PREVALENCE OF STREPTOCOCCUS AGALACTIAE AND

STAPHYLOCOCCUS AUREUS IN CAMEL (Camelus dromedarius)

MILK IN GARISSA AND WAJIR DISTRICTS OF KENYA, THEIR

SENSITIVITY TO ANTIBIOTICS AND ACCEPTABILITY OF

CAMEL MILK AND ITS PRODUCTS

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

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Declaration

I hereby declare that this thesis represents my own work a	
submitted to any university or any other Institution for aw	ard of degree, diploma or any other
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Dedication

I dedicate this thesis to my son Simeon who missed me when busy working on the thesis.

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List of Abbreviations

ASALS Arid and Semi-Arid Lands

CMT California Mastitic Test

FAO Food Agriculture Organization

GBS Group B Streptococus

JETACAR Joint Expert Technical Advisory Committee on Antibiotic

Resistance

MoLFD Ministry of Livestock Fisheries and Development

MRSA Methicillin-resistant Staphylococcus aureus

UHT Ultra Heat Treated

VCML Vital Camel Milk Limited

VISA Vancomycin – Intermediate staphlococcus aureus

VRSA Vancomycin-resistant Staphylococcus aureus

Abstract

Camel milk is commonly consumed raw by pastoralists in arid areas who may be unaware of the risks posed by such milk. It was therefore very important to determine the prevalence of *Staphylococcus aureus* and *Streptococcus agalactiae*, which are some of the most common pathogens in such milk.

Camel milk samples from Garissa and Wajir were analyzed to determine the prevalence of *Staphylococcus aureus* and *Streptococcus agalactiae*. The antibiotic resistance of the bacteria was also studied. Milk samples (n =207) were aseptically obtained from primary marketing agents. Samples were analyzed for the presence of *Staphylococcus aureus* and *Streptococcus agalactiae*. The confirmed *Staphylococcus aureus* and *Streptococcus agalactiae* were subjected to diffusion sensitivity test. Resistance was determined by measuring the diameter of the zone cleared by the antibacterial and the isolates were reported as susceptible, intermediate, or resistant. Questionnaires were administered to evaluate camel milk and milk product acceptability.

The prevalence of *Staphylococcus aureus* and *Streptococcus agalactiae* in the two districts differed with Garissa having higher percent incidence both for *Staphylococcus aureus* (34.95%) and *Streptococcus agalactiae* (37.79%). Wajir, had lower prevalence of *Staphylococcus aureus* (10.58%) and *Streptococcus agalactiae* (7.69%). *Staphylococcus aureus* and *Streptococcus agalactiae* were resistant to most of the antibiotics except Gentamicin.

Although camel milk and milk products were acceptable, each had different quality parameters that attracted customers. The most important purchasing criterion for raw camel milk was taste (27%, 19% and 18%) for Wajir, Garissa and Nairobi respectively. While packaging (18%, 18% and 16%) was more important for pasteurized milk also in the same order. For yoghurt the most important purchasing criteria were taste (18%) and aroma (19%). The taste of sour camel milk is the most important attribute in both Garissa (30%) and Nairobi (24%).

The results indicate the potential health risk of consuming raw camel milk and increasing incidences of resistance of mastitis organisms to the common antibiotics. There is need to educate camel milk producers on hygienic milk production as well as inform the raw camel milk consumers on dangers involved. Marketing of camel milk and products can be enhanced using the attributes appropriate for each product in the respective district.

CHAPTER ONE

INTRODUCTION

Camel meat and milk products are the key foods in arid and semi arid areas (ASALS) of Africa and Asian countries. The Food Agriculture Organization (FAO) has reported that 18 million camels around the world support the survival of millions of people in ASALS (FAO, 2003). The camel is special because it is able to survive the severe drought conditions in the deserts and semi-deserts due to its low water turnover when compared with other ruminants (Macfarlane, 1964; Macfarlane and Howard, 1992; Schmidt-Nielsen, 1956; Yagil, et al, 1974). Camel milk not only contains higher amount of nutrients compared to cow milk but it has also medicinal properties (Barbour et al., 1985). An insulin-like protein has been detected in camel milk (Beg et al., 1986). Clinical trials in human diabetes type 1 have shown that camel milk reduces the need for insulin medication by an average of 30% (Agrawal et al., 2005). In addition physiological studies carried out in Israel (Zagorski et al, 1998) demonstrated the ant diabetic properties of camel milk. FAO speaks highly of the health benefits of camel milk with vitamin C, being three times more than the cow's milk, iron content ten times and B vitamins present in large amounts. The health promoting properties of camel milk are a strong boost for sales and driver for intensification of camel dairying. The camel milk is popular among pastoralists and has been steadily gaining popularity among urban dwellers in many countries. With ground water levels dropping rapidly, United Nations (UN) spell the end of water-intensive

agriculture. In this scenario, camel husbandry represents a perfect solution to the chronic water shortage of many countries since it can go for many days without water. The milk is going to be abundant and easily accessible to many although there is no substantial data on its acceptability.

Under pastoral conditions the camel milk is mostly consumed raw without any heat treatment and this can pose a health hazard to consumers. Milk and milk products derived from any animal can harbor a variety of microorganisms and can be important source of food borne pathogens. The bacterial content of freshly drawn milk is significantly increased by infectious mastitis.

Mastitis is defined as inflammation of the udder indicated by a rise in milk somatic cell count (SCC) (International Dairy Federation, 1979).

It is a multifactorial disease that results when management and environmental factors interact to increase exposure reduce udder resistance and aid deposition of organisms into the teat canal (Philpot, 1984). The teat duct especially the region adjacent to the orifice, can be colonized by mastitis organisms, which persist for many weeks, shedding into the outgoing milk, but not penetrating to the teat sinus.

The most common isolates from camel mastitis are *Strept. agalactiae* and *Staph. aureus*. However other isolates such as *Strept. ubevis*, *Strept. dysgalactiae*, *Strept. pyogene,s Diplococcus pneumoniae*, *E. coli, Bacillus cereus*, *Corynebacterium bovis Pseudomonas aeruginosa*, *Pasteurella spp.*, *Pasteurella haemolytica* (chronic suppurative mastitis), *Klebsiella spp.*, *Corynebacterium pseudotuberculosis*, *Corynebacterium equi* and

corynebacterium pyogenes, Candida albicans have also been reported (Almaw and Molla, 2000; Bekele and Molla, 2001 and Barbour et al., 1985).

Staph. aureus is the cause of serious infections in humans, including endocarditis, deep-seated abcesses, and osteomyelitis (Brakstad et al., (1992). Brooks et al., (2001) and Gillespie et al., (2000) associate Strept. agalactiae, with Group B streptococcal infection which occur as early onset disease of the neonate on day 1-7 after birth or late onset disease on day 7-90. Prenatal infection causes septicemia, pneumonia or meningitis, which are associated with a high mortality. During delivery, a baby can acquire Strept. agalactiae and develop neonatal sepsis, neonatal meningitis, and/or neonatal pneumonia. Invasive Group B Strept. disease of the adult is seen in pregnant women and immunocompromisied individuals such as diabetics or in elderly (Schuchat, 1999).

Mastitis eradication programs have been successful in dairy cattle herds and are justified economically (Radostits *et al.*, 1997; Edmondson, 1989).

Strept. agalactiae and Staph. aureus have been isolated from intramammary infections (IMIs) in different camel populations (Karamy, 1990; Abdurahman et al., 1995; Obied and Bagadi, 1996). However few studies appear to have been done on the prevalence of Strept. agalactiae and Staph. aureus in camel herds in Kenya. There is limited information on the rate that bacteria are developing resistance to antibiotics commonly used to treat mastitis in camel population.

1.1 Justification of the Study

Camel milk is an important source of food in drought areas as other ruminants can not survive severe drought conditions (Seaman and Rivers, 1978; Sweet, 1965). In rain fed agriculture the camel is a better producer of milk than any other domestic animal (Knoess, 1979).

The problem of mastitis has been reported from almost all camel rearing countries (Abdurahman and Younan, 2004). The prevalence of mastitis causing organisms in camel milk is a concern of Public Health. Early problem recognition and improved hygiene can reduce the milk loss due to mastitis resulting in high economic gain (Abdurahman, 2006). Better herd management of mastitis can also increase milk production and thus income for the pastoralists (Abdurahman and Younan, 2004).

Staph. aureus is the most-frequent cause of intramammary infections (IMIs) in dairy cows, but Strept. agalactiae can cause greater production losses (Radostits et al.,1997). Strept. agalactiae is a highly infectious contagious obligate parasite of the bovine mammary gland and remains a significant cause of chronic mastitis in many herds, even though it can be readily eliminated (Jain, 1979). An overview of the annual reports of food-borne diseases from seven countries indicated that milk and milk products were implicated in 1-5% of the total bacteria outbreaks and Staph. aureus was by far the most frequent pathogen associated with the outbreaks (85.5%). On the other hand, Strept. agalactiae can spread widely within a herd, causing immediate loss due to reduced milk yield, and eventual large losses, when it is

finally recognized. The greatest economic loss of mastitis in both sub clinical and clinical mastitis is reduced milk production (Wood and Booth, 1983). Mastitis may also decrease the length of lactation.

Camel milk although highly valued in terms of nutritional quality, is easily contaminated by its handlers and along the marketing chain with pathogenic organisms making the milk unsafe to the public. Incidences of pathogenic organisms in camel milk have been reported in Kenya (Younan *et al.*, 2000).

Veterinary services are rare in camel-herding areas and there is lack of professional personnel to detect mastitis in camels. According to Anderson (1989), Staphylococcus spp. and Streptococcus spp cause more than 80% of clinical mastitis. Antibiotics are the basis of most treatment regimes. Treatment of subclinical mastitis with antimicrobials has been suggested by several authors. However overuse of these drugs raises serious public health concerns of the development of antibiotic resistance (Younan, 2002; Woubit et al., 2001; and Al-Ani and Al-Shareefi, 1994). If a full course of antibiotic treatment is not used in full as specified by product manufacturer, there is a likelihood of an infection recurring and development of antibiotic resistant strains of bacteria. Determination of Strept. agalactiae and Staph. aureus organisms in camel milk in Kenya and their antibiotic resistance will help to advise the pastoralists on management of mastitis in order to curb the incidences of the disease.

For these reasons it was important to identify the prevalence of *Strept*. agalactiae and *Staph. aureas* in camel milk in order to safeguard public health.

Camels supply up to 70% of the milk consumed by pastoralist communities in northern Kenya (Schwartz, 1992). The milk is becoming popular due to its claimed therapeutic property. This is attributed to the fact that camels browse on various plant species (Muli et al., 2008). This feed material can also have an effect on the flavor and general composition of camel milk, which can make it acceptable or unacceptable. Information about camel milk and milk products acceptability will be of value to the producers and marketers.

1.2 Main Objective

To determine the prevalence of the main mastitis pathogens, the antibiotic resistance and acceptability of camel milk in Garissa and Wajir districts of Kenya.

1.2.1 Specific Objectives

- 1. To determine the prevalence of *Streptococcus agalactiae* and *Staphylococcus aureus* in camel milk.
- 2. To determine the antibiotic resistance of *Streptococcus agalactiae* and *Staphylococcus aureus* isolated from camel milk.
- 3. To determine the acceptability of camel milk and milk products in the market.

CHAPTER TWO

LITERATURE REVIEW

2.1 Milk Borne Diseases

According to Jones *et al.*, (1998), the presence of food borne pathogens in camel milk is due to direct contact with contaminated sources in the camel farm environment and to excretion from the udder of an infected camel. Bovine mastitis is considered the major cause of economic loss to the dairy industry. Pathogenic microorganisms that most frequently cause mastitis can be divided into two groups based on their source: environmental pathogens and contagious pathogens. The major contagious pathogens are *Strept. agalactiae*, *Staph. aureus* and *Mycoplasma* spp.

Clinical mastitis is relatively uncommon in camelids when compared with cattle. However, the incidence of mastitis may increase in dairy camels due to hand milking and teat malformation (Almaw and Molla, 2000). *Staph. aureus* has been ranked as the most frequent (Karamy, 1990; Al-Ani and Al-Shareefi, 1994) or second most-frequent (Barbour *et al.*, 1985; Obied and Bagadi, 1996) micro-organism involved in IMI in camels. With the exception of some mycoplasmal infections that may originate in other body sites and spread systemically; these organisms gain entrance into the mammary gland through the teat canal. *Strept. agalactiae* is highly infectious and causes mainly subclinical infections, which are not identified by the herdsman (National Mastitis Council, 1998). As a result, *Strept. agalactiae* can spread widely

within a herd, causing immediate loss due to reduced milk yield, and eventual large losses, when it is finally recognized.

The camel industry should be concerned about the microbial quality of bulk tank milk because of two main reasons: (1) outbreak of disease in humans have been traced to the consumption of raw milk, (2) raw milk is consumed directly by camel milk producers, farm employees, and their families, neighbors, and raw milk advocates, Furthermore, some pathogens can contaminate the camel milk along marketing chain.

These pathways pose a risk to the consumer from direct exposure to food borne pathogens present in raw camel milk and environment (Oliver *et al.*, 2005).

2.2 Streptococcus agalactiae and Staphylococcus aureus.

Intramammary infections (IMIs) or 'mastitis' caused by *Streptococcus* agalactiae (group B streptococcus (GBS)), *Staphylococcus aureus* and *Escherichia coli* are common in East African camels (Abduraham et al., 1995; Abduraham, 1996; Obied and Bagedi, 1996; Younan et al., 2002). *Staphylococcus aureus* is the most frequent or second most frequent cause of IMIs in dairy cows (Karamy, 1990; Al-Ani and Al-Shareefi, 1994; Barbour et al., 1985; Obied and Bagedi, 1996) but *Strept. agalactiae* causes the greatest milk production losses in dairy cattle (Radostits et al., 1997). *Strept. agalactiae* causes up to 30% decrease in milk yield in cattle on an individual animal basis and a 15% decrease on herd basis. *Strept. agalactiae* eradication programmes have been successful in dairy cattle herds and are justified

economically (Radostits *et al.*,1997; Edmondson, 1989) and therefore there is no reason to assume that the effect of *Strept. agalactiae* on milk production in camels differs substantially from the situation in dairy cattle.

Control of mastitis has been estimated to result in a 9% increase in milk yield in Somali camel breeds in Kenya (Abdurrahman and Younan, 2004). *Strept. agalactiae* and *Staph. aureus* are very common in raw camel milk from mastitis camels and presents a potential health risk to raw camel milk consumers, a common practice in most camel-keeping communities (Younan *et al.*, 2002).

The phenotypic properties of Strept. agalactiae and Staph. aureus isolated from camel milk indicate that these bacteria species may be responsible for a significant problem of zoonotic disease in humans in areas where raw milk is important source of nutrition. Strept. agalactiae isolated from camels seem to be more closely related to the human type than the bovine biotype and may survive for up to seven days in souring camel milk showing no significant decline in viable numbers down to pH of 4.5 (Abdurrahman and Younan, 2004). Younan et al., (2001) reported IMIs prevalence of 12% of Strept. agalactiae and 11% of Staph. aureus in six Kenyan camel herds. In two separate studies on Sudanese camels, Strept. agalactiae was isolated from 26.7% of composite vendor milk samples (Obied and Bagedi, 1996) and from 17.6% of quarter milk samples (Abduraham et al., 1995); while Staph. aureus was isolated from 17% udder milk samples and 5.4% quarter milk samples. Pooling of different camel milk batches along the collection and marketing chain can result in increased prevalence of Strept. agalactiae. Abduraham and

Younan, (2004) found *Strept. agalactiae* in 50% of milk transport containers coming from producing herds, 62% of milk containers sampled at primary collection sites, 70% of milk containers sampled from an urban market of the same region and 89% of raw milk batches received at a dairy processing plant. These findings indicate a very wide spread occurrence of the pathogens in milk producing camel herds and in the milk collection and distribution systems. Clinical mastitis is self-evident and can be detected without special tests. There are changes in the secreted milk (colour, consistency, floccules etc) and/or the udder (red, swollen) and other generalized signs exhibited by the animal (fever, anorexia, deteriorating body conditions).

However sub-clinical mastitis is difficult to diagnose. It is characterized by less milk production but the animal does not have a swollen udder or 'abnormal' milk. The infection can only be detected with help of indirect methods. These include California Mastitis Test (CMT); Whiteside Test; Milk Electrical Conductivity Test (Milner et al., 1996) and Direct Microscopic Somatic Cell Count (DMSCC). The CMT is a simple and rapid test that can be applied in the field. It is useful for sub-clinical udder infections caused by either *Strept. agalactiae* or *Staph. aureus*. The DMSCC requires only simple laboratory equipments and produces results on the same day. The sensitivity and specificity of the CMT has been reported to be 95.4% and 30.4% respectively (Abduraham et al., 1995), while Younan et al., (2001) reported a sensitivity and specificity of 60% and 95% respectively which shows a very wide discrepancy.

The CMT scores for Staph. aureus infected quarters have been reported to be significantly higher than CMT scores for quarters infected by environmental mastitis pathogens (Abduraham, 1996). On exclusion of environmental mastitis pathogens, the overall CMT sensitivity of 60% increased to 77% and 68% quarters infected with Strept. agalactiae and Staph. aureus respectively (Younan et al., 2001). The difference in CMT sensitivity between IMIs caused by Strept. agalactiae and Staph. aureus may result from a less intensive inflammatory response to Staph. aureus as compared to Strept. agalactiae (Rodostits et al., 1997). Sub-clinical mastitis causes an increase in the Total Viable Bacteria Count (TVBC) in milk. Aseptically sampled milk from noninfected bovine udders contains 10^2 to 10^3 cfu/ml but the TBC increases to 10^5 cfu/ml for samples from cattle with sub-clinical mastitis. Milk from subclinically tested camel udders (with confirmed Strept. agalactiae infections) ranged in TVBC from 1.0 to 6.5×10³ cfu/ml (Abdurrahman and Younan, 2004).

In a longitudinal study, Abdurrahman and Younan, (2004) reported that, of the 207 lactating camels tested; only 3.4% were affected by clinical mastitis while 21.3% were affected by sub clinical mastitis. The practice of tying off two teats during the daytime (to prevent the calf from suckling) has been cited as a contributing factor to the development of IMIs in camels (Abdurahman *et al.*, 1995; Obied and Bagedi, 1996; Younan *et al.*, 2001) and ultimately leads to loss of intact teats by destruction of the gland tissue (Radostits *et al.*, 1997). Chronic mastitis finally leads to loss of intact quarters by destruction of the gland tissue. Loss of quarters has been reported from one third of Gabra and

Somali breeds of camels in Northern Kenya with between 10% and 50% of female camels having less than four intact quarters (Abdurahman and Younan, 2004). Mastitis can be prevented or controlled by improving animal health and udder hygienic measures.

Currently there is almost complete absence of mastitis control measures practiced by camel-keepers. There is also little evidence of effective ethnoveterinary interventions in managing mastitis.

It is cheaper and easier to prevent mastitis by improving hygienic measures and culling chronically infected camels to eliminate important reservoirs of causative organisms than to treat by therapeutic drugs. Great caution is also necessary when applying intramammary treatment to camels.

The teat of the camel udder contains two sometimes three separate teat canals that open independently into the teat sphincter. The separate canals drain separate gland complexes, which imply that for intramammary treatment of mastitis not only that each quarter must be treated but also each gland complex must be treated separately that is, one intramammary tube per gland complex. The teat canal openings are also smaller than those of the cow and thus require smaller connula. The available literature shows limited research information and knowledge regarding prevalence of mastitis among camel herds in Kenya and its impact on milk quantity, quality and safety.

Mastitis caused by *Staph. aureus* is extremely difficult to control by treatment alone. Once established, *Staph. aureus* usually does not respond to antibiotic treatment, and infected camels eventually must be segregated or culled from

the herd. Successful control is gained only through prevention of new infections and camel culling.

Staph. aureus organisms colonize abnormal teat ends or teat lesions. Milkers' hands, wash cloths, teat cup liners, and flies are means by which the infection can be spread from camel to camel. The organisms probably penetrate the teat canal during milking (Jones et al., 1998). Camels infected with Staph. aureus do not necessarily have high Somatic Cell Counts (SCC). In some herds with SCC below 200 000, dairy managers have not been able to eradicate Staph. aureus, even when they practiced standard milking time hygiene techniques (Roberson et al., 1994). It has been reported that during 1978-1980, Jones et al., (1984) collected 26 739 milk samples aseptically from cows in 28 herds and found 10% infected with Staph. aureus.

2.3 Camel Treatment

According to Victorian Dairy Industry Authority, (1999), *Staphylococcus spp.* and *Streptococcus spp.* cause more than 80% of clinical mastitis cases. Antibiotics are the basis of most treatment regimes and are administered by infusion into the affected quarter (intramammary route) or by intravenous, intramuscular or subcutaneous injection (parenteral or systemic routes). Other support therapies such as oral or intravenous fluids and anti-inflammatories may be used in very severe cases. Frequent stripping out and use of oxytoxin to aid milk let down are important adjuncts. Farmers should always be encouraged to remove milk from mastitic quarters, despite the fact that antibiotics have been administered.

Most cases of clinical mastitis are treated without the benefit of bacteriological examination of the milk before treatment is commenced. The treatment selected is based on the severity of the mastitis, the history of the farm (including previous milk culture results and responses to treatment), and the field experience of the farmer and the prescribing veterinarian (Ziv, 1997). Treatments should always be administered according to the directions given on the label and by the prescribing veterinarian. Recommended withholding periods must be observed for milk and meat (Victorian Dairy Industry Authority, 1999).

2.3.1 Antibiotics

Baytril, Naxcel, Ampicillin, and Penicillin are good injectable antibiotics. The use of Amikacin is recommended instead of Gentocin if an amino glycoside is necessary. Albon is a good oral antibiotic. It is dissolved in water and mixed with grain but should not be given to a camel that is dehydrated or ill (used for injuries, etc.), and should probably not be given for more than five days and not in conjunction with any other drugs that are processed through the kidneys (Manefied and Tinson, 2005).

Withholding periods (WHP) for antibiotics must be observed. These refer to the minimum period that must elapse after the last administration of a drug before an animal or its products are sold for human consumption (Rogers *et al.*, 1992). According to Nicholls *et al.*, 1994, pharmaceutical companies provide recommended WHP for their products. Antibiotic residues in milk or meat will not exceed the relevant residue limit if treatments are used according

to the label directions and milk and meat are withheld for the specified WHP. Recommended WHP are based on trials that specify the: class of livestock, e.g. lactating camel; dose rate, e.g. milligrams of drug per kilogram live weight of animal; dose interval; duration of treatment course; route of administration, e.g. intramammary infusion or intramuscular injection. Any deviation from these registration specifications may lead to changes in the WHP for a product (Whittem 1997). When giving systemic treatments for mastitis it is important to calculate the correct dose, as WHP for milk and meat change markedly when drugs are used at higher dose rates than specified on the label. Traces of antibiotic in milk may cause allergic reactions in people and inhibit some starter cultures used in fermented milk products. National and international regulations stipulate the maximum levels of antibiotics that may be present in milk and these thresholds are often extremely low (Victoria Dairy Industry Authority, 1999).

2.4 Antibiotic Resistance of the Organisms

2.4.1 Staph. aureus Resistance to Antibiotics

Staph. aureus has become resistant to many commonly used antibiotics. Staphylococcal resistance to penicillin is mediated by penicillinase (a form of β-lactamase) production, an enzyme which breaks down the β-lactam ring of the penicillin molecule. Penicillinase-resistant penicillins such as methicillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin are able to resist degradation by staphylococcal penicillinase.

The mechanism of resistance to methicillin is mediated via the *mec* operon, part of the staphylococcal cassette chromosome mec (SCCmec) (Carter et al., 2000). Resistance is conferred by the mecA gene, which codes for an altered penicillin-binding protein (PBP2a or PBP2') that has a lower affinity for binding β-lactams (penicillins, cephalosporins and carbapenems). This allows for resistance to all β-lactam antibiotics and obviates their clinical use during methicillin-resistant S. aureus (MRSA) infections. As such the glycopeptide, vancomycin, is often deployed against MRSA (Jevons, 1961). Glycopeptide resistance is mediated by acquisition of the vanA gene. The vanA gene originates from the enterococci and codes for an enzyme that produces an alternative peptidoglycan to which vancomycin will not bind. MRSA infections are commonly treated with non-β-lactam antibiotics such as clindamycin (a lincosamine) and co-trimoxazole (Baker, 2006). Resistance to these antibiotics has also led to the use of new, broad-spectrum anti-Gram positive antibiotics such as linezolid. First-line treatment for serious invasive infections due to MRSA is currently glycopeptide antibiotics (vancomycin and teicoplanin). Vancomycin-resistant Staph. aureus (VRSA) is a strain of S. aureus that has become resistant to the glycopeptides (Blot et al., 2002). The first case of vancomycin-intermediate Staph. aureus (VISA) was reported in Japan in 1996 (Hiramatsu et al., 1997) but the first case of Staph. aureus truly resistant to glycopeptide antibiotics was only reported in 2002 (Chang et al., 2003).

One of the few published studies on the change in prevalence of resistance mastitis bacteria was in Finland, where Myllys *et al.*, (1998) reported an increase of 27% in the proportion of *Staph. aureus* strains resistant to at least one antibiotic (mostly due to strains capable of producing beta-lactamase). There is currently limited data that enables comparisons of these findings with what is happening in the camel population.

An expert advisory committee (Joint Expert Technical Committee on Antibiotic Resistance - JETACAR), considering the future management of antibiotic use in food producing animals, recommended that a mechanism for measuring the rate of development of resistance in Australia be established. A surveillance system to measure the incidence and prevalence of antibiotic-resistant bacteria and resistance genes in all areas of antibiotic use (including medical and veterinary applications) may be appropriate (JETACAR 1999).

2.4.2 Strept. agalactiae Resistance to Antibiotics

Tetracycline resistance is common in GBS and usually is due to ribosomal protection encoded by *tet*K or *tet*L (Culebras *et al.*, 2002; Speer *et al.*, 1992). To better understand the emergence and transmission of antibiotic-resistant *Streptococcus agalactiae*, phenotypic and genotypic characteristics of 83 bovine *Strept. agalactiae* isolates were studied by Belgin *et al.*, (2005). Serotypes found among isolates from bovine hosts included III and II (53 and 14.5%, respectively). Twenty one different ribotypes were found among bovine isolates. Resistance to tetracycline and erythromycin in bovine isolates

was reported as 14.5% and 3.6%, respectively. *tet()* was the predominant resistance gene and *ermB* the only erythromycin resistant among isolates from bovine hosts. Emergence of erythromycin and tetracycline resistance appears to largely occur independently among bovine isolates and occasional cross-species transfer of resistant strains or transmission of resistance genes between human- and bovine-associated subtypes may occur. Dissemination of antibiotic-resistant *Strept. agalactiae* appears to include both clonal spread of resistant strains as well as horizontal gene transfer

2.5 Camel Milk Products

While the greatest amount of camel milk in the world is consumed fresh or as a naturally fermented product, camel milk has a wide range of products that include the following: pasteurized milk, Fermented milk generally called *Susca* found in North Eastern Africa — Egypt, Somalia and North Eastern Kenya, Yoghurt, Cheese, Butter, Ice creams, Puddings and Chocolates of different flavors (Farah and Fischer 1990).

2.5.1 Fermented Milk

According to Farah *et al.*, (1990), camel milk in pastoral societies, is mainly consumed in naturally fermented form. In east Africa, however, where 60% of the world camel populations are found, there is a long tradition in preparing the fermented camel milk. The milk is either home-consumed or sold. No starters are used and acidification develops after about three days, either from natural flora of raw milk or inoculums from the containers. The milk is left un-

disturbed, often in a covered container sheltered from dust at ambient temperatures (25-35°C) usually for 24-48 hours until it becomes sour to taste. Due to spontaneous nature of the fermentation, this traditional method results in a product with varying taste and flavor and often of poor hygienic quality.

To improve this spontaneous traditional fermentation, controlled fermentation using mesophilic lactic acid bacteria starter culture have been developed and successfully introduced in camel milk processing plants in different eastern African countries.

2.5.2 Heat treated camel milk products

There are very few studies on the effect of heat treatment on the proteins of camel milk. Kacem (1989), studied on the effect of heat treatment on the protein of camel milk. Research work in their laboratory indicated that the whey proteins in camel milk are more heat resistant than in cow milk. Under the selected experimental conditions the rate of heat denaturation of camel milk whey proteins was approximately twofold lower than cow milk whey proteins. There are commercial small and middle scale camel milk processing plants for production of pasteurized milk in Mauritania, Kenya and Somalia (Farah et al., 1992).

2.5.3 Ultra Heat Treated Camel Milk

An investigation by Farah and Fischer, (1990), to study the ability of camel milk to withstand ultra-high processing temperatures (UHT) showed heat instability of camel milk. Bulk camel milk collected from camels in Kenya was UHT heat treated applying both direct (150 °C/ 2 sec.) and indirect (138

°C/8-10 sec.) method. After processing the milk was stored at 5, 10, 25 and 30 °C for five weeks. After 3 weeks, milk stored at 25 and 30 °C separated forming fine deposit which was more in milk processed by indirect method then the direct. No deposit formation was observed in milk stored at 5 and 10 °C even after 5 weeks storage. This heat instability of camel milk at high processing temperatures can be due to the low content of K-casein and the total absence of a- Lacto globulin in camel milk. Both proteins play an important role in the heat stability of bovine milk.

Their conclusion so far was that camel milk can not be UHT treated following the same procedure as in cow milk.

2.6 Camel Yoghurt Acceptability

Quality and acceptability of set-type yoghurt made from camel milk was done by Hashim *et al.*, (2009). Camel milk set yoghurt was formulated with gelatin, alginate, and calcium. Titratable acidity, pH, sensory properties, and acceptability of camel milk yoghurt were studied. Twelve treatments were prepared; 3 using gelatin at 0.5, 0.75, and 1% levels and 9 with combinations of alginate and calcium at different levels. Titratable acidity and pH of fresh yoghurt were not affected by the addition of gelatin or the alginate and calcium combinations. Trained sensory panel results showed that camel milk yoghurt containing 1% gelatin or 0.75% alginate + 0.075% calcium had the highest intensities for firmness and body. Consumer results indicated that the hedonic ratings of the sensory attributes and acceptability of camel milk yoghurt containing 0.75% alginate + 0.075% calcium were similar to that of

cow's milk yoghurt. The camel milk yoghurt containing alginate + calcium and flavored with 4 different fruit concentrates (15%) had similar hedonic ratings and acceptability. Addition of 0.75% alginate + 0.075% calcium could be used to produce acceptable plain or flavored CM yoghurt.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 The Population of Camels in Kenya

Figures 1(a) and 1(b) comprises a bar graph and pie chart respectively, showing the population of camels in Kenya between 1960 and 2007 and their distribution in districts. This statistics were collected by the Ministry of Livestock Development (MoLD) which indicates the relative camel populations in the country. As per this set of data, the leading district in camel populations is Wajir followed by Mandera and Turkana. Garissa is ranked 4th with a population of about 101000 camels in 2007. It is because of this ranking that little study on camel milk seems to have been done in Garissa.

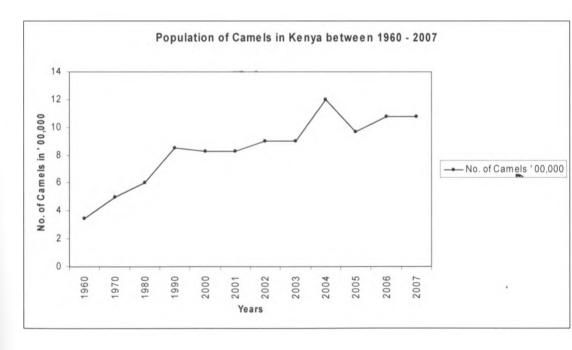


Figure 1(a): Camel Population in Kenya

Source: FAO Statistics, 2008 Source: MoLD, 2007

Camel Population by District 1960 - 2007

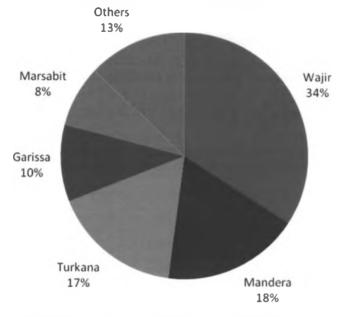


Figure 1(b): Camel Population by District

Source: FAO Statistics, 2008 Source: MoLD, 2007

3.2 Study Area (Garissa and Wajir Districts)

Garissa and Wajir districts are two of the four districts comprising the expansive North Eastern Province where few studies appear to have been done on camel milk (Younan *et al.*, 2001; Schwartz and Schwartz, 1985). They lie in the Arid and Semi-Arid Lands (ASAL) of the country. The rainfall pattern is erratic and unreliable. It is always less than 600 mm annually. Temperature ranges between 22 °C to 42 °C. The districts are flat covered by trees shrubs with grass undergrowth.

Water is scarce and the main sources are pans, borehole, dams and shallow wells. The main economic activity of the two districts is livestock keeping, under pastoralism system. The livestock include cattle, sheep, goats, camels, donkeys and poultry (Table 1). Nomadic pastoralist communities in ASAL regions largely depend on milk produced by camels which contribute 80% of

the household needs (Schwartz-1992, Guliye-2006), especially during the dry periods when other animals are not as productive. The estimated human population of Garissa district (area of 44 952 km²) is 329 939 while Wajir district (area of 55 501 km²) has a population of 319 261 (1999 census)

Table 1: Livestock Population in Garissa and Wajir Districts

Species	Garissa District	Garissa District	
	2006	2007	2006
Cattle	246,488	254,538	251,349
Sheep	535,370	628,526	345,500
Goats	257,336	305,186	379,500
Camels	100,168	101,170	279,549
Donkeys	61,759	67,925	33,147
Poultry	33,450	45,000	28,680

Sources: Ministry of Livestock Fisheries and Development (MoLFD): annual report of 2006/2007 and District Veterinary Officer (DVO): annual report of 2006/2007.

Wajir District

Wajir district lies between latitudes 3° 6'N and 0° 20'N and longitudes 39 °E and 41 °E. 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.

Garissa District

Garissa is located in a desert region of Kenya, along the Tana River. Garissa has a very warm/hot climate, due to the low elevation and distance away from the cooler coastal areas. The daytime temperature typically rises above 33° C

(91° F), every day, but returns to a mild temperature, every night. Most of the inhabitants of Garissa are ethnic Somalis.

3.3 Sampling

Milk samples (20-50 ml) were collected in the morning from primary marketing agents and put into sterile screw bottles, which were kept in cool boxes until transported to the laboratory. The milk samples were stored at 4 °C until analysis on the day that followed. 1 calibrated loop of milk sample was streaked on nutrient media slants for transportation to Nairobi.

3.4 Sample size

Sample size was determined using the formula below:

$$Ss = \frac{Z^2 \times (P) \times (1-P)}{C^2}$$
 Where

Z = z value (e.g. 1.96 for 95% confidence level)

P = % picking a choice, expressed as decimal

C = the margin of error expressed as a decimal

Previous studies on prevalence of *Staph. aureus* and *Strept. agalactiae* in camel herds in Kenya and other places have been in the range of 11%-20% (Younan *et al.*, 2001, Abdurahman, 2006, Benkerroum *et al.*, 2003; Semereab and Molla, 2001; Buyser, 2001) . 16% was determined as an average from these reported cases.

Sample size for camel milk was therefore determined as below:

$$Ss = \underline{1.962 \times 0.16 \times (1-0.16)}$$
$$0.05^{2}$$

= 206.5244. This is approximately 207 samples.

3.5 Sampling Frame

The study was a cross-sectional survey of potential public health hazards and associated health risks with an aim of establishing the prevalence of selected hazards in camel milk. The sampling frame was constructed with the assistance of key stakeholders. Due to resource constraints, 103 random samples of camel milk were collected from Garissa and 104 from Wajir to make a total of 207 giving a margin of error of approximately 7 instead of the desired 5 units.

3.6 Field Survey

Acceptability of camel milk and camel milk products were assessed by using a single-visit multiple-subject diagnostic survey (ILCA 1990). Random samples of 138 households were purposefully chosen for interview (50 each from Garissa and Wajir towns and 38 from Nairobi- Eastleigh). Camel milk products (bought from women groups processing camel milk in Garissa and Wajir towns) were presented to the interviewee for sensory evaluation. Information about consumption pattern, preference, frequency of camel milk consumption, main house hold users and factors affecting purchasing criteria were obtained from households in three urban centres by means of a semi-structured questionnaire. Research assistants were hired to assist in administering the questionnaires due to language barrier.

3.7 Sample Analysis

The camel milk samples were subjected to Bacteriological analysis.

3.8 Bacteriological Analysis

The bacteria growth from nutrient media slants were streaked out on Blood Agar (BA; Oxoid No. CM 271) containing 5% defibrinated sheep blood and on Mannitol Salt Agar (MSA; Oxoid No. CM 85). The BA and MSA cultures were examined after 24 hours at 37 °C and reincubated for another 24 hours if there was no growth. Type of haemolysis, colony morphology, Gram stain (3.8.1) and catalase reaction (3.8.2) were recorded. Catalase-positive, Grampositive cocci were tested for coagulase reaction (Oxoid Staphylase Test No. DR 596, DR 597, DR 500) (3.8.3) Catalase negative Grampositive cocci were subjected to CAMP TEST (Christie, Atkins, Munch-Petersen, 1944) (3.8.4).

All isolates referred to as *Strept. agalactiae* (Lancefield group B) produced a characteristically shaped (arrow head) clear zone of haemolysis in the cloudy zone of haemolysis due to *Staphylococcus* beta toxin on BA. All isolates referred to as *staph. aureus* reacted positive in the Oxoid Staphylase test and negative with the negative control and showed beta – haemolysis on BA.

3.8.1 Gram Stain.

Thin smears of BA and MSA cultures were made on slides and heat fixed. The slides were flooded with crystal violet and left for one minute. The slides were washed under a stream of tap water and excess water drained off. Gram's iodine solution was applied for one minute. The slides were washed with water and excess water also drained off. Decolourisation was followed with acetone-alcohol (50:50), adding drop wise on tilted slides until all free colour (violet) had been removed. The slides were washed with water as above. Counter

staining was done using safranin for two minutes. The slides were washed under a stream of tap water, drained and blot dried. Examination was done with the dry (x40) objective to find a satisfactory area, and then an oil immersion was used (Dey *et al.*, 1999).

3.8.2 Catalase Test

A drop of 3% hydrogen peroxide was placed on a microscope slide. A portion of bacterial colony was transferred from an agar plate to the drop of hydrogen peroxide using a clean sterile platinum wire loop (Arbeit, 1988).

3.8.3 Coagulase Test

Two drops of normal saline was put onto a clean glass slide. A loopful of bacteria culture from the BA and MSA agar plate was emulsified in each drop of saline. One drop of undiluted rabbit plasma was added to each drop and mixed gently (Arbeit, 1988). The second suspension served as a control on the degree of granularity of the strain.

3.8.4 CAMP Test

A line was drawn a cross the centre of the blood agar plate. The toxin-producing strain of *Staph. aureus* culture was streaked across the plate i.e. (directly over the guide line drawn above). Three known *Streptococcus* cultures were streaked at right angles to the Staphylococcal streak on one slide, and similarly the unknown *Streptococcus* isolate was streaked on the opposite side. Incubation was done at 37°C for 24 hours (Christie, Atkins. Much- Peterson 1944).

3.9 Antibiotic-Sensitivity Test

The confirmed *Strept. agalactiae* and *Staph. aureus* isolates were subjected to agar diffusion sensitivity test (NCCLS, 2002), using Nutrient Agar (Oxoid No. CM 3) with 5% defribrinate sheep blood for *Strept. agalactiae*. The antibiotic test discs used were: Ampicillin 25μg, Tetracycline 100μg, Nitrofurantoin 200μg, Nalidixic acid 30μg., Streptomycin 25μg, Sulphamethoxazole 200μg, Co-Trimoxazole 25μg and Gentamicin 10 μg. The plates were incubated at 35°C and examined after 18 hours and the zones of inhibition were measured to the nearest millimeter.

3.10 Statistical analysis

Descriptive and correlation analysis between the acceptability parameters were performed using SPSS software (Version 10, SPSS Inc., Chicago).

Analysis of variance was used to analyze the results for antibiotic susceptibility.

CHAPTER FOUR

RESULTS

4.1 Prevalence of Staph. aureus and Strept. agalactiae

Table 2 shows the prevalence of the two organisms in Garissa and Wajir districts. Out of 207 milk samples examined, 49 (24%) were contaminated with *Staph. aureus* while 48 (23%) with *Strept. agalactiae*. The incidence of *Staph. aureus* in Garissa was almost three and half times more (34.95%) than in Wajir (10.58%). *Strept. agalactiae* prevalence in Garissa was even higher with 37.79% which is about five times that of Wajir (7.59%). In Garissa district, 23 (22.33%) of camel milk samples were contaminated by the two organisms.

Table 2: Prevalence of Staph. aureus and Strept. agalactiae in Percentage

District	Mastitis	organisms s	Prevalence of both the				
			two organisms in same				
					sample		
	Staph. av	ıreus	Strept. ag	alactiae	Staph. aureus and		
						Strept. Agalactiae	
	Absent	Present	Absent	Present	Absent	Present (%)	
	(%)	(%)	(%)	(%)	(%)		
Garissa	65.05	34.95	62.13	37.79	77.67	22.33	
Wajir	89.42	10.58	92.31	7.69	0.00	0.00	

4.2 Antibiotic Sensitivities of Staph. aureus and Strept. agalactiae

Table 3: Analysis of Variance tables

	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Staph.						
aureus	Antibiotic	7	12804.20	1829.17	42.96	<.001
	Residual	312	13284.78	42.58		
	Total	319	26088.97			
Strept.			ĺ	ĺ		
agalactiae	Antibiotic	7	12220.68	1745.81	42.5	<.001
	Residual	296	12145.92	41.03		
	Total	303	24366.60			

Where: d.f. = degrees of freedom s.s. = sum of squares, m.s. = mean square v.r. = variance ratio

Table 4: Means of antibiotics for each organism

	Means in mm			
Antibiotic	Staph. aureus	Strept.agalactiae		
Nalidixic acid	1.35 a	9.16 ° ~		
Sulphamethoxazole	2.15 a	0.42 a		
Co-Trimaxazole	3.95 ab	0.95 a		
Ampicilin	4.95 b	0.63 a		
Nitrofurantoin	10.10 °	5.79 b		
Streptomycine	11.80 °	5.58 b		
Tetracycline	17.00 d	14.47 d		
Gentamicin	19.00 d	18.71 e		
LSD (0.05)	2.87	2.89		

The Analysis of Variance indicates that the antibiotics are highly effective (p value < 0.01). However the susceptibility of the two organisms to the antibiotics is different: *Staph. aureus* is highly susceptible to both Gentamicin

and Tetracycline. *Strept. agalactiae*, is also very susceptible to Gentamicin. The antibiotics Nalidixic acid, Sulphamethoxazole, Co-Trimaxazole and Ampicilin are not so effective against the two organisms.

4.3 Acceptability of Camel Milk

Camel Milk products known and consumed in order of preference from each town are shown in figures 2, 3 and 4.

Knowledge of camel milk and milk products in Garissa is as follows; raw 27%, sour 25%, yoghurt 24%, pasteurized 17%, sweets 4% and sour mixed with herbs 3 %. The consumption percent rate of raw and sour camel milk is higher than other products (17% and 20%). Raw and sour camel milk products are the most popular, with sour milk (20%) being the highest product consumed in Garissa town. Although yoghurt is popularly known in Garissa by 24% its consumption is lower with 13%.

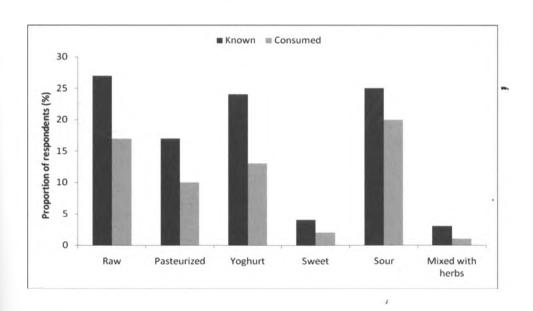


Figure 2: Camel Milk Products Known and Consumed in Garissa Town

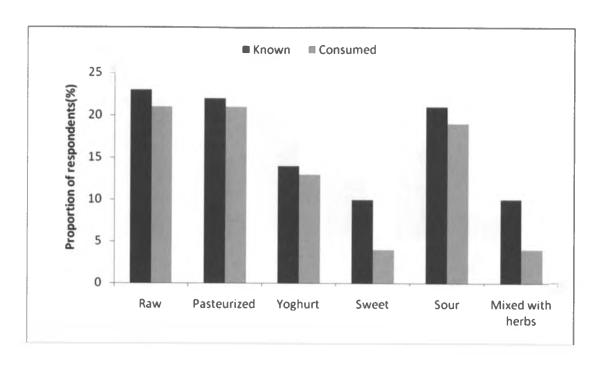


Figure 3: Camel Milk Products Known and Consumed in Wajir Town

In Wajir town raw, pasteurized and sour camel milk are popular and highly consumed, with percent knowledge and consumption of the order (23, 21), (22, 21) and (21, 19) respectively. Knowledge about yoghurt is 14% and consumption is 13%. Sweets and sour milk mixed with herbs are least known products each 10% and consumption rate of 4% for each respectively.

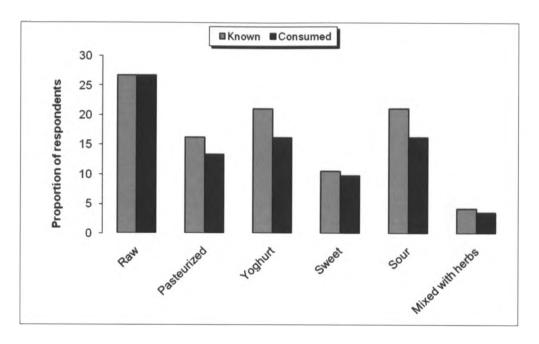


Figure 4: Camel Milk Products Known and Consumed in Nairobi

Figure 4 shows knowledge and consumption of camel milk and its products in Nairobi (Eastleigh). Consumption of raw camel milk (27%) and knowledge about it (27%) is higher compared to other products. For processed camel milk products i.e., pasteurized, yoghurt and sour, the percentage values of consumption versus knowledge are lower (Figure 4). Milk processing adds extra cost to the product which affects its consumption.

4.3.1 Frequency of Consumption of Camel Milk and Milk Products in Each Town

Garissa Town

Frequency of consumption of camel milk and its products in Garissa town is shown in figure 5. Seventy five percent of the respondents generally take camel milk or the milk products everyday. Almost everybody consume camel milk or products from this town. Those who rarely take are 14%, sometimes

take (1-3 times per month) 8%, about once a week and more than twice a week 2% each, and every day 75%.

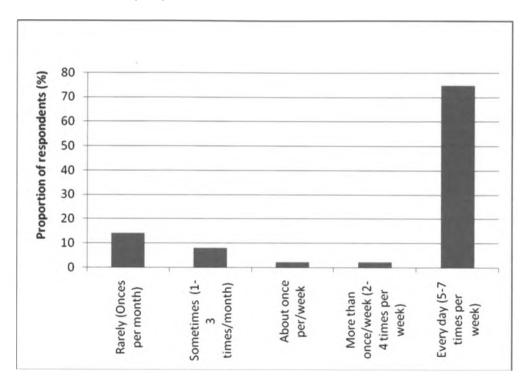


Figure 5: Frequency of Camel Milk Consumption in Garissa Town

Wajir Town

Figure 6 shows the frequency of consumption of camel milk and its products in Wajir District. The proportion of people who take camel milk and its products everyday are 31%, rarely 29%, sometimes 21%, about once per week 11%, more than once per week 8% and those who do not take 5 %. Frequency of consumption is widely distributed with high percentage of respondents taking camel milk products every day.

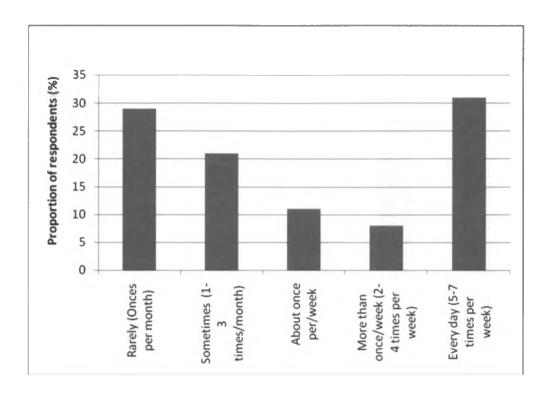


Figure 6: Frequency of Consumption of Camel Milk and its Products in Wajir District

Nairobi-Eastleigh

As figure 7 shows, 38% of respondents in Nairobi- Eastleigh take camel milk and the milk products everyday and more than twice a week (31%). Those who rarely take the milk are 15%, about once a week 10% and sometimes 6%. This indicates that frequency of camel milk consumption is generally low.

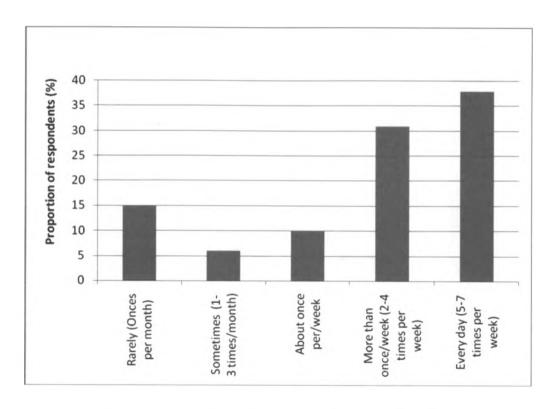


Figure 7: Frequency of Camel Milk and Products Consumption in Nairobi

Most respondents from all the districts consume camel milk every day, some products especially yoghurt is consumed thrice monthly, once a week or more than once per week.

4.3.2 Main Household Users of Camel Milk and Milk Products

Figure 8 shows household users of camel milk and its products in Garissa town.

The respondents who indicated that camel milk products are consumed by all members of the family were 74%. This is followed by young adults with 11% respondents, 9% for children and 6% for adults.

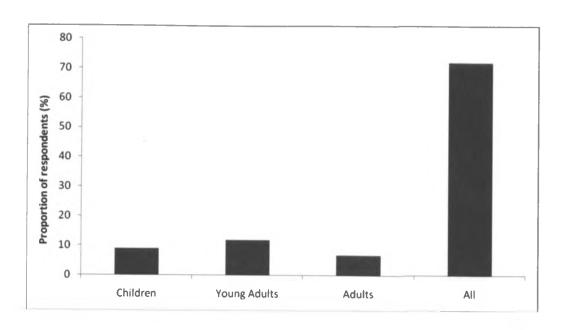


Figure 8: Main Household Users from GarissaTown

Figure 9 shows fifty one percent (51%) of respondents in Wajir town indicated that camel milk and milk products are consumed by all family members, 20% by adults and children and 9% by young adults.

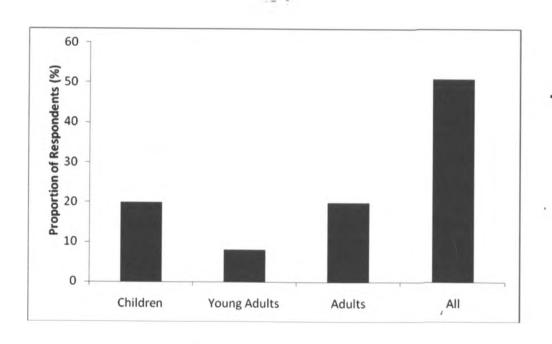


Figure 9: Main Household Users from Wajir Town

Majority of respondents (73%) in Nairobi (Eastleigh) claimed that camel milk and milk products are used by all family members, 11% by adults, 9% by children and 7% by young adults (figure 10).

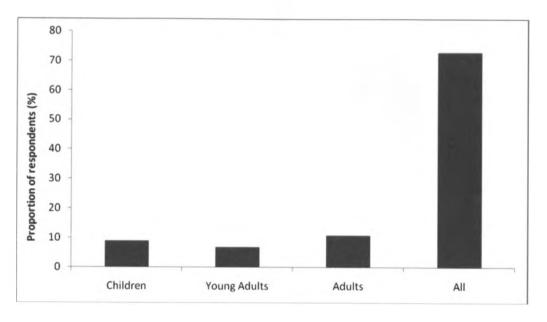


Figure 10: Main Household Users from Nairobi-Eastleigh

From the above three figures on main household users, it can be agreed that camel milk is consumed or used by all family members.

4.3.3 Purchasing Criteria for Camel Milk and Milk Products

Camel milk and milk products were bought from camel milk processors in Garissa and Wajir town. Samples of those products were given to consumers to taste and rate them according to their purchasing criteria. Figures 11 to 13 show the quality attributes that affect the purchasing of each product in Garissa, Wajir and Nairobi (Eastleigh) respectively

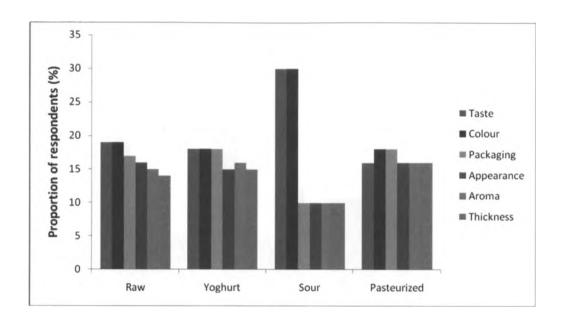


Figure 11: Purchasing Criteria for Camel Milk Products in Garissa Town

In Garissa, figure 11, the most important purchasing criteria for sour milk are taste and colour (30% each). The two are also important for raw camel milk with percent respondents of 19% each. For yoghurt, taste, colour and packaging all have same measure of 18%. These three are the quality attributes that can influence the purchase of yoghurt from this district. Colour (18%) and packaging (18%) measures highest for pasteurized milk.

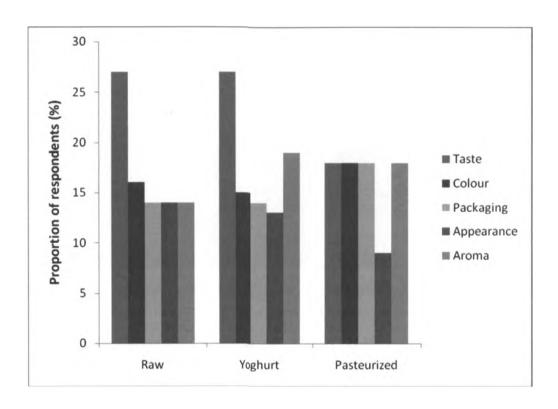


Figure 12: Purchasing Criteria for Camel Milk Products in Wajir Town

In Wajir, figure 12, the most important quality attributes for raw camel milk are taste (27%) and colour (16%). The other attributes for the raw milk have same measures of 14%. Taste is also an important attribute for yoghurt (27%). This is followed by aroma (19%), colour (15%), packaging (14%), appearance (13%) and thickness (11%) in that order. Pasteurized camel milk got same measure for taste, colour, packaging aroma and thickness of 18% each as quality attributes. The least attribute in the group is appearance (9%), for pasteurized milk.

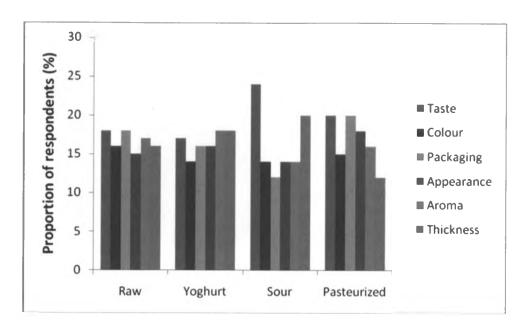


Figure 13: Purchasing Criteria for Camel Milk Products in Nairobi-Eastleigh.

In Nairobi, figure 13, taste and packaging each 18%, are the important purchasing criteria for raw camel milk. These are followed by aroma (17%), colour and thickness each (16%) and appearance (15%) in that order. For yoghurt, aroma and thickness (each 18%) are the highest measures of quality attributes, followed by taste (17%), then packaging and appearance each 16% and colour 14%. The taste of sour camel milk is a very important attribute (24%). Thickness (20%) is ranked second followed by appearance, aroma and colour each 14% and lastly packaging (12%). Taste and packaging (each 20%) are the important quality attributes for pasteurized camel milk. These are followed by appearance (18%), aroma (16%), colour (15%) and thickness (12%).

Respondents gave several reasons why they preferred camel milk. The pie chart in figure 14 summarizes these reasons.

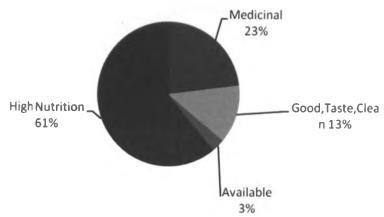


Figure 14: Main Reasons for Camel Milk Preference

Sixty one percent of the respondents preferred camel milk because of its high nutritional value, twenty three percent medicinal, thirteen percent good taste and clean while three percent take it since it is available.

4.3.4 Factors Affecting Consumption of Camel Milk and its Products

Figure 15 shows the factors that affect consumption of camel milk and its products. The main factors limiting camel milk sub sector are high and fluctuating prices (27%), poor quality products (24%) and product unavailability (24%).

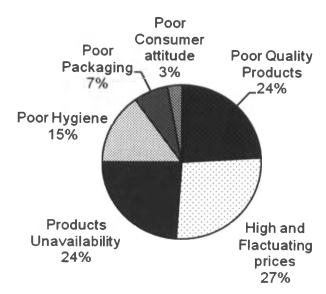


Figure 15: Factors Affecting Consumption of Camel Milk and its Products.

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of Staphylococcus aureus and Streptococcus agalactiae in Camel Milk

5.1.1 Garissa

In this study Staph, aureus and Strept, agalactiae prevalence in Garissa had values of 34.95% and 37.79% respectively (Table 2). A study by Abdurahman (2006) in the Errer Valley of Eastern Ethiopia indicated a prevalence of 12.7% for Staph. aureus while 8.8% for Strept. agalactiae. Intramemmary infections udder – prevalence were 12% for Strept. agalactiae and 11% for Staph. aureus in six Kenyan camel herds (Younan et al., 2001). In two other studies on Sudanese camels, Strept. agalactiae were isolated from 26.7% of composite udder-milk samples (Obied and Bagadi, 1996) and from 17.6% of quarter-milk samples (Abdurahman et al., 1995) while Staph. aureus were isolated from 17% (udder milk sample) and 5.4% (quarter milk samples). The results of composite udder-milk samples are close to the findings in Garissa. The results are also in agreement with those of Younan (2001) which showed a higher percentage of multiple quarter infections for Strept. agalactiae infected udders (44%) compared to udders infected with Staph. aureus (23%). The incidence of the two organisms existing together in the same sample was found from this study to be 22.33%, (Table 2). There is currently no data to compare this value with.

Muli *et al.*, (2008), from their survey, found that small scale camel milk producers who do not wash the camel udders before milking usually contaminated the milk with dirt, the camel's urine and other debris. In this study all camel milkers stated that they do not use water for milking. In many cases, use of little scarce water or dirty water may result in spreading of microorganisms.

Garissa being a more vibrant business environment compared to Wajir, pooling and exchange of the camel milk in many hands could be a contributing factor to the high figures. Pooling of different camel milk batches along the collection and marketing chain can result in increased prevalence of *Strept*. agalactiae. Abduraham and Younan, (2004) found Strept. agalactiae in 50% of milk transport containers coming from producing herds, 62% of milk containers sampled at primary collection sites, 70% of milk containers sampled from an urban market of the same region and 89% of raw milk baths received at a dairy processing plant. These findings indicate a very widespread occurrence of the pathogens in milk producing camel herds and in the milk collection and distribution system. From the results, Garissa district showed higher values especially for Strept. agalactiae indicating there is actually pooling of milk. The same study showed that, farmers with camel herds of up to 500 will have only up to 35% lactating camels and with the general low yield per camel, even the largest farmers will have milk production of not more than 500 litres per day at the best of times. The majority of farmers, with an average of 20 lactating camels are able to produce only up to 10 litres of milk per day. None of these levels of production are large enough for farmers

to operate on their own. It is therefore essential that milk from different farmers is assembled together for economies of scale in transportation and market access.

According to a study by Dargent *et al.*, (1988), high *Staph. aureus* prevalence generally increased with increasing herd-size among herds infected with *Strept. agalactiae*. There was no consistent pattern within market or district category, but a high prevalence of *Staph. aureus* and *Strept. agalactiae* is more likely in a wider market. The current study cannot explain the variations in results, however poor management and unhygienic milking practices prevalent in the traditional husbandry systems including tying the teats with soft parks to prevent the calf from suckling, tick infestations and casterisation of the udder skin (Abdurrahman *et al.*, 1995, Obeid and Bagadi, 1996 and Woubit *et al.*, 2001) seem to be common in Garissa. Although there is no data, from the researcher's observation, pastoralists in this region have poor hygienic practices.

The Garissa market serves a wide market, including Nairobi, from where some milk is forwarded to other traders, particularly Kakuma refugee camp, Kisumu and Kampala. A number of wholesale traders also have occasional buyers who take up to 10 litres every time they are traveling abroad (especially Turkey). The high levels of *Staph. aureus* and *Strept. agalactiae* in the "highly esteemed" camel milk, potentially reach and expose a wide and diverse market beyond the Garissa market to pathogens. From this study 28% (92 383) of Garissa population takes raw camel milk contaminated, with *Staph. aureus*

(34.95%) and *Strept. agalactiae* (37.79%)(Table 2). The milk is consumed daily (75%) and by all members of the family (75%). These values are worrying to any one who understands the harm the pathogens can cause to the people in the district thus serious measures should be taken.

5.1.2 Wajir

Wajir had lower prevalence for both *Staph. aureus* (10.58%) and *Strept. agalactiae* (7.69%), Table 2. These values look impressively small in the eyes of camel milk handlers from this district; however, Kenya Bureau of Standards indicates that pasteurized camel milk for human consumption should have "Nil value" for the two organisms. *Staph. aureus* has been ranked as the most frequent (Karamy, 1990; Al-Ani and Al-shareeti; 1994) or second most-frequent (Barbour *et al.*, 1985; Obied and Bagadi, 1996) microorganism involved in intramammary infection in camels. This was reflected in the results from Wajir (Table3). Other studies (Benkerroum *et al.*, 2003; Semereab and Molla, 2001; Buyser, 2001 and El-ziney and Al-Turki, 2007) have indicated *Staph. aureus* as the most frequent pathogen associated with many disease outbreaks.

Staph. aureus and Strept. agalactiae are common in the environment, their presence in the bulk milk is often attributed to unhygienic practices in milking. Generally it was not determined if the pathogens originated from the camel or the environment. This would have been possible by comparing the strains found in the milk. The variations in results from Garissa and Wajir are a clear indication that environmental contamination may play a great role. The mean prevalence in both districts for Staph. aureus was 22.77% while for Strept.

agalactiae was 22.74% (Table 2). These are almost twice the finding of Younan *et al.*, 2001 in Kenya. According to their study, intramammary infection udder prevalence was 12% for *Strept. agalactiae* and 11% for *Staph. aureus*. Since their research was based on milk from camel herds, it is expected that the percent prevalence be lower than what is expected from bulk or pooled milk.

Camels affected by mastitis are not treated in traditionally managed camels and will often take a natural course to chronicity resulting in a permanent loss of milk production (Obied and Bagadi, 1996). The potential danger of this is that before the loss, the pathogenic mastitis organism will have caused much damage to the humans, including hospitalization and even death of many individuals. Neonatal mortality is primarily due to diarrhea following failure of passive transfer and exposure to *Escherichia coli*, rotavirus, Corona virus, Coccidia and Salmonella. Now that this milk is becoming popular, the problem of mastitis in camels should not be looked at single handedly as affecting not only pastoralists but the whole public.

5.2 Antibiotic Sensitivity

The antibiotic sensitivity screening showed that high proportions of *Staph. aureus* isolates were resistant to Ampicilin, Co-Trimoxazole, Nalidixic acid (, Nitrofurantoin and Sulphamethoxazole while high proportions of *Strept. agalactiae* were resistant to all but susceptible to Gentamicin (Table 3). Significantly (P < 0.001), high proportions of *Staph. aureus* and *Strept. agalactiae* isolates treated with Gentamicin were susceptible. This likely indicates differences in the usage pattern of the antibiotics in camel herds.

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Because there was little information regarding the actual use of drugs and treatment regimens on the different camel herds, a relationship between antimicrobial usage and resistance could not be conclusively established in this study.

A study by Mody *et al.*, (1998) showed that all mastitis bacterial isolates were resistant to Nitrofurantoin, Furazolidone and Penicillin. Antimicrobics against which the bacterial isolates showed good sensitivity were Gentamicin and Chlorophenicol. Cotrimoxazole, Sulphamethoxazole and Streptomycin were also found to be highly effective against some bacterial isolates. Antimicrobial drugs against which bacterial isolates showed moderate susceptibility were Oxytetracycline, Co-trimoxazole and Chlorophenicol. In yet another study by Azmi *et al.*, (2008), Gentamicin, Ampicillin and Tetracycline were the most effective drugs against mastitis bacterial isolates. The bacteria flora showed greatest resistance to Penicillin and Streptomycin. These two drugs were said to be the most commonly used for domestic animals in Jordan.

According to the study done in Kenya, by Younan et al., (2000), 56% of the tested Strept. agalactiae and 50% of the tested Staph. aureus isolates were fully sensitive to Tetracycline. Nitrofurantoin and Streptomycin affected the two organisms differently. In this study, Staph. aureus was sensitive to Gentamicin and Tetracycline while Strept. agalactiae was less sensitive to all antibiotics but very sensitive to Gentamicin.

There is limited information on the rate at which bacteria are developing resistance to antibiotics commonly used to treat infections in camels. The

likelihood of antibiotics resistance developing broadly depends on the: prevalence of resistant bacteria in the animal population, frequency of antibiotic use and type of exposure to the antibiotic e.g. short treatment courses of high doses of antibiotic confer less selective pressure than long term exposure to low doses of antibiotic (Guterbock *et al* .1993 and Hallberg *et al.*, 1994). Myllys *et al.*, (1998) reported an increase of 27% in the proportion of *Staph. aureus* strains resistant to at least one antibiotic (mostly due to strains capable of producing beta lactamase). There is currently no substantial data set in Kenya that enables comparison of these findings with what is happening in Garissa and Wajir camel population.

The population of mastitis bacteria changes over time. This is true for the occurrence of bacteria species and for the occurrence of antimicrobial resistance within bacterial species. According to a study done in 1985, and 2000 in New York Dairy Herds by Linda et al., 2004, there was a significant decrease in the susceptibility of Streptococcus Spp to Ampicillin, Cloxacilin, Penicillin, Erythromycin, Pirlimycin and Tetracycline. The trend for Staph. — aureus was very different than the trend for Strept. Spp. Where Streptococcus Spp showed a decrease in susceptibility, Staph. aureus showed significant increase in susceptibility to both Ampicillin and Penicillin. Susceptibility to. Amoxicillin (94%) and Cephalothin (98%) remained stable. Susceptibility to Erythromycin, Eirlimycin and Tetracycline did not change significantly over time either. In 1975, Davidson performed a similar analysis that covered 10 years, on antimicrobial resistance (Davidson, 1980). The contrast between the

95% of *Strept. Spp* tests were susceptible to Ampicillin (Davidson, 1980) while Linda *et al.*, data indicated as little as 26% susceptibility of *Strept. Spp* in 1999. By contrast only 49% of *Staph. aureus* isolates tested in 1975 were susceptible to Ampicillin, in 1999, the overall susceptibility to Ampicillin was 79%. These changes in susceptibility reflected changes in mastitis treatment used over the years. The data suggests that long-term trends in antimicrobial resistance may be different from trends measured over a limited number of years.

5.3 Camel Milk Acceptability

Knowledge of raw camel milk from the three districts is almost proportional to its consumption. Camel milk is predominantly consumed raw (figures 2, 3 and 4). This observation is in agreement with what is reported earlier by Yagil (1982), Alhadrami (2003) and Eyassu Seifu (2007), who indicated that camel milk is consumed fresh in most camel rearing societies. According to Muli *et al.*, (2008), camel milk is generally consumed in raw form, either fresh or naturally fermented. The demand for camel milk is largely driven by perceived superior quality compared to cow milk (in terms of flavor and need for little milk: water ratio when making tea) as well as the acclaimed medicinal value. Pastoralists claim that cows' milk causes obesity but camel milk gives strength, endurance and stamina, an attribute that they need in order to pursue a nomadic life style. Besides, camel milk keeps fresh a longer time and it quenches thirst. The milk is highly valued among the pastoralist community, it does not only contain higher amount of nutrients as compared to cow milk, but it also has

medicinal properties (Barbour *et al.*, (1985), Elagamy *et al.*, (1992). The survey actually confirmed these two parameters as the main reasons why people consume the milk, with high nutrition having (61%) followed by medicinal having (23%). One peculiar characteristic of camel milk is its therapeutic value against a number of human diseases (Eyassu, 2007). In his study, pastoralists claimed that camel milk was used to treat a number of illnesses in human beings including jaundice, malaria and constipation. According to the pastoralists view, the claimed therapeutic property of camel milk was attributed to the fact that camels browse on various plant species and active agents with therapeutic properties from these plant species are secreted into the milk of camels. From traditional point of view, anything medicinal is taken fresh or raw without any heat treatment for better effectiveness. This could suggest part of the reason why the raw milk is highly preferred raw. In contrast, raw milk consumption poses the highest risk of exposure of pathogens to humans.

Consumption of other camel milk products varied among the three districts. Sour milk is known and consumed more in both Garissa(25%, 20%) and Wajik (21%, 19%) districts. In Nairobi, although known its consumption is lower. In Garissa and Nairobi district respondents who know about pasteurized camel milk and yoghurt are more than those who consume them (Figures 2 and 4). Though the difference is small, the cost of purchasing the products could be affecting consumption. In Wajir, the case is different for the knowledge and consumption of the two products. Though these statistics are seen to be higher in Wajir than in Garissa and Nairobi districts, the daily frequency of consumption of camel milk products is least in Wajir (31%), Figure 6,

compared to Garissa (75%), Figure 5 and Nairobi (38%), Figure 7. Nairobi (Eastleigh) respondents seem to be price sensitive and will only go for the cheapest that is probably why yoghurt and sour camel milk are not consumed at the same measure as their knowledge. Sweets and sour milk mixed with herbs are not popular in all the districts.

There is a clear indication that there are other factors, which influence consumption of camel milk products. The average prices that respondents were willing to pay per litre for raw camel milk in Nairobi are Ksh 80 and Ksh 70 for sour milk. In Garissa district the average price for all camel milk products is Ksh 120. The amount respondents were willing to pay in Wajir is as follows; raw camel milk and yoghurt Ksh 54 for each and Ksh 40 for pasteurized milk. Looking at these prices, there is a clear indication that raw camel milk is highly valued among the consumers. All the above prices are low compared to the real market prices of camel milk products in the Kenyan market. From a study by Muli et al., (2008) farmers who take their milk to the camel milk processing plant - the Vital Camel Milk Limited (VCML) based in Nanyuki are paid prices of Ksh 40 per litre. Although the study team did not access detailed costing information, estimates by the Managing Director indicated that, at the level of production then, operational costs amounted to about Ksh 30 – 40; packaging Ksh 35, and transportation Ksh 80 per litre. The milk is supplied to wholesalers in Nairobi and other parts of the country at Ksh 84 per half litre (i.e. Ksh 168 per litre) with a recommended retail price of Ksh 94 per half litre. From a survey of retail outlets however all retailers with VCML milk were retailing the milk at Ksh 120 per half litre. These prices

were quite high compared to Ksh 35 for the same quantity of cow's milk. The survey found a significant mix between customers seeking camel milk for its health qualities with those valuing the milk from a food perspective (and substitute to cow milk). Nairobi-Eastleigh has consumers from camel keeping communities, mainly the Somali. The main market is in Eastleigh estate with business hub for medium and low income people. Muli *et al* (2008) study indicated that camel milk from Eastleigh is also forwarded onward to other large urban areas in Kenya, including Nakuru, Mombasa, Kisumu and as far as Kampala in Uganda. Some milk is also sent to Kakuma refugee camp (currently 60 litres per week) and some even exported once in a while to Turkey and other parts of the World through customers who buy the milk when traveling to these countries. There are indications that a significant portion of milk from Garissa is sold in Dadaab refugee camp (Muli *et al.*, 2008).

Three sample products were given to consumers to taste and rate each according to their purchasing criteria. The most important purchasing criteria for raw camel milk is the taste; it should have distinctive taste characteristic to camel milk. In Wajir, taste and aroma are the important quality attributes for yoghurt. Taste, colour and packaging are important for yoghurt in Garissa; while for Nairobi aroma thickness and taste. Pasteurized camel milk quality attributes are almost similar in all the districts; however in Nairobi district taste and packaging are highly valued. The unscrupulous businessmen should not take this as an advantage to exploit consumers. There is currently no substantial data to compare these results with. Quality and acceptability of a

set –type yoghurt made from camel milk was determined by Hashim *et al.*, (2009). Consumer results indicated that the hedonic ratings of the sensory attributes and acceptability of camel milk yoghurt containing 0.75% alginate+ 0.075% calcium were similar to that of cow's milk yoghurt. The camel milk yoghurt containing alginate + calcium and flavored with 4 different fruit concentrates (15%) had similar hedonic ratings and acceptability. This research indicates that although camel milk and milk products are acceptable, each has different quality parameters that attract customers.

The main factors limiting the growth of camel milk sub-sector (figure 15) are high and fluctuating prices, poor quality products and products unavailability. Muli et al., (2008) survey revealed that, although there are many factors constraining the development of the camel milk sub sector, the main ones were: low milk productivity, low quality of milk, poor organization of actors in the chain, poor business orientation of producers, inadequate physical and institutional support infrastructure and poor market development. From a combination of these factors, the majority of smallholder milk producers are unknowingly making losses in their activities related to camel milk. Production and profitability among all other factors in the value chain are low. From these factors also, the high growth potential of pasteurized camel milk channel is struggling to survive and massive losses have so far been incurred which threaten collapse of the entire chain. Their analysis of the constraints facing the camel milk sub-sector showed that most of them were cross-cutting and could not be effectively addressed through piecemeal interventions which do not take a holistic view of the interconnectedness of actors in the value

chain. At the initial value chain segment of production, low milk productivity among farmers is tied to issues of market access, poor organization of the producers as well as traders to collect the milk, and also to poor development of support infrastructure. This in turn has resulted to only small volumes of milk getting through the value chain which is adversely affecting profitability among all other factors in the chain. A similar situation also applies to the issue of poor milk quality and so do the other constraints. There is likely to be an increasing demand for clean milk from consumers and by regulatory agencies (food safety authorities) in the near future.

CHAPTER SIX

CONCLUSIONS

The prevalence for *Staph. aureus* was found to be 22.76% and 22.74% for *Strept. agalactiae*. The two districts however differed with Garissa having high percent incidence both for *Staph. aureus* (34.95%) and *Strept. agalactiae* (37.79%). Wajir, on the other hand, had low prevalence of 10.58% and 7.69% respectively. It was only Garissa district which had samples (22.33%) contaminated by the two pathogenic organisms.

High percentage of the tested *Staph. aureus* and *Strept. agalactiae* were resistant to most antibiotics except Gentamicin. Although camel milk and milk products are acceptable, each has specific quality attributes that affect their demand and these should be considered when processing the same.

Raw camel milk consumption is favored more but on the other hand poses the greatest risk to human health through exposure to *Staph. aureus* and *Strept. agalactiae*. There should be promotion of processed camel milk and products to non-conventional consumers to increase their consumption.

CHAPTER SEVEN

Recommendations

- The prevalence of Staphylococcus aureus and Streptococcus agalactiae
 differed in Garissa and Wajir, there is need to find out the causes of
 those variations.
- It would be very useful to investigate the impact of these levels of milk contamination on human health by surveying clinical and sub clinical infection in humans consuming camel milk
- Health intervention strategies need to be put in place in Garissa market since it serves a wide and diverse market including Nairobi, Kakuma refugee camp, Kisumu, Kampala and other countries.
- The trend in *Staphylococcus aureus* and *Streptococcus agalactiae* susceptibility to antibiotics in camels over time needs to be studied. This is necessary in order to come up with an effective program for fighting mastitis in camels.
- There is need for broad research in camel milk sector to elaborate the constraints as this sector provides an entry point for development in the ASALs.
- Capacity building of camel milk production groups in terms of training:
 provision of inputs e.g. aluminium cans, cooling points, credit and milk
 testing units would greatly enhance quality camel milk production
 capacity.

The vast camel population in the ASALs and their adaptability to live and thrive in the ASALs can be used as a resource by exploring and expanding the camel milk market if hygiene issues are taken care of.

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APPENDICES

Appendix I: A herd of camels near a watering well in Wajir



Appendix II: Camels browsing in the rangelands in Garissa



Appendix III: Camels in Wajir (Griftu) watering in a well



Appendix IV: Camels watering in River Tana Garissa District



Appendix V: Camel Milk being off-loaded from a truck in an open air market in Wajir Town

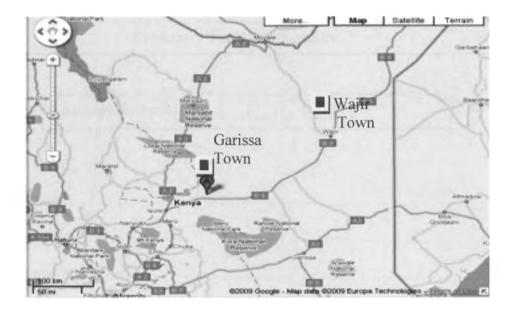


Appendix VI: Map of Kenya Showing Nairobi, Garissa and Wajir Towns.



Source: ©1996-2004 Timothy F. Bliss

Appendix VII: Map of Garissa and Wajir Towns



Appendix VIII: Test Fair Questionnaire on Camel Milk and Camel Products Acceptability

	Consumer Test Questionnaire
	Enumerator code _ _ _
Section A: Basic l	Data
Reg	gion
Dis	trict
Tov	wn/City
Res	pondent
nan	ne
Mo	bile
con	tact
Dat	e of interview _ / _ / _ /

SECTION B: Data (Respondent's)

Sex of respondent	☐ 1 Male	
PSEX	☐ 2 Female	
What does the respondent	☐ 1 Farmer	
consider to be his/her	☐ 2 Teacher	
main occupation?	☐ 3 Non-farm casual service	
POCC	☐ 4 Private service	
	☐ 5 Business	
	☐ 6 Government service	
	☐ 7 Professional	
Age group of respondent	1 🗆 10-18	5 35-40
PAGR	2 🗆 18-25	6 🗆 40-45
	3 🗆 25-30	7 🗆 45-50
	4 □ 30-35	8 🗆 50-55
		9 □ 55 and
		above
Highest level of education	☐ 1. None	
of the respondent	☐ 2. Primary	
PHEL	☐ 3. High school	140
	☐ 4. Technical inst.	
	□ 5. College	
	☐ 6. University	
	☐ 7. Other (specify)	

SECTION C: Consumer Knowledge and Preferences on Milk and Milk Products

1.	Do yo	u consume milk and milk products?
	Yes	
	No	
2.	If no p	please explain why?
	* * * * * * * *	
3.	(a) l	If yes what type of milk do you consume? (Rank in order of
	I	preference)
	i	i. Camel
	ii	i. Goat
	iii	i. Cattle
	iv	Other (please specify)
	(b) I	Please give reasons for preference

4. What products and forms of camel milk do you know?

(Please indicate the ones consumed in order of preference)

Camel milk products and forms known	Camel Milk Products Consumed
1= raw	1= raw
2= pasteurized	2= pasteurized
3= yoghurt	3= yoghurt
4= sweet	4= sweet
5= sour	5= sour
6= mixed with herbs	6= mixed with herbs
7= other (please specify)	7= other (please specify)

5. How often do you consume milk/milk products? Please indicate the main household users.

Milk/Milk Product	Frequency of use	Main
(specify type e.g. camel,		household users
goat)		
1= raw	1= never	1= Children
2= pasteurized	2= rarely (<1 per month)	2=Young
3= yoghurt	3=sometimes	3=Adults
4= sweet	(1-3 times/month)	4= All
5= sour	4= about once/week	5= others
6= mixed with herbs	5= more than once	
7= other(please specify)	per week(2-4 times per	
P+ :	week)	
	6= every day(5-7 times	
	per week)	
		-

6. Place of purchase

Where do you buy your milk and milk	Super market
products in order of frequency?	Kiosk \square
Number appropriately	Farm
	Open air Market
	Wholesalers
	Others
	Any other (Specify)

7. What factors influence your purchasing decision criteria on milk and milk products? Please rate from 1(not important) to 4 (extremely important) and 5 don't know.

	Not	A little	Important	Extremely important	Don't know	Please describe your preferred attribute of each trait
Colour						
Appearance						
Tiste						
Aroma						
Availability						
Shelf life						
Manufacture						
Packaging						
Nutritional value						
Thickness		8-				
Origin of products						
Point of sale						
e.g. Supermarket,						
open air						*
Price						
Hygiene						
Labeling						
Others (specify)						,

SECTION D: Product Test.

6= mixed with herbs

7=other (please specify)

	Have you ever consumed camel milk/camel milk product?					
(☐ Ye	S				
[□ No					
((a)	If No please explain v	vhy?			
			******************		••••••	*****
		***************************************	***************************************	******************	•••••	****
((b)	If yes please fill as per	r table below			
	For	m in which	Last date	Price per	What	Dislikes
	con	sumed	of	Unit	impressed	
			consumpti	(specify	you most	
			on	units)		
	1= r	aw				
	2= p	pasteurized				
	3= y	oghurt				
	4= s	sweet				
	5= s	sour				

9. Please sample our products and rate them according to your purchasing criteria.

Indicate product	Purchasing criteria	1. Excellent
sample code	1=Taste	2. Good
	2=Colour	3. Fair
	3= Packaging	4. Poor
	4= Appearance	5.No comment
	5= Aroma	
	6= Thickness	
	7=Other (please specify)	

10. Purchasing information

Indicate	Willingness	If no	If yes	What can
product	to buy	please	please state	be done to
sample code	1=yes	explain why	the amount	improve
	2=no		you would	the product?
			be willing	
	1 10 7 90000 20		to pay per	
			unit volume	

11.	What are the major factors limiting the growth of the camel milk sub
secto	r?
	Please rank them in the box below
	1= Poor quality products
	2= High and fluctuating prices
	3= Product unavailability
	4=Poor hygiene
	5= Poor packaging
	6= Poor consumer attitude
	7= other (Please specify)
12.	What general comments would you like to make on camel milk and
prod	acts?
For a	official use only
	Received _
	Approved _
	Signed

