proportion of β-haemolytic E. coli isolates along the chain for cow and camel milk*

Milk type	Udder milk	Post-udder milk	*p*-value
Cow milk, *n*=32	21.9%	78.1%	<0.0001
Camel milk, *n*=42	26.2%	73.8%	<0.0001

n is the number of β-haemolytic *E.coli* isolates along the chain. *p* -values in the rows, and columns are for proportions in the rows and columns respectively.

CHAPTER FIVE

DISCUSSION

5.1 Exploratory study and establishment of stakeholder platforms

Stakeholder platforms were set up in the study areas. Three levels of stakeholder platforms were formed. The platforms at the micro-level were the farmer groups and milk traders associations. The farmers formed co-operatives with the intention of improving production and marketing the milk. These co-operatives were more vibrant in the small holder rural value chain than in the smallholder peri-urban and pastoral value chains. This was attributed to the fact that the co-operatives in the Smallholder rural value chain met the need of the members due to the lack of alternative profitable marketing channels. The high production of milk coupled with lack of access to urban markets was a push/pull factor for farmers to be part of the co-operative. This happened despite the presence of milk collection centres for private milk processors that also offered competitive prices. The pricing for the private processors was however not negotiated by the farmers but imposed by the processor. Farmers who supplied milk to the co-operative had the advantage that the co-operative would negotiate pricing with the processors. Some farmer/traders however supplied milk to both the co-operative collection centre and to the private processor collection centre. This was meant to keep membership to the co-operative as well as take advantage of spike in prices offered by the private processors during dry seasons when the supply of milk was limited. The co-operatives in the Small holder rural dairy value chain was also made up of one ethnic group, compared to the peri-urban groups, which made it easier for group dynamics.

In the smallholder peri-urban dairy value chain, formation of farmer groups was equally driven by interest to promote production and marketing of the milk. However, the co-operatives were faced by real competition from milk traders who bought milk from the farmers to supply to urban consumers either in homesteads, milk bars, hotels, schools, or hospitals. Famers co-operative in the peri-urban value chain also sold milk to urban consumers just like the traders. With the regulation by Kenya Dairy Board to sell only pasteurized milk or milk products to consumers, the co-operatives were investing in milk value addition to produce yoghurt and *mala*, a fermented product. Essentially, the farmers' co-operatives were being driven by Kenya Dairy Board regulations to be licensed as mini-dairies. On the issue of post-harvest losses, the managers of the co-operatives reported that no losses occurred. However, USAID-KAVES, 2014 reported losses as a result of milk spoilage ranging from 0.2% to 5% for eight bulking centres in Kenya (USAID, 2014).

Along the pastoral dairy value chain, there were no farmer/herder groups. The herd

owners sold milk to the Co-operatives, which bulked and chilled the milk for onward sell to the urban consumers in Nairobi. The lack of these herder co-operatives was as a result of the herds being geographically distant from each other. At the production level therefore, the stakeholders were the herders who also doubled up as the milking persons. The camel owners themselves lived in Isiolo town, and could visit the herders only once in a while.

Platforms for milk traders were commonly found in the peri-urban and urban centres. The traders formed groups according to the routes they take from the farms. Most of these groups also sold their milk in the same geographical location. The group members met once every week, usually for table banking. With the new regulation that requires that milk be pasteurised before selling to consumers, the groups were also raising funds to acquire premises and purchase pasteurisers. Raising funds, through groups to purchase pasteurisers, to a large extent was driven by Kenya Dairy Board. In the Smallholder rural dairy value chain, there were no platforms for milk traders. All the milk traders delivered milk to either the farmer co-operative collection centre, or the collection centre owned by private processor. Similarly, there were no groups for milk traders in the pastoral value chain, apart from Anolei women group and Tawakal groups that collected milk destined for Nairobi. Milk sold for instance at the Isiolo milk market, or at the road sides was done by individual traders.

At the macro level, government ministries at the county government level in both smallholder and pastoral value chains were involved in livestock vaccination and control of animal diseases. Kenya Dairy Board which is a primary government agency for promotion of dairy farming, and trade, was only active in the peri-urban value chain. The impact of the Board was not felt in both the Small holder rural value chain and the Pastoral dairy value chain. In the peri-urban value chain, the traders did not welcome the regulation of pasteurizing milk before sale. This was because of the initial and running cost of milk pasteurization. In addition, the price of pasteurised milk would increase beyond the reach of their regular consumers. Kenya Dairy Board also does not have mandate over milk that is not cow milk according to the Dairy Industry Act, 1958. Its licencing camel milk processing facility in Nanyuki was extrajudicial, and calls for a quick review of the Dairy Industry Act.

5.2 Description of the Small-holder dairy value chain in Kenya

5.2.1 Smallholder farm characteristics

Smallholder dairy farming is not unique to Kenya. It is estimated that, globally, the average dairy herd size is 2.4 cows per farm. In most countries, especially in Africa, Asia, Eastern Europe and parts of Latin America, the vast majority of dairy farms comprise less

than ten cows (FAO, 2010). Smallholder dairy farming is an important economic activity in many African countries (Bebe, 2003; Moll *et al*., 2007; Tegegne *et al.*, 2013), and in Sub Saharan Africa, Kenya has the most developed Smallholder dairy farming (Muriuki & Thorpe, 2001). The development of the vibrant smallholder dairy sector has been brought about by many factors. The most important was the Swynerton plan of 1954 (Thurston, 1987) which allowed Africans to own land and participate in commercial agriculture. This was enhanced by removal of milk delivery quotas by Kenya Co-operative Creameries in 1971 to allow Smallholder farmers to supply milk to the processor (Muriuiki & Thorpe, 2001). Recently, the animal husbandry system has been shifting from free range to zero-grazing especially in peri-urban areas due to shrinking land holding (Bebe, 2003) with increased use of commercial feeds especially in peri-urban areas (Migose *et al*., 2018).

The government's role in support of smallholder dairy farmers decreased from the 1980s (Muriuki & Thorpe, 2001) and this situation has not been reversed. The government has restricted itself to mass vaccinations of livestock for notifiable diseases such as East Coast Fever (Muriuki, 2001), issuance of animal movement permits, and declaration of quarantine (GOK, 2012). The void left by government in provision of veterinary services has to a large extent been filled by private veterinary operators or veterinary operators employed by farmers' co-operative societies or milk processors.

5.2.2 Smallholder milk production and marketing channels

Milk production was 9.1 kg and 12.3 kg/cow/day for rural and peri-urban farms respectively due to higher intensification in the peri-urban farms compared to the rural farms (Kashongwe *et al*., 2017). Stall-feeding and provision of concentrates and supplements was practiced more in peri-urban farms compared to the rural farms as reported by Kashongwe *et al*. (2017) and Migose *et al*. (2018). Sourcing for animal feed in peri-urban farms was the greatest constrain faced by the farmers, and this affected milk production. Along the peri-urban chain, most of the raw milk was sold directly to urban consumers' homesteads, or via milk bars and hotels compared to rural value chain where most of the marketed milk was collected in bulking centres where it was taken for processing. Sale of raw milk to urban consumers by smallholder peri-urban farmers was driven mainly by milk price. Higher prices were offered by the urban consumers compared to co-operative societies. On the contrary, demand for raw milk in the rural areas was low based on the fact that every household had at least a cow. The smallholder rural farmers were left only with the option of selling their milk to the processors, either directly or through their co-operative societies. Milk processing was

dominated by two processors, Brookside dairy ltd and New Kenya Co-operative Creameries (New-KCC). Both had a market share of 40% and 35 % respectively (Kenya Dairy Board website). The two processors are oligopolistic and determine farm-gate prices with no regard to the cost of milk production of the smallholder farmers.

5.2.3 Transportation of milk along the smallholder dairy value chain

Transport of milk has revolutionised over the past 15 years. Over the years, transport of milk was commonly done using vehicles and bicycles. Milk transportation has now shifted to use of motorbikes because the price of motorbikes has fallen to as low as USD 600, from at least USD 1850 per piece years ago following the setting up of several motorcycle plants by Asian manufacturers in the country supported with tax exemptions by government. This has made the motorbikes the most dominant transport mode for milk from the farms to either the collection centres, milk bars, hotels or other outlets. The operators find it more convenient to use plastic receptacles compared to Aluminium churns because of the cost, weight and the risk of milk spillage associated with Aluminium churns. The use of plastic receptacles is thus dominant in the rural value chain because of lack of surveillance by law enforcers. In the peri-urban and urban areas however, surveillance and enforcement of use of Aluminium churns is done by Kenya Dairy Board inspection officers. The motorbike operators have modified their motorbikes to make them suitable to transport milk in Aluminium churns. Vehicle transport using insulated containers is useful for transporting large quantities of milk from collection centres to the dairy processing plants. In such circumstances, milk quality testing is done before bulking the milk.

5.2.4 Milk collection, bulking and chilling along the Smallholder dairy value chain

Milk collection from farm-gates, bulking and chilling is one of the most important processes to ensure that milk reaches the processor in a wholesome condition for processing into various products. It is generally agreed that milk should be cooled within 2 hours to 4^{o}C after milking to reduce the rate of microbial proliferation (Jay *et al.,* 2005). The long-time taken before cooling the milk in this study is a contributing factor to deterioration of milk quality and post-harvest losses. It was however observed that milk quality tests were done at the collection centre before the milk was bulked. The milk was cooled to 4^{o}C while awaiting night transport in insulated containers to the processing plant. Transport at night ensured minimum temperature change, and milk reached the process plant before morning of the following day at a temperature of less than 6^{o}C. The milk was then immediately cooled again to 4^{o}C while awaiting processing.

5.3 Description of the Pastoral dairy value chain in Kenya

5.3.1 Pastoral *boma* characteristics and milk production

The climatic conditions in the arid and semi-arid lands, can only be suitable to camel rearing because of their resilience (Watson *et al.,* 2016). Camel keepers essentially practice nomadism, moving from one place to another in search of pasture, water and often security. They cannot then put up permanent residence. Though the government has provided watering points, these are not enough especially during the dry season, where lack of water sometimes results to clashes between the herders. It is therefore necessary for county governments to priorities provision of water for watering camels, and maintenance of milking hygiene.

5.3.2 Pastoral camel milk marketing channels- from *boma* to consumer

The milk leaving the *bomas* in Isiolo had two main outlets. The first channel is where the milk was sold at the milk market in Isiolo town. This milk was bought by individuals who take the milk home for consumption, or sell the milk by the road side within Isiolo town. The second channel is where the milk was bulked, chilled overnight and transported to Nairobi. The milk delivered in Nairobi was sold to hotels and house-holds, the major consumers being people of Somali origin.

Camel milk is a high value product, and the price does not reduce just because the milk is sour. Milk handled at ambient temperature for a long time undergoes natural fermentation but it was noticed that the price per litre of fresh milk and *suusa* was the same at both the Isiolo market and Nairobi market. There is therefore loss in quality due to increased microbial numbers and increased acidity, but no loss in monetary value. Post-harvest loss for camel milk would then only apply for milk destined for processing because the high acid milk cannot stand heat processing, and therefore would be rejected by the processor. The loss of value would only be valid if the processor offers a higher price for fresh camel milk than what the informal market would offer for *suusa.*

5.3.3 Transportation of milk along the Pastoral dairy value chain

Collection and transport of milk from the primary collection centres to the secondary collection centres, also referred to as bulking or cooling centres was done using motorbikes, and four-wheel drive pick-up trucks. This was due to the absence of access roads to the production areas. Distant production areas such as Kulamawe depended on pick-up trucks or lorries to transport milk to Isiolo town.

5.3.4 Milk collection, bulking and chilling along the Pastoral dairy value chain

The pastoralists often moved from one place to another in search of grazing land and water for their animals. Milking of lactating camels occurred at the *boma* (kraal) where the pastoralists pitched camp. The milk would then be taken to the nearest primary collection centre which is usually temporary and only existed till the pastoralists moved with the herd to another grazing area. Milk transportation from the *bomas* to the primary collection centre was usually on foot. The primary collection centres were a walking distance from *bomas.* Primary collection centres generally had milk from one or more herds. The nomadic lifestyle of herders created a challenge in milk collection because the traders and transporters sometimes had challenges in tracking the herders. The lack of roads made it even more difficult for the milk to be collected. Motorbikes therefore were the most effective means of transport from the hinterland to the secondary collection centres. Vehicle transport was used to transport milk from distant milk production areas such as Kulamawe and Gotu. Milk was usually held at ambient temperature before and during transport to Isiolo town. This exposed milk to quality deterioration. Sometimes, evaporative cooling was practiced where a wet gunny bag was used to cover the milk containers, which were put under a shade awaiting collection. This was found to be effective in reducing milk spoilage (Ogolla *et al*., 2017).

Transport of milk from bulking centres in Isiolo to Nairobi was done using public service vehicles. Transport was done under ambient temperature resulting in increased microbial counts in milk delivered to Nairobi. The milk was received by milk agents who then apportioned the milk to the traders. The traders quickly took away the milk for sale at the roadside or to restaurants. The whole value chain is informally controlled by a few people in Isiolo and their associates in Nairobi. Any activities to improve the quality of the milk along the chain will have to involve these actors, who incidentally, benefit at the expense of producers.

5.4 Microbial loads and associated risk factors along the Smallholder dairy value chain in Kenya

5.4.1 Microbial loads in milk along the Smallholder dairy value chain in Kenya

The study found that there was no significant difference between TVC in udder milk from peri-urban and rural value chains. However, CC in udder milk in the peri-urban farms was higher than in the rural areas. High coliform counts were associated with dirty udder surfaces and faecal contamination of the milk (Orwa *et al*., 2017). Kashongwe *et al.* (2017) found that the cow sheds in rural farms were cleaner compared to those in peri-urban farms,

which demonstrates why coliforms counts was higher in milk from peri-urban farms compared to rural farms. Comparison of the TVC and CC between collection centres at the rural and peri-urban value chains found that the counts were not significantly different. This is accounted for by the time taken to deliver milk to the collection centre. In the peri-urban value chain, despite recording higher CC counts than the rural chain, all the milk was delivered by 10.00am to the collection centre compared to deliveries that stretched to late afternoon in the rural chain. It is demonstrated in this study that time had an influence on microbial counts, as has been demonstrated by Jay *et al*. (2005).

Along the peri-urban value chain, the microbial counts were not significantly different between udder milk and milk from milk bars. This was due to short time the milk was delivered to the milk bars. Some milk bars received milk as early as 6am. This was due to the fact that milk bars needed to sell the milk to their consumers, most of them who buy milk for preparation of breakfast. All the milk bars under this study had milk cooling facilities. Milk sold by milk bars had counts not significantly higher than udder milk. However, there was a significant difference in microbial counts between udder milk and milk delivered to collection centres in the rural value chain. The change in microbial count from production to market was less in peri-urban chain compared to change from production to collection centres in the rural value chain. This could be attributed to stringent surveillance by Kenya Dairy Board inspection officers to ensure milk transporters and traders comply with licencing requirements such as use of food grade milk receptacles.

5.4.2 Compliance of cow milk along the smallholder Dairy Value chain in Kenya to East African quality specifications

The East Africa Standards (EAS) have requirements for both TVC and CC for grading milk. At the production level, 100% of the milk at the rural farms was classified as ‘grade 1’ based on TVC while 77% of the milk from the peri-urban value chain was classified as ‘grade 1’. However, based on CC, only 53% the same milk at the rural farms was classified as ‘very good’ and only 3 % from peri-urban value chains was classified as ‘very good’. This trend is observed in all the nodes in both rural and peri-urban value chains. This means that the milk is rated highly based on TVC but poorly based on CC. The problem therefore appears to be hygiene both during milking and along the value chain. High microbial counts are often noticed along the value chain, which incidentally is populated by informal traders. This has led many researchers to conclude that milk handled by informal traders has higher microbial counts than the formal channels. The problem could as well be at

the farm and not in subsequent nodes of the value chain. Some authors have indicated that informal traders are illegal traders (EADD, 2008). This is not necessarily true because most of them especially in peri-urban and urban areas are licenced by Kenya Dairy Board.

For pasteurised milk, none of the milk brands fully complied with the quality requirements with regard to TVC and CC. Two brands met the quality requirements for CC and one brand met the quality requirement for TVC. Failure of brands to satisfactorily meet the requirements of the standard could be due to high microbial counts in raw milk before pasteurization. Improving the quality of raw milk meant for processing would certainly improve the quality of the final products.

5.4.3 Risk factors and their relationship to microbial loads in milk along the Smallholder dairy value chain in Kenya

a) Effect of milk receptacles, cleaning and disinfection on microbial loads of milk during transport

There was a significant difference in microbial counts of milk contained in Aluminium churns compared to ordinary plastic containers in the rural value chain. This is because the ordinary plastic containers have rough surfaces and thus categorised as 'unhygienic' (Tamburini *et al*., 2015). The containers usually have blind corners that are prone to biofilms which resist cleaning and disinfection as demonstrated by Wafula *et al*. (2016). The difference in microbial counts was thus significant in spite of the fact that all transporters disinfected the milk containers using either hot water or chemical disinfectants (as noted in the focussed group discussion). The disinfectant was supplied by the processor and some transporters told to retain a little to add to the container before filling it with milk, which is in contravention of the quality requirements for raw milk with regard to antibiotic residues (EAS, 2006a). Along the smallholder peri-urban chain, all the milk transporters and traders reported that they disinfected the milk containers with hot water. Microbial quality of milk receptacles' surfaces has been demonstrated by Wafula *et al*. (2016) on their study on effect of different sanitation regimes of milk receptacles on microbial counts in milk.

b) Effect of Source of farm water and Udder hygiene on microbial loads in milk

The microbial counts of milk after milking was not dependent on the source of water. This was because all milking persons boiled the water before using it to clean the udder. It was however reported by Orwa *et al*. (2017) that udder hygiene is the major contributing factor to microbial counts in milk at the farm level. Since water was boiled before being used

to clean the udder, it is now evident that the effectiveness of udder cleaning, in addition to animal housing conditions (Kashongwe *et al*., 2018) play a major role in microbial quality of udder milk.

Accordingly, the water source did not have significant effect on microbial quality of milk along the value chain. The traders/transporters along the peri-urban value chain used a particular brand of soap which they claimed did not impart undesirable odour to the milk, and rinsed the milk containers (Aluminium churns) with hot water, before airing them to dry. This explains why the difference in counts was not significant. In addition, some transporters used antibiotics to sanitise the containers as reported in the focused group discussions though our study did not confirm this claim. None of the transporters admitted to use of antibiotics in milk.

c) Effect of time on microbial loads of milk along the dairy value chain

The relationship between microbial counts and time was positive. It has been demonstrated that milk should be cooled within 2h after milking to 4^{o}C. The longer the time taken before milk is cooled, the higher the microbial count (Jay *et al*., 2005). Long delivery time was affected by transporters and traders who collected milk from various farms to deliver to the bulking centres. Poor state of roads in the milk production areas contributed to delay in milk delivery to the market. Along the smallholder rural value chain, poor roads in some cases restricted milk transport to use of donkeys which took a long time to deliver milk to the collection centres.

d) Effect of milk temperature on microbial loads of milk along the dairy value chain

The temperature of milk traded within Nakuru town was positively related to microbial counts. The lower the temperature of storage, the lower the microbial count (Jay *et al*., 2005). Morning milk for marketing, in both rural and peri-urban chains, was only chilled on delivery to the bulking centres, or the milk bars. It was however found during the study that some milk bars switched off coolers to save on energy. The cost of energy in Kenya is USD 15 cents per KWh compared to USD 0.4 and 0.6 cents per KWh in Ethiopia and Egypt respectively (ESI Africa, 2017). Electricity coverage from the national grid is still low, and some of the milk producing areas especially in the extensive pastoral areas are not serviced. Some of the areas that are serviced still suffer from frequent power outages. This is because Kenya Rural Electrification Programme is under-resourced and cannot deliver services

efficiently (USAID-KAVES, 2014). High cost of energy is thus a risk factor to microbial spoilage of milk. There is urgent need to explore alternative cheaper sources of energy such as solar energy to reduce the cost of cold storage of milk.

e) Effect of Volume of milk handled on microbial loads in milk along the dairy value chain

Pooling of milk without quality checks was expected to increase microbial load of the milk (Younan & Abdulrahman, 2004). However, this study demonstrated that the higher the volume of milk in the container, as a result of pooling, the lower, though not significantly, the microbial load. This was also similar to camel milk. This is explained by the fact that the traders had sufficient experience to notice milk of lower quality. The assessment was done either visually or by smelling the milk. The traders either carried milk of doubtful quality separately or avoided collecting such milk altogether. In addition, large containers have a small surface area to volume ratio compared to small containers. More microbes are likely to contaminate milk if the surface area to volume ratio is large. In the event that the milk quality did not improve, the trader ceases to collect milk from the farmer. Such milk could be from a cow milked beyond the lactation period of 305 days, and/or milk from a cow suffering from sub clinical mastitis. These two are common causes for recurrent failure of milk to pass alcohol test because of elevated salt levels which destabilise protein in milk.

f) Effect of training of milk handlers on microbial load of milk

In the smallholder rural dairy value chain, none of milking persons had received formal training on milk quality. On the contrary, the farmers were the ones trained with the belief that they would pass over the knowledge to the milking persons. In the Peri-urban value chain, microbial counts for the milk handlers who had training on milk quality was significantly lower than those who had no training. Training of milk handlers therefore has a significant effect on milk quality at production. However, training of milk transporters along the peri-urban value chain had no effect on microbial counts.

5.5 Microbial quality of camel milk along the Pastoral dairy value chain and associated risk factors

5.5.1 Microbial loads in milk along the Pastoral dairy value chain in Kenya

The microbial counts of udder milk were not significantly different from the *boma* bulk milk. This is basically because the *boma* bulk milk quality is the mean of quality of milk from the herd. There was no delay between milking and bulking at the *boma*. Bulking was

done immediately after milking to enable transport of the milk to the primary collection centre. However, from the primary collection to the secondary collection centre in Isiolo town, there was a significant increase in TVC possibly due to high ambient temperature and time taken to deliver the milk. However, coliform counts did not increase significantly from the *boma* bulk to the secondary collection centre. Research has demonstrated die off of coliforms, this being the reason as to why faecal streptococci is used as in indicator organism in foods (Motlagh & Yang, 2019). There was however significant increase in microbial numbers, both TVC and CC from boma bulk to the Nairobi market. This was essentially as a result of multiple reasons. These include transporting milk at high ambient temperature for a long time to the Nairobi market. Hygiene in milk handling along the value chain, with indiscriminate use of worn out and tainted plastic containers being a contributing factor to the high microbial count.

5.5.2 Compliance of camel milk along the Pastoral Dairy Value chain in Kenya to East African quality specifications

Compliance of camel milk along the value chain to Kenya Standards for udder milk based on TVC and CC was fairly high at 78% despite the myriad challenges in maintaining hygiene during milking. The proportion was equally high for both TVC and CC for *boma* bulked milk. The proportion meeting the Kenya Bureau of Standards standard continued to decrease as the microbial counts increased along the value chain. The Nairobi market had the lowest compliance to the Kenya Bureau of Standards standard. In essence, camel milk reached Nairobi at least 28 hours after harvesting. Milk is chilled overnight in Isiolo but again exposed to ambient temperature during transport to Nairobi. On average, about 40% of the milk along the chain failed the standard based on coliform counts and this represents Post harvest loss along the camel milk value chain. This requires intervention by the regulatory agencies, particularly the Public health departments domiciled at the counties of Isiolo and Nairobi City County. Unlike the Cow milk value chain in the peri-urban value chain, where regulation is carried out to protect public health, the pastoral camel milk value chain is not regulated at all and presents a health risk considering that most consumers of camel milk prefer to consume the milk without heat treatment.

5.6 Risk factors and their relationship to microbial loads in milk along the Pastoral dairy value chain in Kenya

a) General hygiene (udder hygiene, milking personnel hygiene, milking container type and cleanliness, and excessive human handling of milk)

Many studies have linked poor udder hygiene to high microbial counts in milk (Gleeson *et al*., 2009). The microbial load at production continues to increase in subsequent nodes up to the market as was also reported by Matofari *et al*. (2013). The non- food grade plastic milking containers as well as failure to clean hands and the camel's udder contribute to milk contamination, and this study also isolated *E. coli* from both the udder surface and the hands of the milking persons. The identified challenge was poor hygiene of the milking parlour, especially in wet weather, the lack of appropriate milk containers and unavailability of water for maintenance of udder and milking persons' hygiene.

At the Anolei and Tawakal secondary collection centres, group members are involved in most activities from milk reception to record taking. The collection centre was therefore a beehive of activities and the inordinate human presence and excessive manual handling increased the risk of milk contamination. To reduce excessive human handling, exposure of milk by pooling it into chill-tanks, and transferring the milk back to the plastic receptacles, the alternative is to cool milk in their original containers in a cold store, before transporting it to Nairobi the following day in the morning. Non-Governmental Organizations such as the Netherland Development organization (SNV) have supported the groups to acquire Aluminium churns for milk handling. Despite this, milk is still transported in unhygienic plastic containers since Aluminium churns are a challenge for transport of milk by bus to Nairobi due to milk spillage and weight. The bus operators therefore only accept to transport milk in plastic containers. The use of food grade plastic containers would be an alternative to Aluminium churns to overcome this challenge.

b) Milk holding time and temperature

Time and temperature are two of the most important extrinsic factors affecting microbial growth, and generally, milk should be cooled to 4^{o}C within 2 hours of milking (Jay *et al*., 2005). In this study, between production and secondary collection, milk was held at an ambient temperature of between 28^{o}C and 32^{o}C for up to 12 hours. This substantially increases microbial counts of the milk and accounts for significant increase in microbial loads from production to the secondary collection centre. There was indeed a significant relationship between time and TVC between production and the secondary collection centre

despite the result having a low adj. R^2. Since the secondary collection centres have cooling facilities, microbial growth could be curtailed if milk was delivered less than 2 hours after milking. Cooling the milk to 4^{o}C overnight at the secondary collection centre, then transporting it to Nairobi at ambient temperature, a journey of 7 hours, compounds the problem of increased microbial counts. In this study, regressing microbial counts against temperature was not found to have a significant relationship because of a small range in the milk temperature from production to secondary collection centres.

c) Relationship between volume of milk and microbial counts

This relationship between microbial load and volume of milk in the receptacle was not found to be significant. Smaller containers have a large surface area to volume ratio and were expected to have a higher microbial count. However, milk in large containers was likely pooled from different sources which is considered a risk factor for increased microbial load (Younan, 2004). It was noted that transporters and traders did not use objective means of milk quality assessment like lactometer test, alcohol test or clot on boiling because of rush against time to collect and deliver the milk to different destinations without delay. It was observed during the study that transporters and traders had sufficient experience to notice milk of doubtful quality and did not mix it with the rest of the milk. In the event that the milk was tested during delivery at the milk bar and failed the test, the trader would return the milk to the farmer.

d) Level of training of operators

Training of milk handlers has always been advocated as a way of improving milk quality (GoK, 2010b). In this study however, at the secondary collection centre, there was no significant difference in microbial load of milk handled by individuals who have received training on milk quality and those who have never been trained. This is because microbial contamination starts at production, where none of the herders, who also milk the camels, had ever been trained on milk quality. It was also observed that the milk handling practices of individuals who had received training on milk quality was not different from those who had never been trained. Therefore, for the training to be effective, it has to be done along the value chain beginning from production. In addition, the actors need support in co-operative management to reduce milk ownership as is the case, but rather own the cooperative and entrust its operations to elected directors and employees. This would reduce congestion and excessive handling of milk at the collection centre where everybody wants to follow up on

their milk. However, as discussed earlier, there are a few people in Isiolo and Nairobi who benefit from the current arrangement at the expense of producers, and they should be brought on board for any effective change to be achieved.

5.7 General Discussion of the Smallholder and Pastoral Value chains

Milk production for cow milk in the smallholder farms was higher than production from camels in the pastoral value chain. Camels however survive very harsh climatic conditions and limited fodder and water, conditions which cow milk production is almost impossible. The benefits of camels in this ecology has been described by Huelsebusch and Kaufmann (2002). Cows also benefit from provision of concentrates and supplements, a practice not observed in the pastoral value chain. Both smallholder and pastoralists however report animal feeding as the greatest challenge to dairy farming (Kashongwe *et al*., 2017).

Peri-urban cow milk producers sold the milk to the urban and Peri-urban consumers who offered a ready market and a better price compared to delivering milk to the co-operative societies. This was not so in the rural area where most households owned cows. The rural producers therefore sold their milk to the co-operative societies which sold to processors. For the pastoral producers, some milk was sold to urban consumers in Isiolo town while the rest was sold to urban consumers in Nairobi. The milk reaching Nairobi market took more than 24h, with cooling only done overnight at the bulking centres in Isiolo town. This milk was sold raw for consumption in either raw form, semi fermented form (*orawa-kandi*) or fermented form (*suusa*). On the other hand, milk from the smallholder farms that is sold to the processors through co-operative societies is processed into various dairy products including pasteurised milk, yoghurt, mala, butter, cheese and dairy ice cream. The major processors are Brookside dairy ltd and New Kenya Co-operative Creameries (New-KCC) having market share of 40% and 35 % respectively (Kenya Dairy Board website) which are oligopolistic and determine farm-gate prices with no regard to the cost of milk production of the smallholder farmers. Happy Cow is fairly small processor sourcing milk from the same study area.

Transport of milk from the farms and herds to primary and secondary collection centres in both value chains dominated by motorbikes. This is mainly influenced by cost. The price of motorbikes has reduced by about 70% over the last 15 years. The motorbikes also have the ability to navigate difficult terrains that characterize the milk production areas compared to vehicle transport. Donkeys are used where the topography and road conditions are not favourable to motorbikes. Transport from the secondary collections centres to the

processor for cow milk is done using chilled containers owned or contracted by the processors. For camel milk, transport from the secondary collection centres to Nairobi market is done using public transport buses.

Milk transporters using motorbikes find it more convenient to use plastic jerry-cans because of the low cost of acquiring them and the low risk of milk spillage compared to Aluminium cans. Hygienic plastic containers are also available that have been customised for motorbike use (Appendix H). However, they are only available in 10L. This is not favourable to transporters who prefer 20 to 25 L containers. Use of unhygienic plastic containers is however discouraged by Kenya Dairy Board and offenders are apprehended. Unfortunately, Kenya Dairy Board is limited in its operations and it is only active in urban areas where cow milk is sold. Camel milk in Nairobi is sold in unhygienic plastic containers and it appears Kenya Dairy Board is blind to this situation. In both value chains, milk transporters and traders are well experienced to detect milk quality by sensory evaluation. Milk of doubtful quality is not pooled with the rest of the milk. This milk however will still be sold to unsuspecting consumers. Such milk is not delivered to the secondary collection centres because it will fail quality test.

Milk collection from farm-gates to bulking centres is done by farmers themselves, transporters or traders. No contracts are signed between the farmer and the trader or transporter. The relationship between the farmer and transporter or trader is oral and based on trust. Usually, milk should be cooled within 2 hours to 4^{o}C after milking to reduce the rate of microbial proliferation (Jay *et al.*, 2005). Milk along the camel milk value chain took a longer time to reach the consumers compared to cow milk. The long time at elevated temperatures contributes to microbial spoilage of milk. However, unlike cow milk where there is loss of value for soured milk, this was not observed for camel milk where fresh, semi-fermented and fermented milk fetched the same price. Chilling was only done at the secondary collection centres using either horizontal or vertical (Appendix M and N) coolers run on electricity. At primary collection centres, along the pastoral value chain, cooling effect is achieved using evaporative cooling (Ogolla *et al.,* 2017). This is done using wet gunny bags to cover milk containers (Appendix O).

Milk delivered at the secondary collection centres is tested and bulked in a chill tank at a temperature of 4^{o}C awaiting transportation to either processing for cow milk, or to Nairobi market for camel milk. Transport to the processors is done at night in insulated tanks for cow milk to take advantage of low night temperatures which can be as low as 10^{o}C. For camel milk, transport is done during the day at ambient temperature which accelerates

microbial proliferation of in the milk.

5.8 Microbial quality of milk along the Smallholder dairy value chain and associated risk factors

It is recommended that udder hygiene practices such as udder washing and drying reduce microbial counts in milk (Orwa *et al*., 2017). There was neither hand cleaning, udder washing nor drying before milking camels. This was attributed to the scarcity of water in production areas. Herders in the pastoral areas depended on water that was brought by milk traders from Isiolo town for their personal use. This water is not enough for udder hygiene. In addition, the herders are nomadic and live on limited supplies. Microbial counts for camel milk increased significantly for milk delivered to Nairobi market because of the long distance and high ambient temperatures. Microbial loads in milk long the smallholder value chain for milk is lower than camel milk value chain due to high temperature and long time to reach the market in pastoral environment, domination of unhygienic plastic containers, in addition to poor udder hygiene. Ironically, there is no loss of value in milk for camel milk compared to cow milk. However, loss would be experienced if camel milk were to be processed because of technological challenges associated with processing low quality milk.

5.9 Compliance of cow and camel milk along the Dairy Value chain in Kenya to East African quality specifications

The level of compliance for cow milk with regard to TVC was higher compared to CC. The challenge is attributed to udder hygiene of lactating cows along the Smallholder value chain. Coliform count for camel milk was lower compared to the smallholder system. The irony is that camel udders are not washed prior to milking. Improper washing only helps to loosen microbes from the udder surface into the milk.

Failure of cow pasteurised milk to comply with the standard is attributed to lack of Good Hygiene Practices for the processors. Two brands recorded zero CC despite one of the brands sharing milk supply with one of the brands that recorded high CC counts. However high microbial counts in raw milk can compromise the microbial quality of pasteurised milk. Log reduction of microbes during pasteurization to meet requirements of the standard presumes good quality raw milk.

5.10 Risk factors and their relationship to microbial loads in milk along the dairy value chain in Kenya

Ordinary plastics contributed to milk contamination compared to aluminium churns. This is attributed to the difficulty in cleaning the containers. However, transporters still find the containers cheaper and more convenient to use. Determination of source of water as a source of contamination was not possible in this study because all the milking persons for cow milk boiled water before use. The transporters and traders for cow milk used the same type of bar soap for cleaning milk containers that they claimed imparted no scent on the milk

The relationship between microbial counts and time was positive. It has been demonstrated that milk should be cooled within 2h after milking to 4^{o}C. The longer the time taken before milk is cooled, the higher the microbial count (Jay *et al*., 2005). Long delivery time was affected by transporters and traders who collected milk from various farms to deliver to the bulking centres. Poor state of roads in the milk production areas contributed to delay in milk delivery to the market. Along the smallholder rural value chain, poor roads in some cases restricted milk transport to use of donkeys which took a long time to deliver milk to the collection centres.

Milk temperature played a role in microbial counts of milk. The lower the temperature of storage, the lower the microbial count (Jay *et al*., 2005). Morning milk for marketing, in both rural and peri-urban chains, was only chilled on delivery to the bulking centres, or the milk bars.

Younan and Abdulrahman (2004) identified pooling of milk without quality tests as a risk factors for microbial spoilage of milk. It was found during this study that traders and transporters did not pool milk of doubtful quality but carried it separately. This was observed in both smallholder and pastoral value chains. The actors depended on their experience with organoleptic tests. Higher volumes of milk due to pooling had no significant effect on microbial counts.

This study found that training on milk quality never targets the milking persons. Ironically, it is the household heads who are trained assuming that they will train the milking persons. This approach is not successful. Many milking persons reported that they have never received any training at all. For those who were trained, their milk was of better microbial quality. Training of handlers along the chain had no added benefit because the quality of milk for actors along the chain who are trained was not different from those who are not trained. This was observed for both smallholder and pastoral value chains. This is because milk contamination starts at production, and the milk quality continues to deteriorate along the

chain.

5.11 Role of regulatory agencies in reducing PHL along the dairy value chain in Kenya

The study found that many provisions of the Dairy Industry Act are outdated and not serving the needs of the dairy industry in Kenya. According to the Kenya Dairy Board regional manager, the Act was designed to serve the interests of the colonial masters. The provisions that are no longer tenable include Section 2 of the Act which lists the regions where Kenya Dairy Board has jurisdiction. This excludes many regions in the country that have an active Smallholder dairy system such as Olenguruone, which was part of this study. Section 3 of the Dairy Industry Act defines milk as 'milk from a cow'. This definition limits the activities of Kenya Dairy Board to only cow milk. It was not envisioned in 1958 that other dairy animals such as camels and goats would play an important role in the Kenyan dairy industry. In 1961, camel milk accounted for only 1% of the total milk produced, which increased to 18% in 2017. Kenya Dairy Board however has licenced Vital Camel milk plant in Nanyuki and a goat milk plant in Meru. This was however done without strict adherence to the law, because, essentially, the colonial Dairy Industry act does not recognise milk from other sources other than cows. Section 32 of the Act requires dairy producers to be registered. However, Kenya Dairy Board is not able to implement this requirement for Smallholder dairy farmers, because the requirement was essentially for large scale dairy farmers. Subsequent sections, 33 and 34, which are about penalty for failure to register and maintenance of register respectively cannot be administered. The Act also does not ban use of unhygienic plastic containers because only Aluminium churns were used as milk receptacles at the time the Act came into effect. The code of practice for hygienic milk production only prohibits handling of milk under unsanitary condition. Individuals conveying milk in plastic receptacles were therefore prosecuted with the offence of handling milk in unsanitary condition.

The major policy change in the Kenyan dairy sector post-independence was the liberalization of milk marketing in 1992 (Dairy Development Policy, 1993), which followed recommendations contained in the Dairy Master Plan (1991). This led to the proliferation of dairy processors, diminishing the market share of KCC. The collapse of KCC towards the end of the last century, together with collapse of the upcoming dairy processors due to low operational scales contributed to increased milk hawking in urban areas. This overwhelmed Kenya Dairy Board which was not ready for this challenge. Kenya Dairy Board was

understaffed with Nakuru region, which covers Nakuru and Baringo counties having only 5 staff members with only 3 being qualified inspectors. It was not possible for them to carry out scheduled inspections on time to root out unlicensed operators. Kenya Dairy Board estimated that unlicensed operators accounted for about 50% of the milk marketed within Nakuru. The unlicensed operators most often invest very little in their trade. Along the Smallholder dairy value chain, most traders used motorbikes to supply milk to milk-bars, shops, homesteads, and sometimes sell milk by the roadside. Some unlicensed traders operate milk-bars without pasteurizers, while others sell raw milk in shops and some even in butcheries. Some unlicensed traders include farmers who deliver milk to urban areas, especially hotels, as they go to work. The number of unlicensed operators increases during the wet season because of the milk glut and low prices offered by processors. It is also during the wet season that proportion of milk rejected by the processors, based on quality, increases. Most of the milk harvested during the wet season has low density due to high moisture content in the pasture predisposing milk to fail the lactometer test. Physical and microbial contamination of the milk is also high during the wet season and milk is likely to fail the alcohol test. It is this milk then that finds itself to the urban consumers. Research on quality of milk of unlicensed traders along the smallholder value remains a challenge since the operators are not co-operative.

Kenya Dairy Board therefore works together with the Public Health department and Kenya Police service to regulate the sector. Public Health (Milk and Dairies) rules under The Public health Act, CAP 242 specifies rules guiding handling of milk. The rules require that dairy cows be groomed every day and be free from manure or any kind of filth. It requires hairs on cow flanks to be cut and udders to be cleaned before milking, and the first lets of milk rejected. The Act also requires cleanliness of the milking person. The Act also requires milk operators to be licenced. The Act also describes the requirements of a milk receptacle as being bottle, or can or churn, or any other receptacle which must be of a material and pattern approved by the local authority. The material must be able to be cleaned and disinfected by steam, boiling water or any other approved disinfectant. It is upon these rules that offenders are prosecuted. Since Kenya Dairy Board and Public Health officers do not have the power to arrest, the Kenya Police Service officers are enlisted to assist in arresting and prosecuting offenders. Kenya Dairy Board intends to partner with County enforcement officers to increase compliance with the regulations. This is expected to yield results since Veterinary services, including dairy are devolved functions under the 2010 constitution of the republic of Kenya.

The challenges along the Smallholder dairy value chain that contribute to Post harvest loss of milk are quite complex. Kenya Dairy Board therefore uses a two pronged approach. The first approach is speaking to the actors and encouraging them to form groups to mobilise funds for purchase of pasteurizers and coolers, as well as training them on milk quality. The second approach is arresting and prosecuting offenders. The first approach is time-taking, resource intensive but yields long term results. The second option stimulates instant behaviour change, uses less resources, but relapse of behaviour takes only a week. Due to complexities of problems experienced by actors along the chain, single cause-effect factors have little effect on results because of interlinked problems. The systems theory (Lai & Lin, 2017) would be the most appropriate approach. A system is composed of elements related to each other. The system is looked at as a whole, because the whole is more than the sum of parts, which is the concept of *holism*. In particular, the *complex adaptive systems and self-organizing systems theory* would be helpful. In complex adaptive systems, spontaneous order arises out of chaos through self-organization. Complex adaptive systems are open systems which reflect the active role played by components of a system in adopting to external changes, in this case brought about by the need of dairy actors to align to regulations, through emergent self-regulation (Lai & Lin, 2017). Kenya Dairy Board should therefore create closer collaboration with the actors along the value chain, encourage them to form groups that would then steer self-regulation.

Activities of other government agencies along the value chain were not visible. Kenya Bureau of Standards has developed Kenya Standards for raw cow and camel milk; and their products. The Standards body was keener on enforcing standards for processed and packaged products than the raw milk along the value chain. Public Health authorities were only involved when invited by Kenya Dairy Board inspectors for the purpose of inspecting and apprehending offenders.

5.12 Biochemical profiles and nutrient utilization patterns of coliforms isolated from milk along the dairy value chain in Kenya

5.12.1 *Escherichia coli*

All *E. coli* isolates tested positive for beta-galactosidase utilization. ONPG is an analog of lactose, except that orthonitrophenyl has been substituted for glucose. Lactose fermenting bacteria possesses both lactose permease and β-galactosidase, two enzymes required for the production of acid in the lactose fermentation test. The permease is required for the lactose molecule to penetrate the bacterial cell where the β-galactosidase can cleave

the galactoside bond, producing glucose and galactose. Non-lactose fermenting bacteria are devoid of both enzymes and are incapable of producing acid from lactose (Brenner and Farmer III, 2005). Up to 10% of *E. coli* have been shown historically to either ferment lactose slowly or not ferment it at all (Yaratha *et al*., 2017). Manufacturers notice for API20E indicates 10% and ID 32E with 9% for non-lactose fermenting *E. coli* (Leclercq *et al*., 2000). It has been demonstrated by Yaratha *et al*. (2017) that lactose non-fermenting *E. coli* are most likely to be pathogenic but less likely to be resistant to later generation cephalosporins such as cefepime and less likely to have Extended Spectrum Beta Lactamase enzymes. The absence of the lactose non-fermenting *E. coli* in milk is a likely indicator of low prevalence of pathogenic microbes. The results for ADH were 7.1% and 20% for camel and cow respectively, consistent with various studies reported by Leclercq *at al*. (2001). The manufactures notice for API20E was equally low at 1%. Reasons for this deviation between the manufacturer and the researchers is not known to this author. However, the high positive result is concordant with the inferred low incidence/absence of enterohaemorhagic *E. coli* in milk from the study areas. Leclercq *et al*. (2001) reported that *E. coli* O157:H7 and *E. coli* 0157: H (-) microbes record higher positive for ADH at 98.2% and 89.5% respectively. This result infers either absence or low prevalence of these two microbes in the study area. Indeed, Omore *et al*. (2005), found only 2 out of 261 milk samples collected in Nairobi, Nakuru and Narok, positive for *E. coli* O157:H7. For URE test *E coli* from both sources tested negative for urease activity, against manufactures notice of 3%. *E. coli* O157:H7 and *E. coli* O157: H (-) usually have a higher score of 18.2 and 5.3% respectively (Leclercq *et al*., 2001). For IND test, all isolates from both camel and cow milk tested positive against a manufacturer's notice of 89%. Studies have reported 98 and 98.6 % against a manufacturer's notice of 84% for ID32E (Lecrecq *et al*., 2001). Leclercq *et al*. (2001) also found that all isolates of *E. coli* O157:H7 and *E. coli* O157: H (-) tested positive for indole. Indole production promotes bacterial toxicity, drug defense as well as biofilm adhesion (Hu *et al*., 2010) which enhances pathogenicity and antibiotic resistance. Indeed, Wafula *et al*. (2016) demonstrated that plastic containers used in the same study area to carry milk have higher microbial counts on their surfaces than aluminium churns.

This study found that none of the isolates tested positive for inositol against a manufacturer's notice of 1%. This is in agreement with Uzolgwe *et al*. (2007) in their study on microbial source tracking based on nutrient utilization patterns. The researchers found that the source of inositol utilizing *E. coli* was soil, and not cattle. This points at the possibility that soil was not a contaminant for the milk along the value chain. For SOR test, *E. coli* from

both sources tested 100% positive. It has been reported that shiga toxin producing *E. coli* O157:H7 does not ferment sorbitol (Brenner & Farmer III, 2005) while only 5.3% of *E. coli* O157: H(-) test positive for sorbitol. It can therefore be stated that *E. coli* O157:H7 was not isolated from milk in the study area. These microbes either don't utilize sorbitol or utilize it very slowly.

The SAC test revealed that the proportion of Sucrose fermenting *E. coli* were not significantly different for isolates from camel milk and cow milk. The isolates from the two sources testing positive for sucrose utilization was significantly higher than the manufactures notice. The ability to metabolize sucrose as a carbon source is a highly variable feature among *E. coli* strains. Sucrose-fermenting strains include the enteropathogenic strains (Sabri *et al*., 2013) which are an important cause of diarrheal diseases worldwide (Clarke *et al*., 2013). It is not however evident the role played by sucrose utilization genes in pathogenicity of the enteropathogenic strains. Brenner and Farmer III (2005) found sucrose utilization of 50% which is higher than the manufactures notice of 36%, and closer to the proportion found in this study. This variation could not be explained by this study. Last, Melibiose utilizing *E coli* are found to be ubiquitous in nature (Uzolgwe *et al*., 2007). However, this study did not find a reason why all the isolates from both camel and cow milk tested positive against a manufacturer's notice of only 75%, and a similar proportion by Brenner and Farmer III (2005).

5.12.2 *Enterobacter cloacae*

E. cloacae isolates from both camel and cow that tested positive for ADH were significantly higher compared to manufacturers notice. The result of this study was however consistent with findings of Brenner and Farmer III (2005) of 97%. For LDC, the test is negative in *E cloacae* clinical isolates (Traub *et al*., 1970). The manufacturers notice gives a conservative value of 1%. The high values found in this study with regard to cow milk could indicate that a high proportion of the isolates were not pathological.

URE tests on *E. cloacae* clinical isolates have 94% of isolates testing positive for urease (Traub *et al*., 1970). There is evidence that urease production is associated with pathogenesis in *K. pneumoniae* (Traub *et al*., 1970), and this is also possible for *E. cloacae*. None of the Isolates from camel milk tested positive while only 4.6% of the isolates from cow milk tested positive while the proportion positive for GLU was low pointing at the possibility of slow glucose fermenters among *E. cloacae* strains. All isolates tested were negative for INO against a manufacturer's notice of 12%. This is consistent with Traub *et al*.

(1970) who found 0.8% clinical isolates of *E. cloacae* utilizing inositol. The reason for the absence of INO utilising *E cloacae* is probably the same as for *E coli*. Inositol utilizing *E cloacae* may not be natural inhabitants of milk. Similarly, do not demonstrate pathogenesis. It is possible that they are natural inhabitants of soil, similar to inositol utilizing *E coli*. A significantly higher number of isolates fermented/oxidized sorbitol, sucrose and melibiose. It has been observed that pathogenic strains use a limited number of carbon sources, and this points at the strains in milk being less pathogenic (Brenner & Farmer III, 2005).

5.12.3 *Klebsiella pneumoniae*

The proportion of *K. pneumoniae* isolates from both camel and cow milk testing positive for LDC is similar to proportions from clinical isolates (Alves 2006; Hansen *et al*., 2004). This demonstrates the importance of this isolates in Gram negative mastitis, calf pneumoniae, calf mortality as well as risk to consumers of the milk (Younan *et al*., 2013). For CIT test, all *K. pneumoniae* isolates in cow milk were citrate utilizing compared to 93.3% of isolates from camel milk; against the manufacturer's notice of 95.65%. Chen *et al*. (2009) found that half the clinical isolates of *K. pneumoniae* have a genomic region containing the citrate fermentation gene *gltA* with ability to grow anaerobically on citrate. The citrate gene interacts indirectly with innate immune molecule Lipocalin 2 (Lcn2) whose function is to sequester iron limiting bacterial growth. In turn, *K pneumoniae* circumvents Lcn2 activity by expressing alternative hemophores, which acquire iron from the host, increasing pathogenesis (Vornhagen *et al*., 2019). Indeed, Younan *et al*. (2013) has related calf pneumonia and morbidity in camels in Kenya to *K. pneumoniae*. *K. pneumoniae* isolates from camel milk, with urease activity were 87.7% while all the isolates from cow milk had urease activity, against a manufacturer's notice of 75%. Urease production is a virulent factor. Higher frequency of urease positive isolates are from clinical samples (Traub *et al*., 1970). A high frequency of urease positive isolates in milk is a strong indicator of clinical mastitis and possibility that the isolates are contagious (Langoni *et al*., 2015; Sharmal *et al*., 2015). Urease production allows bacteria to grow in acidic environment. The higher prevalence in isolates from cow milk compared to camel milk could be an indicator of severity of gram-negative mastitis attack in cow populations from the study area compared to camel populations. A parallel study by Kashongwe *et al*. (2017) in the same study area found 60% prevalence of mastitis in smallholder cows and 93 % in extensive pastoral camels. However, the same study identified *Staphylococcus aureus* and *Streptococus* spp being involved in less than 60% in cow and less than 42% in camel mastitis cases. Wanjohi *et al*. (2013) identified *Klebsiella*

and *Enterobacter* as dominant microbes in milk from camels suffering from clinical mastitis. This is enhanced by the fact that mastitis treatment in the study area focusses on gram positive microbes by use of Penicillin based antibiotics which have been demonstrated to be ineffective against Gram negative microbes. There was lower utilization of glucose by isolates from camel milk while it is expected that all isolates be positive for glucose. However, it has been reported that pathogenic *K. pneumoniae* use less carbon sources than commensal microbes (Brenner & Farmer III, 2005). For INO test, there were statistically low proportions of isolates utilizing inositol from camel milk compared to cow milk, and the manufacturer's notice. It is possible, but not certain that high INO utilizing *K. pneumoniae* are environmental as reported by Uzolgwe *et al*. (2007) for *E. coli.*

5.12.4 *Klebsiella oxytoca*

Proportionately fewer *K. oxytoca* isolates from camel milk tested positive for LDC compared to cow and manufacturers notice. The difference was marginal, and could be attributed to the test itself. In a study of comparison of LDC test on same isolates of *K. oxytoca* between three labs, Hansen *et al*. (2004) reported that one lab scored 96.7% and the other two labs scored 100%. Such differences are therefore possible with LDC test on *K. oxytoca*. For CIT test, *K. oxytoca* isolates from camel milk had higher percentage of positive isolates than those from cow milk, while cow milk isolates had lower proportion of positive isolates compared to manufacturers notice. This study did not find a reason for this difference. Hansen *et al*. (2004) however did not find the test useful in differentiating between *Klebsiella* species due to its variability. For URE test, the proportion of positive isolates from cow milk for URE test was very low at only 13.3% compared to isolates from camel (77%), and manufacturer's notice (78%). Equally, Brenner and Farmer III (2005) reported 90% positive. It is possible that since *K. oxytoca* are closely related to *K. pneumoniae*, the proportion of *K. oxytoca* pathogenic microbes in cow milk is low, and this species may not be a problem in Gram negative mastitis in lactating cows. For INO test, both camel and cow milk isolates posted lower positive results than the manufacturers notice. As noted for *E. coli* and *E. cloacae*, it appears that inositol utilizing *K. oxytoca* inhabit soil, which would possibly indicate that soil is not a serious contaminant of milk along the value chain (Uzolgwe *et al*., 2007). The source of low inositol utilizing *K. oxytoca* is probably the udder of infected lactating animals.

5.13 Phylogeny and distribution of coliforms along the dairy value chain based on 16s rRNA

Sequencicing results show that *E. coli* and *Klebsiella sp* isolates were ubiquitous in their existence. Their main entry into milk in the smallholder and pastoral herds is through contamination from the udder surface (Orwa *et al*., 2017). For *E. cloace*, it is possible that some isolates may be associated more with udder swabs. The sequences of genes coding for 16S rRNA are generally highly conserved among bacteria although some variable regions are present within these sequences (Wolska & Szweda, 2012). Use of 16s rRNA is however not robust enough in tracking sources of microbes.

5.14 Haemolytic activity of *E. coli*

E. coli cause infections due to the presence of virulent factors which include β-haemolysins, ESBL's and biofilms which endow it ability to survive, multiply and cause disease (Kukanur *et al*., 2015). The presence of *E. coli* with these virulent factors in milk and not on the udder surface, milking persons' hands and water therefore indicate the adaptation and colonization of these microbes in the lactating animals' udder. It has been shown that *E. coli* is one of the major microorganisms isolated from camel mastitis milk (Abdelgadir, 2014), and in Kenya, Wanjohi *et al*. (2013) reported that the prevalence of *E. coli* isolated from camel milk was 60%.

In human subjects, β-haemolytic *E. coli* act as virulent factors for pathogenesis in patients with urinary tract infection. It is therefore probable that the β-haemolytic *E. coli* in this study were from infected udder tissues. This is because β-haemolytic *E. coli* are known to be mostly associated with diseased tissues because they have selective advantage by releasing iron from erythrocytes and enhances pathogenicity by destroying phagocytic and epithelial cells (Kukanur *et al*., 2015).

The predominance of β-haemolytic *E. coli* in the udder milk could be because the protein haemolysin, which is the virulence factor, is only produced upon receiving certain signals from the host (China & Goffaux, 1999), which in this study points at the udder of the lactating animal. In the environmental isolates, the genes of virulence may be present in the bacterial genome or the plasmid, even though the virulence factor is not expressed (Harel & Martin, 1999).

5.15 Antibiotic sensitivity of *E. coli*

The emergence and spread of resistance of pathogens to antimicrobials is a worldwide phenomenon which may arise from their overuse, inadequate dosing, poor adherence, and substandard antimicrobials (GARP, 2011; Patel & Levitin, 2014). These antimicrobials are used in the control of diseases in human, animals and crops. In Kenya, penicillins are the most prescribed antibiotics in human subjects (GARP, 2011; Mitema *et al*., 2001) despite the fact that it is rarely recommended for treatment of serious Gram negative infections (CDC, 2013). In this study, although the resistance of AMP to β-haemolytic *E. coli* from cow and camel milk was 25% and 52.4% respectively, it was lower than 89% reported by GARP (2011) for *E.coli* isolates from healthy human subjects in Kenya. The higher resistance in isolates from human sources is due to overuse of the antibiotic in human populations. It was found during this study that penicillin was the drug of choice for mastitis control in both cows and camels in the study areas, which could be the reason for the resistance levels witnessed in this study. However, the significantly higher resistance of isolates from camel milk compared to cow milk could be due to lack of antibiotic stewardship in administration of antibiotics to camels in the study area.

Indeed, Lamuka *et al*. (2017) reported that 46% of the pastoralists administer veterinary drugs to sick camels without seeking services of veterinary officers. This results to either under dosing, overdosing, or administering the wrong drug to the animal and lack of observation of withdrawal periods. Their study also reported that the common antibiotics in common use were adamycin (33%), ampicillin (27%) and penicillin (14%). This resistance to AMP can also be used to predict resistance to amoxyllin (CLSI, 2016) which is another drug that is frequently prescribed to human populations in the country. From our data however, it was not possible to tell the cause for resistance of the pathogens to CTX and FEP because the drugs are not used in the study area for animal disease management. It could however be due to inherent ESBL's in the microbes. This is because resistance to CTX is a good marker of ESBL prevalence (Wiedemann *et al*., 2014), and demonstrates the ability of the *E. coli* strains to be resistant to other 3rd generation cephalosporins (Paterson, 2005). The level of resistance to Cefepime is of great concern being that it was only introduced into clinical practice in the 1990s and recommended for treatment of *Enterobacteriaceae* infections. More so, in previous studies, FEP has been demonstrated to have '*in vitro*' activity against ESBL-producing *Enterobacteriaceae* (Nogueira *et al*., 2011). This finding therefore demonstrates the prevalence of ESBL producing *E.coli* in the study area reducing treatment options for both infected persons and animals. This is because some ESBL's are known to destroy AMP,

2nd, and 3rd generation oxyimino-cephalosporins (Shaikh *et al*., 2015). It is however positive that none of the isolates was resistant to the second-line and last line agent, ciprofloxacin and Imipenem respectively. Ciprofloxacin is however restricted to high-level facilities, in this case, district (sub-county) hospitals and above (GARP, 2011) which implies that individuals infected with these microbes cannot get treatment at lower medical facilities such as dispensaries. In addition, the use of ciprofloxacin in paediatric patients has been limited due to the possibility of arthropathy (Adefurin, 2011) and therefore children remain at great risk if infected. The use of last line carbepenems (Imipenem) would result in selection of carbepenem resistant *Enterobacteriaceae* which is an emerging global time-bomb.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

6.1.1 Factors driving microbial spoilage of milk along the Smallholder dairy value chain in Kenya

This study mapped the smallholder value chain from production to the market with respect to microbial quality of milk. It was demonstrated that smallholder Peri-urban value chain was distinct from the smallholder rural value chain. The Peri-urban chain was characterised by delivering most of the raw milk to urban consumers while the rural value chain delivered most of the milk to cooling centres. The processors then transported the milk from the cooling centres to the dairies for further processing into dairy products. The study found that the main factors that influence microbial quality are training of milking persons, duration of holding milk at ambient temperature during transport, and type of container used for milk handling.

6.1.2 Effect of predisposing factors on microbial loads in camel milk along the pastoral dairy value chain in Kenya

The study found that practices at production and along the chain contribute to high microbial counts in milk. The long time that milk is held at ambient temperature while awaiting collection at primary collection centres, or during transport contributes to increased microbial load in the milk. Most importantly, training of milk handlers should include all actors starting with the herdsmen at production. This should be complemented by policy to support provision of water not only to support camel husbandry but also hygiene during milking. Supporting the producers, transporters, and traders to acquire low cost food grade plastic containers with a capacity of 20 to 25L of milk will not only reduce microbial counts but also be convenient during handling and transportation. Most importantly, design of cheaper and more practical cooling facilities based on solar energy or evaporative cooling using charcoal housing would greatly reduce milk spoilage. Processing of the milk would add value by improving its shelf-life, and therefore access distant markets. In addition, the Kenya Bureau of Standards, a government agency established under the Kenyan law (Standard Act, CAP 496), and responsible for quality and standards should intervene and regulate this industry. Similarly, the Kenya Dairy Board (established under the Dairy Act, CAP 336) has a direct mandate over the dairy industry and it is therefore required to streamline it. There is need however to revise the age-old Dairy Industry Act to recognise other sources of milk

such as camel milk, whose production and demand is growing tremendously, and the highly priced goat milk as well as donkey milk that recent media reports have indicated that it is being produced and consumed in Naivasha, within Nakuru county. This would greatly improve the livelihoods of the pastoral camel milk producers, and other emerging markets for novel milk such as goat milk and donkey milk.

6.1.3 Biochemical profiles of coliforms isolated from milk along the dairy value chain in Kenya

Coliforms in milk are not only indicators of hygiene and opportunistic pathogens, but they also contribute to deterioration as demonstrated by their ability to break down lactose. *E. coli:* There is great concern that all *E. coli* isolates from both camel and cow milk produce indole which is related to bacterial toxicity, drug defence, as well as biofilm adhesion. *E. coli* with high biofilm adhesion not only compromises attempt to meet milk quality requirements but also poses a risk to consumers of milk especially when coliform counts are high in raw milk meant for processing.

E. cloacae: Recent studies have pointed at *E. cloacae* as an emerging pathogen. This is not yet a concern in isolates from both camel and cow milk from the study area due to evidence of LDC and URE tests. However, continuous effort should be taken in management so that this emergence does not reach the milk value chain in future.

K. pneumoniae: The high proportion of *Klebsiella pneumoniae* that test positive for CIT and URE in milk is a matter of concern. CIT positive *K. pneumoniae* are likely to be pathogenic while Urease positive *K. pneumoniae* are associated with gram negative mastitis. *K. pneumoniae* are also associated with calf septicaemia and death and efforts should therefore focus on heard management to reduce gram negative mastitis which appears to be the source of the pathogens. Reduction of Gram negative mastitis would not only reduce the risk posed by urea hydrolysing *K. pneumoniae* on calves but to consumers of the milk too.

K. oxytoca: This study has not expressly established a relationship between biochemical profiles of K. oxytoca in milk with pathogenicity. The close relationship of *K. oxytoca* with *K. pneumoniae,* and the fact that it is the second most isolated *Klebsiella* pathogen, it is important to manage it the same way *K. pneumoniae* would be managed to reduce its proliferation in milk

6.1.4 Genetic distribution of coliforms in milk along the value chain in Kenya based on 16s rRNA

Use of 16s rRNA gene sequencing does not distinguish micrbobes according to their sources in milk. It is however a useful tool for confirming identity of micrbes that software for biochemical profiles mis-identify. A good example is the misidentification of *E. coli* ATCC 25922 as *Salmonella ozaenae* by API 20E software.

6.1.5 Pathogenicity and antibiotic sensitivity of *E-coli* isolated from milk along the dairy value chain in Kenya

This study established that the source of β-haemolytic *E. coli* in milk was inside the lactating animals' udder. There's therefore need for antibiotic stewardship so that the right antibiotic is administered in the right way for the right infection. In this case, there should be a shift from blanket administration of penicillins to treat mastitis infections in the study areas to pathogen-specific antibiotic prescription based on microbial culture results. Meanwhile, the veterinary officers should change from penicillin drugs to *E. coli* (Gram negative) sensitive antibiotics for the treatment of mastitis in case the infected animals do not respond to antibiotics targeting Gram-positive microbes. However, there is still controversy on whether treatment regimens for mastitis caused by Gram-negative microbes is effective or not.

6.2 Recommendations

6.2.1 Factors driving microbial spoilage of milk along the Smallholder and Pastoral dairy value chain in Kenya

To reduce Milk Post-harvest losses along the Smallholder and Pastoral dairy value chains in Kenya, this study recommends;

i) Train milk handlers' right from milking persons and along the value chain for both Peri-urban and rural value chains. Training of household heads (farmers) only does not necessarily transfer the skills to the milking persons, while training traders and transporters without training milking persons and milk handlers a the farm is futile. Emphasis should be given to proper maintenance of hygiene especially for stall fed cows to reduce microbial levels.

ii) Milk coolers to be closer to the producers to reduce time taken to deliver milk to the cooling centres.

iii) Reduction in the cost of energy for milk cooling by considering alternative energy

sources of energy such as solar energy.

iv) Improving roads in milk producing areas to facilitate faster transport of milk to the cooling centres.

v) Sink boreholes or provide other appropraite form of water suppy to camel milk producing areas to provide water for camels as well as water for milking hygiene

vi) Empower value chain operators to self-regulate to enhance quality assurance along the chain. For pasteurised milk, although the East African Standard has a more severe heat treatment requirement for pasteurization, processing of only good quality raw milk would result in a high quality product that meets the standards.

vii) Revise the Dairy Induatry Act to include camel milk, goat milk and the new emerging donkey milk production and trade, in order to increase the mandate of Kenya Dairy Board

viii) Policy intervention similar to the United States of America Common Agricultural Policy, and the Dairy Product Price Support in the European Union which centred on price support to farmers, stabilization of markets as well as trade protection would spur investment in the dairy sector with increased production and reduction of losses (Abdulssamad & Gereffi, 2016). A structered determination of milk prices, right from the producer to the processor should be put in place. This should consider production cost of milk, and costs along the value chain. This should be structured similar to the Energy and Petroleun Regulatory Authority that regulated fuel prices in Kenya. This is the only way, smallholder farmers can be enpowered, even to be able to adopt measures to improve hygiene, and ultimately reduce losses.

6.2.2 Biochemical profiles of coliforms isolated in milk along the dairy value chain in Kenya

This study recommends the use of biochemical profiles for microbial source tracking in dairy systems in under-resourced countries. It is possible to use biochemical profiles to know the probable source of microbes that infect milk along the chain. This is especially useful at the production level because the possible sources of contamination are known. These being soil, water, and animal waste. Further study is recommended to develop a reference data base of microbes based on their Carbon Utilization Profiles and Nutrient Utilization Patterns with reference to their sources.

6.2.3 Genetic diversity of coliforms in milk along the value chain in Kenya based on 16s rRNA

This study recommends the use of cultivation independent methods based on nucleic acid analysis for study of genetic diversity in microbial communities, including distribution of coliforms along the dairy value chain. A review of these methods has been done by Fakruddin & Mannan, 2014.

6.2.4 Pathogenicity and antibiotic sensitivity of *E. coli* isolated from milk along the dairy value chain in Kenya

To prevent shedding of pathogenic microbes into milk, this study recommends;

i) Prioritising mastitis prevention and hygiene of lactating cows and camels since this study has demonstrated that pathogenic *E. coli* in milk are shed with milk.

ii) That interventions for microbiological contamination of milk should start from production instead of focusing only on nodes along the value chain. It has been demostrated that most contamination occurs during production, and microbial numbers increase along the value chain.

iii) Pastoral communities should desist from tasting and consuming raw milk due to the presence of β-haemolytic *E. coli* strains in the milk. More concerted efforts should be deployed towards periodic tracking of resistant patterns of *E. coli* not only in milk, but also in human populations.

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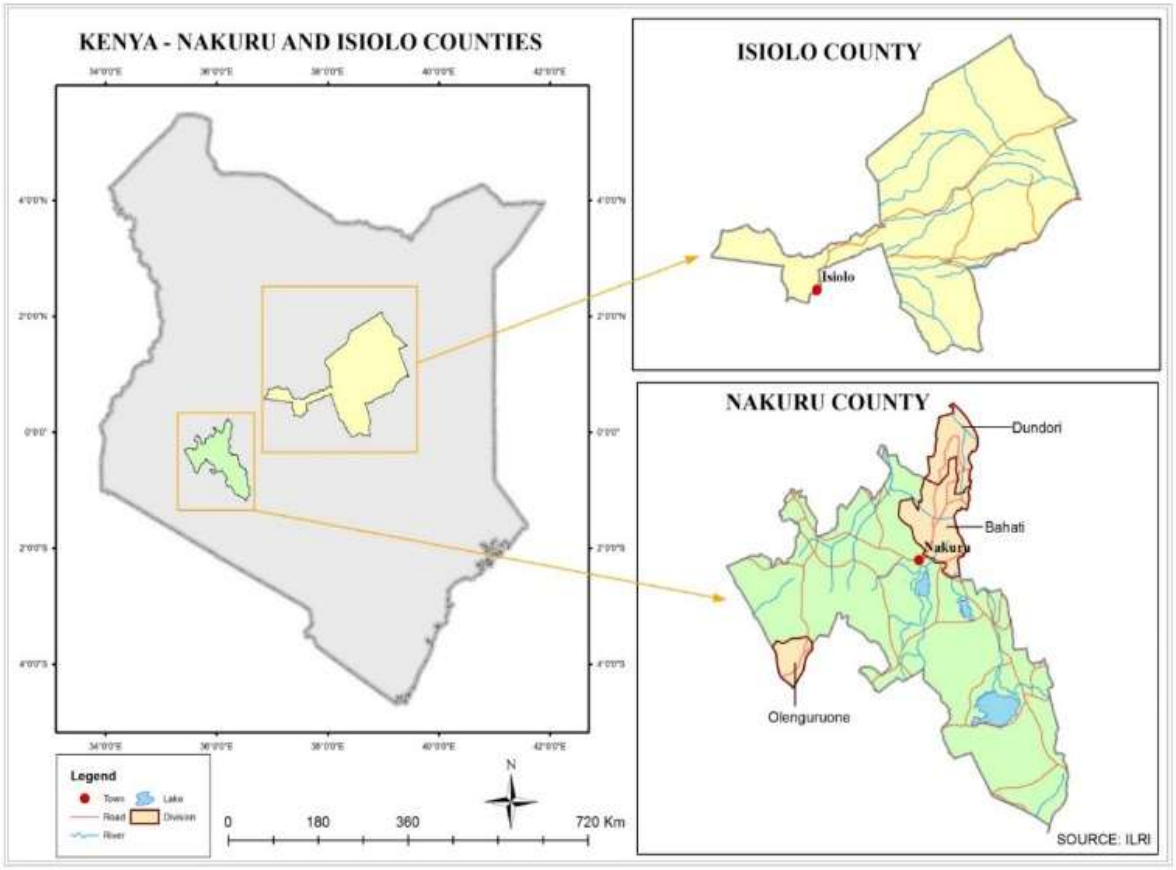
APPENDICES

Appendix A: Map of Kenya showing the Study area



Appendix B: Checklist for determination of risk factors for high microbial load in milk at the farm level

Egerton University

Faculty of Agriculture

Department of Dairy and Food Science and Technology

Checklist for determination of risk factors for high microbial load in milk

Preliminaries

Questionnaire No…….

Name of enmerator…….

Date of interview…….

Introduction

Hallo. My name is…….

I am conducting a survey for determining the risk factors for microbial milk loss. This information will be useful in developing appropriate intervention measures. I would like to inform you that the information you provide will be completely confidential.

Preliminary information	**Description/Score**
1. DVC	1-Smallholder periurban 2. Smallholder rural 3. Pastoral
2. Location	
3. Name of respondent	
4. Sex of respondent	1-male. 2-female.
5. Age of respondent	
6. Telephone No. of respondent	
Variables	
7. Udder washing	1-Yes 2-No
8. Udder drying	1-Yes. 2-No
9. Hand washing	1-Yes. 2-No
10. Source of farm water	1-Rain catchment. 2-Shallow well 3. River. 4-Municipal supply. 5-Anyother
11. Treatment/Boiling of water before use	1. Yes. 2-No

Appendix C: Interview schedule for determination of risk factors for high microbial load in milk along the dairy value chain in Kenya

Egerton University

Faculty of Agriculture

Department of Dairy and Food Science and Technology

Interview schedule for determination of risk factors for high microbial load in milk

Preliminaries
Questionnaire No…….
GPS ……..
Name of enumerator…….
Date of interview…….
Introduction
Hallo. My name is…….
I am conducting a survey for determining the risk factors for microbial milk loss. This information will be useful in developing appropriate intervention measures. The interventions will not only be technological but will also influence policy to increase gains of all actors along the value chain.
I would like to inform you that the information you provide will be completely confidential.
If it is agreeable, start the interview.
Starting time…….Ending time……

Preliminary information	**Description/Score**
1. DVC	1-Smallholder periurban 2. Smallholder rural 3. Pastoral
2. Node	1-farmgate. 2-collection centre. 3-processor. 4. Milk-bar. 5-trader (hawker)
3. Location	
4. Name of respondent	
5. Sex of respondent	1-male. 2-female.
6. Age of respondent	
7. Telephone No. of respondent	
Variables (By observation or measurement)	
8. Milk temperature	……..^{o}C
9. Transport means	1-foot. 2-Donkey. 3-Camel. 4-Bicycle. 5-Motorbike. 6-Vehicle
10. Container type	1-Aluminium churn. 2-Plastic jerrican. 3-anyother……
11. Cleanliness of container	1-Clean. 2-Dirty. 3-Very dirty
12. Method of	1. Pouring. 2-Scooping

dispensing milk	
13. Volume of milk in the receptacle	…….Litres
Variables (To be answered by respondent)	
14. What is your education level?	1- None. 2-Primary. 3-Secondary. 4- Certificate. 5-Diploma. 6-Degree
15. Have you ever been trained on milk quality?	1-Yes. 2-No
16. For how long have you been engaged in this activity (experience)?	……..years
17. Do you mix morning and evening milk?	1-Yes. 2-No
18. Do you pool milk from different sources?	1-Yes. 2-No
19. Do you carry out quality test on milk quality?	1-Yes. 2-No If yes, which ones……..
20. How much time do you take from milk source to this point?	……..hours
21. What is the source of water for cleaning milk containers?	1-Rain catchment. 2-Shallow well 3. River. 4-Municipal supply. 5-Anyother
22. Do you disinfect milk containers after cleaning?	1-Yes, how…….. 2-No.
23. Volume of milk handled in a day	…….litres

Appendix D: Guidelines for semi-structured interview with group milk collection centre manager

Themes

- History and motivation for formation of group
- Milk sourcing, handling and sale
- Major challenges faced
- View on Post harvest losses

Appendix E: Guideline for semi-structured interview with macro-level value chain actors

Themes

- History of institution
- Mandate of institution
- Major challenges faced
- View on Post harvest losses

Appendix F: Major statistical outputs

i) Total Viable Counts (Log_{10} cfu/ml) in milk along the Smallholder Peri-Urban Value Chain

The GLM Procedure

Class Level Information

Class	Levels	Values
Node	4	MB PUCC PUUM TR

Number of Observations Read	80
Number of Observations Used	77

The GLM Procedure

Dependent Variable: TVC log base 10 of TVC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	45.0007408	15.0002469	17.11	<.0001
Error	73	63.9830603	0.8764803		
Corrected Total	76	108.9838012			

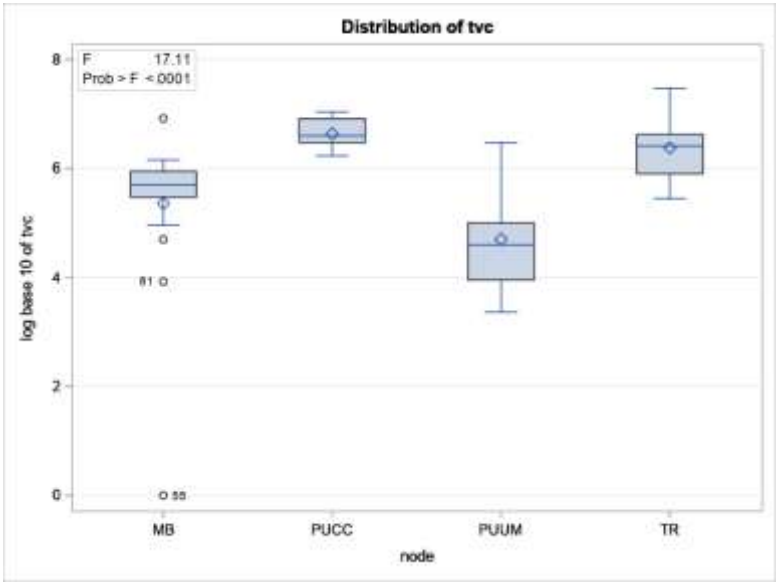
R-Square	Coeff Var	Root MSE	TVC Mean
0.412912	17.11862	0.936205	5.468930

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Node	3	45.00074084	15.00024695	17.11	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Node	3	45.00074084	15.00024695	17.11	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	6.381721029	B	0.22706313	28.11	<.0001
node MB	-1.023081783	B	0.30883924	-3.31	0.0014
node PUCC	0.255399357	B	0.38593310	0.66	0.5102
node PUUM	-1.681351770	B	0.28254419	-5.95	<.0001
node TR	0.000000000	B	.	.	.

Note: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

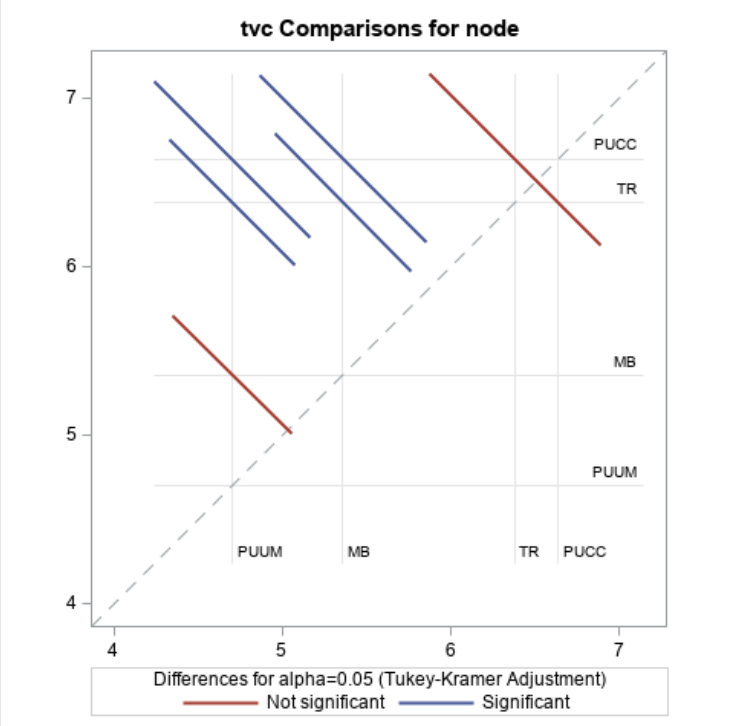


The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

NODE	TVC LSMEAN	LSMEAN Number
MB	5.35863925	1
PUCC	6.63712039	2
PUUM	4.70036926	3
TR	6.38172103	4

Least Squares Means for Effect NODE
t for H0: LSMean(i)=LSMean(j) / Pr > |t|
Dependent Variable: TVC

i/j	1	2	3	4
1		-3.40221	2.451567	-3.31267
		0.0059	0.0765	0.0077
2	3.402205		5.463549	0.661771
	0.0059		<.0001	0.9110
3	-2.45157	-5.46355		-5.95076
	0.0765	<.0001		<.0001
4	3.312668	-0.66177	5.950757	
	0.0077	0.9110	<.0001	



ii) Total Viable Counts (Log10 cfu/ml) in milk along the Smallholder Rural value chains

The GLM Procedure

Class Level Information

Class	Levels	Values
Node	2	RCC RUM

Number of Observations Read	41
Number of Observations Used	41

The GLM Procedure

Dependent Variable: TVC log base 10 of TVC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	46.53615601	46.53615601	190.87	<.0001
Error	39	9.50843739	0.24380609		
Corrected Total	40	56.04459339			

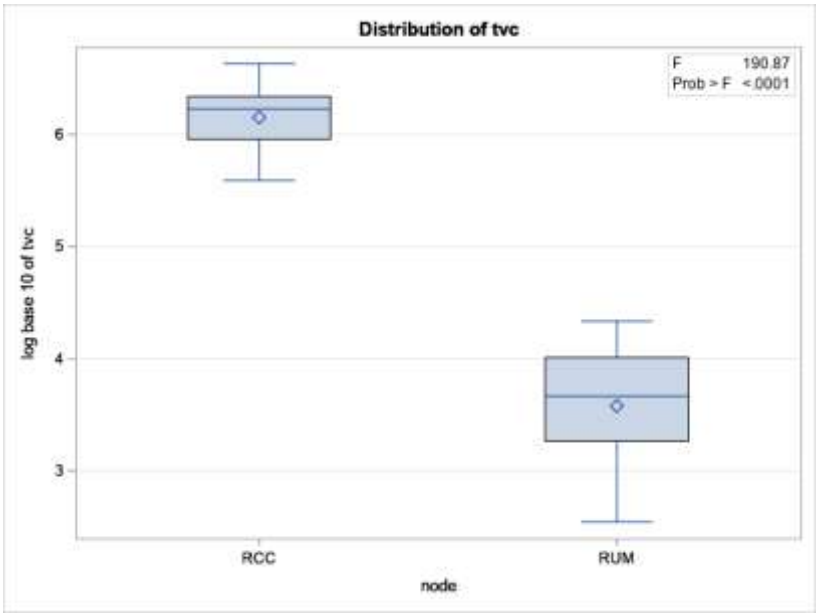
R-Square	Coeff Var	Root MSE	tvc Mean
0.830342	11.91791	0.493767	4.143069

Source	DF	Type I SS	Mean Square	F Value	Pr > F
node	1	46.53615601	46.53615601	190.87	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
node	1	46.53615601	46.53615601	190.87	<.0001

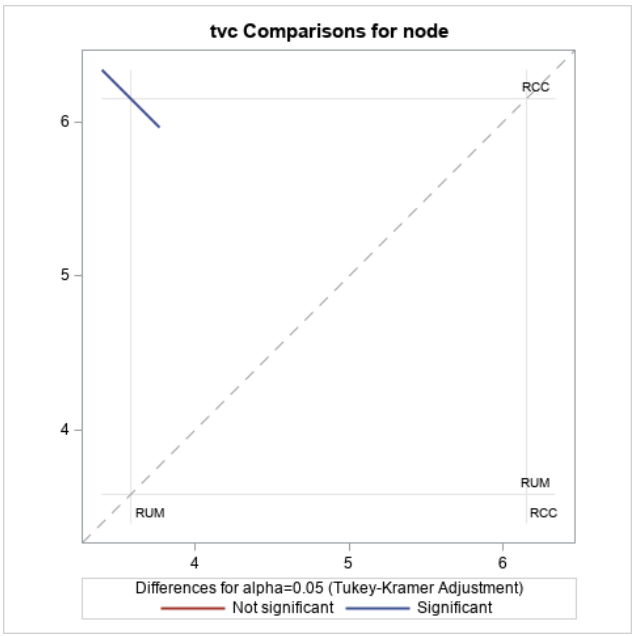
Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	3.578067964	B	0.08728654	40.99	<.0001
node RCC	2.573895661	B	0.18630219	13.82	<.0001
node RUM	0.000000000	B	.	.	.

Note: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.



The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

node	tvc LSMEAN	H0:LSMean1=LSMean2	
		t Value	Pr > |t|
RCC	6.15196363	13.82	<.0001
RUM	3.57806796		



iii) Coliform Counts (TVC, Log10 cfu/ml) in milk along the Peri-urban (P-urban) value chains

The GLM Procedure

Class Level Information

Class	Levels	Values
node	4	MB PUCC PUUM TR

Number of Observations Read	80
Number of Observations Used	68

The GLM Procedure

Dependent Variable: cc log base 10 of cc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	46.0034228	15.3344743	10.74	<.0001
Error	64	91.3670820	1.4276107		
Corrected Total	67	137.3705047			

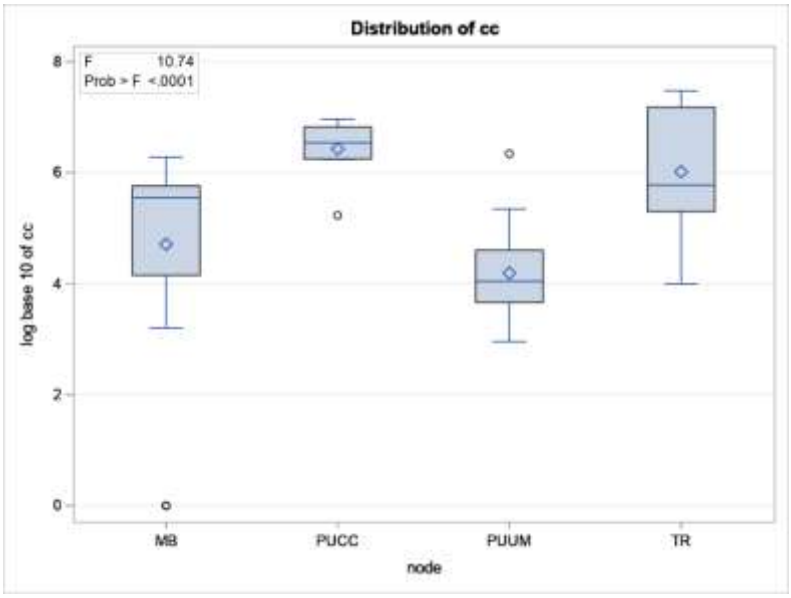
R-Square	Coeff Var	Root MSE	cc Mean
0.334886	24.64822	1.194827	4.847516

Source	DF	Type I SS	Mean Square	F Value	Pr > F
node	3	46.00342278	15.33447426	10.74	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
node	3	46.00342278	15.33447426	10.74	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	6.015663654	B	0.39827554	15.10	<.0001
node MB	-1.306692094	B	0.47958726	-2.72	0.0083
node PUCC	0.410897495	B	0.58058138	0.71	0.4817
node PUUM	-1.825398586	B	0.45241061	-4.03	0.0001
node TR	0.000000000	B	.	.	.

Note: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

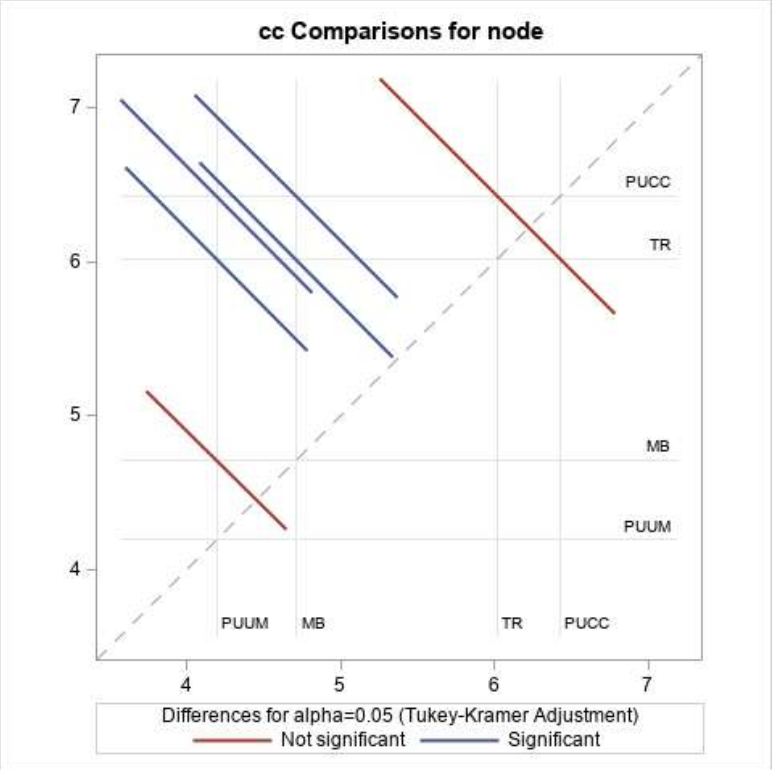


The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

node	cc LSMEAN	LSMEAN Number
MB	4.70897156	1
PUCC	6.42656115	2
PUUM	4.19026507	3
TR	6.01566365	4

Least Squares Means for Effect node
t for H0: LSMean(i)=LSMean(j) / Pr > |t|
Dependent Variable: cc

i/j	1	2	3	4
1		-3.43633	1.513657	-2.72462
		0.0056	0.4354	0.0403
2	3.436335		4.719739	0.707735
	0.0056		<.0001	0.8937
3	-1.51366	-4.71974		-4.03483
	0.4354	<.0001		0.0008
4	2.724618	-0.70773	4.034827	
	0.0403	0.8937	0.0008	



iv) Coliform Counts (Log10 cfu/ml) in milk along the Smallholder Rural value chains

The GLM Procedure

Class Level Information

Class	Levels	Values
Node	2	RCC RUM

Number of Observations Read	41
Number of Observations Used	40

The GLM Procedure

Dependent Variable: cc log base 10 of cc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	62.36737466	62.36737466	138.37	<.0001
Error	38	17.12816327	0.45074114		
Corrected Total	39	79.49553793			

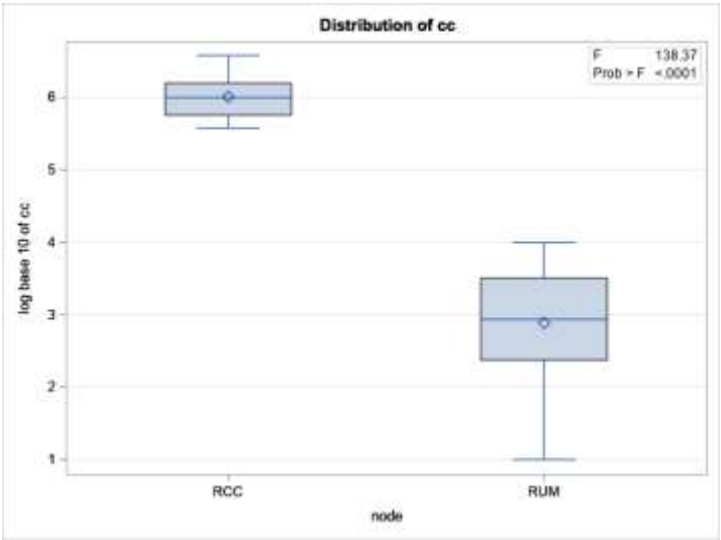
R-Square	Coeff Var	Root MSE	cc Mean
0.784539	19.11869	0.671373	3.511604

Source	DF	Type I SS	Mean Square	F Value	Pr > F
node	1	62.36737466	62.36737466	138.37	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
node	1	62.36737466	62.36737466	138.37	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	2.887267210	B	0.11868303	24.33	<.0001
node RCC	3.121682606	B	0.26538331	11.76	<.0001
node RUM	0.000000000	B	.	.	.

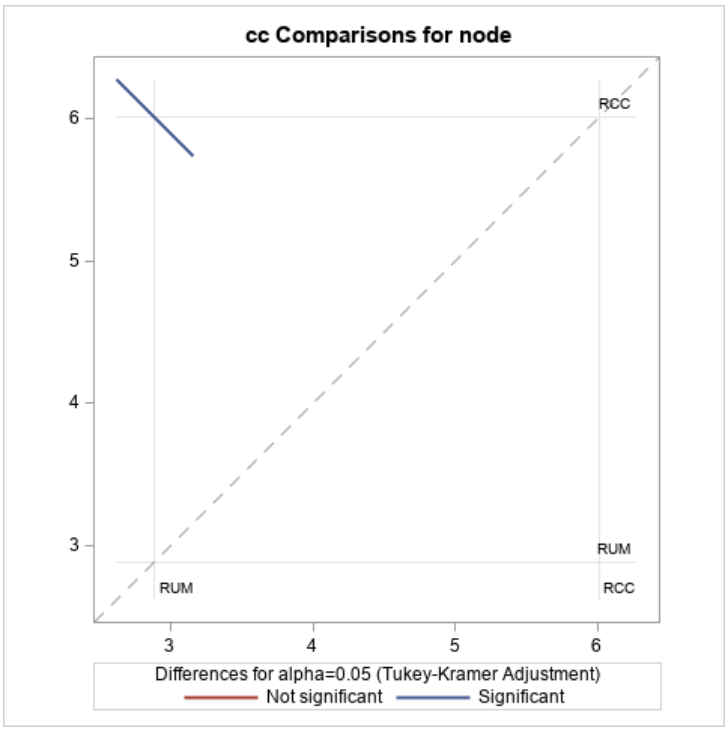
Note: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.



The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Node	cc LSMEAN	H0:LSMean1=LSMean2	
		t Value	Pr > |t|

Node	cc LSMEAN	H0:LSMean1=LSMean2	
		t Value	Pr > |t|
RCC	6.00894982	11.76	<.0001
RUM	2.88726721		



Compliance of raw milk (% by volume) along the smallholder Rural DVC based on TVC according to EAC standards

The FREQ Procedure

Table of node by grade

Frequency / **Percent** / **Row Pct** / **Col Pct**	**node**	**grade**				
		1	**2**	**3**	**poor**	**Total**
	RCC	0	5	2	2	9
		0.00	12.20	4.88	4.88	21.95
		0.00	55.56	22.22	22.22	
		0.00	100.00	100.00	100.00	
	RUM	32	0	0	0	32

	78.05	0.00	0.00	0.00	78.05
	100.00	0.00	0.00	0.00	
	100.00	0.00	0.00	0.00	
Total	32	5	2	2	41
	78.05	12.20	4.88	4.88	100.00

Statistics for Table of node by grade

Statistic	DF	Value	Prob
Chi-Square	3	41.0000	<.0001
Likelihood Ratio Chi-Square	3	43.1558	<.0001
Mantel-Haenszel Chi-Square	1	30.5927	<.0001
Phi Coefficient		1.0000	
Contingency Coefficient		0.7071	
Cramer's V		1.0000	

Sample Size = 41

Compliance of raw milk (% by volume) along the smallholder peri-urban DVC based on TVC according to EAC standards.

The FREQ Procedure

Table of node by grade

Frequency
Percent
Row Pct
Col Pct

node	**grade**				
	1	**2**	**3**	**poor**	**Total**
MB	4	12	4	0	20
	5.19	15.58	5.19	0.00	25.97
	20.00	60.00	20.00	0.00	
	14.29	54.55	40.00	0.00	
PUCC	0	0	1	8	9
	0.00	0.00	1.30	10.39	11.69
	0.00	0.00	11.11	88.89	
	0.00	0.00	10.00	47.06	

PUUM	24	5	0	2	31
	31.17	6.49	0.00	2.60	40.26
	77.42	16.13	0.00	6.45	
	85.71	22.73	0.00	11.76	
TR	0	5	5	7	17
	0.00	6.49	6.49	9.09	22.08
	0.00	29.41	29.41	41.18	
	0.00	22.73	50.00	41.18	
Total	28	22	10	17	77
	36.36	28.57	12.99	22.08	100.00

Statistics for Table of node by grade

Statistic	DF	Value	Prob
Chi-Square	9	73.6545	<.0001
Likelihood Ratio Chi-Square	9	81.2746	<.0001
Mantel-Haenszel Chi-Square	1	0.5973	0.4396
Phi Coefficient		0.9780	
Contingency Coefficient		0.6992	
Cramer's V		0.5647	

Sample Size = 77

Compliance of raw milk (% by volume) along the smallholder peri-urban DVC based on CC according to EAC standards

The FREQ Procedure

Table of node by grade

Frequency **Percent** **Row Pct** **Col Pct**	**Node**	**grade**			
		good	**poor**	**v-good**	**Total**
	MB	4	14	2	20
		5.88	20.59	2.94	29.41
		20.00	70.00	10.00	
		12.90	41.18	66.67	

PUCC	0	8	0	8
	0.00	11.76	0.00	11.76
	0.00	100.00	0.00	
	0.00	23.53	0.00	
PUUM	25	5	1	31
	36.76	7.35	1.47	45.59
	80.65	16.13	3.23	
	80.65	14.71	33.33	
TR	2	7	0	9
	2.94	10.29	0.00	13.24
	22.22	77.78	0.00	
	6.45	20.59	0.00	
Total	31	34	3	68
	45.59	50.00	4.41	100.00

Statistics for Table of node by grade

Statistic	DF	Value	Prob
Chi-Square	6	32.3208	<.0001
Likelihood Ratio Chi-Square	6	37.0852	<.0001
Mantel-Haenszel Chi-Square	1	7.8964	0.0050
Phi Coefficient		0.6894	
Contingency Coefficient		0.5676	
Cramer's V		0.4875	

Sample Size = 68

Compliance of raw milk (% by volume) along the smallholder Rural DVC based on CC according to EAC standards

The FREQ Procedure

Table of node by grade

Frequency
Percent **node** **grade**

Row Pct / Col Pct		good	poor	v. good	Total
	RCC	0	8	0	8
		0.00	20.00	0.00	20.00
		0.00	100.00	0.00	
		0.00	100.00	0.00	
	RUM	15	0	17	32
		37.50	0.00	42.50	80.00
		46.88	0.00	53.13	
		100.00	0.00	100.00	
	Total	15	8	17	40
		37.50	20.00	42.50	100.00

Statistics for Table of node by grade

Statistic	DF	Value	Prob
Chi-Square	2	40.0000	<.0001
Likelihood Ratio Chi-Square	2	40.0322	<.0001
Mantel-Haenszel Chi-Square	1	0.0306	0.8612
Phi Coefficient		1.0000	
Contingency Coefficient		0.7071	
Cramer's V		1.0000	

Sample Size = 40

Microbial quality (Log base 10 TVC) of different brands of pasteurized milk from different processors in Kenya. (1) Fresha (2) KCC (3) Kinangop, and (4) Molo

The UNIVARIATE Procedure
Variable: tvc (log base 10 of tvc)
brand=Fresha

Moments

N	10	Sum Weights	10
Mean	8.51745306	Sum Observations	85.1745306
Std Deviation	1.83706111	Variance	3.37479352
Skewness	0.19581252	Kurtosis	-0.7287651
Uncorrected SS	755.843208	Corrected SS	30.3731417

Moments

Coeff Variation	21.5681976	**Std Error Mean**	0.58092973

The UNIVARIATE Procedure
Variable: TVC (log base 10 of TVC)
brand=KCC

Moments

N	10	**Sum Weights**	10
Mean	3.32656518	**Sum Observations**	33.2656518
Std Deviation	3.18906043	**Variance**	10.1701064
Skewness	1.32430744	**Kurtosis**	3.02226212
Uncorrected SS	202.191317	**Corrected SS**	91.5309576
Coeff Variation	95.8664643	**Std Error Mean**	1.00846945

The UNIVARIATE Procedure
Variable: TVC (log base 10 of TVC)
brand=Kinangop

Moments

N	10	**Sum Weights**	10
Mean	6.21743231	**Sum Observations**	62.1743231
Std Deviation	2.39103607	**Variance**	5.71705347
Skewness	1.69217546	**Kurtosis**	3.27966762
Uncorrected SS	438.018126	**Corrected SS**	51.4534812
Coeff Variation	38.4569698	**Std Error Mean**	0.75611199

The UNIVARIATE Procedure
Variable: TVC (log base 10 of TVC)
brand=molo

Moments

N	10	**Sum Weights**	10
Mean	4.530765	**Sum Observations**	45.30765
Std Deviation	2.81076405	**Variance**	7.90039454
Skewness	0.59979971	**Kurtosis**	0.49425474

Moments

Uncorrected SS	276.381865	**Corrected SS**	71.1035509
Coeff Variation	62.0372951	**Std Error Mean**	0.88884164

Microbial quality (Log base 10 coliform counts) of different brands of pasteurized milk from different processors in Kenya. (1) Fresha (2) KCC (3) Kinangop, and (4) Molo

The UNIVARIATE Procedure
Variable: cc (log base 10 of cc)
brand=molo

Moments

N	10	**Sum Weights**	10
Mean	3.1067049	**Sum Observations**	31.067049
Std Deviation	4.25187914	**Variance**	18.0784762
Skewness	0.78156102	**Kurtosis**	-1.5572857
Uncorrected SS	259.22244	**Corrected SS**	162.706286
Coeff Variation	136.861378	**Std Error Mean**	1.34456224

Basic Statistical Measures

Location		**Variability**	
Mean	3.106705	**Std Deviation**	4.25188
Median	0.000000	**Variance**	18.07848
Mode	0.000000	**Range**	9.22039
		Interquartile Range	8.74846

Tests for Location: Mu0=0

Test		**Statistic**	**p Value**	
Student's t	**T**	2.31057	**Pr > |t|**	0.0462
Sign	**M**	2	**Pr >= |M|**	0.1250
Signed Rank	**S**	5	**Pr >= |S|**	0.1250

Quantiles (Definition 5)

Level	**Quantile**
100% Max	9.22039
99%	9.22039

Quantiles (Definition 5)

Level	Quantile
95%	9.22039
90%	9.10386
75% Q3	8.74846
50% Median	0.00000
25% Q1	0.00000
10%	0.00000
5%	0.00000
1%	0.00000
0% Min	0.00000

Extreme Observations

Lowest		Highest	
Value	**Obs**	**Value**	**Obs**
0	40	0.00000	40
0	38	4.11087	34
0	37	8.74846	39
0	36	8.98732	32
0	33	9.22039	35

Proc GLM procedure for pasteurized milk (Log base 10 TVC)

The GLM Procedure

Class Level Information

Class	Levels	Values
brand	4	Fresha KCC Kinangop molo

Number of Observations Read	40
Number of Observations Used	40

The GLM Procedure

Dependent Variable: tvc log base 10 of tvc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	153.0676688	51.0225563	7.49	0.0005

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	36	245.2144166	6.8115116		
Corrected Total	39	398.2820854			

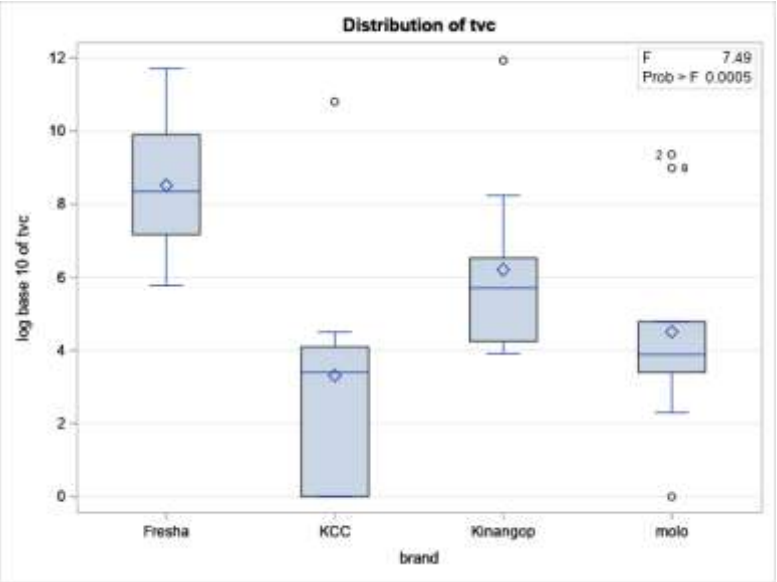
R-Square	Coeff Var	Root MSE	tvc Mean
0.384320	46.29941	2.609887	5.636978

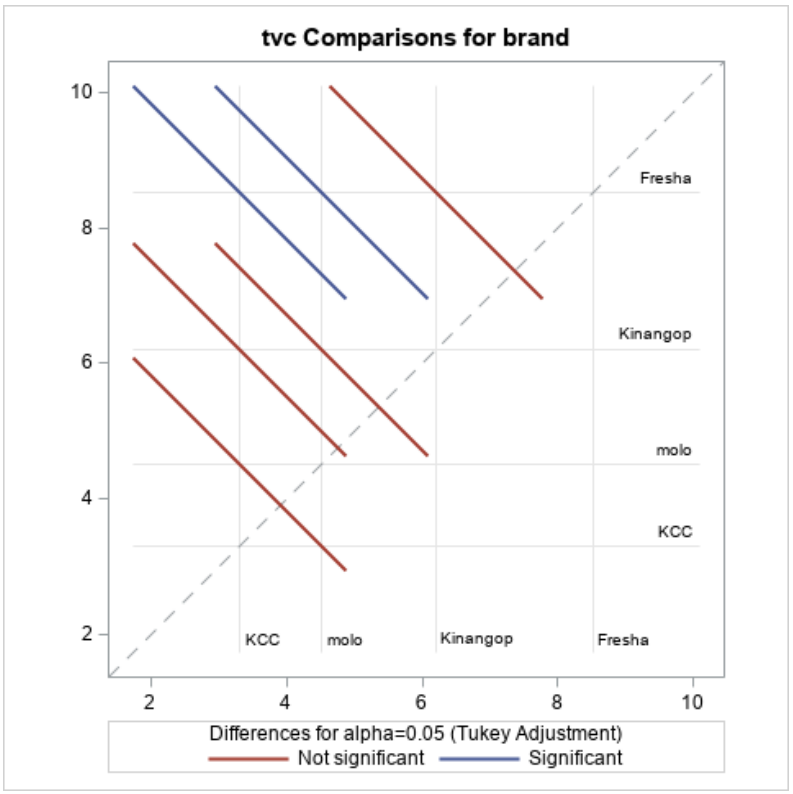
Source	DF	Type I SS	Mean Square	F Value	Pr > F
brand	3	153.0676688	51.0225563	7.49	0.0005

Source	DF	Type III SS	Mean Square	F Value	Pr > F
brand	3	153.0676688	51.0225563	7.49	0.0005

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	4.509682663	B	0.82531882	5.46	<.0001
brand Fresha	4.007123780	B	1.16717707	3.43	0.0015
brand KCC	-1.199294678	B	1.16717707	-1.03	0.3110
brand Kinangop	1.701350337	B	1.16717707	1.46	0.1536
brand molo	0.000000000	B	.	.	.

Note: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.





Proc GLM procedure for pasteurized milk (Log base 10 coliform counts)

The GLM Procedure

Dependent Variable: cc log base 10 of tvc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	64.3224559	21.4408186	3.87	0.0170
Error	36	199.6765785	5.5465716		
Corrected Total	39	263.9990344			

R-Square	Coeff Var	Root MSE	cc Mean
0.243647	232.0486	2.355116	1.014924

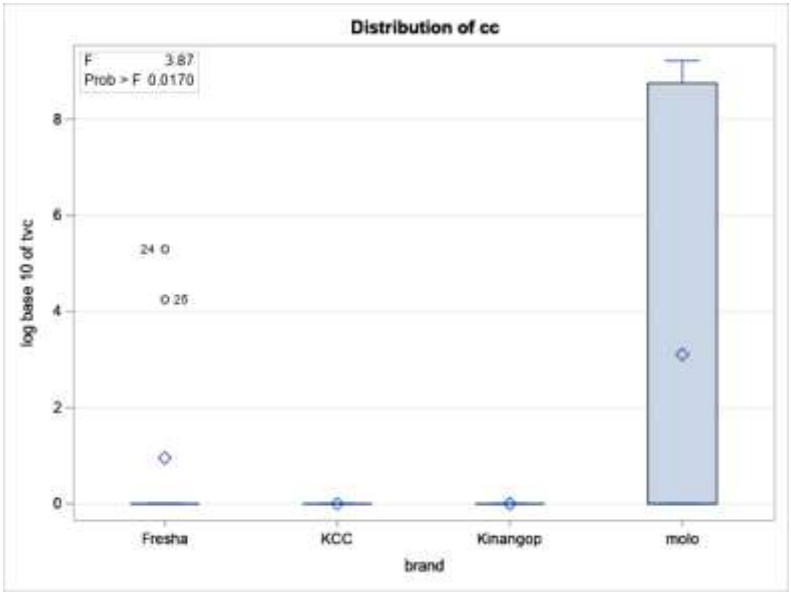
Source	DF	Type I SS	Mean Square	F Value	Pr > F
brand	3	64.32245592	21.44081864	3.87	0.0170

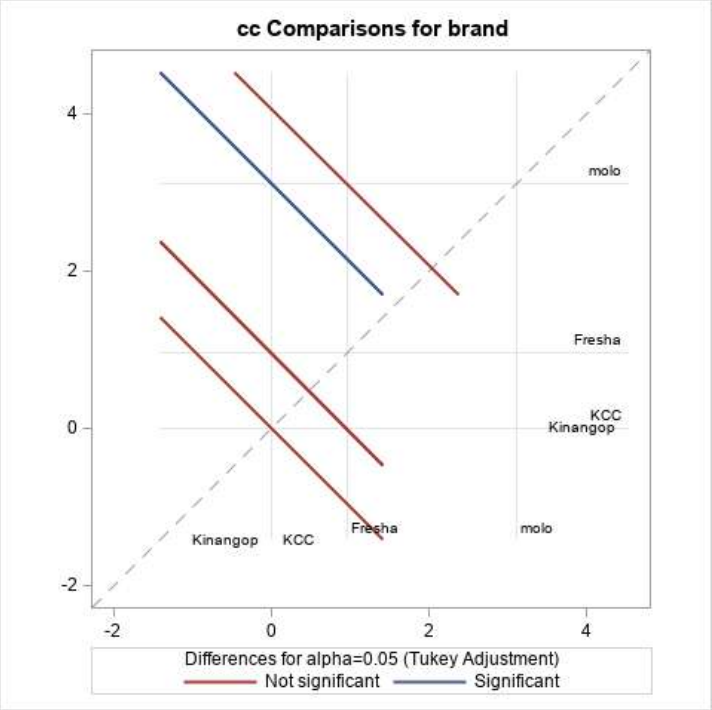
Source	DF	Type III SS	Mean Square	F Value	Pr > F
brand	3	64.32245592	21.44081864	3.87	0.0170

Parameter	Estimate	Standard Error	t Value	Pr > |t|

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	3.105013700	B	0.74475309	4.17	0.0002
brand Fresha	-2.150332439	B	1.05323992	-2.04	0.0486
brand KCC	-3.105013700	B	1.05323992	-2.95	0.0056
brand Kinangop	-3.105013700	B	1.05323992	-2.95	0.0056
brand molo	0.000000000	B	.	.	.

Note: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.





Quality compliance of selected pasteurized milk brands from different processors to EAC standards based on TVC. (1) Fresha, (2) KCC, (3) Kinangop, and (4) Molo

Table of TVC by brand

Frequency / Percent / Row Pct / Col Pct	TVC	brand				
		Fresha	**KCC**	**Kinangop**	**molo**	**Total**
	No	1	1	1	0	3
		2.50	2.50	2.50	0.00	7.50
		33.33	33.33	33.33	0.00	
		10.00	10.00	10.00	0.00	
	Yes	9	9	9	10	37
		22.50	22.50	22.50	25.00	92.50
		24.32	24.32	24.32	27.03	
		90.00	90.00	90.00	100.00	
	Total	10	10	10	10	40
		25.00	25.00	25.00	25.00	100.00

Statistics for Table of TVC by brand

Statistic	DF	Value	Prob
Chi-Square	3	1.0811	0.7816
Likelihood Ratio Chi-Square	3	1.8058	0.6137
Mantel-Haenszel Chi-Square	1	0.6324	0.4265
Phi Coefficient		0.1644	
Contingency Coefficient		0.1622	
Cramer's V		0.1644	

Sample Size = 40

Quality compliance of selected pasteurized milk brands from different processors to EAC standards based on coliform counts. (1) Fresha, (2) KCC, (3) Kinangop, and (4) Molo

Table of cc by brand

Frequency
Percent
Row Pct
Col Pct

CC	brand				
	Fresha	**KCC**	**Kinangop**	**Molo**	**Total**
No	2	0	0	4	6
	5.00	0.00	0.00	10.00	15.00
	33.33	0.00	0.00	66.67	
	20.00	0.00	0.00	40.00	
Yes	8	10	10	6	34
	20.00	25.00	25.00	15.00	85.00
	23.53	29.41	29.41	17.65	
	80.00	100.00	100.00	60.00	
Total	10	10	10	10	40
	25.00	25.00	25.00	25.00	100.00

Statistics for Table of cc by brand

Statistic	DF	Value	Prob
Chi-Square	3	8.6275	0.0347
Likelihood Ratio Chi-Square	3	10.3484	0.0158
Mantel-Haenszel Chi-Square	1	1.3765	0.2407
Phi Coefficient		0.4644	
Contingency Coefficient		0.4212	
Cramer's V		0.4644	

Sample Size = 40

Effect of time of delivery of milk to collection centres in the smallholder rural value chain on TVC of the milk

The REG Procedure
Model: MODEL1
Dependent Variable: tvc log base10 of tvc (cfu/ml)

Number of Observations Read	32
Number of Observations Used	32

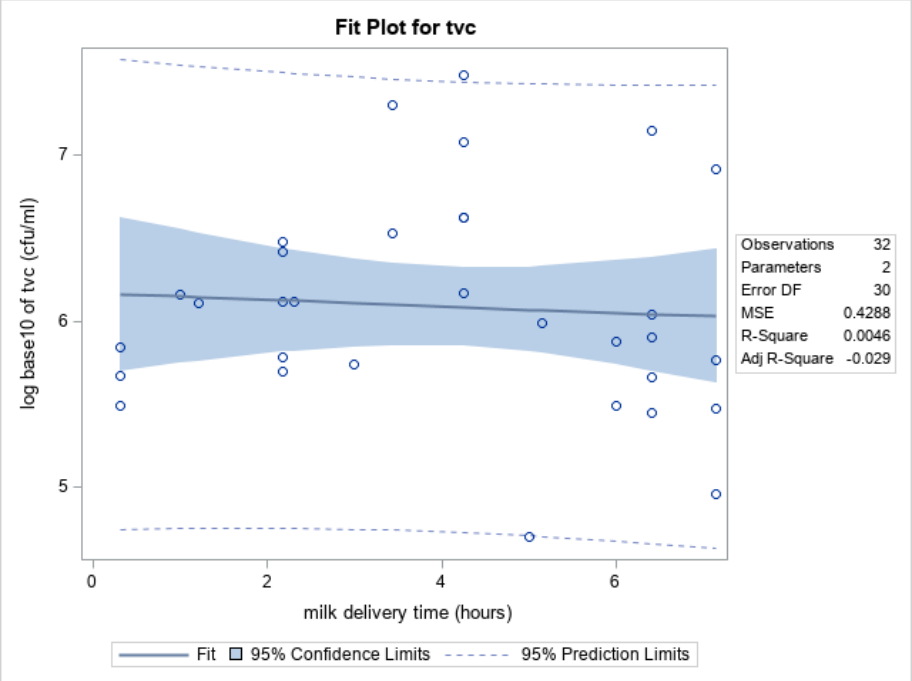
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.05985	0.05985	0.14	0.7113
Error	30	12.86385	0.42879		
Corrected Total	31	12.92370			

Root MSE	0.65482	**R-Square**	0.0046
Dependent Mean	6.08734	**Adj R-Sq**	-0.0285
Coeff Var	10.75716		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	**1**	6.16659	0.24165	25.52	<.0001
Time	milk delivery time (hours)	**1**	-0.01947	0.05211	-0.37	0.7113



Effect of temperature on microbial loads of milk along the Smallholder dairy value chain

The REG Procedure
Model: MODEL1
Dependent Variable: TVC log base 10 of TVC

Number of Observations Read	37
Number of Observations Used	35
Number of Observations with Missing Values	2

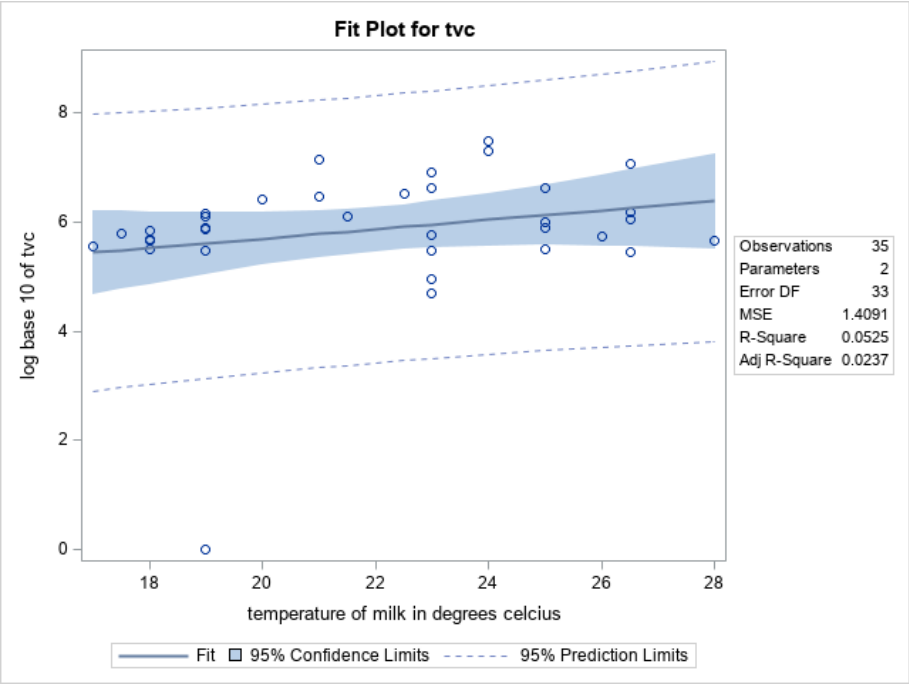
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.57447	2.57447	1.83	0.1857
Error	33	46.50029	1.40910		
Corrected Total	34	49.07476			

Root MSE	1.18705	**R-Square**	0.0525
Dependent Mean	5.87478	**Adj R-Sq**	0.0237
Coeff Var	20.20596		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	**1**	3.98874	1.40969	2.83	0.0079
Temp	temperature of milk in degrees celcius	**1**	0.08545	0.06322	1.35	0.1857



Effect of Volume of milk handled on microbial loads in milk along the dairy value chain

The REG Procedure
Model: MODEL1
Dependent Variable: TVC log base 10 of TVC

Number of Observations Read 29

Number of Observations Used 29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.40638	0.40638	0.23	0.6374
Error	27	48.29015	1.78852		
Corrected Total	28	48.69652			

Root MSE	1.33736	**R-Square**	0.0083
Dependent Mean	5.89144	**Adj R-Sq**	-0.0284

Coeff Var	22.70001

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	**1**	6.05818	0.42900	14.12	<.0001
Vol	volume of milk in litres	**1**	-0.00533	0.01117	-0.48	0.6374

Microbial loads (TVC, $Log_{10,}$ Mean ±SD) along the pastoral camel milk value chain

The GLM Procedure

Class Level Information

Class	Levels	Values
node	5	PBB PPCC PRSTN PSCC PUM

Number of Observations Read	113
Number of Observations Used	113

The GLM Procedure

Dependent Variable: TVC log base 10 of TVC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	100.6186178	25.1546545	21.97	<.0001
Error	108	123.6704625	1.1450969		
Corrected Total	112	224.2890803			

R-Square	Coeff Var	Root MSE	tvc Mean
0.448611	17.75922	1.070092	6.025558

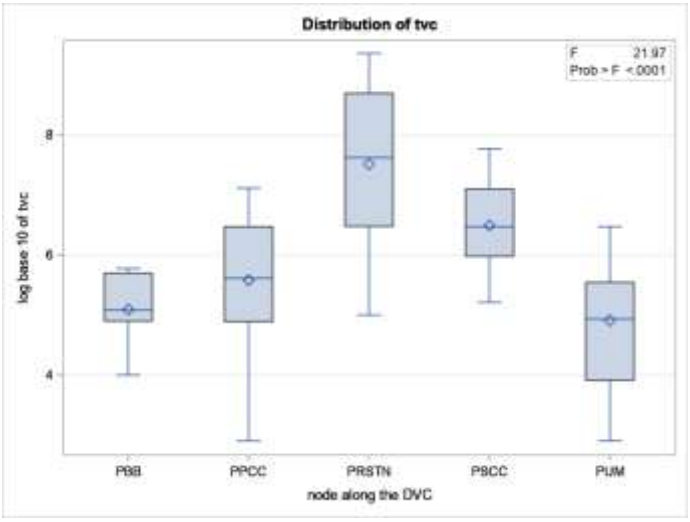
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Node	4	100.6186178	25.1546545	21.97	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Node	4	100.6186178	25.1546545	21.97	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	4.911200086	B	0.22814438	21.53	<.0001
node PBB	0.182089217	B	0.42341805	0.43	0.6680
node PPCC	0.670528289	B	0.29453313	2.28	0.0248

Parameter	Estimate		Standard Error	t Value	Pr > |t|
node PRSTN	2.607039963	B	0.31585159	8.25	<.0001
node PSCC	1.583490991	B	0.31281581	5.06	<.0001
node PUM	0.000000000	B	.	.	.

Note: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.



The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Node	tvc LSMEAN	LSMEAN Number
PBB	5.09328930	1
PPCC	5.58172838	2
PRSTN	7.51824005	3
PSCC	6.49469108	4
PUM	4.91120009	5

Least Squares Means for Effect node
t for H0: LSMean(i)=LSMean(j) / Pr > |t|
Dependent Variable: tvc

i/j	1	2	3	4	5
1		-1.21379	-5.79765	-3.36894	0.430046
		0.7434	<.0001	0.0091	0.9928
2	1.213788		-6.74566	-3.2177	2.27658
	0.7434		<.0001	0.0144	0.1605
3	5.797646	6.745656		3.347072	8.254003
	<.0001	<.0001		0.0097	<.0001
4	3.368941	3.217695	-3.34707		5.062055
	0.0091	0.0144	0.0097		<.0001
5	-0.43005	-2.27658	-8.254	-5.06206	
	0.9928	0.1605	<.0001	<.0001	

Microbial loads (Coliform counts, $Log_{10,}$ Mean ±SD) along the pastoral camel milk value chain

The GLM Procedure

Class Level Information

Class	Levels	Values
Node	5	PBB PPCC PRSTN PSCC PUM

Number of Observations Read 96

Number of Observations Used 96

The GLM Procedure

Dependent Variable: cc log base 10 of cc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	195.0202652	48.7550663	38.87	<.0001
Error	91	114.1306496	1.2541830		
Corrected Total	95	309.1509148			

R-Square	Coeff Var	Root MSE	cc Mean
0.630825	23.45569	1.119903	4.774547

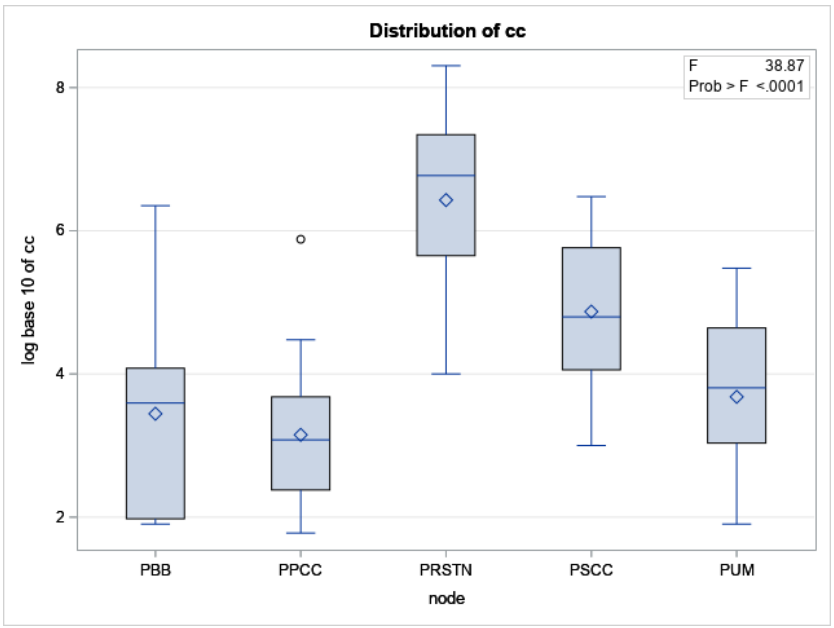
Source	DF	Type I SS	Mean Square	F Value	Pr > F

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Node	4	195.0202652	48.7550663	38.87	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Node	4	195.0202652	48.7550663	38.87	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	3.680094471	B	0.37330103	9.86	<.0001
node PBB	-0.235911505	B	0.54417509	-0.43	0.6657
node PPCC	-0.530703219	B	0.43311831	-1.23	0.2236
node PRSTN	2.748799570	B	0.41623371	6.60	<.0001
node PSCC	1.190465641	B	0.46662629	2.55	0.0124
node PUM	0.000000000	B	.	.	.

Note: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.



The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Node	cc LSMEAN	LSMEAN Number

Node	cc LSMEAN	LSMEAN Number
PBB	3.44418297	1
PPCC	3.14939125	2
PRSTN	6.42889404	3
PSCC	4.87056011	4
PUM	3.68009447	5

Least Squares Means for Effect node
t for H0: LSMean(i)=LSMean(j) / Pr > |t|
Dependent Variable: cc

i/j	1	2	3	4	5
1		0.651069	-6.83536	-2.94139	-0.43352
		0.9660	<.0001	0.0329	0.9925
2	-0.65107		-11.4431	-4.83688	-1.22531
	0.9660		<.0001	<.0001	0.7367
3	6.835361	11.44313		4.650536	6.603981
	<.0001	<.0001		0.0001	<.0001
4	2.941395	4.836877	-4.65054		2.551219
	0.0329	<.0001	0.0001		0.0885
5	0.433521	1.225308	-6.60398	-2.55122	
	0.9925	0.7367	<.0001	0.0885	

Compliance of raw camel milk along the Pastoral DVC to Kenya standards (Coliform Counts)

The FREQ Procedure

Table of node by grade

Frequency / **Percent** / **Row Pct** / **Col Pct**	**Node**	**grade**			
		I	**II**	**poor**	**Total**
	PBB	3	4	1	8
		3.13	4.17	1.04	8.33
		37.50	50.00	12.50	

	14.29	16.00	2.00	
PPCC	15	9	2	26
	15.63	9.38	2.08	27.08
	57.69	34.62	7.69	
	71.43	36.00	4.00	
PRSTN	0	2	35	37
	0.00	2.08	36.46	38.54
	0.00	5.41	94.59	
	0.00	8.00	70.00	
PSCC	1	5	10	16
	1.04	5.21	10.42	16.67
	6.25	31.25	62.50	
	4.76	20.00	20.00	
PUM	2	5	2	9
	2.08	5.21	2.08	9.38
	22.22	55.56	22.22	
	9.52	20.00	4.00	
Total	21	25	50	96
	21.88	26.04	52.08	100.00

Statistics for Table of node by grade

Statistic	DF	Value	Prob
Chi-Square	8	64.7019	<.0001
Likelihood Ratio Chi-Square	8	74.8447	<.0001
Mantel-Haenszel Chi-Square	1	11.2733	0.0008
Phi Coefficient		0.8210	
Contingency Coefficient		0.6345	
Cramer's V		0.5805	

Effect of time of delivery at the secondary collection centre in isiolo town on microbial

counts

The REG Procedure
Model: MODEL1
Dependent Variable: TVC log base 10 of TVC

Number of Observations Read	52
Number of Observations Used	47
Number of Observations with Missing Values	5

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	11.02002	11.02002	8.94	0.0045
Error	45	55.44435	1.23210		
Corrected Total	46	66.46436			

Root MSE	1.11000	**R-Square**	0.1658
Dependent Mean	5.82685	**Adj R-Sq**	0.1473
Coeff Var	19.04972		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	**1**	0.13601	1.90974	0.07	0.9435
Time	milk delivery time (24hr clock reading)	**1**	0.48712	0.16288	2.99	0.0045

Effect of milk volume on microbial counts in camel milk along the pastoral dairy value chain

Model: MODEL1
Dependent Variable: tvc log base 10 of tvc

Number of Observations Read	84
Number of Observations Used	47
Number of Observations with Missing Values	37

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.95922	0.95922	0.69	0.4117

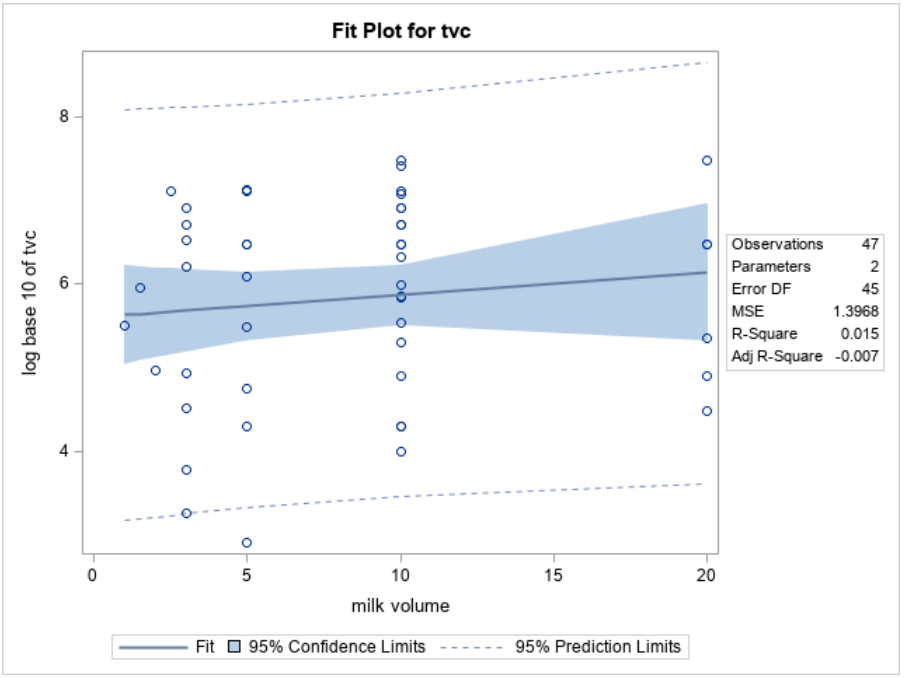
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Error	45	62.85766	1.39684		
Corrected Total	46	63.81689			

Root MSE	1.18188	**R-Square**	0.0150
Dependent Mean	5.82334	**Adj R-Sq**	-0.0069
Coeff Var	20.29554		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	**1**	5.60072	0.31920	17.55	<.0001
Vol	milk volume	**1**	0.02642	0.03188	0.83	0.4117



Appendix G: Plastic milk containers in at a milk collection centre in Isiolo

Appendix H: Hygienic plastic containers customised for motorbike carriage in Olenguruone

Appendix I: A milk collection centre attendant taking a milk sample for quality analysis in Olenguruon**e**

Appendix J: A milk collection centre attendant taking an alcohol test on milk in Olenguruone

Appendix K: Unloading of milk from a donkey in Olenguruone

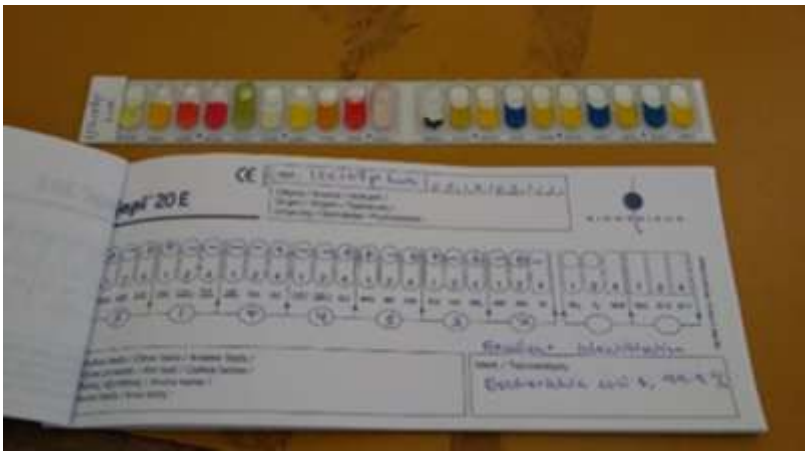
Appendix L: Milk reception at a collection centre in Olenguruone

Appendix M: A horizontal milk cooler at a collection centre in Isiolo

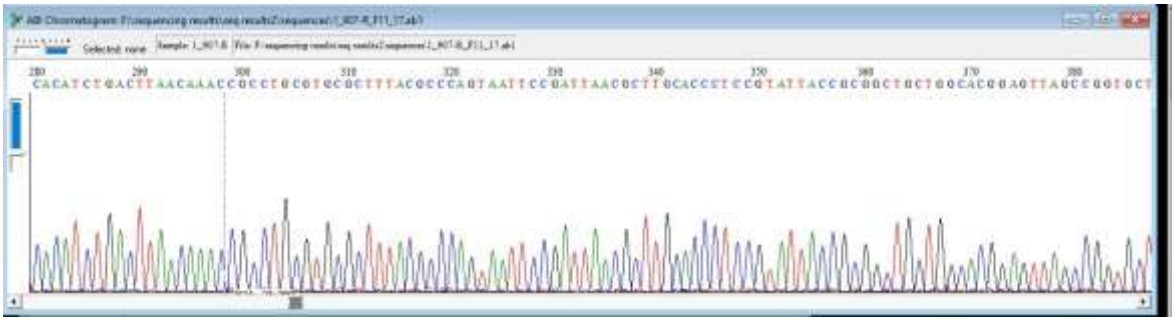
Appendix N: A vertical milk cooler at a collection centre in Nakuru

Appendix O: Milk in plastic containers covered with wet gunny bags, under a shade at a Primary collection centre in Isiolo

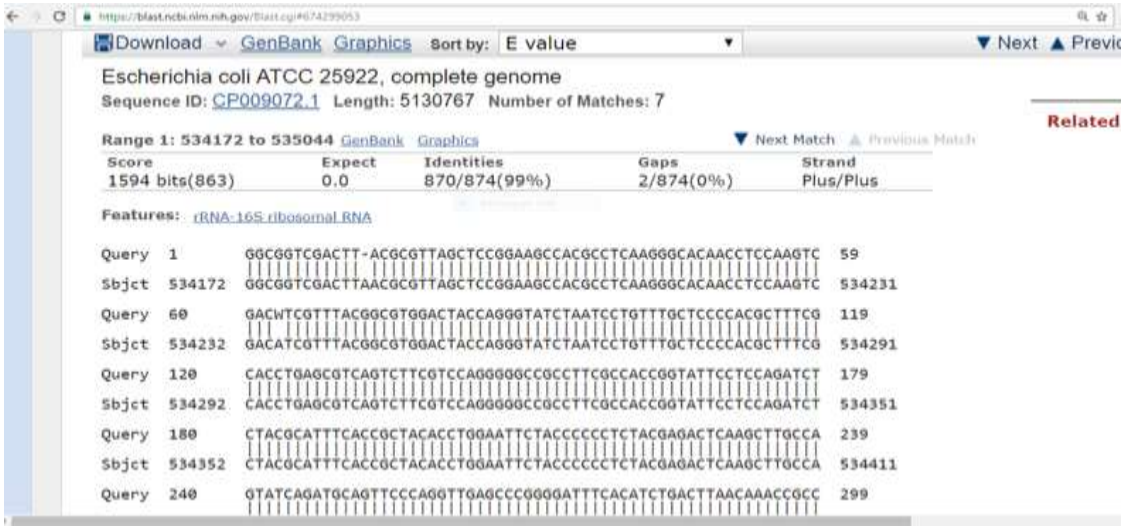
Appendix P: API20E Strip and test result for *E. coli*



Appendix Q: Section of 16s rRNA sequence profile for *E. coli*



Appendix R: *E. coli* ATCC 25922 BLAST result



Appendix S: **List of publications, conference papers, posters and Newspaper articles**

1. Nato, S.M., Matofari, J. W., Bebe, B.O., Huelsebusch, C. (2018). Effect of predisposing factors on microbial quality of camel milk along the pastoral dairy value chain in Kenya. *Pastoralism Research, Policy and Practice*. 8:16 https://doi.org/10.1186/s13570-018-0123-7
2. Nato, S.M., Matofari, J. W., Bebe, B.O., Huelsebusch, C 2019. Prevalence of β-haemolytic multi-drug resistant *E. coli* in cow and camel milk in Kenya.*J Consum Prot Food Saf*.14 (1):55-61. DOI 10.1007/s00003-018-1187-4
3. Nato S.M., Matofari J.W., Bebe B. O., Huelsebusch C . G. Quality of Pasteurised milk in Kenya. Kenya Institute of Science and Technology, 2016: Nairobi, Kenya:
4. Nato S.M., Matofari J.W., Bebe B. O., Huelsebusch C . G. Predisposing factors for microbial loads in camel milk along the pastoral dairy value chain in Kenya. Tropentag 2016: Theme; Solidarity in a competing world-Fair use of resources: Organised by the University of Natural Resources and Life Sciences (BOKU Vienna), Austria: September 18-21, 2016.
5. Nato S.M., Matofari J.W., Bebe B. O., Huelsebusch C . G. Quality of Pasteurised milk in Kenya. Tropentag 2016: Theme; Solidarity in a competing world-Fair use of resources: Organised by the University of Natural Resources and Life Sciences (BOKU Vienna), Austria: September 18-21, 2016.
6. How to keep milk clean, fresh and marketable. Daily Nation, January 23, 2015.
7. Four dairy products you can make at home. Daily Nation. February 6, 2015.
8. Wood smoke that gives long life and health to your milk. Daily Nation, May 29. 2015

Appendix T: Paper abstracts

Effect of predisposing factors on microbial loads in camel milk along the pastoral dairy value chain in Kenya

Samuel Muyoma Nato, Joseph Wafula Matofari, Bockline Omedo Bebe and Christian Huelsebusch

Abstract

The aim of this study was to map the camel milk value chain and establish the predisposing factors for increase in microbial counts in milk along the chain. Isiolo County was chosen for the study. Data collection was done through key informant interviews, structured interview schedules, observation and microbial analysis of milk samples. During milk sampling, milk temperature, environmental temperature, time and volume of milk from which the sample was taken were recorded. Along the value chain, microbial counts in milk increased significantly from log10 4.91 ± 1.04 CFU/ ml at production to log10 7.52 ± 1.32 CFU/ml at Nairobi market for total viable counts and log10 3.68 ± 1.28 CFU/ml at production to log10 6.42 ± 1.13 CFU/ml at the Nairobi market for coliform counts. At production, milking persons neither washed their hands nor cleaned the camels' udder before milking, and plastic, non-food grade containers were the only form of receptacles used for milk along the chain. The relationship between microbial counts and time taken to transport milk along the chain was significant while the volume of milk in the receptacle had no effect on microbial counts. The milk was held at a temperature of between 28 and 32.5 °C before delivery to secondary collection centres from 10:15 am to 6:30 pm for cooling. Training on milk quality for milk handlers at the collection centre had no effect on microbial counts. Affordable access to low-cost food grade plastic containers as well as cooling milk in the individual receptacles within two hours of milking, without bulking and refilling again into the receptacles for transportation, as is the practice, would reduce microbial counts. Similarly, training on milk quality should start at production where milk contamination is initiated. Finally, milk value addition would improve milk shelf-life enabling access to distant markets. This would greatly improve the livelihoods of the pastoral camel milk producers.

Keywords: Camel milk, Isiolo, Microbial load, Pre-disposing factor

Nato et al. Pastoralism: Research, Policy and Practice (2018) 8:16 https://doi.org/10.1186/s13570-018-0123-7

Prevalence of b-haemolytic multi-drug resistant *E. coli* in cow and camel milk in Kenya

Samuel M. Nato, Joseph W. Matofari, Bockline O. Bebe and Christian Huelsebusch

Abstract

The aim of this study was to find the prevalence of b-haemolytic Escherichia coli in milk, as well as their sources, and their sensitivity to antibiotics. E. coli was isolated from samples of cow and camel milk, cow and camel udder surfaces, and milking persons' hands. The organisms were identified using API20E biochemical kit. Haemolytic activity was tested on 7% defibrinated sheep blood agar while antibiotic sensitivity was tested using the Kirby-Bauer disc diffusion method. The prevalence of b-haemolytic isolates from cow and camel milk was 25% and 32% respectively. None of the isolates from the udder swabs, the milking persons' hands, or water was b-haemolytic. In cow milk, the prevalence of isolates resistant to Ampicillin, Cefotaxime, and Cefepime was 25, 37.5 and 12.5% respectively, while in camel milk it was 52.4, 23.8 and 28.6% respectively. Prevalence of b-haemolytic and multidrug resistant isolates to the three antibiotics was 12.5% for cow milk and 19% for camel milk. None of the isolates was resistant to Ciprofloxacin, Piperacillin/Tozobactam, Amikacin, and Imipenem. The prevalence of E. coli resistant to Cefotaxime and Cefepime indicates growing resistance of the microorganisms to drugs that are supposed to be effective against them. The presence of b-haemolytic isolates in milk and their absence on the animals' udder surface and hand swabs could indicate their better survival in the udder from which they are shed into the milk. This is a public health concern especially for pastoral communities who have a preference for consumption of raw milk.

Keywords: *E. coli*, Milk, Antibiotics, Haemolysis, Multidrug resistance

Journal of Consumer Protection and Food Safety (2019) 14:55–61 DOI 10.1007/s00003-018-1187-4