ANALYSIS OF MICROBIAL INFECTIONS IN CAMEL (*Camelus dromedarius*) MILK AND IMPLICATIONS IN KENYA

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DECLARATION

This Thesis is my original work and has not been presented for an award in any University.

Candidate: **JOSEPH W. MATOFARI** **Date** _____

Supervisors Approval

This Thesis, having been prepared with our supervision, meets our approval.

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DEDICATION

This thesis is dedicated to my late Father,

Dismas Matofari Wabwile

1918-2006

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ABSTRACT

Raw camel milk production and marketing chain in Kenya was investigated for microbial infections and implications. Milk samples were taken using simple random sampling method in a clustered sampling plan. There were three cluster levels, the production, processing and market levels. Analysis of samples in the laboratory for enumeration and characterization was by standard methods as described in the methodology. Data analysis was done by Pearson correlation coefficient and chi-square. At production level, 66% of the 107 samples taken had bacterial load ranging from 10^3-10^5 colony forming units per ml (cfu/ml). Over 90% of the samples from the processing and market levels ranged from 10^6-10^8 cfu/ml. The total viable counts were higher ($P < 0.05$) than coliform counts at production level. There were more spores at production than at market level. All the isolated organisms did not survive temperatures above 55^0 C. *Salmonella enterica* was prevalent at production and processing level. There was no *S.enterica* isolation at market level. Gram-negative rods (GNR) occurred at every level of the camel milk chain with an incidence of 54% of the 254 samples taken. Gram-positive cocci (42% incidence) were highest at production level. From the study, the microbial load in raw camel milk chain increased from production to the market. GNR were the majority and included the genera *Escherichia*, *Enterobacter* and *Pseudomonas*. *S.enterica* contamination of raw camel milk chain exists at production and collection level and not at the market level. The *S. enterica* serovars involved were *S. enterica Typhi* and *S. enterica Paratyphi C.* Since camels, pastoralists and camel milk handlers may act as carriers of *S. enterica* in the causation web, it is recommented that another study be done to determine host specificity for the serovars identified.

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LIST OF ACRONYMS

ASAL	Arid and Semi Arid Lands
BGA	Brilliant green agar
CBO	Community based organization
CC	Coliform counts
CCP	Critical Control Point
Cfu/ml	Colony forming units per millitre
CMT	California Mastitis test
EU	European Union
FAO	Food Agricultural Organization
GNR	Gram Negative Rods
GIT	Gastro intestinal tract
GIS	Geographical information systems
GLM	General linear model
HACCP	Hazard Analysis Critical Control Point
ICSMF	International commission on microbiological specifications for Foods
IDF	International Dairy Federation
ILRI	International Livestock Research Institute
KARI	Kenya Agricultural Research Institute
KEBS	Kenya Bureau of Standards
LA	Lactic acid
LPS	Lactoperoxidase system
NGO	Non-Governmental Organization
PACODEO	Pastoral community develop. Organization
PCA	Plate couny Agar
PFGE	Pulsied-Field Gel Electrophoresis
PHAC	Public Health Association of Canada
PMN	Polymorphonuclear lymphocytes
SAS	Statistical analysis for Scientists
SPI 1&2	Salmonella pathogenicity island 1&2
STE	Salmonella translocated effectors
TA	Titratable acidity
TBC	Total bacterial counts
TSI	Tripple sugar iron
TTSS	Two type three secretion system
TVC	Total Viable Counts
VBNC	Viable but not culturable
XLD	Xylose lysine desoxycolate

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Camel milk is traditionally consumed raw by the pastoralists. For a long time, a very limited amount was being sold. Due to the changing life style in the ASAL as a result of urbanization, population increase and insecurity in the low lands where camels are concentrated, the demand for camel milk has increased. The pastoralists now sell camel milk as alternative for income generation (Farah, 1996). The bulk of marketed milk reaches consumers through informal marketing. Camel milk marketed informally is usually sold raw and in small quantities over varying distances from source to market, ranging from 20 to 400km, especially for those middlemen supplying the Nairobi market. The means of transport include walking, donkeys, bicycles and motor vehicles. The increase in marketing of camel milk for herders' household income generation has raised concern over the hygienic management and preservation of the milk. The time taken to reach the market due to long distance, the milk containers used, the means of transport and the infrastructure in the camel milk catchment areas contribute to the hygienic concerns in raw camel milk production and marketing chain.

1.2 Health hazards of milk with respect to poor hygiene

Milk is an excellent culture medium for the growth of microorganisms. Their rate of multiplication depends mainly on storage temperature and handling conditions. The handling of milk during informal marketing has been reported to affect the quality of the milk (Bachmann, 1992). It influences bacteriological quality by adding to the milk some externally acquired microbial contaminants. The external sources of such microbes include the equipment, the

personnel and water. The time taken and temperature at which milk is kept influences generation time of microbes, hence the rate of multiplication of bacteria in the milk. The most important external source of pathogenic organisms in milk is contaminated water (Heeschen, 1992). Water is used as a cleaning agent for equipment that store milk. It is also used by animals and human for drinking and if it is contaminated with pathogenic organisms it will cause disease to both the animals and humans. The unavailability of water in ASAL is a problem and if available it is normally surface water, which comes from erratic rainfall that characterizes these areas. The bacteriological quality of this surface water is unknown.

The ability of microorganisms to cause disease depends upon the type of microorganisms present, the initial load of contamination of the milk, handling conditions and the time lapse from production before consumption. The potential health hazards associated with raw camel milk are well documented. The genera *Salmonellae, Shigellae, Brucellae, Yersinia, Listeria, Escherichia, Mycobacterium, Campylobacter* and *Staphylococci,* have been reported to be transmitted through milk (IDF, 1981, Heeschen, 1992). The presence of *Salmonellae* in raw milk has been reported in many studies. McManus and Lanier (1987) and Humphrey and Hart (1988) reported 0.2% and 4.7%, respectively of raw cow milk samples in UK were Salmonellae positive. *Listeria monocytogenes* has also been found in raw cow milk (James *et al*., 1985, Bannister (1987) and Siliker (1987).

The growth of contaminating bacteria in raw camel milk poses a threat to consumer health when milk of unknown microbial quality is sold. There is no documented study about the microbial

infections and implications of camel milk production from the udder to the table in Kenya (Matofari, 1999).

1.3 Camel milk production in Kenya

More than 60% of the world's camel population is found in East Africa. Kenya has about 850,000 camels (*Camelus dromedarius*) occupying over 70% of the arid and semi arid lands (ASAL) (FAO, 1994). The camel production systems in Kenya are both transitional (i.e from pastoral to income generating systems like peri-urban systems) and ranching where superior bulls have been imported from Pakistan to improve milk production (Trevor, 1998). Camel milk contributes up to 12% of the 3 billion litres of total domestic milk production, and 70% of the camel milk is consumed by pastoral communities in Northern Kenya (Schwartz and Doili, 1992).

It is estimated that camel milk produced per annum in Kenya is in the order of 0.36 billion litres per year valued at 3 billion Kenya shillings assuming the average price per litre during the drought is Ksh. Thirty (Field, 2001). The herds supplying camel milk to urban markets are concentrated around Moyale, Isiolo, Garissa and Nanyuki in Laikipia district. Isiolo supplies about 600 litres, Garissa 500 litres and Nanyuki 500 litres to Nairobi market daily (Field, 2001). The camel milk movement routes from production areas to market centres are as indicated in Figure 1.1 below.

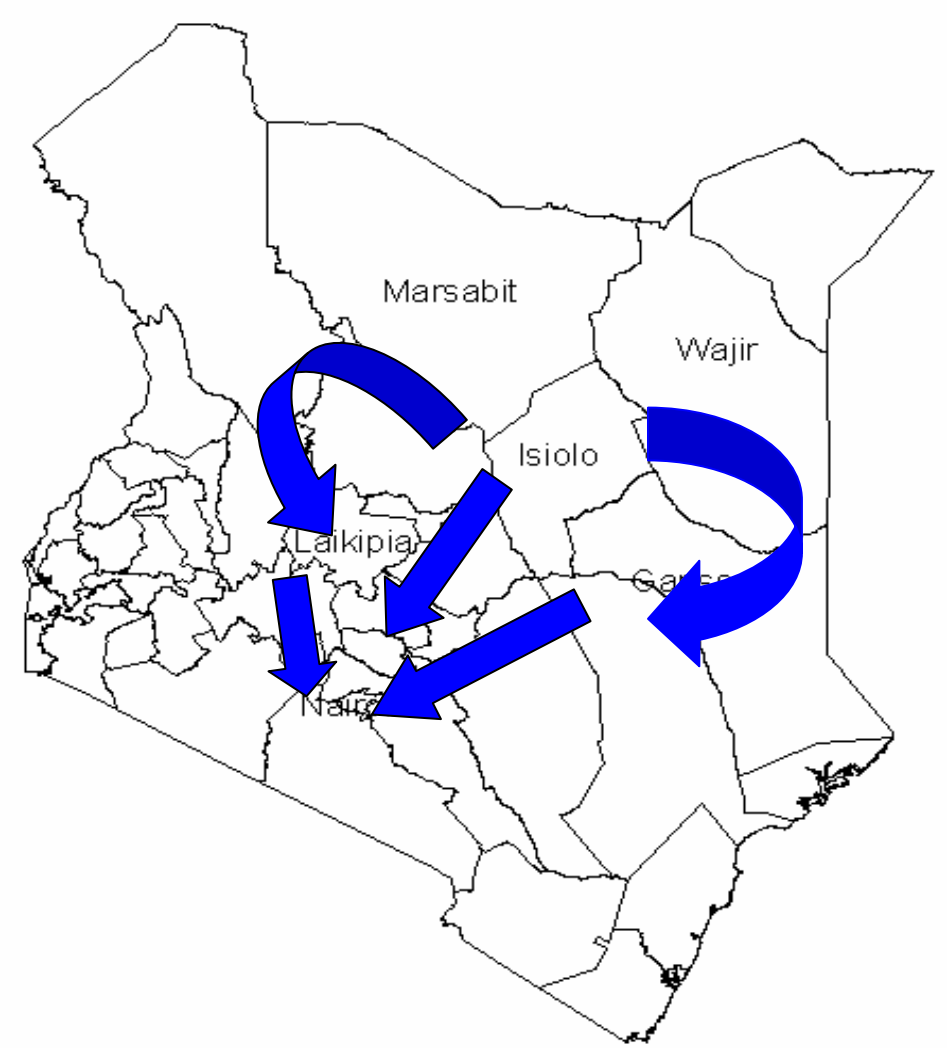


Figure 1.1 Map of Kenya showing camel milk movement routes from production to market centers.

1.4 Camel milk as an income generation activity for household

The consumption of camel milk is no longer limited to the pastoral nomads, but has been commercialized to urban areas (Schwartz and Dioli, 1992). The commercialization of camel milk in Kenya has been stimulated by several factors that have come to exist in the recent past. Population pressure has caused people to move from high to low potential lands for settlement and agriculture. The population of the pastoralists in the low potential lands has grown by an estimated 25% of the national population that is now about 31 million people (CBS, 1994). Camel milk is preferred by pastoralists and is more available in these ASAL (Field, 1999). Continued droughts have usually eliminated other livestock and left the camel since it is more adapted to the ASAL. The camel produces milk almost all the year round (8-18 months) and in quantities greater than any other milking animals living under the same conditions (Trevor, 1998). Therefore, people in ASAL, resort to camel milk for food security and livelihood under these conditions. The nomads have continued to drift to urban centers in search of alternative sources of income, better schools and hospitals and generally better quality of life. Others do this due to insecurity in the ASAL where cattle rustling and banditry is rampant. Wherever these camel keeping pastoralists move to, they prefer camel milk.

The nomadic people who have settled in urban centers prefer to consume camel milk instead of bovine milk. Several reasons are given for this preference: - the nomads believecamel milk has medicinal value and does not get spoiled as quickly as cow's milk; they are used to camel milk from birth. They also claim that a small amount of camel milk can make tea for more people than the same amount of cow's milk. It is also said that one can take more camel milk at once and walk for a long distance without causing stomach discomfort, as the case would be with cow's milk (Personal communication). Milk production potential of the improved cows in high

potential areas is decreasing due to scarcity of feeds. The demand for milk on the other hand has increased. It is projected that the demand for milk and milk products will double by the year 2020 in the developing countries (ILRI, 1995). Therefore, the solution is to focus on alternative technologies and research into viable solutions indigenous to the arid lands and resources like camel milk.

1.5 Problem statement

Camel milk production and consumption in Kenya was confined to the pastoral (ASAL) areas. In the recent past, it was introduced in the urban centres through informal marketing. Other communities have taken up the consumption of camel milk. There are no adequate hygienic practices in the camel milk production and processing since there are no quality standards set for camel milk in Kenya. This poses a high risk of microbial contamination and possible transmission of pathogenic microorganisms. The informal marketing of camel milk is a risk to consumers. Information on microbial quality and safety of camel milk procurement and marketing chain in peri-urban and urban markets is lacking.

This study attempted to fill the knowledge gap on microbial infections and implications of raw camel milk production and marketing in Kenya.

1.6 Conceptualization of the objectives

The pastoral communities in Northern Kenya are becoming less normadic as urbanization and demorgraphic changes set in. The demand for camel milk has increased. Camel milk is being produced and marketed as an income generating activity informally. Kenya Agricultural Research Institute (KARI) in collaboration with the European Union (EU) developed a general objective of adding value to the camel milk with the aim of making camel milk products that

could be used during the drought period to reduce food deficiencies. Pilot milk processing and preservation programmes were launched. They included mini dairies of *Karare women group* in Karare and *Salato women group* in Ngurnit in Marsabit District and the Pastoral community development organization (PACODEO) in Moyale District called PARMCO. These value addition activities needed good quality milk.

This proposal was developed to study microbial profile of raw camel milk and possible presence of pathogens like *Salmonella enterica*.

1.7 Objectives

1.7.1 The general objective

The overall objective of this study was to analyse the microbial infections, their profile and possible presence of pathogens as exemplified by *Salmonella enterica* in raw camel milk.

1.7.2 Specific objectives

1. To determine the microbial load and profile in raw camel milk at different levels of handling from production to the market.
2. To investigate the presence of *Salmonella enterica*, a potential health hazard in raw camel milk from production to marketing chain.

1.8 Hypothesis

H_o. The conditions under which camel milk is produced and handled in the chain of production upto marketing makes it to be of poor microbial quality and unsafe to the consumer.

H_{o1}. Camel milk in the ASAL is of poor quality.

H_{o2}. Camel milk in the ASAL can transmit microbial pathogens.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

2.1.1 Composition of Camel milk

Camel milk is white and, although it has a pH of 6.5, has a slightly salty taste. The changes in taste are influenced by the type of fodder consumed and availability of water to the animal (Yagil and Etzion, 1980). The density of camel milk is between 1.025-1.032 g/ml with an average of 1.029g/ml. Both the pH and density are lower than those of the cow milk (Farah, 1996). The total solids in camel milk range between 11 and 14%. The fat content is between 3-5%, Protein ranges between 2.7-5.4% while lactose is 3.4-5.6% as compared to 4.6% of the cow. The mineral content of camel milk is not well known but calcium is said to be lower than that of the cow's milk. The milk is rich in vitamin C but contains very little carotene (Farah, 1996). On the whole, camel milk contains more lactose and ash than cow's milk and more ash than buffalo milk. Casein is lower in camel milk than in cow's milk but camel milk has a higher content of whey proteins (Trevor, 1998).

The total free fatty acids (FFA) concentration in camel milk is 1.36 μmol/ml. Saturated fatty acids content is 62.5% of FFA and is the same as that of the cow milk. That of the goat milk is 74.5%. Camel milk lacks short chain (C_4- C_8) fatty acids (FA) while the middle chain (C_9 – C_{14}) FA are lower than those of goat and cow milk. The long chain (C_{16} –C_{20}) FA content of the camel milk is higher than that of both goat and cow milk (Cardak *et. al*., 2003). The natural antimicrobial proteins like lysozyme in camel milk, is higher (648 μg/100ml) than the cow's milk, which is 120 μg/100ml (Farah, 1996).

Table 2.1: Some physical and chemical properties of camel milk compared

Property	Camel milk	Cow milk
PH	6.6	6.5
Density	1.029g/ml	1.032g/ml
Lysozyme	648µg/100ml	120 µg/100ml
Lactose	5.5%	4.6%
Vitamin C	Very high	Low
Water	86.5%	87.3%
Casein	2.7%	2.6%
Whey proteins	0.9%	0.6%
Fat	4.0%	3.9%
Ash	0.8%	0.7%
Short chain fatty acids	None	Present
Total free fatty acids conc.	13.6 µmol/10ml	
Saturated FA	62.5%	62.5%
Carotine	Very little	High

Source: Farah, 1996 and Cardak *et al*., 2003

Camel milk antimicrobial properties

Barbour *et al*., (1984), studied the ability of camel milk to inhibit the growth of bacteria, especially pathogenic ones and the relatioship of its lysozyme content to the inhibitory effect. He used four protective milk proteins, lysozyme, Lactoferin (lf), lactoperoxidase (lp) and immunoglobulin G (IgG) and assayed them against *Lactococcus lactis* sub sp. *cremoris*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhymurium* and rotavirus. The antibacterial activity spectrum of camel milk lysozyme was similar to that of eggwhite lysozyme but higher than bovine lysozyme. Bovine and camel milk lactoferin antibacterial spectrums were similar. The camel Lactoperoxidase system (LP) was bacteriostatic against Gram-positive species of bacteria and bactericidal against Gram-negative species of bacteria. Antibody titre against rotavirus was higher in camel than cow milk. Lysozyme has bactericidal effect, as it is capable of degrading the gram-positive bacterial cell wall (Farah, 1996). Preservation of raw camel milk may possibly be due to lysozyme, which naturally occurs in camel milk in large amounts.

2.1.2 Temperature based associations of microorganisms in milk

Mesophilic and Psychrotrophic organisms are important in food microbiology because they often include foodborne pathogens and spoilage organisms respectively. Examples include pathogens like *Salmonella*, *Staphylococcus aureus* and *Clostridium perfringens*. This makes them important in the spoilage of foods. Psychrotrophs include *Pseudomonas*, *Bacillus*, *Klebsiella*, *Proteus*, *Listeria*, *Vibrio*, *Micrococcus*, *Lactobacillus*, *Enterobacter*, *Clostridium*, *Aeromonas*, *Acinetobacter* and *Alcaligenes* among others. Psychrotrophic species of yeasts belong to the genera *Candida*, *Torulopsis*, *Cryptococcus* and *Rodotorula*. In molds, the genera *Penicillium*,

Cladosporium, *Trichothecium* and *Aspergillus* are Psychrotrophs. Thermophilic sporeformres such as *Bacillus stearothermophilus* and *Clostridium botulinum* are important in pasteurized and sterilized foods because they multiply, produce toxins and cause poisoning (Angelotti *et al*., 1961, Barnes, 1968, Goepfert and Kim, 1975, Hobbs, 1978).

Thermal death of organisms in any food will mostly depend on the initial load of organisms. The higher the initial load, the more the heat required. But heat resistance of any group or species of microorganisms is gene dependent (Burning *et al*., 1990, Chatfield *et al*., 1992). However, it is known that most coliforms are killed at temperatures between 45^0 C and 55^0 C (Warsen and Strauch, 1976; Van Soest, 1982, Mackey *et al*., 1986 and 1990).

2.1.3 Sources of microbial contamination

Foods are contaminated with soil, air and waterborne microorganisms during harvesting, processing, distribution and preparation. High numbers of microorganisms are found in animal intestinal tract and on the skin surface. These find their way to animal products like meat and milk (Bryan, 1979). Handling of animal food products like meat and milk through the food distribution channels increases the number of microorganisms from the environment (Guthrie, 1988). Dairy products may be contaminated from udders of animals and milking equipment. Contamination of equipment occurs during production and when the equipment is idle. It can collect microorganisms from air and personnel during production. The udders harbour external and internal microorganisms as a result of mastitis. Unsanitary raw milk or improper sanitation of equipment will lead to contamination. It has been suggested that because not all dairy products are heat-treated, the presence of pathogens, especially *Listeria monocytogenes* in the dairy industry has increased (Varnan *et al*., 1994, Buchanan *et al*., 1997). It is also reported that

sanitation for dairy products is mostly associated with drainage and waste disposal. Therefore, the environment in which raw milk originates should have proper drainage and reduced contamination (Troller, 1993).

Personnel handling food products can introduce microorganisms to food and they form the largest contamination source. The hands, hair, nose and mouth harbour microorganisms that can be transferred to food during processing, packaging and serving by touching, breathing, coughing or sneezing (Bryan, 1979, Guthrie, 1988). Water serves as a source of contamination. When raw sewage flows into drinking water sources like wells, rivers, lakes or dams, the water gets contaminated. Insects and rodents are always associated with food processing equipment as well as with the toilets and garbage and they transfer microorganisms from contaminated areas to food (Bryan, 1979, Todd, 1980, Guthrie, 1988).

2.1.4 Microorganisms commonly found in raw milk

The bulk of information on microorganisms in raw milk is associated with cattle. Cattle milk has been used in research on microorganisms in most countries. The organisms commonly encountered include *Bacillus*, *Clostridia* and enterobactericeae especially coliforms. These may go up to 10^2- 10^3 cfu/ml. (Burriel, 1997). Microorganisms originating from equipment that are contaminated due to insufficient sanitation include, *Streptococci*, especially the lactis group such as *S. lactis*, *S. cremoris*, *S. lactis diacetylactis*, coliforms and gram-negative psychrotrophs such as *Pseudomonas*, *Alcaligenes*, *Flavobacterium* and *Chromobacterium*. Those organisms related to hand milking and handling milk after milking that might contaminate the milk via the skin, nose and mouth include Micrococci, Staphylococci, coliforms and enteric pathogens like

Salmonella enterica. Spores of *Bacillus* and *Clostridium* that survive heat processes may also be present (Frazier, 1977).

A few organisms have been isolated from raw camel milk in Ethiopia, Libya, Sudan, Saudi Arabia and Israel. They included mostly coagulase-negative *Staphylococci*, *S. aureus, Streptococcus agalactiae* and other *Streptococcal species, Micrococci* and coliforms (Donchenko *et al*., 1975, Barbour *et al*., 1985, Ramadan *et al*., 1987). However, most of these isolates were related to udder infections and not normal commensals of the raw camel milk. In Kenya, in a study conducted by Matofari (1999), group D (non-enterococci) *Streptococci*, group D-enterococci and viridans were mostly isolated. These were mainly environmental *Streptococci* and were associated with subclinical mastitis in camel udders.

2.1.5 The concept of spoilage

Spoilage of food may be defined as any organoleptic change that the consumer considers to be an unacceptable departure from the normal state. Spoilage can be of microbial, physical or chemical origin (Adams and Moss, 1997). Microorganisms will produce changes in appearance, flavour, odour and other qualities of foods. The degradation takes three forms; putrefaction, fermentation and rancidity or lipolysis. In putrefaction, proteins are broken down to amino acids, amines, ammonia and hydrogen sulphide. In fermentation, carbohydrates are broken down to acids, alcohols and gases while in lipolysis the fats are broken down to fatty acids and glycerol. The changes that microbes cause in foods are not limited to the results of degradation but may also be caused by products of microbial synthesis. For example, production of dextrans or levans that is slimelike on microbial metabolism of sugar which causes ropiness in milk. Pigmented bacteria

can be observed in changing colour of the foods. Enzymes produced by microorganisms in storage may decompose the food and cause spoilage (Frazier, 1977).

Spoilage is associated with large numbers of microorganisms. Therefore, the organisms that cause spoilage are those that can multiply and become dominant. The milk can be contaminated with organisms from various sources. The main sources of organisms are the contaminated equipment used to handle, transport, store and process milk. In cold stored milk, the genera commonly found are *Pseudomonas, Acinetobacter, Enterobacter, Alcaligenes, Flavobacterium* and members of the enterobacteriaceae (Frazier, 1977). These cause spoilage of milk under refridgiration.

Spoilage of milk is caused by psychrotrophs that recontaminate the milk after pasteurization and the thermoduric psychrotrophs, which survive pasteurization as well as heat stable proteases that are produced before pasteurization by oxidase positive psychrotrophs like *Pseudomonas*.

2.1.6 Spoilage of milk by Bacteria

Proteolysis in milk usually causes alkalinity as a result of products of protein decomposition. The major organisms responsible include *Micrococcus, Alcaligenes, Pseudomonas, Proteus, Achromobacter* and *Flavobacterium*, all of which are non-sporing bacteria. The spore formers encountered most commonly are *Bacillus* and *Clostridium* species. These spoil milk at a later time after pasteurization when they become vegetative. Some of the species of the genera *Micrococci, Pseudomonas, Achromobacter* and *Flavobacterium* will grow well at low temperatures and cause some proteolysis and bitterness in milk held at chilling temperatures. None of these bacteria except micrococci are thermoduric and therefore should not be found in

pasteurized milk. Some of the spoilage due to high microbial content in pasteurized milk includes; ropiness, changes in butterfat, alkali formation and flavour changes. Common organisms associated with the spoilage are mostly coliforms, *Micrococcus* and gram positive spore forming bacilli (Frazier, 1977).

2.1.7 Contamination of milk by *Salmonella enterica* Pathogen

The most important reservoirs or carriers of *Salmonella enterica* as a microbiological agent of foodborne illness are humans and other warm-blooded animals. It is transmitted through water, food or from person to person. The organism multiplies in foods; the most incriminated foods being dairy products, especially raw milk and meat products. Food borne illness accounts for an estimated one billion episodes, mostly diarrhoeal diseases in children in the world per year (Adams and Moss, 1997). The risk factors associated with *Salmonella* infection leading to out breaks of food poisoning involve people and common sources of the food and the contributing factors are common faults in food hygiene. Outbreaks result from distribution of a contaminated food product or products produced for large numbers of people. The reasons for this include lack of hygienic management, lack of knowledge of hygiene and use of inappropriate facilities in processing the food products. Specific failures in food hygiene have been attributed to temperature and time conditions used in food processing (Adams and Moss, 1997).

The genus *Salmonella* belongs to the family enterobactericeae. It is composed of bacteria related to each other both phenotypically and genotypically. The *Salmonellae* DNA base composition is 50-52 mol % G+C, similar to that of *Escherichia, Shigella* and *Citrobacter* (Ulrich and Oscar, 2002). The genus *Salmonellae* are also related to each other by DNA sequence based on numerical taxonomy and 16S ssRNA analysis. The principal habitat of the Salmonellae is the

intestinal tract of humans and other warm-blooded animals. *Salmonella enterica* serovars can be found predominantly in one particular host (host-specific). An example is *Salmonella enterica* Typhi and *Salmonella enterica* Paratyphi A that are strict human serovars. They can be ubiquitors (non-host adapted) or can have unknown habitat (Kenneth, 2005).

Salmonellae are Gram-negative, non-sporing rods, which are aerobic or facultatively anaerobic, catalase positive, oxidase-negative and motile with peritrichous flagella. Their growth temperature ranges from 5^0 C to 47^0 C with the optimum being 37^0 C (Wolfgang and Gunter, 1988). *Salmonellae* are heat sensitive and are easily destroyed by pasteurization temperatures; for example *S. typhimurium* D_{72}= 0.003 min. when the water activity (a_w) is high. Heat resistance increases with decrease in a_w, for example *S. typhimurium* has D_{70} of 11.3 to 17.5 h in some foods. The minimum a_w for growth is 0.93 but cells survive well in dried foods of between 0.96 and 0.93 a_w. The pH for growth ranges from 5.4-8.05. Optimal pH is 7.0. *Salmonellae* are inactivated below pH of 4 and above pH of 9. However, *Salmonellae* survive freezing (-2^0 C to -10^0 C) and chilling temperatures of -1^0 C to $+7^0$ C.

2.1.8 Classification of Salmonellae

The original classification of *Salmonellae* was not based on DNA, the names were given according to clinical signs of the infection. For example, *Salmonella typhi*, *Salmonella cholera-suis, Salmonella abortus-ovis* etc. Kauffman and White introduced serological classification where *Salmonella* species were defined as a group with related fermentation phage type, hence *Salmonella* serovar was considered a species. Names derived from geographical regions of origin of the first isolated strain of newly discovered serovar were given, for example *S. london, S. panama, S. copenhagen* etc. It is now known that all *Salmonella* serovar form a single DNA

hybridization group, ie a single species composed of several subspecies. To avoid confusion of serovars, the species name *Salmonella enterica* was proposed and names of subspecies were given, for example enterica I, salamae II and Arizona IIIa. Each subspecies contains various serovars defined by a characteristic antigenic formula (Kenneth, 2005).

The genus *Salmonella* has three major antigens namely; somatic, surface and flagella. The somatic (O) or cell wall antigens are heat stable, alcohol resistant and has 67 cross-absorption antigenic factors used for serological identification. The surface or envelope antigens are often observed in other genera of enteric bacteria such as *Escherichia coli* and *Klebsiella*. Surface antigens in *Salmonella* may mask O antigens and the bacteria will not be agglutinated with O antisera. One such surface antigen in *Salmonellae* is the Vi antigen. The Vi antigen occurs in only three *Salmonella* serovars out of 2,200. These are Typhi, Paratyphi C and Dublin. Strains of these three serovars may or may not have the Vi antigen. The flagella (H) antigens are heat labile proteins. Only few *Salmonella enterica* serovars produce flagella, which always have the same antigenic specificity, such as Enteritidis and Typhi. Such H-antigen is monophasic. Other serovars can produce flagellae with two different H-antigen specificity, such as Typhimurium. These H-antigens are diphasic (Kenneth, 2005).

There are several techniques used to distinguish serovars and their variants. Serological techniques and Phage typing are used to distinguish serotypes and their variants based on susceptibility to a set of bacteriophages. The phage type system was developed to distinguish definitive phage types (DT) and is being used for *Salmonella* epidemiological surveys (Kenneth, 2005). Molecular finger printing and modified biotyping schemes are presently being used to

further differentiate between isolates of the same phage type (Wolfgang and Gunter, 1988). These classification schemes based on antigenic profiles like multilocus enzyme electrophoresis and comparative nucleotide sequence analysis have come up with two species; *Salmonella bongori*, which colonizes the gut of poikilothermic animals and *Salmonella enterica*, one that causes gastrointestinal or systemic disease in both cold and warm blooded hosts (Boyd *et al*., 1996).

2.1.9 Association with food

Salmonellae cause two diseases called salmonellosis in humans. One disease is enteric fever (Typhoid) that results from bacterial invasion of the bloodstream and the other is acute gastroenteritis that result from a foodborne infection, which is an intoxication (Kenneth, 2005). It is a zoonotic infection because infected animals are a source of human illness. Consumption of raw milk contributes to the outbreak of this disease (Adams and Moss, 1997). *Salmonella* organisms may contaminate the milk by releasing endotoxins in milk or through faecal material entering the milk. Faecal contaminated water, when used to wash milk containers, is also a source of contamination. Animals suffering from salmonellosis can also secrete viable organisms in their milk (Hobbs and Gilbert, 1978). Some serious food poisoning outbreaks through consumption of raw milk have been reported and the causal organisms found to be *S. dublin* and *S. typhimurium* (Kenneth, 2005).

Food poisoning due to consumption of raw camel milk and meat has been reported (El-Nawawi *et al*, 1982). In the United Arab Emirates, identical *Salmonella* serotypes were isolated from human stool and camel stool (Wernary and Makarem, 1996). *Salmonellae* produce special

toxins, which are responsible for the systemic and enteric forms of salmonellosis. The toxins produced include lipopolysaccharides (LPS), endotoxins, enterotoxins, cytotoxins and plasmids. The endotoxins of *Salmonella* are complex LPS derived from bacterial cell walls and liberated when bacteria lyse. The LPS are heat stable with molecular weight of between 100,000 to 900,000 Daltons (Jawetz *et al*., 1980). Prevention of *Salmonella* toxic infection relies on avoiding contamination, thus improving hygiene, preventing multiplication of *Salmonella* in food and using pasteurized and sterilized milk and milk products. It is reported that the incidence of foodborne *Salmonella* infection and toxification in developing countries is known to be high because of poor hygienic conditions in their environment (Kenneth, 2005).

2.1.10 Pathogenesis of *Salmonella enterica* infections

Salmonella enterica infection varies with the serovar, the strain, the dose of cells, the nature of the contaminated food and the status of the host. Strains of the same serovar are known to differ in their pathogenicity. *Salmonella enterica* enter the host's digestive tract as food or waterborne contaminants. They survive the acidic environment of the stomach and the degradative action of bile salts in order to reach the primary site of colonization, the small intestines (Cotter and DiRita, 2000). They penetrate the intestinal mucosa without causing any lesion and then enter the lymphatic system. They accumulate in the mesenteric lymph nodes where they multiply. Part of the bacterial population lyse here releasing the endotoxin- lipopolysaccharides (LPS). From the lymph nodes, viable bacteria and LPS (endotoxin) are released into the bloodstream resulting into septicaemia. The release of the endotoxin is responsible for cardiovascular collapse and stuper state ("collapsus and tuphos", a stuporous state- the origin of the name typhoid) due to

action on the ventriculus neurovegetative centers (Kenneth, 2005). Typhoid is strictly a human disease.

2.1.11 Survival tactics of *Salmonella*

Salmonellae have several modes of survival; including formation of biofilms (microcolonies of bacteria closely associated with an inert surface attached by a matrix of complex polysaccharides in which nutrients and other microorganisms may be trapped). This may become an environment for microbial multiplication and establishment. Resistance to low water activity, formation of rugose and the capability of entering into a viable but non-culturable (VBNC) state. Other techniques include gene manipulation and acid development in the milk. Each one of these modes has factors that will make the organism revert to it as explained below.

2.1.12 Acid development in milk

Weak acids produced by microorganisms themselves contribute to acid stress (Foster and Spector, 1995). The specific role played by lactic acid bacteria (LAB) as a probiotic against *Salmonellae* and other enterics like coliforms has been discussed extensively by Juven *et al*., (1991). Lactic acid bacteria produce many kinds of metabolites, which affect other microbes. Lactic acid (LA), acetic acid and hydrogen peroxide produced, both by homofermentative and heterofermentative strains, reduce the pH of the food and are inhibitory against *Salmonellae*, coliforms and clostridia *in vitro* (Nousiainen, 1993). Bacteriocins produced by these LABs are only inhibitory against gram-positive bacteria and inhibition against gram-negatives has not been demonstrated (Abee *et al.,* 1995).

2.1.13 Resistance to cationic peptides in milk

Various animal species synthesize small cationic peptides that have antimicrobial properties against gram-negative and gram-positive bacteria. Many of these peptides form voltage-gated channels in lipid bi-layers, suggesting that they kill bacteria by depolarizing the cytoplasmic membrane therefore allowing foreign materials into the bacterial cells which kill them (Christensen *et al.,* 1988, Kagan *et al.,* 1990). *Salmonellae* could have been killed by amphipathic, cationic and antimicrobial peptide molecules that are present in the phagosomes, neutrophil granules and macrophages in the camel milk. These molecules kill bacteria by ionically binding to lipopolysaccharides that form the surface coating of GNR including *Salmonella* and mediating permeability of the outer and inner membranes resulting in bacterial cell death (Cotter and DiRita, 2000).

2.1.14 Viable but non-culturable state (VBNC)

Salmonella is known to enter into a VBNC state (Chmielewski *et al.,* 1995, Anviany *et al.,* 2001 and Erikson *et al.,* 2001). This is a state in which the salmonella cells cannot be detected by standard culture on enriched agar media although they remain viable and capable of resuscitation under favourable conditions. It mainly applies to gram-negative bacteria as a strategy for survival in natural environment (Roszak and Cowel, 1987). This behaviour of *Salmonellae* is of importance because they can remain viable and retain their reproductive potential outside the living animal but in food materials like milk, surface water and sewage (Wolfgang and Gunter., 1988). This causes public health concern. *Salmonellae* do this in milk by invading and penetrating phagocytic cells, including macrophages and polymorphonuclear neutrophils, which are also in camel milk. When they interact with activated macrophages, this results in

Salmonella-induced apoptosis (Fig 2.1) (Chen *et al.,* 1996, Lindgren *et al.,* 1996 and Monarck *et al.,* 1996,). When *Salmonellae* encounter resisting macrophages, they induce their own macropinocytotic entry into the spacious phagosomes (Alpuche-Aranda *et al.,* 1994). Intramacrophage survival is facilitated by *Salmonella*-mediated altered membrane trafficking and prevention of phagosome-lysosome fusion (Ishibashi and Arai, 1990, Buchmeier and Heffron, 1991, Rathman *et al.,* 1997 and Uchiya *et al.,* 1999) (Fig 2.1). Within these macrophages, *Salmonellae* are not culturable but remain viable and can multiply. The same scenario may take place in milk because it contains polymorphonuclear neutraphils and macrophages on transit.

2.1.15 Escape from the Extracellular Environment

Salmonella infections start with the invasion of columnar epithelial cells and/ or M cells overlaying the payer's patches in the distal Ileum and proximal colon of the bowel. This step makes *Salmonella* species inaccessible to the humoral response and becomes a survival strategy. Since epithelial cells are not normally phagocytic, *Salmonella spp*. initiate an endocystic or phagocytic event to potentiate uptake. They do this by subverting existing host–signal transduction pathways (Gallan *et al.,* 1992, Rosenshine and Finlay, 1993). Initial association between *Salmonella spp*. and the apical surface of intestinal epithelial cell results in degeneration of the microvilli. Contact between bacterium and host cell triggers the synthesis of thin appendages on *Salmonella species* that may signal the host cell to begin engulfment and facilitate invasion (Ginnochio *et al.,* 1992) (Fig.2.1) The host membrane begins to bleb and swell as a result of local reorganization of the cytoskeleton (Finlay *et al.,* 1991) and the resultant membrane ruffle encloses and internalizes the invading bacterium (Francis *et al.,* 1993). Several different

invasion genes (*inv*) and loci participate in this process (Galan and Curtis, 1989 and 1990 Altmeyer *et al.,* 1993).

2.1.16 Macrophage survival

Once the *Salmonella* species gain entry into a phagocytic cell, survival within this macrophage is paramount to its virulence. Though little is known of the mechanisms *Salmonella* uses, it is thought to be sequestered within macrophages at all stages of the infection. *Salmonellae* may use two pathways to internalize themselves into macrophages. One is normal phagositosis and the other is *Salmonella*-induced pathway as described above for epithelial cells. This way, most *Salmonellae* are found in polymorphonuclear leucocytes (PMNs) (Dunlap *et al*., 1992). Many genes associated with survival in macrophages have been identified (Baeumler *et al*., 1994, Libby *et al*., 1994). *Salmonella enterica* uses two type III secretion systems (TTSS) for different modes of interaction with the infected host during pathogenesis. Both TTSS are encoded by pathogenicity islands. The TTSS encoded by salmonella pathogenicity island 1 (SPI 1) mediates for the invasion by salmonella of non-phagocytic cells, such as epithelial cells of intestinal mucosa (Galan *et al*., 1992, Wallis and Galyor, 2000). The second TTSS encoded by SPI 2 is required for intracellular survival and replication of *S.enterica* (Hensel, 2000). A cluster of effector proteins of SPI 2 called salmonella translocated effectors (STE) were identified in *S. enterica* and they indicate that intracellular salmonella translocate STE into host cells via the TTSS of SPI 2. STE genes are all outside the SPI 2 locus and several of these loci are associated with prophage genes, indicating that the STE genes are part of the virulence factors of *Salmonella enterica*.

2.1.17 Host specificity

The host ranges of different *Salmonella enterica* serotypes vary between highly host adapted forms and serotypes that can cause infections in a wide range of hosts. The molecular basis of the different disease outcomes and host specificity is not yet understood (Figueroa-Bosi and Bossi, 1999). At the moment, the genome for Paratyphi C is being analysed for this phenomenon and other virulent determinants. It is suspected that possession of various sets of *Salmonella* translocated effector (STE) loci in the genome of Paratyphi C may contribute to the different host ranges and pathogenic potentials that this serovar may have (Imke Hansen-Wester *et al.*, 2001). The pathogenicity implications as a food hazard, Paratyphi C is among the three serovars out of 2,200 serovars that have the surface or envelope antigens associated with virulence. One such surface antigen is the Vi antigen that it may be having.

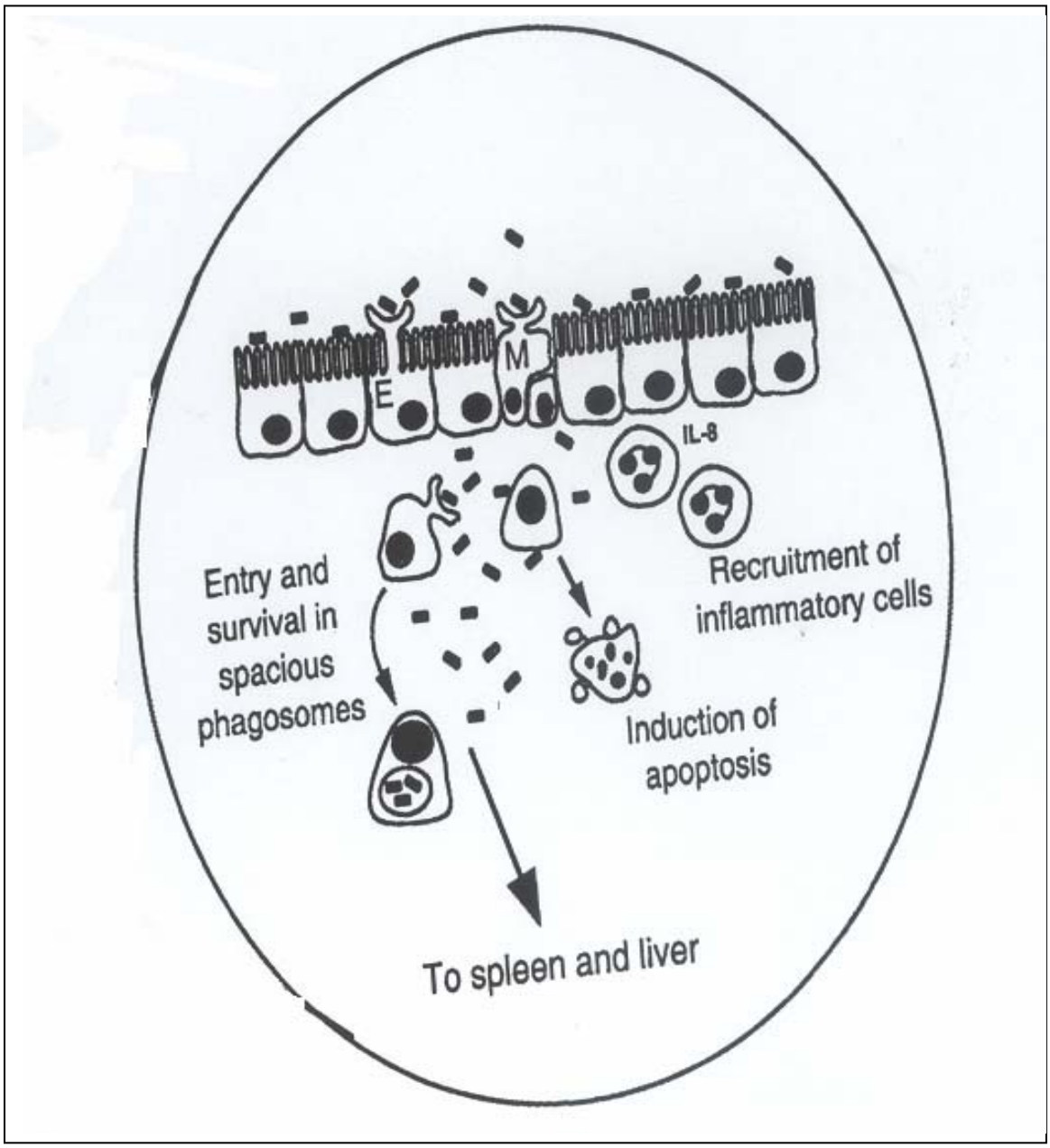


Fig 2.1 *Salmonella enterica* invading enterocytes (E) or M cells (M) and encountering PMNs neutrophils and macrophages to be carried to spleen and liver. Also shows *Salmonella* phagosome-lysosome fusion (Apoptosis) Escaping the extracellular environment. (Source: Cotter and DiRITA, 2000)

2.2 General materials and methods for the two objectivess

2.2.1 Site of study

The study was conducted in 4 provinces of Kenya, namely; North Eastern, Eastern, Rift Valley, and Nairobi Province. The Districts covered included Garissa, Isiolo, Marsabit, Moyale, Laikipia and Nairobi (Fig 2.2). Apart from Nairobi, these areas lie between longitudes 39^0 E 41^0 E and latitudes 2^0 S 4^0 N and at an altitude of between 200-500 m, an average of 350 metres above sea level. This is the arid and semi arid land of Kenya with rainfall of about 250-510 mm. The mean minimum and mean maximum temperatures are 30^0 C and 34^0 C respectively (Kenya Atlas, 1979).

2.2.2 Sampling design

The technique of sampling was simple random sampling laid down in cluster sampling design. The measurements were based on ratings, occurrences and loads of targeted organism. Quality cases were assessed and classified as acceptable, partially acceptable and rejected. Data base analysis was by correlation coeficient and Chi-square.

Five categories of sampling units namely; camel udder milk, bulk milk, faeces, water and soil were used. A representantive composite milk sample was taken from camel udders by mixing the milk from each quarter and then taking one sample. Bulk milk was collected at colletion centers. The milk in the container was mixed by shaking the container and a cup (normally used by the milker) was used to take the sample. The sample was then poured into a sterile glass bottle with a screwcap. The same method was used to take samples from the market. All samples for each category were collected asceptically and kept below 10^0 C in a coolbox that had cooling

elements. The samples were transported to the laboratory within 8 h and analysis started immediately.

The microbiological analysis of the samples was done at KARI-Marsabit station and Egerton University, Department of Dairy and Food Science and Technology. This study was undertaken between September 2002 and April 2004 on pastoralists living in the ASAL of the districts given in Fig 2.2 and the vendors of camel milk in market centres. The camels were kept under shifting conditions in search of forage. They were milked twice daily. Feeding was based on shrubs without any supplementation.

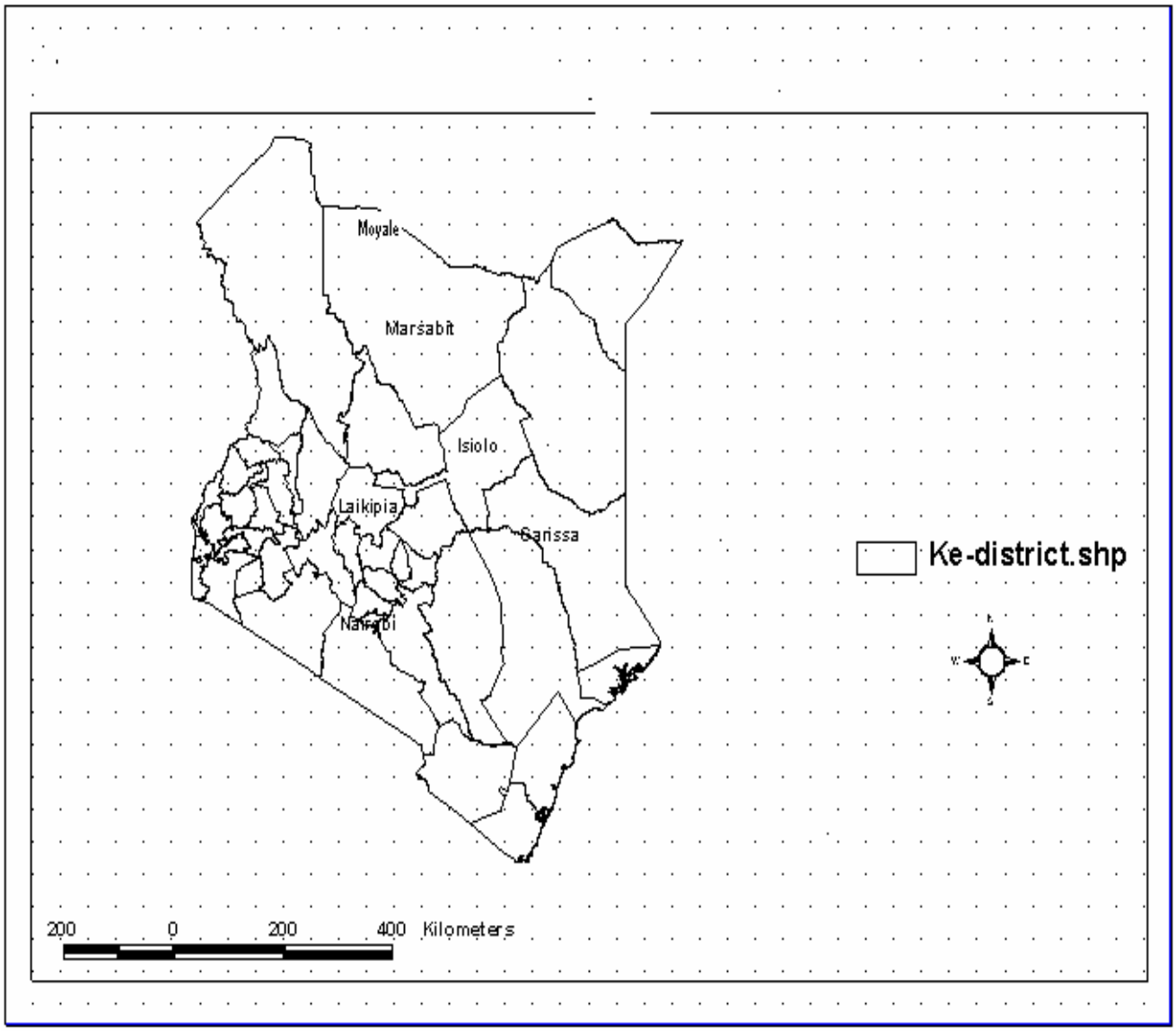


Fig 2. 2 Republic of Kenya showing Districts where the study was conducted

CHAPTER THREE

THE MICROBIAL LOAD AND PROFILE OF MICROFLORA IN RAW CAMEL MILK FROM PRODUCTION THROUGH THE MARKETING CHAIN

3.1 Introduction

The initial load of microorganisms present in any food will originate from the natural microflora of the raw materials and those introduced into it in the course of production and handling. Production refers to the harvesting, processing, storage and distribution of the food product. In most cases, these microorganisms have no discernible effect on the food. However, in some cases, they may manifest themselves in several ways. Some of the known ways include; causing spoilage, causing food borne illness or transforming the food material in a beneficial way like in food fermentation. They develop under the influence of a number of internal factors of the food like the pH, redox potential, water activity, level of nutrients and presence of some antimicrobial protein cations, external factors like temperature, humidity and presence of gases like carbon dioxide and oxygen and implicit factors like processing. In considering the possible sources of microorganisms as agents of food spoilage or food borne illness, it is necessary to examine the natural flora of the food materials themselves, the flora introduced by processing and handling and the possibility of chance contamination from the environment (atmosphere, soil or water) (Adams and Moss, 1997).

Camel milk in pastoral areas (ASAL) is traditionally consumed raw and is presently marketed in raw form. The traditional milking and storage containers, the poor infrastructure in ASAL and the transportation means of raw milk to market centres contribute to the deterioration of the quality and safety of the camel milk. The chemical composition of camel milk supports the

growth of many bacteria and fungi. With a pH of 6.5, protein content of upto 4%, lactose of upto 5.5% and a diversity of mineral content and rich in vitamin C (Trevor, 1998, Farah, 1996), camel milk has all the growth factors required by the microrganisms.

3.2 Materials and Methods

3.2.1 Milk Sampling

Composite and representative sample

This sample was taken at the *Boma* (production) level. Only lactating camels were sampled. Each of the four quarters of the camel udder was milked and emptied into a traditional milking container. Before taking the sample, the container was shaken to mix the milk. A sample of 10ml of milk was poured into a sterile labelled screw-cap tube and stored in a coolbox maintained at 8^0 – 10^0 C using cooling elements. The collection of camel milk samples was done in the morning and evening at normal milking time. Composite milk samples were collected from 107 lactating camels.

Bulk sample

This sample was taken from the collection centers where milk from various production farms was pooled together for transportation to market centers. This was taken as a processing point. The milk was brought in various containers including gourds and plastic cans. Milk was poured into 20 litre plastic cans. Milk in each can was stirred to produce a homogeneous mixture before taking a representative sample. About 10ml of milk sample was taken and poured into a sterile labelled screw-cap tube and stored in a coolbox at 8^0 – 10^0 C. The sampling was done at random. In locations where the milk was less, all bulk milk containers were sampled. Fifty two (52) bulk milk samples were taken from five collecting centres in five locations of Northern Kenya. The

collection points included Moyale town, Ngurnit and Manyatta Lengima in Marsabit, Isiolo town and Nanyuki town. The samples were transported to KARI-Marsabit laboratory within six hours and analytical work started immediately.

Market samples

These were taken at roadside displays, bus termini, make shift shops (Kiosks) and market places in urban centres. The market points included; Moyale town, Ngurnit and Manyatta Lengima in Marsabit, Isiolo town and Nairobi-Eastleigh. The sampling was the same as for bulk milk at collecting centers. The samples were transported in a coolbox to KARI-Marsabit for those from Moyale and Marsabit and to Egerton University for those from Isiolo and Nairobi for laboratory analysis. The procedure was the same as for bulk milk at collecting centers. Fifty nine (59) milk samples were collected from the market and other sales outlets.

Water samples

Water samples from milk production areas were taken from the nearby wells, boreholes dams and rivers that were used as source of water for domestic and animal drinking. For well and borehole samples, 50ml samples were taken. The water was collected by the same means the people at the well used. It was then poured directly from the container which was used to collect the water into a sterile screw cup glass bottle. The sampling container cup was asceptically replaced by wiping the cup and neck of the container with a paper towel that was soaked in 70% ethanol. The sample was kept in a coolbox. For river water, the sterile aluminium cover was removed from the sterile sampling bottle container. The lid was asceptically removed and the mouth of the bottle was faced upstream. The neck of the bottle was tilted down into the water to

the middle depth of the river. It was then tilted slightly upwards to let the water fill completely. Where there was no current, it was pushed forward horizontally until it was full. The lid was asceptically replaced by wiping it and the neck of the container with a paper towel that was soaked in 70% ethanol. The sample was kept in a coolbox that had cooling elements. Transportation of the samples to the laboratory for bacteriological examinations took less than 20h

3.2.2 Laboratory analysis

The flow chart below is a schematic procedure used for culturing of camel milk samples for microbial isolation and identification.

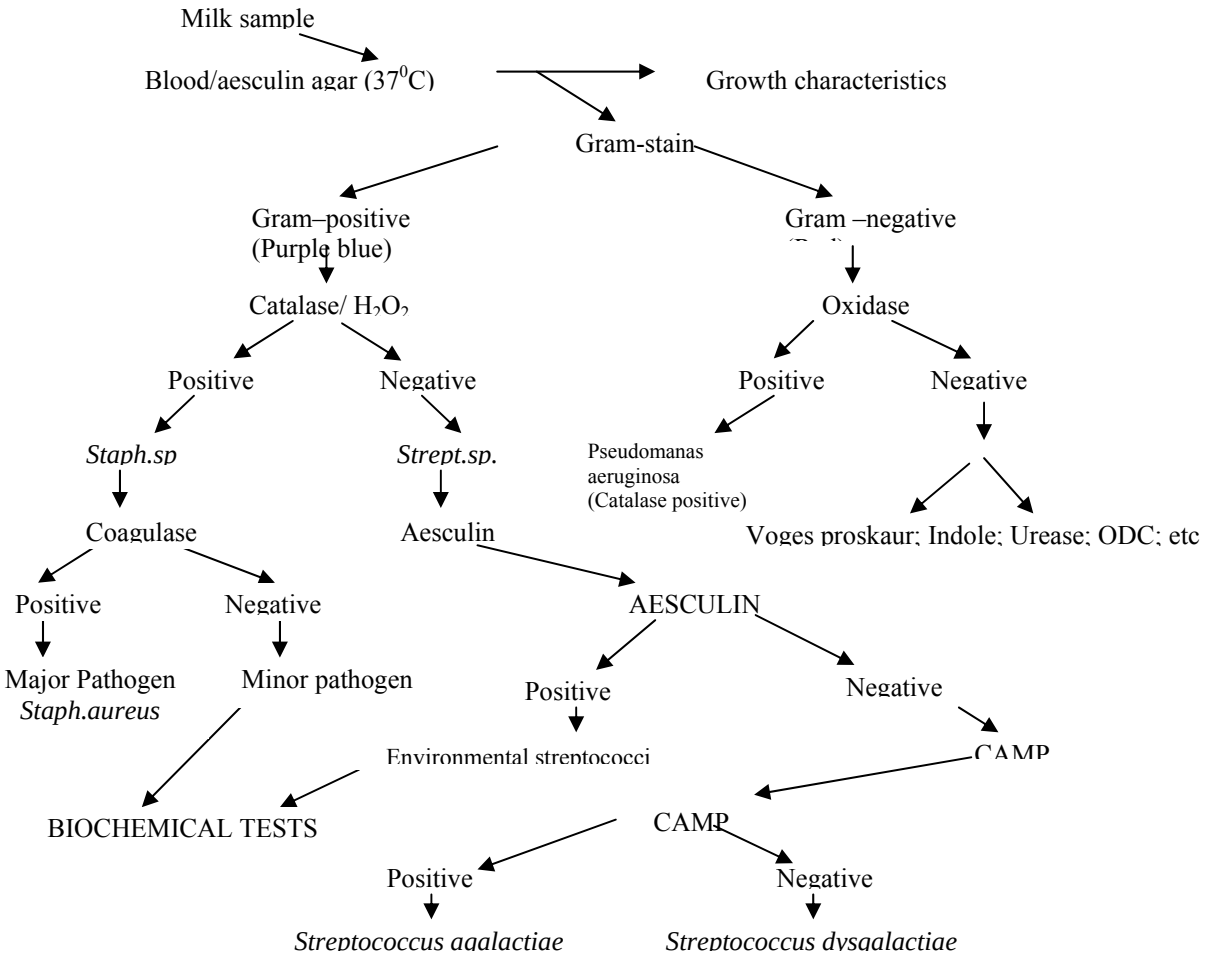


Fig 3.1 A flow chart indicating laboratory Identification of Bacteria in Camel milk. (Method according to Carter (1990))

Examination of samples for bacteria

Each milk sample was cultured in two ways; one loopful of milk sample was streaked directly on 5% sheep blood agar (Oxoid, blood agar base). Another 0.3 ml of the same milk sample was transferred by a sterile 1ml pipette into 10 ml of enriched nutrient broth, which consisted of 0.3% yeast extract (Difco) and Nutrient broth (Oxoid). The remaining milk sample was stored at 4^0C for other tests.

Direct and enrichment cultures were incubated at 37^0C for 12 h. Growth of direct cultures was examined and sub-cultured. Enrichment broth cultures were sub-cultured by streaking on 5% sheep blood agar and incubated at 37^0C for 24 h. Selection of colonies from subcultures was done according to their predominance and homogeneity throughout the streak, type of haemolysis and the comparison of growth characteristics in both direct and indirect cultures. Special emphasis was given to the scrutiny to the slow growing and more fastidious colonies. All blood agar plates that showed none or scarce growth were re-examined after 48h and 72 h of incubation. Haemolytic colonies were sub-cultured onto blood agar whereas fast growing non-haemolytic colonies were sub-cultured on nutrient agar (Oxoid).

Storage of pure isolates

Pure colonies from the sub-cultures were harvested using a sterile cotton swab and suspended in sterile 0.25 molar sucrose solution in plastic vials with screw caps and stored at -23^0C in a deep freezer. The sucrose was prepared by taking 8.6g of sucrose and dissolving in 100 ml distilled water. The solution was then dispensed at 0.5ml amounts in each vial and autoclaved at 121^0C for 15 minutes. It was stored at 4^0C until the time of use. When required, the organisms were

removed from the freezer, thawed and a loopful of them sub-cultured on nutrient agar or blood agar. The remaining was stored again for future use.

Biochemical Identification of colonies from cultures

Pure cultures from the subcultures were screened for identification based on colony morphology and haemolysis on blood agar, Gram stain, and catalase and coagulase tests done according to Carter (1990) (Fig. 3.1) above. Gram-positive, catalase positive cocci in clusters (*Staphylococcus*) were further differentiated from the other catalase positive cocci according to their ability to ferment glucose and mannitol. The gram-negative, oxidase-negative rods were further differentiated by the reactions in indole, voges proskauer, citrate and methyl red (IMVIC) (Fig. 3.1).

Catalase test

This was done according to Carter *et al*. (1990). Three to four colonies of the 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 test organisms on the glass slide using a pasteur pipette at room temperature. Bubbles indicated a catalase positive reaction.

Oxidase test

This test was done to separate the oxidative and fermentative gram negative organisms. Pure colonies of the isolates (about 3 colonies) were smeered on the test oxidase strip. Colour change to deep blue was positive for the test.

The Voges Proskauer (V.P) test

This test was used for the detection of acetylmethylcarbinol. Some gram positive and gram negativeorganisms ferment some carbohydrates to produce acetylmethylcarbinol instead of mixed acids, which in the presence of an alkali is oxidised to diacetyl and gives a pink to brick-

red colour. This was used to differentiate these types of isolates that do not produce this compound on fermentation of some sugars. This was done alongside the sugar tests and was carried out as described by the manufacturer (Oxoid). The media used was M.R.V.P (methyl-red voges proskauer) (Oxoid). It was prepared by weighing 7.5g of the media and dissolving in 500ml of distilled water by shaking gently with the hand. The solution was dispensed in test tubes at the rate of 3 ml per tube. The tubes were capped and autoclaved at 121^0C for 15 minutes.

The medium was left to cool at room temperature before inoculation was done. The medium was inoculated with 0.1 ml of the culture suspension as in the sugars. The cultures were incubated for 9 days. The results were read after adding 3 ml of 6g α-naphthol dissolved in 100ml absolute ethanol, and 3ml of 40% potassium hydroxide. After adding the two reagents mixing was done by slight shaking. The mixture was incubated for 30 minutes at 37^0C for the pink to brick red colour to develop for positive cases. *Enterobacter aerogenese* was used as positive control and similarly inoculated as the test organisms.

Indole test

Pure colonies of isolates were suspended in peptone water medium and incubated 37 ^{0}C for 24 h. One ml of Kovac's reagent was added and hsken gently. It was left to stand for 5 min. A pink to red colour development was positive for indole production.

Biochemical differentiation of the cocci spp.

All *Staphylococcus spp*.were further differetiated and confirmed as *Staphylococcus aureus* from the rest of the coagulase negative S*taphylococci* according to their fermentation of carbohydrates especially mannitol and glucose. Organisms were inoculated into the sugar medium as described below in the case of streptococcal biochemical differentiation. They were incubated at 37^0c for 12 h. Change of colour from red to yellow indicated positive fermentation.

Colonies that were Gram-positive cocci in chains and catalase negative were further differentiated according to the following biochemical reactions:

Salt broth

This test was used to differentiate non-enterococci from enterococci streptococci. The enterococci streptococci will grow in 6.5% salt broth but not other streptococci. The salt broth used was 6.5% sodium chloride.

Sodium chloride (6.5%) in Nutrient broth (Oxoid), yeast extract (Merck) and a spatula-tip of phenol red as an indicator were used. 32.5g of sodium chloride, 12.5g Nutrient broth, 3g of yeast extract and a spatula-tip of phenol red were weighed and dissolved in 500ml of distilled water. The mixture was autoclaved at 121^0C for 15 minutes. The media was left at room temperature for 2 h to cool and then kept at 4^0C till it was reguired. The test organisms were inoculated into the broth at room temperature and incubated for 12 h at 37^0C. Colour change from red to yellow indicated growth.

Bile aesculin agar

The test is used to differentiate group D-*Streptococci.* Some *Streptococci* are able to split the glycoside aesculin into glucose and aesculin. *Streptococcus agalactiae* and *Streptococcus dysgalactiae* do not split aesculin while *Streptococcus uberis* does. Bile aesculin agar (Oxoid) was used in this test according to the manufacturer's instruction. 22.25g was weighed and dissolved in 500ml of distilled water. The mixture was first boiled gently to dissolve completely and autoclaved at 121^0C for 15 minutes. It was dispensed into sterile glass petri- dishes. The media was left to cool and solidify at room temperature for 2-3 h. The test organism was streaked on the solid media and incubated at 37^0C for 24 h. Growth on bile aesculin and production of colour was recorded.

CAMP test

Streptococcus agalactiae produces an extracellular diffusable protein referred to as CAMP (Christie, Atkins and Munch Peterson) factor. This interacts with Staphylococcal ß-haemolysin on Sheep red blood cells. A known *Staphylococcus aureus* culture was streaked across a 5% Sheep blood agar plate using a cotton swab. The test *Streptococcus* organisms were inoculated at right angles to the streak. The cultures were incubated at 37^0C for 12 h to 24 h. Enhanced haemolysis indicated positive identification of *Streptococcus agalactiae*

Fermentation of carbohydrates and biochemical identification of GNR

Casein Triptic Agar (CTA) (BBL) was used as the basic medium and with added carbohydrates; fermentation reactions of microorganisms were tested. CTA consists of L-cystine, pancreatic digest of casein, agar, sodium chloride, sodium sulphate and phenol red. The sugars used were

dissolved in 20ml distilled water, sterilised using a Pro-X filter unit 0.22μm hydrophilic cellulose acetate membrane (Lida Co). Each sugar was treated separately and was filtered into the base media after these had been cooled down to 50^0C in a water bath. The mixture was shaken gently and then dispensed into sterile test tubes at the rate of 5 to 6 ml per tube using a sterile 50ml pipette. The tubes were left at room temperature for the media to solidify ready for inoculation. The inoculum (pure isolate) was made by making a suspension of overnight culture in 3 ml of sterile water in a test tube. Using a 1ml pipette, 0.1 ml of the culture suspension was dispensed into each reagent tube of the sugar medium. Since the medium was solid, a straight sterile wire was used to stab the medium for the culture to percolate. These were incubated at 37^0C for 48 h. The reading was taken after 24 h, and the culture re-incubated for a further 12 h to 24 h for the second reading.

3.2.3 Determining the microbial load

Total viable counts (TVC)

The TVC was done using standard procedures as described by Carter (1990) using pour plate method. Plate count agar (Oxoid) was used. This test was carried out to determine the content of microbial contamination of milk before any processing was done. One millilitre (1ml) of milk sample was serial diluted six-fold using buffered peptone water (Oxoid) then one ml of the sample milk was diluted in 9 ml of peptone water (ratio of 1:10) up to six dilutions. Sterile duplicate glass petri dishes were labelled according to the dilution index. One ml of the dilutions was aseptically withdrawn using a sterile 1ml pipette and delivered into an opened and sterile petri dish and closed. The same was done for a duplicate petri dish. This was repeated till all the dilutions were pipetted into their corresponding plates up to 10^{-6}. This was followed by pouring

about 15 ml of plate count agar (PCA) (Oxoid), which had been autoclaved at 121^0 C for 15 min, cooled and tempered in a waterbath at 45^0 C. The sample and the agar were gently mixed by alternate clock and anti-clockwise rotations for about 3 min. and left to solidify on the bench for about 30 min. The plates were inverted and incubated at 37^o C for 48 h. Because the first dilutions are expected to have heavier growth, they were not used; instead the last three dilutions (10^{-4}, 10^{-5}, and 10^{-6}) were used for total viable counts.

The coliform count (CC)

The procedure used was similar to total viable counts as described by Carter (1990). MacConkey agar (Oxoid) and Violet red bile agar (VRBA) (Oxoid) were used to select for lactose fermentors One millilitre (1ml) of milk sample was serial diluted six-fold using buffered peptone water (Oxoid) then one ml of the sample milk was diluted in 9 ml of peptone water (ratio of 1:10) up to six dilutions. Sterile duplicate glass petri dishes were labelled according to the dilution index. One ml of the dilutions was aseptically withdrawn using a sterile 1ml pipette and delivered into an opened and sterile petri dish and closed. The same was done for a duplicate petri dish. This was repeated till all the dilutions were pipetted into their corresponding plates up to 10^{-6}. This was followed by pouring about 15 ml of violet red bile agar (VRBA) (Oxoid) or MacConkey agar (Oxoid), which had been autoclaved at 121^0 C for 15 min, cooled and tempered in a waterbath at 45^0 C. The sample and the agar were gently mixed by alternate clock- and anti-clockwise rotations for about 3 min. and left to solidify on the bench for about 30 min. The plates were inverted and incubated at 37^o C for 48 h. Because the first dilutions are expected to have heavier growth, they were not used for counting, instead the last three dilutions (10^{-4}, 10^{-5}, and 10^{-6}) were used for total viable counts.

The spore formers

The sporeformers are heat resistant and they vegetate later in milk that is pasteurized and cause spoilage. Milk samples for testing for presence of spore formers were taken in 10ml amounts using 10 ml capacity test tubes. The tubes were labelled and placed in a rack, which was placed in a water-bath set at 80^{o} C. In one of the test tubes with milk sample, a thermometer was inserted to determine actual temperature of the sample to ensure the milk samples attained 80^{o} C. The samples were removed and cooled immediately after attaining 80^{o}C. Each sample was serially diluted using buffered peptone water and pour plated using PCA (Oxoid) from 10^{-1} to 10^{-6}. After solidifying for 45 min. on the bench, the plates were incubated at 37^{o} C for 24 h. The colonies were counted and expressed as total sporeform counts.

Development of acidity and total viable counts in spontaneous fermentation

Spontaneous fermentation of raw milk occurs naturally due to mixed microflora inherent in the milk. Depending on the initial load of these microflora in the milk, rate of spoilage of milk due to acid development will be an indication of the activity of the numbers of microorganisms present. Apart from the normal acidity of raw camel milk (pH of 6.5-7), there is additional acidity as a result of microbial multiplication called developed acidity. This developed acidity was monitored at producton and market levels using standard procedure as described by International Dairy Federation (1990) to determine its effect on microbial load (TVC) of raw camel milk. Acid development was measured in percent lactic acid (% LA) against time of incubation in spontaneous fermentation of raw camel milk as follows. Nine millitres (9 ml) of the milk sample was pippetted into a conical flask. One millitre of 0.5% alcoholic phenolphthalein indicator was added and then titrated against 0.1N sodium hydroxide (NaOH) until a faint pink colour appeared. The number of ml of sodium hydroxide solution or titre was divided by ten and expressed as percent lactic acid.

Temperature association of microorganisms

The pour plate technique was used as in the case of TVC above. The plates were incubated at different temperatures for different durations (Table 3.1)

Table 3.1 Temperature and time incubation regime for microorganisms

Type of microorganism	Temperature	Duration
Aerobic Psychrophilic bacteria	20^0 C	72 h
Aerobic Mesophilic bacteria	32^0 C	48 h
Aerobic Thermophilic bacteria	55^0 C	48 h

After incubation, visible colonies were counted and the number of colonies on each duplicate plate and dilution were recorded. The number of colonies was multiplied by the reciprocal of each dilution. The counts of the duplicate plates were added and the arithmetic mean was calculated. The final count was recorded as colony forming units per millilitre (cfu/ml).

3.3 Statistical analysis

The results for TVC, CC and spore formers were logarithmically transformed into $\log_{10}$ for statistical analysis. The statistical analysis was done using ANOVA and Chi-square. The package used for Anova was General linear model (GLM) of SAS version 8.0. Significance measurements were determined at $\alpha = 0.05$.

3.4 RESULTS

3.4.1 Initial microbiological load

Of the 107 samples collected at production level, 66% of raw milk samples had microbial load of less than 10^5 cfu/ml (Table 3.2). The coliforms and sporeform counts increased from production to market level.

Table 3.2 Initial microbial load in raw camel milk samples (n= 382)

	Production				**Collection centers**				**Market**			
Range	≤ 30	≤ 10^5	> 10^6	**N**	≤ 30	≤10^5	> 10^6	**N**	≤30	≤10^5	> 10^6	**N**
TVC (cfu/ml)	18	71(66%)	18	**107**	0	2	50(96%)	**52**	0	1	54(92%)	**55**
CC (cfu/ml)	2	38(73%)	12	**52**	0	2	19(90%)	**21**	0	0	50(100%	**50**
Spores (cfu/ml)	25	7	0	**32**	0	14	2	**16**	0	1	0	**6**
Total				**191**				**80**				**111**

3.4.2 Comparisons of the initial load of bacteria from different sources

Table 3.3 shows that the ranches had lower microbial content (TVC and CC) than the pastoral areas at production level. The $\log_{10}$TVC and $\log_{10}$ of coliform counts between pastoral areas and ranches were significantly ($P< 0.05$) different. At the market level, the initial load of both TVC and Coliforms were different. The coliforms were lower than TVC at production than market level. But at market level, especially at the Nairobi market, the coliforms dominated as shown by the difference in the mean values (Table 3.3).

Table 3.3 Mean values with their standard deviation (SD) of total viable counts and coliforms from different sources

	Production (n=165)		Market (n=137)	
Source	**Log_{10}TVC**	**Log_{10}Coliforms**	**Log_{10}TVC**	**Log_{10}Coliforms**
Pastoral				
Marsabit	4.52 ± 0.25^{a}	2.77 ± 0.19^{bc}	-	-
Isiolo	5.40 ± 0.41^{a}	3.42 ± 0.24^{b}	8.48 ± 0.22^{a}	7.82 ± 0.25^{a}
Moyale	4.49 ± 0.53^{a}	4.23 ± 0.31^{a}	8.68 ± 0.21^{a}	7.74 ± 0.21^{a}
Garissa	-	-	8.66 ± 0.22^{a}	8.20 ± 0.22^{a}
Ranches	2.84 ± 0.41^{b}	2.66 ± 0.24^{c}	7.73 ± 0.14^{b}	8.11 ± 0.17^{a}
Nairobi mkt	-	-	7.73 ± 0.14^{b}	8.11 ± 0.17^{a}
Corr. Coef	0.24 (P<0.05)		-0.15 (P>0.05)	

Mean values in the same column with the same superscript are not significantly different (***P*** > 0.05).
R (corr. Coef) – is between log_{10}TVC and log_{10} coliforms at production and marketing level.

The microbial activity at post harvest handling of the raw camel milk is indicated in Table 3.4. The coliforms increasesd at collection centers before transportation to the market. However, there was no difference between TVC and CC but the mean values of both TVC and CC showed an increase from the initial count with a positive correlation coefficient of 0.06. Table 3.7 shows that most of the microorganisms making the TVC at collection centers are coliforms.

Table 3.4 Milk from collection centers (bulk) in the production environment

	Mean values with SD	
Collection center (n=80)	**Log_{10}TVC**	**Log_{10}Coliform**
Isiolo	5.70 ± 0.35^{a}	7.19 ± 0.40^{a}
Marsabit	6.54 ± 0.22^{ab}	6.47 ± 0.31^{a}
Moyale	6.81 ± 0.31^{b}	0
Corr. Coef between log_{10}TVC and log_{10} CC	0.06 (P>0.05)	

Mean values of in the same column with the same superscript are not significantly different (***P*** > 0.05).

3.4.3 Spores

The spore content at production and market levels were significantly (P<0.05) different as shown in Table 3.5.

Table 3.5 Comparison of total viable countsT(VC), coliforms and spores between production and market centers (n = 54)

Point along chain	Mean values with SD		
	$Log_{10}TVC^{*}$	**$Log_{10}Coliforms$**	**Spores**
Production	4.37 ± 0.17^{a}	3.10 ± 0.18^{a}	43.60 ± 4.20^{a}
Market	6.44 ± 0.28^{a}	4.29 ± 0.37^{a}	11.84 ± 5.27^{b}

Mean values in the same column with the same superscript are not significantly different ($P > 0.05$).

3.4.4 Effect of acid development on (TVC) in fermentation of raw camel milk at production level.

Development of acidity due to microbial activity in the raw camel milk had an effect on the load of microflora (Figures 3.2 A and B). As the acid increased, the TVC decreased. An interaction between acid development and TVC showed that as acidity increased from an initial value of 0.17 to 0.24 %L.A in 5 h, there was a steady decrease in TVC by 2 log cycles from a high of 6.5 log_{10} cfu/ml to 4 log_{10} cfu/ml (Figure 3.3). However, a dynamic (stable) population that was reached at the second hour of incubation and the acidity started increasing but microbial population remained constant only to start reducing further at the fourth hour.

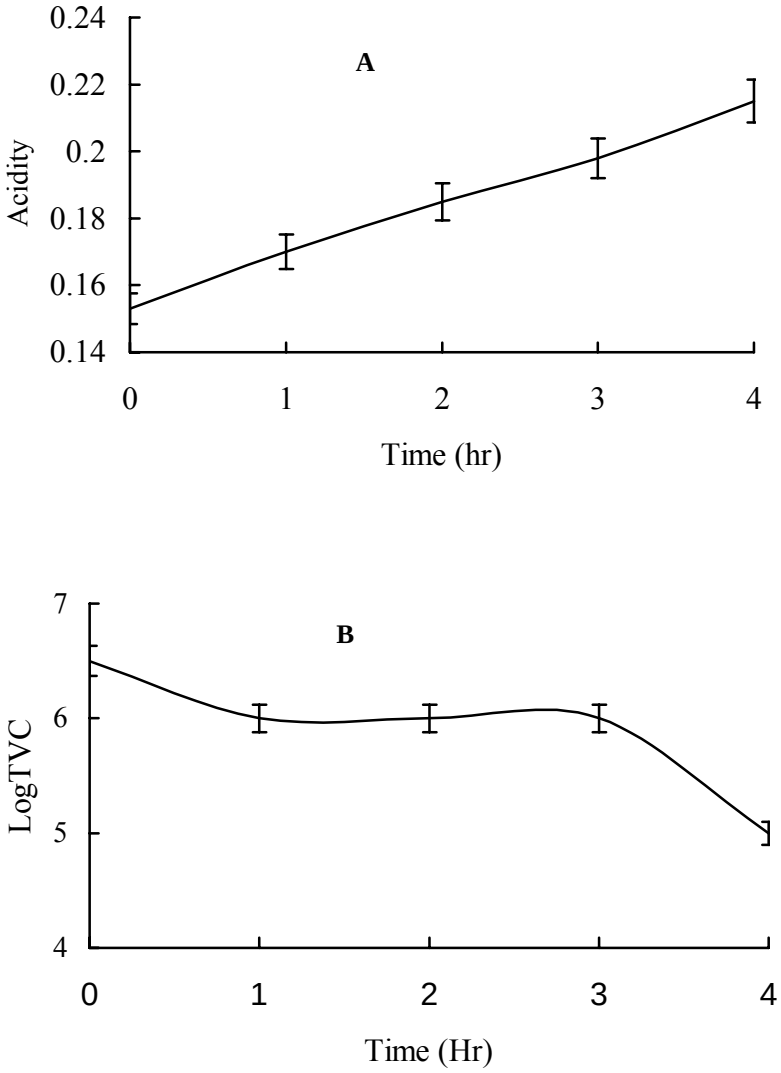


Fig 3.2: (a) and (b): standard deviations (error bars) associated with acid development and log_{10}TVC with incubation time respectively.

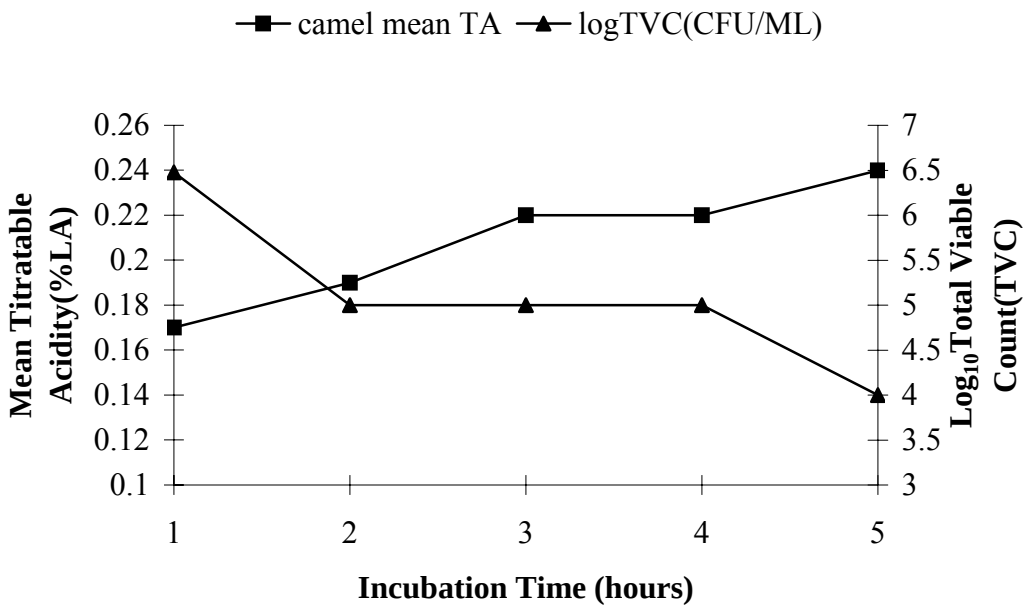


Fig 3 3 Acid development in relation to total viable counts (TVC) in raw camel milk during spontaneous fermentation at production environment.

3.4.5 Acid development and total viable counts (TVC) at market level

The acid development of market camel milk in relation to TVC is shown in figure3.3.

About 29% of the TVC reduction at market level can be associated with developed acidity as predicted by a uniform regression of TVC ($R^2 = 0.2909$) eguastion. This is a very low reduction in TVC.

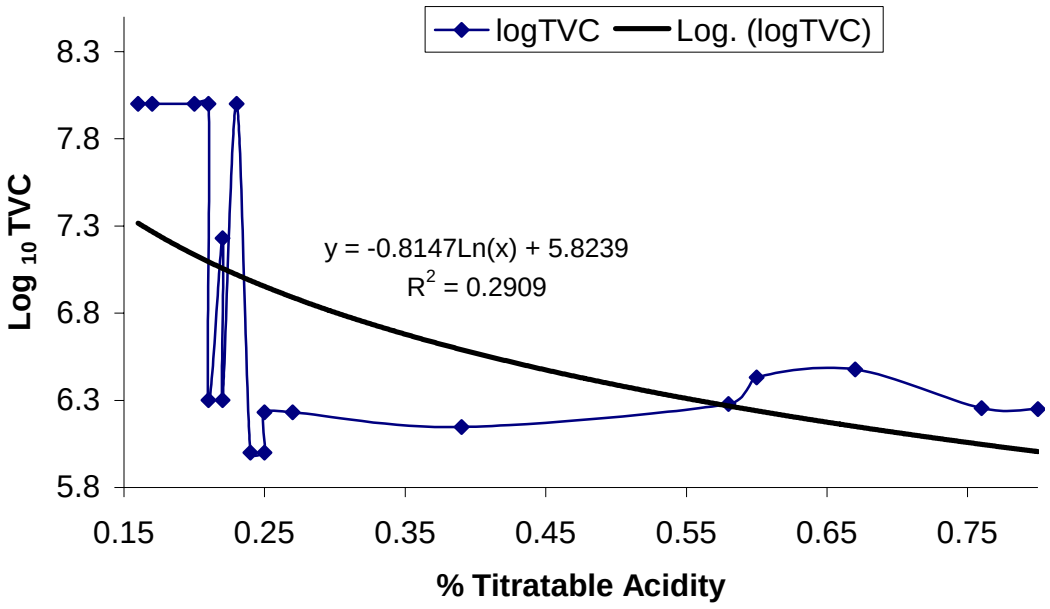


Fig 3.4 The relationship between log_{10} total viable counts (TVC) and titratable acidity for market camel milk samples

3.4.6 Types of microorganisms in raw camel milk

The occurence of various groups of microorganisms isolated and identified from raw camel milk at various levels of handling based on culture characteristics and morphology is shown in Table 3.6. The gram-negative rods had the highest incidence (55%) followed by gram-positive cocci with 31%. Yeasts and molds had the least incidence. The GNR were higher (54%) than gram-positive (42%) at production level. The group incidences at each level are shown in brackets against the number of isolates. The yeasts and moulds were the laest in incidence (3.3%).

Table 3.6 Incidence of main groups of microorganisms in raw camel milk

	N	Gram+ve cocci	Gram–ve rods	Gram+ve rods	Spores	Yeasts/molds
Production	107	45 (42%)	58 (54%)	2	1	1
Bulk	52	12 (23%)	28 (54%)	7	3	2
Market	55	10	32 (54%)	7	6	4
Total	214	67	118	16	10	7
Incidence		**31%**	**55%**	**7.5%**	**4.7%**	**3.3%**

3.4.7 Profile of isolates from the main groups of microorganisms

The isolates were confirmed based on biochemical tests. A total of 72 isolates were identified out of 214 samples taken (Table 3.7). The confirmation was based on gram reaction, cultural characteristics on selective media and fermentation of glucose and lactose. Other biochemical tests included catalase, indole, methyl red, voges proskauer and citrate utilization (Appendix 1). The typical coliforms (*E. coli* and *Ent. aerogenes*) had the highest incidence 36% and 22% respectively at the market. At production level, micrococci had the highest incidence (20%).

Table 3. 7 Main organisms isolated from raw camel milk in chain of production and marketing (n=72)

	E.coli	***E.aerogenes***	***Micrococci***	***Pseudomonas***	***Flavobm***	***Fungi***
Production	0	0	15	4	4	7
Market	26	16	0	0	0	0
Incidence(%)	36	22	20	5.5	5.5	10

3.4.8 Oxidase identification of main isolates

The separation of the identified isolates based on oxidase test showed more oxidase negative organisms at the market than at the production level (Table 3.8).

Table 3. 8 Incidence of main isolates identified in raw camel milk based on oxidase test (n=214)

Oxidase test	E.coli	E. aerogenes	Micrococci	Pseudomonas	Flavobact	Fungi
Oxidase –ve						
Production	10	8	10	0	0	-
Market	25	16	2	0	0	-
Oxidase +ve						
Production	0	0	3	4	4	7
Market	0	0	0	0	0	0
Total	35	24	15	4	4	7

3.4.9 Microbial associations in camel milk based on temperature

The groups of microbial isolates exposed to the various temperature regimes are shown in Table 3.9. Only three (3) isolates grew at 55^{o} C. The rest of the isolates did not. The three that grew were *Bacillus* type and fungi (Appendix 2). The growth at 25 ^{o} C and 32 ^{o} C was the same. Based on temperature regimes, psychrophilic types were dominant to the rest followed by mesophilic type.

Table 3. 9 Groups of microbial isolates based on temperature (n=37)

Growth Temp ^{0} C	Mean cfu/ml	Count/ml	Temp Classification
25	> 300	> 300	Psychrophilic
32	210 x 10^{4}	2 x 10^{6}	Mesophilic
55	3	3	Thermophilic

3.5 Discussion

3.5.1 Initial microbial load in camel milk

Camel milk harvesting at *Boma* level in the pastoral areas has low microbial content of less than 10^5 cfu/ml (Table 3.2). The Kenyan standard for good raw bovine milk is put in the range of 0-10^6 cfu/ml (KEBS).

The increase in TVC and CC at collection and market centres can be associated with post harvest handling of the milk. External sources that influence the increase in content of TVC and CC are water, personnel and equipment. There is scarcity of water in ASAL due to recurrent prolonged droughts. The little that may be available is not chemically treated, filtered or boiled. This is the water used to clean milk containers. Camel milk is mostly transported and stored in plastic cans which are difficult to clean because of their interior design. This is contrary to the recommended seamless containers that are easy to wash and sanitize. They provide suitable sites for microbial multiplication. *E. coli* is known to multiply fast in such environment (ICSMF, 1980) outside the colon of humans and animals. This explains the high count of CC at collecting and market centers (Table 3.2). Since coliforms are indicators of hygienic status in a particular environment, it means that at production level at both pastoral and ranch farms, there is less contamination. Camel milk has low exogenous contamination at production level. The subsequent increase of coliforms in the camel milk from production up to the market level can only be associated with poor post harvest handling of the product. For example mixing of raw milk of low microbial load with that of high microbial load between production and market encourages growth of coliforms. This increases the TVC. Data produced from collection centers (Table 3.4) within the production area supports this inference. It shows no significant difference ($P>0.05$) between the $\log_{10}$TVC

and $\log_{10}$ Coliforms. It was also noted that the majority of organisms making the TVC at collection and market centres are coliforms (Table 3.7).

3.5.2 Source comparisons of the initial load

The TVC is higher than the coliforms at production level (Table 3.3) because the environmental microflora, the majority being coliforms, have not, at this time gained entry into the milk as the milk is coming directly from the udder. The high TVC could be associated with udder infections like mastitis as reported in earlier investigations by Matofari *et al*., (2001). About 42% of cocci group of microorganism was isolated at production level (Table 3.6) and these are mostly associated with mastitis infected camel udders (Matofari *et al*., 2001 and Younan, *et al*., 2001). The organisms involved are type B Streptococci *(Streptococcus agalactiae*) and group D (non-enterococci) *Streptococci*. This is also reported in studies done in the Sudan, Kenya and Israel by Abdurahman (1995), Obeid and Bagadi (1996), Younan *et al*., (2001) and Guliye *et al*., (2002). They reported that the cocci group of bacteria, especially *Streptococcus agalactiae*, causes subclinical mastitis commonly encountered in camel udders.

The coliforms are indicator bacteria that give a signal, when present in any food, that there is the possibility of the presence of enteric pathogens. They also show the hygienic conditions under which the commodity was produced and handled. The increase in coliforms in the market raw camel milk could be associated with contaminated containers, water and the soil including dust and mud. The coliform organisms are found in the soil, on plant materials and can be dispersed into the atmosphere by dust. Rainwater carries the surface contamination to the water sources (Banwart, 1989). Since coliforms are both of faecal and non-faecal origin, they are capable of

multiplying outside the animal body, hence their presence in high numbers in camel milk at the market centres. This is therefore not indicative of original contamination by coliforms but of improper handling of camel milk. Transfering of milk from container to the next during bulking towards the market makes milk sweep over wide container surfaces, thus collecting the microorganisms from container surfaces. These are coliforms in most cases.

The observation from the results (Table 3.6) is that the handling of camel milk from production through to market level enhances accumulation of coliforms because they are known to dominate over other organisms in milk with time (Christina and Bramly, 1983). How these coliforms evade the natural antimicrobial proteins in the camel milk and the organic acids produced after substrate breakdown and other products of microbial synthesis could be explained as follows: Coliforms are gram-negative rod (GNR) organisms that can adapt to several survival strategies in any food material. These survival strategies range from temperature evasions, acid tolerances and production of probiotics to forming complex patterns or cooperative organizations of colonies (Abee *et al*., 1995). The other strategies are genetically controlled (Eisenbach, 1996).

The high content of lysozyme enzyme in camel milk will have no effect on GNR (coliforms) because these organisms have their peptidoglycan layer (murein) covered at the surface by lipoproteins and lipopolysaccharides. These shield the murein from attack by lysozyme. Lysozyme only acts on gram-positive organisms, which have their outermost surface covered with peptidoglycan layer (murein) (ICSMF, 1980).

The GNR are fermentative organisms in their metabolism. They use heterofermentative pathways, which produce a mixture of organic acids, including lactic acid, acetic acid, formic

acid and propionic acid among others. These acids are produced in various concentrations and the level of concentrations may not have any effect on the coliforms, especially those from the gastrointestinal tract (GIT). It has been reported that variability in organic acid concentration affects their effectiveness on enteric organisms, especially coliforms. For example less than 0.8% propionic acid concentration in a food material does not kill enterobacteriaceae species. This effect is achieved at 1% of food material (Van der Wal, 1979). This implies that individual groups of microflora in raw camel milk have different susceptibility to different organic acids, hence the variability in their population reduction.

Coliforms are also known to produce substances called probiotics (colicins) that inhibit the growth of other microbes in their environment (Abee, *et al*., 1995). Probiotics are metabolites of bacteria that affect the growth of other competing bacteria in the same environment. For example, some lactic acid bacteria of the coliform group produce hydrogen peroxide, acetic acid and others produce bacteriocins. Bacteriocins are very active against gram-positive but not gram-negative organisms (Abee, *et al*., 1995).

Coliforms, just like other microbes use several gene products to control their movement within the environment. The cell "senses" the concentration of chemo-attractant (or repellant) by measuring the fraction of receptors occupied by the signaling molecules. At high concentrations, the chemotactic response vanishes because of receptor saturation. At lower limit of attractant, the response is masked; hence it is negligible (Ben-Jacob *et al*., 1998). The movement of motile bacteria (chemotaxis) involves changes in the movement of the cell in response to a concentration gradient of certain chemical fields (Adler, 1969), Berg and Porcell 1977, Lackiie,

1986, Berg, 1993). Thus chemotaxis enables microbial cells in a variety of natural environments to obtain more favourable conditions, such as movement towards nutrients, escape from predators, movement towards specific surfaces and protection by cellular aggregation (Eisenbach, 1996). Therefore, chemotaxis implies a response to an externally produced field eg acidity or nutrients. However, self generated bacterial chemotactic signaling by secretion of amino acids and peptides, organic acids and other probiotics have been reported (Budrene and Berg 1991 and 1995, Blat and Eisienbach 1995, Woodward *et al*., 1995).

In the GNR, the mode of chemoattraction involves membrane receptors such as the Tar receptors (These are bacterial gene controlled self generated amino acids and peptides that respond to externally produced fields such as attraction) for chemotaxis (Ben-Jacob *et al*., 1998). This chemotaxis based adaptive self-organization may also explain the GNR coliforms dominance in the market raw camel milk despite the acidity development as shown in fig. 3.3.

All isolates in this study, both gram-positive and gram-negative did not survive temperatures > 55^0 C on incubation (Table 3.9). It is known that the majority of coliforms are killed at temperatures between 45 and 55^0 C (Wassen and Strauch, 1976; Van Soest, 1982). Heat production during fermentation also contributes to the inhibition of coliform bacteria and some pathogens like *Salmonella* and *Shigella* (Chung and Coepfert, 1970). This suggests that pasteurizing or boiling may eliminate the contaminants in raw camel milk. However, thermal death of organisms will mostly depend on initial load of microorganisms in the milk. The higher the initial load, the more the heat is required to reduce the load by 1-log cycle. Heat resistance of microorganisms is gene dependant (Foster and Hall 1990, Chatfield *et al*., 1992 and Rutz, *et al*.,

1992). Temperatures above an organism's optimum for growth will stimulate synthesis of heat-shock proteins and this will lead to the cells developing increased resistance to higher temperatures; hence thermotolerance (Ben-Jacob *et al*., 1998). Therefore, the long distances and high environmental temperatures in the ASAL may have contributed to the survival of the coliforms and other GNR at market level.

3.5.3 Association of acid development and total viable counts in raw camel milk

The increase in acidity as a result of the production of organic acids like lactic acid may be associated with the inhibition of TVC in camel milk (Fig 3.3). The weak organic acids produced by the multiplication of microorganisms (Fig.3.2 B) in the milk do not dissociate hence increase in acidity (Fig 3.2 A). Coliform organisms have been reported to be sensitive to organic acids (Garotte, *et al*., 2000; Gran, *et al*., 2003; Tezira *et al*., 2004). Bacterial isolates (TVC) from raw camel milk were mostly GNR and composed mostly of coliform group (Table 3.6). Weak organic acids limit microorganisms, especially when such acids are at or below their pKa values. The acids become potent inhibitors of amino acid transport pathways in microorganisms. This way, the organisms do not metabolize, hence do not multiply. The un-dissociated form of these weak organic acids may diffuse freely through the cell membrane and ionize inside the cell to yield protons that acidify the alkaline cell interior. This activity of weak acids, eg lipolytic acids, mostly free fatty acids like butyric acid, caproic acid, capric acid, palmitic acid, stearic acid and loric acid have been used as preservatives (Adams and Moss., 1997). Coagulation of raw camel milk by acid development appeared when the TVC was > 10^5 cfu/ml with a titratable acidity of 0.26% LA (Fig. 3.3).

The explanation as to why the acid development continued while the TVC remained constant (Fig 3.2) may be associated with the intrinsic characteristics (pH, level of nutrients, redox potential, antimicrobial proteins and water activity) of the camel milk and the dominant species of the microorganisms present in the TVC. The GNR were the main isolates as the camel milk is produced and handled up to the market. Animal products like milk are rich in proteins. Protein content of camel milk is in the range of 2.7-5.4% (Farah, 1996). Proteins in nature have great buffering capacity since they are amphoteric, i.e they resist change in pH as they act both as acids and bases due to the charges in amino acids. The carboxylic amino acids predominate in cow milk; hence the acidic properties of cow milk. However, there is no information whether this has been determined in camel milk. The fresh camel milk has a pH of 6.5, thus tends towards neutral.

Antimicrobial peptides in the camel milk are thought to kill the GNR bacteria by ionically binding to the lipopolysaccharide (LPS) called lipid A. Lipid A is a component of all gram-negative microorganisms' cell walls (Jawetz *et al*., 1980). A C_{14} fatty acid is always present and is unique to this lipid. The other fatty acids vary according to the bacterial species (Costerton *et al*., 1974, DiRienzo *et al*., 1978). After binding, the peptides mediate permeabilization of the outer and inner membranes resulting in bacterial cell death or inhibition (Bearson *et al*., 1997, Guo *et al*., 1998). GNR have lipopolysaccharides and lipoproteins at their surface coating. These protect them from attack by some of the antimicrobial proteins like lysozyme, whose content is high in camel milk (Barbour *et al*., 1984).

The property of inhibiting or killing of GNR in camel milk seems to be the main cause of the long shelf life of camel milk despite the initial high counts of bacterial cells in the chain from production to marketing. The gram-positive organisms were inhibited or killed at collection centres of the camel milk production and marketing chain. The gram-positive organisms are killed by lysozyme enzyme that is high in content in camel milk.

At the market level, the acidity increased from 0.27 to 0.57% LA, corresponding to a uniformly reducing TVC (R^2 = 0.2909) (Figure 3.2). This regression predicts that 29% of the decreasing microbial load is due to acid development in the raw camel milk. However, the percentage decrease is very low. The reason for the low decrease could be associated with the survival tactics of the coliforms against acid development at this level. That is why acidity rose with a decreasing microbial load. The microbial load at the market was dominated by the GNR of fermentative type (Incidence of 55%) (Table 3.6) that was oxidase negative (Table 3.8) and they are catalase positive. The oxidase negative reaction indicates presence of enteric bacteria, especially coliforms and specifically *E.coli* (Wolfgang and Gunter, 1988).

The high acid content seems to have had an effect on the yeast content and other groups of microorganisms apart from the GNR of the market centres milk. Also, the presence of faecal coliforms in market milk indicates poor hygienic conditions in handling. The major contributing factors at this level are the sanitation of the milk container, temperature and time taken for the camel milk to reach the market. The yeasts and molds are expected to multiply fast at this acid level but this is not so as shown by results shown in Table 3.6 that shows an incidence of 3%. The organic acids produced by heterofermentation of lactose and other sugars in milk by the

GNR are said to have an inhibitory effect on the growth rate of fungi in foodstuffs (Hinton and Linton, 1988). Therefore, the low incidence may be associated with slow rate of growth of the yeasts and molds at market level.

3.5.4 Spores

Ecologically, spore forming bacteria are environmental microorganisms. At production level of camel milk, they may originate from water used to wash equipment or the dust in the milking area. Table 3.5 shows the spores at production and market levels had a significant ($P< 0.05$) difference. This agrees with reports by Alderton and Snell (1963). The gram-positive rods that are spore formers are mesophilic and common mesophilic organisms are sensitive to high acidity hence occurrence at production level before acidity increased. Fig 3.1 shows that acidity increased with time and hence confirms the decreasing number of spores as camel milk is handled to the market centers. Spores are easily inactivated at low pH because the environmental pH alters the ionic environment within the spore coat. Spores act as weak cation exchangers so that low pH permits replacement of other ions in the spore wall by H^+, thus lowering the stability of the spore (Alderton and Snell 1963). Spore formers like *Bacillus subtilis* spoil milk by producing microbial polysaccharides like dextrans, levans and amyloses from disaccharides in milk. These polysaccharides form unpleasant slime. An example is the ropy texture of milk infected with *B. subtilis*. Therefore, camel milk at production level has more spore forming bacteria as compared with market milk. Market milk will have developed acidity due to microbial activity because of the long time taken in transportation and the time-temperature combination and the initial load of microorganisms.

3.5.5 Types of microorganisms in raw camel milk

The coliforms were the majority of the GNR with *E. coli* having an incidence of 36% and *Enterobacter aerogenes* 22% (Table 3.7). They occurred mostly at market level. *Micrococci* had the highest incidence (20%) at production level. When *E. coli* is the dominant isolate, it is a clear indication of faecal contamination of camel milk. It could be from faeces of humans or other warm-blooded animals (Elmund *et al*, 1999). The other GNR, *Pseudomonas* and *Flavobacterium* were mostly oxidase-positive. Based on oxidase test, oxidation on one hand correlates with high cytochrome oxidase activity of some bacteria where carbohydrates are broken down oxidatively without formation of gas such as *Pseudomonas*, while on the other hand a negative oxidase shows presence of enteric bacteria. These enteric bacteria are mainly fermentative and produce gas. Some like *Pseudomonas* are protease producing. The proteases are heat stable enzymes and can survive pasteurization to cause spoilage. These organisms are mostly spoilage types and they break down carbohydrates oxidatively without producing gas (Wolfgang and Gunter, 1988).

The external source of contamination of milk by coliforms is water (Heeschen, 1992). Water sources in ASAL are boreholes, shallow wells, ponds, seasonal rivers and storm water. All these sources get a supply of surface runoff water when erratic rains come. This is where the coliforms associated with faecal origin may be coming from. Pastoralists deposit their faecal material in the bush on the ground surface, and these find their way into the water supply sources through runoff. They do not have the habit of constructing pit latrines. Contamination of water supplies by coliforms of human origin may be steming from here. However, because the water in ASAL is scarce, may be the camel milk handlers are the biggest source of faecal coliforms. This has to be investicated.

The GNR are known to predominate other organisms at any level of total viable count in raw bovine milk (Christina and Bramley, 1983). The most commonly occurring GNR in fresh raw bovine milk are Psychrotrophic (grow in a wide range of temperature, -5 to 35^0 C) types and include *Pseudomonas spp*., *Alcaligenes*, *Flavobacterium*, *Acinetobacter* and *Achromobacter*. Coliforms comprise most of the other psychrotrophs like *Escherichia, Enterobacter,* and *Proteus*. Most of the GNR are killed at pasteurization temperature except those that produce proteases like the *Pseudomonas spp*. especially *Pseudomonas fluorescens*. Since camel milk is consumed raw, these organisms may be the major cause of spoilage of the milk. The gram-positive rods are aerobic spore formers that occur in fresh raw milk in low numbers (< 10 cfu/ml) (Table 3.2). They include *Bacillus cereus*, *B. subtilis*, *B. stearothermophilus*, *B. coagulans* and *Clostridium spp*., such as *Cl. butyricum*, *Cl. tyrobutyricum* and *Cl. saccharolyticus*. They cause food poisoning by production of toxins (Christina and Bramley, 1983).

3.5.6 Microbial associations in camel milk based on temperature

All isolates, both gram-positive and GNR could not grow at temperatures > 55^0 C as shown on Table 3.9. Organisms that formed the psychrophilic (*Pseudomonas, Flavobacterium*) and mesophilic (*E. coli, E. aerogenes*) classes were coliforms (Table 3.7). They dominated the growth at 25-32^0 C. The other organisms that grew within this range of temperatures were the cocci. The few that grew at 55^0 C and above were mainly fungi and some spore forming bacillus type. These were both at production and market level. Temperature is one of the environmental factors that affect the growth and viability of microorganisms (Olson *et al*., 1952). This is due to temperature influence on properties like solubility of molecules, viscosity, density, osmotic

properties of cell membranes, surface tensions and hydrogen bond among other factors on the organisms. The actual temperature that permits growth of any specific organism rarely exceeds 35^0 C (ICMSF, 1980). This could be the reason for the heavy growth at 25-32^0 C. However, each organism exhibits a minimum, optimum and maximum temperature at which growth occurs. These cardinal temperatures are influenced by the foods intrinsic factors like nutrient availability, pH and water activity (a_w). This suggests that pasteurizing raw camel milk or simply boiling may eliminate most organisms that are contaminants or pathogens in raw camel milk.

3.6 Conclusion

The microbial load in raw camel milk increased gradually from 100,000 microbial cells per ml at production to over 1,000,000 cells per ml at market level. Most of the bacteria forming the load are gram-negative rods that are of coliform type. The major isolates were of the genera *Escherichia*, *Enterobacter* and *Pseudomonas.*

Camel milk standard is yet to be determined. The work on the determination of camel milk standards is in progress at KEBS at the moment and the Guildford Dairy Institute is a member of the committee through this study. The Kenya Bureau of Standards (KEBS) categorises bovine raw milk as very good when the total viable counts (TVC) are between 0-10^6 cfu/ml and coliform counts between 1-10^3 cfu/ml. Gram-negative rods are the major isolates in raw camel milk and they come mostly from the environment unlike the gram-positive cocci that may come from infected udders. Majority of the GNR are Psychrotrophs of mesophilic and psychrophilic origin. Coliforms increase from collection center to the market centres. *E. coli* is the major coliform and increases in number with increase in time lapse at high ambient temperature. Presence of *E. coli* is an indication of faecal contamination of the raw camel milk by handling from collection centers. This indicates the possibility of the presence of enteric pathogens. Camel

milk has certain properties that make it resist coagulation for more than three hours despite acid development and microbial load. These properties may not necessarily be the natural antimicrobial proteins in camel milk but may be related to the structural nature of these proteins. Further investigation is needed in this area.

CHAPTER FOUR

THE OCCURRENCE OF *SALMONELLA ENTERICA* IN RAW CAMEL MILK IN CHAIN OF PRODUCTION TO MARKET LEVEL

4.1 Introduction

Raw camel milk may occasionally play a role in transmission of pathogenic bacteria like *Mycobacterium* species, *Brucella spp* and enteric pathogens like *Salmonella, Shigella*, and *Klebsiella*. Tuberculosis is rare among camels under nomadic conditions. Almost all reports on tuberculosis in camels originate from non-pastoral situations where camels are kept in confinement and/or in close contact with other livestock (Younan and Abdurahman, 2002). Brucellosis in pastoral areas of Kenya on the other hand is more associated with goat milk than camel milk. Pastoralists in Northern Kenya consider raw camel milk as safe and raw goat's milk as potentially unsafe (Personal communication). This empirical experience could be related to both lower *Brucella* excretion rates in camels and a higher *Brucella melitensis* prevalence in goats (Ulrich and Oskar, 2002). There is no documentation to prove this either way. However, in other countries like Pakistan, Somalia, Sudan and Mauritania, Brucellosis prevalence in camels varies widely ranging from 1% to 30% positive reactors in the Rose Bengal Plate Test (Ulrich and Oskar, 2002). *Salmonella* infections are common in camels, but human *Salmonella* infections originating from consumption of raw camel milk have not been documented It should be noted that enterobacteriaceae related infections were very common in the area where this study was undertaken. This is based on the diarrhoea cases that the health centers were reporting (Personal communication). The reports concur with the isolation of *E. coli* in this study from the camel milk, which is part of their diet.

Why *Salmonella enterica* was picked for this study

Risk factors associated with *Salmonella* infection in foods are based on people feeding on food from common source. The informal distribution of camel milk has common faults in hygiene which poses a threat of outbreaks of salmonellosis. One of the faults is the use of inappropriate containers. *Salmonella* is also infectious as it can multiply in the intestine and invade the intestinal mucosa causing enteric fever. It can also produce toxins in food and when such food is ingested causes food poisoning. The time/temperature condition in which camel milk is handled from production to the market favours the organism's proliferation.

4.2 *Salmonella enterica* in Camels

Salmonella enterica infection in camels has been reported in various countries including Sudan (Curasson, 1918), Palestine (Olitziki and Ellenbogen, 1943), French North Africa (Donatien and Boue, 1944), USA (Bruner and Moran, 1949) and more recently from Somalia (Cheyne *et al.,* 1977), Ethiopia (Pegram and Tareke, 1981), Egypt (Refai *et al.*, 1984, Yassiem, 1985 and Osman, 1995) and the United Arab Emirates (UAE) (Wernery, 1992). In camels, *Salmonella* can cause enteritis, septicaemia and abortion. Faye (1997) reported that *S. typhimurium*, *S. enteritidis*, *S. Kentucky* and *S. St. Paul* are the most important in camels. Healthy camels can be health carriers of *Salmonella* (Selim, 1990). The *Salmonella* organisms have been isolated from faeces, milk and lymph nodes (Zaki, 1956, Hamada *et al*, 1963, El-Nawawi *et al*., 1982, Refai *et al*, 1984 and Yassiem, 1985). The salmonella isolated from healthy camels and those from camels with enteritidis are similar. Camels that are chronic carriers of *Salmonella* present a human health hazard through consumption of food products like meat and milk from the camel. There has been very limited foodborne infection surveillance work done in Kenya The surveillance of *Salmonella enterica* in the chain of raw camel milk production and marketing in

Kenya is one among many to come. Risks of foodborne infection from raw camel milk must be considered in view of the traditional preference for raw camel milk by the pastoralists and the general public that is taking up the consumption of camel milk on the market. (Personal communication)

This study investigated the presence of *Salmonella enterica* in raw camel milk as a potential hazard and its serotypes that may be present in camel milk being produced and marketed in Kenya.

4.3 Materials and methods

4.3.1 Sampling

The samples for isolation included the composite milk sampled from the individual camel udders, bulk milk from collection and market centers, faeces, soil and water samples. For the composite and bulk milk, the containers were shaken to mix the samples well. A cup (normally used by the farmer to distribute milk) was used to take the sample after shaking the container. About 25ml of the milk sample from the cup was poured into a sterile screw cap universal bottle and then capped. This was then put in a coolbox that contained cooling elements. Faecal samples were taken using sterile cotton swabs wrapped on splint wood sticks. The cotton swab stick was pushed into the rectum in a screwing manner of the lactating female camel whose milk sample had been taken. The swab was immediately transferred into a sterile Stuart transport medium in a screw cup bijou bottle. The handle stick was broken and the swab remained in the transport medium. The bottle was capped and put in the coolbox. Water samples were taken from the boreholes or wells that were being used as sources of drinking water for the camels. Borehole samples were taken by pumping the water out for five minutes and then taking the sample by slanting the mouth of a sterile glass bottle towards the nozzle of the water pipe. Five hundred

millitres of water were taken and the bottle capped. Well water was taken by lowering a bucket on a rope into the well, when the bucket reached the water level, it was swirled to stir the water and then lowered to scoop the water. The water from the bucket was then poured into a sterile 500ml capacity glass bottle and capped. It was then placed in the coolbox. About 200g of soil was scooped in the middle of the boma and wrapped in clean polythene papers, then transferred to the coolbox. The coolbox was maintained at 8-10^0 C using cooling elements. All samples were transported to the laboratory at KARI-Marsabit in a coolbox within 12 h of sampling and tested within 24 h. A total of 196 samples were taken along the chain of production to marketing and analysed for the isolation of *Salmonella*.

4.3.2 Isolation of *Salmonella enterica* from camel milk

4.3.2.1 Cultural method for genus *Salmonella* identification

Twenty-five millilitres of milk and water and 25g of faecal and soil samples were pipetted and weighed, respectively and inoculated into 225ml of buffered peptone water for pre-enrichment. The mixture was incubated at 37^o C for 24 h. After the incubation, the mixture was shaken gently to mix well, then using a sterile pipette, 1ml was transferred into 10ml Rappaport Vassiliadis medium (Difco). This was incubated in a water bath at 42^{o}C for 24h. After incubation, a loopful of the Rappaport Vassiliadis broth (Difco) culture was streaked on both the xylose lysine desoxycolate (XLD) agar (Oxoid) and brilliant green agar (BGA) (Oxoid) simultaneously. These were incubated at 37^{o}C for 24 h. The slow growers were incubated for 48 h. Colonies that appeared dark on XLD and those that appeared pink on BGA were taken to be non-lactose fermenters and were purified on MacConkey agar (Oxoid). The purified colonies on MacConkey agar were inoculated into the triple sugar iron (TSI) agar (Oxoid) slants by stubbing the butt and

streaking the slant. The colonies that appeared shiny - yellow-mucoid on both BGA and XLD agar were taken to be lactose fermenters and were then purified on MacConkey agar (Oxoid) as illustrated in Fig. 4.1.

The flow chart of cultural diagnosis

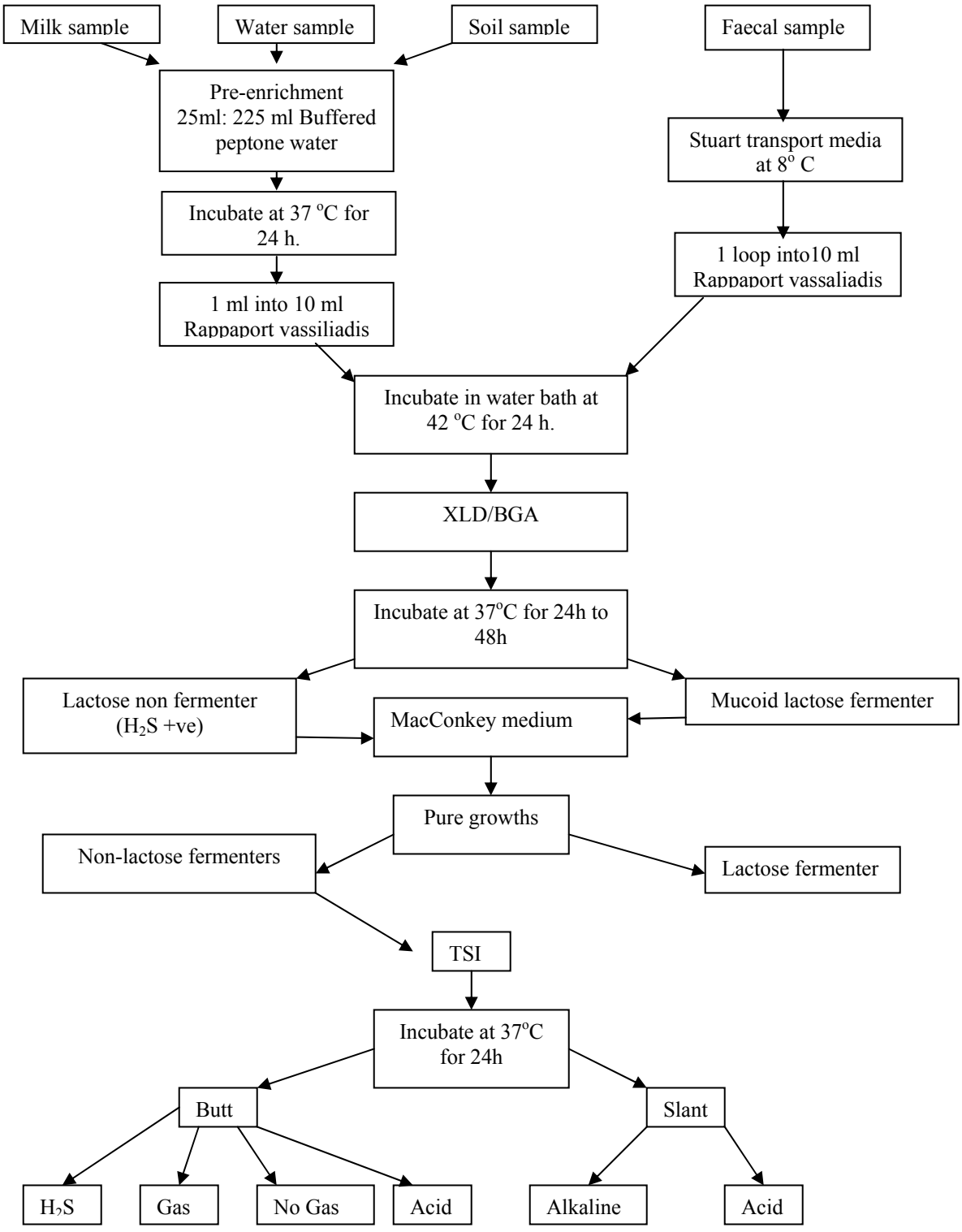


Figure 4.1: Isolation of *Salmonella enterica* from samples (Flow chart as described by Wolfgang and Gunter, 1988)

4.3.2.2 *Salmonella* species identification

This was done by standard procedure as outlined by Wolfgang and Gunter (1988). The TSI (Oxoid) reactions and sugar fermentations formed the biochemical identification tests as shown in Fig 4.1.

4.3.2.3 Biochemical identification of *Salmonella species*

Pure isolates that were lactose negative on culture, purified in an overnight culture on MacConkey agar (Oxoid) were inoculated in 10ml preparations of fermentable sugars with phenol red as the indicator and incubated at 37^{o} C for 24 h. The results were recorded based on acid and gas production after fermentation, colour changes of the indicator and H_2S production.

4.3.2.4 Serological identification of *Salmonella species*

This was done using a standard procedure as outlined by Wolfgang and Gunter (1988) using specific antisera from Biotec Laboratories limited, UK. When the specific antisera are mixed with the corresponding salmonella antigens, an antibody-antigen reaction occurs, which is observable microscopically. *Salmonella* serotypes, Somatic (O), Vi (surface) and H (Flagella) were examined.

Specimen Collection

The specimens for serological tests were isolates already determined morphologically and biochemically as *Salmonella species*. The suspected salmonella organisms were cultured overnight on nutrient agar, a non-selective media prior to testing.

Determination of O-group

A slide agglutination test using O-grouping polyvalent sera and Vi serum was used. All the reagents were left to attain room temperature. On a clean microscope slide, a drop of antiserum was placed at one end and a drop of sterile normal saline (0.85% Nacl) was placed at the opposite end of the same slide. 3-4 colonies from the non-selective media were suspended in 0.3ml sterile normal saline and a dense cell suspension was made by stiring using a wood splinter. One loopful of the cell suspension was put onto each of the drops of serum and normal saline and mixed well. The cell suspension and normal saline acted as control. The slide was gently shaken for 1min. Agglutination within one minute was regarded as positive for polyvalent O-group.

O grouping using single factor O-sera

Whenever any isolate agglutinated with one of the polyvalent O- sera, it was again tested against the corresponding monovalent antisera, which is the O-antigen structure, using the same procedure as above. The reason for testing with monovalent O was to avoid cross-reaction with non *Salmonella* genera like *Escherichia, shigella, Citrobacter* and *Proteus*. The serum that produced agglutination was considered as the name of the O-antigen possessed by the tested *Salmonella spp*.

Determining Vi group

Whenever there was no agglutination after 1min from the above test, the procedure above was carried out using Vi-serum instead of the polyvalent sera. When a positive reaction was found, a dense suspension of the organism in sterile saline solution was made and autoclaved at 121^0 C for 15min. After cooling to room temperature, the agglutination test was repeated with

polyvalent serum and Vi serum using the inactivated cells. If live cells that were negative with polyvalent serum and positive with Vi serum, were heated and became positive with polyvalent serum and negative with Vi serum, then they were taken to be *Salmonella enterica* Typhi.

Determining the H antisera

Tube agglutination test was used to determine the presence of H antigens. H polyvalent and monovalent sera were left at 20^0 C. A pure culture of suspected *Salmonella spp* (cultured 8 h before at 37^0 C in non-selective broth) was diluted with equal volume of saline containing 1% formalin. The mixture was dispensed at 0.05ml of each specific H-serum into a small test tube. Antigen suspension of 0.4 – 0.5ml quantities was added. A control was prepared which contained the antigen suspension only. The tubes were mixed well for 2min and allowed to stand in a water bath at 50-52^0 C for 1h. They were observed for agglutination. The name of the serum that produced agglutination corresponds to the name of the H-antigen possessed by the test organism.

4.4 Results

Out of 196 milk samples taken along the chain of production and marketing, 26 samples yielded *Salmonella enterica* species on laboratory analysis, forming 13.2% of the total milk samples taken as presented in Table 4.1.

Table 4.1 Salmonella isolation from different samples obtained from the environment of production through to marketing

Level	n	+ve	%	Chi-test
Environment	31	6	19.35	**
Production	120	15	12.50	***
Processing	19	5	26.32	NS
Market	26	0	0	0
Total	196	26	13.2	-

Key: ** $P < 0.05$, *** $P < 0.01$, NS not significant (Environment-soil & water, Production- camel udder & faeces, Processing- pooled milk and market- selling points.

Table 4.1 shows the incidence of *S. enterica* in the sampled herds and the market outlets. Nairobi is the major market in this case. *S. enterica* occurence was highly significant ($P < 0.01$) at production level followed by environmental level ($P < 0.05$). At processing level, the occurrence was not significant ($P > 0.05$). There was no *Salmonella enterica* isolation at the market level.

Serological identification of Salmonella in raw camel milk is presented in Table 4.2.

Table 4.2 Serological identification of *Salmonella enterica* in camel milk

Serovar isolate	n	Polyv.O Ag	S/factorO.Ag	Subgroup	Serotype
Paratyphi	15	A-G	C-factor 6, 7	C_1-anti-O-sera 7	Paratyphi C
Typhi	11	A-G	D-factor 9 (Vi)	D_1-anti-O-sera 9	Typhi

S/factor O. Ag -- single factor O Ag, Polyv.—polyvalent

Salmonella enterica serovars isolated from camel milk were polyvalent O antigen, reaction group A-G. Those with single factor O antigen C-factor 6, 7 in subgroup C_1-anti-O-sera 7 were identified as *Salmonella enteritica* serovar Paratyphi C. Those with single factor O antigen D-factor 9 (Vi) in subgroup D_1-anti-O-sera 9 were identified as *Salmonella enterica* Typhi (Table 4.2). *Salmonella enterica* was found in all the sample categories that represented the camel milk production environment, thus the milk, faeces, water and soil (Table 4.3). Two serovars were represented (Table 4.2). The serovar Typhi was found in three of the four sample categories accounting for 42.3% of the total isolates while serovar Paratyphi C was also found in three categories accounting for 54%, but missing in soil category as shown in Table 4.3.

Table 4.3 *Salmonella enterica* serovar occurence from camel milk production environment and market

Sample Category	N (+ve isolate)	% Positive	S. enterica serovar (n)
Milk(Udder &Pooled)	89 (15)	16.85	Paratyphi C (7), Typhi (8)
Water	25 (5)	20	Paratyphi C (3), Typhi (2)
Faeces	50 (5)	10	Paratyphi C (4), Typhi (1)
Soil	6 (1)	16.66	Paratyphi C (0), Typhi (1)
arket	26 (0)	0	Nil
Total	**196 (26)**	**13.26**	**Paratyphi (14) (7.14%), Typhi (12) (6.12%)**

Key: (n)- indicates the number of positive isolates and type of serovar

Table 4.3 shows the occurrence of *Salmonella enterica* serovar types in the various sample categories analysed. The serovar Paratyphi C had 7.14% occurrence in the total samples that were analysed while serovar Typhi had 6.12%.

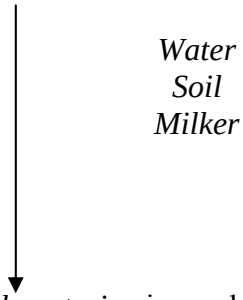
4.5 Discussion

The presence of *S. enterica* serovars Typhi and Paratyphi C in camel milk confirms faecal contamination of the camel milk through the production and marketing chain in Kenya (Table 4.1). Even if the numbers of *S. enterica* cells are few in the samples analysed and may not necessarily cause a foodborne disease, they should be considered a potential health hazard. The environment in which camel milk is produced supports the existence of these *S.enterica* serovars, as indicated by the occurence significance level of $P < 0.05$. For the pathogens to contaminate the camel milk they must go through the chain of contamination where the pathogens have a

source and there exists a mode of transmission to the food (Norman, 1999). The links in the infectious process will include transmission of the causative mirobial agent from the environment in which the food is produced, processed or prepared to the food itself, a source and reservoir of transmission of the causative agent, transmission of the causative agent from source to the food and growth support of the microorganism through the food or host that has been contaminated (Fig 4.3).

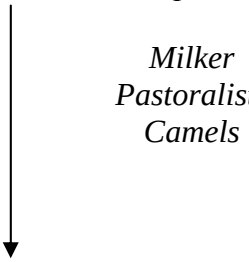
S. enterica from camel milk must have originated from the environment in which camel milk is produced and processed. The environment includes the camel itself, the camel herders, camel milk handlers, water and soil. This is supported by the results (Table 4.3) where *S. enterica* milk contamination accounted for over 16.85% while water and faeces accounted for 20% and 10% respectively. It is possible that the camel milk is harvested from the camel udder when already contaminated through secretion of the pathogen into the milk cistern. The camel may be a healthy carrier and acts as a host of the pathogen. The camel herders and milkers could also be heathy carriers and shed the *Salmonella enterica* in the environment through deposition of faeces in the environment, as is the case with the pastoralists in the ASAL. The herders and milkers also act as hosts for the pathogen. These deposited faeces eventually contaminate the water sources for human and animal drinking and the cycle of infection continues in a web of causation (Fig 4.4). Other Salmonella studies on the camel have always focused on disease assessment, especially camel calf diarrhoea (Malik *et al*., 1967, Ambwani and Jaktar, 1973, Wernery, 1992, Nation *et al*., 1996, Salih *et al*., 1998a and b). *Salmonella* in camels has also been isolated from lymphnodes and intestines of slaughtered dromedaries in Egypt (Refai *et al*., 1984, Yassiem, 1985).

Transmission of *Salmonella enterica* from the environment to the camel milk



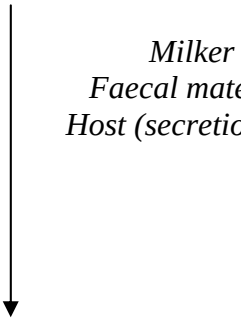
Water
Soil
Milker

Source and reservoir of *Salmonella enterica* in production environment



Milker
Pastoralists
Camels

Transmission of *Salmonella enterica* from source to camel milk



Milker
Faecal material
Host (secretion into milk) camel

Growth support of *Salmonella enterica* through the camel milk that is contaminated

Chemical composition of camel milk (nutrients),
Other intrinsic factors of camel milk,
Temperature
Host specificity (Host preference)

Figure 4.2: Suggested schematic representation of the links to camel milk contamination by *Salmonella enterica* (Personal view)

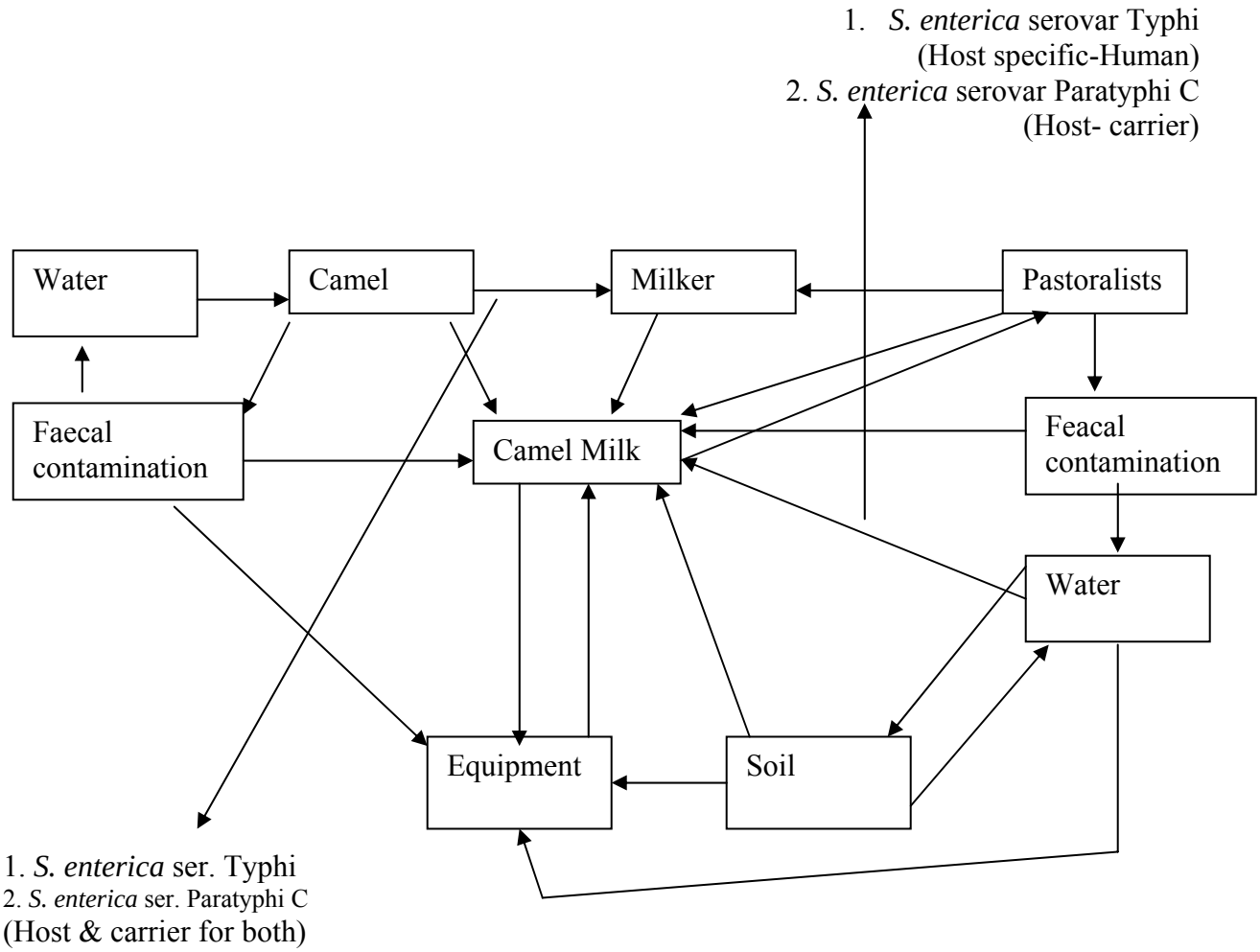


Figure 4.3: Suggested web of causation showing factors that influence contamination of camel milk by *Salmonella enterica* at the production level (Personal view)

The isolation of *S. enterica* was only in samples from the production level ($P < 0.01$) and environment ($P < 0.05$). The isolation was not significant at pooled centres (Table 4.1). Therefore, the critical point for control of this organism is at the production level. When the milk reached the market, there was no *S. enterica* isolation from market samples as indicated in Table 4.1. The disappearance of *Salmonella* organisms from raw camel milk at market level can only be explained by the survival tactics embraced by these organisms. They include, resistance to cationic peptides and acid developments in the milk, entering a viable but non-cultural state, escaping from the extracellular environment and surviving as macrophage in a phagositic cell of the milk. The details of these tactics are explained in the literature review. Any of these tactics may explain why there was no *Salmonella enterica* at the market level.

4.5.1 The main *Salmonella enterica* serovars isolated from camel milk

Only two serovars of *Salmonella enterica* were isolated from camel milk as reported in Table 4.2. These were *Salmonella enterica* Paratyphi C and *Salmonella enterica* Typhi. *S. enterica* Typhi is a strict human serovar that causes diseases associated with invasion of the blood stream. These narrow range or host-adapted serovars are transmissible through faecal contamination of water or food (Kenneth, 2005). Camel milk is produced in the environment where human faecal waste is deposited within the environment (Fig 4.4). The pastoralists do not construct pit latrines. Whenever rains come, the faecal waste is carried as surface run-off to the nearest water body like a river, dam, borehole or shallow well. These are used as sources of drinking water for both camels and humans. Since there is no controlled water and human sewage system, there is a likelihood of human faecal contamination of water. The human healthy carriers like the herders, milkers and those who handle the camel milk in the chain of procurement are the main source (host) of the Typhi serovar but also carriers of Paratyphi C.

The paratyphi group occurs in almost all domestic animals and this means they are transmissible to man. They are known to cause food poisoning in man (Pietzsch, 1981). The epidemiology of serovar Paratyphi C is such that it occurs sporadically. In North America, it is extremely rare as compared to serotype A and B. Its mode of transmission is either direct or indirect contact with faeces or contaminated food, especially milk and milk products (PHAC, 2001). As a health hazard, serovar Paratyphi C causes bacterial enteric fever with an abrupt onset. The infection is clinically similar to typhoid fever, but milder with a lower fatality rate (PHAC, 2001). Kariuki *et al*. (1999) in a study on typing of serotype Paratyphi C isolates from various countries using plasmid profiles and pulsied-Field Gel Electrophoresis (PFGE) showed that Paratyphi C is a resistant serotype to most antimicrobials.

4.6 Conclusions

This study has shown that camel milk production and marketing chain is contaminated with *Salmonella enterica*, a clear indication of faecal contamination of camel milk. The highly susceptible level of contamination is at the production level. The serovars mainly involved were *S. enterica* serotype Typhi and *S. enterica* serotype Paratyphi C. *Salmonella enterica* serotype Typhi is highly host pecific for humans. This suggests that there is direct and indirect human and animal faecal contamination of the camel milk through the chain of production and marketing. For both pathogenic serovars, the reservoirs are pastoralists and camels and they keep excreting them into the environment. The molecular basis of the different disease outcomes they cause and host specificity is not yet well understood. At the moment, the genome for Paratyphi C is being analysed for this different disease outcomes and other virulent determinant phenomenon. Therefore, camel milk being produced and marketed in Kenya is not safe for human

consumption in the raw state since it is contaminated with *Salmonella enterica*, a potential hazard.

CHAPTER FIVE

GENERAL DISCUSSION

5.1 Rationale for the study

Camel milk production and marketing in Kenya has initiated the need to understand the the commodity's microbial characteristics. The raw camel milk can be contaminated with pathogenic and spoilage microorganisms if proper handling practices are not followed.

5.2 Methodological approaches

In this study, the monitoring of camel milk microbial infections from pastoral production areas using total bacterial counts and *Salmonella enterica* isolation and identification had serious logistical problems because of the distance from the farms (Bomas) to the laboratory and environmental temperatures. The bacterial counts in camel milk from pastoral areas may reflect a higher count than originally present in the samples taken. However, this problem was minimized by having proper sampling equipment, especially the sterile sampling bottles and the coolbox that was maintained at 8-10^0 C using ice packs. Also the counts were converted into $\log_{10}$ form for statistical analysis to avoid dealing with large numbers.

5.3 The Results

In the first objective, the initial load of microbial contamination of raw camel milk at production through to market indicated a gradual increase in contamination. At production level, there was a smaller load of upto 100,000 microbial cells per ml of raw camel milk. At collection (bulk) and market level, the microbial cells per ml increased to above 1,000,000. This suggests that raw camel milk handling practices after harvesting lacks proper sanitary care.

Most of the bacterial isolates from raw camel milk were GNR. Some were oxidase positive and others oxidase negative. The oxidase negative ones were more than the oxidase positive and were mostly of coliform type. The oxidase negative coliforms suggest the presence of faecal coliforms (Wolfgang and Gunter., 1988). The coliforms were isolated mostly at market level. This concurs with the findings of Christina *et al*., (1983) who found out that at market level, coliforms comprised 10-30% of the total counts of the GNR isolated. The other GNR that were oxidase positive occurred at production level. They included *Pseudomonas spp*. The gram-positive rods were mostly spores of *Bacillus spp* and occurred at production. The coliforms comprised mostly of *Escherichia, Enterobacter* and *Pseudomonas spp*. These genera are known to be psychrotrophic, thus they grow in a wide range of temperatures from –5 to 35^0 C. *Pseudomonas spp* are known to produce the protease enzyme that is heat stable and this makes them important in milk spoilage (Hobbs, 1973, Goepfert and Kim, 1975).

The sources of these coliforms in raw camel milk are likely to be contaminated containers, water and the soil. The time taken for the raw camel milk to reach the market from production and the ambient temperatures of the milk also contribute to the multiplication of these coliforms, hence dominating the rest of the microorganisms at market level. All organisms isolated in raw camel milk were killed or inhibited at temperatures upto 55^0 C and below. This is below pasteurization temperature. This suggests that if raw camel milk is pasteurized at production level these organisms will be eliminated from the milk, and this would ensure long shelve life as long as temperature is controlled.

By understanding the microbial quality and safety of camel milk, the standards for raw camel milk will be realized. Processing it into other products like yoghurt and cheese as a value addition activity will meet the public health legal requirement. This will improve livelihoods of camel farmers in the ASAL of Kenya. The opening of 'Vital Camel milk' processing plant in Nanyuki, the recognition of camel milk in the draft Dairy policy of 2006 and the formulation of standards for whole camel milk by the Kenya Bureau of Standards that is going on is a development in the right direction for the camel keepers in the ASAL.

In the second objective, the results have shown that the raw camel milk being produced and marketed is contaminated with *Salmonella enterica* pathogen. It indicates that there is faecal contamination of the raw camel milk in the chain of production and marketing. *S. enterica* causes Salmonellosis as a food infection through ingestion of the organism by humans. About 1 million cells of *Salmonella* organisms are required to be ingested for the infection to occur (Norman, 1999). The number of *Salmonella* cells isolated in this study was less than 1 million, suggesting that they may not cause the infection to the consumer. However, despite the small numbers of *Salmonella* organisms isolated, the situation should be seen as potentially hazardous. Celum *et al*., (1987) reported that Salmonellosis is harmful to persons with AIDS. Archer (1988) stated that AIDS patients are quite susceptible to Salmonellosis.

Two *S. enterica* serovars were isolated in this study. These were *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi C. *S. enterica* serovar Typhi causes enteric fever (Typhoid), which is strictly a human disease. *S. enterica* serovar Paratyphi C causes food poisoning (Pietzsch, 1981) but may also cause bacterial enteric fever (PHAC, 2001, Kariuki *et al*., 1999).

5.4 Conclusion

Generally, the results of the two objectives suggest that the raw camel milk being produced and marketed has a high load of microflora ranging from 10^3 cfu/ml at production level to 10^8 cfu/ml at collecting (bulk) and market centers within the study area. The milk is also contaminated with the enteric pathogen *Salmonella enterica*. The serovars identified indicate the existence of healthy carriers in both camels and humans.

The approach to improving camel milk hygiene should be based on the principles of food sanitation. The principles emphasize the need for food safety based on improved sanitary practices like avoiding contamination and use of appropriate equipment for processing. The following recommendations have been suggested in this study based on the results obtained to improve the hygiene of camel milk production and marketing chain and hence its safety to the consumers.

CHAPTER SIX

RECOMMENDATIONS

Based on the findings in this study, the following recommendations are made.

1. The risk factors in the camel milk production environment that were associated with camel milk microbial infections in this study included equipments, water, soil, animals and humans. It is suggested that another study be done to understand the interaction of these risk factors to come up with point sources and non-point sources of both pathogenic and spoilage microbial infections of camel milk production and marketing chain. This will help in understanding the approach to pathogen or spoilage organisms tracking and have implications for production, processing and marketing level monitoring of camel milk chain.
2. The presence of *S. enterica* serovars Typhi and Paratyphi C in raw camel milk highlights the potential health hazards of *Salmonellae* infections to consumers of camel milk. The serovars may originate from camels, cattle, poultry, birds or humans that occur in the pastoral ecosystem. Two studies are recommended to be done;
    (i) A large scale study involving the collection of detailed data needed to obtain information at production level of risk factors and the multiple sources of *Salmonella enterica* serovars.
    (ii) Compare or link the *S. enterica* serovars sources at molecular level to determine host specificity. This information could be used during outbreaks and to develop camel milk chain safety plans and hence reduce the exposure of camel milk consumers

to *S. enterica* serovars.

3. The output of the data generated can be used to develop a hazard analysis critical control point (HACCP) system in the camel milk production and marketing chain. Control points will be located at particular points in camel milk production and marketing chain where the hazard of *S. enterica* serovars and spoilage organisms should be destroyed or controlled.
4. Currently, there is the practice of mixing evening milk and morning milk at collection centres. This was reflected in the increase in TVC at collection and market levels. This practice increases growth of coliforms that are introduced at post harvest handling level, hence spoilage of the milk. Therefore, evening milk should not be mixed with morning milk, each batch should be treated separately and be sold separately.

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APPENDICES

Appendix 1. Main isolates in (Oxidase negative and positive) from production and market centres

1. Market (oxidase negative) isolates

S/no	Gramrxn	Rxn on EMB	Lact	Cata	Ind	MR	VP	Cit	Glu	Spp
Cm1-I	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	10%	100%	*Ent. aerogenes*
Cm1b-I	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	100%	*E. coli*
Cm2-I	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	80%	100%	*Ent. aerogenes*
Cm3-I	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	50%	100%	*Ent. aerogenes*
Cm4-I	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	40%	100%	*Ent. aerogenes*
Cm5-G	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	50%	100%	*Ent. aerogenes*
Cm6-I	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	40%	*E. coli*
Cm7-I	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	100%	*E. coli*
Cm8-I	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	50%	100%	*Ent. aerogenes*
Cm9-I	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	100%	*E. coli*
Cm10-I	-ve	Metallic sheen	+ve	+ve	+ve	+ve	-ve	0%	100%	*E. coli*
Cm11-Ib	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	0%	100%	*Ent. aerogenes*
Cm12-I	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	30%	100%	*Ent. aerogenes*
Cm13-I	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	20%	100%	*E. coli*
Cm14-I	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	30%	100%	*Ent. aerogenes*
Cm15-I	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	20%	100%	*E. coli*
Cm16-G1	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	100%	*E. coli*
Cm16-G2	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	0%	100%	*E. coli*

Cm17-G	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	0%	100%	*Ent. aerogenes*
Cm18-G	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	100%	*E. coli*
I1-26/4	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	20%	100%	*Ent. aerogenes*
I2-27/4	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	30%	100%	*E. coli*
I3	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	20%	100%	*Ent. aerogenes*
G1-27/4	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	10%	100%	*Ent. aerogenes*
2-27/4	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	30%	100%	*Ent. aerogenes*
G2b-27/4	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	20%	100%	*E. coli*
G3-26/4	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	30%	100%	*E. coli*
Cm1	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	100%	*E. coli*
Cm2	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	20%	100%	*Ent. aerogenes*
2^{nd} east	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	30%	100%	*E. coli*
20^0 C	-ve rod	Metallic sheen	+ve	+ve	+ve	+ve	-ve	20%	100%	*E. coli*
30^0 C	-ve rod	Mucoid pink	+ve	+ve	-ve	-ve	+ve	40%	100%	*Ent. aerogenes*
2. Production (oxidase-negative and coagulase-negative isolates)										
AMA2	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	100%	*E. coli*
AMA7	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	0%	100%	*E. coli*
AMA9	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	20%	100%	*E. coli*
AMAp	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	100%	*E. coli*
AMO1	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	30%	100%	*E. coli*
AMO2	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	100%	*E. coli*
AMO5bf1	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	10%	100%	*E. coli*
AMO6bf1	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	0%	100%	*E. coli*

AMO6bf9	+ve cocci		-ve	+ve						*micrococci*
AMO4s	+ve cocci		-ve	+ve						*micrococci*
AMO5s	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	0%	100%	*E. coli*
AMOR	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	0%	100%	*E. coli*
AMO3bf1	-ve rods	Metallic sheen	+ve	+ve	+ve	+ve	-ve	0%	100%	*E. coli*

3. Production (Oxidase-positive) isolates

S/no	Gram rxn	Cata	Coag	Lac	Glu	ind	MR	VP	Cit	
AMA3	Yeast	+ve	+ve	+ve						
AMA10	-ve rod	+ve	-ve	+ve	+ve	+ve	+ve	-ve	30%	*E. coli*
AMAc	Yeast	+ve	+ve	+ve						
AMO2bf1	-ve rod	+ve	-ve	-ve						
AMO2bf2	-ve rod	+ve - ve								
AMO2bf9	+ve cocci	+ve	-ve	-ve						
AMO9	-ve rod	+ve	-ve	-ve						
AMOp	-ve rod	+ve	-ve							
AMO3bf1	-ve rod	+ve	-ve							
CM12-mg	-ve rod	+ve	-ve	+ve	+ve	+ve	+ve	-ve	30%	*E. coli*

Appendix 2 Temperature associations of isolates from both production and market Levels

S/No	20^0C(72h)	32^0C(48h)	55^0C(48h)	Gram rxn	lac	cata	coag	Oxida	Genus/Spp
AMA2	+	+	-	-ve rod	+	+	-	-	*E.coli/coliform*
AMA3	+	+	+	Yeast					*Yeast*
AMA7	+	+	-	-ve rod	+	+	-	-	*E.coli/coliform*
AMA9	+	+	-	-ve rod	-	+	-	-	*E.coli/coliform*
AMA10	+	+	-	-ve rod	-	+	-	+	*Pseud/Flavobact*
AMAp	+	+	+	+ve rod	-	+	-	-	*Bacillus*
AMAR	+	+	+	Yeast					*Yeast*
AMO1	+	+	-	-ve rod	-	+	-	-	*E.coli/coliform*
AMO2	+	-	-	-ve rod	+	+	-	-	*E.coli/coliform*
AMO2bf1	+	+	-	-ve rod	-	+	-	-	*Coliform*
AMO5bf1	+	+	-	-ve rod	-	+	-	-	*Coliform*
AMO6bf1	+	+	-	-ve rod	-	-	-	-	*Coliform*
AMObf9	+	+	-	+vecocci	-	+	-	-	*CNS/Micrococci*
AMO9	+	+	-	-ve rod	-	+	-	+	*Pseud/Flavobact*
AMO4s	+	+	-	+vecocci	+	-	-	-	*CNS/Micrococci*
AMO5s	+	+	-	-ve rod	+	-	-	-	*Coliform*
AMOR	-	+	-	-ve rod	+	-	-	-	*Coliform*
AMOp	+	+	-	-ve rod	+	+	-	+	*Pseud/Flavobact*
AMO3bf1	+	+	-	-ve rod	-	-	-	-	*Coliform*
Cm1MI	++	+	-	-ve rod	+	+			

Appendix 3. Biochemical identification of Salmonella species

TSI Reaction				Sugar Fermentation					Possible serovar
Isolate/no	**Butt**	**Slant**	**H_2S**	**Glu**	**Lac**	**Man**	**Urea**	**Cit**	
MKF 7	AG	A	+	AG	-	+	-	-	*S. paratyphi*
MKM 12	AG	Alk	+	AG	-	+	-	-	*S. paratyphi*
MNM11	A	Alk	+	A	-	+	-	-	*S. typhi*
GW1	A	Alk	+	AG	-	+	-	-	*S. paratyphi*
PHW	A	Alk	+	AG	-	+	-	-	*S. paratyphi*
MNM7	A	Alk	+	AG	-	+	-	-	*S. paratyphi*
MKF3	AG	Alk	+	AG	-	+	-	-	*S. paratyphi*
MKF5	A	Alk	+	AG	-	+	-	-	*S. paratyphi*
MKM7	A	Alk	+	AG	-	+	-	-	*S. paratyphi*
MKM10	AG	Alk	+	AG	-	+	-	-	*S. paratyphi*
KW1	A	Alk	+	AG	-	+	-	-	*S. paratyphi*
MKF11	A	Alk	+	AG	-	+	-	-	*S. paratyphi*
MKM13	A	Alk	+	A	-	+	-	-	*S. paratyphi*
MGM2	A	Alk	+	A	-	+	-	-	*S. typhi*
LW	A	Alk	+	A	-	+	-	-	*S. typhi*
MGM1	A	Alk	+	A	-	+	-	-	*S. typhi*
GW2	A	Alk	+	A	-	+	-	-	*S. typhi*
MNM2	A	Alk	+	A	-	+	-	-	*S. typhi*
MGM10	A	Alk	+	A	-	+	-	-	*S. typhi*
GS	A	Alk	+	A	-	+	-	-	*S. typhi*
MGM13	A	Alk	+	A	-	+	-	-	*S. typhi*
MNM3	A	Alk	+	A	-	+	-	-	*S. typhi*
MNM4	A	A	+	A	-	+	-	+	*S. typhi*
MNM2T	A	Alk	+	A	-	+	-	-	*S. typhi*
Allif II	A	Alk	-	A	-	+	-	-	*S. typhi*
MKM5	A	Alk	+	AG	-	+	-	-	*S. paratyphi*

Appendix 4: Camel breeds in Kenya

Somali breed

Body size: Large body size (average 550kg)

Milk Yield : higher than other indigenous Kenyan camels (average 6litres)

Feed Requirements : heavy feeder compared to other indigenous camels

Suitable Terrain : smooth (bulky body)

Genetic Makeup : different from other indigenous breeds

Coat Colour : predominantly cream

Udder Size : fairly large

Pakistan breed

Body size: smaller in body size than Somali but larger than Gabbra Rendille (average 400kg)

Milk Yield : superior to all Kenyan Camels (average 15litres)

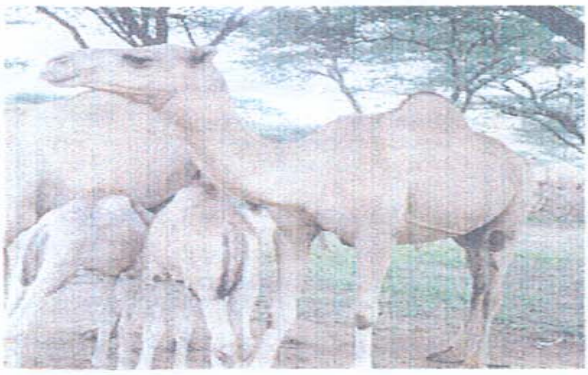
Feed Requirements : gross feeder

Suitable Terrain : yet to be tested

Genetic Makeup : totally different from other Kenyan camels

Coat Colour : predominantly gray

Udder Size : large and pendulum with large teats



Body size: smaller than Somali (average 350kg)

Milk Yield : Lower than Somali (average 3.5 litres)

Feed Requirements : reputed to do well under poor pasture conditions

Suitable Terrain : rough (small body size)

Genetic Makeup : genetically the same

Coat Colour : similar to Somali

Udder Size : medium

Rendille/Gabra breed

Body size: Reputed to be smaller than Gabbra Rendille breed (average 300kg)

Milk Yield : lower in milk production than Rendille Gabbra (average 1.5litres)

Feed Requirements : reputed to have a lower forage requirement than all camel breeds in Kenya

Suitable Terrain : similar to Rendille Gabbra

Genetic Makeup : results not yet out yet

Coat Colour : tend to be hairy an grayish in colour

Udder Size : similar to Rendille Gabbra

Turkana breed