SAFETY AND NUTRITIONAL QUALITY OF PASTORAL TRADITIONAL FERMENTED CAMEL MILK (*SUUSA*)

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DEDICATION

I dedicate this thesis to my parents Mr and Mrs Joseph Mwangi and to my siblings Victor, Nancy, Lucy and Kennedy. God bless you for your support.

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ABSTRACT

Suusa is spontaneously fermented raw camel milk prepared by pastoral women and informally marketed in Kenya. Processors, traders and consumers of *suusa* rely on organoleptic testing for quality and safety along the *suusa* value chain. Handling practices in raw milk and *suusa* products potentially exposes the product to microbial contamination which is associated with reduced shelf life and risk of infections to consumers with milk borne and zoonotic diseases such as brucellosis and tuberculosis. Furthermore, spontaneous fermentation results in a product of inconsistent quality. This study analysed the microbial safety and nutritional quality of *suusa* along its value chain. Simple random sampling was used to select women group processors, traders and consumers in the value chain. The samples analysed had microbial load increasing significantly ($p<0.05$) from production to market with total viable counts by 1 log cycle, coliform count by 1 log cycle, spore count by 3 log cycles and yeast and moulds by 1 log cycle while lactic acid increased from 0.07% to 0.23%. The overall microbial load comprised of 67% gram negative rods, 62% gram positive cocci and 28% yeast and moulds from production, processing and marketing. *Brucella* species and *Mycobacteria tuberculosis* complex (MTBC), a human tuberculosis causing strain, were detected at production and marketing nodes. Fat content of *suusa* (2.45 to 3.26%) decreased significantly ($p<0.0.5$) along the value chain. Handling practices affects microbial load and fat content of *suusa* along the value chain. The *suusa* value chain presents risk of infection to consumers with zoonotic pathogens making the product a potential public health risk.

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ARBAT	Anigen rapid *Brucella* antibody test
ASAL	Arid and Semi-arid Lands
BPW	Buffered Peptone Water
CC	Coliform count
CCP	Critical control point
DNA	Deoxyribonucleic acid
EU MRL	European Union Maximum Residue Limit
GTMD	Genotype *Mycobacteria* Direct
IKFP	Indigenous Knowledge Food Processing
IMViC	Indole Methyl Red Voges-Proskauer Citrate Test
LAB	Lactic Acid Bacteria
LSD	Least Significant Difference
MRT	Milk Ring Test
MTBC	*Mycobacteria tuberculosis* complex
NASBA	Nucleic acid sequence based amplification
PCA	Plate Count Agar
RBPT	Rose Bengal Precipitation test
RCBD	Randomized Complete Block Design
RNA	Ribonucleic acid
RT	Room temperature
SC	Spore count
TMA	Transcription mediated amplication
TVC	Total Viable Count
YM	Yeasts and molds

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Pastoralists in Northern Kenya depend on camel milk and milk products to provide diet to the population in the arid and semi-arid lands (ASAL). Camel milk accounts for 15% of the total national milk production (FAOSTAT, 2012). Camel milk products produced by pastoralists include: cheese, butter, sweetened condensed milk, fermented camel milk and yoghurt. In Kenya, the fermented camel milk (*suusa*) has found way to the market at local urban centres and cities like Nairobi.

Suusa is spontaneously fermented raw camel milk. The fermentation is carried out at room temperature ranging from 26–29^{o}C, for days in a gourd. The product is a white, low-viscosity product with a distinct smoky flavour and astringent taste (Tezira *et al*., 2005). However, due to high demand, the gourds have become too small to produce the amount needed and the women pastoralists now use plastic jericans with larger volumes. *Suusa* is prepared by the Borana and Somali people in North and Eastern Kenya, by storing milk in plastic containers which slowly curdles over a period of 1-3 days. Therefore two types of *suusa* exist; one intentionally prepared for home consumption and the other from high acid coagulated fresh milk either on transit to the market or to the bulking centre due to long distances from the production area due to lack of cooling facilities.

In both types of *suusa*, spontaneous fermentation takes place where no known starter culture is used. The inherent and environmentally acquired microorganisms set the fermentation. In such fermentation, the attributes of the products are not consistent and may not attract many consumers, especially the non-pastoralists. Spontaneous fermentation of raw milk takes advantage of the action of naturally occurring mixed microflora inherent in the milk, in the plastic containers as well as environmental factors such as temperature and pH that provide the necessary selective factors for evolution of lactic acid bacteria (LAB) strains like *Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus salivarius, Lactococcus raffinolactis* and *Leuconostoc mesenteroides subsp. Mesenteroides* that impart desirable attributes to the product (Tezira *et al*., 2005).

The production processes of indigenous foods have several handicaps; these include unpredictable production environment, unknown microbiology in processing, lack of process control and, unknown nutritional and toxicological status, and inefficiency (Chinyere and Onyekwere, 1996). Under pastoral production of *suusa*, the production environment has

factors such as the udder health, milking equipment usually plastic, milking personnel who may act as carriers of some zoonoses like *Brucella* and *Mycobacteria*, practices like tying the quarters to prevent suckling by the calf and the milking environment, usually dusty and without water that may act as points of contamination (Mulwa *et al*., 2011).During transportation, either by walking, on donkeys or occasionally on open pickups, long distance from milking point to collection or storage point, poor roads and lack of cooling facilities affects the microbial load by providing conditions for rapid multiplication in the milk (Mulwa *et al*., 2011).During bulking, milk from different suppliers is pooled without prior quality control tests and this acts as a source of contamination and affects the safety and quality of the milk (Momanyi and Jenet, 2010). During processing, fermentation technology is used and the variables involved include lack of heat treatment of the milk, storage at ambient temperature, lack of known culture, pH and hence lack of process control which results in a product of variable quality (Eyassu, 2007). The product is finally sold in open air markets and this has an effect on the microbial load and quality of the product due to environmental pollution from dust. *Suusa* produced under pastoral environment faces these challenges along the value chain from production to consumption.

This study aimed at evaluating the effect of handling practices by pastoralist women on microbiological and nutritional quality of *suusa* along the value chain with the aim of enhancing food and income security among the pastoral communities especially women by ensuring market access of this product.

1.2 Statement of the problem

Processors, traders and consumers of fermented camel milk *suusa* rely on organoleptic testing for quality and safety yet handling practices along the *suusa* value chain faces several challenges with keeping standards of hygiene. Handling practices in raw milk and *suusa* product potentially expose the product to microbial contamination which is associated with reduced shelf life and risk of infections to consumers with milk borne and zoonotic diseases such as brucellosis and tuberculosis. Furthermore, spontaneous fermentation of *suusa* results in a product of unknown nutritional quality. The study addressed safety and nutritional quality of *suusa*.

1.3 General objective

To enhance food safety, quality and market acceptability of traditionally processed *suusa* for consumers and income security for the pastoral women processors by evaluating its handling practices, microbiological and nutritional quality.

1.3.1 Specific objectives

i. To map out the flow of fermented camel milk along the value chain in Kenya.
ii. To determine the influence of handling practices of *suusa* production on microbial load along the value chain.
iii. To determine the presence of *Brucella* and *Mycobacteria* species in *suusa* along the value chain.
iv. To determine the influence of handling practices and microbial load on nutritional content of *suusa* along the value chain.

1.4 Research questions

i. What is the flow of fermented camel milk in Kenya?
ii. What is the influence of handling practices of *suusa* production on microbial load along the value chain?
iii. Is there risk of occurrence of *Brucella* and *Mycobacteria* species in *suusa* along the value chain?
iv. What is the influence of handling practices and microbial load on nutritional content of *suusa* along the value chain?

1.5 Justification

Estimates suggest that demand in the urban market for raw and fermented milk (*suusa*) is four times higher than the supply (Musinga *et al*., 2008). This is a result of growing consumer demand for traditionally processed foods in urban areas. However, this is being done in the informal way as the value chain does not have standards. The regulators and consumers are concerned with the quality of the camel milk and its products being sold on the market. This is a barrier to economic development of the pastoralists, especially the women who are the major players in this value chain. There is the danger of pathogens being transmitted through the raw milk and the fermented product-*suusa*. Some of the major zoonotic milk pathogens like *Brucella* and *Mycobacteria* species are likely to be transmitted through the raw milk and the *suusa* because the pastoral communities in Eastern and North Eastern Kenya consume raw camel milk. There is need to assess the entire *suusa* value chain with the aim of ascertaining quality for standard development for *suusa*. This will be in line with Kenya's

strategy of improving livelihoods through adding value of farm and livestock products to fetch higher prices at local and international markets. This will also improve food security.

1.6 Operational definition of terms

Value Chain- The term Value chain has been used in this context to mean the value addition chain from raw material to the product (*suusa*). It includes production (from the udder), processing, bulking, storage and marketing.

Household- In this context, the term refers to those women who are involved in *Suusa* production, distribution and marketing. These women are those who are in groups. It also includes their milkers because the women are not the same people who do the camel milking.

Intended *suusa* – This has been used to refer to homemade fermented camel milk prepared by women specifically for home consumption and sometimes though rarely for sale as it fetches a lower price as compared to raw camel milk. This product is left to ferment at ambient temperature for 3 days.

Unintended *suusa*– This refers to raw camel milk that undergoes coagulation while on transit from the '*bomas'* (camel milk production areas) to the cooling centres or from the cooling centres to the market (Nairobi Eastleigh). This milk is not discarded and is sold as *suusa*, fermented camel milk.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of fermented camel milk (*suusa*) in pastoral livelihoods

Camel population in Kenya is estimated to be 2.9 million (Noor *et al*., 2012). They are all one-humped (*Camelus dromedarius*), found mainly in the low lands of Northern Kenya. The camel lives in areas not suitable for crop production and where other livestock species can hardly thrive (Noor *et al*., 2012). Due to its outstanding performance in arid and semi-arid areas (ASAL) of northern Kenya, camels play a central role to the livelihoods and culture of nomadic pastoralists (Guliye *et al.,* 2007). They provide milk, meat and are a means of transport. The annual camel milk production is estimated at 338.3 million litres, valued at USD 107.1 million, and this represents 12% of the national milk production (Musinga *et al*., 2008). During prolonged droughts, camel milk may contribute up to 50% of total nutrient intake of some pastoralists groups (Wayua *et al*., 2012).

Camel milk as well as *suusa* is low on cholesterol, sugar, high in minerals (Potassium, Sodium, copper, iron, magnesium and zinc), high vitamin C content, low protein and high concentration of insulin (Mona *et al.,* 2010). This nutritional profile of fresh and fermented camel milk (*suusa*) contributes to nutrition security of the pastoral communities in the ASALs. The sale of *suusa* and fresh camel milk provides income to the pastoralists to purchase other commodities such as fruits and vegetables, drugs, cereals and cereal products.These essential roles of camel milk and *suusa* emphasise its importance for food security to the pastoral people. Therefore, camels play an important part in food and income security of communities in the ASALs of Kenya (Noor *et al*., 2012).

2.2 Sources of microbial contamination in foods

Spoilage of a food is caused by infection and subsequent decomposition by bacteria and fungi. These microorganisms are borne by the animal itself, people handling the food and equipment. Milk contains few bacteria when it leaves the udder of a healthy animal. The milk is then subjected to contamination from the exterior of the animal, the atmosphere of the farm, the milk containers and equipments, the hands of the milker and other dairy workers (Matofari *et al*., 2007). The informal marketing of milk and milk products without regulation, thus hygienic standards not observed can be a source of milk contamination (Omore *et al*., 2001; Donkor *et al*., 2007). The pooling of milk from different suppliers and mixing evening and morning milk could be a source of contamination and possible transmission of food borne pathogens like *Listeria monocytogenes.* The pathogens may enter the food chain

through carrier animals that shed the organisms in the milk or the milkers who are infected (Wiedmann, 1998).

Contamination could also be as a result of the water used for cleaning the equipment. Availability of water in the ASAL is normally surface water from erratic rainfall. The microbiological quality of this water is unknown and is a potential source of external microbial contamination. Contaminated water is a vital source of pathogens in foods (Heeschen, 1992; Matofari, 2007). Handling practices such as the milking procedures, pooling of milk from different sources and storage carry the risks of further contamination from the environment and growth of inherent pathogens (Momanyi and Jenet, 2010). Pooling milk from different suppliers without prior testing leads to occurrence of milk-borne pathogens like *Streptococcus agalactiae* and *Staphylococcus aureus* (Younan *et al*., 2001). *S. aureus* has been associated with several human infections and is a major cause of food-borne disease while *S. agalactiae* is considered a major cause of neonatal sepsis and meningitis (Schuchat *et al*., 1992; Harvey and Gilmour, 2004). *Brucella abortus* antibodies and antimicrobial residues have been detected in informally marketed raw and pasteurized milk samples (Omore, 2001).

2.3 Microorganisms associated with fermented camel milk

Suusa is a spontaneously fermented camel milk product that has no specific starter culture. The fermentation is mixed culture fermentation where several microorganisms are involved. The major microorganisms associated with fermentation of camel milk include; lactic acid bacteria like *Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus salivarius, Lactococcus raffinolactis* and *Leuconostoc mesenteroides subsp. Mesenteroides* (Tezira *et al*., 2005). The commonest species include *L. mesenteroides subsp. mesenteroides*, *C. krusei*, *L. plantarum*. Other organisms that have been isolated from camel *suusa* include *Enterococcus faecium* and *Enterococcus faecalis* (Christoph *et al*., 2012). *Streptococcus infantarius* subsp. *Infantarius* in *gariss* (similar to *suusa*) have been isolated in *suusa* (Schlegel *et al*., 2000; Christoph *et al*., 2012).

Raw milk and unpasteurized cheese have been found to contain *Mycobacterium bovis* (Jaros *et al*., 2008). This shows that pathogens are likely to survive fermentation. High levels of total aerobic count, enterococci, fecal coliforms and *Staphylococcus aureus* have been detected in raw camel milk and this suggests the potential hazard associated with consumption of raw camel milk and milk products (Benkerroum *et al*., 2003). Similarly, *Bacillus*, *Corynebacterium*, *Micrococcus*, *Streptococcus*, *Staphylococcus* and *Pseudomonas*

species have been isolated from raw camel milk in the Middle East (Zahran and Al-Saleh, 1997).The fate of the pathogens during fermentation or storage of the fermented product (such as whether or not the pathogens grow to infectious levels or produce toxins) also depends on the initial levels of contamination of either the raw materials or the final product (Ogwaro *et al*., 2002). Some food borne pathogens grow to high levels during the early stages of fermentation due to the low acid levels. They may develop resistance to acid through a mechanism referred as acid tolerance response (ATR). For example, *Escherichia coli* O157:H7 and *Bacillus cereus* are acid resistant and can survive below pH 4.0 (Gadaga *et al*., 2004).

Raw milk is contaminated with a wide range of bacteria, including *Yersinia* species, *Klebsiella* species, *Proteus* species, *Enterobacter* species, *Escherichia coli*, *Staphylococcus* species, *Bacillus* species and *Mycobacterium* species. *Bacillus* and *Staphylococcus* are associated with food borne intoxication through production of enterotoxins, and the main agents involved are *Bacillus cereus* and *Staphylococcus aureus* (Ryser, 1998). There is the emergence of new milk-borne bacterial pathogens such as *Escherichia coli* O157:H7 that have serious challenges for public health and the dairy industry (Sivapalasingams, 2004). *Streptococcus infantarius* has been reported in *gariss* (a Sudanese product comparable to *suusa*), and fermented cow milk products *roab* in Sudan. *S. infantarius* subsp. *infantarius* is a member of the *Streptococcus bovis/Streptococcus equinus* complex (SBSEC), of which some members are associated to different degrees with human and animal infections, endocarditis, bacteremia, and colonic cancer (Schlegel *et al*., 2000;Corredoira *et al*., 2008, Abdelgadir *et al*., 2008, Hamza *et al*., 2009). Yeast and moulds organisms have been isolated from these fermented milk products.

2.4 Factors affecting microbial growth in foods

Inadequate time/temperature exposure during initial cooking, and inadequate reheating to kill pathogens in retail food service establishments or homes are often contributing factors to food poisoning outbreaks (Roberts, 1991). The food safety concerns are magnified because of the consumer demand for fresh tasting, high-quality, low salt, preservative-free meals which require minimal preparation time. Cases of presence of pathogens such as *Salmonella enterica* in camel milk and *E. coli* in indigenous fermented dairy products have been reported (Matofari *et al*., 2007; Kumbhar *et al.,* 2009).

A variety of factors such as the pH, moisture content, oxidation reduction potential, nutrients, influence microbial activity in foods. Most yeasts favour a pH around 4.0-4.5 while fungi can tolerate a pH much lower than that. Most bacteria favour a pH around 7.0. Thus, both the growth as well as their survival in foods depends on the pH of the food material, pH of camel milk is 6.5 and *suusa* is pH 6.4 (Attia *et al*., 2001). The pH of foods varies; some may be neutral while others may be acidic or alkaline. Microorganisms have an absolute, demand for water and the optimum level, of moisture required for growth varies with the organisms. Most bacteria grow well in a medium of water activity around 0.995 to 0.998. Moulds differ considerably in the optimal a_w requirement (Matofari, 2007). Nutrients in food, their kind and proportions determine the type of organism that will grow. Also, microorganisms vary in their ability to use nutrients. The presence of easily utilizable nutrients will encourage faster growth and quicker damage as is the case with milk. For example, a food with easily utilizable sugars will allow faster growth than one which contains polysaccharides (Gadaga *et al*., 2004).

2.5 Public health risks associated with dairy products

There has been public concern and debate on health risks posed by the sale of raw milk by middle traders (hawkers) since the liberalization of the dairy industry in Kenya. There is an increased risk of human brucellosis as a result of milk bulking and failure to pasteurize (Omore *et al*., 2001). To protect the public against milk-borne infections, there are regulations that require proper hygienic handling of milk and its pasteurization. However, such regulations are not usually adhered to in developing countries especially in the informal sector, making milk borne health risk higher in developing countries. Some risks in traditionally fermented and handled milk have not been investigated and established (Omore *et al*., 2001).

2.5.1 Safety of fermented milk

2.5.1.1 Legislation

The Foodstuffs, Cosmetics and Disinfectant Act (54/1972) (GOK, 1972) states *inter alia* that ‘no person shall use or sell any raw milk intended for further processing which:

i. Contains pathogenic microorganisms, extraneous matter and any inflammatory product or other substance that for any reason whatsoever may render the milk unfit for human consumption;

ii. Exceeds the most probable number (MPN) of 10 coliform bacteria per 1 ml of milk; or if the number exceeds 20 coliform per ml of milk using the dry rehydrated film method for coliform and *E. coli* counts;

iii. Gives a standard plate count of more than 500 000 colony forming units per 1 ml of milk when subjected to the dry rehydrated film method for standard colony count test.

The Kenya standard (DKS 05-941:2013) gives the following microbiological requirements for fermented milk:

Table 1: Microbiological requirements for fermented milk

S/no	Microorganism	Requirement/limit
1	*E. coli*	Nil/g
2	*Salmonella*	Nil/25g
3	*Listeria monocytogenes*	Nil/25g
4	*Stapylococcus aureas*	Nil/g
5	*Clostridium botulinum*	Nil/g
6	Yeast and molds	100 cfu/g
7	Coliforms	10 cfu/g

Key: cfu = colony forming units

2.6 Brucellosis

2.6.1 Disease transmission.

Animal brucellosis can be transmitted by both vertical and horizontal transmission. Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, via conjunctiva, inhalation and udder contamination during milking (Radostits *et al.,* 2007). Spread of the disease is due to movement of infected animals to disease free herds. Proximity of infected herds to non-infected herds occurs at watering points where camels come together. The important epidemiological risk factors are large herd size, poor management, abortion, milking more animals by a single person and herding with other ruminants. Survival of the organisms in the environment may also play a role in epidemiology of the disease. (Abbas *et al*., 1987; Radwan *et al.,* 1992; Abuo-Eisha, 2000). Animal health workers, butchers, farmers, and those who are habitually consume raw milk and come in contact with animals are at high risk for brucellosis (Chukwu, 1987). In man, transmission occurs as a result of ingestion of milk, contact via skin abrasion, mucous membranes and inhalation (Seifert, 1996; Radostits *et al*., 2007).

2.6.2 Brucellosis in camels

The genus of *Brucella* are gram-negative, non-motile, non-sporulating cells having a coccus or cocco bacillus morphology. They are fastidious in their growth requirements; most require complex media containing serum and an atmosphere enriched to 5–10% carbon dioxide for growth. A unique feature of *B. abortus* is its use of erythritol, a four-carbon sugar alcohol, as an energy source. This substrate is abundant in the uterus of pregnant cows, stimulating the localization of the organism at that site (Weimer, 2001).

Brucellosis in camels has been reported in Somalia, Iraq, Iran, Sudan, Egypt, Oman, Libya Kuwait, Kuwait, Jordan and Saudi Arabia. Racing camels in the United Arab Emirates have also been reported to have brucellosis. *Brucella melitensis* biovar 3 is the most commonly isolated species from animals in Egypt, Jordan, Israel, Tunisia and Turkey. *B. melitensis* biovar 2 was reported in Turkey and Saudi Arabia, and *B. melitensis* biovar 1 in Libya, Oman and Israel. *B. abortus* biovar 1 was reported in Egypt. Most human cases of brucellosis are caused by *B. melitensis*, particularly biovar 3 (Mayada *et al*., 2012).

2.6.3 Survival of *Brucella*

Following exposure, the organisms penetrate intact mucosal surface. In the alimentary tract the epithelium covering the ileal Payer's patches are the preferred sites of entry. After penetration, the organisms are engulfed by phagocytic cells and transported to regional lymph nodes (Walker, 1999). They proliferate, disseminate haemogenously and localize in the reticulo endothelial and reproductive tract. Various mechanisms are employed by *Brucella* organisms to survive inside the phagocytic cell: inhibiting phagolysosome fusion, blocking bactericidal action of phagocytes and suppressing the myeloperoxidase H_2O_2 halide system (Frenchick *et al.*, 1985; Harmon *et al.*, 1988; Tizard, 1992; Walker, 1999). In ruminants, *Brucella* organisms bypass the most effective host defense by targeting embryonic and trophoblastic tissue. In cells of these tissues, the bacteria grow not only in the phagosome but also in the cytoplasm and the rough endoplasmic reticulum (Anderson and Cheville, 1986).

In the absence of effective intracellular microbicidal mechanisms, these tissues permit exuberant bacterial growth, which leads to fetal death and abortion. The presence of erythritol in the placenta further enhances growth of *Brucella*. Products of conception at the time of abortion may contain up to 10^{10} bacteria per gram of tissue (Anderson *et al*., 1986). When septic abortion occurs, the intense concentration of bacteria and aerosolization of infected body fluids during parturition often result in infection of other animals and humans. Naturally infected and vaccinated animals can be serological reactors. After infection, the level of

immunoglobulin IgM, IgG and IgA significantly increases in serum (Radostits *et al.*, 2007). IgM antibodies, which appear initially after infection and low levels of IgG will cause complement-mediated lysis of *Brucella.* Secretory IgA tends to be abundant in milk, whereas IgG is high in serum (Walker, 1999). The O-chain of the smooth lipo-polysaccharide complex of the bacterial cell envelope together with the outer proteins are potent immunogens. On the other hand, the immunogenicity of the non-smooth cell variant is relatively low (Corbel *et al*., 1980). The O-chain specific antibodies play a major role in protective immunity, but do not eliminate the bacteria as they are protected by their intracellular niche (WHO, 1997).

2.6.4 Occurrence of *Brucella* in dairy products

As *Brucella* are facultative intracellular, organisms characteristic chronic granulomatous lesions develop in infected tissue where macrophage, neutrophils and lymphocytes respond to *Brucella* antigens. Phagocytes play a key role in initiating Tcells by processing and presenting antigens. Sensitized T-cells release cytokines that activate macrophages which in turn combat *Brucella* by reactive oxygen intermediates. Both CD4 and CD8 subsets are involved in cell-mediated protection. Cytokines also play a role in controlling *Brucella* infections (WHO, 1997).

Neutrophils effectively utilize the myeloperoxidase, H_2O_2, halide system in killing *Brucella*. However, the organisms inhibit degranulation and the respiratory oxidative burst and they are able to survive within the cell (Riley and Robertson, 1984). Macrophages readily ingest *Brucella* when opsonized with either complement or specific antibodies. The survival of the organisms in macrophages may result from a failure of phagosome-lysosome fusion and resistance to oxidative killing by producing superoxide dismutase and catalase (Frenchick *et al.,* 1985; Harmon *et al.,* 1988; Quinn *et al.,* 2002). Tatum *et al.* (1992) suggested that anti-oxidant Cu-Zn superoxide dismutase plays a role in the survival of *Brucella* in phagocytic cells. The organism is then shed in milk in form of somatic cells. Somatic cells are white blood cells, the basic function of which in the mammary gland is defence against bacterial invasion. In normal milk from non-infected quarters, the somatic cell count is typically <100 000 ml. This total count represents the sum of the numbers of several different types of somatic cells: macrophages (typically _60±70%), lymphocytes (_20±30%) and polymorphonuclear leucocytes (PMN; 10±30%) (Kelly, 2002).

Various inocula are used for direct culture, particularly uterine discharge, colostrum, or milk (from live animals); supramammary lymph nodes (from slaughtered animals); and lung, stomach, and liver (from aborted foetuses and full-term calves). The simplest test to detect the organism is the milk ring test in which killed *Brucella* cells are added to a fresh milk sample. If the milk is infected, a bluish ring will form around the cream line as the cream rises. Other tests involve the reaction of serum antibodies with antigens stained with Rose Bengal, the reaction of milk fat antibodies with stained *B. abortus* cells, or the complement fixation test, which is regarded as the most definitive of the antibody tests (Huber and Nicoletti, 1986). Under normal storage conditions *Brucella* survived in UHT milk for 87 days, for 60 days in water and less than a week in yogurt. When milk was inoculated with low bacterial numbers, *Brucella* multiplied by five log units within three weeks and survived in 3.5% and 10.0% fat yogurt for four and two days, respectively (Falenski *et al*., 2011)

2.7 Tuberculosis

2.7.1 *Mycobacteria* species in dairy products

Non-tuberculosis *Mycobacteria* (NTM) including *M. avium*, *M. kansasii*, *M. aquae*, *M. fortuitum* and *M. smegmatis* have been isolated from tuberculosis like lesions in dromedaries. This implies that these organisms can also be secreted in milk.*M. intracellulare* typically causes pulmonary disease, followed by lung damage or reduced lung function, or cervical lymphadenitis, and occasionally affects children (Falkinham, 2002). Species from MTBC have been isolated from milk; 16 isolates of *M. bovis* and 8 isolates of *M. tuberculosis* were obtained from 543 milk samples collected from bulk tanks (Franco *et. al*., 2013). Furthermore, *M. bovis* strains have been isolated in Russia from bulked samples of raw dromedary milk (Zerom *et al*., 2012).

Paratuberculosis is a systemic infection, with clinically diseased animals shedding up to 5 x 10^{12} (5 trillion) bacteria per day. The organism is pervasive in tissue and body fluids. *Mycobacteria* species may exist in milk in fat droplets. Research has shown that MAP, *M. arbotus* has an affinity for the fat layer in milk. Thus, after milk has been taken from an infected cow, *Mycobacteria* in free suspension will migrate to fat droplets in the milk, and conceal themselves within these droplets (Crohns). *Mycobacteria* are also shed in urine and faeces. Therefore fecal contamination of the milk and udder during milking introduces the bacteria in milk. Results from this study agree with those reported by new world camelids (NWC) where M. *avium, M. kansasii* and *M. bovis* were isiolated (Kiine *et al*., 2006). *M. kansasii* and *M. malmoense* causes chronic pulmonary disease similar to tuberculosis in

immunocompetent patients. They also cause pulmonary, extrapulmonary, or disseminated disease in patients with various immunodeficiencies, particularly human immunodeficiency virus (HIV) infection (Taillard *et al*., 2003). *M. avium* and *M. intracellulare* (known together as *M avium-intracellulare* complex) are the most common causes of pulmonary disease, lymphadenitis, and disseminated disease in healthy and immune suppressed persons (Ashford *et al*., 2001).

2.7.2 Principle of Genotype *Mycobacteria* Direct (GMTBDR)

GenoType *Mycobacteria* direct (GTMD) is a qualitative *in vitro* test for diagnostic purposes based on nucleic acid sequence based amplification and DNA strip technologies. It permits the genetic detection direct from decontaminated samples of five *Mycobacteria* species: *Mycobacteria avium, Mycobacteria kansasii, Mycobacteria intracellulare, Mycobacteria malmoense* and *Mycobacteria tuberculosis* complex.

2.7.2.1 Nucleic Acid Sequence Based Amplification (NASBA).

Nucleic acid sequence-based amplification (NASBA), also known as "self-sustained sequence replication" (3SR) **(**Guatelli *et al*., 1990**)** and Transcription mediated amplification (TMA) (Gill and Ghaemi, 2008). It is a sensitive transcription-based amplification system (TAS) for the specific replication of nucleic acids *in vitro*. The complete amplification reaction is performed at the predefined temperature of 41^{o}C. Three enzymes are involved in this homogeneous isothermal reaction: avian myeloblastosis virus (AMV), reverse transcriptase (RT), RNase H and T7 DNA dependent RNA polymerase (DdRp). Due to the integration of RT into the amplification process, the method is especially suited for RNA analytes like mRNA, rRNA or genomic RNA (Deiman *et al*., 2002).

2.7.2.2 Principle of NASBA

Isothermal nucleic acid amplification of RNA is achieved by the simultaneous action of avian myeloblastosis virus reverse transcriptase (AMVRT), T7 RNA polymerase and RNase H (Guatelli *et al*., 1990). Nucleic acid sequence based amplification (NASBA) was developed in the early 90s to amplify nucleic acids without the help a thermal cycler (Compton, 1991). This technique obtains many copies of RNA starting from a few RNA molecules. This approach makes use of two specific primers flanking the sequence to be amplified. The first primer (P1) carries the binding sequence for the T7 RNA polymerase at its 5' end and is used to initiate the RNA reverse-transcription (RT) reaction, catalyzed by a reverse-transcriptase. RNA strand in the RNA– DNA hybrid molecules resulted from the RT reaction is then degraded by RNAse H. The remaining cDNA is then accessible to the second primer (P2)

which initiates the synthesis of the complementary strand. A third enzyme, the T7 RNA Polymerase, docks the double strand DNA on the sequence at the 5' end of P1, transcribing many RNA copies of the gene. This process is repeated starting from the newly transcribed RNA (Fig. 1). The double-stranded promoter sequences can again be produced, and simultaneously, newly synthesized RNA fragments will be formed by the polymerase, thus initiating the next round of the cyclic phase (Chia-Chen *et al*., 2012). RNA and double strand cDNA accumulate exponentially. The reaction is performed at a single temperature, normally 41°C. At this temperature, the genomic DNA from the target microorganism remains double-stranded and does not become a substrate for amplification (Fakruddin *et al*., 2012; Chia-chen *et al*., 2012). The kinetics of the reaction are mainly determined by the efficiency of primer binding, which is dependent on the sequence and structure of the target RNA, and the extent of nonspecific product synthesis due to mispriming events (Fakruddin *et al*., 2012).

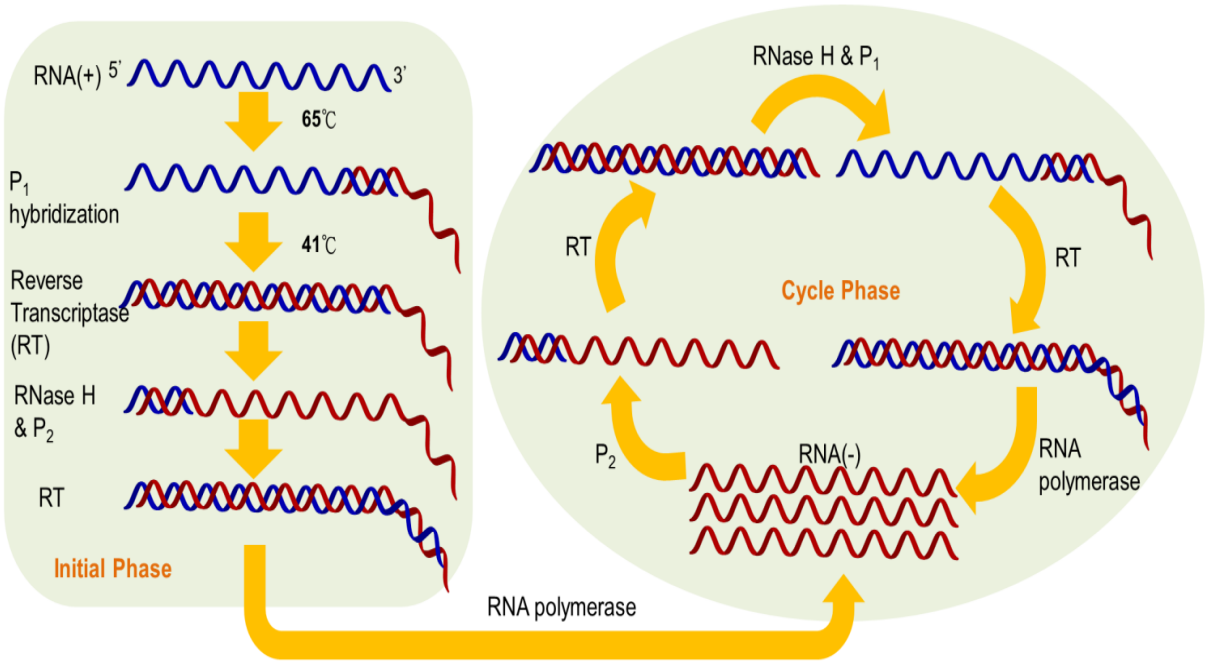


Figure 1: Principles of NASBA (source: Chia-Chen *et al*., 2012)

2.7.3 Detection of NASBA products

2.7.3.1 Reverse hybridization

To monitor the amplified target during the NASBA process molecular beacon probes (a labelled nucleic acid molecule necessary for the detection of complementary (or highly similar) target DNA or RNA molecules) are used (Fakruddin *et al*., 2012). Line-probe assays also referred to as reverse hybridization, are a family of novel DNA strip-based tests that use

PCR and reverse hybridization methods for the rapid detection of mutations associated with drug resistance using an automated system. (Angkana, 2009).

Line Probe assays were developed to expand the range of mycobacterium species identified using nucleic acid probes since those were only available for *Mtb* complex, *M. avium*, *M.intracellulare*, *M. kansasii* and *M. gordonae* (Gen-Probe, San Diego, CA). Commercially available LineProbe assay, the INNO LiPa *Mycobacteria* (Innogenetics, Ghent,Belgium) and the Genotype MTBC DNA strip assay (Hain LifeScience,), use reverse hybridization technology in which probes are immobilized as parallel lines on a membrane strips (figure 1). Amplified, biotinylated DNA fragments of the 16-23S rRNA space region of *Mycobacteria*l organisms are incubated with the labeled strips; addition of streptavidin alkaline phosphatase and chromogenic substrates results in the formation of a precipitate on the membrane where hybridization has occurred (Scarparo *et al*., 2001; Tortoli *et al*., 2001). The coloured precipitate when hydrolysed, reveals positive hybridization observed by eye (fig 2) (WHO, 2008). The biotin streptavidin system used is based on the high affinity between biotin (a naturally occurring vitamin) which is incorporated in the probe, and the bacterial protein streptavidin, which binds to it. Biotin and streptavidin bind together with an affinity constant of 10^{14}, one of the strongest described in biological systems.

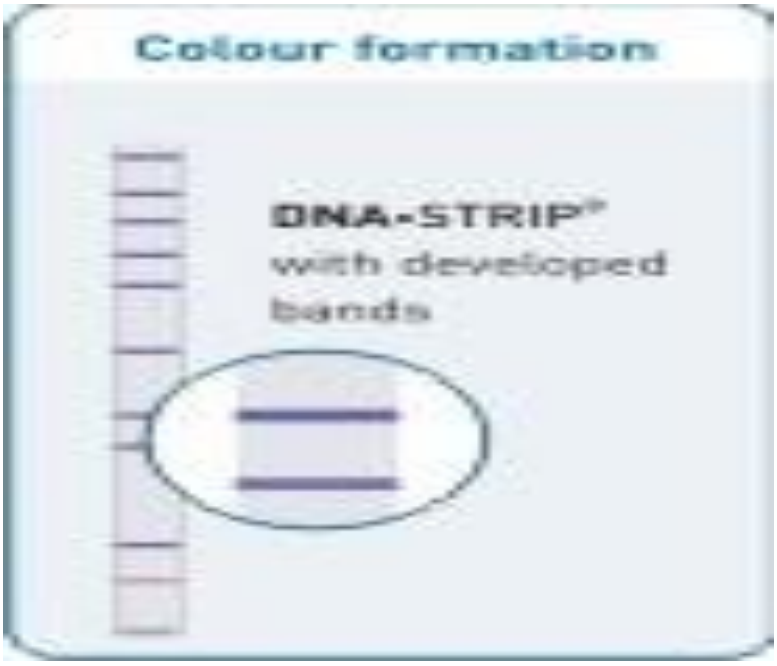


Figure 2: Colour formation. Source (Hainlifescience, Germany)

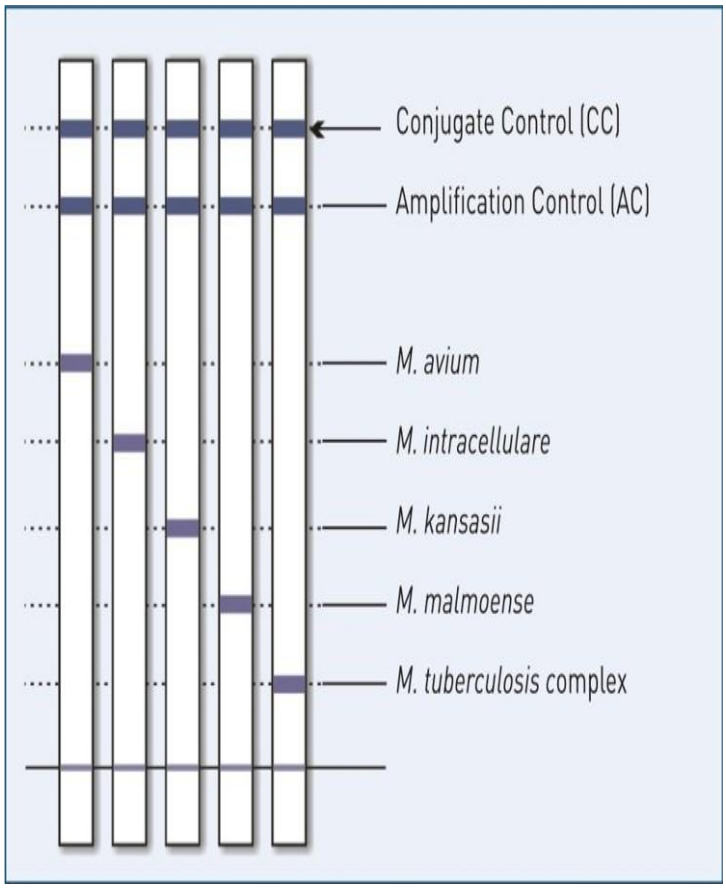


Figure 3: Immobilized probes on a membrane strip (Source: Hainlifescience, Germany)

2.8 Nutritional composition of camel milk

Variability in camel milk composition depends on geographical region, physiological stage, feeding conditions, milk production, genetic or health status. The camel milk composition is different from other ruminants. The camel milk is low in fat 2% which consists mainly of polyunsaturated fatty acids which are completely homogenized to give the milk a smooth white appearance. It contains lactose 4.8% that is easily digestible by lactose intolerant people. The protein content ranges from 2.15% to 4.90%. The proteins do not contain beta-lactoglobulin and have a low quantity of beta-casein the two components present in cow milk that are responsible for causing allergies (Konuspayeva *et al*., 2008).

References from Asia have given results with higher values in almost all the components, except ash, probably linked to camel species; the Bactrian camel is predominant in the area. Milk composition reported in East African references was higher in fat matter content compared to other references in Africa and Western Asia (Konuspayeva *et al.*, 2008). Camel milk has high mineral content (Potassium, Sodium, copper, iron, magnesium and zinc), vitamin C and insulin like protein (Mona *et al.,* 2010). Relatively higher ash content was

found in fermented camel milk compared to fresh camel milk which could be greatly affected by drought conditions (Yagil and Etzion, 1980). Camel milk is low on cholesterol, sugars and protein (Mona *et al*., 2010).

Chemical composition is affected by factors based on the physiological and environmental conditions such as the species of camel, age, health condition, differences in genotypes, seasonal conditions (wet or dry), parity of camel's, ecological localities for camels, water availability, availability of the green fodder, fermentation period and stage of lactation. Increased fermentation time lowered ash content (Abdulaziz *et al*., 2014). Variation in the nutritional composition from other authors could be as a result of difference in factors such as management, location, environment and processing conditions (El Zubeir and Ibrahium, 2009, Eilaf *et al*., 2014).

A review by Lu *et al.* (2013) reported that lowest percentages of lipid and protein content in milk occur when *Pseudomonas* levels are at their highest 10^7cfu/ml. Gerber method was reported to be sufficient to determine the fat content in milk (Michael *et al*., 2013). *Micrococcus* and *Staphylococcus* produce lipase enzyme and degrade milk fats at refrigeration temperature (7^oC) and affects the quality of milk and fermented milk during their preservation at refrigeration storage (Patil and Gandhi, 2013).

The presence of spoilage as well as pathogenic microorganisms in the milk can result in hydrolytic rancidity. The food is hydrolysed, first to diglycerides, then monoglycerides and finally to fatty acids. The enzyme lipase is a risk factor, which can come from bacteria and from the cells of the animal tissue. Animal tissue and bacterial contamination should therefore be avoided. Sterilisation can be used to reduce the risk of lipolytic activity.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study was conducted along the camel milk value chain from Isiolo camel producing areas to Nairobi urban consumption centre. The selected areas are among those with the highest number of camels and high production and consumption of camel milk and *suusa*. Isiolo County has a prominent peri-urban camel population and thriving camel milk business. The growth and demands for camel milk is a result of the influx of Somali refugees into Nairobi, progressive migration of people from a camel keeping background (i.e. Kenyan Somalis, Gabra and Rendille tribes) into urban cities like Nairobi in search of business and employment opportunities.

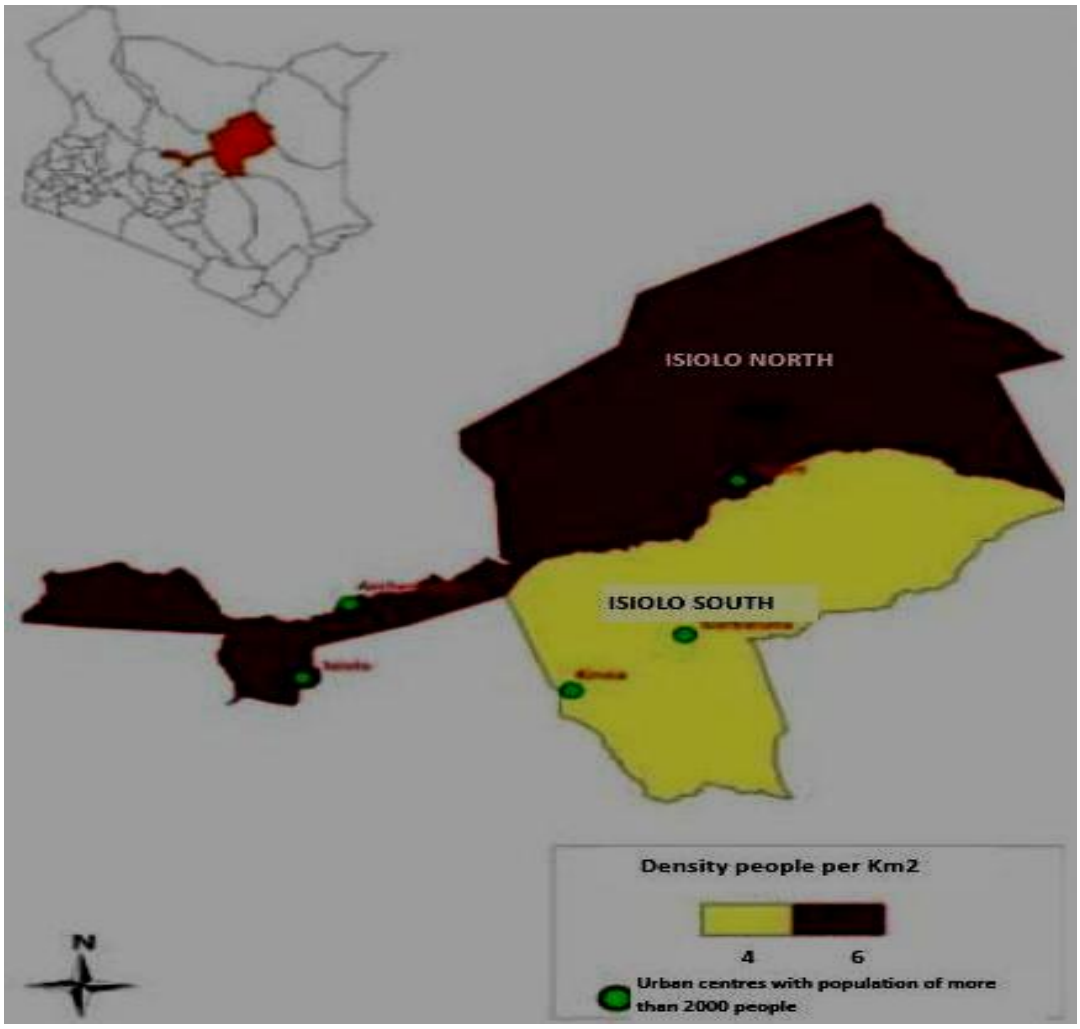


Figure 4: Map of Isiolo County

3.2 Experimental design

A cross sectional survey was conducted and simple random sampling was used to select producers, herders, transporters, women group processors, traders and consumers in the value chain. Focus group discussions and questionnaires were used to supplement the survey. Due to the high mobility and scattered nature of the pastoral camel herds as well as necessity to obtain consent from herd owners, sampling was limited to accessible herds whose owners agreed to participate in the study. Sample size was determined according to Kothari (2004):

$n = K^2R\ (1\text{-}R)/D^2$ where n = sample size

R = proportion of population containing major attribute

D = Margin of error in percentage

K = confidence level (z)

$n = 1.96^2 x\ 0.5\ (1\text{-}0.5)/0.1^2$

n = 96 samples

3.3 Mapping camel milk *suusa* value chain

Semi structured questionnaires were administered and focus group discussions (Appendices 2 and 3) held with 5 women group members. Key informant interview was also conducted. The sampling unit consisted of camel milk producers, herders, milk traders, traders and consumers in local urban towns, centres and cities where camel milk and *suusa* is consumed.

3.4 Determining the association between microbial load with the handling hygiene practices of *suusa* along the value chain

A semi-structured questionnaire (Appendix 2) was administered to randomly selected women groups involved in the production, processing, trading and marketing of *suusa* together with other actors in the *suusa* value chain. The researcher also made observations at each value chain node of obtaining milk and *suusa* samples in order to capture details of hygiene and handling practices.

Samples of fresh camel milk and *suusa* were collected at each representative point of the value chain. Pooled samples were collected from *bomas* (production) level, cooling hubs (collection/processing) and roadside displays in urban centres (market). A sample of 10 ml of both fresh and fermented milk was poured into sterile labelled screw cap tubes and stored in a cool box maintained at 4^oC by cooling elements. The collected field samples of milk and *suusa* were transported to Egerton University food microbiology laboratory for microbiological and nutritional analysis. Samples of collected milk and *suusa* were classified into two quality classes: fresh milk processed into *suusa* and high acid milk sold as *suusa.*

3.4.1 Estimating the level of contamination and health risks.

All the microbiological and biochemical analysis were carried following standard procedures as described by Harrigan and MCcance, 1982.

a) Total viable count (TVC)

TVC gives an indication of the initial microbial load which is related to initial microbial contamination. One millilitre (1ml) of the milk sample was serially diluted six-fold (10^{-6}) using 9ml buffered peptone water. One millilitre (1ml) of homogenate sample was transferred using a sterile pipette into sterile labelled petri dishes. Approximately 20ml of plate count agar (PCA) which had been autoclaved at 121^{o}C for 15min, cooled and tempered in a water bath at 45^{o}C, was poured into the petri dish. The media and sample were mixed gently by swirling in a figure eight manner. The petri dish was left to solidify at room temperature and incubated at 37^{o}C for 48h in an inverted manner. Plates containing 30-300 colonies were selected and counted. An average count of the duplicate plates was calculated and converted into logarithm for recording and analysis.

Discrete colonies grown on plate count agar were selected randomly and purified by repeated plating on the same agar. The colonies were subjected to morphological (cell shape, cell grouping and endospores), biochemical (catalase, oxidase, indole, and Methyl red-Vosges-Proskauer) and physiological tests and identified to genus level.

b) Biochemical Tests of selected colonies

Catalase Test

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

Oxidase Test

This was done to differentiate oxidative and fermentative gram negative organisms. Pure colonies of the isolates (4 colonies) were smeared on the oxidase test strip. Colour change to deep blue was positive for the test.

Indole Test

Pure colonies were suspended in peptone water and incubated at 37^{o}C for 24hrs. One millilitre (ml) of Kovac's reagent was added and shaken gently. It was left to stand for 5min. A pink to red colour development was positive for indole production.

Voges-Proskauer (VP) test

A tube of Methyl Red Voges Proskauer (MR-VP) broth was incubated for 48hrs at 35°C. One ml was transferred to 13 x 100 mm tube and 0.6 mL α-naphthol solution and 0.2 mL 40% KOH was added and shaken. A few crystals of creatine were added, shaken and let stand for 2hrs. Test was positive if eosin pink colour developed.

Methyl red test

After VP test, MR-VP tube was incubated for 48hrs at 35°C. Five drops of methyl red solution was added to each tube. Distinct red color was positive. Yellow was a negative reaction.

Citrate utilization

After Methyl red test, a sample was collected using a wire loop and lightly inoculated in a tube of Koser's citrate broth while avoiding detectable turbidity. It was incubated for 96 hrs at 35°C. Development of distinct turbidity was a positive reaction.

Gas from lactose

A tube containing lauryl sulphate tryptose broth was inoculated with a loopful of the test organism and incubated for 48hrs at 35°C. Gas production (displacement of medium from inner vial) or effervescence after gentle agitation was a positive reaction.

c) Total coliform count (TCC)

Coliforms were enumerated using pour plating technique on violet red bile agar (VRBA) selective media. Pink to red colonies were counted. Serial dilution was done to samples up to 10^{-6} and 1ml of homogenate sample was transferred using a sterile pipette into a sterile petri dish. Approximately 20 ml of VRBA was poured into the petri dish. The media and sample were mixed gently by swerving in a figure eight manner. The petri dish was left to solidify at room temperature and incubated at 37^{o}C for 48h in an inverted manner.

Colonies from VRBA were streaked onto Eosin Methylene Blue agar (EMB) and incubated for 24 hours at 37^{o}C to observe for a green metallic sheen formation (*Escherichia coli*). Colonies were picked aseptically using an inoculating loop for gram staining. All cultures

appearing as Gram-negative, short rods were tested for the indole, methyl red, Voges Proskauer and citrate (IMViC) reactions.

d) Spore count

Spore count was determined by heat treating milk samples in a water bath at 80^{o}C for 10 minutes and 1 ml volume of the appropriate dilution was pour plated on plate count agar and incubated at 37^{o}C for 24 hours.

e) Yeast and mould count

Yeast and mould count was determined using spread plating technique of diluted samples on potato dextrose agar and incubated at 25^{o}C for 7 days.

3.5 Determining the occurrence of zoonotic pathogens in *suusa* along the value chain.

Blood samples from individual lactating camels were collected for detection of *Brucella* in serum. Raw and fermented camel milk (*suusa)* from each representative point of the value chain were collected for detection of *Brucella* and *Mycobacteria* species presence. The protocols used for analysis are described in the next sections.

3.5.1 Detection of *Brucella* species

Isolation and detection of *Brucella* in fresh camel milk and *suusa* samples was done using milk ring test (MRT). Due to the insensitivity of MRT, Rose Bengal precipitation test (RBPT) was done on serum samples collected from 42 individual lactating camels at production. Confirmatory tests of the positive results were done using the Anigen Rapid *Brucella* Antibody Test kit (Bionote).

Milk Ring Test (MRT)

The milk ring test (MRT) is a rapid screening test for detecting antibodies to *Brucella* infection in milk. It is a test that is cheap, simple and requires no specialised equipment to perform it. MRT detects anti-*Brucella* IgM and IgA (*Brucella* antibodies) bound to milk fat globules. MRT antigen is prepared from concentrated, killed *B. abortus*S99 or S1119-3 cell suspension. Antigen and milk samples were brought to room temperature prior to performing the test. Approximately 30 µl of antigen was added to 2 ml of milk in a narrow test tube and mixed thoroughly. The tubes were then incubated at 37°C for 1 hr together with positive and negative working standards. Results were read after 1 hour. The samples were further incubated at 4^{o}C for 12 hours and results recorded. A strongly positive reaction was indicated by formation of dark blue ring above a white milk column. The test was considered to be

negative if the blue colour of the underlying milk exceeded that of the cream layer (Nielsen *et al*., 1996).

Rose Bengal Precipitation Test

The RBT is a simple agglutination technique. Since the test does not need special laboratory facilities and is simple and easy to perform, it is used to screen sera for antibodies to *Brucella*. RBPT antigen is prepared by depositing killed *B. abortus* S99 or S1119-3 cells. The antigen, control and test sera were placed at room temperature 1 hour prior to the test. Approximately 20 µl (one drop) of the antigen was placed on each square of the plate. Approximately 20 µl (one drop) of the test serum was placed alongside the antigen. With an applicator stick, the antigen and the sera were mixed thoroughly. The plate was then shaken gently for 5 minutes. The results were read immediately after 5 minutes by examining for agglutination. Any degree of agglutination was taken as a positive result.

Anigen Rapid *Brucella* Antibody Test kit (Bionote).

The test kit is a solid phase chromatographic immunoassay for the qualitative detection of *Brucella arbotus* antibody in whole blood, plasma, serum and milk. This test was used as confirmatory. The test kit was removed from the foil pouch and placed on a flat, dry surface at room temperature. Approximately 20 µl (one drop) of the test serum was slowly added using a capillary tube to the sample well and then 4 drops of the assay diluent was added. The test results were interpreted after 20 minutes. The presence of only one purple colour band within the result window indicated a negative result while the presence of two colour bands (Test band and Control band) within the result window, no matter which band appears first, indicated a positive result.

3.5.2 Detection of *Mycobacteria*

To detect the presence of *Mycobacteria* species in camel milk, direct microscopy and Genotype *Mycobacteria* direct with DNA strip technology was used. These tests were used to examine raw and fermented milk samples collected at production and at the market.

Direct Microscopy

Decontamination of fresh camel milk and *suusa*: Fresh milk and *suusa* samples (5 ml) were decontaminated using 5% oxalic acid and concentrated by centrifugation (10 min, 1200 x g, 4°C). The supernatant was used to make smears for microscopic examination.

Direct smears: Direct smears were prepared with 200 µl of each of the final decontaminated volumes smearing the specimen on the slide over an area approximately (1.5 X 1.5mm).The smears were left to air dry and later heat fixed by holding the slide with forceps and passing over a flame 5 times for 4 seconds.

Ziehl-Nielsen staining: The slides were placed on a staining rack 1cm apart, covered with 1% filtered carbolfuchsin and heated from underneath until vapour started to rise but not boiling. They were then covered with the hot steaming carbolfuchsin for 7 mins and rinsed gently with distilled water to remove the excess stain. They were then covered with 25% sulphuric acid solution and allowed to stand for 3 mins until the red color disappeared completely. Sulphuric acid was then washed off with distilled water and the slides tilted to remove excess water. They were then covered with 0.1% Methylene blue counter stain for 1min and later rinsed with distilled water to remove excess stain. Slides examination was later conducted under a microscope at ×100.

Genotype *Mycobacteria* direct (GMTD) with DNA strip technology

GenoType *Mycobacteria* direct (GTMD) is a qualitative *in vitro* test for diagnostic purposes based on nucleic acid sequence based amplification and DNA strip technologies. It permits the genetic detection direct from decontaminated samples of five *Mycobacteria* species: *Mycobacteria avium, Mycobacteria kansasii, Mycobacteria intracellulare, Mycobacteria malmoense* and *Mycobacteria tuberculosis* complex. The working areas were decontaminated using freshly prepared 1.5% sodium hypochlorite solution and a residence time of 15 min observed. The procedure involved 4 steps which included ribonucleic acid (RNA) extraction, amplification, reverse hybridization and interpretation of results.

1. RNA extraction(Magnetic beads capture technique)

Lysis buffer (LB) was pre-incubated at 37^{o}C for 20 min until the reagent was free of precipitates. Positive control RNA (PC-RNA) lyophilisate was dissolved by adding 15 µl control RNA buffer (CRB) and vortexing the mixture. PC-RNA was diluted by mixing 5 µl of the PC-RNA with 500 µl of molecular water and vortexing. After dilution, PC-RNA was handled like a normal sample. Magnetic beads (MB) and binding buffer (BIN) mix was prepared by mixing 33 µl MB and 0.22 ml BIN for 1 sample. The mixture was mixed thoroughly by carefully pipetting up and down.

Approximately 230 µl MB/BIN mix was pipetted into a 1.5 ml screw cap tube and 1000 µl of the sample was added and mixed by pipetting up and down. It was incubated at room

temperature for 15 min. The mix was then transferred to a magnetic separator with the magnetic bar inserted on position A (Attached in the appendices) for 2 min. The supernatant was removed using a pipette without losing the MB pellets. The magnetic bar was removed from the magnetic separator and 50 µl of the dissolved LB was added and mixed by pipetting up and down. The mixture was incubated at room temperature for 5 min. 150 µl of 96% ethanol was added and mixed by vortexing and incubated for 5 min at room temperature. The magnetic bar was inserted at position A in the magnetic separator, the sample was incubated for 2 min at room temperature (RT) in a separator and the magnetic separator was flipped upside down including the sample shortly. Incubation lasted for another 1min and the supernatant was removed without losing the MB pellets. The magnetic bar was removed and 1ml of 70% ethanol added and mixed by vortexing. The sample was incubated in the separator for 2 min (with the magnetic bar inserted at position A) and subsequently flipped upside down. Incubation lasted for another 1min in the separator and supernatant removed without losing the MB pellets. Incubation was then done at RT for 5 min. Residual liquid was aspirated and subsequently magnetic bar was removed from the separator. 100 µl BB was added and re-suspended by pipetting up and down. Incubation followed at 85^{o}C for 20 min in a water bath with the lid open. The sample was transferred to the magnetic separator (with the magnetic bar inserted at position B) and incubated for 2 min at RT. The supernatant (purified RNA solution) was transferred to a new tube.

2. Amplification

Nucleic acid sequence based amplification (NASBA) technique of RNA fragments was used. NASBA reaction is an isothermal amplification at 41^{o}C. EML was dissolved by adding 40 µl enzyme dilution buffer (EDB) to each enzyme mix lyophilisate (EML). Internal control RNA (ICR) lyophilisate was dissolved by adding 70 µl EDB and vortexing. From the ICR, amplification control (AC) product is generated. To each enzyme mix (40 µl), 2 µl of dissolved ICR was added and mixed by pipetting up and down. 3.5 µl DMSO was added to 11.5 µl PNM and vortexed (the mix is reffered as PNM^{D}). For each reaction, 15 µl PNM^{D} was added to 10 µl of purified RNA to a PCR tube. The samples were then transferred to a thermal cycler. The amplification program was then started. The amplification profile was for 5 min at 65^{o}C for denaturation, 3 min at 41^{o}C for cooling, 5 min at 41^{o}C for addition of enzyme and 5 µl enzyme/ICR mix and 60 min at 41^{o}C for amplification.

3. Hybridization

Hybridization included the following steps: chemical denaturation of the amplification products, hybridization of the single-stranded biotin labelled amplicons to membrane bound probes, stringent washing, addition of streptavidin/alkaline phosphatise (AP) conjugate, and an AP mediated staining reaction.

The twincubator was pre-warmed to 50^{o}C. Hybridization buffer (HYB), stringent wash solution (X-STR), conjugate buffer (CON-D) and substrate buffer (SUB-D) were then warmed to 37^{o}C until they were free from precipitates. Conjugate concentrate (CON-C) and substrate concentrate (SUB-C) were diluted at a ratio of 1:100 with the respective buffer in the amounts needed. They were mixed and brought to room temperature (RT). Approximately 20 µl of denaturation solution (X-DEN) was dispensed in a corner of each of the wells. 20 µl of the amplified sample was added, mixed by pipetting up and down and incubated at RT for 10 min. 1 ml of the pre-warmed HYB was added to each of the wells and shaken gently until the solution had a homogenous colour. Labelled strips were placed using sterile tweezers in each well until they were completely covered by the solution. The tray (of wells) was placed in the twincubator and incubated for 20 min at 50^{o}C. The HYB was later completely aspirated using a Pasteur pipette. 1ml of stringent wash solution (X-STR) was added to each strip and incubated for 10 min at 50^{o}C in the twincubator. X-STR was then completely aspirated. 1ml of diluted conjugate was added to each strip and incubated for 20 min at 37^{o}C on the twincubator. The solution was then removed and each strip washed twice for 1min with 1ml rinse solution (X-RIN) and once for 1min with 1ml of distilled water on the twincubator at 25^{o}C. Each solution was poured out each time. 1ml of diluted substrate was added to each strip and incubated at 25^{o}C on twincubator for between 3-15 min thus, the time until the bands are clearly visible and protected from light. The reaction was stopped as soon as the bands were clearly visible by rinsing twice with distilled water. The strips were removed from the tray with sterile tweezers and dried between two layers of absorbent paper.

4. Evaluation and interpretation of results

An evaluation sheet was provided with the kit. When using the evaluation sheet, the developed strips were pasted in the designated fields by aligning the conjugate control (CC) band with the respective line on the sheet. An interpretation chart (attached in the appendices) was used to identify the species.

3.6 Determining the influence of handling practices and microbial load on nutritional changes of *suusa* along the value chain

In triplicates, collected samples of fresh camel milk and *suusa* from each representative point of the value chain were analysed to determine any nutritional changes. Titratable acidity, protein, ash and fat content in fresh camel milk and *suusa* samples were carried out.

3.6.1 Determination of titratable acidity

Developed acidity in the samples was determined according to the method described by the International Dairy Federation (1990). Approximately 9 ml of the milk samples was measured into the conical flasks, and 1 ml 0.5 % alcoholic phenolphthalein indicator added then titrated with 0.1M sodium hydroxide (NaOH) until a faint pink colour appeared. The results were expressed as % lactic acid where 1/10 ml NaOH is equal to 0.09 % w/v lactic acid.

3.6.2 Determination of protein content

Crude protein content in camel milk and *suusa* samples was determined using micro-kjeldahl method according to AOAC 1990. 0.2 grams of the sample was weighed and digested using 10 ml concentrated sulphuric acid and selenium tablets (catalyst) at 445^{o}C for 3hr in a digester (Foss Tecator). The digested samples were cooled to room temperature and then distilled using kjeldhal unit. The distillate was collected in a 15 ml 0.1N hydrochloric acid (HCL) in which a mixed indicator of methyl red and methylene blue had been added. Titration was later done of the HCL against sodium hydroxide (NaOH). Crude protein was determined using the formula below:

$$\% \text{ crude protein} = \frac{(V1-V2) \times N \times 1.4 \times 6.25}{W}$$

Where V1 is the volume of HCL used for the blank test, V2 is the volume of HCL used for the sample, N is the normality of the acid and W is the weight of the sample.

3.6.3 Determination of Ash content

Dry ashing was used as described by AOAC (1990). This involves incineration at high temperature (550^{o}C) to completely decompose organic matter and carbonates. The empty silica crucible was first weighed on a weigh balance. Five millilitre (5 mls) of the sample was then weighed into a silica crucible. The sample was first ashed in a fume chamber until it stopped smoking, ashed in a muffle furnace at 550^{o}C for 3hrs, cooled in a dessicator to room temperature and weighed. Ash content was calculated as a percent of the dry sample using the formula below:

$$\% \text{ ash} = \frac{(\text{Weight of crucible + ash}) - (\text{weight of empty crucible})}{(\text{Weight of crucible + sample}) - (\text{weight of empty crucible})} \times 100$$

3.6.4 Determination of fat content

The modified Majonnier method was used as described by AOAC (1990). Approximately 10 ml of milk (fresh and fermented) was weighed into a round bottomed flask. Ten millilitres (10 ml) concentrated hydrochloric acid was added into the sample and refluxed for 1 hr and then cooled to room temperature. The contents were then transferred into 100ml separating funnel. Two millilitres (2 ml) of ammonium hydroxide solution was added to neutralize the sample then 3 drops of phenophthalein dye was then added. Ten millilitres (10 ml) of ethanol was added to the sample to end the gel formation and shaken for 2mins. Twenty five millilitres (25 ml) of diethylether was added and shaken for 2 mins to dissolve the lipids. To the extract, 25 ml petroleum ether was added to dissolve more non polar lipids and shaken for 2 mins. The mixture was left to separate for 1 day. It was later decanted and transferred to a weighed round bottomed flask. The clear ethereal layer was evaporated on a hot plate at < 100^{o}C. The flask was then dried in an air oven at 102^{o}C for 2hrs to a constant weight, cooled in a dessicator and reweighed to obtain the weight of the oil. The percent amount of oil was determined using the formula below:

$$\% \text{ fat} = \frac{\text{Weight of the fat}}{\text{Weight of the sample}} \times 100$$

3.7 Statistical Analysis

3.7.1Determining the association between microbial load with the handling hygiene practices of *suusa* along the value chain

The microbiological counts indicated by the total viable count (TVC), total coliform count (TCC), spore count, yeast and mould count were converted to base-10 logarithm of colony forming units (cfus) per millilitre (ml) of the milk samples (log cfu/ml). Means and standard errors were calculated of TVC, CC, SC and YM in milk and *suusa* were the dependent variables subjected to general linear model procedure GLM of SAS version 9.1 (2007). The independent variables were the class of milk quality (intended and unintended) and value chain points (production, bulking, processing and marketing). The significant differences between the means of TVC, CC, SC and YM at different points along the value chain were analyzed using Tukey's HSD test at α=0.05. Incidence of occurrence of microorganisms along the value chain was determined according to Matofari *et al*., 2007:

$$\% \text{ incidence} = \frac{\text{Number of positive samples} \times 100}{\text{Total samples collected}}$$

3.7.2 Quantifying the risks of *Brucella* and *Mycobacteria* species presence in *suusa* along the value chain

Prevalent risk and likelihood ratio was used to quantify risk of occurrence of zoonotic pathogens *Brucella* and *Mycobacteria* species along the value chain. Chi square was also used to determine significant difference along the value chain. Prevalent risk/incidence and likelihood ratio (McGee, 2002) was determined as follows:

$$\text{Incidence} = \frac{\text{Positive cases}}{\text{Total observed cases}}$$

$$\text{Likelihood ratio} = \frac{\text{Probability of finding positive cases}}{\text{Probability of same finding negative cases}}$$

3.7.3 Determining association between nutritional changes of *suusa* with handling practices and microbial spoilage along the value chain

Means and standard errors were calculated of titratable acidity, protein, ash and fat contents in milk and *suusa*. Titratable acidity, protein, ash and fat contents in milk and *suusa* were the dependent variables subjected to general linear model procedure GLM of SAS version 9.1 (2007). The independent variables were the class of milk quality (intended and unintended) and value chain points (production, bulking, processing and marketing).The significant differences between the means of acidity, protein, fat and ash at different points along the value chain were analyzed using Tukey's HSD test at $\alpha=0.05$.

CHAPTER FOUR

RESULTS

4.1 Mapping camel milk *suusa* value chain

Figure 5 shows the mapped value chain of both intended and unintended *suusa.* Women source camel milk from herds and traders. Fresh milk is bought by women groups or individuals from production (herds), transporters (traders) or bulking centres to make *suusa* or sell in open air market. Coagulated milk due to acid development on transit is sold as unintended *suusa*. Pastoral women have formed groups that transport the *suusa* to bigger cities like Nairobi and Nakuru. One observation is that consumption of *suusa* is being taken up by non-pastoralists.

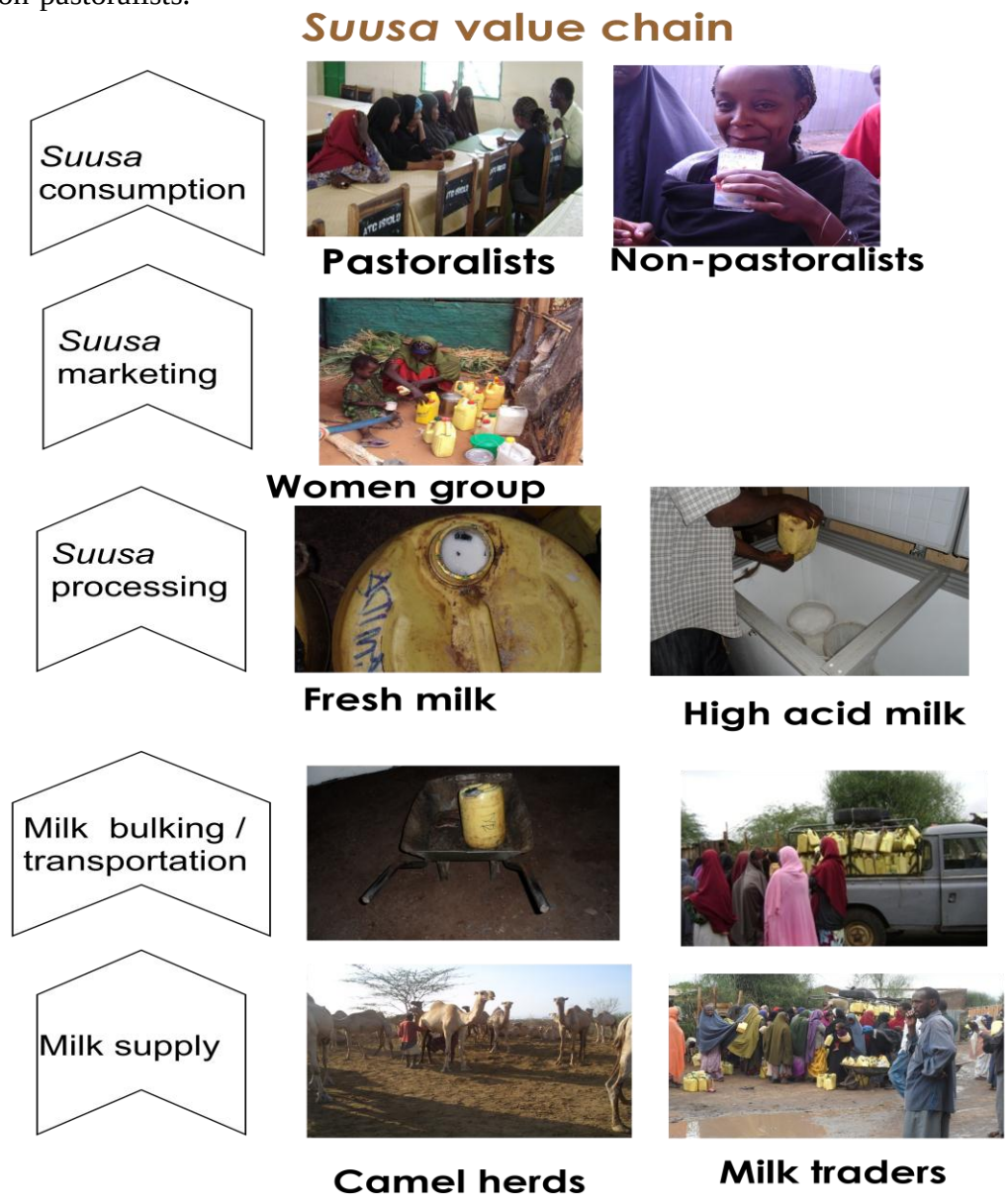
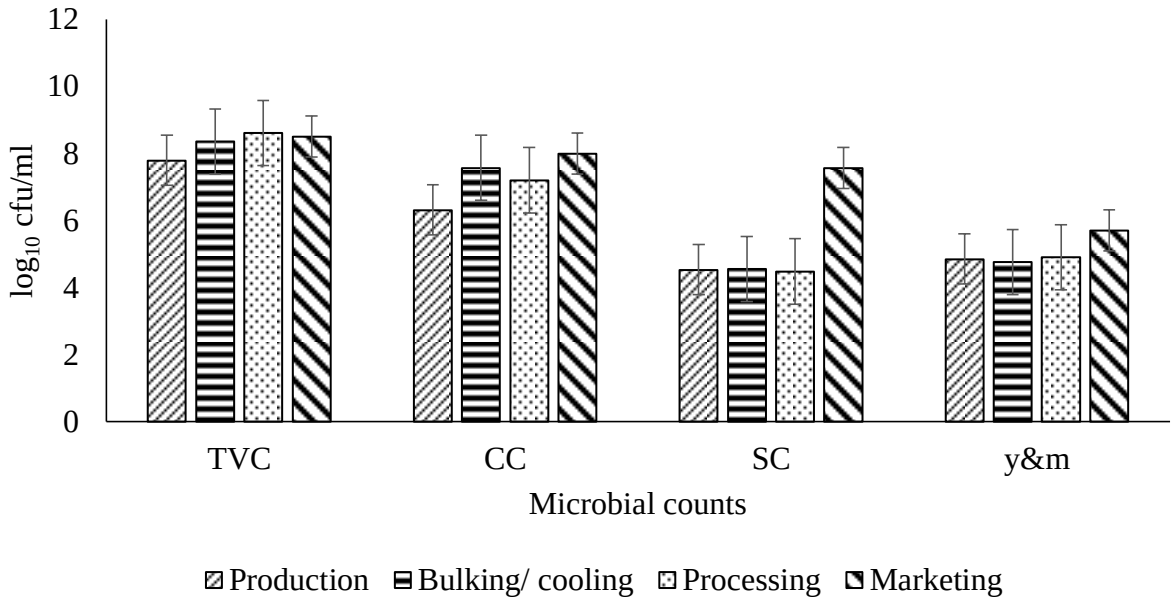


Figure 5: Map of fermented camel milk (*suusa)* value chain

4.2 The microbial load as a result of handling practices of *suusa* along the value chain

a) Un-intended *suusa*

Un-intended *suusa* is fresh camel milk that gets sour on transit to bulking or market centres from the production area-*Boma.* It is sold as *suusa*, while the intended *suusa* is the homemade spontaneously fermented camel milk. Figure 6 shows the microbial load for unintended *suusa* along the value chain. For unintended *suusa*, the total viable count (TVC) increased from $\log_{10}$ 7.79 at production to $\log_{10}$ 8.51 at the market, coliform counts (CC) from $\log_{10}$ 6.31 to $\log_{10}$ 7.99, spore count (SC) from $\log_{10}$ 4.53 to $\log_{10}$ 7.56 and yeast and moulds (Y&M) from $\log_{10}$ 4.85 to $\log_{10}$ 5.70 cfu/ml.



Key: tvc=total viable count; cc=coliform count; sc=spore count; ym=yeast and molds; cfu=colony forming units

Figure 6: Microbial load for unintended *suusa* along the value chain

Figure 7 shows the change in developed lactic acid in unintended *suusa*. Lactic acid increased significantly ($p<0.05$) along the value chain from 0.07 to 0.23%.

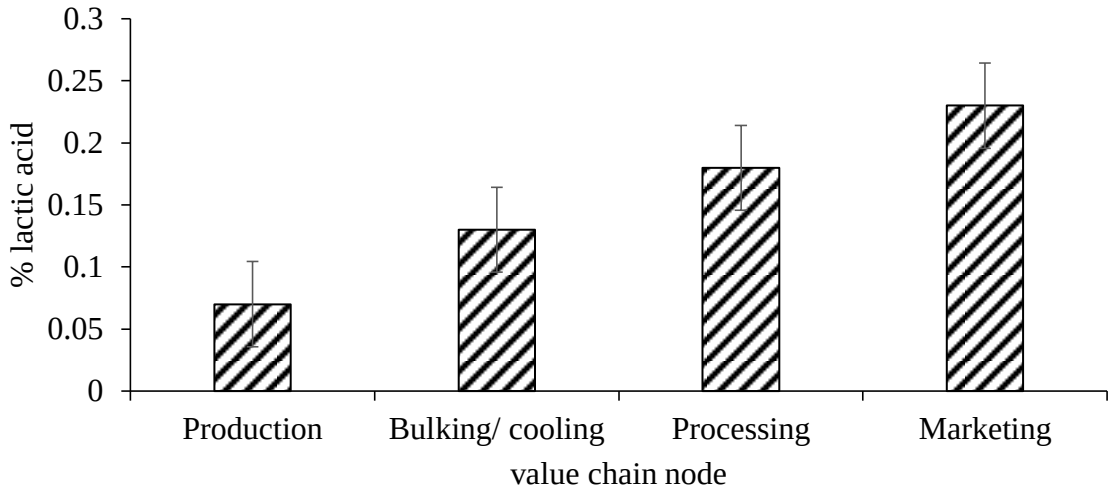
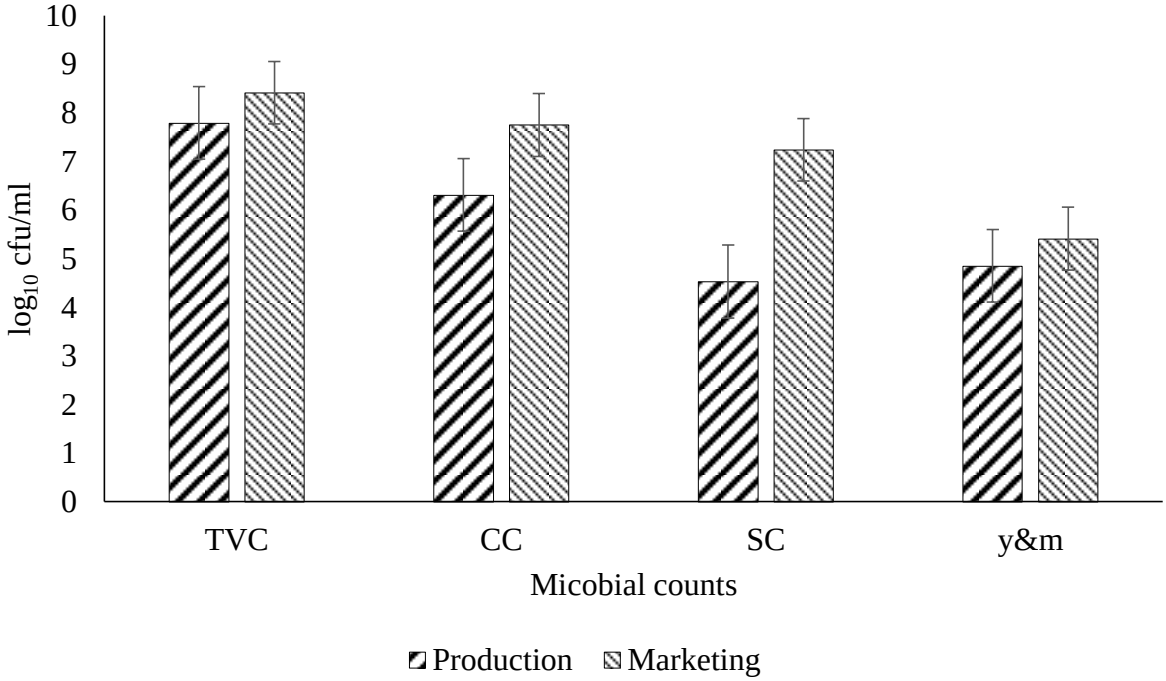


Figure 7: Lactic acid in unintended *suusa* along the value chain

b) Intended *suusa*

For intended *suusa* TVC increased significantly ($p<0.05$) from $\log_{10}$ 7.79 at production to $\log_{10}$ 8.41 at the market, CC from $\log_{10}$ 6.31 to $\log_{10}$ 7.75, SC from $\log_{10}$ 4.53 to $\log_{10}$ 7.24 and yeast and moulds (Y&M) from $\log_{10}$ 4.85 to $\log_{10}$ 5.41 cfu/ml.



Key: tvc=total viable count; cc=coliform count; sc=spore count; ym=yeast and molds; cfu=colony forming unit

Figure 8: Microbial load for intended *suusa* along the value chain

Figure 9 shows developed lactic acid in intended *suusa* along the value chain. Lactic acid (% LA) increased significantly ($p<0.0.5$) along the value chain from 0.07 to 0.60%.

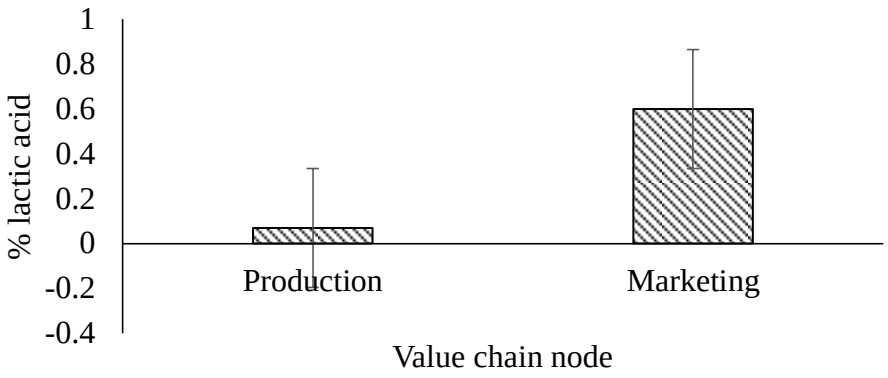


Figure 9: Lactic acid development for intended *suusa* along the value chain

Table 2 shows the main type of microorganisms that were isolated from unintended *suusa* value chain. Gram negative rods had the highest incidence of 67% from production to the market followed by gram positive cocci with an incidence of 62%.Yeast and moulds had the least incidence at 28%.

Table 2: Main groups of microorganisms isolated from the unintended *suusa* value chain and their incidence

Chain node	**N**	**G+ rods [a]**	**G- rods [a]**	**G+ cocci [a]**	**Spores [a]**	**Y&M [a]**
Production	10	2	6	7	2	2
Cooling\bulking	12	6	8	7	3	2
Processed product	7	4	4	4	3	4
Market	10	8	8	6	5	3
Total	39	20	26	24	13	11
Incidence (%)		**51**	**67**	**62**	**33**	**28**

Key: N is the number of samples; G+: Gram positive, G-: Gram negative, Y&M: yeast and moulds Figures in parenthesis represent incidence of occurrence: [a] is the number of positive observed for a specific group of organisms.

Table 3 shows the main groups of organisms isolated from intended *suusa*. Gram negative rods and gram positive cocci had the highest incidence at 60% and 50% respectively.

Table 3: Main groups of microorganisms isolated from the intended *suusa* value chain and their incidences

Value chain node	N	G+ rods [a]	G- rods [a]	G+ cocci [a]	Spores [a]	Y&M [a]
Production	10	2	5	6	2	2
Processing/ marketing	10	3	7	4	3	3
Total	20	5	12	10	5	5
Incidence (%)		**25**	**60**	**50**	**25**	**25**

Key: N is the number of samples; G+: Gram positive, G-: Gram negative, Y&M: yeast and moulds: Figures in parenthesis represent incidence of occurrence, [a] is the number of positive samples for a specific group of organism

Table 4 shows the organisms isolated from the *suusa* value chain. Gram negative rods were identified to be *E. coli, Pseudomonas* and *Enterobacter*. Gram positive rods included *Bacillus* and *Lactobacillus* species while the gram positive cocci included *Micrococcus*, *Streptococcus* and *Staphylococcus* species.

Table 4: Types of microorganisms isolated from the unintended *suusa* value chain

Chain node	N	*E. coli*	*Enterobacter*	*Pseudomonas*	*Micrococcus*	*Staphylococcus*	*Streptococcus*	*Bacillus*	Yeast and molds
Production	10	5	6	-	5	2	3	2	2
Bulking/ cooling	12	4	4	8	3	2	4	3	2
Processing	7	4	4	-	2	2	3	3	4
Marketing	10	4	3	2	6	2	2	5	3
Total	39	17	17	10	16	8	12	13	11
Incidence (%)		**44**	**44**	**27**	**41**	**21**	**31**	**33**	**28**

Key: N is the number of samples analysed.

Table 5 shows the main organisms isolated from intended *suusa*. The gram negative rods: *E. coli* and *Enterobacter* had the highest incidence of 50 and 55% respectively. Staphylococcus had the least incidence of 20% followed by *Streptococcus, Bacillus* and Yeast molds with an incidence of 25%.

Table 5: Types of microorganisms isolated from intended *suusa* value chain

Chain node	N	*E. coli*	*Enterobacter*	*Micrococcus*	*Staphylococcus*	*Streptococcus*	*Bacillus*	Yeast and molds
Production	10	5	6	5	2	3	2	2
Marketing	10	5	5	4	2	2	3	3
Total	20	10	11	9	4	5	5	5
Incidence (%)		**50**	**55**	**45**	**20**	**25**	**25**	**25**

Key: N is the number of samples

4.3 Prevalence of *Brucella* and *Mycobacteria* species in *suusa* value chain

4.3.1 Occurrence of *Brucella* in *suusa* value chain

Table 6 shows the results from Rose Bengal Precipitation test (RBPT), milk ring test (MRT) and Anigen Rapid B. *Brucella* antibody test (ARBAT). There was no significance (p=0.05) difference in the number of positive samples along the value chain.

Table 6: Incidence of *Brucella* species along the *suusa* value chain

Chain Node		Screening		Confirmatory
	N	**MRT**	**RBPT**	**ARBAT**
Production	42	11	6	6
Bulking	35	15	NA	8
Market	19	11	NA	5
Total	96	37	6	19
Incidence (%)		**39**	**17**	**20**

Key: MRT is milk ring test; RBPT is Rose Bengal Precipitation test and ARBAT is Anigen rapid *Brucella* antibody test NA means not applicable. Significance level p=0.05

Table 7 shows the prevalent risk of occurrence of *Brucella* organism in the *suusa* value chain. The odds of obtaining samples positive with the *Brucella* organism at production, processing and marketing are 0.69, 1.22 and 1.50 times higher than obtaining samples negative with the organism respectively.

Table 7: Prevalent risk of *Brucella* in *suusa* along the value chain

Chain node	Total samples	Positive sample	Negative samples	Prevalence risk	Likelihood ratio
Production	42	6	36	0.17	0.69
Processing	35	8	27	0.30	1.22
Marketing	19	5	14	0.36	1.50
Total	**96**	**19**	**77**		

4.3.2 Prevalence of *Mycobacteria* along the *suusa* value chain

4.3.2.1Detection of *Mycobacteria* species using direct microscopy

It is the initial screening step in the diagnosis of tuberculosis. A total of 19 milk samples were examined using direct microscopy. All samples showed negative result.

4.3.2.2 Detection of *Mycobacteria* species using Genotype *Mycobacteria* direct with DNA strip technology

A total of 38 milk samples were analysed. Figure 10 shows a DNA strip from milk samples that showed mixed infection

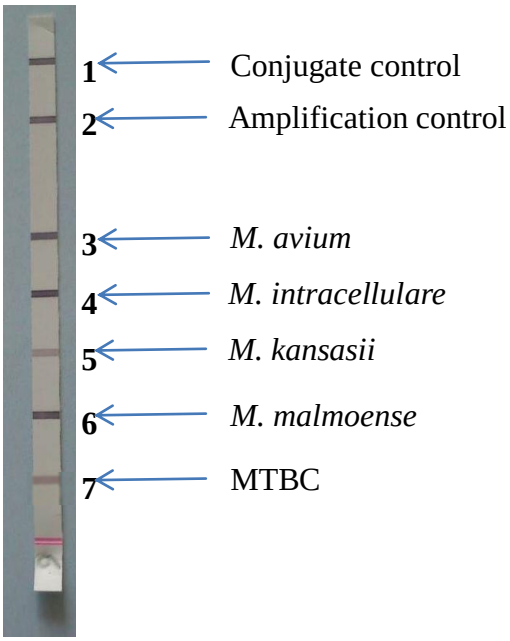


Figure 10: DNA strip showing the different *Mycobacteria* species from one milk sample.

Figure 11 shows a DNA strip stained at position 7 with *Mycobacteria tuberculosis* complex (MTBC) which is a human tuberculosis strain.

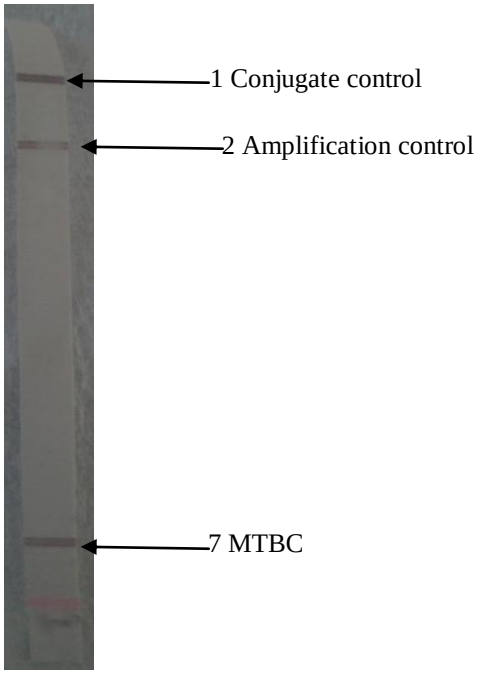


Figure 11: DNA strip showing MTBC from *suusa* sample

Table 8 shows the occurrence of *Mycobacteria* species in the *suusa* value chain based on the frequency of occurrence of each species as detected on DNA strip technology above. There was no significant difference (p=0.05) between frequency of occurrence of the *Mycobacteria* organisms at production and the market using Chi square test. *M. kansasii* had the highest incidence of 42% and 21% at production and marketing respectively followed by *M. avium* with an incidence of 37% and 16% at production and marketing respectively. Incidence of MTBC, a human tuberculosis strain, was 26% and 21% at production and marketing respectively.

Table 8: Occurrence of *Mycobacteria* species along the camel *suusa* value chain

Chain Node	**N**	***M. avium***	***M. intracellulare***	***M. kansasii***	***M. malmoense***	**MTBC**
Production (Fresh milk)	19	7 **(37)**	6**(32)**	8**(42)**	6**(32)**	5 **(26)**
Marketing (*suusa*)	19	3**(16)**	3**(16)**	4**(21)**	3**(16)**	4 **(21)**
Fishers exact test		0.269	0.447	0.295	0.447	1.00

Key: n is the number of samples; MTBC is *Mycobacteria tuberculosis* complex; Figures in parenthesis represent the percentage incidence of occurrence. Significance level p=0.05

Table 9 shows the prevalent risk of *Mycobacteria* occurrence in the *suusa* value chain. The likelihood of obtaining samples positive with the *Mycobacteria* organism at production and marketing are 1.38 and 0.73 times higher than obtaining samples negative with the organism respectively.

Table 9: Prevalent risk of *Mycobacteria* in *suusa* along the value chain

Chain Node	Total samples	Positive samples	Negative samples	Prevalence risk	Likelihood ratio
Production	19	11	8	1.38	1.38
Marketing	19	8	11	0.73	0.73
Total	38	19	19		

4.3.3 Nutritional changes of *suusa* as a result of handling practices and microbial spoilage along the value chain

Figure 12 shows the results for nutritional content of raw and fermented camel milk along the value chain. There was a significant decrease ($p<0.05$) in fat content for unintended *suusa* at production, bulking and processing. Ash and crude protein content remained constant along the value chain.

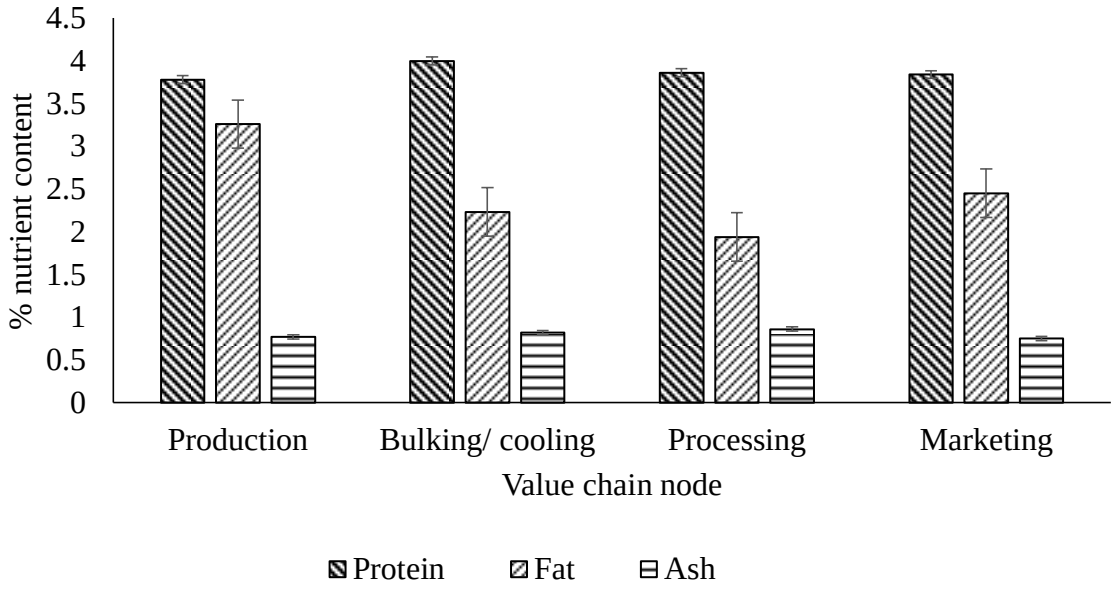


Figure 12: Nutritional composition of unintended *suusa* along the value chain

Figure 13 shows the nutritional content for raw and fermented camel milk along the value chain. There was a significant decrease ($p<0.05$) in fat content for intended *suusa* at production, bulking and processing. Ash and crude protein content remained constant along the value chain.

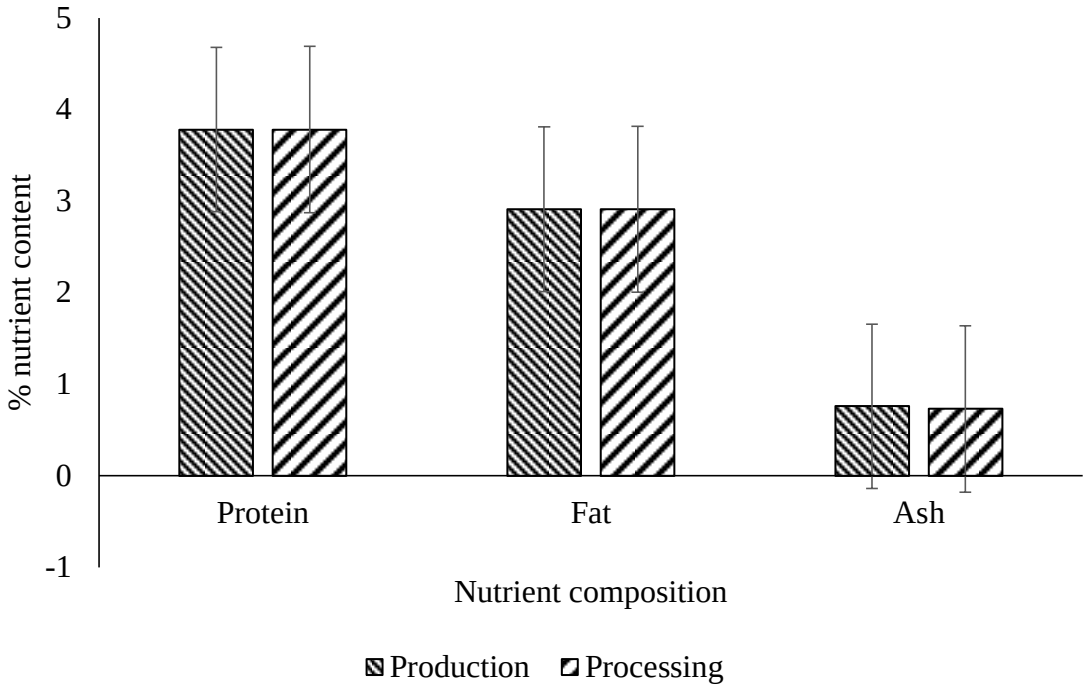


Figure 13: Nutritional composition of intended *suusa* along the value chain

CHAPTER FIVE
DISCUSSION

5.1 Mapping of the *suusa* value chain

Value chain mapping has many objectives and in this study, mapping was conducted in order to understand the physical flow of the camel milk product, *suusa*, as well as the hygiene practices while handling both fresh camel milk and *suusa* at each node. At production, milking of the lactating camels was done by the herders. Water at production was mostly unavailable. The milking environment was observed to be dusty. The herders used plastic containers for milking which are difficult to clean**.** Some of this milk from the farm is the same that is used to prepare *suusa* for home consumption which in this study is referred as intended *suusa* as it is collected with the intention of fermentation at home. At production, veterinary officers did not participate in camel health management as the producers and herders treated their own camels as was reported by camel producers, herders and the Isiolo veterinary office. Mode of transport to the primary milk collection centre at production was by foot (30% of herders) and by donkeys (70% of herders).

At the cooling/bulking centres, milk took up to 3 to 4hrs to arrive from the production areas (‘bomas’).Milk from different suppliers was pooled in some cooling hubs and organoleptic tests were used for quality assessment of milk. Organoleptic tests used included the use of smell to determine if milk had soured and use of sight. These tests are insufficient to determine spoilage or occurrence of pathogenic bacteria. The cooling hubs were owned by cooperatives and women groups and even individuals. Power failure was sighted as a contributor to milk spoilage. Fresh milk meant to be sold in Nairobi sometimes soured at the cooling hubs and was sold as *suusa.* In this study it has been referred to as unintended *suusa* since it has undergone unintentional fermentation. Consequently, the distant markets remain inaccessible to producers and the milk either fetches low prices or is wasted because of deterioration of quality. This point of the value chain was also considered as a critical control point (CCP) because contamination of milk can occur due to bulking milk from different suppliers, inadequate use of quality control tests insufficient to detect pathogens and unintentional fermentation of raw milk under uncontrolled environment.

At the market, the quality of fresh camel milk was affected by various factors such as hygiene, use of not easy to clean plastic containers, delayed milk delivery and lack of refrigeration as reflected by total viable count. Milk is transported directly to urban markets of Isiolo by special milk collection vehicles (vans), 100% of the traders relied on public

transport ferrying passengers and other goods (buses) to Nairobi. Inadequate transport services plus poor milk handling result in contamination, rapid souring and, sometimes, spoilage of the milk before it reaches urban consumers. All the traders of *suusa* had neither shops nor stalls and sold their *suusa* directly at the roadside, under dusty and hot conditions. Interesting observation was that milk that spoiled during transportation to Eastleigh market, was sold as *suusa*. Retail prices are directly linked to milk freshness as fresh milk was sold at 150Kshs/litre while *suusa* was sold at 100Kshs/litre. The value chain for *suusa* mapped from surveys and focus group discussions reveals the handling practices along the value chain. Camels are milked at "boma" by herders. Milk traders buy camel milk from different "bomas" bulk it and transport to processors or market directly. Fresh milk is bought by women groups or individuals to make *suusa* or sell in open air market. High acid (coagulated) milk is downgraded and sold as un-intended *suusa*. Fresh camel milk and *suusa* is consumed by both pastoralists and non-pastoralists.

5.2 Influence of hygiene practices of *suusa* along the value chain on the microbial load and species.

5.2.1 Unintended *suusa*

Currently, camel milk standards have not yet been gazetted; therefore reference will be with regards to cow milk standards. Kenya Bureau of Standards (KEBS) regards raw bovine milk as good when the total viable counts (TVC) are between $0\text{-}2\times10^5$cfu/ml (East African Standard 67, 2007). The raw camel milk at production was above the recommended range. This milk can be regarded as of poor quality as TVC values are beyond what is recommended. High TVC at production can be attributed to handling practices like not washing hands before milking, no washing of the camel's udder before milking, use of plastic containers for milking and storage of milk in plastic containers which are not easy to clean. Use of recycled plastic containers which are not easy to clean harbours spoilage microorganisms, unrefrigerated transportation, long distance as a result of poor roads to cooling centres and pooling of milk from different suppliers at the cooling centres are risk factors to the growth and multiplication of the indigenous microflora, resulting in reduction of milk quality and safety. There was significant increase ($p<0.0.5$) in TVC from production to marketing which is attributed to absence of heat treatment of milk prior to fermentation coupled with spontaneous fermentation.

Lactic acid increased significantly at $p<0.0.5$ from production to the market with no effect on microbial load reduction. Increased acid development in camel milk during spontaneous

fermentation despite high microbial contamination may be associated with the antimicrobial components and chemistry of camel milk. Camel milk is known to have high antimicrobial components such as lactoferin, lysozyme, lactoperoxidase and immunoglobulin A (Stefan *et al.,* 1999).These components elicit a variety of inhibitory effects against microorganisms such as bacteria, viruses, fungi, comprising stasis, cidal, adhesion-blockade, cationic, synergistic, and opsonic mechanisms. They have inhibitory activity against *Escherichia coli, Streptococcus agalactiae, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Alaa *et al*., 2013).

Coliforms increased significantly ($p<0.05$) from production to the market by 1 log cycle. Coliforms are found in the soil, mud, dust, plant materials and can be dispersed into the atmosphere by dust into the product. Coliforms also have adaptation strategies that range from temperature evasions, acid tolerance) and production of probiotics like colicins that inhibit growth of other microorganisms (Abee *et al*., 1995; Gadaga *et al*., 2004). These adaptation strategies could explain the high coliform count despite increase in acidity. Isolation of coliform bacteria along the *suusa* value chain is an indication of presence of enteric pathogens in the *suusa* value chain. This shows hygienic conditions during handling and processing of camel milk into *suusa* are low.

There was a significant ($p<0.05$) increase in spore counts from production to market samples by 3 log cycles. Spore forming bacteria are environmental microorganisms such as *Bacillus* and *Clostridium* species. At production, they may originate from water used to wash the milking equipment and dust from the milking area. Spore formers like *Bacillus cereus* display a mechanism of acid tolerance response (ATR) and can survive below pH 4.0 favourable for spore formation (Gadaga *et al*., 2004). This explains the existence of increased spore forming bacteria in *suusa* at the market level. Gram positive spore forming rods were identified as *Bacilli.* High incidence at the market could be attributed to the marketing environment characterised by sale in the open with heaps of waste material, dust and mud close to where the product is sold. Spores are carried by wind into the atmosphere and into the product. *Bacilli* are aerobic whose typical habitat is soil although they are widely distributed in nature and gain access to milk and *suusa* through air, water, fodder and feed. Spore-forming bacteria are known to cause food spoilage and food-poisoning by producing heat labile enterotoxins.

Yeast and moulds increased by 1 log cycle production to the market. Yeasts and moulds belong to the kingdom of fungi whose habitat is mainly soil or plant material. High contamination by yeasts and moulds may be due to poor processing and marketing conditions

and uncontrolled fermentation which led to contamination. During spontaneous fermentation of *suusa*, organic acids such as lactic, acetic and propionic acids are produced which lower the pH. The lower pH is favourable for growth of yeast and mould species which causes these species to become competitive in the immediate medium (*suusa*) hence the significant increase at $p<0.05$ in yeast and mould count in marketed *suusa* (Lefoka, 2009).

5.2.2 Intended *suusa*

Intended *suusa* refers to homemade spontaneously fermented camel milk which has been prepared from raw milk with the sole intention of fermentation for consumption. This product is prepared from spontaneous fermentation of camel milk in a smoked plastic container at ambient temperature (26-29^{o}C) for 3 days or more. This is prepared by women at the household level for home consumption and rarely sold. It fetches lower income (100Ksh/litre) as compared to fresh camel milk (150Kshs/litre). There was a significant increase in at $p<0.05$ TVC in fresh camel milk at production to fermented product by 1 log cycle. This can be explained by spontaneous fermentation which results in growth and multiplication of inherent microflora. Increase in TVC is also a result of handling practices before processing such as not washing hands before milking, unsuitable milking and storage containers, lack of cooling facilities at production and long time to reach the processor due to poor roads. Handling practices caused high initial microbial load hence increased final load in the product (*suusa*) above the recommended limit of $0\text{-}2\times10^{5}$ (EAS, 2007).

Coliform count increased significantly at $p<0.05$ from production to processed product. *E. coli* was isolated from both *suusa* and raw camel milk.Use of recycled plastic containers which are not easy to clean harbours microorganisms, unrefrigerated transportation, long distance as a result of poor roads to cooling centres and pooling of milk from different suppliers at the cooling centres are risk factors to the growth and multiplication of the indigenous microflora, resulting in reduction of milk quality and safety. With natural fermentation, the pathogens will multiply and cause problems in the final product because lactic acid bacteria will initially be very low (Gadaga *et al*., 2004). During fermentation, organic acids such as lactic acid, acetic acid and propionic acid are produced. Undissociated organic acid is able to penetrate the cytoplasm of the pathogen and later dissociate causing inhibition of the organism (Gadaga *et al*., 2004).The undissociated acid will diffuse into the bacterial cell thereby reducing the intracellular pH and slow down metabolic activities. However, the extent to which pathogens are inhibited by low pH will depend on the organism concerned, temperature, amount of undissociated acid produced, buffering capacity of the

food and presence/absence of any additional hurdles. Exposure of *E. coli* to acidic conditions induces acid tolerance of the organism (Bearson *et al*., 1997). Proton leakage into the cell lowers the internal pH of the organism and subsequently induces amino acid decarboxylases in the cell. Low pH also increases RpoS (RNA polymerase sigma S) and PhoP (Transcriptional regulatory protein) which control production of acid shock proteins. Acid shock proteins enhance survival of *E. coli* through neutralization of the external environment, adjusting catabolism to the new environment, performing DNA repair and membrane biogenesis and contribute to microbial pathogenesis (Bearson *et al*., 1997). Karagozlu *et al*. (2007) found that stationary phase cells of *E. coli* strains were able to survive and multiply in *kefir* (Caucasian fermented camel milk). It has also been found to survive in fermented goat milk, *amasi* (Bearson *et al*., 1997). Low refrigeration temperature at the cooling centres has been shown to enhance the survival of *E. coli*. Low temperature causes alteration of the cell membrane fatty acid composition by increasing the unsaturated fatty acids to prevent fluid components from formation of gel. Formation of gel-like compounds hinders proper functioning of proteins and membrane content leakage. Low temperature also causes expression of acid shock proteins by the organism which enhances its survival.

Yeasts and moulds increased significantly at $p<0.0.5$ by 1 log cycle with an increase in lactic acid content from 0.07% to 0.6%. Increase in the count is a result of handling practices along the value chain probably from external contamination. The source of yeast and molds in traditional fermented milk could be contamination from the environment and from equipment used in milking and processing especially the fermentation vessel (Bitutu *et al*., 2014). Fungal cells when confronted with an acidic condition rely on proton transporters to maintain pH gradient. The plasma membrane bound and ATP dependent proton pump expel protons out of the cell at the expense of energy (Bearson *et al*., 1997) hence they are able to survive pH change in the product.

Spore counts increased significantly at $p<0.0.5$from by 3 log cycles. Spore forming bacteria are environmental microorganisms (Gadaga *et al*., 2004). At production they probably originate from water used for washing equipment and soil or dust in the milking environment. Spore formers like *Bacillus cereus* display a mechanism of acid tolerance response (ATR) and can survive below pH 4 favourable for spore formation (Gadaga *et al*., 2004).This ATR could explain the significantly higher spore count of the final product. *Bacillus* species was identified to be the main spore former.

Lactic acid increased significantly at $p<0.05$ from 0.07% to 0.6%. With fermentation, lactic acid bacteria break down lactose into lactic acid. Presence of gram negative rods which are fermentative organisms had an influence on increased acid content. Intended *suusa* is fermented over a period of 3 days (72hrs) and this further explains why percent lactic acid was higher than unintended *suusa* which takes less than 48 hours.

5.2.3 Main organisms isolated from *suusa*

Gram negative rods and gram positive cocci had the highest incidence in both types of *suusa*. High number of coliforms quantified in this study accounts for the high incidence of gram negative rods. Some of the gram negative rods identified include: *E. coli,* and *Pseudomonas* species. These are commonly found in water, soil, plants, humans and animals (Lefoka, 2009). Occurrence of faecal coliforms, *E. coli* and *Enterobacter* along the chain implies that camel milk handling is not hygienic hence possible contamination with fecal material from human and animals (Matofari *et al*., 2013). Occurrence of *E. coli* indicates possible occurrence of other enteric pathogens which are known to cause food borne illnesses. *Pseudomonas* species produce heat stable proteases and lipases keeping their activity even after pasteurization thereby producing off-flavours in milk as well sweet curdling of pasteurized milk (Perko, 2011). *Pseudomonas* are the main psychrotrophic bacteria isolated from refrigerated raw milk, being among the major spoilage agents in the dairy industry (Paula, 2011). Presence of the genus *Pseudomonas* indicates improper cooling and refrigeration of camel milk.

Gram positive cocci isolated from camel milk and *suusa* included *Streptococcus* and *Micrococci* species. *Streptococci* species especially the *lactis* group originate from equipment that is contaminated due to insufficient sanitation. Organisms like *Micrococci,* coliforms and enteric pathogens originate from hand milking and milk handling that might contaminate the milk via the skin, nose and mouth. *Micrococcus* and *Staphylococcu*s produce lipase enzyme and degrade milk fats at refrigeration temperature (7^{o}C) and may affects the quality of milk and fermented milk during their preservation at refrigeration (Patil and Gandhi, 2013).Risk factors identified in the field of study that have contributed to the specific organisms associated with *suusa* are: not washing hands or camels' udder before milking, dusty milking environment, use of organoleptic tests for quality assessment of milk, bulking milk from different suppliers, use of not easy to clean plastic containers, delayed milk delivery, lack of refrigeration during transport and sale of *suusa* in open air markets.

Spores of *Bacillus* species as well as the organism were also detected. The genus *Bacillus* are typical habitats of the soil and are widely distributed in nature and may gain access to milk and dairy products through the air, water, fodder and feed thereby present on the skin and hair of cattle (Loralyn and Robert, 2009). They also produce heat stable extracellular protease and lipase which cause spoilage of milk and milk products. Both types of enzymes are thermostable at all temperatures of milk heat treatment and therefore remain active in the all dairy product (Samaržija *et al.,* 2012). They are important in the dairy industry due to production of toxin from ingestion of contaminated dairy products by *Bacillus cereus*. Spores increased significantly at $p<0.05$ along the value chain. This is attributed to handling practices observed along the value chain that have led to contamination of both raw and fermented product. Risk factors identified include: dusty milking environment, use of plastic milking and storage containers and unrefrigerated transport of raw milk from production to cooling centres. These factors led to the growth and multiplication of spore forming bacteria which could have changed from vegetative form to spores with developed acidity hence high counts.

5.3 Quantifying the risks of *Brucella* and *Mycobacteria* species presence in *suusa* along the value chain

Brucella and *Mycobacteria* species are important zoonotic pathogens in animal products. Even if the numbers of these pathogens are few in the samples analysed and may not necessarily cause a food borne illness, they should be considered a potential health hazard.

5.3.1 Incidence of *Brucella* species in *suusa* along the value chain

Camels are susceptible to *Brucella* species especially *B. arbotus* and *B. melitensis*. Camels are infected through contaminated feeds and water, through the respiratory tract by inhaling dust or droplets. The bulls that are infected may pass the organisms through semen to females (Mayada *et al*., 2012). The *Brucella* organisms, once in lactating camels localize themselves in mammary tissues and are excreted in milk when the camel is milked. The pastoralists consume camel milk in raw form with a belief that in that state, the milk is therapeutic to other health complications in their community such as asthma and diabetes (Akweya *et al*., 2012).With such beliefs and practices, the pastoral community is potentially exposed to brucellosis, a zoonotic infection.

Incidence of *Brucella* organism in raw camel milk as well as *suusa* was high at 20%. The increase in incidence may be associated with the intensive production system practiced in the

area. With intensification, *Brucella* organisms may form a web of circulation in re-infections from the fields, water and the handlers. Around the area of study in Northern Kenya, pastoralists keep dairy camels and dairy goats around Isiolo to supply milk to residents. Under this kind of mixed system, *Brucella* organisms may be shed in the environment by smaller ruminant stock that are known to be carriers and cause infection to lactating camels (Abbas and Agab, 2002, Musa *et al*., 2008). An interview conducted in the study area with the herders, camel owners and veterinary office revealed that vaccination programmes are not carried out to camels thus the risk of occurrence of *Brucella* amongst camel herds.

Brucella organisms are Gram-negative rod shaped bacteria that do not have flagella or pili, nor do they create capsule slime. They also do not produce endospores (Weimer, 2001). *Brucella* species are intracellular pathogens with the ability to avoid the killing mechanisms and proliferate within macrophages like other intracellular pathogens, not only resist killing by neutrophils following phagocytosis and but also replicate inside macrophages and nonprofessional phagocytes. These macrophages are shed in milk from lactating camels (Kelly, 2002). Additionally, survival in macrophages as well as virulence is due to the presence of a smooth LPS (lipopolysaccharide) membrane with the O antigen side chain. With LPS being the most prominent component of the outer cell membrane of *Brucella* and dominating the antibody response of the host, it has been suggested that LPS may be important for the extracellular survival of *Brucella* in the host for the intracellular survival in the host cells, or both (Rittig *et al*., 2003).

Animals are known reservoirs of human brucellosis. The infection is mainly transmitted to humans through the ingestion of raw milk or unpasteurized dairy products contaminated with one of the *Brucella* species pathogenic to humans. *Brucella* organism has been known to survive dessication and starvation outside the host cell for years due to presence of LPS in their cell membrane. *Brucella* is known to survive in soil in an inactive form and will only be reactive when in host cells. Handling practices observed in this study that act as predisposing factors for infection with *Brucella* organism include consumption of raw milk at production by herders, processing dairy products (*suusa*) with unpasteurized milk and dusty milking environment. Another predisposing factor is lack of vaccination of the camels against brucellosis in the study area. This is because camels are kept in a pastoral system where the herders move from one place to another making access and monitoring of the camel herds difficult for the veterinary officers. Therefore, it is important to pasteurize milk as it does not affect the chemical composition (Farah, 1996).

5.3.2 Incidence of *Mycobacteria* species along the *suusa* value chain

5.3.2.1 Detection of *Mycobacteria* species using direct microscopy

Direct microscopy is a conventional technique that can be used to detect *Mycobacteria* species in milk samples through acid fast staining.It is the initial step in the diagnosis of tuberculosis. The most common specimens examined are sputum, Alternative samples include laryngeal swab, gastric lavage (in children), induced sputum, invasive techniques (bronchial brush sampling, trans tracheal aspirate, lung biopsy), milk and others.

The direct microscopic examination did not detect any *Mycobacteria* species. Microscopic examination has a specificity of almost 100% but sensitivity is 45-75% because the specimen must contain between 5000-10,000 bacilli/ml therefore a sample with less bacilli/ml may not be detected. Owing to the low sensitivity and specificity, a direct smear from the milk gives low sensitivity. However, a negative smear does not rule out infection, and therefore other techniques should follow to confirm the results (Al-Saqur *et al*., 2009). A more sensitive technique was employed.

5.3.2.2 Identification of *Mycobacteria* species using Genotype *Mycobacteria* Direct with DNA strip technology

The atypical *Mycobacteria* isolated from this study were *M. avium, M. intracellulare, M. kansasii* and *M. malmoense.* The non tuberculous/atypical species of *Mycobacteria* detected in this study were *M. avium, M, intracellulare, M. kansasii* and *M. malmoense* while the tuberculous/typical *Mycobacteria* detected was MTBC. All the detected *Mycobacteria* species from this study were slow growers. Therefore, the decrease in incidence of both the atypical and typical *Mycobacteria* species in fermented milk can be attributed to their slow growing nature and thus are not able to compete for nutrients with other microorganisms such as Lactic acid bacteria (Simone *et al*., 2008).

The high lipid content in the cell wall of *Mycobacteria* species prevents its destruction by acidic, alkaline and attack by antimicrobial compounds like lysozyme in both the intracellular and extracellular environment. This explains the presence of *Mycobacteria* species in fermented camel milk (*suusa*) despite its acidic nature. The most significant risk factor for the occurrence of *Mycobacteria* in the *suusa* value chain is the lack of pasteurization of camel milk before processing into *suusa* as well as consumption of unpasteurized product exposes the population to infection with tuberculosis. *Mycobacteria* species are intracellular organisms where they colonize and multiply within the white blood cells of the host. Since

milk contains white blood cells, *Mycobacteria* may be shed in milk from an infected camel. *Mycobacterium avium paratuberculosis* may be present in milk in free suspension.

Of significant importance was the occurrence of MTBC in both fresh and fermented camel milk with an incidence of 10.2% and 8.2% respectively as shown in. *Mycobacterium tuberculosis* complex (MTBC) which is a group of pathogenic *Mycobacteria* species that includes *M. tuberculosis, M. bovis, M. africanum, M. canetti* and *M. microti* responsible for causing tuberculosis infection in humans. *Mycobacterium bovis* causes tuberculosis in a broad range of mammalian hosts including cattle and other ruminants, felids, canids, lagomorphs, porcids, camelids, cervids and primates including humans. Although fermentation results in formation of lactic acid and hence lowers the pH which is detrimental to many pathogenic microbes, *Mycobacteria* species are known to survive in soured milk for up to 14 days (Kazwala, 1998). *Mycobacteria* species have a cell wall that is composed of over 60% of lipids which comprises of three major components i.e., mycolic acid, cord factor and wax-D (Alderwick *et al*., 2007; Brennan, 2003). Mycolic acids are unique alphabranched lipids which determine the permeability of *Mycobacteria* cell wall due to its strong hydrophobic nature. It forms a lipid shell around the organism and thus affects permeability properties at the cell surface. Mycolic acid is an important factor responsible for virulence in MTBC, because they defend *Mycobacteria* from the attack of cationic proteins, lysozyme and oxygen radicals in the phagocytic granule. They are also known to protect extracellular *Mycobacteria* from complement deposition in serum.

An important finding was that the milk samples showed mixed infection where 2 or more bands specific for a certain type of *Mycobacteria* species were observed as shown in (figure 4). This is an indication of contamination from both the environment as well as from the animal. Environmental opportunistic/atypical *Mycobacteria* are normal inhabitants of natural waters, drinking waters, and soils (Falkinham, 2002). *M. avium*is an environmental organism that has been isolated from natural water, soil and dust, drinking water, plant and plant products (Falkinham, 2002). *M. intracellulare* has also been isolated from soil and water. *M. kansasii* is frequently isolated from tap water aswell river and lake water (Taillard *et al*., 2003). Environmental reservoir for *M. malmoense* is soil and water (Falkinham, 2002). Production site was characterised by milking using unsuitable containers, dust and soil. Marketing of fermented camel milk (*suusa*) was characterised by sale in the open air markets along the road sides. These act as risk factors for contamination with the *Mycobacteria*

species from dust. Animals with pulmonic lesions from MTBC will excrete the organism in exhaled air, in the sputum and in the faeces and milk (Kinne *et al*., 2006).

Unpasteurized contaminated milk and other secretions or tissues from any of the isolated species can serve as the source of infection for humans. In cows, one cow can secrete enough viable bacilli to contaminate milk of up to 100 cows, when their milk is pooled (Kazwala, 1998).Mixing of milk from different suppliers as is practiced in the cooling hubs in the study area increases the chances of gross contamination of camel milk with *Mycobacteria*. Tuberculosis in the study area has been reported to have a prevalence of 338 and incidence of 137 people per 100,000 people (Ministry of health, 2012).The habit of drinking raw camel milk and preparing spontaneously fermented milk from unpasteurized milk among the pastoral community greatly increases the chances of acquiring tuberculosis disease.

The contamination of raw camel milk by *Mycobacteria* species is apparently inevitable, even under sanitary conditions, due to the ubiquitous nature of these microorganisms. Only the heat treatment of raw milk using commercial pasteurization protocols can ensure the adequate destruction of *Mycobacteria* contaminants. The increasing number of individuals worldwide who are infected with HIV in the world, Kenya inclusive predisposes the increase in the number of cases of emerging and re-emerging, opportunistic agents, such as *Mycobacterium* species. For example, in many regions of the world, tuberculosis is a major cause of death in HIV-infected individuals. Such *Mycobacteria* infections in HIV patients are frequently disseminated, although the transmission route of the bacteria remains controversial. In particular, unpasteurized, *Mycobacteria* contaminated milk poses a serious risk to HIV patients (Falkinham, 2002).

5.4 Association between nutritional changes of *suusa* with microbial spoilage along the value chain

Crude protein in *suusa* found in both classes of milk was between 3.78-3.84%. Crude protein remained constant along the *suusa* value chain despite the presence of proteolytic bacteria *Bacillus, Pseudomonas* and *yeasts and molds*. Protein in milk is degraded by protease inherent in milk and from microbial source, to form peptides and amino acids. Protein degradation can be quantified using the Kjedahl method which is broad and sensitive to detect milk spoilage. Acid digestion of samples used in this experiment was not able to detect proteolytic changes along the *suusa* value chain. Amino acid, peptides and proteins all have Nitrogen which will be quantified using Kjedahl method. However, constant crude protein content in *suusa* value chain does not rule out proteolysis. Therefore other techniques that

measure soluble protein are recommended to determine protein hydrolysis in camel milk and *suusa.* Therefore, crude protein is not affected by souring of camel milk under intended and un-intended fermentation using acid digestion of samples as detected in this study.

Fat content along the value chain decreased significantly in unintended *suusa*. This can be attributed to microbial lipases produced by *Micrococcus*, *Pseudomonas*, *Bacillus* species detected in milk samples. Lipase produced by microbial activity leads to spoilage through the hydrolysis of triglycerides, with the preferential release of medium and short-chain fatty acids. Even at low concentrations, production of heat stable lipase by *Pseudomonas*, hydrolysis of as little as l% of the milk triglycerides, hence reducing the fat content and causes rancid off-flavors thereby affecting the quality of camel milk and *suusa*. Lipase from the cells of the animal tissue may also be a risk factor for reduction of fat content in milk, hence *suusa*.Lower fat content for market samples of *suusa* can be attributed to lipolytic activity of *Bacillus* spp. that were isolated along the *suusa* chain which is significant at temperatures higher than 40°C. Lecithinase and phospholipases are groups of lipases of psychrotrophic bacteria, especially *Pseudomonas* that are able to disrupt membrane structure of fat globules and milk fat making it available to the milk lipases resulting in physical degradation of the emulsion in milk (Weimer, 2001). Milk pasteurization aims at destroying pathogenic microorganisms as well enzymes inherent in milk like lipase. In the production of *suusa*, milk is not exposed to any heat treatment and possibly explains the reduction in fat content. There is the possibility of adulteration of milk with extraneous water which significantly reduces fat content (Hossain and Sima, 2013).

Ash content remained constant in both classes of *suusa* and along the value chain. Ash content is a measure of the mineral present within a food. Fermentation process has little to no effect on the content of minerals in fermented milk, though (Yaman *et al*., 2005; Sahlin, 1999) undesirable metals may enter yogurt, and the levels of nutritional metals may increase in the fermentation procedure depending on conditions such as the container material used (aluminium, plastic, or steel). The time of storage as well as transportation to the consumer may increase undesirable metals in yogurt, due to the probability of turning sour. Therefore fermentation has little to no effect on the mineral content of fermented milk (Leo and Fidel, 2010). Mineral content be affected when some salts are added to the product during fermentation or by leaching when the liquid portion is separated from the fermented food (Sahlin, 1999).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSIONS

1. The *suusa* value chain follows an informal channel and with the following functions: production, cooling/bulking, processing and marketing. Intended *suusa* has only two functions which include production and processing/marketing.
2. Handling practices along the *suusa* value chain influences microbial load and consequently, contributes to spoilage of camel milk and *suusa.*
3. The *suusa* value chain presents the risk of infection with zoonotic pathogens *Brucella* and *Mycobacteria* species.
4. Handling practices and microbial load is associated with decreased fat content in the *suusa* value chain.

6.2 RECOMMENDATIONS

1. Extension, training and regular monitoring to improve handling practices along the *suusa* value chain is essential. Statutory (legal) institutions like Kenya Bureau of Standards need to address handling practices of camel milk and milk products along the chain to improve safety, quality and acceptability hence better market.
2. Pasteurization of camel milk is recommended before processing into *suusa* which is sufficient to destroy *Brucella* and *Mycobacteria* species. There is need to carry out more studies at molecular level to identify the specific *Brucella* strain and other *Mycobacteria* strains in Kenya's informal camel milk value chain to fill the gap of information. Research need to be carried to determine presence of other zoonotic pathogens like *Coxiella burnetti.*
3. Spontaneous fermentation results in a product of lower fat content. It is recommended that starter culture be used in the production of *suusa* for improved sensory and nutritional properties. Further studies need to be carried out to identify ideal yeast lactic starter culture for production of *suusa*. There is need for more research to determine effect of spontaneous fermentation on individual minerals.

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APPENDICES

APPENDIX 1.0 CONSENT FORM

Form code _____

Dear Respondent,

CONSENT STATEMENT

Hello, I am a student from Egerton University pursing Master of Science degree in Food Science. As part of course requirements, am currently conducting a research study to assess the microbial quality and safety of fermented camel milk for rural and urban markets in Kenya. To meet this objective, am kindly requesting you to participate in this study by volunteering to provide information asked. The results of this survey will provide a clear understanding of the concerns about milk products. The results will also provide baseline information to help stakeholders come up with interventions that will be used in removing the barriers to urban niche markets that are satisfactory to chain actors.

Thank you very much in advance.

Will you volunteer to participate in this study?

YES

NO

For the Respondent

Consent granted _____

Signature

APPENDIX 2.0: SURVEY QUESTIONNAIRE FOR INDIGENOUS KNOWLEDGE FOOD PROCESSED MILK PRODUCTS PRODUCED IN ISIOLO, KENYA.

Questionnaire Code_____

Please answer the following questions. (Translate in Kiswahili where necessary)

Tick (√) where necessary.

SECTION 1: PRODUCTION

Objective: To identify the activities at production that may act as sources of contamination of camel milk

1. Do you wash your hands before milking?

1. Yes 2. No

If yes, how do you do it?

1. With warm water and disinfectant
2. Cold water and disinfectant
3. Warm water
4. Cold water

2. Do you wash the camel's udder before milking?

1. Yes 2. No

If yes, how do you do it?

1. Warm water
2. Cold water

If yes, do you use a towel to dry the udder?

1. Yes 2. No

Others (specify) _____

3. Where do you obtain water for milking (Source of water)?

1. Borehole
2. River
3. Pipe water
4. Others (Specify)? _____

4. Do you treat your water before use?

1. Yes 2. No

If yes, explain how?

5. What type of container do you use to store your milk?

1. Plastic containers

2. Gourds
3. Calabashes
4. Others (Specify)_____

6. What do you do with the remaining milk and milk products at the end of the day?

_____

_____

7. How and where do you keep the milk after harvesting awaiting transportation?

_____

_____

_____

_____

_____

8. How is milk transported to the collection centres from the farm?
    1. Donkey
    2. Car
    3. By foot
    4. Others (specify)
9. How long does it take to transport milk from the farm to the collection centre?

10. Are you aware of any information regarding food quality and safety?
    1. Yes 2. No
11. If yes, what is the level of awareness?

12. What challenges do you experience at production?

13. What are the common ailments the camels suffer from?

14. How do you treat your camels?

SECTION 2: COOLING CENTERS

Objective: To determine the activities during transportation and at the cooling centres

15. How is milk transported to the cooling centres?
    1. By van
    2. By donkeys
    3. Others (specify)
16. How long does it take for the milk to reach the cooling centres?

17. How is the milk stored during transportation?

18. Do you perform quality control tests on milk before cooling?

    1. Yes 2. No

    If yes, explain

19. What challenges do you experience at the cooling centres?

SECTION 3: PROCESSING

Objective: To determine the practices involved during processing of *suusa*

20. Do you boil the milk before processing and consumption?
    1. Yes 2. No
21. How do you confirm that the milk is of good quality to make *suusa*?

    _____

    _____
22. How is *suusa* prepared (explain the procedure)?

    _____

    _____

    _____
23. What is the shelf life of *suusa*before spoilage?

    _____
24. When is *suusa* regarded as spoilt?

    _____

    _____

25. What challenges do you experience during processing of *suusa*?

SECTION 4: MARKETING

Objective: To determine how *suusa* is marketed with regards to safety and quality of the product

26. Where do you sell *suusa*?
    a) In a shop
    b) At a dairy
    c) By the road side
27. What type of container do you use to store the *suusa* for sale?
    1. Plastic containers
    2. Gourds
    3. Calabashes
    4. Others (Specify)_____

28. How much *suusa* do you sell in a day?

29. How do you package the *suusa* for sale?

30. What do you do with the remaining milk and milk products at the end of the day?

    _____

    _____

31. What challenges do you experience during marketing?

SECTION 5: CONSUMPTION

Objective: To identify consumer concerns with regards to indigenous processed *Suusa*

32. Do you make your own *suusa*?
    1. Yes 2. No

    If yes, how do you prepare it?

33. Do you buy the readymade *suusa* from the market?

If No, explain why?

34. Why do you like *Suusa*?

35. Which one do you prefer? Homemade or from the market

36. How do you store it in the house (storage environment, type of container used)?

37. How long does it keep to your liking (Number of days)?

38. What don't you like about *Suusa* of either type (concerns)?

OBSERVATION CHECKLIST

APPENDIX 3.0: GUIDE TO FOCUS GROUP DISCUSSION

1. What hygienic practices are observed before and during milking?
2. Where is the source of water?
3. How do you store your milk?
4. How do you preserve your milk?
5. What products do you make from the raw milk?
6. How are they prepared?
7. What is the shelf life of *Suusa*?
8. Describe *Suusa* that has spoiled (appearance, taste, smell).
9. Where do you sell your products?
10. How do you transport the product to the market?
11. How long does it take to reach the market?
12. What do you do with left over milk or product?
13. Who are involved in the trade of *Suusa* (value chain actors)?
14. What challenges do you experience during manufacture of *Suusa*?
15. Are you aware of any information regarding food quality and safety standards? If yes, what is the level of awareness?

APPENDIX 4.0: SUMMARY OF ANOVA TABLES

Objective 2: Generalized Linear Model (GLM) outputs

a) Intended *suusa*

Dependent Variable: Total Viable Counts (TVC)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	5.60592667	5.60592667	22.18	<.0001
Error	58	14.65786667	0.25272184		
Corrected Total	59	20.26379333			

R-Square	Coeff Var	Root MSE	TVC Mean
0.276647	6.206607	0.502714	8.099667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VC	1	5.60592667	5.60592667	22.18	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VC	1	5.60592667	5.60592667	22.18	<.0001

Dependent Variable: Coliform Count (CC)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.49322667	0.49322667	1.92	0.1717
Error	58	14.93496667	0.25749943		
Corrected Total	59	15.42819333			

R-Square	Coeff Var	Root MSE	CC Mean
0.031969	6.624307	0.507444	7.660333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VC	1	0.49322667	0.49322667	1.92	0.1717

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VC	1	0.49322667	0.49322667	1.92	0.1717

Dependent Variable: Spore Count (SC)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	110.2699267	110.2699267	181.98	<.0001
Error	58	35.1452467	0.6059525		
Corrected Total	59	145.4151733			

R-Square	Coeff Var	Root MSE	SC Mean
0.758311	13.22211	0.778430	5.887333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VC	1	110.2699267	110.2699267	181.98	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VC	1	110.2699267	110.2699267	181.98	<.0001

Dependent Variable: Yeast and moulds (Y&M)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	4.82233500	4.82233500	135.13	<.0001
Error	58	2.06975000	0.03568534		
Corrected Total	59	6.89208500			

R-Square	Coeff Var	Root MSE	YM Mean
0.699692	3.682730	0.188906	5.129500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VC	1	4.82233500	4.82233500	135.13	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VC	1	4.82233500	4.82233500	135.13	<.0001

Dependent Variable: Lactic acid

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	4.20290667	4.20290667	161.36	<.0001
Error	58	1.51072667	0.02604701		
Corrected Total	59	5.71363333			

R-Square	Coeff Var	Root MSE	ACID Mean
0.735593	48.66056	0.161391	0.331667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VC	1	4.20290667	4.20290667	161.36	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VC	1	4.20290667	4.20290667	161.36	<.0001

Tukey's Studentized Range (HSD) Test for Total Viable Count

Tukey's Studentized Range (HSD) Test for TVC

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square	0.252722
Critical Value of Studentized Range	2.83093
Minimum Significant Difference	0.2598

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	VC
A	8.4053	30	2
B	7.7940	30	1

Tukey's Studentized Range (HSD) Test for Coliform counts

Tukey's Studentized Range (HSD) Test for CC

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square	0.257499
Critical Value of Studentized Range	2.83093
Minimum Significant Difference	0.2623

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	VC
A	7.7510	30	2
A	7.5697	30	1

Tukey's Studentized Range (HSD) Test for Spore count

Tukey's Studentized Range (HSD) Test for SC

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square	0.605953
Critical Value of Studentized Range	2.83093
Minimum Significant Difference	0.4023

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	VC
A	7.2430	30	2
B	4.5317	30	1

Tukey's Studentized Range (HSD) Test for Yeast and molds

Tukey's Studentized Range (HSD) Test for YM

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square	0.035685
Critical Value of Studentized Range	2.83093
Minimum Significant Difference	0.0976

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	VC
A	5.41300	30	2
B	4.84600	30	1

Tukey's Studentized Range (HSD) Test for Lactic acid

Tukey's Studentized Range (HSD) Test for ACID

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square	0.026047
Critical Value of Studentized Range	2.83093
Minimum Significant Difference	0.0834

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	VC
A	0.59633	30	2
B	0.06700	30	1

The Mixed Procedure outputs

b) unintended *suusa*

Dependent Variable: Coliform Count

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	3	113	45.85	<.0001

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
VC	1	7.5697	0.1121	113	67.53	<.0001
VC	2	6.3103	0.1023	113	61.67	<.0001
VC	3	7.2025	0.1373	113	52.46	<.0001
VC	4	7.9903	0.1103	113	72.46	<.0001

Differences of Least Squares Means

Effect	VC	_VC	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1	2	1.2594	0.1518	113	8.30	<.0001	Tukey-Kramer	<.0001
VC	1	3	0.3672	0.1772	113	2.07	0.0406	Tukey-Kramer	0.1687
VC	1	4	-0.4207	0.1572	113	-2.68	0.0086	Tukey-Kramer	0.0420
VC	2	3	-0.8922	0.1712	113	-5.21	<.0001	Tukey-Kramer	<.0001
VC	2	4	-1.6800	0.1504	113	-11.17	<.0001	Tukey-Kramer	<.0001
VC	3	4	-0.7878	0.1761	113	-4.47	<.0001	Tukey-Kramer	0.0001

Dependent Variable **Total Viable Count**

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	3	113	29.37	<.0001

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
VC	1	7.7940	0.06508	113	119.77	<.0001
VC	2	8.3558	0.05941	113	140.66	<.0001
VC	3	8.6140	0.07970	113	108.08	<.0001
VC	4	8.5148	0.06402	113	133.01	<.0001

Differences of Least Squares Means

Effect	VC	_VC	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1	2	-0.5618	0.08811	113	-6.38	<.0001	Tukey-Kramer	<.0001
VC	1	3	-0.8200	0.1029	113	-7.97	<.0001	Tukey-Kramer	<.0001
VC	1	4	-0.7208	0.09129	113	-7.90	<.0001	Tukey-Kramer	<.0001
VC	2	3	-0.2582	0.09941	113	-2.60	0.0107	Tukey-Kramer	0.0514
VC	2	4	-0.1590	0.08733	113	-1.82	0.0713	Tukey-Kramer	0.2691
VC	3	4	0.09916	0.1022	113	0.97	0.3341	Tukey-Kramer	0.7668

Dependent Variable **Spore Count**

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	3	113	329.73	<.0001

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
VC	1	4.5317	0.08406	113	53.91	<.0001
VC	2	4.5508	0.07674	113	59.30	<.0001
VC	3	4.4815	0.1030	113	43.53	<.0001
VC	4	7.5613	0.08270	113	91.43	<.0001

Differences of Least Squares Means

Effect	VC	_VC	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1	2	-0.01917	0.1138	113	-0.17	0.8666	Tukey-Kramer	0.9983
VC	1	3	0.05017	0.1329	113	0.38	0.7066	Tukey-Kramer	0.9816
VC	1	4	-3.0296	0.1179	113	-25.69	<.0001	Tukey-Kramer	<.0001
VC	2	3	0.06933	0.1284	113	0.54	0.5903	Tukey-Kramer	0.9491
VC	2	4	-3.0105	0.1128	113	-26.68	<.0001	Tukey-Kramer	<.0001
VC	3	4	-3.0798	0.1321	113	-23.32	<.0001	Tukey-Kramer	<.0001

Dependent Variable **Yeast and Molds**

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	3	113	17.41	<.0001

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
VC	1	4.8460	0.1064	113	45.55	<.0001
VC	2	4.7603	0.09711	113	49.02	<.0001
VC	3	4.9005	0.1303	113	37.61	<.0001
VC	4	5.6981	0.1047	113	54.45	<.0001

Differences of Least Squares Means

Effect	VC_VC		Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1	2	0.08572	0.1440	113	0.60	0.5530	Tukey-Kramer	0.9334
VC	1	3	-0.05450	0.1682	113	-0.32	0.7465	Tukey-Kramer	0.9882
VC	1	4	-0.8521	0.1492	113	-5.71	<.0001	Tukey-Kramer	<.0001
VC	2	3	-0.1402	0.1625	113	-0.86	0.3900	Tukey-Kramer	0.8239
VC	2	4	-0.9378	0.1428	113	-6.57	<.0001	Tukey-Kramer	<.0001
VC	3	4	-0.7976	0.1671	113	-4.77	<.0001	Tukey-Kramer	<.0001

Dependent Variable **Lactic Acid**

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	3	113	310.90	<.0001

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
VC	1	0.06700	0.004008	113	16.72	<.0001
VC	2	0.1283	0.003659	113	35.08	<.0001
VC	3	0.1825	0.004909	113	37.18	<.0001
VC	4	0.2313	0.003943	113	58.66	<.0001

Differences of Least Squares Means

Effect	VC	_VC	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1	2	-0.06133	0.005427	113	-11.30	<.0001	Tukey-Kramer	<.0001
VC	1	3	-0.1155	0.006337	113	-18.23	<.0001	Tukey-Kramer	<.0001
VC	1	4	-0.1643	0.005622	113	-29.22	<.0001	Tukey-Kramer	<.0001
VC	2	3	-0.05417	0.006122	113	-8.85	<.0001	Tukey-Kramer	<.0001
VC	2	4	-0.1030	0.005379	113	-19.14	<.0001	Tukey-Kramer	<.0001
VC	3	4	-0.04879	0.006296	113	-7.75	<.0001	Tukey-Kramer	<.0001

Objective 3: Chi square test outputs

a) To determine significance difference of occurrence of *Brucella* species along the value chain using ARBAT values

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	2.393[a]	2	.302	.338		
Likelihood Ratio	2.464	2	.292	.304		
Fisher's Exact Test	2.561			.304		
Linear-by-Linear Association	2.177[b]	1	.140	.171	.097	.046
N of Valid Cases	96					

b) To determine significance difference of occurrence of *Mycobacteria* along the value chain

i) *Mycobacterium avium*

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	2.171[a]	1	.141	.269	.135	
Continuity Correction[b]	1.221	1	.269			
Likelihood Ratio	2.219	1	.136	.269	.135	
Fisher's Exact Test				.269	.135	
Linear-by-Linear Association	2.114[c]	1	.146	.269	.135	.103
N of Valid Cases	38					

ii) *Mycobacterium intracellulare*

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	1.310[a]	1	.252	.447	.224	
Continuity Correction[b]	.582	1	.445			
Likelihood Ratio	1.330	1	.249	.447	.224	
Fisher's Exact Test				.447	.224	
Linear-by-Linear Association	1.276[c]	1	.259	.447	.224	.161
N of Valid Cases	38					

iii) Mycobacterium kansasii

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	1.949[a]	1	.163	.295	.148	
Continuity Correction[b]	1.096	1	.295			
Likelihood Ratio	1.977	1	.160	.295	.148	
Fisher's Exact Test				.295	.148	
Linear-by-Linear Association	1.897[c]	1	.168	.295	.148	.108
N of Valid Cases	38					

iv) Mycobacterium malmoense

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	1.310[a]	1	.252	.447	.224	
Continuity Correction[b]	.582	1	.445			
Likelihood Ratio	1.330	1	.249	.447	.224	
Fisher's Exact Test				.447	.224	
Linear-by-Linear Association	1.276[c]	1	.259	.447	.224	.161
N of Valid Cases	38					

v) *Mycobacterium tuberculosis* complex

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	.146[a]	1	.703	1.000	.500	
Continuity Correction[b]	.000	1	1.000			
Likelihood Ratio	.146	1	.703	1.000	.500	
Fisher's Exact Test				1.000	.500	
Linear-by-Linear Association	.142[c]	1	.707	1.000	.500	.276
N of Valid Cases	38					

OBJECTIVE 4: SUMMARY OF ANOVA

a) Unintended *suusa* value chain

Mixed Procedure Outputs

Dependent Variable Protein

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	3	113	94.68	<.0001

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
VC	1	4.0793	0.04057	113	100.56	<.0001
VC	2	3.6942	0.03703	113	99.76	<.0001
VC	3	3.6157	0.04849	113	74.57	<.0001
VC	4	3.1197	0.04057	113	76.90	<.0001

Differences of Least Squares Means

Effect	VC	_VC	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1	2	0.3852	0.05493	113	7.01	<.0001	Tukey-Kramer	<.0001
VC	1	3	0.4636	0.06322	113	7.33	<.0001	Tukey-Kramer	<.0001
VC	1	4	0.9597	0.05737	113	16.73	<.0001	Tukey-Kramer	<.0001
VC	2	3	0.07845	0.06101	113	1.29	0.2011	Tukey-Kramer	0.5738
VC	2	4	0.5745	0.05493	113	10.46	<.0001	Tukey-Kramer	<.0001
VC	3	4	0.4960	0.06322	113	7.85	<.0001	Tukey-Kramer	<.0001

Dependent Variable Ash

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	3	113	24.64	<.0001

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
	VC	1	0.7740	0.01047	113	73.95	<.0001
	VC	2	0.8233	0.009555	113	86.17	<.0001
	VC	3	0.8619	0.01251	113	68.90	<.0001
	VC	4	0.7353	0.01047	113	70.25	<.0001

Differences of Least Squares Means

Effect	VC	_VC	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1	2	-0.04933	0.01417	113	-3.48	0.0007	Tukey-Kramer	0.0039
VC	1	3	-0.08790	0.01631	113	-5.39	<.0001	Tukey-Kramer	<.0001
VC	1	4	0.03867	0.01480	113	2.61	0.0102	Tukey-Kramer	0.0494
VC	2	3	-0.03857	0.01574	113	-2.45	0.0158	Tukey-Kramer	0.0736
VC	2	4	0.08800	0.01417	113	6.21	<.0001	Tukey-Kramer	<.0001
VC	3	4	0.1266	0.01631	113	7.76	<.0001	Tukey-Kramer	<.0001

Dependent Variable **Fat**

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	3	113	27.61	<.0001

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
VC	1	3.2597	0.1031	113	31.61	<.0001
VC	2	2.2344	0.09414	113	23.74	<.0001
VC	3	1.9438	0.1233	113	15.77	<.0001
VC	4	2.4493	0.1031	113	23.75	<.0001

Differences of Least Squares Means

Effect	VC	_VC	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1	2	1.0252	0.1396	113	7.34	<.0001	Tukey-Kramer	<.0001
VC	1	3	1.3159	0.1607	113	8.19	<.0001	Tukey-Kramer	<.0001
VC	1	4	0.8103	0.1458	113	5.56	<.0001	Tukey-Kramer	<.0001
VC	2	3	0.2906	0.1551	113	1.87	0.0635	Tukey-Kramer	0.2451
VC	2	4	-0.2149	0.1396	113	-1.54	0.1266	Tukey-Kramer	0.4178
VC	3	4	-0.5055	0.1607	113	-3.15	0.0021	Tukey-Kramer	0.0112

b) Intended *suusa* value chain

Dependent Variable **Protein**

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	1	58	13.94	0.0004

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
VC	1	3.9273	0.05025	58	78.15	<.0001
VC	3	3.6620	0.05025	58	72.87	<.0001

Differences of Least Squares Means

Effect	VC	_VC	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1	3	0.2653	0.07107	58	3.73	0.0004	Tukey	0.0004

Dependent Variable Ash

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	1	58	8.62	0.0048

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
VC	1	0.7630	0.007868	58	96.97	<.0001
VC	3	0.7303	0.007868	58	92.82	<.0001

Differences of Least Squares Means

Effect	VC	_VC	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1	3	0.03267	0.01113	58	2.94	0.0048	Tukey	0.0048

Dependent Variable Fat

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VC	1	58	0.00	0.9971

Least Squares Means

Effect	VC	Estimate	Standard Error	DF	t Value	Pr > |t|
VC	1	2.9070	0.06505	58	44.69	<.0001
VC	3	2.9073	0.06505	58	44.70	<.0001

Differences of Least Squares Means

Effect	VC_VC	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
VC	1 3	-0.00033	0.09199	58	-0.00	0.9971	Tukey	0.9971

APPENDIX 5.0: PICTURES SHOWING HANDLING PRACTICES

Picture 1: Plastic milk storage container

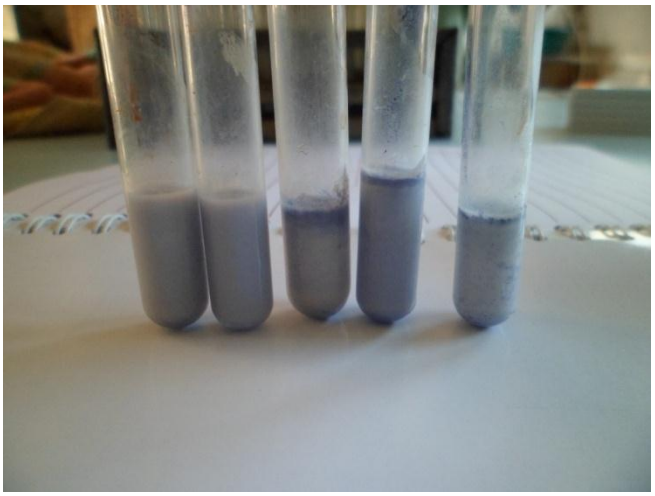
Picture 2: Milking container

Picture 3: Milking environment

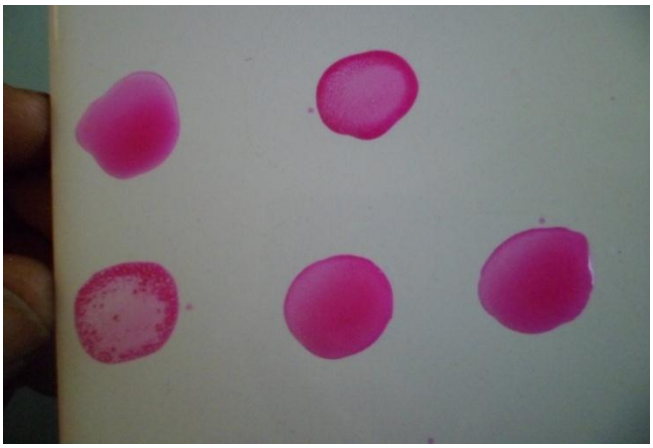
Picture 4: Public transport of camel milk to Nairobi that unintentionally ferments

Picture 5: Open air market of *suusa* at Eastleigh

APPENDIX 6.0: PICTURES SHOWING RESULTS FROM *BRUCELLA* SCREENING TESTS.



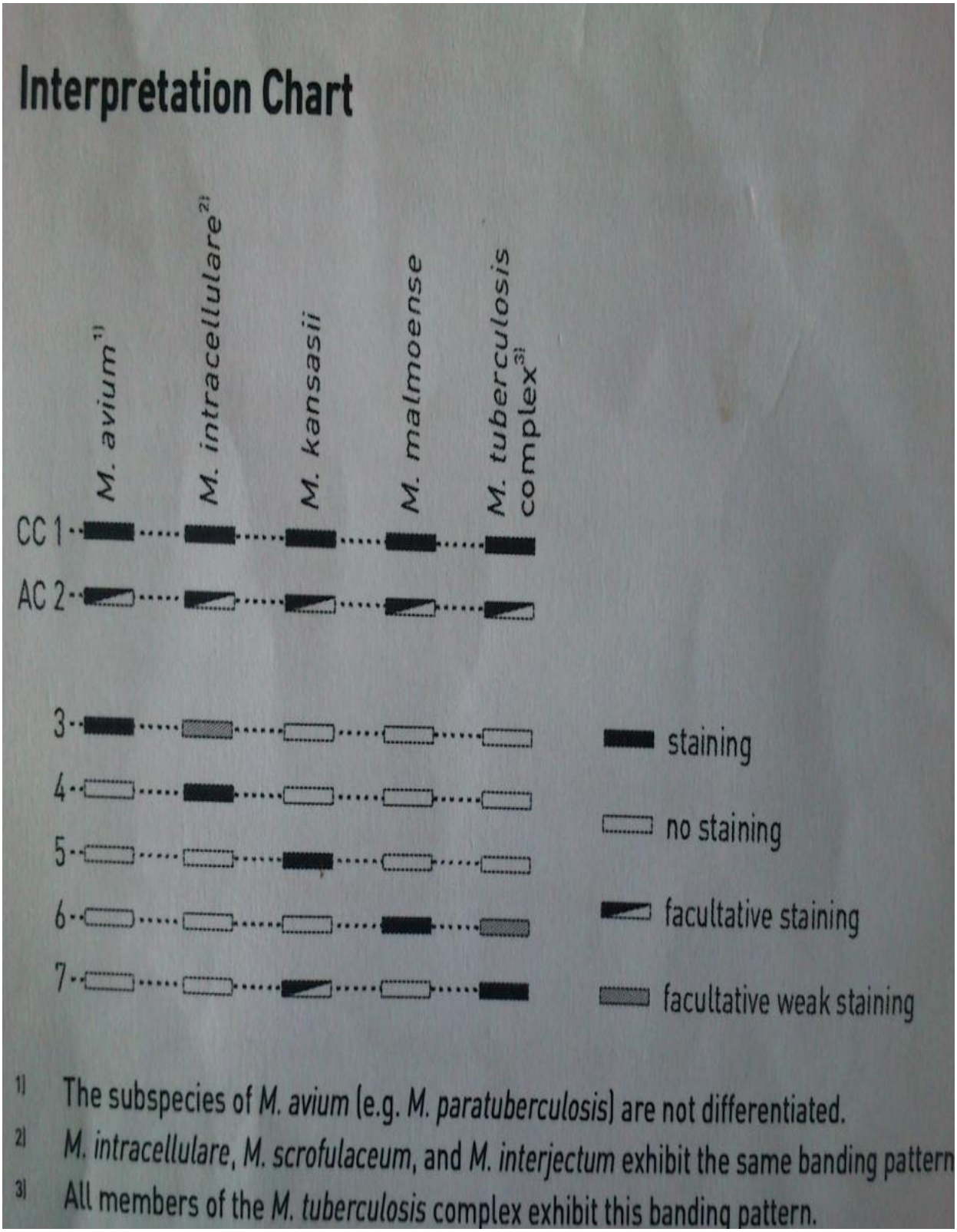
Picture 6: Milk ring test results



Picture 7: Rose Bengal precipitation test results showing slight agglutination, no agglutination and complete agglutination of serum sample

Picture 8:Anigen Rapid *Brucella* Antibody test results. Sample labelled 6 was positive.

APPENDIX 7.0: INTERPRETATION CHART USED IN GENOTYPE *MYCOBACTERIA* DIRECT



APPENDIX 8.0: AUTHOR'S PUBLICATION

Managing Editor
AJAFS <ajafs@ajouronline.com> May 19 (7 days ago)
to Patrick, me, Joseph

Dear Dr. Patrick Muliro:

We have reached a decision regarding your submission to Asian Journal of Agriculture and Food Sciences, "Characterization of Mycobacterium tuberculosis and Brucella spp from raw and fermented camel milk along the camel milk value chain in Kenya".

Our decision is to:
accept the manuscript for publication.

You need to pay USD 100 towards publication fee of your paper. You can click â€œPay Processing Feeâ€ on the top menu of AJAFS homepage (available at http://www.ajouronline.com/index.php/AJAFS) and put "1" in the first textfield, then "0" in the second one. The total will come up as USD 100. We advise you to use either credit card or Paypal as payment methods for fast processing as wire transfer sometimes take 4-6 weeks to process and additional $25 for the wire processing.

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