OCCURRENCE OF SUBCLINICAL MASTITIS, BRUCELLOSIS AND FACTORS RESPONSIBLE FOR CAMEL MILK CONTAMINATION IN GARISSA AND WAJIR DISTRICTS OF NORTH-EASTERN KENYA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR MASTER OF SCIENCE DEGREE OF THE UNIVERSITY OF NAIROBI [APPLIED MICROBIOLOGY (BACTERIOLOGY OPTION)]

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This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

This thesis is dedicated to my family; my loving and caring wife Monica Mumbi, sons Lewis Wanjohi and Dennis Kireru and daughters Maryann Wanjira and Joy Wangechi.

ACKNOWLEDGEMENT (AS WORDED BY LATE DR WANJOHI)

I express my heartfelt gratitude to my supervisors Dr. C.G. Gitao , Prof. L.C. Bebora and Dr. G M. Muchemi for their constant and untiring encouragement, guidance and stimulating discussions during this study. Special thanks also go to Dr.C.G Gitao for accommodating me in his project (Camel milk value chain enhancement: The case of Garissa and Wajir pastoral communities of North Eastern Kenya) from where this study was done; Prof. L. C. Bebora for introducing me to Kenya Medical Research Institute (KEMRI), Centre for Microbiology Research (CMR) from where some important tests were done; and to Dr. G.M. Muchemi (Department of Public health, Pharmacology and Toxicology- PHPT) who together with Dr. Joshua O. Onono (PHPT) assisted me in statistical analysis of my data. I am grateful to the University of Nairobi for allowing me to pursue my studies and research work in that institution. I am deeply indebted to the Department of veterinary pathology, microbiology and parasitology, for providing all the necessary support in terms of manpower and facilities throughout my study period.

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TRIBUTE TO LATE DR WANJOHI

This work was mostly done by Dr Wanjohi who died in 2009 with support from Dr. Gitao C. G, and Prof Bebora L.C. It is published in his memory. The work was part of a bigger project called “Camel milk value chain enhancement: The case of Garissa and Wajir pastoral communities of North Eastern Kenya KAPP CGS 06/1RC-LVST and the project leader was Dr. Gitao C.G.. Most of the laboratory work was done at the Dept of microbiology and parasitology, University of Nairobi. Some of the preliminary laboratory work was done at Garissa veterinary Investigation laboratory who are acknowledged.

TABLE OF CONTENTS

TABLE OF PLATES

TABLE OF FIGURES

ABSTRACT

Camel milk is one of major food components for the pastoralists in North-Eastern Province, Kenya. This milk is widely marketed in the Garissa and Wajir districts and is currently being sold in distant markets in Nairobi and other far places. The demand for camel milk is thus fast growing, necessitating the need to establish safety level for the milk; more so since these people have a traditional preference for raw milk. The casual way that farmers and traders in Garissa and Wajir districts handle milk exposes the milk to contamination along the market chain. This is more so considering that milk is a very nutritious medium; readily supporting growth of microorganisms. Other managemental practices, like tying of camel teats with a soft bark as an effort to prevent the calf from suckling, may contribute to the development of mastitis in the camels. This study was, therefore, geared towards establishing the safety of camel milk, through establishment of extent of subclinical mastitis, incidences of brucellosis and factors responsible for camel milk contamination in the two districts. The main objectives of the investigations were: (1) to collect baseline data on socio-economic practises of the respective people, (2) to establish the extent of subclinical mastitis in the two areas, (3) to determine milk quality and bacterial contamination along market chain, and (4) to check for occurrence of brucellosis in the camels.

This study was cross-sectional, conducted on livestock grazing units, watering points and along market chains within the two districts. The methods used included: participatory approaches, questionnaire administration, and laboratory analysis (physical, bacteriological and serological) using standard methods. From questionnaire analysis, the main reason for keeping camels was for economic, domestic and socio-cultural purposes, like selling of milk and meat to make money. Camels were also kept as draft animals, and as a sign of wealth. Farmers kept more female adults than males and growers. They preferred to drink raw milk –

apart from it being a traditional preference, they believed that the milk was nutritious and a source of vitamin C. They also preferred to consume sour milk. They were aware of the various diseases affecting their camels, listing diarrhoea, camel pox, brucellosis and mastitis as the common ones. Sick animals were treated by owners or herdsmen using conventional medicine or herbs. Milk from mastitic and treated camels was given to calves, some sold and some consumed; although most of it was poured off. The farmers also kept other animals – cattle, goats, sheep, donkeys and chickens – all were grazed together in the rangelands and watered at the common points. There was, therefore, a high chance of spread of diseases, like mastitis and brucellosis, among the animals. The farmers practised dry hand-milking and kept the milk in traditional gourds, some of which are difficult to clean properly.

Using California Mastitis Test, 61.2% of the samples were positive for subclinical mastitis; All the milk samples also yielded mixed types of bacteria on culture, which included Staphylococcus (90.1%), Streptococcus (84.9%), Escherichia (59.9%), Klebsiella/ Enterobacter (95.8%) and Bacillus (45.8%). Both direct smear and culture did not demonstrate presence of Brucella organisms.

On establishment of milk quality, apart from specific bacterial isolations, as given above, Total Coliform Counts ranged between 1.3x106-1.9x108 coliform forming units (cfu)/ml and Total Viable Bacterial Counts ranged between 1.2x106-1.6x108 cfu/ml. Sixteen E. coli isolates were sero-typed, of which one tested positive for serotype O157:H7; this is significant considering the fact that the organism multiplies very fast and could easily attain infective levels. Overall, assessment of physical characteristics showed that 289 samples (75.3%) had gross dirt/particulate matter including grass/leaves, sand/soil particles and/or black charcoal particles. Thirty four samples had abnormal yellowish colour. Generally,

formation of flakes in the Alcohol test was recorded in 128 samples (33.3%), indicating they were either acidic, mastitic or colostrum milk. Seventy samples (18.2%) had offensive/bad odour/smell (sour or foul smell). Generally, results of pH determination of the milk samples indicated that 119 of them (31.0%) had a pH of "6", 203 (52.9%) had a pH of "7" and 62 samples (16.2%) had a pH of "8". The range of specific gravity of the tested samples was between 1.019 gm/litre to 1.032 gm/litre; this gives an indication of adulteration with water since the mean specific gravity of normal camel milk is 1.0305 gm/litre. Thus, so far, the study indicated that milk samples from the two study areas were substantially contaminated by both physical substances and bacteria, either at the farm or along the market chain .However Resazurin test, which is an indicator of microbial load/quality of milk, demonstrated 72.4%of the tested camel milk samples as being of good quality.

Much as direct smear and culture did not demonstrate presence of Brucella organisms, as given above, 15.4% of the samples tested positive using Milk Ring Test, while 52.2% tested positive using a combination of Rose Bengal Plate Test, Tube Agglutination Test and Complement Fixation Test. This study has, therefore, confirmed presence of camel brucellosis in North-Eastern Province;.

In view of the above findings, with respect to Garissa ans Wajir districts, it is concluded that there is need to create awareness on subclinical mastitis and brucellosis in camels, and to implement respective control measures. In order to minimise milk contamination, the farmers need to be trained on good milking practices, proper milk storage and transport systems. The Government needs also to intervene and facilitate the famers to attain milk safety.

1. GENERAL INTRODUCTION

The camel (*Camelus dromedarius*) is the dominant livestock in North-Eastern province of Kenya where it provides sustenance to many people especially during the frequent droughts when other animals either die or are unthrifty. This is because the camel is highly suited for hot desert, semi-desert, arid and semi-arid areas. Camel population in Kenya is over 1 million and about 54% of them are kept in Garissa and Wajir counties (National Census, 2009). Inhabitants of these arid areas are mostly of Somali origin and are pastoralists. They use camels mainly for milk production and transport purposes; also as draft animals (Schwartz and Dioli, 1992). Camel plays a major role in the daily diet (meat and milk) and socio-economic well-being of these people. It contributes about 80% of the household food needs (Schwartz and Dioli, 1992; Guliye, 2006). The respective people prefer camel milk to other types of milk. They consider it the most precious valuable product and describe it as being "nutritious, thirst-quenching, easily digestible, and one that can be preserved for much longer time". Camel milk has been shown to contain all nutrients, similar to those of cow milk (Farah, 1993). It has also been shown to be rich in Vitamin C, which comes in handy for the people living in deserts, where vegetables and fruits are not readily available (Schwartz and Dioli, 1992; Wilson, 1984). Sales of camels/camel meat and milk, therefore, contribute towards the economic status of these people.

Currently, camel milk is also sold in Nairobi and other far places. There is also a campaign for camel milk consumption; in an effort to increase sales. There is, therefore, a fast-growing demand for raw camel milk. People of North-Eastern Kenya and other camel-keeping areas consume raw camel milk as one of the components of their diet (Schwartz and Dioli, 1992). This poses a health risk since the milk could contain disease-causing organisms, like those of the genus *Brucella*. Apart from the organisms originating from clinical and subclinical

mastitis, they can be introduced by handlers along the market chain. This study, therefore endeavored to establish the magnitude of mastitis, in particular subclinical mastitis, and the extent of bacterial contamination along the market chain, in the North-Eastern province. It also, by design, gave particular attention to prevalence of brucellosis, a zoonotic disease, because, if present, the *Brucella* organisms would be excreted through the milk. This was necessitated by observance of cases of abortions in the area in camels and other livestock. Thus, overall, this study was geared towards establishment of two aspects: (1) the safety of camel milk in North-Eastern province, Kenya, and (2) prevalence of brucellosis in the camels. The thesis is presented in 4 chapters, covering: (1) Collection of baseline data on socio-economic practices of the people living in the province – their behavior and practices towards camel keeping, milk production and handling, (2) Determination of the current status of subclinical mastitis in camels in the areas studied, (3) Determination of milk quality and bacterial contamination along market chain, and (4) Determine the prevalence of brucellosis in the camels

1.1 Objectives

1.1.1 Overall objective

To investigate occurrence of subclinical mastitis, brucellosis, and factors responsible for camel milk contamination in Garissa and Wajir counties of North-Eastern Kenya.

1.1.2 Specific objectives

1. To collect baseline data on socio-economic practices of the people of North-Eastern province.
2. To establish the magnitude of subclinical mastitis in camels in the areas studied,
3. To determine milk quality and bacterial contamination along market chain
4. To determine the prevalence of brucellosis in the camels

1.2 Justification for the study

Camel milk is one of major food components for the pastoralists in North-Eastern province, Kenya. This milk is also widely marketed in the local areas and is currently being sold in distant markets in Nairobi and other far places. There is, therefore, a fast growing demand for raw camel milk. Since milk is a very nutritious medium that presents a favorable physical and biochemical environment, it readily supports multiplication of microorganisms, some of which are zoonotic such as *Brucella* and shiga-producing *E. coli* serotype O157:H7. It was, thus, found necessary to establish the level of bacterial contamination of the milk produced in North-Eastern province.

This contamination could occur at udder level (mastitis – clinical or subclinical), during milking or along the transport chain. Currently, there are no mastitis control measures practiced by the camel keepers in the area. These people also carry out traditional husbandry practices including tying of the camel teats with soft bark as an effort to prevent the calf from suckling; this may contribute to the development of mastitis in the camels. The casual way that farmers and traders in Garissa and Wajir counties handle the milk, including the hand-milking process, and transportation under low hygienic conditions, exposes the milk to contamination. Also, since there is high temptation for farmers to adulterate their milk, in order to increase their sales, parameters like milk density, pH, were studied to determine the extent of adulteration, if any.

2 GENERAL LITERATURE REVIEW

2.1 Mastitis in camels

2.1.1 Introduction and Definition of Mastitis

Mastitis can be defined as inflammation of the mammary gland regardless of the cause and is characterized by physical, chemical and, usually, bacteriological changes in the milk. It is also characterised by pathological changes in the glandular tissue. The most important changes in the milk include: discoloration, presence of milk clots and presence of a large number of leucocytes (Blood and Radostits, 2007). While clinical cases are easy to detect by manual palpation and by visual examination of the milk using a strip cup (there is swelling, heat, pain and induration in the mammary gland, and the milk is discoloured and clotted), a large proportion of mastitis cases are not readily detectable; such cases are referred to as "subclinical mastitis". In the latter cases, the diagnosis has become dependent largely on indirect tests which depend in turn on the leucocyte content of the milk (Radostits *et al*, 2000).

In all types of subclinical mastitis, the typical change observed in milk is increased number of somatic cells, particularly white blood cells. This is brought about by the migration of these leucocytes into the udder and milk as the body's natural defence forces fight the infection (Schalm *et al*, 1971). Subclinical mastitis is detected by the use of indirect tests which depend on the leucocyte content of the milk. The test counts used are: Direct microscopic somatic cell count (DMSCC) and Electronic somatic cell count (ESCC), Cell counts are usually performed on the same sample as that used for cultural examination and serious errors are avoided if samples are always taken at the same stage of milking (Blood and Radostits, 2007).

2.1.2 Overview of mastitis in camels

The frequency of occurrence of mastitis in camels depends on the ability of the bacteria (etiological agent) to set up infection in the mammary tissue. The difference between bacteria, with respect to their ability to set up a mastitic state, is dependent on at least two important groups of factors: bacterial characteristics and transmission mechanisms. Bacterial characteristics include: the ability of the micro-organism to survive in the camel's immediate environment; the ability to colonize the teat duct (Bramley *et al*, 1979); the ability to adhere to mammary epithelium and set up a mastitic reaction (Brook and Barnum, 1984); and its resistance to any antibiotic therapy. Transmission mechanisms depend on the bulk of the infection in the environment, including: infected quarters; efficiency of milking personnel; susceptibility of the animal/camel, which is related to the stage of lactation, age of the camel (older animals more susceptible) and level of inherited resistance (possibly related to teat shape and anatomy of the teat canal); lesions on the teat skin especially the orifice; and the immunological status of each mammary gland (Bramley, 1978). Physiology and diseases of the udder are an important facet of reproduction and milk production of camels. Camel milk has been a source of nutrients for millions of people in Africa, middle-eastern and Asian countries (Schwartz and Dioli, 1992; Wilson, 1984). Mammary gland function is also very important for the health and growth of the new-born since udder diseases (the primary one being mastitis) are known to have a negative effect on both of these factors and can pose public health risks for populations consuming camel milk (Raymond, 1994, Knoess *et al*, 1986).

Mastitis is a relatively infrequent disease in camels compared with cattle, but the incidence of mastitis may increase in dairy camels due to hand milking and teat malformation (Almaw and Molla, 2000). Acute mastitis has been reported to occur during the first few days following

parturition, dystocia or cesarean section in the dromedary (Kapur *et al*, 1982, Quandil and Qudar, 1984). Mammary secretions in these cases are watery, yellowish or blood-tinged and bacteria isolated have included *Klebsiella pneumoniae* and *Escherichia coli* (Kapur *et al*, 1982). Milk from mastitic female is common source of infection for the newborn calf. Subclinical or chronic mastitis is suspected when the young fail to grow normally and when an anomaly of the conformation of the udder is observed, such as atrophy of one or more quarters, asymmetry or presence of pustules on the surface. The presence of pus can also be observed in milk (Saad and Thabet, 1993; Barbour *et al*,1984).The percentage of milk samples from CMT positive quarters yielding a positive bacteriological result can vary between 10 to 50% (Almaw and Molla, 2000; Abdurahman *et al*, 1995). Treatment of chronic mastitis is very difficult and the condition often results in the loss of the affected quarter (Saad and Thabet, 1993; Barbour *et al*, 1985).

Other conditions affecting the udder include traumatic lesions and lacerations. In the dromedary, the udder skin can show typical lesions of camel pox and it is also the site of choice for tick infestation. In one study in Ethiopia, 72% of udders were infested by ticks (Almaw and Molla, 2000). The incidence of mastitis was higher (30%) in heavily infested udders than in non-infested udders (9%).

2.1.3 Economic losses due to mastitis in camels

In terms of economic loss, mastitis is undoubtedly the most important disease with which the dairy industry has to contend. This loss, as in dairy cattle, is occasioned much less by fatalities, although fatal cases do occur, than from the reduction in milk production from affected quarters. The clinical syndrome may vary from peracute inflammation with toxemia to a fibrosis which develops in chronic mastitis until most of the secretory tissue has been

destroyed. There is the additional danger that the bacterial contamination of milk from affected camels may render it unsuitable for human consumption or interfere with manufacturing process or in rare cases, provide a medium of spread of diseases to humans. Streptococcal sore throat and brucellosis may be spread this way, through drinking unpasteurized milk (Blood and Radostits, 2007). Treatment of chronic mastitis is very difficult and the condition often results in loss of the affected quarter (Saad and Thabet, 1993; Barbour *et al*, 1985). Subclinical mastitis is a major factor in depressing milk yield and has a much greater impact on the productivity of lactating animals than the sporadic clinical forms of the disease. Chronic inflammatory infection ultimately leads to loss of intact quarter by destruction of the gland tissue. Loss of teat is reported from one third of Gabra and Somali camels in Northern Kenya (Younan *et al*, 2001).

2.1.4 Causes of camel mastitis

Many infective agents have been implicated as causes of mastitis in camels, the commonest being bacterial infection. The documented common causes of bacterial mastitis in camels are *Streptococcus, Staphylococcus*, *Micrococcus* and *Aerobacter* species and *Escherichia coli* in that descending order of importance (Obied *et al*, 1996) **(Appendix 10.1).** *Streptococcus agalactiae* and *Staphylococcus aureus* have been documented as the two most important mastitis pathogens in camels **(Table 2-1).**

Table 2-1 prevalence of Streptococcus agalactiae and staphylococcus mastitis in different camel populations

Mastitis pathogen			
Country	*Streptococcus agalactiae*	**Country**	references
Kenya (n= 1305)	12.1% Individual herd prevalence 25%	Kenya (n = 1305)	Younan *et al* (2001),
Sudan (n = 757)	26.7%	Sudan (n = 757)	Obied *et al* (1996),
Sudan (n = 391)	17.6%	Sudan (n = 391)	Abdurahman *et al* (1995)

n = number of milk samples

2.1.5 Subclinical mastitis

Subclinical mastitis causes an increase in the total bacterial count in milk. It is a major factor in depressing milk yield and has a much greater impact on the productivity of lactating animals than the sporadic clinical forms of the disease. In a longitudinal study of 207 lactating camels, only 3.4% were affected by clinical mastitis while 21.3% were affected by subclinical mastitis. The prevalence of mastitis in camels in Kenya has been reported to be about 25% (Younan *et al*, 2001).

2.1.6 Signs and diagnosis of clinical and subclinical mastitis in camels

Clinical mastitis is characterized by anorexia, fever, general depression, swelling, severe inflammation and pain of the udder **(Plates 2-1 and 2-2)**, which can cause rejection of the newborn by the female (Schalm, 1977). Clinical mastitis is self evident and can be detected without special test (Abdurahman, 2006). There are changes in the secreted milk (color,

consistency, floccules etc) and/or the udder (red, swollen) and other generalized signs exhibited by the animal (fever, anorexia, deteriorating body condition). Subclinical mastitis, on the other hand, is difficult to diagnose and depends on various test procedures aimed at detecting the cause or products of inflammation in milk [IDF (International Dairy Federation)**,** 1987]. A camel with subclinical mastitis produces less milk, but does not have a swollen udder or abnormal milk. Infection is present but can only be detected with the help of indirect methods (Abdurahman, 1995a, b; Abdurahman *et al,* 1995; Abdurahman, 1998). These include the California mastitis test (CMT), and Direct microscopic somatic cell count(DMSCC) a simple and rapid test that can be applied in the field; it is particularly used to detect subclinical udder infections caused by either one of the two major mastitis pathogens: *Streptococcus agalactiae* and *Staphylococcus aureus*. The direct microscopic somatic cell count (DMSCC), which requires only simple laboratory equipment and produces results on the same day. However there has been a problem in interpretation of results of these tests because the basal levels of cells and their physiological variations in the camel are still not yet established (Abdurahman et *al*, 1992).

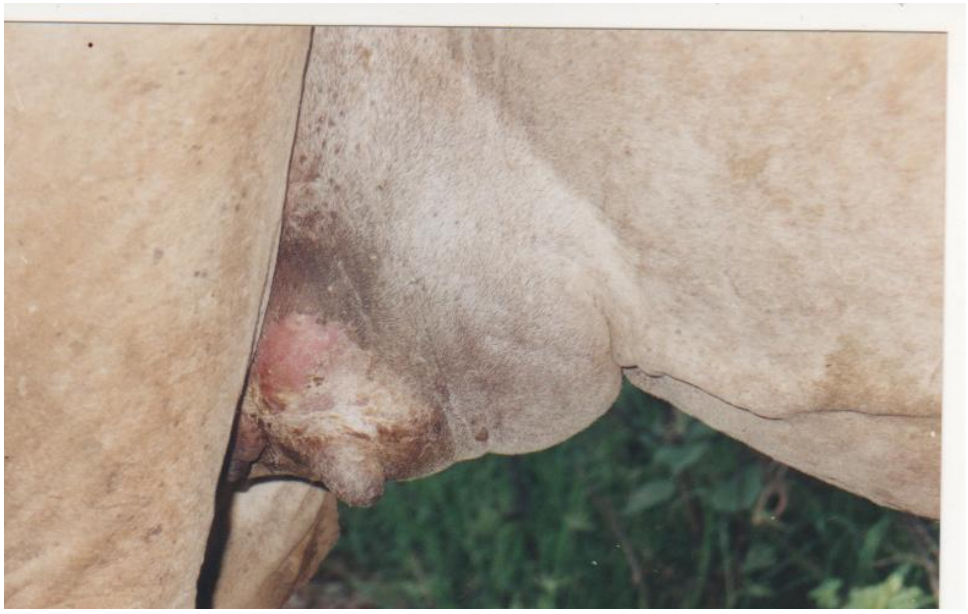

Plate 2-1Camel with severe clinical mastitis

Notice the evident swelling and hyperemia on the inflammed right quarters.

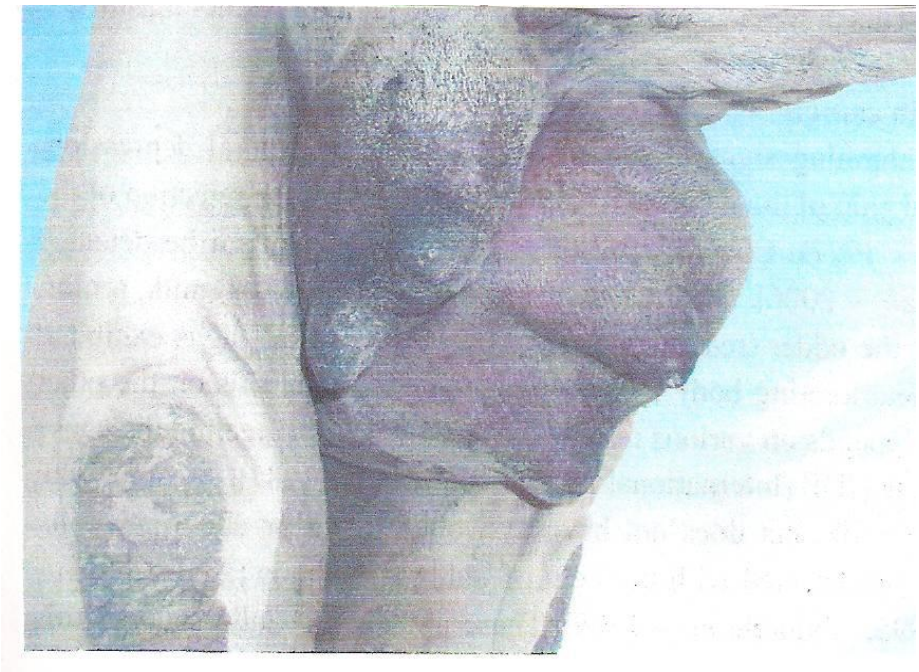

Plate 2-2 Mastitis with Swollen Left quarters

2.1.7 Pathogenesis of mastitis and risk of traditional practices

Except in the case of tuberculosis, where the method of spread of the etiological agent may be hematogenous, infection of the mammary gland always occurs via the teat canal and the development of inflammation can be most satisfactorily explained in terms of the three stages – invasion, infection and inflammation (Schalm, 1977). Invasion is the stage at which microorganism passes from the exterior of the teat to the milk inside the teat canal. Infection is the stage at which the organisms multiply rapidly and invade the mammary tissue. After invasion, a bacterial population may be established in the teat canal and using this as a base, a series of multiplication and extension into the mammary tissue may occur with infection of the mammary tissue occurring frequently or occasionally depending on its susceptibility. This in turn causes inflammation, the stage at which clinical mastitis appears or a greatly increased leucocyte count is apparent in the milk. Of the three phases, prevention of the invasion phase offers the greatest potential for reducing the incidence of mastitis. This is done through good

management, notably in the use of good hygiene procedures. Management practices prevalent in traditional husbandry system include tying the teats with soft bark to prevent the calf from suckling **(Plate 2-3)** and cauterization of the udder skin; this leaves behind scar tissue, blind teats and permanent loss of milk production (Woubit *et al*, 2001). The traditional practice of tying teats may contribute to the development of mastitis in camels (Mohammed *et al*, 2005). As the udder is a predilection site for tick infestation (*Amblyoma, Hyalomma,* and *Rhipicephalus* species) (Woubit *et al*, 2001) thereby facilitating bacterial entry and leave behind permanent tissue damage. In a limited study in Kenya, 22% of tick bite lesions were shown to harbor *Streptococcus agalactiae* (Younan *et al*, 2001).

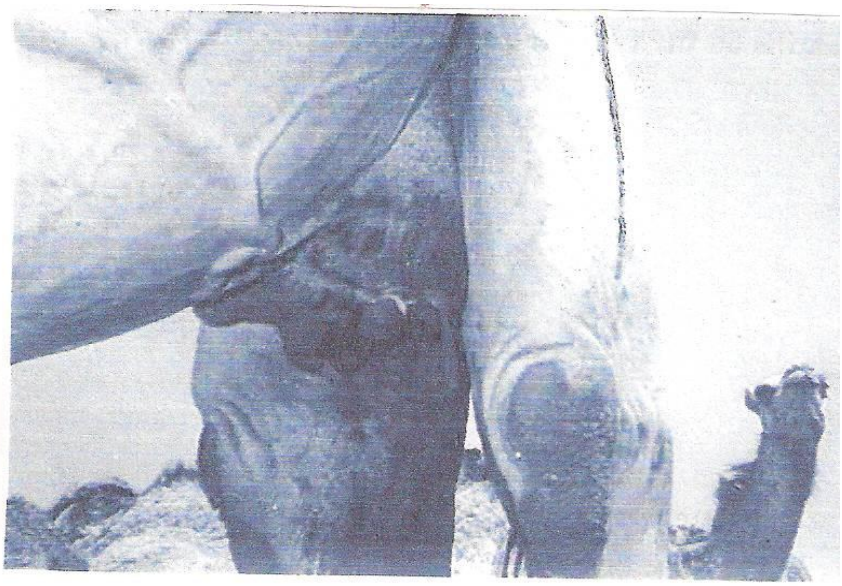

Plate 2-3 An anti-suckling device in a lactating camel

The above picture shows an anti-suckling device used by camel pastoralists in the Northeastern Kenya to prevent calves from suckling and secure milk for themselves. Notice the two left teats have been tied with a rope/string (arrow). This practice is a major predisposing factor to camel mastitis.

2.1.8 Clinical pathology of mastitis in camels

In the diagnosis and control of mastitis in camels, laboratory procedures are of value in the examination of milk samples for cellular, bacterial and chemical changes. Much attention has been given to the development of field tests based on physical and chemical changes in milk (Barbour *et al*, 1984; Saad and Thabet, 1993). These tests are indirect and detect only presence of inflammatory changes; they are of value only as screening tests and may need to be supplemented by bacteriological examination for determination of the causative organism (Almaw and Molla, 2000; Qaundil and Qudar, 1984; Saad and Thabet, 1993; Barbour *et al*, 1985). The physical tests carried out on milk in a mastitis examination are limited to the cell count and its immediate development; it is normally a bulk milk cell count. Indirect tests are also limited entirely to tests such as the California mastitis test (CMT) and the white slide test which are dependent on the cell count. Other indirect tests are the chloride content test, electrical conductivity test and test for camel serum albumin (Abdurahman, 1998; Abdurahman, 1995a, b; Abdurahman *et al*, 1995). The latter tests are more accurately diagnostic of damage to mammary epithelium as in bovine mastitis (Blood and Radostits, 1989). Since present day emphasis in mastitis control is on maintaining a particular programme of hygiene and continuous monitoring of subclinical mastitis, clinico-pathological tests are the best choice – they are practical for quick screening.

2.1.9 Bacteriological culturing of milk

Culturing of milk samples is a standard method of examination of mastitis. It may be carried out on individual quarter samples or on composite samples including milk from all four quarters. In a mastitis control program, the cost of bacteriological culture in the laboratory can be greatly reduced by screening the camels with the indirect tests first and then culturing the positive reactors (Almaw and Molla, 2000; Abdurahman *et al*, 1995).

2.1.10 Treatment of mastitis in camels

Although some authors have suggested daily intramammary infusion with antibiotic preparations as used in cattle, there is opposition to this practice because of the particular anatomy of the camelidae udder and because of the difficulty in administering such treatment (Tibary and Anouassi, 2000). Therapeutic approach in treating acute mastitis is via systemic antibiotics (e.g. trimethoprim – sulfamethoxazole or penicillin/ Aminoglycoside) and anti-inflammatory drugs (flunixin meglumine), with regular stripping of the mammary glands. Hydrotherapy is beneficial in reducing local edema. The teat of the camel udder contains sometimes three separate teat canals that open independently into the teat sphincter. The separate canals drain separate gland complexes (Nosier, 1974; Smuts and Bezuidenhout, 1987). This implies that for intramammary treatment of mastitis, not only must each quarter but also each gland complex be treated separately, that is, one intramammary tube per gland complex. Great caution is necessary when applying intramammary treatment to camels. The teat canal openings in camel are smaller than those of the cow and thus require smaller canula. Unhygienic and traumatic application of intramammary treatment is very likely to do more harm than good.

Intramammary infections (IMI) with *Streptococcus agalactiae* (Lancefield type B) in camels are common and have been diagnosed in the United Arab Emirates (Quandil and Qudar, 1984), Egypt (Karamy, 1990), Sudan (Abdurahman *et al,* 1995; Obied *et al,* 1996) and Somalia (Younan *et al,* 2002). In Northern Kenya, *Streptococcus agalactiae* prevalence of up to 50% in market oriented camel dairy herds (Younan *et al,* 2001) has become a concern to camel owners. One case of successful parenteral treatment of mastitis in a camel is reported in the literature (Barbour *et al,* 1985). However, published treatment recommendations for mastitis in camels have not been validated (Faye, 1997; Youssef, 1992).

2.1.11 Udder health management programs

Specific steps of all udder health management programs must be devised to fulfill three basic principles, which are: - elimination of existing infections, prevention of new infections and monitoring of udder health status (Radostits *et al*, 1994 b). Mastitis can be prevented or reduced by improving animal health and udder hygiene. Currently there is almost a complete absence of modern mastitis control measures practiced by camel keepers. Attention must be paid to udder health and hygiene, not only during lactation, but continuously, even when the animal is dry. Animals suffering from any contagious disease, including mastitis, should be separated from the healthy animals and milk from diseased camels should be kept separate and disposed off safely. It is cheaper and easier to prevent mastitis by improving hygienic measures and culling chronically-infected camels, to eliminate important pathogen reservoirs, than to treat by medication. The cost of treatment includes veterinary fees, medicine, and costs of milk losses. Treatment also contributes to the build up of antibiotic resistance.

2.2 Camel milk hygiene (quality)

2.2.1 General comments

Nowadays public health concern associated with microbial food safety has arisen. Numerous epidemiological reports have implicated non-heat-treated milk and raw-milk products as the major factors responsible for illness caused by food-borne pathogens (De Buyser *et al*, 2001; Hanington *et al*, 2002). Milk contamination with pathogenic microorganisms can occur either through fecal contamination or by direct excretion from the udder into the milk. Camel meat and milk are the key foods in arid and semi-arid areas of the African and Asian countries. Food and Agriculture Organization (FAO) has reported that more than 18 million camels around the world support the survival of millions of people (FAO, 2003). Camel milk not

only contains more nutrients compared to cow milk (Agrawal *et al,* 2005), but also has therapeutic and antimicrobial agents (Barbour *et al,* 1984; Elagamy *et al,* 1992).

Most of camel milk in the pastoral areas is consumed in the raw state, without any heat treatment or acid fermentation. It is also kept at high ambient temperatures due to lack of refrigeration facilities during milking and transportation. These conditions make milk unsafe, capable of causing food-borne diseases and even spoils fast (De Buyser *et al,* 2001; Hanington *et al,* 2002). In the pastoral areas of Eastern Africa and Middle East (Younan *et al,* 2002), as in many regions around the kingdom of Saudi Arabia, camel milk is traditionally produced by way of hand milking, handled and transported under low hygienic measures. However, there are no reports tracing any outbreak to unpasteurized (raw) camel milk (Al-Mohizea, 1994; Semereab and Molla, 2001; Benkerroum *et al,* 2003). This work on subclinical mastitis affecting milk hygiene in camels was first reported in 2013 (Wanjohi *et al.,* 2013)

The monitoring of camel milk hygiene (quality) from pastoral production areas by performing total bacterial counts (TBCs) has serious logistical problems because of the distance to the laboratories. Hence bacterial counts in milk from pastoral regions must be interpreted with caution. Spoiled camel milk has been found to have TBC of 10^7 - 10^8 colony forming units per milliliter (cfu/ml) of milk, although milk with lower TBC is occasionally perceived as spoiled, by organoleptic testing (**Appendix 10.2)**. The results of coliform counts (CCs) are even more affected by the delays before laboratory testing. Coliform counts of less than 10^2 cfu/ml have been found in milk samples from traditional milking buckets. This shows that, at collection, the milk is normally of.good quality; it tends to deteriorate rapidly as it enters the informal marketing chain (Younan *et al,* 2002).

The influence of pooling of different camel milk batches along the collection and marketing chain is illustrated by the increase in prevalence of *Streptococcus agalactiae*, a mastitis pathogen that originates from the udder (Younan *et al,* 2002). This pathogen was found in 50% of transport containers coming from producing herds, in 62% of milk containers sampled at primary collection sites, and in 70% of milk containers sampled from an urban market of the same region (Younan *et al,* 2002). Under pastoral production conditions, environmental contamination is thus likely to play a bigger role in the hygiene of raw camel milk than mastitis bacteria. Adulteration of marketed camel milk also occurs. Addition of up to 15% water to marketed camel milk has been reported from Southern Somalia (Younan *et al,* 2002); the quality of the water added to the milk representing an additional hygienic risk. The specific gravity of camel milk tested in three large commercial herds in Kenya over a two months' period varied between 1.026 and 1.029 grams/liter (**Appendix 10.3)**, indicating differences in specific gravity; which is indicative of adulteration.

2.2.2 Consumer Health and pathogens of great concern

Milk contaminants including faecal organisms pose threats to consumers of marketed camel milk. Isolation of coliforms (especially *Escherichia coli*) is taken as indicator of faecal contamination, which is one of the milking hygiene conditions. This is important because the faeces may have contained pathogenic organisms. Also, some of the *E. coli* organisms are pathogenic, including those that produce potent toxins like serotype O157:H7. *E.coli* serotypes O157:H7 and O157:non-motile (NM) (O157 STEC) produce one or more Shiga toxins, also called verocytotoxins and are the most frequently identified diarrheagenic *E.coli* serotypes in North America and Europe (Mead *et al,* 1999). Shiga toxin-producing *Escherichia coli* O157:H7 and other STEC serotypes cause human illness that can present as mild non-bloody diarrhoea, severe bloody diarrhoea (haemorrhagic colitis), and haemolytic-

uremic syndrome (HUS) (Griffin *et al,* 2002). Additional symptoms of *E. coli* O157:H7 infections include abdominal cramps in absence of high fever. Serotype O157 STEC colonizes dairy and beef cattle; it is, therefore, not surprising that ground beef has caused more O157 STEC outbreaks than any other vehicle of transmission (Griffin *et al,* 2002). Carriage of this organism by camels is not documented. Arimi *et al,* (2000) and Griffin *et al,* (2002) have shown that human infection is associated with consumption of a number of contaminated foods among them meat, undercooked beef, raw milk, yoghurt, salamis, cheese and unpasteurized apple cider. Contamination of milk and water can be at different stages of handling (farm level, market level and consumer level). Zoonotic organisms need to be considered in view of the traditional preference for consumption of raw milk.

Other zoonotic risks include brucellosis; its prevalence in camels varies widely ranging from 1% to 30% positive reactors to Rose Bengal plate test (RBPT) (Younan *et al,* 2002). Brucellosis prevalence seems to be higher in regions where camels are kept under more stationary conditions and close together with other livestock (cattle, sheep and goats) (Younan *et al,* 2002). The two most common mastitis pathogens in camels, *Streptococcus agalactiae* and *Staphylococcus aureus,* are both potential human pathogens (Younan *et al,* 2001; Abdurahman *et al,* 1995). While toxin producing *Staphylococcus aureus* may cause food poisoning, *Streptococcus agalactiae* is known to cause human infection, particularly in newborn children. *Salmonella* infections are also common in camels, but human *Salmonella* infections originating from raw camel milk have not been documented (Younan *et al,* 2002). Numerous other zoonotic diseases including plague and Rift valley fever have been recorded from camels but the literature provides no detailed information on their transmission (Younan *et al,* 2002).

2.2.3 Effect of high temperatures and water scarcity on milk hygiene

Milk is a very nutritious medium which is rich in carbohydrates, proteins, fats, vitamins and minerals (Blowery and Edmondson, 2000) and presents a very favourable physical and chemical environment for the multiplication of microorganisms. Being an animal product subject to differing production methods, it can be contaminated by a broad spectrum of microbial types. High ambient temperatures in ASAL areas (including Garissa and Wajir counties) also enhance the rapidity of multiplication of microorganisms in milk once out of the lag phase: 1 – 3 hours after milking (during this period lactoperoxidase system inhibits multiplication of microorganisms). This accelerates spoilage of milk and greatly compromises the milk hygiene (quality). High temperatures favour quick multiplication of bacteria and other microorganisms **(Table 2-2).**

Table 2-2 Effect of temperature on microbial load

Temperature(Degrees Celsius - ^{o}C)	Standard plate count (cfu/ml)
0 ^{0}C	(2.4×10^{3})
4 ^{0}C	(2.5×10^{3})
10 ^{0}C	(1.16×10^{4})
16 ^{0}C	(1.8×10^{5})
20 ^{0}C	(4.5×10^{5})
30 ^{0}C	1 (1.4×10^{9})
35 ^{0}C	(2.5×10^{11})

Typical bacterial growth over a 24 hour period in milk in different temperatures. Source: Institute DeTechnology Agroalimentaire (ITA) - 1998

Scarcity of water in these ASAL areas also compromises hygiene (quality) during and after milking due to inadequate washing of the udder and milking equipment. Protection of milk is supposed to start as soon as the end of the previous milking when the equipment is washed

and sanitized to avoid build up of bacteria when equipment is standing idle between milking. Any bacteria that may have grown by this time is normally removed by rinsing and sanitization of equipment (teat cups, buckets etc) prior to the start of milking helps in removing any. In regions of water scarcity this may be difficult to practise (Younan *et al,* 2002).

2.3 Brucellosis in camels

2.3.1 General comments

Disease is one of the major constraints to camel production. In the past, the camel was thought to be resistant to diseases commonly affecting livestock in the same ecozone. Recent studies (Abbas and Ohmer, 2005, Wernery and Kaaden, 1995), however, show the contrary and in fact the camel was found to be more susceptible to some diseases, including brucellosis, than other animals in the same ecozone. Published studies on relative occurrence of brucellosis in camels are largely confined to serological surveys. Teshome *et al*,(2003) reported a sero-prevalence of camel brucellosis of 5.7 % using Rose Bengal Plate Test (RBPT) and 4.2% using Complement Fixation Test (CFT) in three camel-rearing regions in Ethiopia. An incidence of approximately 7% has been reported in camels from eastern region of Sudan (Yagoub *et al,* 1990; Damier *et al,* 1984), whereas a prevalence of 4.1% was reported from 967 camels in Libya using RBPT, serum agglutination test (SAT) and CFT (Gameel *et al,* 1993). There are no recent studies conducted in camels in Kenya; however Waghela *et al,* (1978) reported a prevalence of 14% in 172 camels in North Eastern province using RBPT, SAT and CFT. This work on brucellosis in camel milk was first reported in 2012(Wanjohi et *al.,* 2012)

Brucellosis is widely distributed in Sub-Saharan Africa and many regions of the world (FAO Guidelines, 2003). There are many reasons for this, including: expansion of livestock herds, uncontrolled animal movements, lack of veterinary support services and vaccines and husbandry practices favouring infections. In general, disease is highest in pastoral production systems and decreases as herd size or land holding system decreases (McDermott and Arimi, 2002). Brucellosis is a zoonotic disease affecting humans and various domesticated and wild animals. Transmission to humans may be food-borne, mainly through ingestion of raw milk, or by direct contact (through the skin) with infected material, for example aborted fetuses, placenta or vaginal discharges from infected animals (FAO/WHO, 1986; Kiel and Khan, 1987; Dawood, 2008). Methods of prevention include health education to reduce occupational and food-borne risk. However ultimate prevention of human infection remains pegged on elimination of infection among animals. This can be achieved by vaccination of all breeding stock to reduce risk of abortion and raise herd immunity, followed by elimination of infected animals or herds by segregation (FAO Guidelines, 2003).

2.3.2 Epidemiology of Brucellosis in camels

Brucellosis in camels is caused by different biovars of *Brucella abortus* and *Brucella melitensis* (Abbas and Agab, 2002; Agab *et al,* 1996). Camel brucellosis has been reported in many camel rearing countries including Libya (Gameel *et al,* 1993), Saudi Arabia and the near East region (Radwan *et al,* 1992; Refai, 2002), Sudan (Damier *et al,* 1984; Yagoub *et al,* 1990; Agab, 1993), Ethiopia (Teshome *et al,* 2003) and Kenya (Waghela *et al,* 1978) **(Appendix 10.4)**. Both vertical and horizontal transmissions exist in animal brucellosis. Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, via conjunctiva, inhalation and udder contamination during milking. Congenital infection that happens during parturition is frequently cleared and only few animals remain infected as

adult (Radostits *et al*, 1994a). Spread of the disease is due to movement of infected animals to disease free herds.The risk of exposure is increased when infected animals encounter clean herds at the watering points. Epidemiologically important risk factors are: large herd size, poor management, abortions, milking more animals by single person and herding with other ruminants. Common sources of infection include infected pasture, contaminated by fetal membranes from infected animals and aborted fetuses (Blood *et al*, 1994).

Brucellosis in camels is related to contact with large and small ruminants (FAO Guidelines, 2003; Abbas and Agab, 2002). Camel-pastoralists keep relatively large flocks of sheep and goats alongside camels. Frequent isolation of *Brucella melitensis* from camels in North Africa and Arabia suggests possible active transmission between small ruminants and camels (Abbas and Agab, 2002; Dafni *et al*, 1991)). The sero-prevalence of camel brucellosis seems to follow two distinct patterns: a low prevalence (2 - 5%) in pastoralist camels and a rather high prevalence (8 - 14%) in more intensively kept camels (Abbas and Ohmer, 2005). Even under pastoral conditions, individual herds could have appreciably higher prevalence of brucellosis than the regional risk. Agab (1993) recorded a sero-prevalence in certain camel herds in Sudan ranging between 26.5% and 30%. Thus, the chance of transmission is higher during parturition and abortion when most of the *Brucella* contamination occurs (Abbas and Agab, 2002). Long term chronic infection provides a steady supply of infectious organisms to maintain transmission and a constant supply of new infections.

Survival of the organisms in the environment may also play a role in the epidemiology of the disease (Abbas *et al*, 1987; Radwan *et al*, 1992; Abou–Eisha, 2000). *Brucella* does not multiply in the environment but merely persists. Temperature, humidity and pH of the environment influence the survival of *Brucella melitensis* as well as that of *Brucella abortus*.

Brucella organisms *are* sensitive to direct sunlight, disinfectants and pasteurization. In dry condition, they survive only if embedded in protein. In optimal conditions, *Brucella* organisms survive in tap water, damp soil, urine, aborted fetuses and uterine exudates and in frozen tissues (Ellen *et al*, 1994).

The relationship between *Brucella* infection and abortion in camels is well established (Agab, 1993; 1997). Nomadic camels have a rather lengthy inter-calving interval estimated at between 2 and 3 years with a mean of 2.4 years (Abbas *et al*, 1992). Since most brucellosis contamination occurs following an abortion or delivery by an infected female, the long inter-calving interval might contribute to the low incidence of brucellosis in extensively kept animals (Abbas *et al*, 1992).

2.3.3 Pathogenesis and clinical findings in camels

Brucella has a predilection for the gravid uterus, udder, testicles, accessory sex male glands, lymph nodes, joint capsule and bursa (Jubb *et al*, 1993, Enright, 1990; Nicoletti, 1980; Fensterbank, 1978; Braude, 1951). After initial invasion of the body, localization occurs initially in the regional lymph nodes draining the area before spreading to other lymphoid tissues including the spleen, mammary and iliac lymph nodes (Enright, 1990; Thoen and Enright, 1986). In adult non-pregnant cow, localization occurs in the udder and if it becomes gravid, the uterus is infected from periodic bacteremic phases originating from the udder. Invasion of the uterine wall and lumen leads to ulcerative metritis. Allanto-chorionic and fetal fluids are invaded and villi destroyed. The infected udder remains clinically normal but is a source of re-infection of the uterus and a source of infection to calves and humans drinking the milk (Blood *et al*, 1994). *Brucella* is a facultative intracellular parasite capable of multiplication and survival within the host phagocytes (Kohler *et al*, 2002). Various

mechanisms are employed by *Brucella* organisms to survive inside the phagocytic cells: inhibiting phago-lysosome fusion, blocking bactericidal action of phagocytes and suppressing the myelo-peroxidase H_2O_2 halide system (Frenchick *et al,* 1985; Harmon *et al,* 1988; Tizard, 1992; Walker, 1999).

The inability of the leucocytes to completely kill virulent *Brucella* at the primary site of infection is a key factor in the dissemination to the regional lymph nodes, the reticulo-endothelial system and other organs such as uterus and udder (Araya *et al*, 1989; Araya and Winter, 1990). The organism is also capable of survival within the macrophages. This ability to survive within the host phagocytes can be utilized for protection from humoral and cellular bactericidal mechanisms during hematogenous spread (Araya *et al,* 1989; Araya and Winter, 1990). The main clinical picture observed is abortion, occurring in mid to late pregnancy. Retention of fetal membranes and endometritis are common sequels to abortion (Coetzer and Tustin, 2004). In subsequent pregnancies, the fetus is usually carried to term, though abortions may occur in the same cow. In the bull, epididymitis and orchitis occur occasionally. One or both scrotal sacks may be affected showing acute and painful swelling. The swelling will normally persist and testis undergoes liquefactive necrosis, and such bulls become sterile. Carpal hygromas, non-suppurative synovitis and infertility are associated with chronic *Brucella* infections (Coetzer and Tustin, 2004). Necrotizing placentitis and disseminated inflammatory reactions in aborted fetal tissue are characteristic changes. Granulomatous lesions and focal necrosis in several organs, edema of the subcutis and skeletal muscles, sero-haemorrhagic lesions in body cavities and bronchopneumonia are observed in fetuses. Granulomatous meningitis has also been described (Blood *et al,* 1994).

2.3.4 Diagnosis of Brucellosis in camels

Isolation of *Brucella* organisms from a patient is not always possible, even though isolation and identification of the organism is required for definitive diagnosis of Brucella infections. Serological tests, thus, play a major role in the routine diagnosis of the disease (Alton *et al,* 1975). However, normally, there is no single serological test that will pick all positive cases; making it a common practice to run a number of serological tests together. This increases the chance of picking most of the positive cases. In fact, the most-sure way to diagnosis of this disease is to combine bacteriological and serological methods (OIE Manual, 2004). In camels (*Camelus bactrianus* and *Camelus dromedarius*), *Brucella* infection follows a course similar to that in cattle. The same serological procedures may thus be used for these animals, but it is recommended that each test is validated in the animal under study (OIE Manual, 2004).

2.3.4.1 Culture

Samples for culture include aborted fetuses (stomach contents, spleen and lungs), fetal membranes, vaginal secretions/swabs, milk, semen and arthritis or hygroma fluids (Alton et al, 1988; Crawford *et al,* 1990). From animal carcasses, the preferred tissues for culture are those of the reticulo-endothelial system (i.e. head, mammary and genital lymph nodes and spleen), the late pregnant or early post parturient uterus, and the udder (Agab *et al,* 1994). Growth normally appears after 2 - 3days, but cultures should not be discarded as negative until 8 – 10 days have elapsed.

Direct culture and isolation and culture of *Brucella* are usually performed on solid media so that developing colonies can be isolated and recognized clearly. Commercially available media include *Brucella* Agar medium base, and Tryptose (Trypticase) soy-Agar (TSA) (Alton *et al,* 1975). The addition of 2 - 5% bovine or equine serum is necessary for the

growth of strains such as *Brucella abortus* biovar 2. Selective media may be prepared from the above basal media through addition of specific antibiotics (cyclohexidine, bacitracin, polymyxin B or crystal violet dye) to suppress growth of other organisms (OIE, 2000; Walker, 1999). As the number of *Brucella* organisms is likely to be lower in milk, colostrum and some tissue samples than in abortion material, enrichment is required. In the case of milk, results can be improved by centrifugation of milk at 5900 to7700 x g for 15 minutes and culture made from the cream and the pellet (Walker, 1999). The enrichment medium should be incubated at 37^0 C in air supplemented with 5 – 10% (v/v) carbon dioxide (CO_2) for up to 6 weeks (Alton *et al*, 1975; Gameel *et al*, 1993; Agab *et al*, 1994), with weekly subcultures on to solid selective medium (Songer and Post, 2005).

On suitable solid media, *Brucella* colonies are visible after a 2-day incubation period. After 4 days incubation, *Brucella* colonies are round, 1 – 2 millimetres (mm) in diameter, with smooth margins. They are translucent and a pale honey colour when plates are viewed in the daylight through a transparent medium. When viewed from above, colonies appear convex and pearly white (Gameel *et al*, 1993; Agab *et al*, 1994). Later colonies become larger and slightly darker (Songer and Post, 2005).

2.3.4.2 Staining methods

Brucella are cocco-bacilli measuring 0.6 to 1.5µm long and from 0.5 to 0.7µm wide. They are non-motile, do not form spores, and flagella; pili or true capsules are not produced. *Brucella* species are Gram negative and usually do not show bipolar staining. They are not truly acid-fast, but are resistant to decolorization by weak acids and thus stain red by the Stamps modification of the Ziehl-Neelsen method (Quinn *et al*, 2002). For smears of organs or biological fluids that have been previously fixed with heat or ethanol, *Brucella* organisms

stain red against a blue background (Holt *et al*, 1994; OIE Manual, 2004). However, these methods have a low sensitivity in milk and dairy products where *Brucella* organisms are often present in small numbers and interpretation is frequently impeded by the presence of fat globules.

2.3.4.3 Identification and typing

Identification of *Brucella* organism can be carried out by a combination of the following characteristics; Colony morphology and Gram or Stamps staining, macro morphology, growth characteristics, urease, oxidase and catalase tests, and the slide agglutination test with an anti-*Brucella* polyclonal serum. Species and biovar identification requires elaborate tests such as phage lysis and agglutination with A-, M- or R- specific antisera (Holt *et al*, 1994).

2.3.4.4 Serological tests

The major objective in laboratory diagnosis is to identify animals that are infected and are potentially shedding the organisms; thus spreading the disease. This is possible using standard serological tests. However, latent infections occur in some animals which are serologically negative and vaccinated animals may be serologically positive, and these can interfere with interpretation of results. Presumptive diagnosis may be made on presence of antibodies in serum, milk, vaginal mucus or seminal plasma. Test for the detection of specific immunoglobulin includes: Milk Ring Test (MRT), Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Mercaptoethanol Test (MET), Complement Fixation Test (CFT), and Enzyme Linked Immunosorbent Assay (ELISA) (Alton *et al*, 1988; Anon, 1986; Anon, 2000).

2.3.4.4.1 Milk Ring Test (MRT)

Milk Ring Test is used to detect antibodies in milk. The development of a positive reaction is dependent on two reactions (i) fat globules in the milk are aggregated by milk antibodies (fat-globule agglutinins) and (ii) Stained *Brucella* cells (antigens), which are added to the milk, are agglutinated by the *Brucella* antibody/fat globule complexes which rise to form a coloured cream layer at the top (Alton *et al,* 1988; Anon, 1986). This is a sensitive screening test used on bulk milk samples either to detect infected animals on a herd basis or to monitor clean herds. Factors that may cause false positive results include a high prevalence of mastitis, a high proportion of animals in early or late lactation, recent (within three to four months) vaccination with strain 19 vaccine, and souring of milk (Coetzer and Tustin, 2004). Milk samples may be preserved for testing by adding 0.5ml of a formalin solution (prepared by mixing 7.5ml of 37% formaldehyde with one litre of distilled water) to a 10 ml milk sample. The duration and temperature at which milk samples are stored (45^0 C > for more than 5 minutes) may cause false negative reactions. Pasteurized milk cannot be effectively tested by the MRT (Alton *et al,* 1988).

2.3.4.4.2 Rose Bengal Plate Test (RBPT)

Rose Bengal Plate Test is a modification of the plate agglutination test. The antigen, which has been stained with Rose Bengal stain, is buffered at a pH of 3.65 (Alton *et al*, 1988; Anon, 1986). At this level of activity, non-specific agglutinins are destroyed and immunoglobulin G (IgG), normally the most abundant antibody in the serum of infected animals, agglutinates strongly (Anon, 1986; Brinley, 1977). Equal volumes (30µ) of test serum and antigen are mixed, shaken for four minutes and viewed over a white tile and any degree of agglutination is recorded as positive (Alton *et al,* 1988). This test is inexpensive and easy to perform. False positive reactions occur, usually due to the presence of IgM as a result of strain 19

vaccinations (Aguirre *et al,* 2002). Accordingly, RBPT is considered as a satisfactory screening test (Nicoletti, 1980; OIE, 2000). This test is prescribed for international trade in cattle by the OIE (Office International des Epizooties) (Anon, 2000).

2.3.4.4.3 Serum Agglutination Test (SAT)

This test is positive 7 - 10 days after infection (Godfroid and Kasbohrer, 2002). During this stage of the disease the level of agglutinins associated with both immunoglobulins M (IgM) and IgG continue to rise. Sensitivity is rather low ranging from 61– 69%. High titre sera may not cause agglutination in low dilution (the prozone effect). Therefore a range of serum dilutions from 1 to 10 to over 1000 should be made (Herr and Brugge, 1985; Herr *et al,* 1982; Herr *et al*, 1986).

2.3.4.4.4 Mercaptoethanol (ME) Test

Low titre agglutinins due to residual IgM may persist for several months after the infection has cleared. The agglutinating ability of IgM and IgA is destroyed by 2-ME, therefore agglutination in this test is indicative of presence of IgG and likelihood of persisting infection (Holt *et al,* 1994).

2.3.4.4.5 Complement Fixation Test (CFT)

This test is regarded throughout the world as being the confirmatory test for the serological detection of infected animals. It has been modified, standardized and adapted to a microtitre system (Alton et *al*, 1988; Anon, 2000). Some researchers reported its superiority over the other mentioned tests (Mohammed *et al,* 1981; Gameel *et al,* 1993; Asfaw *et al,* 1998). Complement Fixation Test detects predominately IgG antibodies as most of IgM ones are destroyed during serum deactivation; this is why it is used as a confirmatory test (FAO,

2003). The test distinguishes reaction caused by other factors like vaccines and other bacterial infections. *Escherchia coli* O157*, Yersinia entrocolitica O:9, Vibrio cholerae, Pseudomonas mallophilia and Salmonella* serotypes share common chain of lipopolysaccharide (LPS) antigen with smooth *Brucella* strains and do cross react. *Fransiscella tularensis* also cross reacts for unknown reason. Rough *Brucella* strains also cross-react with *Actinobacillus equuli, Pasteurella multocida and Pseudomonas aeruginosa* (Corbel, 1990; Cloeckaert *et al*, 1992; Garin-Bastuji *et al*, 1999). These organisms contribute to false positive reactions for brucellosis in animal herds. Thus, the use of highly specific test such as monoclonal antibody-based competitive - Enzyme linked Immunosorbent Assay (c-ELISA) and CFT minimizes the risk of cross-serological reactions between *Brucella* and these groups of bacteria (Vizcaino *et al*, 1991; OIE, 2000).

Complement Fixation Test is important in distinguishing calf-hood vaccination from those due to infection. The CFT titres do not wane as the disease becomes chronic and often CFT reaches diagnostic levels sooner than the serum agglutination test (SAT) following natural infection (Anon, 1986; Seagerman *et al*, 1999).

2.3.4.4.6 Enzyme linked Immunosorbent Assay (ELISA)

Competitive ELISA (c-ELISA) and Indirect ELISA (i-ELISA) tests can be used as supplementary tests to CFT. They have an advantage over other serological tests of being more sensitive, economic and rapid (Anon, 2000). The ELISA test is also capable of differentiating acute from chronic infections. Recently, ELISA has been used not only for detecting *Brucella* antibodies in sera but also in camel milk (Straten *et al*, 1997; Azwai *et al*, 2001). Besides its higher sensitivity than other conventional tests, ELISA is found to detect sera as positive about 2 to 4 weeks earlier (Gameel, 1983). It can also be used both for

screening and confirmatory tests (FAO, 2003). These tests are prescribed for international trade in livestock (cattle) by the OIE (Anon, 2000).

2.3.5 Control and Prevention

The control and prevention of brucellosis in farm animals depend on animal species involved, *Brucella* species, management practices and availability and efficacy of vaccines. The options to control the disease include immunization, testing and removal, and improving management practices and movement control (Hunter, 1994; WHO, 1997; Wernery and Kaaden, 2002). Control of camel brucellosis should suit conditions in particular countries where camels are raised. In most of the developing countries where camels are raised by pastoralists, brucellosis prevalence is low. Thus control by herd immunization and vaccination of calves at 4 to 8 months of age is helpful. On the other hand, test and slaughter policy can be followed in countries where intensification is practiced (Abbas and Agab, 2002).

2.3.5.1 Immunization

The live attenuated *Brucella abortus* S19 and *Brucella melitensis* Rev-1 have proved to be effective vaccines against the disease in camels and other ruminants. Both vaccines have disadvantages of causing abortion, being pathogenic to human-beings and interference with serological tests (WHO, 1997; Wernery and Kaaden, 2002). The non-smooth strains of *Brucella abortus* RB51 and *Brucella melitensis* M111 have recently been introduced into some countries. These vaccines are said to be safe and do not interfere with serological tests (WHO, 1997).

2.3.5.2 Management practices and control of movement of herds

Improving management practices is one way of attempting to control brucellosis. This would aim at improving hygiene and reducing the chances of contact between infected and non-infected animals. Although it would not be easy under many circumstances, where resources are lacking and the movement of livestock is difficult to restrict, the following points can be attempted in reducing infection rates (Hunter, 1994; Radostits *et al,* 1994a):- isolation of infected animals, isolation of females at parturition; proper disposal of aborted fetus, placental tissue and uterine discharge, and disinfection of contaminated areas. Public awareness is of vital importance in successful control and prevention of brucellosis.

3 SOCIAL BEHAVIOUR AND PRACTICES

3.1 Introduction

The way nomadic people relate to their animals is generally known but that of North-Eastern province inhabitants with their camels is not documented yet. The intimacy that exists between the nomadic people and their animals, i.e. the closeness to them to the extent of sharing water points; and the consumption of raw blood and milk, exposes the people to various zoonotic diseases. This study, by design, endeavors to document the behavior of the North-Eastern province inhabitants generally and, specifically, towards camels.

3.2 Materials and Methods

3.2.1 Study area and animals kept

The study was done in Garissa and Wajir counties of North-Eastern province, Kenya. These are two of the four counties making up the expansive North Eastern province of Kenya. They lie in the Arid and Semi-Arid Lands (ASAL) of the country **(Appendices 10.5, 10. 6 and 10.7)**. The rainfall pattern is erratic and unreliable; it is always less than 600 mm annually.

Temperature ranges between 22^0 C and 42^0 C. The counties are flat, covered by trees and shrubs with grass undergrowth. Water sources are rivers (permanent and seasonal), pans, boreholes, dams and shallow wells. The mainstream activity of the two counties is livestock keeping. The animals are kept under pastoralist system. They include cattle, sheep, goats, camels, donkeys and poultry **(Table 3.1)**. Nomadic pastoralist communities living in ASAL regions largely depend on milk produced by camels which contribute 80% of the household needs (Schwartz and Dioli, 1992; Guliye, 2006).

Table 3-1 Livestock population in Garissa and Wajir counties for year 2006

Species	**Garissa district**		**Wajir district**
Cattle	246,488		251,349
Sheep	535,370		345,500
Goats	257,336		379,500
Camels	**100,168**		**279,549**
Donkeys	61,759		33,147
Poultry	33,450		28,680

Sources: **MOLFD** – Ministry of Livestock Fisheries and Development annual report of 2006 and **DVO –** District Veterinary Officer annual report of 2006

3.2.1.1 Wajir County

Wajir County lies between latitudes 3^o 6" N and 0^o 20"N and longitudes 39^0E and 41^0E. It borders the republic of Somalia to the East; Garissa County to the South, Isiolo to the Southwest, Marsabit to the West, Moyale to the Northwest, Ethiopia to the North and

Mandera to the Northeast. The county covers an area of 56,501km^2, divided into 13 administrative divisions. The county population is 533,537 persons (1999 Kenyan census) with annual growth rate of 3.7%. The main form of land use is nomadic pastoralism which is the most efficient method of exploiting the range land. Incidence of insecurity as a result of banditry in the area is quite high because of the porous borders. Locations that were selected for sampling were conveniently chosen; they included those that had large populations of camels. These were: Griftu, Khorl-Haral, Tarbaj and Wajir-Bor **(Appendix 10.8).**

3.2.1.2 Garissa County

Garissa is an administrative countyin Northeastern Province of Kenya. Its capital town is Garissa. Garissa is located near 0^0 27`25" S, $39^0$ 39`30"E. The county has an area of 44,952 Km$^{2.}$ The county has a population of 329,939 (1999 Kenyan census).. Garissa is a town in North Eastern Province, Kenya. It is the head quarters of the province and Garissa County. It has a population of 65,881 according to 1999 Census (Populations of local authorities with towns-1999). Tana River flows through the town of Garissa. Most of the inhabitants of Garissa are ethnic Somalis. Locations that were selected for sampling were also conveniently chosen; they were: Korakora, Kulan**,** and Damajale **(Appendix 10.9).**

3.2.2 Study design

This was a cross-sectional study conducted on livestock grazing units within the two counties and around the watering wells/areas, using participatory approaches.

3.2.2.1 Selection of the study sites

The study sites (manyattas and centres) were conveniently selected based on camel population (availability of camels) in the area, security concerns and accessibility in terms of

physical infrastructure (roads). A full list of these grazing units and wells was obtained from the Local Arid Development projects (ADP) offices in the two counties.

3.2.2.2 Rapid Rural Appraisal (RRA) and Cross-Sectional studies (CSS)

Qualitative and quantitative approaches were carried out together using the respective tools (Okuthe *et al*, 2003; 2006). Rapid Rural Apraisal tools included: secondary data collection, key informants, interviews, semi-structured interviews (community), guided by a check list, transect walks, seasonal calendars and direct observations. Secondary data from the district annual reports (DLPO) were summarized. A check list was then developed to guide the facilitator during the RRA interviews with the community. Transect walks/ drives, were done in selected manyattas and centres, to probe triangulate and confirm some of the unclear issues from the discussions. A semi-structured, pre-tested, questionnaire [**Appendix 10.10** designed following Bekele's format (2004)] was designed to elicit information on the possible presence of mastitis in camels, gauge the level of hygiene in milking and udder health and to elicit information on the possible risk factors for brucellosis in camels. The questionnaire was administered through personal interviews with milk producers (farmers, group leaders or pastoralists). The researcher was accompanied by an interpreter. The information sought included: Knowledge of the disease "mastitis" and other livestock diseases; Appearance of swollen painful udder and teat lesions; Discoloration of milk and change in consistency of milk; Knowledge of mastitis treatment methods; Milking procedure (udder washing with clean water and disinfectant/lack of it and the use/non use of teat dips); Presence or appearance of traumatic lesions on the udder and teats due to various causes (physical injury due to the use of anti-suckling devices and by tick bite wounds); Knowledge of the disease "Brucellosis" and other livestock diseases Incidences of abortion in the herds and how they

are handled when they occur; Grazing systems used ; Inter-calving intervals in the herd; Other livestock kept alongside camels Camel age groups and sexes

Questions to the producer pastoralists included: livestock kept, ownership and management, milking, milk handling, milk disposal, constraints, solution, production levels, income from milk and inputs. Information regarding reported cases of mastitis and brucellosis was also sought from the local District Veterinary Officer (DVO) and District Livestock Production Officer (DLPO). Sixty four (64) camel owners from the two counties were interviewed using the structured questionnaire.

3.3 Results: Questionaire data

3.3.1 Types of livestock kept by the pastoralists

Types of livestock kept by the pastoralists in Garissa and Wajir Counties included cattle, sheep, goats, camels, donkeys, and domestic chicken **(Table 3-2)**. The respective ranges and average numbers are given in the same table. The numbers kept differed considerably from one pastoralist to the other.

Table 3-2 Types of livestock kept by the pastoralists and their average numbers

Type of Livestock kept	**Cattle**	**Sheep**	**Goats**	**Camels**	**Donkeys**	**Chickens**
Average no. of livestock per pastoralist	21	43	62	**25**	3	10

3.3.2 Purpose for keeping Camels

Pastoralists kept camels for various purposes which included: for their meat and milk (17.2% and 23.4%, respectively), which were used for domestic consumption, for sale to earn cash (ie. For economic reasons; 15.6%) and as traditional medicine; for their hides and skins (9.4%), which the owners could sell or use as bedding or roofs of manyatta; and/or for traditional ceremonies (payment of dowry or fund-raising activities; (12.5%). Camels were also kept as a means of transport, especially when the pastoralists were moving from place to place in search for fresh pastures for their livestock. The number of camels possessed by an individual was considered as a wealth status of that individual in the community **(Table 3-3).**

Table 3-3 The various 36urposes for keeping camels

Purpose	Number of respondents	% respondents
Slaughter and meat	11	17.2
Milk production	15	23.4
Sale/cash income	10	15.6
Hides and skins	6	9.4
Traditional ceremonies	8	12.5
Transportation	8	12.5
Wealth status	6	9.4
Total	64	100.0

Number of respondents =64

3.3.3 Feeding and watering of the camels

Table 3-4 gives various data on feeding and watering points, and feed supplementation. People who took care of the camels (feeding and watering them) included herdsboys (35.9%), hired herdsmen (18.8%), and male owners (45.3%). All of them grazed their camels in the

range lands. About 23.4% of them watered their livestock in rivers (seasonal streams or permanent ones like Tana river in Garissa); 15.6% utilized bore holes, 12.5% shallow wells; 23.4% water dams and shallow wells; and 25% used water pans (see pictures on **Plates 3.1** and **3.2**). The livestock were kept under pastoralist system. Camels were watered every 3 to 5 days (34.4%) or once every week (7 days; 45.3%), depending on the distance to the water source from the pastures. In cases of long distances (like 15-30 kilometers), watering intervals could range from 9 to 12 days. Feed supplementation with conventional mineral salts was done by some of the respondents (18.8%). This was done during rainy season, when camels were grazing on pastures on mineral-deficient soils (eg. red soil). The mineral salts were bought from local shops. It was, however, noted that the majority of respondents (81.3%) did not supplement their camels. All those that supplemented their camels with mineral salts said they did so to prevent mineral deficiency.

Table 3-4 Data on feeding and watering of camels

Activity	Respondents(n=64)	No. of respondents	% respondents
People taking care of the camels	Herdsboys	23	35.9
	Hired herdsmen	12	18.8
	Male owners	29	45.3
Watering points for The camels	Rivers (seasonal or permanent	15	23.4
	Boreholes	10	15.6
	Shallow wells	8	12.5
	Water dams	15	23.4
	Water pans	16	25.0
Watering frequency for the camels	Every 3 to 5 days	22	34.4
	Once a week	29	45.3
	Others (9-12 days)	13	20.3
Feed supplementation For the camels	No supplementation	52	81.3
	Supplementation	12	18.8

Plate 3-1A watering point in Garissa county

Notice (1) cattle, sheep, goats, camels and donkeys intermingling at the water-point, and (2) women drawing water for domestic use from the same point where animals are watering

Plate 3-2 Watering camels in a communal trough

The water-troughs are filled with water drawn from a near-by well (seen on the foreground on the right of the plate). The two young men on the right were involved in the filling of the troughs

3.3.4 Profiles of camels kept by pastoralists

Table 3-5 gives various data on age, sex and lactation levels of camels kept by the respondent pastoralists. The respondents kept camels of various ages and sexes, more so adult females (lactating/non lactating, pregnant) which were of ages more than 3 years. The overall mean number of female per respondent for camels was 14; 10 of which (on average) were lactating. Average number of adult was 4; that of growers and calves (either male or female) was 4 and 3, respectively. The grower group consisted camels that were 1-2 years old, while the calves were less than one year old.

Table 3-5 Profile of camels kept by pastoralists

Activity	No. of animals kept per respondent		Average
	Minimum per respndent	Maximum per respondent	
Number of adult males	1	15	4
Number of adult females	2	60	14
Number of lactating females	1	45	10
Number of growers and weaners	0	15	4
Number of calves	0	10	3

Number of respondents = 64

3.3.5 Milk production levels and milking methods used

Table 3-6 gives data on milk production per camel at various milking times, while **Table 3-7** gives data on milk production per camel in various seasons. From the 64 responses, it was noted that the minimum number of milking times per day per camel was 3.and the maximum was 4; the mean number being 3.8 (two times or once in the morning and two times in the afternoon/evening). The morning milking produced an average of 1.3 liters per camel while the evening milking produced an average of 1.5 liters **(Table 3-6).** When observation was made in terms of seasons, the mean amount of milk production per camel during dry season was calculated to be 4.1 liters, while that in wet season was 8.2 liters **(Table 3-7)**. Lactating camels were milked either by herding boys/employed herdsmen (24/64 = 34.4%), women (15/64 = 23.4%), or male owners (27/64 = 42.2%). All the respondents said they practiced

what is known as “dry milking”. That is: the udder is not washed with water, but dust is wiped from the udder and teats with the palms of the milker’s hands and milking started immediately. At times, when not being milked, the camel owners tied one or two teats of the camel with a piece of rope from the bark of a tree to serve as an anti-suckling device **(Plate 2.1)** – to prevent calves from suckling. These ropes were untied before milking commences.

Table 3-6 Milk production at various milking times daily

Time of day	**Amount of milk produced in liters at various milking times**		**Mean amount produced in liters at various milking times**
	Minimum	**Maximum**	
In the morning	1	2	1.3
In the evening	1	2	1.5

Table 3-7 Milk production per day per season

Season	**Amount of milk produced in liters per camel per day**		**Mean amount produced in liters per camel per day**
	Minimum	**Maximum**	
Dry season	3	6	4.1
Wet season	7	10	8.2

3.3.6 Problems encountered during milking and inter-calving intervals

Tables 3-8 and 3-9 give data on problems encountered during milking and intercalving intervals, respectively. According to the respondents, some of the problems encountered during milking of the camels included: swollen and painful udder and teats (25%), milk discoloration (bloody or reddish; 25%), changes in milk consistency (watery, clotted, creamy,

etc; 21.9%) and traumatic lesions on the udder or teats (28.1%) **(Table 3-8)**. The traumatic lesions may have been caused by thorns, teat-tying practice, pox infection, mange infestation and tick-bite wounds. Camels were milked for periods between 2 to 4 years before they could conceive again, ie: calving interval was reported to be 2-4 years **(Table 3-9).**

Table 3-8 Problems encountered during milking

Problem	No. of respondents reporting it	% respondence
Change in milk consistency	14	21.9
Milk discoloration	16	25.0
Swollen and painful udder/teats	16	25.0
Traumatic lesions on udder or teats	18	28.1

Number of respondents = 64

Table 3-9 intercalving intervals (years) among the camels

Calving interval	No. of respondents reporting it	% respondence
2 years	21	32.8
3 years	32	58.0
4 years	11	17.2

Number of respondents = 64

3.3.7 Milk containers used by the pastoralists and how they are cleaned

Containers that the pastoralists used for milking, preservation and transportation of camel milk, and their respective average costs, were as given on **Table 3-10**. They included:

traditional gourds, used at 48.4%; aluminium/steel, cans at 17.2%; and plastic jerrycans, at 34.4%. All those interviewed said the milk containers belonged to them. Traditional gourds, of approximate capacity of 3 liters, and aluminium/steel cans, of 5 liter capacity, were used for milking. Traditional gourds, of 6 liter capacity, and plastic jerrycans, of varying sizes, were normally used for milk transportation; while traditional gourds of 10 liter capacity, aluminium/steel cans of 5 liter capacity, and plastic jerrycans of varying sizes, were used for milk preservation. The traditional gourds were curved from wood. The cost of buying traditional gourds ranged from KShs. 400 to 600; that for aluminium/steel cans (5 liters) was KShs.300; while that for plastic jerrycans ranged from KShs. 20 to 250. The traditional gourds of different capacities seemed to be referred to by different terms: "Amel" for one with approximately 3 liter capacity; "Sulma" for one with approximately 6 liter capacity; and "Gilla" for one with approximately 10 liter capacity.

Table 3-10 Milk containers used by pastoralists and their cost

Containers	**No. of respondents using it**	**% respondence**	**Average costs**
Aluminium/steel cans	11	17.2	KShs. 300 per 5 liter can
Plastic jerricans	22	34.4	KShs. 20 per 3 liter can; 30 per 5 liter can; and 250 per 20 liter can
Traditional gourds	31	48.4	KShs. 400-600 each

Data on how milking containers are cleaned and timing of the cleaning are given on **Table 3.11.** All interviewees cleaned their milk containers using hot or cold water without any

detergent, immediately after milking and just before the next milking. Some (34/64 = 54.7%) used traditional herbs to clean the containers. The Woman on **Plate 3-3** was demonstrating to the researcher how the pastoralist traditional milk containers ("Amel" and "Sulma") were cleaned and smoked using traditional herbs (locally known as "Meril" or "Agel"). Those using traditional herbs normally washed their containers immediately after milking (when they also smoke-treated them with the herbs). Others, 17/64 (26.6%) washed their containers just before the next milking, while 12/64 (18.8%) washed immediately after milking but did not use herbs.

Table 3-11 How and when milk containers were cleaned

Method used		**Number**	**%**
Using hot and cold water immediately after milking andjust before next milking		64(all those interviewed	100
Additional usage of traditional herbs	**Overall number using herbs**	34	54.7
	Those using them imme-diately after milking	17	26.6
	Those using them before next milking	12	18.8

Plate 3-3 Traditional gourds used by pastoralists to milk and store milk

The person was also demonstrating to the researcher how the pastoralist traditional milk containers ("Amel" and "Sulma") were cleaned and smoked using traditional herbs.

3.3.8 How camel milk benefited the camel keeper

The camel milk benefited the camel keepers in several ways: (1) sold to earn some income/cash (economic gain); (2) used for domestic consumption for both children and adults – it could be taken fresh or sour (susa); (3) used to feed calves; and (4) used traditionally as medicine, to treat various ailments including diabetes, diarrhea and hypertension. All the respondents stated similar benefits.

3.3.9 Camel diseases noticed by the pastoralists

When interviewed, the pastoralists claimed to have noticed camel diseases. Their responses, together with respective proportions (except for recumbency and nostril infestation with flies) are given in **Table 3-12.** The most mentioned diseases/conditions were diarrhea and camel

pox (at 14.1%, each), followed by brucellosis and mastitis (at 10.9%, each), and trypanosomosis and udder injury (at 9.4%, each).

Table 3-12 Camel diseases from pastoralists and respective proportions

Camel diseases noticed		Traditional (Somali) name of the Disease	No. of respondents	% respondence
1	Trypanosomosis	Gedi, Kharal, Dhukan	6	9.4
2	Brucellosis	Sevudut	7	10.9
3	Diarrhoea	Adeye	9	14.1
4	Physical Udder injury		6	9.4
5	Pneumonia		2	3.1
6	Foot rot / Lameness		3	4.7
7	Camel Pox	Haday	9	14.1
8	Mange Infestation	Haday	2	3.1
9	Tick infestation	Keril	5	7.8
10	Mastitis (Swollen Udder)		7	10.9
11	Emaciation (Unthriftness)	Shoque	1	1.6
12	Abscesses		6	9.4

Number of respondents = 64

3.3.10 Management decisions on camels

All the respondents had similar answers. Most of the milk produced by the camel during dry season was sold/consumed locally as fresh (unpasteurized) milk; very little was sold/consumed as sour (*susa*) milk. However, during wet/rainy season, when the camels produced a lot of milk, most of it was sold as fresh (unpasteurized) milk, while remainder was consumed or sold as sour (susa) milk.

Table 3-13 gives data on treatment of sick animals and how milk from mastitic and treated camels was disposed off or consumed. Most of the treatment (71.9%) was done locally by the owner or herdsperson; 46.9% using conventional medicine, while 25% used traditional medicine/herbs. Milk from mastitic camels was mainly given to calves (54.7%), while some (23.4%) poured it out and some (15.6%) sold it for cash. Rarely (6.3 %) did they consume the milk. On the other hand, for milk from treated camels, 60.9% was given to calves, 25% was consumed by the people, and only 6.3% was poured off, and 7.8% sold for cash. Taking care of the animals (grazing, watering) was a shared chore among male owners, herdsboys and employed herdsmen, while management decisions were mainly made by the male owners, in consultation with their wives/women.

Table 3-13Treatment of sick camels and disposal of milk

			Number	%
Treatment of sick camels	**By Govt. Vet.**		18	28.1
	By owner	**Using conventional medicines**	30	46.9
		Traditionally, using herbs	16	25
Disposal of milk from mastitic camels	**Domestic consumption**		4	6.3
	Given/fed to calves		35	54.7
	Poured after milking		15	23.4
	Sold for cash		10	15.6
Disposal of milk from treated camels	**Domestic consumption**		16	25
	Given/fed to calves		39	60.9
	Poured after milking		4	6.3
	Sold for cash		5	7.8

3.3.11 Camel milk use for domestic consumption

Most of the camel milk meant for domestic/home use was consumed as raw (unpasteurized) fresh milk (42/64 = 65.6%). However, it was sometimes subjected to Flash-boiling (heating the milk briefly to just before boiling) (22/64 = 34.4%) before human consumption.

3.3.12 Reasons for consumption of raw camel milk

There were many reasons why the pastoralists preferred to consume raw (unpasteurized) fresh camel milk**.** These were; (1) claim that raw fresh camel milk was sweet and palatable, (2) belief that camel milk contained some medicinal substances and vitamins that would be destroyed by heating/boiling/pasteurization (6/64 = 9.4%) and (3) belief that raw camel milk was clean (without any disease-causing microorganisms) and thus there was no need of heating it before consumption (58/64 = 90.6%).

3.3.13 Preservation of camel milk by the pastoralists

All respondents gave same response. They did not put any additives (preservatives) to their camel milk after milking; the milk was collected and stored in traditionally-treated (smoked) containers (gourds) using smoke produced from some special traditional herbs (locally known as "Meril") before the milk was disposed (sold) or consumed domestically. Disposal of camel milk meant for sale was done almost immediately after milking, especially if done during day time. The one that was milked in the evening was disposed off (sold) the following day, early in the morning.

However, the respondents knew that camel milk could be cooled using refrigerator, coolant plants or cold rooms. Since all the above-suggested methods would require the use of some energy (electricity or solar energy), the pastoralists said they were willing to contribute towards meeting the cost of such energy, whenever possible (ie. contribute to pay for and maintain the power supply.

3.3.14 Milk marketing and seasonal variation of demand

Pastoralists, who were the primary producers of camel milk, normally sold the milk to milk hawkers (63/64 = 98.4%) in the local town centres. The hawkers, in turn, transported the milk to larger/bigger/major town centres (Garissa and Wajir towns), where it was sold to bulkers and retailers, who finally sold it to the consumers. Very rarely did the camel keepers sell their milk directly to bulkers (1/64 = 1.6%).

Table 3-14 gives milk prices, with respect to seasons. The milk was sold expensively during the dry season (mean price KShs. 57.95 per litre), due to reduced milk production. The mean price for wet season, when there was plenty of milk, was KShs. 25.06 per litre. All respondents indicated that the demand for milk was low during wet season and very high during dry season.

Table 3-14Milk prices (KShs) per litre with respect to seasons

Season	**Milk prices per liter**		
	Minimum price	Maximum price	Mean price
Dry season	40	68	57.95
Wet season	20	27	25.06

3.3.15 Camel-milk marketing societies and milk processing

Most of the pastoralists interviewed (59/64 = 92.2%) were willing to come together and form a camel-milk marketing society so that they could easily control the producer price of camel milk. They were, however, of the opinion that their camel milk would fetch more money in the market if the following things were done:- (1) be trained; on camel udder hygiene, and how to hygienically handle and produce milk; this way, they would be able to process and package their own milk in organized groups and improve on the milk hygiene (63/64 = 98.4%)**,** (2) receive Government assistance in terms of: disease control and management; provision of coolants (refrigerators) and cold rooms; provision of hygienic aluminium milk cans (containers) that are easy to clean/wash and sterilize; and provision of subsidized transport means to town markets from the production areas (8/64 = 12.5%)

3.3.16 Access to credit facilities to improve livestock keeping

All pastoralists interviewed had no credit access to improve on their camel rearing/keeping activities. They said that they would only be interested in such a credit facility as long as it does not attract any interest at all. If such a credit was advanced to them, they would buy more livestock (camels) to increase their number and also buy pharmaceutical drugs used to treat animal diseases.

3.4 Discussion

The types of livestock kept by the pastoralists in Garissa and Wajir counties included cattle, sheep, goats, camels, donkeys and chickens. Apart from chickens, all other animals were grazed together in the rangeland and were watered at the same points. These livestock, including camels, were kept under pastoralist system. There was, therefore, a high chance of spread of infectious diseases, like mastitis and brucellosis, among them. The other species of

animals, which were kept together with the camels, could serve as sources of udder infection for the camels (Obied *et al,* 1996). The pastoral communities of North-Eastern province of Kenya reported keeping of camels for economic, domestic and socio-cultural purposes. Economically, camels or their products (meat, milk and hides) were sold in order to earn cash income. Camels provided milk and meat for domestic consumption and were also used as a means of transport, especially when the pastoralists relocated, and also for draft power. Socio-cultural purposes included traditional ceremonies like payment of dowry and contribution towards fund-raising activities. Camels were also kept as an indication of the person's wealth status within the community. These attributes to the camel have been reported in other pastoralist communities (Schwartz and Dioli, 1992); milk and meat being taken as the important products. A study in eastern Ethiopia indicated 3 to 6 liters of daily milk yield over 13 to 15 months of lactation length (Getahun and Bruckner, 2000), while Tefera and Gebreab (2001) reported an average daily milk yield of 2.5 liters. These values compare well with the figures obtained for Garissa and Wajir counties in this study (mean value of 4 liters per day). Long lactation and ability to maintain milk production over long dry spells are important facets of camel productivity. It is common practice that, apart from home consumption, households sell at least one-third of the produced milk to generate cash income (Getahun and Bruckner, 2000). With improved management, dairy milk yield has been shown to rise to a high of 20 liters (Schwartz and Dioli, 1992). Until the arrival of motorized transport in the arid and semi-arid areas, camels have been the sole means of transport in the areas where they have been adapted. They are also used for wheel transport, water lifting and source of power for oil mill. Camel-racing and other leisure activities, such as camel safaris and trekking, have recently become tourist attractions and luxurious in some parts of the world (Schwartz and Dioli, 1992; Wilson, 1998).

During milking, the pastoralists practiced what is known as “dry milking”. The pastoralists also tied one or two teats of the camel with a piece of rope from bark of a tree (calves anti-suckling device; **Plate 2.1**). These ropes were first untied before milking commenced. Some of the problems encountered during milking of camels included: swollen and painful udder and/or teats, milk discoloration (bloody or reddish), changes in milk consistency (watery, clotted, creamy, etc), traumatic lesions on the udder and/or teats that could have been caused by thorns, teat-tying practice, pox infection, mange infestation, or tick wounds. These observations are consistent with what other researchers have found in the past. It is documented that poor management and unhygienic milking practices, prevalent in the pastoral traditional husbandry systems, include tying of teats with soft barks to prevent the calf from suckling, tick infestations and cauterization of the udder skin (Abdurahman *et al*, 1995a; Obied *et al*, 1996; Almaw and Molla, 2000; Woubit *et al*, 2001.) The use of anti-suckling devices in North-Eastern province camels was practiced during day time, when young calves older than one year were herded together with their dams. The use of these devices, together with heavy tick infestation, could predispose the udders to bacterial infections (mastitis), which may be chronic. This could result in induration and atrophy of injured quarters (Obied *et al*, 1996). Some of the bacteria that cause mastitis may cause disease in humans; these include: *Streptococcus* species, *Staphylococcus aureus* and those that cause the more important zoonotic diseases: *Brucella* and *Mycobacterium* species (Younan *et al*, 2001; Abdurahman *et al*, 1995).

The respondents indicated that milk produced by the camels was normally sold or consumed domestically as raw-fresh (unpasteurized) milk or as sour (*susa*) milk. Sometimes, the fresh milk was subjected to flash boiling (heating the milk briefly to just near boiling) before consumption; an exercise that hardly killed the bacteria in the milk. Many reasons were given

for the preference to raw milk; most of which were cultural beliefs that had no scientific basis. This practice of drinking raw or under-boiled milk increases the chances of transmission of these organisms to humans. The respondents practiced traditional hand-milking; stored and transported the milk in traditional gourds ["Amel", which carried approximately 3 liters; "Salma", which carried approximately 6 liters; and "Gilla", which carried approximately 10 liters **(Plate 3.1)**]. They also used aluminium/steel cans, which carried approximately 5 liters, and plastic jerrycans, with various capacities of 3, 5 and 20 liters. After washing, the containers were normally smoked using traditional herbs, locally known as "Meril" or "Agel"; this was done to preserve the milk for longer period of time and for flavoring. The storage and transportation of the milk was normally done under low hygienic conditions, which have been demonstrated, in another study, to introduce gross dirt, especially charcoal particles in the milk (Younan *et al*, 2002). Pooling of different raw camel milk batches and usage of unhygienic plastic containers have been shown to accelerate spoilage, with non-refrigerated bulk milk reaching a total bacterial count of 10^4 cfu/ml (Younan *et al*, 2002); this milk turns sour in less than 12 hours under the hot pastoral conditions. Plastic jerrycans of cheap quality used (eg. recycled cooking oil containers) have been shown to have a fast-corroding surface and are not cleaned properly in pastoral areas, due to lack of water. The non-availability of safe clean water makes introduction of common hygiene recommendations difficult; thus adapting hygiene practices and guidelines to the pastoral situation remains a big challenge (Younan *et al*, 2002). The interviewees claimed to have noticed the following camel diseases: trypanosomosis, brucellosis, diarrhea, physical udder injury, pneumonia, foot-rot/lameness, camel pox, mange infestation, tick infestation, mastitis (swollen/painful udder), emaciation (unthriftiness), recumbency, nostril infestation by flies, and abcesses. Published information on diseases reveals that camels may be either carriers of, susceptible to or suffering from a vast array of infectious and parasitic diseases

(K**ö**hler-Rollefson *et al*, 2001). Trypanosomosis, caused by *Trypanosoma evansi* is found to be the major health problem in pastoral areas. Other diseases, such as pox, contagious skin necrosis, pneumonia and parasitic infestations are also known to affect the health of camels in these areas (Demeke, 1998). Some of the diseases such as brucellosis have considerable public health importance in these areas, as camel milk is consumed raw (Abbas *et al*, 1987; Gameel *e al*, 1993).

These pastoralists also treated their own camels (livestock) using various methods, including: usage of conventional medicine obtained/bought from pharmaceutical companies; and usage of traditional herbs. There was, however, some involvement of Government veterinarians, but mostly for disease control and prevention purposes (vaccination campaigns). This habit of pastoralists treating animals on their own, without any professional advice was not desirable, since indiscriminate use of antibiotics could lead to the build-up of bacterial antibiotic resistances both in animals and humans consuming the camel products (milk and meat).

During the wet season, the demand for camel milk was reported as being relatively low, since a lot of milk was produced (surpassing the demand; with a lot going to waste), while during dry season, the demand was relatively high, since little milk was produced (could not meet the demand). Thus, the producer prices during wet season were quoted as being very low (mean price of KShs. 25 per liter), and those during dry season as high (mean price of KShs. 57.95 per liter). It was also reported that, during period of milk surplus (rainy/wet season), transport on earth/dirt roads was unreliable, resulting in vehicle breakdowns and delays in milk deliveries. Storage in unhygienic containers (plastic and traditional gourds), mixing of evening and morning milk collections, pooling of milk from different suppliers, prolonged transport times, high environmental temperatures, and road-side selling of milk in open

containers, have all been recorded as factors that increase contamination and spoilage of milk (Younan *et al*, 2001).

This study has shown that the way the people of North-Eastern province live and relate to their animals and animal products exposes them to milk-borne and other zoonotic diseases. It was, therefore, found interesting to establish the prevalence of clinical and, especially, subclinical mastitis, as one of the sources of bacterial contamination.

4 SUBCLINICAL MASTITIS

4.1 Introduction

Subclinical mastitis is more common in animals than clinical mastitis (Younan *et al*, 2001); it is thus a major factor in erducing milk yield and has a much greater impact on the productivity of lactating animals than the sporadic clinical forms of the disease. Apart from the reduced milk yield, the milk produced, in cases of subclinical mastitis, appears normal; in contrast to that produced in cases of clinical mastitis. There is little information on camel mastitis, compared to that of the cow. This is because the disease was thought to be uncommon in this species of animal (Abdurahman *et al*, 1995). However, according to the current available literature, mastitis in the camel has been reported from a number of camel-rearing countries of the world during the past years: Kenya (Younan *et al*, 2001), Iraq (Al-Ani and Al-Sharreffi, 1997), and Israel (Guliye *et al*, 2002). There is lack of information on the aetiology of subclinical mastitis in Kenya. Therefore, one of the reasons for this study was to determine the bacterial pathogens associated with subclinical mastitis in camels in Garissa and Wajir counties of North-Eastern province of Kenya.

4.2 Materials and Methods

4.2.1 Study area and animals

This was as given in section 3.2.1.

4.2.2 Sample collection

Samples of milk produced by locally-kept camels, were collected from various market points in the two counties. Volumes of 200 to 300 milliliters of bulk camel milk (from producers or hawkers) were collected into labeled sterile bottles **(Plate 4.1)** and kept in an ice box, transported to laboratory for bacteriological culture and identification, which was done either immediately or after keeping them for not more than 24 hours in a refrigerator.

Plate 4-1 Camel milk collected into sterile 200 ml bottles

4.2.3 Sample size (n) calculation

The sample size (n) was determined based on anticipated prevalence of mastitis in camels; in Kenya, it is estimated at 25% (Younan *et al*, 2001). The calculation was done using the formula of Dahoo *et al.* (2003), as given below:

$$\mathbf{n} = \frac{Z\alpha^2 pq}{L^2}$$

Where

n = estimated sample size

Zα = 1.96 = the normal deviate at 5% level of significance

p = estimated prevalence (25%)

q = 1 – p (75%)

L = precision of estimate (considered to be 5%)

This translated to 288 camel milk samples (samples collected 384)

4.2.4 Study design

The study was cross-sectional. The collected milk samples from various producers and hawkers were investigated using California Mastitis Test (CMT), general demonstration of bacterial presence in milk (direct smears), isolation and identification of the bacteria, with specific search for presence of *E. coli* serotype 0157:H7

4.2.4.1 California Mastitis Test (CMT)

California Mastitis Test was done according to the procedure described by Schalm *et al* (1971) to screen the milk samples for subclinical mastitis using a CMT kit. The test was carried out following standard methods (Quinn *et al,* 1994; Schalm *et al,* 1971). The

interpretation of the test was as follows: CMT score **0** was taken as **"negative"** (-ve), while CMT scores **"Trace,"** "positive one" **(1^+),** "Positive two" **(2^+)** and "positive three" **(3^+)** were considered positive. These considerations were based on the amount of gel formation **(Plate 4.2)** in the sample, which formed the basis of interpretation **(Table 4.1)**. All milk samples positive for CMT were subjected to bacteriological examination (culture and identification).

Plate 4-2 California mastitis test(CMT) reaction

Note the slight slime formation **("Trace" score)** in the lower two wells of the paddle. The upper two wells have no gel formation **("Negative" score)**

Table 4-1 CMT Reaction and equivalent Somatic cell count (SCC) in cattle

Test results	Reaction observed	Equivalent milk SCC
Negative	No gel formation	0 – 200,000 cells/ml
Trace	A slight slime formation	150,000 - 500,000 cells/ml
1+	Distinct slime formation immediately	400,000 - 1,500,000 cells/ml
2+	Formed slime settles at the bottom and side	800,000 - 5,000,000 cells/ml
3+	Formed slime is convex and domed up	>5,000,000 cells/ml

Source: Radostits *et al*-2000

4.2.4.2 Gram stain on camel milk smears

Gram stain procedure was performed according to the method described by Forbes *et al* (2002) and Bebora *et al* (2007). It was done for all the 384 camel milk samples collected.

4.2.4.3 Isolation and identification of bacteria

Bacteriological examination was carried out following standard methods (laboratory and field handbook on bovine mastitis, 1987, Quinn *et al*, 1994, Sears *et al*, 1993). Briefly a loopful of each milk sample was streaked on 7% sheep Blood Agar (BA). MacConkey Agar (MaC) plates were used in parallel to detect *Enterococcus* species and any gram-negative bacteria. Inoculated plates were incubated aerobically at 37^0 C for 24 – 48 hours. Presumptive identification of bacterial isolates on primary culture were made based on colony morphology, haemolytic characteristics on blood agar, Gram stain reaction, catalase and oxidation – fermentation tests (Quinn *et al*, 1994, Sears *et al*, 1993, Forbes *et al*, 2002). *Staphylococcus* and *Micrococcus* species were identified based on their growth characteristics on Mannitol Salt agar (MSA), coagulase production, catalase, and oxidase

tests. *Streptococcus* species were evaluated according to CAMP reaction (*Streptococcus agalactiae* potentiates *Staphylococcus aureus* hemolysin leading to complete or Beta (β) haemolysis of the red blood cells on bovine blood agar – a positive CAMP test), growth characteristics on 7% sheep blood agar, catalase production and sugar fermentation tests (Quinn *et al,* 1994, Sears *et al,* 1993, Forbes *et al,* 2002). Gram-negative isolates (Enterobacteriaceae) were sub-cultured on MacConkey agar and further tested using Triple sugar Iron (TSI) Agar, IMViC test (Indole, Methyl red, Voges-Proskauer and Citrate utilization test) and oxidase reaction (Quinn *et al,* 1994, Sears *et al,* 1993, Forbes *et al,* 2002).

Primary bacterial isolation was done in the field laboratory (Garrissa District Veterinary Investigation Laboratory (Garissa VIL; **Plate 4-3**). Bacterial colonies from the two primary isolations (7 % Sheep Blood Agar and MacConkey Agar) were inoculated into Nutrient Agar slants (Transport media), incubated at 37^{0} C for 12 hours, and then stored at 4^{0} C in Garissa VIL. These colonies were later transported in a cool box to the University of Nairobi, Bacteriology laboratory, for secondary bacterial culture and further biochemical testing/characterization, using the same type of media.

All bacterial isolates were preserved in glycerol-Nutrient broth at 0^{o}C until time to work on them.

Plate 4-3 Veterinary Investigation Laboratory (VIL), Garissa

4.2.5 Statistical data analysis for subclinical mastitis in camels

Data collected was entered into Ms-Excel as data package for processing and was analyzed with Instat for windows to obtain frequency distribution for California Mastitis Test (CMT) and isolation of various bacterial microorganisms including *Staphylococcus* species, *Micrococcus* species, *Streptococcus* species, *Bacillus* species, *Escherichia coli*, *Klebsiella* species and *Enterobacter* species.

4.3 Results

4.3.1 Results of California Mastitis Test (CMT)

Table 4-2 and **Figure 4-1** give frequency distributions for CMT positive reactions for Garissa and Wajir counties, separately and collectively, while **Figure 4-2** gives comparison of frequencies, in percentage, of CMT for the 2 areas, separately and collectively. Of the 384 camel samples (230 from Garissa and 154 from Wajir) investigated for subclinical mastitis through cell counts, 235 (61.2%) gave positive reactions. The equivalent somatic cell counts

(SCC/ml) of the positive samples ranged from $1.5x10^5$ to $5x10^6$ leucocytes per milliliter of milk. For Garissa and Wajir counties, separately, 139/230 (36.2%) and 96/154 (25%) gave positive reactions, respectively. For both, the majority of the reactions were traces, followed by 1+ scores. Those with 2+ scores were fewer. It should be noted, however, that Wajir samples gave a higher percentage of 2+ scores and lower percentage of trace scores than Garissa.

Table 4-2 Frequency distribution for Califortnia Mastitis test

	Garissa n = 230		**Wajir n = 154**		**Garissa and Wajir Combined n = 384**	
	No. positive	% positive	No. positive	% positive	No. positive	% positive
Overall positive	139	60.4	96	62.3	235	61.2
2+	4	1.7	10	6.5	14	3.7
1+	58	25.2	40	26.0	98	25.5
Trace score	77	33.5	46	29.9	123	32.0

Key:

Trace (positive) – A slight slime formation

1+ positive - Distinct slime formation immediately

2+ positive - Distinct slime formation which settled at the bottom and sides when the paddle is swirled

Negative - No gel formation

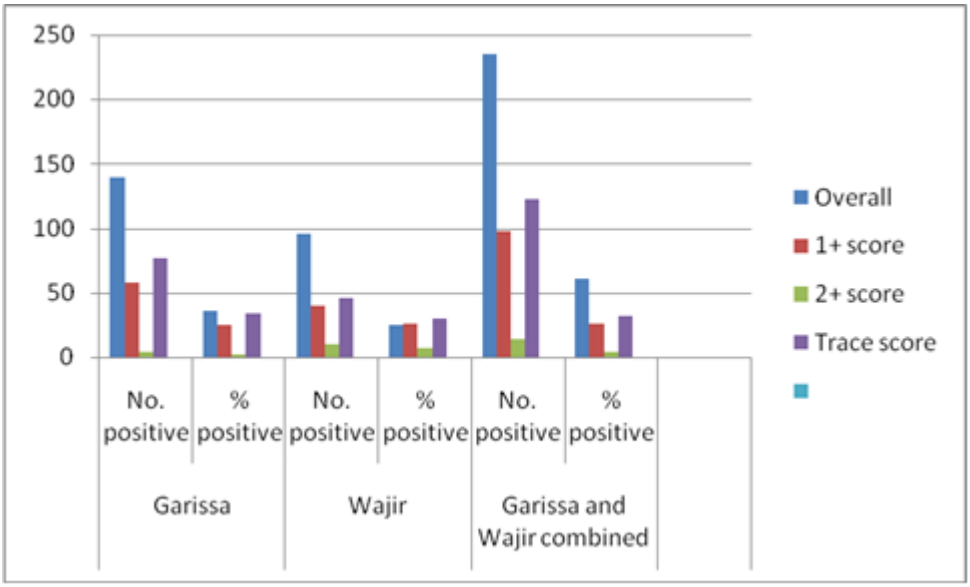

Figure 4-1 Frequency distribution of California Mastitis test

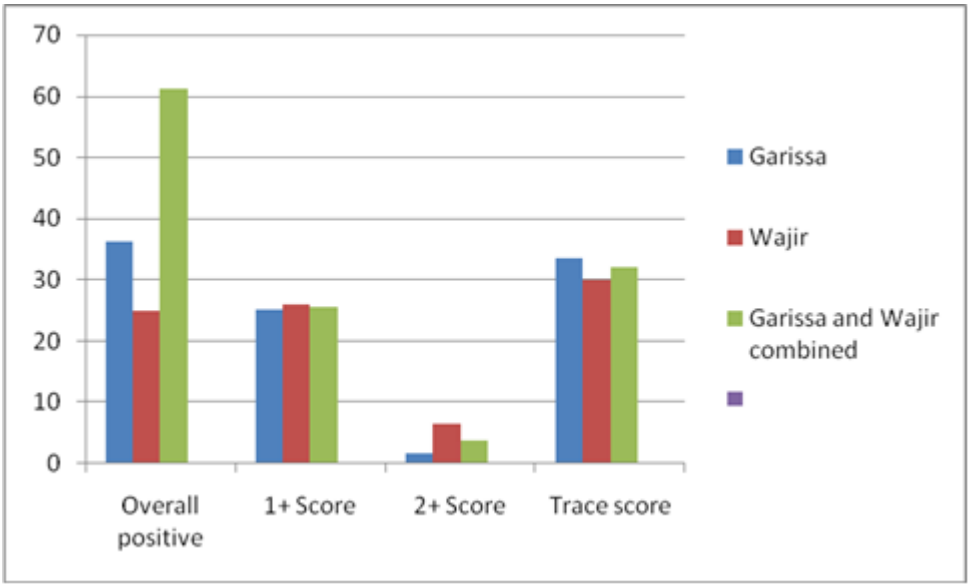

Figure 4-2 Comparison of frequencies for Wajir and Garissa counties

4.3.2 Results of Gram stain on smears of camel milk

Gram stain revealed the presence of both Gram positive and Gram negative bacterial cells in the milk samples tested. The result of Gram stain was used as an indicator of what was to be expected on primary isolation of the bacteria on solid media (culture). The suspected bacteria, based on Gram staining reaction are given on

Table 4-3 Gram stain on smears of camel milk

	Gram stain observation at oil immersion (×1000 magnification	**Bacterial microorganism suspected**	**Samples**	
			No. positive	**% positive**
1	Gram positive cocci, characteristically arranged in grape-like clusters, in pairs and singly.	*Staphylococcus* species	346	90.1
2	Gram positive cocci, occurring singly, in pairs or in small clusters of varying sizes (3 – 6 cells).	*Micrococcus* species	346	90.1
3	Gram positive cocci, characteristically occurring in chains of variable length (4 – 8 cells), singly or in pairs	*Streptococcus* species	326	84.9
4	Gram positive bacillus, sporulated (with terminal or central spores), occurring singly, in pairs or in small clusters (3 – 5 cells).	*Bacillus* species	176	45.8
5	Gram negative (Red/Pink) coccobacillus (straight rods) with rounded end, cells occurring in pairs or singly	*Enterobacteriaceae* including *Escherichia coli, Klebsiella and Enterobacter* species	368	95.8

No of samples = 384

4.3.3 Bacteria isolated from the camel milk samples

Table 4-4 shows bacteria (and their respective prevalences) isolated from the camel milk samples from Garissa and Wajir, respectively, and as combined data, while **Figure 4-3** gives comparison of occurrences, with respect to the various bacteria isolated; **Figures 4-4 and 4-5** give breakdowns of isolated *Staphylococcus* and *Streptococcus*, with respect to coagulase production and CAMP reaction, respectively.

The two areas had similar patterns of bacterial occurrences; the highest across board was *Klebsiella/Enterobacter* group, isolated at 96%, followed, frequency-wise,by *Staphylocccus* (94% in Wajir and 88% in Garissa); *Streptococcus,* at 85% in both areas; *E. coli* (Garissa 59.9% and Wajir 40.3%); and lastly *Bacillus* (Garissa 45.8% and Wajir 50.4%). Thus, Garissa yielded more of *Bacillus* and *E. coli* organisms than Wajir, while Wajir yieded more *Staphylococcus* than Garissa. *Streptococcus* and *Klebsiella/Enterobacter* were isolated at more-or-less the same rate in both areas **(Table 4-4; Figure 4-3).**

Garissa yielded more (30%) coagulase positive *Staphylococcus* than Wajir (14.3%), despite more *Staphylococcus* having been isolated from Wajir (93.5%) as compared to Garissa (87.8%) **(Figures 4-3; 4-4).** Garissa also yielded more (31.3%) CAMP positive *Streptococcus* than Wajir (19.5%); *Streptococcus* was isolated at same prevalence (85%) for the two areas **(Table 4-4; Figure 4-3).**

Table 4-4Bacteria isolated from Wajir and Garissa milk samples

		Garissa n = 230		Wajir n = 154		Garissa and Wajir Combined n = 384	
		No.	%	No.	%	No.	%
Staphylococcus	Total isolated	202	87.8	144	93.5	346	90.1
	Coagulase positive	69	30.0	22	14.3	91	23.7
	Coagulase negative	133	57.6	122	79.2	255	66.4
Streptococcuc	Total isolated	195	84.8	131	85.1	326	84.9
	CAMP positive	72	31.3	30	19.5	102	26.5
	CAMP negative	123	53.5	101	65.6	224	58.3
Bacillus spp.		116	50.4	60	39.0	176	45.8
E. coli		168	73.0	62	40.3	230	59.9
Klebsiella/ Enterobacter		221	96.1	148	96.1	368	95.8

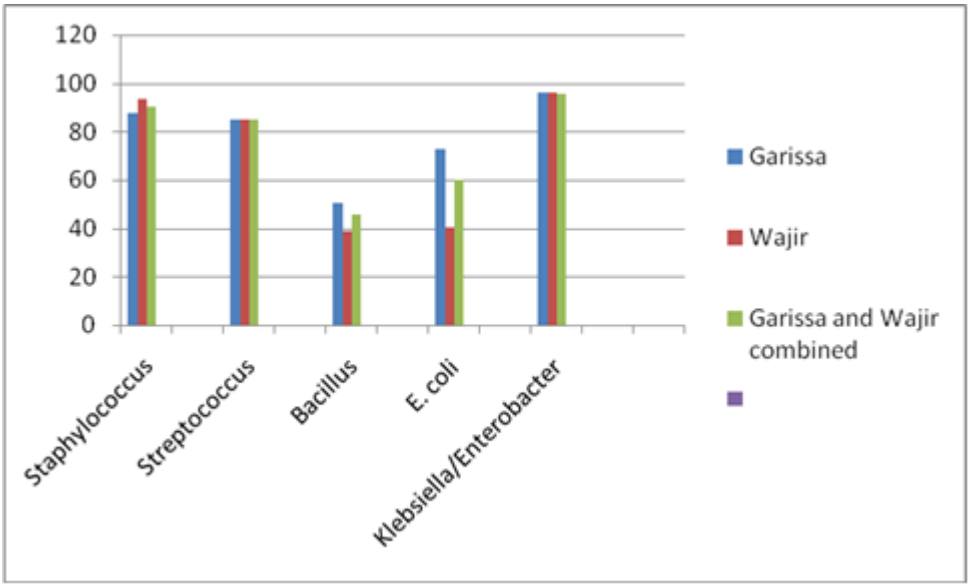

Figure 4-3 Comparison ofpercentage occurences per bacterial organism

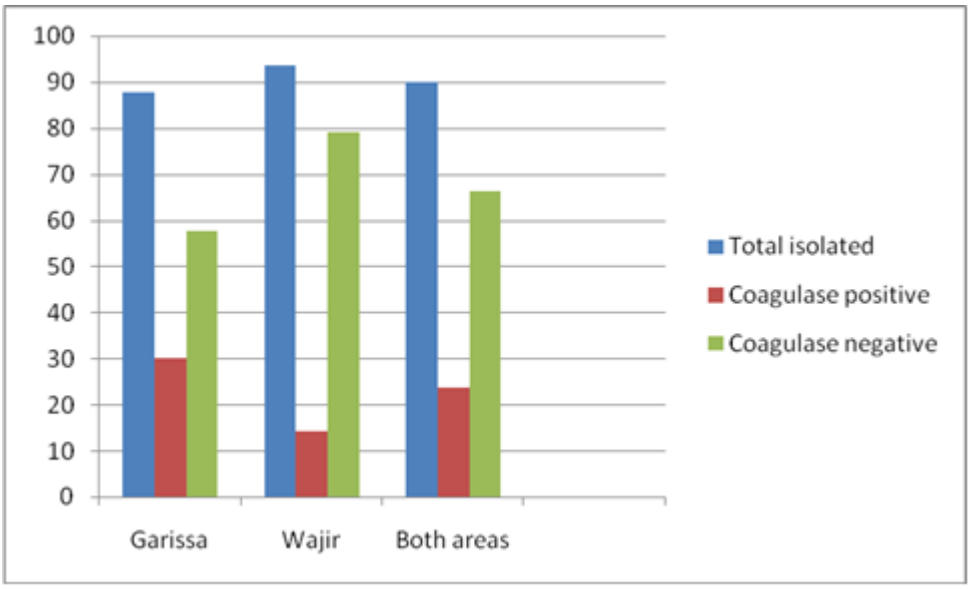

Figure 4-4 Staphylococcus prevalences both coagulase positive and negative

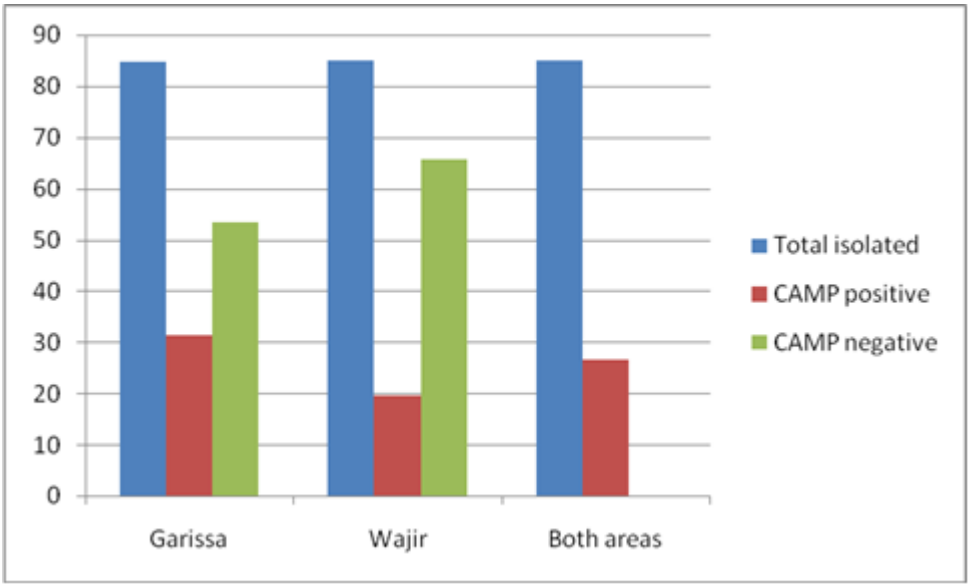

Figure 4-5 Streptococcues prevalences: Both CAMP positive and negative

4.4 Discussion

A total of 384 camel milk samples from the two counties of Garissa and Wajir were collected for laboratory analysis. When CMT was carried out, 235 samples (61.2%) were positive for subclinical mastitis. The equivalent somatic cell counts (SCC/ML) of the 235 positive milk samples ranged from 1.5×10^5 to 5×10^6 leukocytes per millitre of milk. All the milk samples also yielded mixed types of bacteria on culture. These included *Staphylococcus* species (346 samples = 90.1%), *Streptococcus* species (326 samples = 84.9%), *Bacillus* species (176 samples = 45.8%), *Escherichia coli* (230 samples = 59.9%), *KlebsiellaEnterobacter* species (358 samples = 95.8%). Coagulase positive *Staphylococcus* (*aureus* or *intermedius)* was isolated from 91 samples (23.7%) while Coagulase negative *Staphylococcus* (*epidermidis*) was isolated from 255 samples (66.4%). CAMP positive *Streptococcus agalactiae* (Lancefield group B) was isolated from 102 samples (26.6%) while CAMP negative streptococci, which could be Streptococcus *dysgalactiae* (Lancefield group C), *Streptococcus faecalis* (Lancefield group D) or *Streptococcus uberis* (Non lancefield classified) were isolated from 224 samples (58.3%).

A total of 230 camel milk samples were collected from Garissa District, 139 (60.4%) of which tested positive for subclinical mastitis by the use of CMT. The equivalent somatic cell counts (SCC/ML) of these 139 positive milk samples ranged from 1.5×10^5 to 5×10^6 leukocytes per millitre of milk. All the milk samples also yielded mixed types of bacteria on culture. These included *Staphylococcus* species (202 samples = 87.8%), *Streptococcus* species (195 samples = 84.8%), *Bacillus* species (116 samples = 50.4%), *Escherichia coli* (168 samples = 73.0%), *Klebsiella/Enterobacter* species (221 samples = 96.1%). Coagulase positive *Staphylococcus* (*aureus* and *intermedius)* was isolated from 69 samples (30.0%) while Coagulase negative *Staphylococcus* (*epidermidis*) was isolated from 133 samples (57.8%). CAMP positive *Streptococcus agalactiae* (Lancefield group B) was isolated from 72 samples (31.3%) while CAMP negative streptococci, which could be Streptococcus *dysgalactiae* (Lancefield group C), *Streptococcus faecalis* (Lancefield group D) or *Streptococcus uberis* (Non lancefield classified) was isolated from 123 samples (58.5%).

A total of 154 camel milk samples were collected from Wajir District. Out of this total, 96 samples (62.33%) tested positive for subclinical mastitis by the use of CMT. The equivalent somatic cell counts (SCC/ML) of these 96 positive milk samples ranged from 1.5×10^5 to 5×10^6 leukocytes per millitre of milk. All the milk samples also yielded mixed types of bacteria on culture. These included *Staphylococcus* species (144 samples = 93.51%), *Streptococcus* species (131 samples = 85.1%), *Bacillus* species (60 samples = 39.0%), *Escherichia coli* (62 samples = 40.3%), *Klebsiella/Enterobacter* species (148 samples = 96.1%). Coagulase positive *Staphylococcus* (*aureus* and *intermedius)* was isolated from 22 samples (14.3%) while Coagulase negative *Staphylococcus* (*epidermidis*) was isolated from 122 samples (79.2%). CAMP positive *Streptococcus agalactiae* (Lancefield group B) was isolated from 30 samples (19.5%) while CAMP negative streptococci, which could be Streptococcus

dysgalactiae (Lancefield group C), *Streptococcus faecalis* (Lancefield group D) or *Streptococcus uberis* (Non lancefield classified) was isolated from 101 samples (65.6%).

The prevalence of subclinical mastitis in the two counties according to CMT results was 61.2 % with a CMT trace score accounting for 32.0%, 1^+ score accounting for 25.5% and 2^+ score accounting for 3.7%. This result compares closely with the results obtained for she-dromedary camels in Jordan (60.0%) by Hawari and Hassawi (2008), with the most predominant bacterial isolates being *Staphylococcus* species, *Streptococcus* species and *Micrococcus* species.

The high prevalence recorded for the two major mastitis-causing pathogens (Gram positive cocci – *Streptococcus* and *Staphylococcus* species) which originate from the udder, was attributed to bulking or pooling of different camel milk batches from different animals and producers. However, the high prevalence of coliforms (*Escherichia coli*, *Klebsiella/Enterobacter* species) and *Bacillus* species was attributed to contamination of milk containers from the environment due to the poor hygiene of handling milk along the collection and marketing chain. In both counties, bacteria isolated highest were *Staphylococcus* (88% from Garissa and 93.5% from Wajir); *Streptococcus* (about 85% for both counties); and *Klebsiella/Enterobacter* species (about 96% for both counties). Both counties also yielded *Bacillus* species (50% for Garissa and 39% for Wajir) and *E. coli* (73% for Garissa and 40% for Wajir). Garissa district also had higher percentage of coagulase positive staphylococci (30%) and CAMP positive streptococci (31%) than Wajir district (14% and 19.5%, respectively).

Mastitis continues to be the most important economic disease problem among dairy cows worldwide (Radostits *et al* - 2000). Although camels are still multi-purpose animals, they are increasingly kept for milk, but chances are that, soon, there will be specialization into camels kept for milk and those kept for meat (Abdurahman, 2005). Reports of mastitis in traditionally managed camels are on the rise and are likely to increase further as the milk production per individual camel is increased. Two decades ago there was no mention of mastitis problem at herd level; today it is reported from almost all camel-rearing countries (Mohammed *et al*, 2005, Abdurahman and Younan, 2004, Khedid and Soulaimani, 2003, Guliye *et al*, 2002, Bekele and Molla, 2001, Al-Ani and Al-Shareefi, 1997, Al-Mohizea, 1986, Mostafa *et al*, 1987).

This study used bulk camel milk samples collected from milk containers used by the producers and traders. Quarter-milk samples were not available for laboratory analysis since the owners of camels were not willing to have their lactating camels sampled. The influence of pooling of different camel milk batches along the collection and marketing chain was illustrated by the increase in prevalence of *Streptococcus agalactiae* (26.56%), a mastitis pathogen that originates from the udder (Younan *et al*, 2002). The positive correlation of CMT with the bacteriological findings indicated that camel milk, like that of cows (Schalm *et al*, 1971), goats and sheep (Coetzer *et al*, 1994), has phagocytic cells which normally constitute one of the essential defenses against microbial infections. It also indicated that these phagocytic cells constitute one of the essential defenses against microbial infection of the mammary glands. An increase in the number of somatic cells, particularly granulocytes, in camel milk is a good indication of inflammation. As in the cow, the intensity of the cellular reaction correlates with the degree of irritation of the mammary gland. However CMT can be used as a screening test to detect subclinical mastitis in camels (Barbour *et al*, 1985). The

estimates of the somatic cell counts of the milk samples (between 1.5×10^5 to 5×10^6 leukocytes per millitre of milk), found in this study, are in agreement with those of Kospakove (1976; cited by Abdurahman *et al* 1995), who reported a mean score of 1.3×10^6 leukocytes/ml from milk samples in Bactrian camels.

In addition to camels, nomads/pastoralists in Garissa and Wajir counties of Northeastern province of Kenya keep other animals (according to the questionnaire data base). These are kept together with the camels and include cattle, sheep, goats and donkeys. These other animals could serve as sources of mastitis pathogens for the camels. Past researches have also indicated this (Obied *et al*, 1996). The prevalence of mastitis in these study samples (61.2%) was relatively higher than that observed by other investigators elsewhere (Abdurahman *et al*, 1995, Al-ani and Al-Shareefi, 1997, Barbour *et al*, 1985, Obied *et al*, 1996, Quadil and Quadar, 1984). This could be attributed to bulking of the milk samples collected since the figures given by these other investigators were based on quarter-milk samples.

Gram positive cocci (*Staphylococcus* and *Streptococcus* species) were the main pathogens isolated from camel milk samples in addition to environmental coliforms (*Escherichia coli* and *Klebsiella/Enterobacter* species); *Klebsiella/Enterobacter* species were isolated at a much higher rate (96%) than *E. coli* (60%). The relative number of the various pathogens isolated in this study, especially *Staphylococcus aureus* and *Streptococcus* species, is very similar to that reported by Woubit *et al.* (2001) and Abdurahman (2006). *Staphylococcus aureus* and *Streptococcus* species seem to be the major causative agents of mastitis in camels and this is in agreement with observations made by Barbour *et al* (1985) and Woubit *et al* (2001). Various authors have also reported that these Gram positive cocci are major mastitis-causing agents in camels (Abdurahman *et al*, 1995, Barbour *et al*, 1985, Hafez *et al*, 1987,

Karamy, 1990, Mostafa *et al*, 1987, Obied *et al*, 1996), in dairy cows (Coetzer *et al*, 1994, Quinn *et al*, 1994, Radostits *et al*, 1994 b, Roberson *et al*, 1996, Schalm *et al*, 1971), and in goats and sheep (Coetzer *et al*, 1994, Hafez *et al*, 1987). The isolation of *Streptococcus agalactiae* and other major mastitis pathogens could be attributed to the lack of supply and infrequent use of antimicrobials, and inaccessibility of veterinary services for the camel owners, as compared to the dairy cow owners, in urban and peri-urban areas (Woubit *et al*, 2001).

Staphylococcus aureus, Streptococcus agalactiae and coagulase negative staphylococci (*Staphylococcus epidermidis*) seem to be important udder pathogens in the camel (Abdurahman, 1996) as in other dairy animals. However, the camel has not been the subject of experimental mastitis studies and the epidemiology and pathogenicity of mastitis-causing organisms remain unclear. Camels affected by mastitis are reported to have considerably shorter lactation periods (Barbour et al, 1985). The disease is not usually treated in traditionally managed camels and often takes a natural course to chronicity resulting in permanent loss of milk production (Abdurahman *et al*, 1991, Obied *et al*, 1996).

Poor management and unhygienic milking practices prevalent in the pastoral traditional husbandry systems include tying the teats with soft barks to prevent the calf from suckling, tick infestations and cauterization of the udder skin (Abdurahman, 1995a, Obied *et al*, 1996, Almaw and Molla, 2000, Woubit *et al*, 2001). The use of anti-suckling devices in Northeastern province camels was practiced only during daytime when young calves older than one year were herded together with their dams. The use of these devices together with heavy tick infestation could predispose the udders to bacterial infections, which persist as

chronic infections. This could result in induration and atrophy of injured quarters and loss of milk production (Obied *et al*, 1996, Abdurahman *et al*, 1991).

Results of this study showed that subclinical mastitis is prevalent in dromedary camels of Garissa and Wajir counties of Northeastern province of Kenya, and that Gram-positive cocci are the dominant mastitis pathogens isolated. The positive correlation of CMT with the presence of mastitis pathogens in camel milk showed that CMT is a useful screening test in the detection of subclinical mastitis in camels and may serve to segregate camels infected with major pathogens in a subclinical form. Increase in the somatic cell counts of infected mammary glands indicated that camels reacted to inflammation induced by agents of mammary tissue by raising the number of somatic cells in the milk. However, further investigation is needed to determine the infection threshold of somatic cell count (SCC) in camel milk.

5 CAMEL MILK QUALITY ALONG MARKET CHAIN

5.1 Introduction

Most camel milk in pastoral areas is kept and transported at high ambient temperatures due to lack of refrigeration facilities. These conditions make the milk spoil fast and unsafe; i.e. capable of causing food-borne diseases. Being a good source of nutrients, bacteria grow fast on it, hence monitoring of hygiene (quality) of camel milk by performing total bacterial counts only tends to give wrong information of the situation on the ground – the counts tend to be higher than at the time of milk collection. These results also tend to be more affected by delays before laboratory testing. Taking this into consideration, plus the fact that adulteration of the milk can also be done by adding water and/or other substances, additional tests including measuring of specific gravity, determination of pH, and testing of colour and

presence of dirt particles in the milk need to be carried out. These will also give an indication of how the milk was handled along the transport/market chain.

5.2 Materials and Methods

5.2.1 Study area and animals

This was as given in Section 3.2.1

5.2.2 Samples studied and sample collection

This was as given in Section 4.2.2

5.2.3 Sample size calculation

This was done following the format given in section 4.2.3 except for the prevalence, which was estimated at 50% since the hygiene of camel milk was unknown. This translated to 384 samples

5.2.4 Study design

The study was cross-sectional. The collected milk samples from various milk outlets were investigated using various parameters including: assessment of the physical characteristics of milk and assessment of milk quality (bacteriological carriage). Assessment of physical characteristics of milk included: carrying out organoleptic tests, determination of specific gravity, colour and consistency, pH of the milk, and doing alcohol test (Giangiacomo, 2001). Organoleptic tests involved physical observation of the milk for gross dirt, colour, consistency and smell for odours. Assessment of milk quality (bacteriological carriage) was done by determining the Total Viable Bacterial Count (TVBC) and Total Coliform Count

(TCC) and by doing Resazurin test, which is used to determine the microbial load in milk (KEBS, 1976; Giangiacomo, 2001).

5.2.4.1 Assessment of physical characteristics

5.2.4.1.1 Gross dirt assessment

Procedure

The sample of the milk collected (200 – 300 ml) was filtered /sieved using a clean sterile sieve or clean filter paper into a clean container (glass flask) and the gross dirt recovered was noted and recorded.

Reading of the test

A positive sample was denoted by the recovery of gross-dirt which included pieces of grass/leaves, soil or sand particles, charcoal particles (black) or any other particulate matter.

5.2.4.1.2 Colour assessment

Procedure

The colour of the milk sample collected was assessed visually; noting that normal camel milk is white in colour

Reading of the test

Any colour change observed from the normal white was noted and recorded. Colour change mostly noticed was yellowish.

5.2.4.1.3 Odours/smell assessment

Procedure

The milk samples collected were assessed for any bad smell/odours.

Reading of the test

The sample with good normal smell was recorded as negative for bad odours/smell and that with bad smell/odour (sour smell) was recorded as positive for bad odour.

5.2.4.1.4 Determination of Specific Gravity of camel milk

The density of cattle milk ranges between 1.026 g/litre and 1.034 g/litre (Giangiacomo, 2001). The mean specific gravity of camel milk is 1.0305 g/litre, with an average butter fat content of 3.678% (Tibary and Anoussi, 2000, Gitao, 2006). Specific gravity was determined by means of lactodensimeter (lactometer) at 15 - 20^0C.

Procedure

A 250ml glass cylinder was first half-filled with camel milk at 20^0 C, lactodensimeter (lactometer) was then inserted; and more milk was added to fill the glass cylinder to the brim **(Plate 5-1).**

Reading the results

Reading of the specific gravity was taken directly from the lactometer, while kept at temperature of 20^0 C

Plate 5-1 Determination of the Specific Gravity of camel milk

The above picture shows lactodensimeter (lactometer; arrow) inserted in a 250ml glass cylinder with camel milk. Notice that milk has been filled almost to the brim of the glass cylinder so that the readings on the lactometer scale can easily be read.

5.2.4.1.5 Determination of the pH of camel milk

This gave the rough estimate of the acidity of milk. The normal values for milk are 6.6 – 6.8. Lower values generally mean acidification process due to development/growth of bacteria. Higher values mean presence of mastitis (Giangiacomo, 2001).

Procedure

pH of camel milk was measured with a pH indicator paper (Universal-Indikatorpapier – Germany) which was dipped in the milk sample and the resulting colour assessed against the standard values provided on the pack **(Plate 5-2).**

Reading the results

The pH value was read as it matched the respective colour on the standard chart.

Plate 5-2 Determination of the pH of camel milk

The above picture shows universal pH indicator paper used for the determination of the pH of camel milk. Notice the standard values of colours marked 1 – 14 on the left of the picture.

5.2.4.1.6 Alcohol test

Alcohol test was used to determine acidic, mastitic and colostrum milk, which was unsuitable for further processing. This test used 68% alcohol according to the standard method (Giangiacomo, 2001).

Procedure

Five (5) ml camel milk was mixed with 5 ml of 68% alcohol in a clean test tube. Formation of flakes indicated unsuitability.

Reading of the test

A positive alcohol test was denoted by formation of flakes indicating unsuitability while the negative one formed no flakes.

5.2.4.2 Assessment of bacteriological quality

Bacteriological quality of milk was measured using "Total Viable Bacterial Count (TVBC)", "Total Coliform Count (TCC)" and "Resazurin test".

5.2.4.2.1 Total Viable Bacterial Count

Total Viable Bacterial Count (TVBC) was determined using standard Plate Count Agar (PCA) media (Marshall, 1992). Serial dilutions of milk samples were carried out. Briefly, one millilitre (ml) of 10-fold serially diluted milk sample (1ml milk sample in 9ml potassium hydrogen sulphate buffer or normal saline) was placed on a Petri dish, followed by pouring of 20ml molten Plate Count Agar (PCA) cooled to 45^0 C onto the dish (Marshall, 1992). The sample and the agar were then mixed and left to solidify, after which the plates were incubated at 37^0 C for 24 -48 hours. Bacterial colonies [colony forming units **(cfu)** between 30 and 300] were counted using a manual colony counter and multiplied by the dilution factor to get TVBC value in colony forming units per ml **(cfu/ml*)*** of milk (Giangiacomo, 2001; Ombui *et al,* 1995; Kabede, 2005). Four plates were inoculated with each dilution and an average number calculated.

5.2.4.2.2 Total coliform count

Total Coliform Count (TCC) was determined using Violet Red Bile Agar (VRBA) medium, which is selective for coliforms, according to United States (US) standard method (Federal Register, 1990). The TCC served as an indicator of faecal contamination, and therefore poor hygiene and public health risk if numbers present exceeded the Kenya Bureau of Standard (KEBS) set limits (KEBS, 1976).

Procedure

One (1) millilitre of 10-fold serially diluted milk sample (1 ml of milk sample in 9 ml Potassium hydrogen sulphate buffer or normal saline) was placed in a Petri dish, followed by pouring of 20 ml molten Violet Red Bile Agar (cooled to 45^0 C). The sample and the agar (VRBA) were then mixed well and left to solidify, after which the plate was incubated at 37^0 C for 24 - 48 hours. Bacterial colonies [colony forming units **(cfu)** between 30 and 300] were counted using a manual colony counter (four plates were inoculated with each dilution and an average number calculated). The average number of cfu_s was then multiplied by the dilution factor to get TCC value, expressed in colony forming units per ml **(cfu/ml*)*** of milk. .

5.2.4.2.3 Use of Resazurin test to determine microbial load in milk samples

Resazurin test was used to determine the microbial load in milk (KEBS, 1976; Giangiacomo, 2001). Briefly, Resazurin solution was prepared by dissolving one Resazurin tablet in 200 mls of hot distilled water. One milliliter of the dye solution was placed in a sterile 15 ml test tube and 10 mls of the milk sample added. The tube was then stoppered, placed in an incubator at 36^0 C for one hour, examined and classified according to the resultant colour (Atherton and Newlander, 1997; Harrigan, 1998) at the end of the one hour.

Reading of the test

The result of the test was read according to **table 5.1** which gives a relationship of colour and the quality of milk after incubation for a specified time (one hour). **Plate 5.3** demonstrates the various colour changes that were seen, while indicating the respective milk quality grades they are in.

Table 5-1 Accepted Relationships of colour and quality in Resazurin test

Colour of sample	Quality of milk
Blue (No colour change)	Excellent
Blue to Deep mauve	Good
Deep mauve to Deep pink	Fair
Deep pink to Whitish pink	Poor
White	Bad

Source: Atherton and Newlander, 1997; Harrigan, 1998.

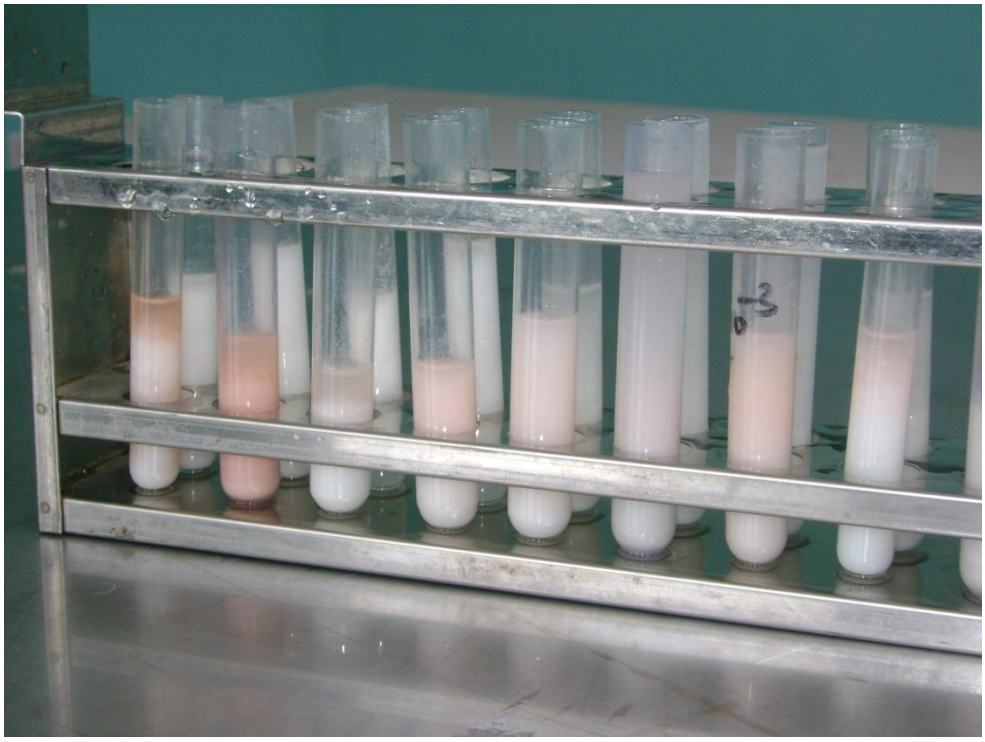

Plate 5-3 Resazurin test

The above picture shows some camel milk samples that have been subjected to Resazurin test for the determination of microbial load in the samples. Notice the different colours in different test tubes, which include white (bad quality), whitish pink (poor quality) and deep mauve (fair quality)

5.2.4.3 Culture and identification of the coliform bacteria

In addition to culturing on Blood agar and MacConkey agar, and carrying out the various biochemical tests, as given in section 4.2.4, *E. coli* were confirmed using Eosin Methylene Blue medium (**Plate 5-4**).

All bacterial isolates were preserved in glycerol-Nutrient broth at 0^{o}C until time to work on them.

Plate 5-4 Bacterial colonies on Eosin Methylene Blue(EMB) agar

The above picture shows bacterial colonies on EMB agar after 24 hours incubation at 37^{o}C. Notice the golden shiny colonies, showing greenish metallic sheen, of *Escherichia coli* and the cream to pinkish and spreading colonies with no metallic sheen, that are coalescing, of *Klebsiella/Enterobacter* group

5.2.4.4 Search for *E. coli* serotype O157:H7

From the *E. coli* bacteria isolated above, an attempt was made to isolate and identify serotype O157:H7, which is capable of producing shiga-like toxin. This was done using the methods described by March and Ratnam, (1986) and Murray *et al*, (2003). Sorbitol MacConkey (SMAC), which is a selective and differential medium for the organism, was used (composition of the medium is given in **Appendix 10.12).** The medium was incubated aerobically at 37^{o} C overnight. Non-sorbitol-fermenting, colourless colonies on this medium were taken as suspect organisms. **Plate 5-5** shows presence of such colonies.Due to limitation of antiserum, only 16 samples were serotyped for O157:H7. These were randomly selected. The serotyping was done at Kenya Medical Research Institute (KEMRI), Centre for Microbiology, using standard procedure.

Plate 5-5 Escherichia coli colonies on Sorbital Mac Conkey(SMAC) agae

The above picture shows *Escherichia coli* on Sorbitol MacConkey agar after 24 hours incubation at 37^{o}C. Notice the colourless colonies (suspected to be *E coli* O157:H7) and the pink colonies (non suspect *E coli* O157:H7)

5.2.5 Statistical data analysis for camel milk hygiene

Data collected was entered into Ms-Excel as data package for processing and analysed with Instat for windows to obtain statistics for each level of Total coliform counts (TCC) and Total viable bacterial count (TVBC) in colony forming units per millilitre of milk (cfu/ml) against the various parameters used. There was no significant difference for these parameters between the two counties. Instat for windows was also used to obtain frequency distribution for presence gross dirt, presence of abnormal colours, presence of bad odour, Specific gravity, pH reading, Alcohol test, Total coliform count (TCC), Total viable bacterial count (TVBC), Resazurin test, and bacteriological isolation of *Escherichia coli*, *Klebsiella* species and *Enterobacter* species. Descriptive statistic was also computed for different variables and used to summarize the data generated from the study.

5.3 Results

5.3.1 Assessment of the physical characteristics of camel milk samples

Results of gross dirt, colour, bad odour, pH and alcohol test reactions, with respect to the milk samples, for the 2 study counties, separately and combined, are given on **Table 5-2** and **Figures 5-1 and 5-2, 5.3,5.4.** Both Garissa and Wajir milk samples showed similar patterns of high gross dirt content (over 70%; slightly more in Wajir than in Garissa), most of the milk being white (over 80%; slightly more in Garissa than in Wajir), while yellowish milk was at less than 10% (slightly more in Wajir than in Garissa), bad odour at about 20% (more in Garissa than in Wajir), alcohol test reaction at about 33% (both areas giving almost the same percentage), more milk samples in Wajir (40.9%) than in Garissa (24,4%) had pH of 6, more milk samples in Garissa (57.8%) than in Wajir (45.5%) had pH of 7, and more milk samples in Garissa (17.8%) than in Wajir (13.6%) had pH of 8. Although most of the milk samples

were at neutral pH, the acid and alkaline pHs denote possibility of bacterial effect or other chemical change.

Table 5-2 Physical and Chemical test results for the two areas

		Garissa n=230		Wajir n=154		Combined n=384	
		Number	**%**	**Number**	**%**	**Number**	**%**
Gross dirt		168	73.0	121	78.6	289	75.3
Colour of the milk sample	White	210	91.3	140	90.9	350	91.2
	Yellow	20	8.7	14	9.1	34	8.9
Bad odour/smell		43	18.7	27	17.5	70	18.2
pH reading	pH 6	56	24.4	63	40.9	119	31.0
	pH 7	133	57.8	70	45.5	203	52.9
	pH 8	41	17.8	21	13.6	62	16.2
Alcohol test reactions		77	33.5	51	33.1	128	33.3

Footnote:

- Gross dirt includes grass, leaves, particles of sand/soil/charcoal
- Alcohol test was detected through flakes formation

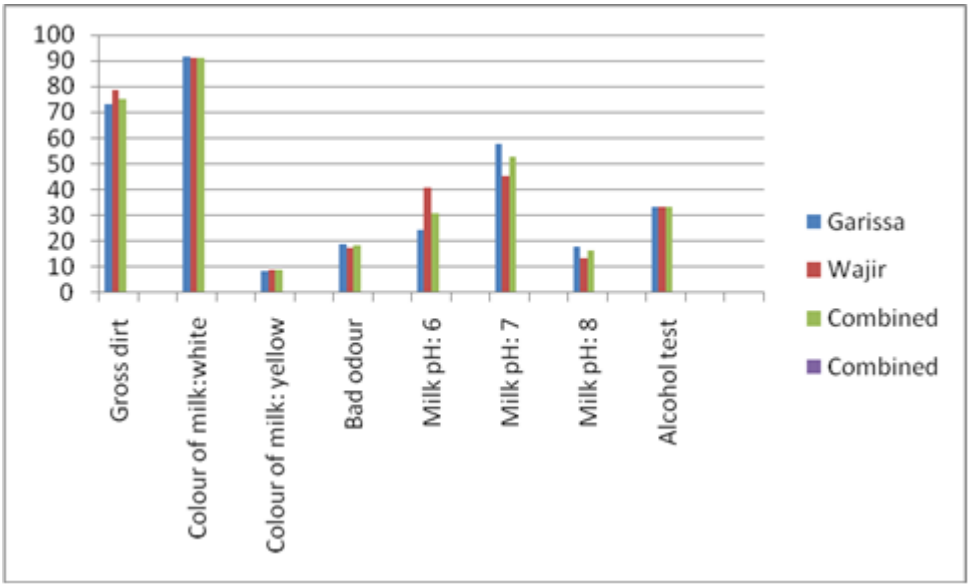

Figure 5-1 Comparison of frequencies of milk sample physical charecteristics

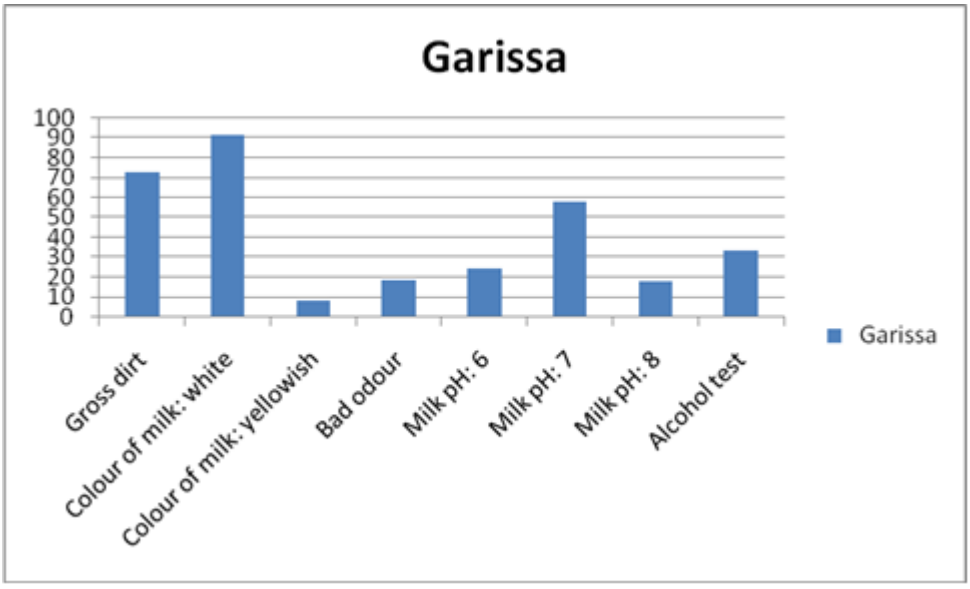

Figure 5-2 Frequencies of physical charecteristics for Garissa County

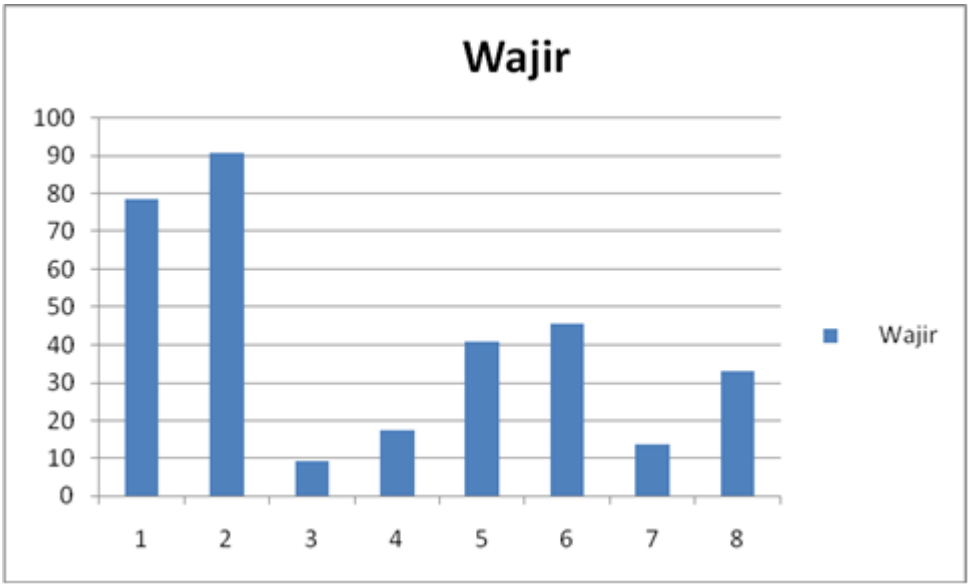

Figure 5-3 Frequncies of physical charecteristics for Wajir county

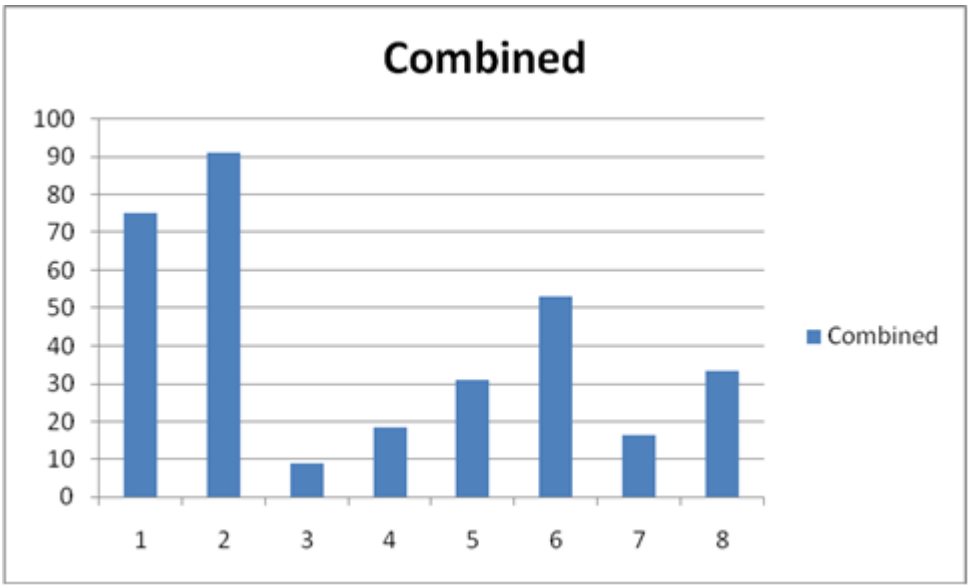

Figure 5-4 Frequencies of physical charecteristics for Wajir and Garissa counties

Table 5-3 gives specific gravity figures/percentages for the milk samples, for the 2 study counties, separately and combined. Respective frequency comparisons are given in **Figure 5.3**. More milk samples in Wajir (44.8%) than in Garissa (37.6%) had specific gravity ranging between 1.019-1.024; more milk samples in Garissa (48.0%) than in Wajir (41.6%)

had specific gravity ranging between 1.025-1.029; while more milk samples in Garissa (14.4%) than in Wajir (13.0%) had specific gravity ranging between 1.030-1.032. Overall, most milk samples (45.4%) had specific gravity ranging between 1.025-1.029; a slightly lower percentage (40.5%) had specific gravity ranging between 1.019-1.024.

Table 5-3 Speicic gravity values for Wajir and Garissa counties

Specific gravity ranges	**Garissa: n=229**		**Wajir: n=154**		**Combined: n=383**	
	Number Within range	**%**	**Number Within range**	**%**	**Number Within range**	**%**
1.019-1.024	86	37.6	69	44.8	155	40.5
1.025-1.029	40	48.0	64	41.6	174	45.4
11.030-1.032	33	14.4	21	13.0	54	14.1

Note: The specific gravity of one sample (from Garrissa District) was not determined since the sample quantity was too small (30 ml) to be determined by a lactometer.

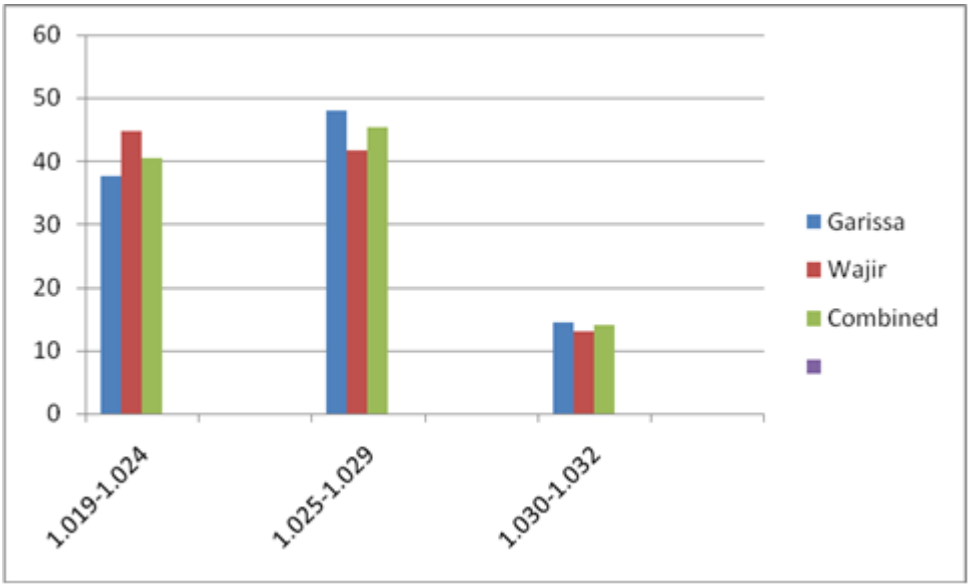

Figure 5-5 Frequencies of specific gravity values for Garissa and Wajir counties

5.3.2 Results on (TBC), coliform and Resazurin test

These are given on **Tables 5.4, 5.5.** and **5.6** as well as **Figures 5.4** and **5.5.** The total coliform count had more samples giving high counts, highest number being within the 110-190x10^5 cfu/ml bracket (47.4% for Garissa and 42.9% for Wajir). The pattern, with respect to concentrations, was similar for the 2 places, although Garissa recorded slightly higher figures than Wajir. The reverse was the case for viable bacterial counts – most of the samples fell within the 110-190x10^5 bracket (73.5% for Garissa; 63.6% for Wajir). The pattern was also similar for both places, being higher for Garissa samples.

Reading of the Resazurin test was by colour change and referred to bacterial quality of the milk sample; blue colour denoting "Excellent", deep mauve colour denoting "Good", deep pink colour denoting "Fair", pinkish-white colour denoting "Poor" and white denoting "Bad". In this study, the bacterial count brackets 120-190x10^4, 110-190x10^5 and 110-120x10^6 cfu/ml gave Resazurin readings of "Excellent", "Good" and "Fair", respectively. Count brackets 130-160x10^6 and above were rated as "Poor" and "Bad" (the actual counts were not

ascertained). The Resazurin test picked other milk qualities which were not covered in the viable cell scaling. These are given in **Table 5-5** and **Figure 5-6**. Sixteen point five percent (16.5%) of the samples in Garissa and 15.6% of the samples in Wajir were rated "Fair" by Resazurin testing, while 9.6% of samples in Garissa and 14.3% of samples in Wajir were rated "Bad".

Table 5-4Total viable count and Resazurin test for Wajir and Garissa counties

		Total viable bacterial count			
		120-190x10^4	110-190x10^5	110-120x10^6	130-160x10^6
Garissa N=230	Number	46	169	10	5
	%	20.0	73.5	4.4	2.2
Wajir N=154	Number	35	98	13	8
	%	22.7	63.6	8.4	5.2
Combined N=384	Number	81	267	23	13
	%	21.1	69.5	6.0	3.4
Resazurin rating per bacterial count		Excellent (Blue colour)	Good (Deep mauve colour)	Fair (Deep pink colour)	Poor (whitish pink)

Table 5-5Total coliform count and resazurin test for Wajir and Garissa counties

		Total coliform count (TCC)			
Garissa N=230	Number	2	109	78	41
	%	0.9	47.4	33.9	17.8
Wajir N=154	Number	1	66	45	42
	%	0.7	42.9	29.2	27.3
Combined N=384	Number	3	175	123	83
	%	0.8	45.5	32.0	21.6
Resazurin rating per bacterial count		Excellent (Blue colour)	Good (Deep mauve colou)	Fair (Deep pink colou)	Poor (whitish pink)

Key for tables 5.4, 5.5 and 5.6

cfu means colony forming units

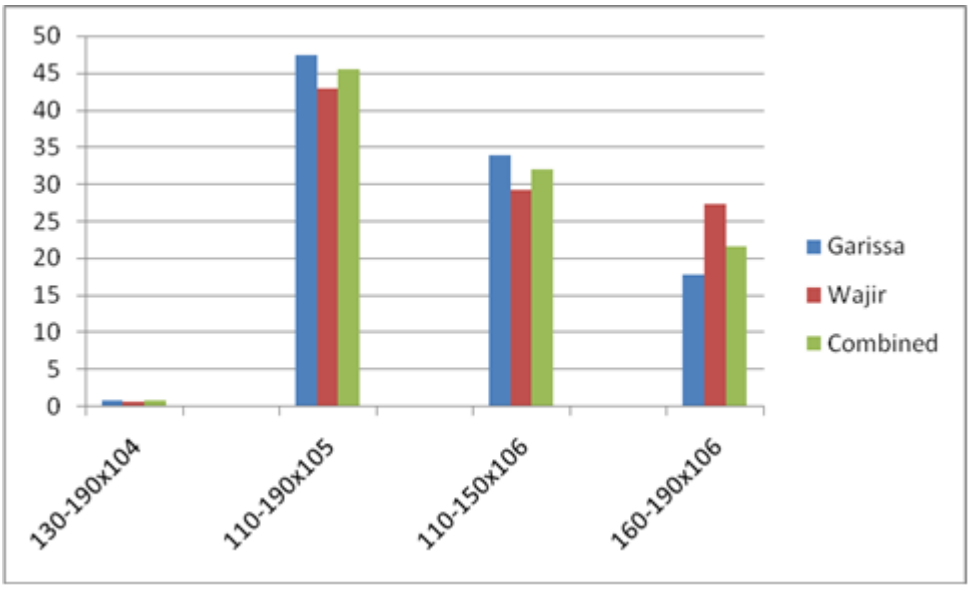

Figure 5-6 Frequencies of coliform isolation for Garissa and Wajir counties

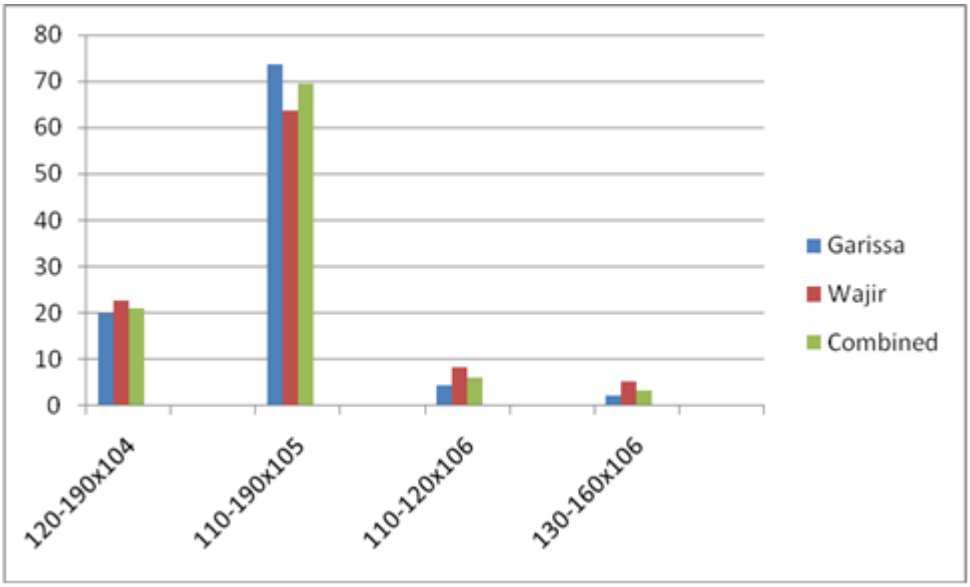

Figure 5-7 Frequencies of viable bacterial isolation for Wajir and Garissa counties

Figure 5-8 Resazurin results for Garissa and Wajir counties

	Poor (pinkish-white		**Bad (white)**		**Total poor/bad quality samples**	
	Number	**%**	**Number**	**%**	**Number**	**%**
Garissa n=230	38	16.5	22	9.6	60	26.1
Wajir n=154	24	15.6	22	14.3	46	29.9
Composite n=384	62	16.2	44	11.5	106	27.6

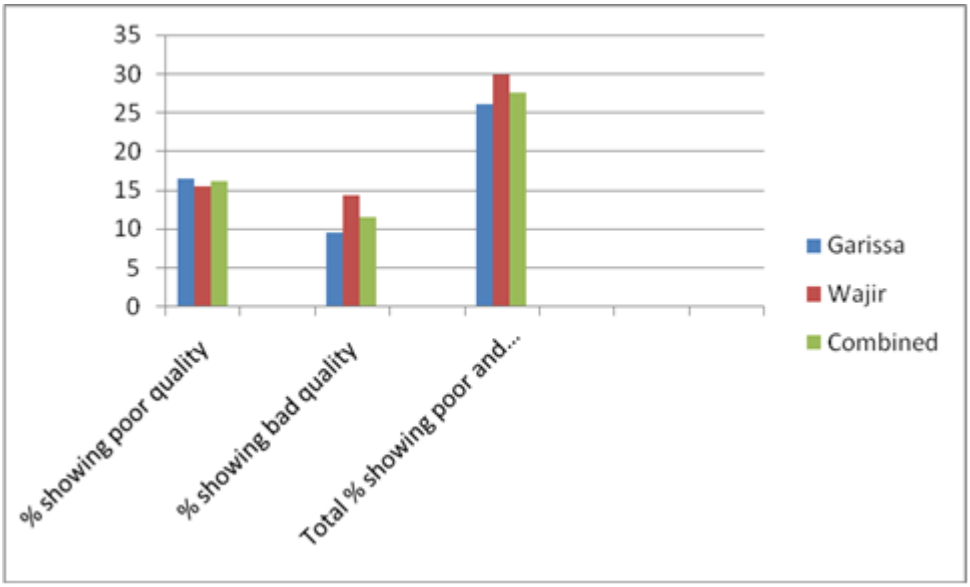

Figure 5-9 Frequencies of Resazurin grading for Wajir and Garissa counties

5.3.3 Coliform bacteria isolated

These were as reported in Chapter 4, Section 4.3.2, and captured under **Table 4.2**. From the 384 samples processed, using various media, 230 (59.9%) of the isolates were *E. coli* while 368 (95.8%) were *Klebsiella/Enterobacter*. This means some samples yielded more than one type of microorganism. Garissa yielded more of *E. coli* organism than Wajir; however, *Klebsiella/Enterobacter* was isolated at the same high rate in both areas. **Plate 5.4**, above, shows greenish metallic sheen as demonstrated by *E. coli* organisms on Eosin Methylene Blue agar.

5.3.4 E. coli O157:H7

Of the 230 *E. coli* isolates streaked onto Sorbitol MacConkey, **Table 5.6** gives a breakdown of suspect and non-suspect strains isolated, per county. When 16 of the suspect samples were serotyped for O157:H7, one sample gave positive reaction; the others were negative. Thus the percent positive cases translated to a minimum of 6.25%.

Table 5-6 Samples with suspect/non suspect E.coli 0157:H7 colonies for Wajir and Garissa counties

		Garissa n=168		Wajir n=62		Combined n=230	
		Number	**%**	**Number**	**%**	**Number**	**%**
Suspect *E.coli* O157:H7	+ve	67	39.9	5	8.1	72	31.3
	-ve	101	60.1	57	91.9	158	68.7
Total number of *E. coli* screened		168	100	62	100	230	100

5.4 Discussion

A total of 384 camel milk samples were collected from Garissa (230 samples) and Wajir (154 samples) counties and were used to determine milk quality, through assessment of physical characteristics and bacteriological carriage. Parameters used to assess the physical characteristics included organoleptic tests (assessment of gross dirt, colour and smell/odour), measuring of specific gravity, determination of pH and alcohol test. Bacteriological parameters included: Total Coliform Count [TCC; done on Violet Red Bile Agar (VRBA)], Total Viable Bacterial Count [TVBC; done on Plate Count Agar (PCA)] and Resazurin test (which gauges the level of microbial load in the milk).

5.4.1 Assessment of physical characteristics of the camel milk samples

A combined assessment of physical characteristics of the 384 camel milk samples from the two counties (Garissa and Wajir), showed that 289 samples (75.26%) had gross

dirt/particulate matter including grass/leaves, sand/soil particles and/or black charcoal particles. This could be attributed to the low level of hygiene in cleaning of the milk containers and lack of milk filters after milking; before packing the milk in containers. The black charcoal particles were attributed to the tradition of smoking milk containers especially traditional gourds as had been observed in this study and by other researchers (Younan *et al,* 2002). Camel milk is traditionally produced by way of hand milking, handled and transported under low hygienic conditions and the common practice of smoking traditional milk containers and milking buckets (made from gourds, natural fibres) contributes to the introduction of gross dirt, especially charcoal particles, in the milk (Younan *et al,* 2002).

Thirty four (34) samples (8.85%) had an abnormal yellowish colour. This is a deviation from the normal white opaque colour of camel milk. Such milk is unsuitable for human consumption; hence unsuitable for further processing. Indeed, there was a strong association (26 out of the 34 samples; 76.47%) between the yellowish colour of milk and flake formation in the Alcohol test, which is used to determine acidic, mastitic and colostrum milk. Apart from being white opaque in colour, normal camel milk has a faintly sweetish odour and a sweet but sharp taste. It is thinner than cow or buffalo milk (Ohri & Joshi, 1961; Abdurahman, 1996). Camel milk has a much slower natural creaming rate than cow milk, both in its raw and heat treated states (Farah & Ruegg, 1991; Farah, 1993). As opposed to subclinical mastitis, where the animal shows no sign of sickness, apart from the abnormality of milk constitution, clinical mastitis is characterized by alarming clinical signs including: anorexia, fever, general depression, swelling, severe inflammation and pain of the udder. Mammary secretions in cases of clinical mastitis are watery, yellowish or blood-tinged and bacteria isolated have included *Klebsiella pneumoniae* and *Escherichia coli* (Kapur *et al,* 1982).

Seventy (70) samples (18.23%) had offensive/bad odour/smell (sour or foul smell); the smell was that of fermenting or souring milk. There was a positive association (26 out of the 70 samples; 37.14%) between the bad smell and the acid pH of "6" found in these milk samples.

The range of specific gravity of the samples tested was from 1.019 g/litre to 1.032 g/litre with 155 samples (40.47%) being from 1.019 – 1.024 g/litre range, 174 samples (45.43%) being from 1.025 – 1.029 g/litre and 54 samples (14.10%) being from 1.030 – 1.032 g/litre range. The mean specific gravity of normal camel milk is 1.0305 gms/litre, with an average butter fat content of 3.678% (Tibary and Anoussi, 2000; Gitao, 2006). When camel milk is adulterated with water, specific gravity will be less than 1.026 g/litre while adulteration with solids like sugars, specific gravity will be higher than 1.034 g/litre (Giangiacomo, 2001). As many as 155 samples (40.47%) had a specific gravity between 1.019 – 1.024 g/litre, an indication of adulteration of marketed camel milk; thus casting great concern on the quality of camel milk supplied to the consumers. Addition of up to 15% water to marketed camel milk has been reported from southern Somalia (Younan *et al,* 2002); the quality of the added water presenting an additional hygienic risk. The specific gravity of camel milk tested in three large commercial herds in Kenya over a two months' period varied between 1.026 g/litre and 1.029 g/litre, indicating a difference to the specific gravity of the camel's milk (Younan *et al,* 2002).

Results of pH determination indicated that 119 samples (30.99%) had a pH of "6", 203 samples (52.86%) had a pH of "7" and 62 samples (16.15%) had a pH of "8". This gave a rough estimate of the acidity of milk. The normal values for milk are 6.6 – 6.8. Lower values generally mean acidification process due to development of bacteria. Higher values mean presence of mastitis (Giangiacomo, 2001).

Formation of flakes in the Alcohol test was recorded in 128 samples (33.33%) indicating they were either acidic, mastitic or colostrum milk. Formation of flakes indicates unsuitability of the milk for consumption; this also means the milk is unsuitable for further processing

5.4.1.1 Comparison of physical properties of milk from Garissa and Wajir counties

Assessment of the physical characteristics from Garissa District (n = 230 samples) showed that 168 samples (73.04%) had gross dirt/particulate matter including grass/leaves, sand/soil particles or black charcoal particles, 20 samples (8.70%) had an abnormal yellowish colour and 43 samples (18.70%) had bad odour/smell (sour or foul smell). The range of Specific gravity of the samples tested was between 1.019g/litre to 1.032g/litre, with 86 samples (37.56%) being between 1.019 – 1.024 g/litre, 110 samples (48.03%) being between 1.025 – 1.029g/litre and 33 samples (14.41%) being between 1.030 – 1.032 g/litre. Results of pH determination indicated that 56 samples (24.35%) had a pH of "6", 133 samples (57.83%) had a pH of "7" and 41 samples (17.82%) had a pH of "8". Formation of flakes in the Alcohol test was recorded in 77 samples (33.48%).

Assessment of the physical characteristics from Wajir County (n = 154 samples) showed that 121 samples (78.57%) had gross dirt/particulate matter including grass/leaves, sand/soil particles or black charcoal particles, 14 samples (9.09%) had an abnormal yellowish colour and 27 samples (17.53%) had bad odour/smell (sour or foul smell). The range of Specific gravity of the samples tested was between 1.019g/litre to 1.032g/litre with 69 samples (44.81%) lying between 1.019 – 1.024 g/litre, 64 samples (41.56%) lying between 1.025 – 1.029g/litre and 21 samples (13.64%) lying between 1.030 – 1.032 g/litre. Results of pH determination indicated that 63 samples (40.91%) had a pH of "6", 70 samples (45.45%) had

a pH of “7” and 21 samples (13.64%) had a pH of “8”. Formation of flakes in the Alcohol test was recorded in 51 samples (33.12%).

Samples from Wajir County had a higher incidence of having gross dirt (by 5.53%) than those from Garissa County. The difference in the presence of abnormal yellowish colour (0.39%) and bad odour/smell (1.17%) in the two counties was not significant. The incidence of adulteration of milk was thus higher in Wajir County (by 7.26%) than in Garissa county. Wajir County had a higher incidence of acidic milk (by 16.56%) than Garissa county. This could be attributed to the long distance travelled by the pastoralist from the grazing fields to the watering wells where the market is available. The difference in flake formation in the Alcohol test (0.36%) in the two counties was not significant.

5.4.2 Analysis for milk hygiene

Total coliform counts (TCC), given as colony forming units per millilitre of milk (cfu/ml), was assessed against the various parameters which included gross dirt in milk, colour of the milk sample, presence of bad odour, Alcohol test, Resazurin test, isolation of *Escherichia coli, Klebsiella* species and *Enterobacter* species. Milk samples with gross dirt had a higher mean (89.11×10^6) of TCC than the negative samples (75.64×10^6). Milk samples having yellowish colour had a higher mean (94.45×10^6) of TCC than the samples with the white colour (84.93×10^6). Milk samples with bad odour had a higher mean (92.96×10^6) of TCC than the negative samples (84.35×10^6). Milk samples with a positive alcohol test had a higher mean (94.38×10^6) of TCC than the negative samples (81.53×10^6). Milk samples with an excellent Resazurin test had the lowest mean (56.63×10^6) of TCC compared with samples with poor Resazurin test (106.30×10^6). Milk samples with no *Escherichia coli* isolation had a lower mean (79.78×10^6) of TCC compared to the ones where sorbitol and non-sorbitol

fermenting *Escherichia coli* were isolated (80.89× 10^6 and 93.74 × 10^6 respectively). Milk samples with *Klebsiella/ Enterobacter* species isolation had a higher mean (87.12× 10^6) of TCC compared to the samples where no *Klebsiella/Enterobacter* species were isolated (85.68× 10^6).

The above results show that there was a strong positive correlation between the parameters used here (i.e. positive gross dirt, positive abnormal colour, positive bad odour, positive alcohol test, poor Resazurin test and isolation of *Escherichia coli, Klebsiella* species, and *Enterobacter* species) and the poor hygiene of camel milk. This phenomenon was largely attributed to poor handling (poor sanitary practices) of camel milk during milking and subsequent transportation/handling to the markets.

Total viable bacterial counts (TVBC), given as colony forming units per millilitre of milk (cfu/ml), was also assessed against the various parameters which included gross dirt in milk, colour of the milk sample, presence of bad odour, alcohol test, Resazurin test, isolation of *Escherichia coli, Klebsiella* species and *Enterobacter* species.

Milk samples with gross dirt had a higher mean (25.24× 10^6) of TVBC than the negative samples (18.00× 10^6). Milk samples having yellowish colour had a higher mean (31.70× 10^6) of TVBC than the samples with the white colour (22.65× 10^6). Milk samples with bad odour had a higher mean (25.01× 10^6) of TVBC than the negative samples (23.11× 10^6). Milk samples with a positive alcohol test had a higher mean (27.23× 10^6) of TVBC than the negative samples (21.57× 10^6). Milk samples with an excellent quality in Resazurin test had the lowest mean (12.11× 10^6) of TVBC compared with samples with poor quality in Resazurin test (35.52× 10^6). Milk samples with no *Escherichia coli* isolation had a lower

mean (16.35× 10^6) of TVBC compared to the ones where sorbitol and non-sorbitol fermenting *Escherichia coli* were isolated (23.54× 10^6 and 26.24 × 10^6 respectively). Milk samples with *Klebsiella/ Enterobacter* species isolation had a higher mean (23.63× 10^6) of TVBC compared to the samples where no *Klebsiella/Enterobacter species* were isolated (18.84× 10^6)

The above results show that there was a strong positive correlation between the parameters used here (i.e. positive gross dirt, positive abnormal colour, positive bad odour, positive alcohol test, poor Resazurin test and isolation of *Escherichia coli, Klebsiella* species, and *Enterobacter* species) and the poor hygiene of camel milk. This phenomenon was also largely attributed to poor handling (poor sanitary practices) of camel milk during milking and subsequent transportation/handling to the markets.

5.4.3 Summary and assessment of camel milk hygiene/quality

Assessment of camel milk quality by bacteriological tests from the two counties (Garissa and Wajir; n = 384 samples) showed that Total Coliform Count (TCC) ranged between 130 × 10^4 - 190 × 10^6 cfu/ml and Total Viable Bacterial Count (TVBC) ranged between 120 × 10^4 - 160 × 10^6 cfu/ml. The total range of TCC and TVBC were divided into four categories each, for ease of interpretation and discussion.

Results of TCC recorded 3 samples (0.78%) in the range 130 × 10^4 - 190 × 10^4 cfu/ml, 175 samples (45.57%) in the range110 × 10^5 - 190 × 10^5 cfu/ml, 123 samples (32.03%) in the range110 × 10^6 - 150 × 10^6 cfu/ml, and 83 samples (21.62%) in the range160 × 10^6 - 190 × 10^6 cfu/ml. Results of TVBC recorded 81 samples (21.09%) in the range 120 × 10^4 - 190 × 10^4 cfu/ml, 267 samples (69.53%) in the range110 × 10^5 - 190 × 10^5 cfu/ml, 23 samples

(5.99%) in the range110 × 10^6 - 120 × 10^6 cfu/ml, and 13 samples (3.39%) in the range130 × 10^6 - 160 × 10^6 cfu/ml.

These findings compare well with those of analysis for Total bacterial count (TBC) done earlier in camel milk in Kenya which indicated a TBC of 10^3 – 10^5 cfu/ml from transport containers, immediately after the end of milking (Younan *et al,* 2002). The same study indicated a TBC of 10^2 – 10^4 cfu/ml for camel milk from udders milked directly into clean containers. The latter results show that good quality raw camel milk is initially produced but it deteriorates rapidly as it enters the informal marketing chain. Pooling of different raw milk batches and unhygienic plastic containers accelerate spoilage, with non refrigerated bulk milk reaching a TBC of 10^8 cfu/ml (Younan *et al,* 2002); this milk turns sour in less than 24 hours when kept at 25ºC. Under hot pastoral conditions (35ºC), this can happen in less than 12 hours

Coliforms isolated from the collected milk samples included *Escherichia coli* (59.90% = 230 samples) and *Klebsiella*/*Enterobacter* species (95.83% = 368 samples), while *Enterobacteriaceae* were detected in all the 384 samples (100%). *Escherichia coli* was identified through growth on MacConkey/Sorbitol-MacConkey and Eosin methylene blue agar plates and carrying out of IMViC tests (+ + - -). The occurrence of total coliforms, in this study, was equivalent to that reported for Ethiopian raw camel milk (100%) by Semereab and Molla (2001). The *Enterobacteriaceae* family is highly reputed among the most pathogenic and most often-encountered organisms in food (El-Ziney and Al-Turki, 2007). *Enterobacteriaceae* family includes coliform group (*Escherichia, Enterobacter, Citrobacter* and *Klebsiella* species) in addition to many other genera (*Salmonella, Shigella, Morganella, Providencia, Edwardseilla, Proteus, Serratia* and *Yersinia*), which are isolated from animal

intestine (Collins *et al*, 1995, Hays *et al*, 2001). The existence of coliform bacteria may not necessarily indicate a direct faecal contamination of milk, but serves as an indicator for poor sanitary practices during milking and further handling processes. However, the presence of faecal coliforms, i.e. *Escherichia coli,* implies a risk that other enteric pathogens may be present in the sample. *Escherichia coli* O157:H7 was identified from one of the samples that were serotyped with *Escherichia coli* antisera O157 and H7. Having one sample yielding *E. coli* O157:H7 is significant especially considering the fact that one organism multiplies very fast. There is also a possibility that there may have been more samples carrying this organism, since serological testing could be rendered negative (false negative) by presence of some non-specified K antigens (Kauffmann 1975). A sure way of determining if the organism has the ability to produce the vero-toxin is through usage of polymerase chain reaction (PCR), where a specific primer for the specific polypeptide chain/gene/plasmid/phage that codes for the toxin is used (Olsvik and Strockbine, 1993; Smith and Scotland, 1993). When infected with respective phage, *E.coli* serotype 0157:H7 and O157:non-motile produce one or more verocytotoxins (Shiga-toxins) and are the most frequently identified diarrheagenic *E.coli* serotypes in North America and Europe (Mead *et al*, 1999). Shiga toxin-producing *Escherichia coli* (STEC), also known as enterohaemorrhagic *E. coli,* is one of the four categories of diarrheagenic *Escherichia coli* (Nataro and Kaper, 1998). Shiga toxin-producing *Escherichia coli* O157:H7 and other STEC serotypes cause human illness that can present as mild non-bloody diarrhoea, severe bloody diarrhoea (haemorrhagic colitis), and haemolytic-uremic syndrome (HUS) (Griffin *et al,* 2002). Additional symptoms of *E. coli* O157:H7 infections include: abdominal cramps and lack of high fever. The organism O157 STEC readily colonizes dairy and beef cattle; thus, not surprisingly, ground beef has caused more O157 STEC outbreaks than any other vehicle of transmission (Griffin *et al,* 2002). Other known vehicles of transmission include raw milk, sausage, roast beef, unchlorinated

municipal water, apple cider, raw vegetables and sprouts (alfalfa and radish) (Griffin *et al,* 2002; Arimi *et al,* 2000). Isolation of the zoonotic serotype in camel milk means that camels could also be a source of infection for humans.

Other bacterial microorganisms isolated from the milk samples alongside the coliforms included: *Staphylococcus* species (90.10% = 346 samples), *Streptococcus* species (84.90% = 326 samples) and *Bacillus* species (45.83% = 176 samples). Of the 346 *Staphylococcus* species isolated, 91 (23.70%) were coagulae-positive. Thus, the existence rate of *Staphylococcus aureus*, in the present study, was relatively high. However, the organism has been detected in all tested samples (n = 12) in Morrocan camel milk (Benkerroum *et al,* 2003). Semereab and Molla (2001) reported that *Staphylococcus aureus* isolates represent 15% of the total bacteria isolated from composite camel udder milk. The reported incidence of mastitis in camel herds (19.5%) and the high frequency of *Staphylococcus aureus* (31.5%) as the causarive agent may explain these results (Obied *et al,* 1996). According to the European Commission (EC) standards for raw cow's milk intended for direct consumption (**European commission, 2001),** 51% (n = 17) of the samples were found to have *Staphylococcus aureus* counts higher than the fixed acceptable limits ($\leq 10^5$ cfu/ml). An overview of the annual reports of food-borne diseases from seven countries indicated that milk and milk products were implicated in 1 to 5 % of the total bacterial outbreaks. *Staphylococcus aureus* was by far the most frequent pathogen associated with these outbreaks (85.5%), followed by *Salmonella* (10%) (De Buyser *et al,* 2001); 45.83% (n = 384) of the collected camel milk samples were contaminated by psychrotrophic *Bacillus* species (*cereus*). The results of psychrotrophs are comparable with the average reported for raw cow milk by Boor *et al,* (1998) and Chye *et al,* (2004). No documentation on the content of psychrotrophs in camel milk was found in the literature. Psychrophilic bacteria are responsible for an

increased production of proteinases and lipases, which can survive heat treatments (i.e. pasteurization) thus affecting the shelf life and quality of milk (Graaf *et al,* 1997).

Resazurin test to determine the microbial load/quality of camel milk showed that a total of 278 samples (72.39%) were of good quality [124 samples (32.29%) were of excellent quality, 135 samples (35.15%) were of good quality, 19 samples (4.95%) were of fair quality], while a total of 106 samples (27.61%) were of poor quality [62 samples (16.15%) were of poor quality and 44 samples (11.46%) were of bad quality]. It was observed from this study that pastoralists of Northeastern province of Kenya, occasionally washed and smoked their milking vessels, but the personal hygiene of the milker was poor; this being due to lack of good hygiene awareness/practice, inaccessibility of soap/disinfectant, and insufficient clean water supply. This resulted in high contamination of milk after milking.

Camel milk possesses superior keeping quality compared to cow milk; a property that makes raw camel milk a marketable commodity even under conditions of high temperatures and very basic hygiene (Younan *et al,* 2001). This is due to its high content of proteins that have inhibitory properties against bacteria. In Somalia and Kenya, camel milk production areas are often located far from markets as observed by Younan *et al* (2001). Distances to provincial markets range from 20 km to 90 km and may be up to 400 km for distant urban markets. During periods of milk surplus (rainy season) transport on dirt roads is unreliable resulting in breakdowns and delays in milk delivery. Storage in unhygienic containers (plastics and traditional gourds), mixing of evening and morning milk, pooling of milk from different suppliers, prolonged transport times, high environmental/ambient temperatures and road-side selling out in open containers, all increase contamination and spoilage of milk. This explains the high values of more than 10^7 cfu/ml of TCC and TVBC observed in most of the samples

(>80%) in the present study. However spoilage does not always equal wastage. Sour milk is part of the traditional diet (Somali "Susa", Arabic "Al-Garss") and sour milk of acceptable quality is sold and consumed comfortably by the pastoralist communities (Younan *et al*, 2001; 2002). However growth of contaminants in raw camel milk poses a threat to consumer health when milk of poor hygiene is sold. Spoilage reduces the market value of the milk causing income losses to producers and vendors. Souring or sour camel milk is also unsuitable for heat treatment in dairy plants.

The common practice of smoking traditional milk containers and milking buckets (made from gourds) with natural fibres achieves high temperatures and appears to have a beneficial effect on the keeping quality of milk, although this has not yet been studied in details.

However, the obvious advantage of plastic containers (cheap, light weight, durable, large volume per container better suited for transport in vehicles) coupled with the limited availability, high costs and small volumes of traditional containers leads to the increasing use of these plastic containers in the camel milk trade. Plastic jerricans of cheap quality (e.g. re-cycled cooking oil containers) have a fast corroding surface and are very difficult to clean in pastoral areas because of the lack of clean water. The non-availability of safe clean water also implies that the introduction of common hygiene recommendations will be difficult and adapting hygiene practices and guidelines to the pastoral situation remains a challenge (Younan *et al,* 2002).

5.4.4 Comparison of milk hygiene from Garissa and Wajir counties

Assessment of camel milk quality by bacteriological tests from Garissa county (n = 230 samples) showed that Total Coliform Count (TCC) ranged between 130×10^4 - 190×10^6

cfu/ml and Total Viable Bacterial Count (TVBC) ranged between 120×10^4 - 160×10^6 cfu/ml. Results of TCC recorded 2 samples (0.87%) in the range 130×10^4 - 190×10^4 cfu/ml, 109 samples (47.39%) in the range110×10^5 - 190×10^5 cfu/ml, 78 samples (33.91%) in the range110×10^6 - 150×10^6 cfu/ml, and 41 samples (17.83%) in the range160×10^6 - 190×10^6 cfu/ml. Results of TVBC recorded 46 samples (20.00%) in the range 120×10^4 - 190×10^4 cfu/ml, 169 samples (73.48%) in the range110×10^5 - 190×10^5 cfu/ml, 10 samples (4.35%) in the range110×10^6 - 120×10^6 cfu/ml, and 5 samples (2.17%) in the range130×10^6 - 160×10^6 cfu/ml. Coliforms isolated from the collected samples included *Escherichia coli* (73.04% = 168 samples) and *Klebsiella*/*Enterobacter* species (96.09% = 221 samples). Resazurin test to determine the microbial load/quality of camel milk showed that a total of 170 samples (73.91%) were of good quality [80 samples (34.78%) were of excellent quality, 82 samples (35.65%) were of good quality, 8 samples (3.48%) were of fair quality], while a total of 60 samples (26.09%) were of poor quality [38 samples (16.52%) were of poor quality and 22 samples (9.57%) were of bad quality].

Assessment of camel milk quality by bacteriological tests from Wajir county (n = 154 samples) showed that Total Coliform Count (TCC) ranged between 130×10^4 - 190×10^6 cfu/ml and Total Viable Bacterial Count (TVBC) ranged between 120×10^4 - 160×10^6 cfu/ml. Results of TCC recorded 1 sample (0.65%) in the range 130×10^4 - 190×10^4 cfu/ml, 66 samples (42.86%) in the range110×10^5 - 190×10^5 cfu/ml, 45 samples (29.22%) in the range110×10^6 - 150×10^6 cfu/ml, and 42 samples (27.27%) in the range160×10^6 - 190×10^6 cfu/ml. Results of TVBC recorded 35 samples (22.72%) in the range 120×10^4 - 190×10^4 cfu/ml, 98 samples (63.64%) in the range110×10^5 - 190×10^5 cfu/ml, 13 samples (8.44%) in the range110×10^6 - 120×10^6 cfu/ml, and 8 samples (5.20%) in the range130×10^6 - 160×10^6 cfu/ml. Coliforms isolated from the collected samples included *Escherichia*

coli (40.26% = 62 samples) and *Klebsiella*/*Enterobacter* species (96.10% = 148 samples). Resazurin test to determine the microbial load/quality of camel milk showed that a total of 108 samples (70.13%) were of good quality [44 samples (28.57%) were of excellent quality, 53 samples (34.42%) were of good quality, 11 samples (7.14%) were of fair quality], while a total of 46 samples (29.87%) were of poor quality [24 samples (15.58%) were of poor quality and 22 samples (14.29%) were of bad quality].

Wajir County had more milk samples (27.27%) with a TCC of between 160×10^6 - 190×10^6 cfu/ml than Garrissa county (17.83%). This could be attributed to the long distance (and hence increased time) pastoralists in Wajir had to travel under hot pastoral conditions (35ºC) from the pastures to the watering wells where the milk is sold. This favoured the multiplication of microorganisms. The incidence of isolating *Escherichia coli* from the milk samples was higher (by 32.78%) in Garrissa county than in Wajir county. This could be attributed to increased use of unhygienic plastic containers, pooling of different milk batches, poor personal hygiene of milkers and handling of milk in unclean environment.

Garissa yielded more (30%) coagulase positive staphylococci than Wajir (14.3%), despite more staphylococci having been isolated from Wajir (93.5%) as compared to Garissa (87.8%). Garissa also yielded more (31.3%) CAMP positive streptococci than Wajir (19.5%); *Streptococcus* was isolated at same prevalence (85%) for the two areas. CAMP-positive streptococci are common causes of mastitis in cows (Buxton and Fraser, 1977)).

5.4.5 Conclusion for camel milk hygiene

This study has shown that there is substantial contamination of milk produced in the two counties of Garissa and Wajir, Kenya. The contaminants include both physical substances

and bacteria. Inadequate availability of water and ignorance on good/hygienic milking practices are the main causes of the contamination, making the milk dangerous to human health. There is, therefore, need for the Government to avail ample clean water to the areas and to undergo training sessions on good/hygienic milking, packaging and transporting practices.

6 PREVALENCE OF BRUCELLOSIS IN CAMELS

6.1 Introduction

Brucellosis is a zoonotic disease affecting humans and various domesticated and wild animals. *Brucella abortus* in cattle and other bovidae, *Brucella melitensis* in sheep and goats, and *Brucella ssuis* in pigs are the major species of concern. They cause abortions in respective animals and septicaemias in humans (Young and Corbel, 1989). *Brucella* organisms have a predilection for the udder of the affected animal (Blood *et al*, 1994; Enright, 1990) hence infected animal normally sheds the organisms through milk. There is also a reaction within the infected udder, leading to production of antibodies, and accumulation of inflammatory cells, which can be detected in the respective milk. Since there have been cases of abortion reported in camels and other animals raised inGarissa and Wajir counties, this study saught to check on the presence and prevalence of brucellosis in resident camels, using various diagnostic parameters. This was found necessary due to the fact that the nomadic residents of Garissa and Wajir countiesnormally consume raw milk. If *Brucella* organisms are shed in the camel's milk, this would pose a major health problem for the people.

6.2 Materials and Methods

6.2.1 Study area and animals kept

This was as given in section 3.2.1

6.2.2 Samples studied and sample collection

Milk and serum samples from camels were used to test for the prevalence of brucellosis in the camels. Volumes of 200ml – 300 millilitres of camel milk (from the producers or hawkers) was collected into labeled sterile bottles and kept in an ice-box, transported to laboratory for immediate Milk Ring Test (MRT) and bacteriological culture (analysis). When not immediately processed, the samples were kept in a refrigerator for a maximum of 24 hours. Volumes of 10 to 15 millilitres of blood were collected from the jugular vein of camels in plain vacutainer tubes after restraining the animals **(Plate 6.1).** Blood was left to stand for 24 hours at room temperature (15 – 30^0C), to allow for serum separation. Serum was harvested by centrifuging at 3,000 to 3,500 × g for 10 minutes and decanting into sterile 2ml-vials labeled appropriately and stored in a freezer at $^-20^0$C at the County (Garrissa) Veterinary Investigation Laboratory (VIL). The serum was then transported in cool boxes packed with ice to the Microbiology Laboratory (Faculty of Veterinary Medicine, University of Nairobi) for further immunological analysis using the Rose Bengal plate test (RBPT) and Serum agglutination (Micro agglutination) test (SAT) (Alton *et al*, 1988, Anon, 1986, Brinley,1977).

Plate 6-1 Restraining camels for bleeding purposes

The above picture shows the researcher collecting whole blood from the jugular vein of a camel into a plain vacutainer tube after the camel has been restrained and pressure applied on the jugular vein with a rope.

6.2.3 Modified Ziehl Neelsen's technique in detecting Brucella organisms

Identification of *Brucella* organisms was carried out using Modified (Stamp's) Ziehl-Neelsen method (Holt *et al*, 1994, Quinn *et al*, 2002, OIE Manual, 2004). The Procedure was as follows:

- A thin camel milk smear was prepared on a microscopic slide and heat fixed.
- The slide was then flooded with dilute Carbol-Fuchsin for 10 minutes (No heating was done). The slide was washed well with tap water, differentiated with dilute acetic acid (0.5 %) for exactly 10 seconds.

- The slide was then washed thoroughly with tap water and counter stained with 1 % methylene blue for 0.5 – 1 minute, washed under tap water, blotted dry and heated gently to remove residual moisture.
- The slide was then examined with the dry objective (× 40) to find a satisfactory area and finally with the oil immersion (× 100) objective).

In a positive test *Brucella* were supposed to stain as pink or red coccobacillus (short rods) occurring singly, in pairs or in short chains. Other bacteria would stain blue.

6.2.4 Isolation of *Brucella* organisms

Camel milk was first centrifuged at 5900 to 7700 xg for 15 minutes, in order to concentrate the bacteria which were expected to be scanty. Culturing through streaking (Laboratory and field handbook on bovine mastitis, 1987; Quinn *et al,* 1994; Sears *et al,* 1993) was done from the resultant pellet (Walker, 1999) onto Tryptose (Trypcase) Soy agar, to which bovine serum was added at 2-5%; the purpose for this was to enhance the growth of *Brucella abortus* (Songer and Post, 2005). The inoculated medium was then incubated at 37^{o} C under 5-10 v/v carbon dioxide (CO_2; using a carbon dioxide jar) for up to one week (Songer and Post, 2005) – the primary isolation of *Brucella* done under high CO_2 concentration (8-10%); growth was expected in 72 hours. *Brucella* colonies were generally expected to become visible after cultures were incubated for 3 to 5 days. Colonies are usually small (o.5-1 mm. in diameter), round with entire (smooth) margin, translucent and have a pale honey colour (Gameel *et al*, 1993; Agab *et al*, 1994). Older colonies are larger (2-4 mm. diameter) and more brown in colour (Songer and Post, 2005). *Brucella* organisms are gram-negative without bipolar staining (Songer and Post, 2005).

6.2.5 Detection of *Brucella* antibodies in milk

6.2.5.1 Milk Ring Test (MRT)

The MRT was used to detect *Brucella* antibodies in milk. Stained *Brucella* antigens (cells) were added to the non-homogenised camel milk. *Brucella* antibodies (when present in milk) agglutinated the *Brucella* antigens added forming fat globule-complexes which rose to form a bluish coloured cream layer at the top (Alton *et al*, 1988, Anon, 1986, Coetzer and Tustin, 2004).

Procedure

- 2.5 ml of camel milk sample was mixed with 1 drop of *Brucella* stained antigens (Urocel®) in a clean sterile 5 ml test tube.
- The mixture was then incubated at 37^0 C for one hour before reading the results.

Reading of the test

Formation of a bluish coloured cream layer at the top of the tube (fat globule-complexes) was interpreted as a positive Milk Ring Test for the presence of *Brucella* antibodies in the milk sample according to **Table 6.1** (Harrigan, 1998)

Table 6-1 Reading Milk Ring test

Colour of the top cream ring	**Colour of the milk column**	**Milk ring test reading**
Blue	White	Positive
White	Blue	Negative

Source: Harrigan (1998)

6.2.6 Detection of *Brucella* antibodies in serum

6.2.6.1 Rose Bengal Plate Test (RBPT)

Equal volumes (30µ) of test serum and antigen were mixed, shaken for four minutes and viewed. Any degree of agglutination was recorded as positive (Alton *et al*, 1988; 1975; Coetzer and Tustin, 2004, Anon, 1986, Brinley, 1977). All serum samples collected (n = 200) were initially screened by RBPT using Rose Bengal Plate Test antigen (Rose Bengal-Antigen, Inaktivert, Flussig, Vor Gebrauch Schuttein, Verw, bls, 31: 05: 4000, In Vit, o Diagnost Skikum Zul – Nrb. BGAF – 146. Belgium). Serum samples were kept in refrigerator at 4^0 C before testing. Sera and antigen were left at room temperature for half an hour before the test, to maintain to room temperature. The test procedure recommended by Alton *et al*, (1975) was followed, as given below:-

- 30 µl (microlitres) of RBPT antigen was added to each circle on the plate,
- 30 µl of test serum was placed alongside the antigen,
- The antigen and test serum were mixed thoroughly by wooden applicator,
- The plate was rocked/shaken for 4 minutes,

A positive control was run concurrently for every 10 samples tested for comparison.

Reading the result of RBPT

After four minutes, the degree of agglutination reactions was read and recorded as positive (+ve) (in case of coarse clumping and clearing or fine agglutination in form of a ring) or negative (-ve) (in case of no agglutinations) in negative reactions **(Plate 6.2).**

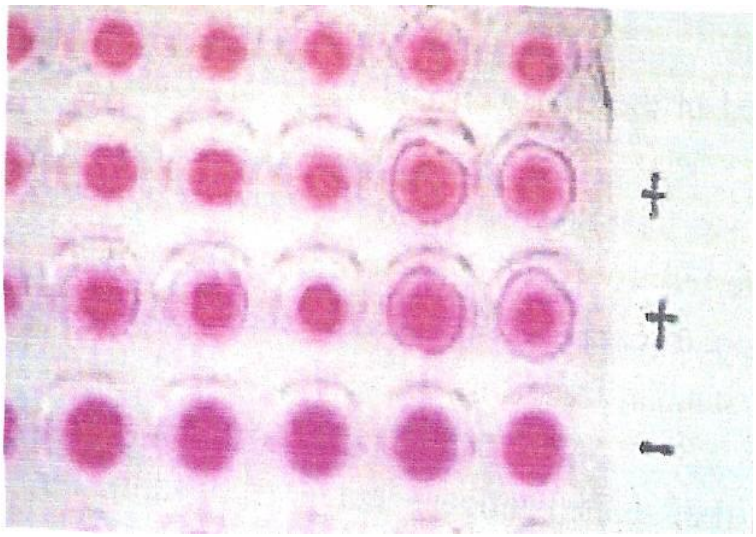

Plate 6-2 Rose Bengal Plate Test (RBPT)

In positive (+) cases an agglutination reaction took place between the antibodies in the serum and the antigen which could be seen as a ring in the well of the plate. Negative cases appear as buttons.

6.2.6.2 Serum Microagglutination Test (SMT)

This test was carried out according to the method described by Bebora (2009) and Tizard (1996). A range of serum dilutions from ½ to 1/4096 was made (Herr and Brugge, 1985; Herr *et al*, 1982; Herr *et al*, 1986).

The test used Rose Bengal stained standardized *Brucella abortus* antigens (Central Veterinary Laboratory – CVL- Weybridge, New Haw, Addtestone, Surrey KT 15 3NB, UK).

Procedure

- 50 µl (microlitres) of saline were placed in all wells of one row of the microtitre plate (96-wells microtitre plate) using a micropipette.
- 50 µl of camel serum (antiserum) was placed in the first well and mixed well using a pipette tip,

- 50 µl of the mixture (saline and antiserum) was then taken from well 1 to well 2. After mixing well, 50 µl of the mixture was transferred from well 2 to well 3, and so on until the last well (12th well) was reached. 50 µl were discarded from the last well.
- To each well was then added 50 µl of the standardized Rose Bengal stained *Brucella abortus* antigens at pre-calculated dilution.
- A negative control was set consisting of 50 µl of stained antigens and 50 µl of saline for each dilution (12 dilutions were used: - ½, ¼, 1/8, 1/16, 1/32, 1/64, 1/128, 1/156, 1/512, 1/1024, 1/2048 and 1/4096).
- The microtitre plate was then shaken well to mix the contents and then left at room temperature overnight (12 – 18 hours).

Reading of the SMT

Negative reaction appeared as a sharp well-defined sedimentation (as a "button") at the bottom centre of the well, while positive reaction appeared as a more diffuse sedimentation (like a "carpet"), covering the well bottom **(Plate 6.3).**

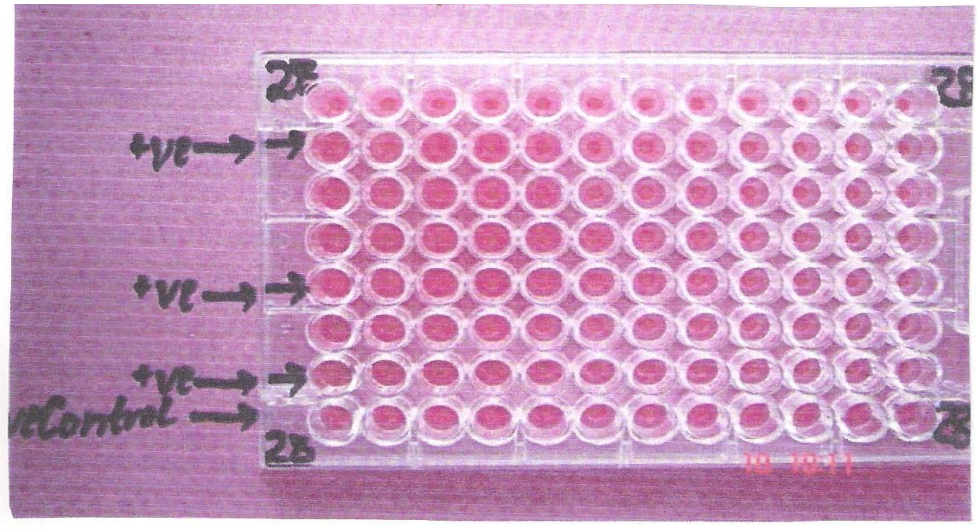

Plate 6-3 Serum Agglutination (Microagglutination) Test (SMT)

Positive reaction appears as a carpet of cells (see bottom row), while negative reaction appears as a button of cells (see top row)

6.2.7 Sample size (n) estimation

The sample size (n) was determined based on anticipated prevalence of brucellosis in camels; in Kenya, it is estimated at 14% (Waghela *et al*, 1978, Abbas and Ohmer, 2005). The calculation was done using the formula of Dahoo *et al.* (2003), as given below:

$$N = \frac{Z\alpha^2 pq}{L^2}$$

Where

n = estimated sample size

Zα = 1.96 = the normal deviate at 5% level of significance

p = estimated prevalence (14% = 0,14)

q = 1 – p (0.86 = 86%)

L = precision of estimate (considered to be 5%)

This translated to 185 camels (The number actually sampled was 200)

6.2.8 Statistical data analysis for brucellosis in camels

Data collected in the questionnaire and from serological tests was scored appropriately and recorded in spread-sheet (Ms-Excel) and analysed using Instat for windows. The seroprevalence for animal level was calculated on the basis of Rose Bengal Plate Test (RBPT) and Serum micro-agglutination Test (SAT) positivity, dividing the number of *Brucella* reactors by total number of tested animals. Descriptive statistics was also used for different variables.

6.3 Results

6.3.1 *Brucella* organisms through direct staining

None of the 384 milk samples cultured yielded *Brucella* colonies after 72 hours' incubation; not even after a further 96 hours' incubation (4 days).

The same was the case when milk smears were stained with modified Ziehl Neelsen staining technique. No *Brucella* organisms were observed.

6.3.2 Milk Ring Test (MRT)

A total of three hundred and eighty four (384) camel milk samples from Garissa and Wajir Counties were tested using the MRT. The results of the test were as recorded in **Table 6.2** Fifty nine (59) samples (15.36%) tested positive while three hundred and twenty five (325) samples tested negative. When compared at percentage level, the two counties had similar reaction rates.

Table 6-2 Results of the Milk Ring Test for Garissa and Wajir Counties

		Garissa n=230		**Wajir n=154**		**Combined n=384**	
		Number	**%**	**Number**	**%**	**Number**	**%**
Milk Ring Test reaction	Positive	35	15.2	24	15.6	59	15.4
	Negative	195	84.8	130	84.4	325	94.6

6.3.3 Rose Bengal Plate Test (RBPT)

A total of two hundred (200) camel serum samples from Garissa and Wajir Counties were tested using the RBPT. The results of the test were as recorded in **Table 6.3.** Four (4) samples

(2.0%) tested positive while one hundred and ninety six (196) samples tested negative. When compared at percentage level, Garissa had a higher percentage of reactors (2.8%) than Wajir (1.6%).

Table 6-3 Results of Rose Bengal Plate Test (RBPT) for Garissa and Wajir Counties

		Garissa n=72		Wajir n=128		Combined n=200	
		Number	**%**	**Number**	**%**	**Number**	**%**
Rose Bengal Plate Test reaction	Positive	2	2.8	2	1.6	4	2.0
	Negative	70	97.2	126	98.4	198	98.0

6.3.4 Serum Micro agglutination Test (SMT)

The agglutination titre of the serum was recorded as the serum dilution factor (well 1 was recorded as ½ and well 12 as 1/4096).

A total of two hundred (200) camel serum samples from Garrissa and Wajir Counties were tested using the SAT. The results of the test were recorded in **Table 6.4**. Twenty one (21) samples (10.50%) tested positive while one hundred and seventy nine (179) samples tested negative. When compared at percentage level, Garissa had a higher percentage of reactors (18.1%) than Wajir (6.4%). The range between the two areas, with respect to positive reactors, was much higher than with RBPT.

Table 6-4 Results of Mico agglutiantion test (SAT)

		Garissa n=72		Wajir n=128		Combined n=200	
		Number	**%**	**Number**	**%**	**Number**	**%**
Serum Micro-agglutination Test reaction	Positive	13	18.1	8	6.4	21	10.5
	Negative	59	81.9	120	93.8	179	89.5

6.4 Discussion

6.4.1 *Brucella* species in milk

All the milk samples examined were negative for *Brucella* Modified Ziehl- Neelsen's stain meaning that no *Brucella* cells were detected in these samples. Likewise, primary isolation of *Brucella* on Tryptose Soy agar (TSA) under high carbon-dioxide (CO_2) concentration yielded no *Brucella* colonies in all the milk samples tested even after incubation of the TSA plates for 7 days at 37^0 C. This could be attributed to the facts that (1) *Brucella* organisms are often present in small numbers in milk and milk products as observed by Walker (1999) and (2) there was a dilution factor since bulk milk was used as samples for culturing.

6.4.2 Presence of *Brucella* antibodies in milk

A total of three hundred and eighty four (384) camel milk samples from Garrissa and Wajir Counties were tested using the Milk Ring Test (MRT) and out of the total, fifty nine (59) samples (15.36%) tested positive while three hundred and twenty five (325) samples tested

negative. From Garrissa County (n = 230), 35 samples (15.22%) were positive for MRT while 24 samples (15.58%) from Wajir County (n = 154) were positive.

The baseline survey covered under Chapter 3 of this thesis, shows that farmers of North-Eastern province practised a high degree of ruminant diversification, i.e., in addition to camels, they kept cattle, sheep and goats. Keeping a mixture of animals is also common in other areas and has economic and ecological advantages (Ayan, 1984; Wilson *et al,* 1990; Getahun and Kassa, 2000). While this may be okay economically, in the event that the other animals are infected, such mixing increases the chances of transmission of brucellosis and other diseases to the camels (Andreani *et al,* 1982; Radwan *et al,* 1992). This is more so since results showed that large numbers of livestock herds normally congregated at water points, facilitating the spread of disease**.** Traditional wells, ponds/dams and few rivers were also documented as major permanent water sources in the area. Unlike traditional wells [water lifted by people and added to trough], animals had direct access to pond/dam water and contaminated it through discharges. However, the exposure rate may not be very high due to the fact that camel herds are mobile; this does not restrict them to a specific category of the water resources (Bekele, 2004). Brucella infection in farm animals is considered a great problem in most countries of the world. Therefore, the early detection of *Brucella* infection in a herd or flock is a pre-requisite for the successful control and elimination of one of the major problems considered to be a predisposing factor leading to infertility and sterility in the herd, along with the possible transmission of infection to man (FAO/WHO, 1986; Wasseif, 1992). Brucellosis exists in stock animals, the disease being an occupational risk for veterinarians, fur workers, abattoir workers as well as laboratory workers (Madkour, 1992). More over, other than occupational contacts, there is high-risk of transmission to humans through consumption of milk or milk products of sero-positive animals (Schelling *et al,* 2003). The

disease can also be a health risk to pastoral households who are exposed to the disease in many ways (Abbas and Agab, 2002). Camel owners (pastoralists) of the study area consume raw camel milk, and do delivery assistance, clean newborns, assist suckling and carry the young from field to home without any protection. The knowledge to the prevailing about brucellosis is nil among the herdsmen. These can put the public health of the area at risk. Abou-Eisha (2000) reported 1% (3 out of 330) brucellosis seroprevalence among nomadic people. The disease in man may be misdiagnosed due malaria infections in dry areas (Abou-Eisha, 2000; El-Ansary *et al*, 2001)

6.4.3 Presence of *Brucella* antibodies in serum

A total of two hundred (200) camel serum samples from Garissa and Wajir Counties were tested using the Rose Bengal Plate Test (RBPT). Four (4) samples (2.0%) tested positive. From Garissa County (n = 72), 2 samples (2.78%), were positive while 2 samples (1.56%) from Wajir County (n = 128) were positive.

The seroprevalence finding of the present study (10.50%) is similar to the previous reports from different countries (Mustafa and Awad El-Karim, 1971; Okoh, 1979; Abou-Damir *et al*, 1984; Abbas *et al*, 1987, Baumann and Zessin, 1992, Abou-Eisha, 2000, Omer *et al*, 2000, Azwai *et al*, 2001, Teshome *et al*, 2003). However, it is lower than some studies in Ethiopia (Domenech,1977), Kenya (Waghela *et al*, 1978), Nigeria (Ajogi and Adamu, 1998), Sudan (Ginawi, 1997; Majid *et al*, 1999), Somalia (Andreani *et al*, 1982), Kuwait (Al-Khalaf and El-Khaladi, 1989) and Saudi Arabia (Radwan *et al*, 1992).

The seroprevalence of brucellosis in camels is low in extensively kept pastoralist camels. Thus, prevalence ranging between 2 and 5% were reported from most countries where camels

are produced by pastoralists (Abbas and Agab, 2002; Wernery and Kaaden, 2002). On the other hand, titres as high as 8 to 15% have been reported in intensively kept camels especially in Saudi Arabia (Radwan *et al*, 1992) and Kuwait (Al-Khalaf and El-Khaladi, 1989). In such production system, large herds, together with overcrowding in restricted area, provide more chances of contact between animals leading to increased likelihood of infection.

Several factors may affect the result of serological findings. Higher seroprevalences of brucellosis have been recorded when multiple serological tests were used in parallel (Waghela *et al*, 1978; Al-Khalaf and El-Khaladi, 1989; Mugambi, 2001) because of sensitivity variations among the tests (Andreani *et al*, 1982). Majid *et al* (1999) reported higher seroprevalence rate (ranging from 14% to 43.9%) using RBPT alone (highly sensitive test). Reported lower prevalence rates by some authors could be a result of using tests with low diagnostic sensitivity (Baumann and Zessin, 1992) or as a consequence of serial multiple tests (Abbas and Agab, 2002). Cross-reacting bacteria such as *Escherchia coli, Yersinia enterocolitica and Salmonella serotypes* (Cloeckaert *et al*, 1992; Garin-Bastuji *et al*, 1999; Mugambi, 2001) have potential to affect serological findings when tests of low specificity are used. *Brucella abortus* may cross-react serologically with *Escherichia coli* sero-group O:157, *Yersinia enterocolitica* serovar O:9, *Salmonella* serotypes of the Kaufmann-white group N, *Francisella tularensis, Pseudomonas maltophilia,* and *Vibrio cholera* (Corbel, 1985) because the immunodorminant O-chain of the smooth lipopolysaccharide (S- LPS) of these bacteria contains antigenic motives (epitopes) that may be detected in brucellosis serological tests that use whole *Brucella abortus* cells or S-LPS extracts (Weynannts *et al*, 1997). Such False Positive Serological Reactions induced by these organisms are probably not of great significance in the early phase of eradication campaigns but when the prevalence of the

disease has been reduced to a very low level, this phenomenon may jeopardize the success of the eradication programme (Godfroid *et al,* 2002).

On the other hand, the immune suppressive effects of trypanosomosis, which is often prevalent where camels are kept, were reported in vaccinated cattle and goats, implying possible impact on serological findings (Chukwu, 1985). One such example is the classical pathway hemolytic complement activity is negatively correlated with parasitaemia caused by the trypanosomes. This phenomenon could also apply in camels although it has not been investigated. Sample selection bias also might affect serological findings. Ajogi and Adamu (1998) recorded seroprevalence as high as 27.8% from camels slaughtered at three camel rearing regions of northern Nigeria. The sample of animals tested may have been affected by the fact that slaughter of animals kept under extensive pastoral management is normally selective – it is the animals whose production performances have declined substantially that are slaughtered.

It is important to note that slide agglutination test and tube agglutination tests have been shown to have poor diagnostic sensitivities compared to RBPT or card test (Alton *et al,* 1975; Quinn *et al,* 2002). Accordingly, RBPT is considered as satisfactory screening test (Nicoletti, 1980; OIE, 2000; Quinn *et al,* 2002). The highest specificity of Complement Fixation Test (CFT) deserved it to be used as confirmatory test in serial testing (OIE, 2000). Improvement of test diagnostic specificity is particularly useful in control programs when test and slaughter policy is adopted. In camels there are yet no standards set for the diagnostic test protocol and diagnostic titre for brucellosis; although OIE (2000) recommends the test procedure outlined for the diagnosis of bovine brucellosis to be applied for camels. It is also not well defined to

what extent biochemical and physiological peculiarities of camelids contribute to the test result variability.

6.4.4 Brucellosis in camels, other animals and man

Brucellosis remains widespread in domesticated and wild animal populations, and it presents a great economic and public health problem in African countries (Chukwu, 1985; 1987). Brucellosis in camels has been reported in Saudi Arabia, Kuwait, Oman, Iraq, Iran, Sudan, Egypt, Libya, Kenya, Ethiopia, and Somalia (Gameel *et al,* 1993; Radwan *et al,* 1992; Refai, 2002; Damier *et al,* 1984; Yagoub *et al,* 1990; Agab, 1993; Teshome *et al,* 2003; Waghela *et al,* 1978). It has been reported even in racing camels in the United Arab Emirates (Afzal and Sakkir, 1994). *Brucella melitensis* biovar 3 is the most commonly isolated species from animals in Egypt, Jordan, Israel, Tunisia and Turkey. *Brucella melitensis* biovar 2 was reported in Turkey and Saudi Arabia, and *Brucella melitensis* biovar 1 in Libya, Oman and Israel. *Brucella abortus* biovar 1 was reported in Egypt, but most human cases are caused by *Brucella melitensis*, particularly biovar 3 (Radwan *et al,* 1992; Gameel *et al*, 1993; Agab *et al,* 1994; Abou-Eisha, 2000; Hamdy and Amin, 2002). According to Chukwu (1985), the high prevalence of the disease in Africa is probably due to the fact that many African countries have not started control or eradication schemes among the camel herds. Vaccination is limited to cattle and small ruminants (Refai, 2002). Camel herd size has been identified to be the major risk factor for brucellosis to occur in relation to other factors (Bekele, 2004). As herd size increases, the chance of contact between animals increases leading to more chances of infection (Abbas and Agab, 2002), which is particularly more important during calving or abortion, when most of brucellosis contamination occurs (Gameel *et al,* 1993; Agab *et al,* 1994). Thus, herd size and density of animal population, together with poor management, are

directly related to infection rate (Abbas *et al*, 1987; Abou – Eisha, 2000; Wernery and Kaaden, 2002).

Zoonotic diseases continue to present an important health risk in most parts of the world, particularly in developing countries (Stohr and Melsin, 1997). Brucellosis is a classical zoonosis and the major sources of infection remain contact with infected animals or the handling of carcasses. Less frequently, it is acquired through food. Camels are not known to be primary hosts of *Brucella* organisms, but they are susceptible to both *Brucella abortus* and *Brucella melitensis* (Cooper, 1991). Consequently, infection rate in camels depends upon the infection rate in primary host animals in contact with them. In the study area of Northeastern province of Kenya, camels are kept in close contact with other animals**.** In Darfur region of western Sudan, which owns over 25% of cattle, sheep and goats in Sudan, brucellosis is widely spread in large and small ruminants, and camels introduced in the area showed high levels of incidence (Mousa, 1995). Mixed herding and frequent contact with small ruminants and cattle are contributing factors to infection rate. There is high chance of brucellosis transmission from these ruminants to dromedaries as they live in free range in promiscuity in the bush and at water points (Andreani *et al,* 1982). Contact between dromedaries and especially small ruminants were more incriminated for the transmission of brucellosis to camels (Ismaily *et al*, 1988; Radwan *et al*, 1992). Abou-Eisha (2000) also observed higher seroprevalence in camels that were in contact with sheep and goats. Moreover, higher frequencies of *Brucella melitensis* isolation from camels (Abbas and Agab, 2002; Wernery and Kaaden, 2002) magnify the role of small ruminants in the transmission of brucellosis to camels.

Brucellosis in camels seems to display less clinical signs and antibody levels than in cattle (Mousa, 1987), probably due to a relative resistance of camels to brucellosis. The disease should be controlled by vaccination of camels and primary hosts (cattle and the small ruminants). Improving management practices is one way of attempting to control brucellosis. This would aim at improving hygiene and reducing the chances of contact between infected and non-infected animals. Although it would not be easy under many pastoral circumstances, where resources are lacking and the movement of livestock is difficult to restrict, the following points can be attempted in reducing infection rates (Hunter, 1994, Radostits *et al,* 1994a): (1) Public awareness, which is of vital importance in successful control and prevention of brucellosis , (2) Isolation of infected animals and female at parturition, (3) Proper disposal of aborted fetus, placental tissue and uterine discharge and (4) Disinfection of contaminated areas.

This survey has thus confirmed the presence of brucellosis in the Northeastern province of Kenya, showing a significant prevalence rate in camels (15.36% with the MRT and 10.50% with SAT). There is, therefore need for control programmes for the disease in the camels and other animals, in the area, so as to improve on production and to minimize risk of transmission to humans. As long as the disease persists in the animal reservoir and the pastoralists continue to drink raw camel milk, prevalence of human brucellosis, in the area, is bound to increase.

7 GENERAL DISCUSSION AND CONCLUSION

The overall objective for this study was to establish the level of safety of camel milk and occurrence of brucellosis in the camels of North-Eastern province of Kenya. In order to do this, the study was carried out through four types of investigations. The first one was to

collect baseline data on socio-economic activities of the people living in the province. The second one was to determine the status of subclinical mastitis in camels in the study area. The third one was to determine milk quality and bacterial contamination of marketed milk. The fourth one was to check for occurrence of brucellosis in the camels.

This study documented that camels were the main and highly-valued livestock kept by people of North-Eastern province; the camel population consisted all age groups, but more so adult females. The people living in this area were pastoralists, who also kept other farm animals, including cattle, sheep, goats, donkeys and chickens. These livestock, including camels, were kept together under pastoralist system; they were grazed together in the rangeland and were watered at the same points. Within such a system there is, therefore, a high chance of spread of diseases, like mastitis and brucellosis, among the animals. The other species of animals, which were kept together with the camels, could serve as sources of udder infection for the camels (Obied *et al,* 1996).

When interviewed, the pastoralist communities of North-Eastern province of Kenya said they kept camels for economic, domestic and socio-cultural purposes. Economically, the camels or their products (milk, meat, hides) were sold in order to earn cash income. Camels also provided milk and meat for domestic consumption, and were used as a means of transport, especially when the pastoralists relocated. Camels were also used for draft power. Socio-cultural purposes included traditional ceremonies like payment of dowry and contribution towards fund-raising activities. Camels were also kept as an indication of the person's wealth status within the community. These attributes to the camel have been reported in other pastoralist communities (Schwartz and Dioli, 1992); milk and meat being taken as the important products. A study in eastern Ethiopia indicated 3 to 6 litres of daily milk yield over

13 to 15 months of lactation length (Gatahun and Bruckner, 2000), while Tefera and Gebrcab (2001) reported an average daily milk yield of 2.5 litres. These values compare well with figures obtained from Garissa and Wajir counties in this study (mean value of 4 litres per day). Long lactation period and ability to maintain milk production over long dry spells are important facets of camel productivity. With improved management, daily milk yield has been shown to rise to a high of 20 litres (Schwartz and Dioli, 1992). Camels are used widely for transport. In fact, before the arrival of motorized transport in arid and semi-arid areas, camels were the sole means of transport in the areas where they have been adapted. They are also used for wheel transport, water lifting and source of power for oil mill. Camel-racing and other leisure activities, such as camel safaris and trecking have recently become tourist attractions and luxurious in some parts of the world (Schwartz and Dioli, 1992; Wilson, 1998). This study has also documented high bacterial loads in milk – this is through bacterial culture, including total coliform count and total viable bacterial count, and through carrying out of Resazurin test. Bacteria in milk could cause spoilage of milk and milk products; they could also cause disease in humans. The course of the heavy bacterial load was placed more on environmental contamination, rather than on subclinical mastitis. This was supported by the fact that, when analysis of total viable bacterial count in the camel milk was compared to the EC standards, it was shown that milk collected directly from the udder was of good bacterial quality but it deteriorated rapidly as it entered the informal marketing chain. It was noted, from the study, that the respective farmers used traditional milking practice, known as "dry milking". In this practice, the udder is not washed with water, but dust is wiped from the udder and teats with the palms of the milker's hands and milking started immediately. The collected milk is then stored and transported in traditional gourds which are normally washed and smoked using traditional herbs. This was aimed at preserving the milk for longer period of time and giving the milk the traditional flavor, preferred by consumers; however this "cold

smoking" of plastic containers is unlikely to have a sanitizing effect. The storage and transportation of the milk was normally done under low hygienic conditions, which have been demonstrated, in another study, to introduce gross dirt, especially charcoal particles into the milk (Younan *et al*, 2002). Pooling of different raw camel milk batches and usage of unhygienic plastic containers have been shown to accelerate spoilage, with non-refrigerated bulk milk reaching a total bacterial count of 10^4 cfu/ml (Younan *et al*, 2002); this milk turns sour in less than 12 hours under the hot pastoral conditions. Plastic jerrycans of cheap quality that were used (e.g. recycled cooking oil containers) have been shown to have a fast-corroding surface and are difficult to clean in pastoral areas, due to lack of water. The non-availability of safe, clean water makes introduction of common hygiene recommendations difficult; thus, adapting hygiene practices and guidelines to the pastoral situation remains a big challenge (Younan *et al*, 2002). However, a suggested simple approach of improving camel milk hygiene is the provision of clean gauze/paper filters for filtration. Receiving milk directly from producers without pooling of different batches, coupled with shorter transport distances, may offer hygiene advantages. This study has shown that approximately 90% of the examined raw camel milk samples were produced and handled under poor hygienic conditions (getting contaminated through handling by the pastoralists). There is, therefore, need for pastoralists to exercise personal hygiene, use clean/disinfected milking vessels, and boil milk before consumption.

At times, when not being milked, the camel owners tied one or two teats of the camel with a piece of rope from the bark of a tree to serve as an anti-suckling device – to prevent calves from suckling. These ropes are untied before milking commences. The use of these devices together with heavy tick infestation could predispose the udders to bacterial infections, which persist as chronic infections.

Subclinical mastitis can also be source of bacterial contamination for the camel milk. When California mastitis test (CMT) was carried out on bulk (pooled) milk (where the effect is normally diluted), a prevalence rate of 61.2% for subclinical mastitis was detected; this means the prevalence could have been much higher. The equivalent somatic cell counts of the 235 positive milk samples ranged from $1.5x10^5$ to $5x10^6$ leukocytes per milliliter of milk. All milk samples also yielded mixed types of bacteria on culture; these included *Staphylococcus* (both coagulase positive and coagulase negative ones), *Streptococcus* (both CAMP positive and CAMP negative ones), *Bacillus*, *Escherichia coli* and *Klebsiella/Enterobacter* group. On grading the CMT positive milk samples, 32.0% had a score of trace, 25.5% had a score of 1+, while 3.7% had a score of 2+. These results compare closely with those obtained for she-dromedary camels in Jordan (60.0%) by Hawari and Hassawi (2008), with the most predominant bacterial isolates being *Staphylococcus* species, *Streptococcus* species and *Micrococcus* species.

The high prevalence for the three major mastitis-causing pathogens (Gram positive cocci - *Staphylococcus*, *Streptococcus* and *Micrococcus s*pecies) which originate from the udder was attributed to bulking or pooling of different camel milk batches from different animals and producers. However, the high prevalence of coliforms (*Escherichia coli* and *Klebsiella/Enterobacter* species) and *Bacillus* species was attributed to contamination of milk containers from the environment due to the poor hygiene of handling milk along the collection *and* marketing chain. In both counties, bacteria isolated highest were *Staphylococcus* (88% for Garissa and 93.5% for Wajir) and *Klebsiella/Enterobacter* species (about 96% for both counties). Both counties also yielded *Bacillus* species (50% for Garissa and 39% for Wajir) and *E. coli* (73% for Garissa and 40% for Wajir). Garissa County also

had higher percentage of coagulase positive staphylococci (30%) and CAMP positive streptococci (31%) than Wajir County (14% and 19.5%, respectively).

Bacterial mastitis pathogens also represent a potential threat to humans if the milk is consumed raw; a common practice in most camel-keeping communities. The two most common mastitis pathogens in camels (*Streptococcus agalactiae* and *Staphylococcus aureus*) are potential human pathogens *(*Younan *et al*, 2001; Abdurahman *et al*, 1995). While toxin-producing *Staphylococcus aureus* may cause food poisoning, *Streptococcus agalactiae* is known to cause human infection, particularly in newborn children. These organisms also reduce productivity of affected camels. Thus, awareness of this should be made among the normadic pastoralists to reduce the problem of mastitis in camels, in order to increase milk production. Consumption of milk that is not boiled or pasteurized can serve as a source of other important zoonotic milk-borne pathogens, such as *Brucella* and *Mycobacterium* species.

Mastitis can be prevented or reduced by improving animal health and udder hygiene. Currently there is almost a complete absence of modern mastitis control measures practiced by the camel keepers. There is also little evidence of effective ethno-veterinary interventions in treating and curing mastitis. Good quality dairy products can only be obtained from healthy camels. Attention, therefore, must be paid to udder health and hygiene, not only during lactation, but continuously; even when the animal is dry. Animals suffering from any contagious disease, including mastitis, should be separated from the healthy ones and milk from diseased camels should be kept separate and disposed off safely. It is cheaper and easier to prevent mastitis by improving hygiene measures than through treatment of clinical cases. The cost of the latter includes veterinary fees, cost of medicine and loss of milk production.

A combined assessment of physical characteristics of the 344 camel milk samples from the two counties (Garissa and Wajir) showed that 289 samples (75.3%) had gross dirt/particulate matter, including grass/leaves, sand/soil particles and/or black charcoal particles. This could be attributed to the low level of hygiene in cleaning of the milk containers and lack of milk filters after milking; before packing the milk in containers. The black charcoal particles were attributed to the tradition of smoking milk containers, especially traditional gourds as has been observed in this study and by other researchers (Younan *et al*, 2002). Where there are organic contaminants in the milk it means that there is a chance of introducing organisms, should the organic substance be contaminated with them. As mentioned earlier, camel milk is traditionally produced by way of hand-milking, handled and transported under low hygienic conditions.

Deviation of the milk from normal constitution has also been demonstrated by: 8.9% of the milk having an abnormal yellowish color (deviation from the normal White color); 18.2% having offensive/bad smell/odour (which was either sour or foul); and pH levels of the milk not being right: 31.0% had pH of 6, 52.9% had pH of 7, while 16.2% had pH of 8 – the normal pH of milk is between 6.6 and 6.8. Lower pH values generally mean acidification process due to development of bacteria, while higher pH values mean presence of mastitis (Giangiacomo, 2001). Formation of flakes in the Alcohol test, recorded in 33.3% of the samples, indicated that they were either acidic, mastitic or colostrum milk. Formation of the flakes indicates unsuitability of the milk for consumption; it also means the milk is unsuitable for further processing.

The range of specific gravity of the samples tested was within the range 1.019 to 1.032 grammes/litre, with 40.5% being within the range 1.019-1.024 grammes/litre, 45.4% being

within the range 1.025-1.029 grammes/litre, and 14.1% being within the range 1.030-1.032 grammes/litre. This gives an indication of some adulteration. The mean specific gravity of normal camel milk is 1.0305 grammes/litre, with an average butter fat content of 3.678% (Tibary and Anoussi, 2000; Gitao, 2006). When camel milk is adulterated with wáter, specific gravity wil be less than 1.026 grammes/litre, while adulteration with solids like sugars, specific gravity will be higher than 1.034 grammes/litre (Giangiacomo, 2001). As many as 155 samples (40.5%) had a specific gravity within range 1.019-1.024 grammes/litre, an indication of adulteration of the marketed milk, thus casting concern on the quality of camel milk supplied to the consumers. Addition of up to 15% wáter to marketed camel milk has been reported from southern Somalia (Younan *et al,* 2002); the quality of the added wáter representing an addtional higiene risk. The specific gravity of camel milk tested in three large comercial herds in Kenya over two months' period varied between 1.026 and 1.029 grammes/litre, indicating a difference to the specific gravity of camel milk (Younan *et al,* 2002).

As mentioned earlier, research exercise on collection of baseline data on behaviour and practices of the people of North-Eastern province showed that these people kept various species of animals together, lived in close association with their animals, and preferred to drink raw milk. All these expose the inhabitants of North-Eastern province to milk-borne diseases. One zoonotic disease that can be transmitted through milk and other exudates is brucellosis.

Brucellosis in camels has been reported to spread from small ruminants to camel herds (Dafni *et al,* 1991). Both vertical and horizontal transmissions exixt in animal brucellosis. Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, via

conjunctiva, through inhalation and through udder contamination during milking. Disease spreads when infected animals are moved to disease-free herds. There is normally mixing of infected herds with clean herds at wáter points, where a number of camels and other animals come together. This is more so in dry pastoral areas, where wáter resources are sparsely distributed (Helland, 1982), leading to congregation of large numbers of mixed ruminants at the wáter points. Epidemiologically important risk factors include: large herd size; poor management; active abortions; milking of a number of animals by a single person; and herding with other ruminants. The incidence of brucellosis in camel population appears to be related to breeding and husbandry practices. Herd sizes, density of animal population, and poor management are directly related to prevalence (Wemery and Kaaden, 2002). Survival of the organism in the environment may also play a role in the epidemiology of the disease (Abbas *et al*, 1987; Radwan *et al*, 1992; Abou-Eisha, 2000). Dafni *et al.* (1991) suggested that small ruminants act as extensive reservoirs of *Brucella melitensis*, which constitutes a threat of infection to large ruminants, including camels, and man, after prolonged contact. The chance of transmission is higher during parturition and abortion when most of the *Brucella* contamination occurs (Abbas and Agab, 2002).

When interviewed, the respondents indicated that they kept more adult female camels than those of other ages. This higher number of adult females recorded could have some significance on the prevalence of brucellosis in the camel herds. Radostits *et al,* (1994b) has reported that although brucellosis may occur in animals of all age groups, it tends to persist more in sexually mature animals. Generally, infection is acquired after 3 years of age, with increase in subsequent age groups (Majid *et al*, 1999; Abou-Eisha, 2000). Some study results have revealed equal distribution of *Brucella* antibodies among males and females (Waghela *et al,* 1978; Abou Damir *et al,* 1984; Abbas *et al,* 1987; Radwan *et al,* 1992). In other

studies, however, it appeared that females were more susceptible to the disease than males (Agab, 1997; Ajogi and Adamu, 1998). Higher susceptibility in female animals is attributed to physiological stresses (Walker, 1999) and presence of erythritol (sugar). Thus, female animals have essential epidemiological importance not only in susceptibility but also in dissemination of the disease via uterine discharges and milk. The role of males in the spread of the disease under natural conditions is not important (Radostits *et al*, 1994b).

Brucellosis in humans represents a major public health risk, which affects social and economic development in various countries. Groups at high risk of contracting brucellosis are: animal health workers, butchers, farmers, and those who habitually consume raw milk and/or come in contact with animals (Chukwu, 1987). In man, transmission occurs as a result of ingestion of raw milk, contact via skin abrasión, mucous membranes and inhalation (Radostits *et al*, 1994b; Seifert, 1996; Masoumi *et al*, 1992). What is worse is that these camel keepers tend to also consume liver without heat treatment; this is often considered a delicacy (Gameel *et al*, 1993). There is also close contact between herdsmen and the animals during watering, grooming, riding, nursing sick ones and delivery assistance (Abbas *et al*, 1987). The isolation of the two major pathogenic *Brucella* species, *Brucella melitensis* and *Brucella abortus*, from milk and other samples of camel origin (Gameel *et al*, 1993; Agab *et al*, 1994; Handy and Amin, 2002) clearly indicates the potential public health risks of camel brucellosis (Straten *et al*, 1997). The disease in man may be misdiagnosed due to the prevailing malaria infections in the dry areas (Abou-Eisha, 2000; El-Ansary *et al*, 2001); the symptoms tend to appear like those of malaria. Misdiagnosis can also occur as a result of cross-reaction serologically with other bacteria, e.g. *Yersinia enterocolitica, Salmonella* serotypes, mainly of group N, *Franciscella tularensis*, *Pseudomonas maltophilia* and *Vibrio cholerae* (Corbel, 1985; Mugambi, 2001).

Camels are the main livestock of the pastoralists; they are the backbone of their wealth and economic status. However, consumer health is of paramount importance when it comes to consumption of milk. Milk contaminants, including faecal organisms, pose an important threat to consumers of marketed camel milk. Zoonotic risks (brucellosis being the most important here) from camel milk must be considered in view of the traditional preference for raw milk for consumption. In an attempt towards reducing the incidence of brucellosis cases, intervention strategies should include safe breeding procedures, good management practices, regular serological testing, slaughtering of infected animals, and vaccination of uninfected herds of camels.

The pastoralists also generally treated sick camels (livestock) on their own, using various methods, including usage of traditional herbs; they rarely engaged veterinarians. This habit of pastoralists treating sick animal on their own, without profesional advice, was not desirable, since indiscriminate use of antibiotics could lead to build-up of bacterial antibiotic resistances both in the camel and humans consuming the camel products (milk and meat).

8 RECOMMENDATIONS

Camel keepers should be educated on camel mastitis, its effect on production and possibility of transmitting pathogens to humans. Also, they should be discouraged from consuming raw or partly-heated milk and be educated the importance of boiling or pasteurizing camel milk before consuming. The camel keepers will thus be encouraged to reduce the problem of mastitis in the camel, so as to benefit from the increased milk production and sales.

Nomadic pastoralists should be encouraged to improve on camel health and udder hygiene, so as to prevent or reduce the incidence of mastitis in camels. Attention must be paid to udder

health and hygiene, not only during lactation, but continuously – even when the animal is dry. This can be done through cleaning of the udder before milking, thorough cleaning of milk containers (preferably using very hot water).

Animals suffering from any contagious disease, including mastitis, should be separated from healthy animals and milk from diseased camels should be kept separate and disposed off safely.

Training on camel udder hygiene, handling and production of milk for better milk safety and quality should be carried out, so as to increase awareness among the pastoralists.

The pastoralists can be encouraged and trained on how to use the CMT screening test for detection of subclinical mastitis in camels. Ultimate improvement of the hygiene (quality) of camel milk lies in selling heat-treated packed milk which would provide a solution to hygiene woes and, at the same time, eliminate zoonotic risks for camel milk consumers.

Pastoralists can only benefit tremendously from the production of good-quality camel milk by coming together and forming camel-milk marketing societies so that they can easily control the quality of their camel milk, thereby improving the producer prices of the milk, and have bargaining power.

Camel milk produced by pastoralists could fetch more money in the market if the following were done and this can be incorporated in an awareness campaign.

1. If they heat-treated their milk to kill bacteria that spoil milk and those that are zoonotic.

2. If they processed and packaged their own milk in organized groups. Decentralized and possibly mobile processing systems relying on simple adapted technology might be more sustainable and should be tested in these pastoral areas.
3. Disease control/treatment and management provision of milk coolants (refrigerators and cold rooms)
4. Introduction of metal containers (such as aluminium/steel cans) that are easy to clean/wash and sterilize; to replace the traditional gourds and plastic milking containers
5. Provision of clean gauze/paper filters for filtration of the milk
6. Provision of subsidized transport means or improving the road infrastructure network to town markets from the production areas.
7. Provision of more public service, in terms of extension services, regular monitoring of progress to these marginal pastoral areas , in terms of livestock industry
8. If milk was received directly from the producers without pooling different batches. This, coupled with shorter transport distances (improved infrastructure) may offer hygienic advantages
9. There is need for more research work to be carried out to identify critical contamination points, so that respective interventory measures can be initiated. It is possible to recommend a control programe for camel mastitis in this region, taking into consideration the use of effective antibiotic therapy during lactation and at drying off – this would be an essential part of such a mastitis control programme. This should, however, be done under professional supervision. The people should be discouraged from treating their animals without consulting a veterinarian, since indiscriminate usage of antibiotics could lead to the build-up of bacterial antibiotic resistances both in the camels and humans consuming the camel products (milk and meat).

In an attempt towards reducing the incidence of brucellosis cases, intervention strategies should include safe breeding procedures, good management practices, regular serological testing, slaughtering of infected animals, and vaccination of uninfected ones.

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9 APPENDICES

9.1 Bacterial etiological agents of mastitis in camels

(Sources: Almaw and Molla, 2000 ; Quandil and Qudar, 1984 ; Sears *et al*, 1993 ; Obied *et al*, 1996 ; Barbour *et al*, 1985 ; Woubit *et al*,2001).

	Bacterial genus	**Species**
1	*Streptococcus*	*agalactiae*
	Streptococcus	*uberis*
	Streptococcus	*dysagalactiae*
	Streptococcus	*pyogenes*
	Streptococcus	*faecalis*
2	*Staphylococcus*	*aureus*
	Staphylococcus	*intermedius*
	Staphylococcus	*hyicus*
	Staphylococcus	*epidermidis*
3	*Micrococcus*	species
4	*Aerobacter* species	species
5	*Escherichia*	*coli*
6	*Bacillus* species	*cereus*
7	*Pasteurella* species	*haemolytica*
8	*Corynebacterium*	*bovis*
	Corynebacterium	*ulcerans*
9	*Enterobacter*	*aglomerans*
	Enterobacter	*faecalis*
10	*Klebsiella*	*pneumoniae*
11	*Actinomyces*	*pyogenes*

9.2 Compilation of (Total Bacterial count)TBC

(source: Younan *et al* - 2002)

MILK SAMPLE	TBC * (cfu/ml)
From udder milk directly into clean containers	$10^2 – 10^4$
From traditional milking bucket	$10^3 – 10^4$
From transport container immediately after end of milking	$10^3 – 10^5$
From bulk milk stored without cooling	$10^5 – 10^8$
From milk purchased in production area (<24 hours old milk	$10^6 – 10^7$
From milk purchased in Nairobi (24-36hours old milk)	10^6 - 10^8

Key: *For comparison of European Union -Standard for Raw Cow Milk (TBC < 10^5 cfu/ml – cfu/ml (Colony forming units per milliliter).

9.3 Milk hygiene risk factors

(Source: Younan et al - 2002)

Camel milk production and Marketing chain	Milk hygiene risk factors
Lactating camel	Unclean udder, subclinical mastitis, zoonotic infections with lactogen transmission
Milker (male)	Unclean hands, personal hygiene and health status, unclean (plastic) milking bucket, unclean milking site
Milk handler (male/ female)	No/unclean filtration, unclean storage container (plastic0, pooling of fresh and old milk
Primary milk collector (mostly female)	No/unclean filtration, unclean (plastic) transportation container, pooling of milk from different producers, high environmental temperatures during intermediate storage, adding unclean water (adulteration)

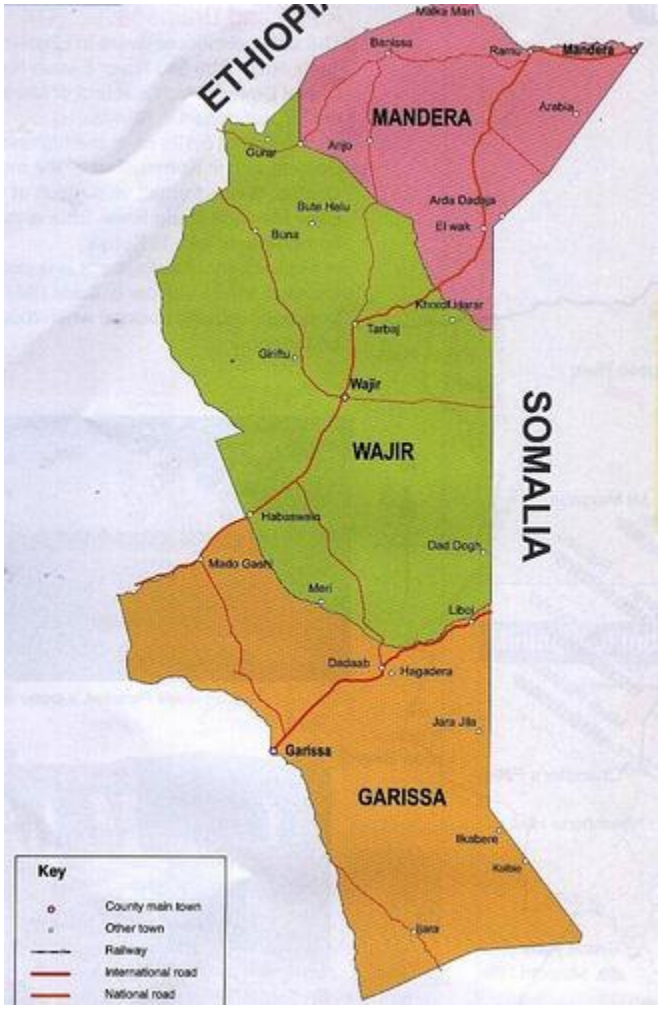
Teansporter (male)	Delayed transport, prolonged exposure to high environmental temperature
Secondary milk collector (mostly female)	Additional pooling, exposure to high environmental temperature, adding unclean water (adulteration)
Milk vendor (female)	Selling from open containers in unclean environment, further exposure to high environmental temperature, adding unclean water (adulteration)
Consumers of camel milk	Traditional preference for consumption of raw (camel) milk

9.4 Summary of *Brucella* isolates from camels (*Camelus dromedaries*) in different countries

(Source: Adopted from Abbas and Agab - 2002, Wernery and Kaaden - 2002)

Country/References/ Authors	**Organ or specimen**	**Isolated organism**
Egypt: Abou-Eisha (2000)	Milk	*Brucella melitensis* biovar 3
Iran: Zowghi and Ebadi (1988)	Lymph node	*Brucella melitensis* biovar 1
Kuwait: Zowghi and Ebadi (1988); Al-Khalaf and El-Khaladi (1989)	Lymph node; Fetal stomach content	*Brucella melitensis* biovar 3
Libya: Gameel *et al.* (1993)	Milk; Aborted fetus; vaginal swab	*Brucella melitensis* biovar 1
Saudi Arabia: Radwan *et al.* (1992); (1995), Ramadhan *et al.* (1998)	Milk; Milk; Carpal hygroma	*Brucella melitensis* biovars 1,2; *Brucella melitensis* biovars 1,2,3; *Brucella melitensis*
Senegal: Verger *et al.* (1979)	Milk	*Brucella abortus* biovars 1,3
Sudan: Agab *et al.* (1994)	Lymph node, Testes, Vaginal swab	*Brucella abortus* biovar 3

9.5 Location of Garissa and Wajir Counties in Kenya



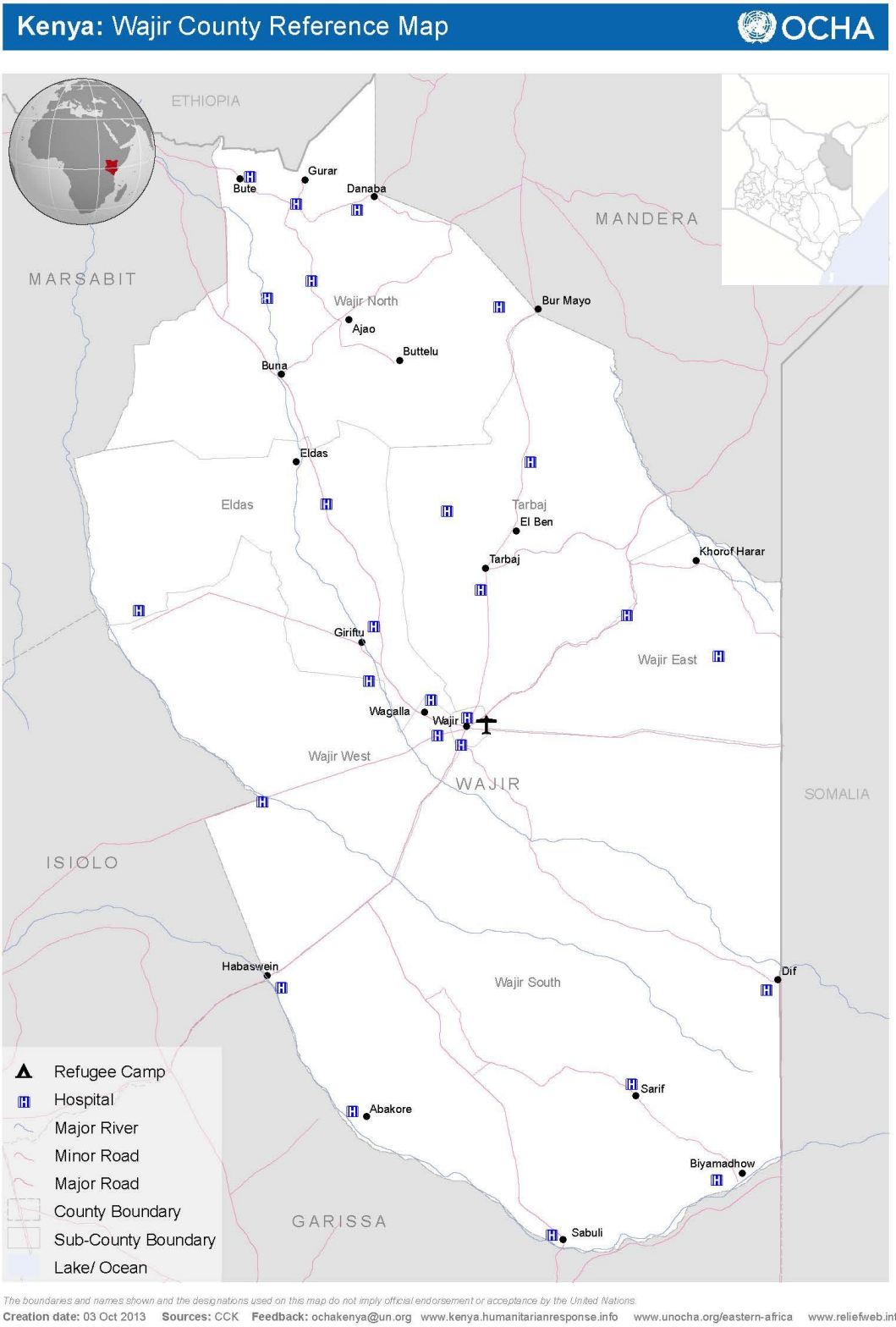
9.6 Kora- Kora Division in Garissa County

Note: The Arid and Semi-Arid Land (ASAL) ecological zone is covered by sand and shrubs.

9.7 Camels browsing in ASAL

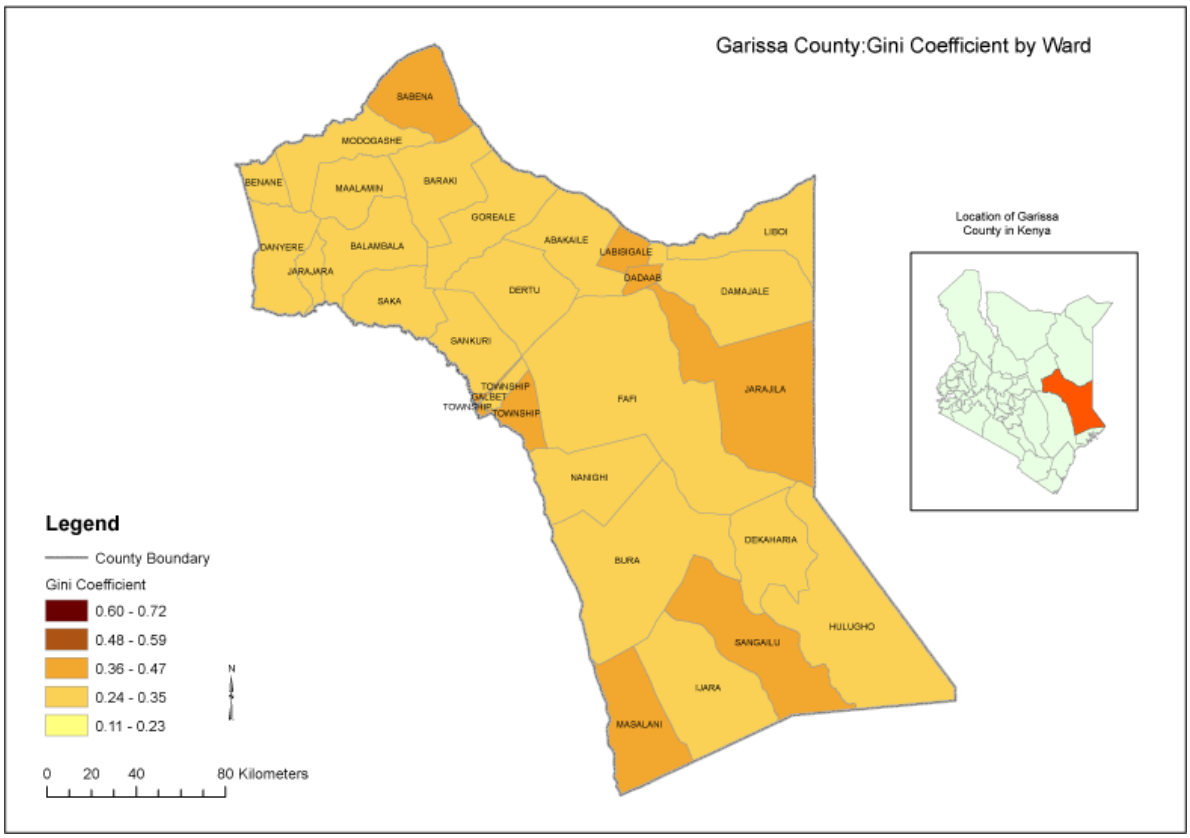
Picture shows camels browsing in the ASAL ecological zone. Notice the ground is covered by sand and no ground vegetation apart from a few shrubs.

9.8 A map of Wajir County showing the locations of the study areas



Source: OCHA

9.9 A map of Garissa County



9.10 Questionnaire for camel milk producers

Camel milk chain enhancement in Garrissa and Wajir County questionnaire for camel milk producers (farmers and pastoralist) - 2008/2009

[I]: Introduction

Good morning /afternoon /evening. My name is DR. WANJOHI. I am an interviewer from the University of Nairobi which is a teaching and a research institution. Today we are conducting a study to identify the constraints in the camel milk chain with a view to designing appropriate solutions and we would be very grateful to get your input. We uphold the secrecy and confidentiality of any information provided. I wish to state that any information provided will be used for the purpose of the study only.

[II]: General

Date: Day / Month / Year. _____ / _____ / _____

1. Name of the respondent._____

2. County._____ Division._____ Location_____

Sub-location_____ Village_____

3. Name of the group _____. Membership _____

4. GIS (Geographical Information System) location._____

5. Number of people in the household._____

[III]: Livestock keeping

1. What livestock do you keep and how many of each?

(i) Cattle_____ (ii) Camels_____ (iii) Sheep_____ (iv) Goats_____

(v) Others (Specify) _____

2. Why do you keep Camels? (i) Consumption_____ (ii) For sale_____

(iii) For traditional ceremonies_____ (iv) For milk production_____

Others (specify)_____

3. Where do you graze and water your Camels? (i) Graze_____ (ii) Water_____

4. How often do you water your camels? (i) Daily_____ (ii) Every 3 to 5 days_____

(iii) Once a week_____ (iv) Monthly_____ (v) Others (specify) _____

5. Do you give extra feed to your camels apart from grazing? _____

6. If yes why do you give these extra additives? _____ . Where do you obtain them from? _____

7. What are the age groups of the camels that you have and what are the numbers?

(i) Adult-females ____ (ii) Adult-males _____ (iii) Wearners & Growers _____

(iv) Calves _____

8. How many adult females are milking? _____

9. How many times do you milk your camels in a day and how much milk is produced per milking? _____ (i) Morning_____ (ii) Evening_____

10. What is the average amount of milk produced by the camel per day? (i) During dry season_____ (ii) Wet season_____

11. Who does the milking? _____

12. What milking procedure do you use? _____

13. What problems are encountered during milking? (i) Swollen painful udder and teats _____ (ii) Milk discoloration (bloody /reddish) _____ (iii) Change in milk consistency (watery, clotted, creamy) _____ (iv) Traumatic lesions on the udder and teats _____

14. For how long do you milk your camels before they deliver again? _____

15. What containers do you use for milking, preserving and transportation of the camel milk and what are their costs?

	Milking	Transportation	Preservation	Cost per container
(i) Plastic containers	_____	_____	_____	_____
(ii) Gourds/Traditional	_____	_____	_____	_____
(iii) Alluminium/steel	_____	_____	_____	_____
(iv) Others (specify)	_____	_____	_____	_____

16. Do any of the above containers belong to any group? _____. If yes what is the name of the group? _____

17. How often do you wash / clean your milk containers?

(i) Immediately after milking _____ (ii) Just before the next milking _____

(iii) Others (specify) _____

18. How do you wash your milk containers? (i) Use of detergent _____ and its name _____ (ii) Use of cold, warm or hot water _____ (iii) Use of disinfectant and its name _____

19. What is the purpose of the milk produced by the camels? (i) Sale _____

(ii) Domestic consumption_____ (iii) Feeding calves_____

(iv) Others (specify) _____

20. What quantity of camel milk is sold? (i) Fresh _____ (ii) Sour _____

21. Who takes care of the camels by grazing and watering them? _____ and who makes management decisions? _____

22. What diseases have you noticed in your herds of camels (i) Mastitis _____ (ii) Trypanosomiasis _____ (iii) Brucellosis/ Abortion _____ (iv) Diarrhoea _____ (v) Udder injury (physical) _____ (vi) Others (specify) _____

23. How do you have your animals treated when they are sick? (i) Gok Veterinarian_____ (ii) Private veterinarian _____ (iii) Traditionally using herbs_____ (iv) Owner treatment using conventional medicine _____ (v) Others (specify) _____

24. What happens to milk from a mastitic camel? (i) Sold _____ (ii) Pour it _____

(iii) Given to calves _____ (iv) Domestic consumption _____

25. What do you do with milk produced from treated camels?

(i) Sold _____ (ii) pour it _____ (iii) Given to calves _____ (iv) Domestic consumption _____

26. How is the camel milk meant for home use consumed?

(i) Consumed raw _____ (ii) Boiled before consumption _____ and for how long boiling _____

27. Why is camel milk consumed raw without pasteurizing first? _____

28. Do you add any additives to camel milk after milking? _____. What are the reasons? _____

29. How do you store your camel milk after milking before disposing? _____

30. How long does it take to dispose of camel milk for sale after milking? _____

31. Who are your customers for the camel milk? (i) Neighbours _____ (ii) Hawkers _____ (iii) Bulkers /Retailers _____ (iv) Co-operatives/Processors _____

32. How much do you sell a litre of camel milk? ,(i) During wet season _____ , (ii) During dry season _____. Are you comfortable with these prices? _____

33. How strong is the demand for camel milk during wet and dry seasons? (i) Wet season _____ (ii) Dry season _____

34. Do you have any intention of coming together and form a camel-milk marketing society? _____

35. What else do you think will help you fetch more money from your camel milk? (i) Process own milk _____ (ii) Improve on milk hygiene _____ (iii) Others (specify) _____

36. Would you or any member of your family / group like to be trained in camel-milk and udder hygiene? _____. For how long would you like to be trained? (i) Three days _____ (ii) Four days _____ (iii) Five days _____ (iv) One week _____

37. Do you have any credit access to improve on your camel rearing / keeping activities? _____.If no would you be interested in some? _____. What would you do with such a credit? _____

38. What do you do to preserve your camel milk for longer periods of time? (i) Boiling _____ (ii) Cooling _____ (iii) Adding Antimicrobials _____ (iv) Adding Hydrogen peroxide _____ (v) Others (specify) _____

39. How would you suggest camel milk be cooled? _____. Would you be willing to pay for cooling services? _____

9.11 California Mastitis Test (CMT), Somatic Cell Counts (SCC) in cattle

(Source: Radostits *et al*, 2005)

Test results	**Reaction observed**	**Equivalent milk somatic cell counts/ml (SCC/ml)**
Negative	The mixture remains fluid without thickening or gel formation	0 – 200,000 cells /ml
Trace	A slight slime formation is observed. This reaction is most noticeable when the paddle is rocked from side to side	150,000 – 500,000 cells /ml
1^+	Distinct slime formation occurs immediately after mixing solution. This slime may dissipate over time. When the paddle is swirled, fluid neither form a peripheral mass nor does the surface solution become convex or "doomed-up	400,000 – 1,500,000 cells /ml
2^+	Distinct slime formations occur immediately after mixing solution. When the paddle is swirled, the fluid forms a peripheral mass and the bottom of the cup is exposed.	800,000 – 5,000,000 cells /ml
3^+	Distinct slime formation occurs immediately after mixing solution. This slime may dissipate over time. When the paddle is swirled, the surface of the solution becomes convex or "doomed-up	>5,000,000 cells /ml

9.12 Composition and Preparation of culture media, Reagents used

9.12.1 Culture media

9.12.1.1 7 % Blood Agar (BA) (Oxoid CM 271)

Manufacturer

Oxoid Ltd, Basingstoke, Hampshire, England

Composition (Typical formula/Ingredients) in grams per litre

Proteose peptone – 15.00, Liver digest – 2.50, Yeast extract – 5.00, Sodium chloride – 5.00, Agar – 12.00: Final PH (at 25^0 C) – 7.4 approximately 70 ml of Sterile Sheep blood added finally.

Uses (Description)

This medium is suitable for cultivation of many fastidious microorganisms and for determination of the typical haemolytic reactions (which are important diagnostic criteria for Streptococci, Staphylococci, and other microorganisms.

Directions

- 40.00 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- Mixed and sterilized by autoclaving at 121^0 C for 15 minutes.
- Cooled to 45^0 C – 50^0 C before adding 7 % sterile Sheep blood.
- Finally mixed with gentle rotation and poured into Petri dishes

Recommendation: Reconstitution and mixing were performed in a flask at least 2.5 times the volume of medium to ensure adequate aeration of the blood.

9.12.1.2 Eosin Methylene Blue Agar (Levine) (IVD) (Oxoid) (CM 0069)

Manufacturer

Oxoid Ltd, Basingstoke, Hampshire, England

Composition (Typical formula/Ingredients) in grams per litre

Peptone - 10.00, Lactose - 10.00, Di-potassium hydrogen phosphate – 2.00, Eosin Y – 0.40, Methylene blue – 0.06, Agar – 15.00: Final PH (at 25^0 C) 6.8 ± 0.2

Uses (Description)

This medium is recommended for the detection, enumeration or differentiation of members of the coliform group (lactose fermenters). It is used for the differentiation of *Escherichia coli* and *Aerobacter aerogenes* and *Klebsiella/Enterobacter* species.

Characteristic colonial morphology

Escherichia coli

Isolated colonies are 2 – 3 mm in diameter, with little tendency to confluent growth, exhibiting a greenish metallic sheen by reflected light and dark purple centres by transmitted light.

Aerobacter aerogenes

Isolated colonies are 4 – 6 mm in diameter, raised and mucoid; tending to become confluent, metallic sheen usually absent, grey brown centres by transmitted light.

Non-Lactose fermenting intestinal pathogens

Isolated colonies are translucent and colourless.

Directions

- 37.50 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- Mixed and sterilized by autoclaving at 121^0 C for 15 minutes.
- Cooled to 60^0 C and shacked the medium in order to oxidize the Methylene blue (That is to restore its blue colour) and suspend the precipitate which is an essential part of this medium.

9.12.1.3 Glucose Phosphate Peptone Water (GPPW)/M.R.V.P- Medium (Oxoid CM 43)

Manufacturer

Oxoid Ltd, Basingstoke, Hampshire, England

Composition (Typical formula/Ingredients) in grams per litre

Peptone (Oxoid L 49) – 5.00, Phosphate buffer – 5.00, Dextrose – 5.00: Final PH (at 25^0 C) – 7.5 approximately.

Uses (Description)

A medium recommended for the Methyl–red (M.R) and Voges–Proskauer (V.P) tests for the differentiation of the Coli-aerogenes group. Methyl – red is employed as a hydrogen–ion concentration indicator in order to differentiate Dextrose, Phosphate, Peptone Water (GPPW) cultures of members of the Coli-typhoid group. This test is known as the Methyl-red test (M.R test).

The Methyl-red test (M.R test) distinguishes between microorganisms which produce and maintain a high acidity, and those producing an initially lower acidity which reverts towards

neutrality. The former type of microorganisms, such as *Escherichia coli*, produces a red colouration and is referred to as Methyl-red positive; the later such as *Aerobacter aerogenes* produce a yellowish colouration, and is termed Methyl-red negative.

Voges and Proskauer described a red fluorescent colouration which appeared after the addition of Potassium hydroxide to cultures of certain microorganisms in Dextrose medium. The colouration was shown to be due to the oxidation of the acetylmethyl-carbinol producing diacetyl, which reacts with the peptone of the medium to give a red colour. It was noted that *Aerobacter aerogenes* gave a positive reaction, but that *Escherichia coli* produced no colouration, and it later became clear that there was negative correlation between Methyl-red (M.R) and Voges–Proskauer (V.P) tests for lactose fermenting Coliform microorganisms.

Directions

- 15.00 grams of the powder was added to 1 litre of distilled water.
- Mixed well and distributed into final containers and sterilized by autoclaving at 121^0 C for 15 minutes.

9.12.1.4 MacConkey Agar (IVD) (Oxoid) CM 007

Manufacturer

Oxoid Ltd, Basingstoke, Hampshire, England

Composition (Typical formula/Ingredients) in grams per litre

Peptone – 20.00, Lactose – 10.00, Bile salts – 5.00, Sodium chloride – 5.00, Neutral red – 0.075, Agar – 12.00: Final PH (at 25^0 C) 7.4 ± 0.2

Uses (Description)

This medium is used for cultivation of coliforms; isolation and differentiation of lactose fermenting and non-lactose fermenting enteric bacilli. It is a differential medium for the detection, isolation and enumeration of coliforms and intestinal pathogens in water, dairy products and biological specimens.

Directions

- 52.00 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- Mixed and sterilized by autoclaving at 121^0 C for 15 minutes.
- The surface of the gel was dried before inoculation.

9.12.1.5 MacConkey Sorbitol Agar (DIFCO)

Manufacturer

Bectone Dickinson, Microbiology systems, Bectone Dickinson and company, Sparks MD 21152 USA

Composition (Typical formula/Ingredients) in grams per litre

Peptone – 15.50, Proteose peptone – 3.00, D-Sorbitol – 10.00, Bile salts – 1.50, Sodium chloride – 5.00, Agar – 15.00, Neutral red – 0.03, Crystal violet – 0.001: Final PH (at 25^0 C) 7.1 ± 0.2.

Uses (Description)

This is a base for isolating and differentiating Entero-pathogenic *Escherichia coli* serotypes (isolation and differentiation of *Escherichia coli* O157:H7 in stool specimen)

Directions

- 50.00 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely with frequent agitation.
- Mixed and sterilized by autoclaving at 121^0 C for 15 minutes.

9.12.1.6 Mannitol Salt Agar (MSA) (USP – Liofilchem) Ref: 610029)

Manufacturer

Liofilchem S.r.L via Scozia, 64026, Roseto, d A (TE) - Italy

Composition (Typical formula/Ingredients) in grams per litre

Beef extract – 1.00, Pepto-special – 10.00, Sodium chloride – 75.00, Mannitol – 10.00, Phenol Red – 0.025, Agar – 15.00: Final PH (at 25^0 C) 7.4 ± 0.2

Uses (Description)

This medium is recommended for the detection and enumeration of coagulase positive Staphylococci in milk, in foods and other specimens (Selective isolation of Staphylococci).

Directions

- 111.00 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- Mixed and sterilized by autoclaving at 121^0 C for 15 minutes.

9.12.1.7 Nutrient Agar (Fluka Biochemika) 70148

Manufacturer

Fluka Chemie Gmbh CH – 9471 Buchs, Tel. 081/7552511, Sigma – Aldrich Chemie Gmbh, Riedstr 2, D – 89555 Steinheim, 07329/970, Product of Spain.

Composition (Typical formula/Ingredients) in grams per litre

Meat extract – 1.00, Yeast extract – 2.00, Peptone – 5.00, Sodium chloride – 5.00, Agar – 15.00: Final PH (at 37^0 C) 7.4 ± 0.2

Uses (Description)

This is a general culture medium for less fastidious microorganisms as well as for permanent cultures. Blood serum or other biological fluids can be added if required.

Directions

- 28.00 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- Mixed and sterilized by autoclaving at 121^0 C for 15 minutes.

9.12.1.8 Plate Count Agar (PCA) (Standard method Agar) (Himedia®)

Ref: M 091

Manufacturer

Himedia laboratories, Prt Ltd, 23, Vadhani, Ind, Est, LBS, Marg. Mumbai – 400086, India

Composition (Typical formula/Ingredients) in grams per litre

Casein enzymic hydrolysate – 5.00, Yeast extract – 2.50, Dextrose – 1.00, Agar – 15.00: Final PH (at 25^0 C) 7.0 ± 0.2

Uses (Description)

This medium is used for determination of Plate counts of microorganisms in foods, water and waste water (used for determination of Total Viable Bacterial Count – TVBC).

Directions

- 23.50 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- Mixed and sterilized by autoclaving at 15 lbs pressure (121^0 C) for 15 minutes.

9.12.1.9 Simmon's Citrate Agar (Oxoid) CM 155

Manufacturer

Oxoid Ltd, Basingstoke, Hampshire, England

Composition (Typical formula/Ingredients) in grams per litre

Magnesium sulphate – 0.20, Ammonium dihydrogen sulphate – 0.20, Sodium Ammonium sulphate – 0.80, Sodium citrate Tribasic – 2.00, Sodium Chloride 5.00, Bromothymol blue 0.08, Agar No. 3 (Oxoid L 13) – 15.00: Final PH (at 25^0 C) – 7.0 approximately

Uses (Description)

Simmon Citrate Agar is recommended for differentiation of the family *Enterobacteriaceae* based on whether or not citrate is utilized as the sole source of carbon.

Technique

The medium may be used either as slopes in test tubes or as plate medium in Petri dishes. In both cases, the surface of the medium is lightly inoculated by streaking and where slopes are

used, the butt of the medium is inoculated by stabbing. Incubation for 48 hours at 37 ^{0} C is recommended.

Positive growth (ie; citrate utilization) produces alkaline reaction and changes the colour of the medium from green to bright blue, whilst in a negative test (ie; no citrate utilization) the colour of the medium remains unchanged.

Directions

- 23.00 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- Mixed and sterilized by autoclaving at 15 lbs pressure (121^{0} C) for 15 minutes.

9.12.1.10 Soyabean Casein Digest Agar (Tryptone Soya Agar) (Himedia®)

Ref: M 290

Manufacturer

Himedia laboratories, Prt Ltd, 23, Vadhani, Ind, Est, LBS, Marg. Mumbai – 400086, India

Composition (Typical formula/Ingredients) in grams per litre

Pancreatic digest of casein – 15.00, Papain digest of Soyabeans meal – 5.00, Sodium Chloride – 5.00, Agar – 15.00: Final PH (at 25^{0} C) 7.3 ± 0.2

Uses (Description)

This is a general purpose medium used for cultivation of a wide variety of microorganisms as per various pharmacopoeia. It is suitable for the cultivation of both aerobes and anaerobes, the later being grown either in deep cultures or by incubation under anaerobic condition. The

medium may also be used as a blood Agar base (for this purpose 7 % sterile Sheep blood should be added to the sterile molten medium which has been cooled to approximately 45^0 C). Bovine or Equine serum (5 %) can be added for the cultivation of *Brucella.*

Directions

- 40.00 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- Mixed and sterilized by autoclaving at 15 lbs pressure (121^0 C) for 15 minutes.
- Aseptically added 5 % v/v Bovine serum to the medium previously cooled to 45^0 - 50^0 C.

9.12.1.11 Sugar Broth (Dextrose Peptone Broth) (Oxoid) CM 11

Manufacturer

Oxoid Ltd, Basingstoke, Hampshire, England

Composition (Typical formula/Ingredients) in grams per litre

Peptone – 20.00, Dextrose – 10.00, Sodium Chloride – 5.00: Final PH (at 25^0 C) – 7.2 approximately

Uses (Description)

This is a simple broth with a carbohydrate source, for use in a routine sterility testing. The medium may also be used as a growth medium or as the basis of carbohydrate fermentation media.

This medium is also available with added Phenol red indicator. The Phenol red medium is orange when neutral, yellow when acid and deep red when alkaline. An inverted Durham's tube is included in the medium for the indication of gas production

Directions

- 35.00 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- The medium was distributed into final containers (Universal bottles) with the Durham' tubes included.
- Mixed and sterilized by autoclaving at 15 lbs pressure (121^0 C) for 15 minutes.

9.12.1.12 Triple Sugar Iron (TSI) (Oxoid) CM 277

Manufacturer

Oxoid Ltd, Basingstoke, Hampshire, England

Composition (Typical formula/Ingredients) in grams per litre

Lab-lemco powder (Oxoid L 29) – 3.00, Yeast extract (Oxoid L 20) – 3.00, Peptone (Oxoid L 37) – 20.00, Sodium chloride – 5.00, Lactose – 10.00, Sucrose – 10.00, Dextrose – 1.00, Ferric citrate – 0.30, Sodium thiosulphate – 0.30, Phenol red – q.s, Agar No. 3 (Oxoid L 13) – 12.00: Final PH (at 25^0 C) – 7.4 approximately

Uses (Description)

This is a composite medium for the differentiation of Enterobacteriaceae, according to their ability to ferment lactose, sucrose and dextrose and to produce hydrogen sulphide (H_2S)

Directions

- 65.00 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- The medium was mixed well and distributed into final containers (Capped test tubes).

- Mixed and sterilized by autoclaving at 15 lbs pressure (121^0 C) for 15 minutes.
- The medium was allowed to set in a slope form with a butt about 1 inch (2.5 cm) long.

9.12.1.13 Tryptone Water (Oxoid) CM 87

Manufacturer

Oxoid Ltd, Basingstoke, Hampshire, England

Composition (Typical formula/Ingredients) in grams per litre

Tryptone (Oxoid L 42) – 10.00, Sodium Chloride – 5.00: Final PH (at 25^0 C) – 7.5 approximately.

Uses (Description)

Tryptone water has been specially evolved as a substrate for the production of Indole. Due to its high content of tryptophan, it is more reliable than Peptone water for this purpose. The ability of certain microorganisms to break down amino acid tryptophan with formation of indole is an important property which is used for the classification and identification of bacteria. Detection of indole can either use Kovac's reagent or Ehrlich's reagent.

Directions

- 15.00 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- The medium was mixed well and distributed into final containers (Universal bottles).
- Mixed and sterilized by autoclaving at 15 lbs pressure (121^0 C) for 15 minutes.

9.12.1.14 Violet Red Bile Agar (VRBA) (Himedia®) Ref: M 049

Manufacturer

Himedia laboratories, Prt Ltd, 23, Vadhani, Ind, Est, LBS, Marg. Mumbai – 400086, India

Composition (Typical formula/Ingredients) in grams per litre

Peptic digest of animal tissue – 7.00, Yeast extract – 3.00, Lactose – 10.00, Bile salts mixture – 1.50, Sodium Chloride – 5.00, Neutral red – 0.03, Crystal violet – 0.002, Agar 15.00: Final PH (at 25^0 C) 7.4 ± 0.2

Uses (Description)

This medium is used for selective isolation, detection and enumeration of Coli-aerogenes bacteria in water, milk and other dairy products/foods.

Directions

- 41.53 grams the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely.
- Mixed and sterilized by autoclaving at 15 lbs pressure (121^0 C) for 15 minutes.
- The medium was cooled to 45^0 C and immediately poured into sterile Petri plate containing the inoculum.

9.12.2 Reagents

12:2:1: Ehrlich Reagent (Ehrlich Rosindole Reagent)

Composition (Typical formula/Ingredients)

Paradimethylamidobenzaldehyde – 4 grams, Absolute alcohol – 380 ml, Concentrated Hydrochloric acid – 80ml.

Directions

- The aldehyde was dissolved in the alcohol and concentrated hydrochloric acid.

9.12.2.1 Kovac's (1928) Reagent for Indole

Composition (Typical formula/Ingredients)

P-dimethylamidobenzaldehyde – 5.00 grams, Amyl alcohol – 75 ml, Concentrated hydrochloric acid (HCL) – 25 ml.

Directions

- The aldehyde was dissolved in the alcohol by gently warming in a water bath (about 50^0–55^0 C)
- The mixture was then cooled and acid added.

9.12.2.2 Oxidase Test Reagent C1: 14

Composition (Typical formula/Ingredients)

1 % Tetramethyl – p – phenylenediamine dihydrochloride aqueous solution

Precaution

The reagent should be colourless and be stored in a glass stoppered bottle, protected from light at 4^0 C. The solution should be used if it becomes deep blue. The auto-oxidation of the reagent may be retained by the addition of 1 % Ascorbic acid.

9.12.2.3 Resazurin Solution (Tablet)

Manufacturer

Made in EC – EMB 45053, Ref: 330884Y, Batch: 36639.

WWR International Ltd, Lutherworth, UK, LF 17, 4 XN, Tel: 01455558600

Composition (Typical formula/Ingredients)

Dye content/tablet, approximately 11 mg, certified by Biological stain commission

Directions

Resazurin solution was prepared by dissolving one Resazurin tablet (Dye content/tablet, approximately 11 mg, certified by Biological stain commission) in 200 ml of hot distilled water.

9.12.3 Test Kits Components

12:3:1: California Mastitis Test (CMT) Kit (Cat No. 170366)

Manufacturer

California Mastitis Test (CMT) Kit made in Denmark by Vkruuse (Technivet, 4 Industry Road, Box 189, Bruswick, ME, 04011)

Description

California Mastitis Test (CMT) Kit (Original Schalm)

9.12.3.1 Escherichia coli Antisera O157

Manufacturer

CE IVD: Denka Seiken Co. Ltd; 3-4-2 Nihorbashikayaba-cho, chuo-ku, Tokyo, Japan: Ref: 2957798 (Lot: 01: 3121, 2005.12)

Description

Escherichia coli Antisera O157 (2 ml vial)

9.12.3.2 Eurocell – A (*Brucella abortus* Antigen)

Manufacturer

Euromedi Equip Ltd, 48 Weibeck Road, West Harrow, Middx, HA 2 ORW, UK (Cat No.: 561005 E, Lot: 56146: GO/DRUGS/356). Preservative: 0.01 % Thimerosal.

Description

Invitro diagnostic reagent for detection of antibodies to *Brucella abortus* and *Brucella melitensis*

9.12.3.3 Rose Bengal Plate Test diagnostic Kit

Manufacturer

Inaktivert, Flussig, Vor Gebrauch Schuttein, Verw, bls, 31: 05: 4000, In Vit, o Diagnost Skikum Zul – Nrb. BGAF – 146. Belgium.

Description

Rose Bengal stained Brucella abortus antigen,

9.12.3.4 Rose Bengal stained *Brucella* Antigens

Manufacturer

Central Veterinary Laboratory – CVL- Weybridge, New Haw, Addtestone, Surrey KT 15 3NB, UK (Product code: PA 0060, Batch: 265).

Description

Rose Bengal stained standardized *Brucella abortus* antigens (100 ml bottle)

9.12.3.5 Universal Indicator PH Paper (PH 1 – 14) (Macherey – Nagel)

Manufacturer

Made in Germany (Macherey – Nagel, Gmbh & Co. P. O. Box 101352 D – 52313, Duren, Germany)

Description

Universal Indicator PH Paper (PH 1 – 14)/ roll (5 metres in length)

Extra information

The camel (*Camelus dromedarius* which was our study animal) is an important livestock species uniquely adapted to hot and arid environment of the ASAL of Kenya. There are 830,000 dromedary type camels in Kenya which form approximately 6% of camel population in Africa (MOLFD - 2004, FAO - 2005). Camel keeping is the main livelihood strategy in Garrissa and Wajir counties. This is because of the ability of the camel to utilize range in marginal areas and survive and produce under harsh environmental conditions. They are a main source of food providing meat and milk (Schwartz and Dioli - 1992, Guliye - 2006). They are also kept as a source of income (Getahun and Bruckner - 2000), provision of transport and for social cultural reasons such as settlement of bride price and compensation for wrong doing.

9.13 Colonial morphology and biochemical reactions for various bacteria

9.13.1 Differentiation between *E. coli, Klebsiella* and *Enterobacter*

All of them are Gram-negative rods

	Growth on MacConkey agar	**Growth on EMB medium**	**IMViC reaction**	**Oxidase**
E. coli	Lactose fermenting, medium-sized colonies (1-3 mm diameter)	Greenish metallic sheen	++--	-
Klebsiella	Lactose-fermenting large mucoid colonies	No metallic sheen - mucoid	--++	-
Enterobacter	Capsulated strains appear like *Klebsiella* Non-capsulated strains appear like *E. coli*	No metallic sheen – mucoid or non-mucoid	--++	-

13a (continued)

	Urease	**Motility**	**Glucose fermentation**	**H_2S production**
E. coli	-	+	Acid and gas	-
Klebsiella	+ (slow)	-	Acid only	-
Enterobacter	-	+	Acid and gas	-

9.13.2 Differentiation between *Staphylococcus* and Streptococcus

	Growth on BA	**Growth on MSA**	**Growth on SAC VBA**
Staphylococcus	Medium-sized colonies showing various degrees of hemolysis	+ Some showing yellow colonies	=
Streptococcus	Pin-point colonies showingvarious degrees of hemolysis	-	+

	Microscopic morphology	**Catalase reaction**	**Nitrate reaction**
Staphylococcus	Normally arranged in clusters, in twos and singly	+	+
Streptococcus	Characteristically forms long chains.. Also seen in clusters, twos and singly	-	-

13c: *Brucella* organisms – they are Gram-negative cocco-bacilli

Generally, they: don't grow on MacConkey medium

: are oxidase positive

: are urease positive

: are non-motile

: produce acid only from sugars

Differentiation between common Brucella species

	Brucella abortus	***Brucella melitensis***	***Brucella suis***
CO_2 requirement	+	-	-
H_2S production	+ (early)	-	V
Growth in:			
Thionine (1:50,000)	-	+	+
Basic fuchsin (1:50,000)	+	+	-
Safranin (1:5,000)	V	+	-

Key for the 3 sub-tables

+	Positive reaction
=	Negative reaction
EMB	Eosin Methylene Blue mediun
IMViC	Indole, Methyl red, Voges Proskauer and Citrate reactions
H_2S	Hydrogen sulphide production
BA	Blood agar
MSA	Mannitol salt agar
SACVBA	Sodium azide crystal violet blood agar
CO_2	Carbon dioxide