ASSESSMENT OF RISKS OF ZOONOTIC *ESCHERICHIA COLI 0157: H7* AND BRUCELLOSIS IN INFORMALLY MARKETED UNPASTEURISED MILK IN NAIROBI AND NAKURU DISTRICTS, KENYA

BY:

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A REPORT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF VETERINARY EPIDEMIOLOGY AND ECONOMICS (M.V.E.E.).

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF NAIROBI

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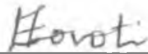
DECLARATION

This project report is my original work and has not been presented for a degree in any other University



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DEDICATION

To my daughter Noel

ACKNOWLEDGEMENT

My sincere appreciation goes to Professor Arimi for his continuous guidance and support through out the course of this study. I would also like to express m y deepest gratitude to my other two supervisors, Dr. Kyule and Dr. Omore for their input in this work that has led to its success. Special thanks goes to Messrs Kaburia for his interest at every stage of my laboratory work. I cannot forget Macharia, Nduhiu, Githua, Grace, Leah, Muruiki and all the technical staff of immunology laboratory for the teamwork that made the workload bearable.

I am deeply indebted to the Dean of the Faculty of Veterinary Medicine, Professor Kagiko, through the Chairman Dept of PHPT, Professor Njeruh, for all the facilitation of the course work and research work. I cannot forget William Sang of Kenya Medical Research Institute (KEMRI) for kindly accepting to carry out PCR test on our isolates. I am very grateful to the University of Nairobi for awarding me a scholarship that enabled me to pursue my course work. I am also indebted to MOARD/KARI/ILRI Smallholder Dairy Project for the additional funds they granted that enabled me to complete project work. My gratitude is also extended to the Public Service Commission for granting me study leave.

My sincere thanks go to my brothers and sisters and especially to my brother Mumia for his continuous challenges that saw me through my entire studies. Last but not the least, my heartfelt gratitude goes to my late husband for his encouragement to take up this course at the time when it was difficult to make that decision and to my late father for his

strong support for girl child education in the family. I cannot forget my mother for being there whenever I needed her. Finally my special thanks go to my daughter, Noel, for being patient when we were both students and for her understanding of the odd situation.

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ABSTRACT

This study investigated the bacteriological quality (total viable counts and coliform counts) and assessed the risk of milk-borne zoonotic *E.coli 0157:H7* and brucellosis infections in humans that may result from consumption of non-heat treated milk. Ways to minimize the risks from these hazards were evaluated and recommendations made.

The study was carried out by questionnaire and laboratory analysis of milk samples obtained from consumer households in Nairobi and Nakuru district s as part of a large study to assess potential milk-borne health risks that may be present in various unpasteurised milk market pathways in Kenya. Between January 1999 and January 2000, survey data and raw milk samples were collected seasonally from households purchasing unpasteurised milk in urban and rural locations. Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. A total of 264 samples were analysed in the laboratory. The samples comprised those from two seasons in Nairobi and one season in Nakuru District. During first seasonal survey in Nairobi (dry season), 49 households were sampled, while during the following wet season, 53 households were sampled. In Nakuru District, a total of 162 households (58 households from Nakuru urban area and 104 households from Nakuru rural) were sampled in the dry season.

The Standard Plate Count (SPC) and Violet Red Bile Agar (VRBA) methods were used to enumerate the total viable and coliform counts and national standards applied to classify quality of the milk. Characterisation of the coliforms was further investigated based on the indole, methyl red, Voges Proskauer and Citrate (IMViC) reaction pattern. Identification of the *E.coli 0157:H7* was by streaking *Escherichia coli* cultures onto Biosynth selective indicator medium (BCM^{TM}0157: H7 (+)(Biosynth International Inc., 1997) and serotyping using the latex agglutination test. Production of Verocytotoxin 1 and 2 was determined using reverse passive latex agglutination test (VTEC-RPLA). All the three samples that were positive on BCM^{TM}0157: H7 (+) medium were screened for genes encoding for virulence on Polymerase Chain Reaction (PCR). All the 264 samples were also screened for brucella antibodies using Milk Ring Test (MRT) and a more accurate Indirect Milk ELISA.

According to Kenya Bureau of Standards (KEBS), 83.7% of the milk samples collected from Nairobi were of "bad quality" based on the threshold of 2 million c.f.u/ml for total bacterial counts, while only 50% of the same samples were classified as such based on the standard of 50,000 c.f.u/ml for coliform counts. Urban milk samples were of worse bacterial quality than rural samples. Nakuru urban had 81% milk samples classified as "bad quality" based on total counts (>2 million c.f.u/ml), and 60% classified as such based on coliform counts (>50,000 c.f.u/ml). In contrast, only 40% of samples from Nakuru rural were classified as "bad milk" based on both total and coliform counts.

Escherichia coli was recovered from 34% (91 out of the 264) of the samples analysed, majority (63%) of which were of faecal origin. Of the 264 samples, two yielded confirmed *E.coli 0157:H7* isolates, one from Nakuru rural and the other one from Nakuru urban. One of these isolates produced verocytotoxin 1 and this isolate was confirmed on PCR to be carrying genes for verocytotoxin production.

Six percent of the samples (16 out of 264) were positive for *Brucella abortus* antibodies on MRT. Four of the positive samples were from Nairobi (dry season) while Nakuru rural and urban had six positive samples each. ELISA detected more samples (21 out of 264 or 7.9%) as positive for brucella antibodies. These two tests for brucella antibodies had moderate agreement (*Kappa*=0.40, 95% confidence interval=0.19-0.60). The presence of brucella antibodies is an indication of possible presence of brucella organisms in milk, which may pose a risk to consumers. Those at high risk are consumers of traditionally fermented milk "maziwa lala", who constituted 6% of the households in Nakuru rural.

The health risk of zoonotic *E. coli* and brucellosis was considered to be minimal due to the common consumer practice of boiling milk (commonly taken in tea). In this study, over 96% of the households reported boiling milk prior to consumption. It is recommended that in the absence of pasteurisation, the practice of boiling raw milk by consumers be reinforced.

CHAPTER ONE

1.0 INTRODUCTION

Since market liberalisation in the early 1990s, the extent of informal milk marketing in Kenya has greatly increased (Omore *et al.*, 1999). This has in turn redistributed and increased the overall socio-economic benefits of smallholder dairy farmers, market agents and consumers. These benefits include regular cash income, creation of employment, increased food security, competitive prices and improved nutrition. These changes continue to be stimulated by the increasing demand for dairy products estimated at 3.8% per year for Sub-Saharan Africa (Delgado *et al.*, 1999). The demand for dairy products in urban centres has led to the establishment of smallholder dairy farms within and immediately around towns.

Given the current liberalisation of milk marketing, the smallholder dairy farms around urban areas are supplying raw milk directly to the consumer without pasteurisation. There are a very high proportion of raw milk sales yet there is no public milk quality control measures put in place. Over 80% of the marketed milk is sold raw to the consumer (Omore *et al.*, 1999). Concerns have been raised over public health hazards that people can be exposed to, particularly zoonotic brucellosis, tuberculosis and *E.coli* 0157:H7. Concerns over general bacterial and physical milk quality including hygiene practices of informal market agents, and drug residues have also been raised. This study was initiated to assess the public health risks associated with the informal marketing of raw milk and specifically

to address the problems of zoonotic diseases spread through milk in selected study areas of Nairobi and Nakuru districts at the consumer level. Besides assessment of bacteriological quality of milk in general, the main focus of this study was on two important zoonoses. First, enterohaemorrhagic *Escherichia coli* that is an emerging zoonosis in the world today having the cow as it's main reservoir. Given market liberalisation and thus increased informal milk marketing in the country, there is need to investigate the importance of informally marketed milk as a source of *Escherichia coli 0157:H7.* Secondly brucellosis as an existing zoonosis in Kenya (since it was first described in detail in humans in 1953 by Wright and others). There is renewed interest in brucellosis as a zoonosis spread through informally marketed milk. Hence, the main objectives of the study were to:

- Assess the bacteriological quality of informally marketed milk in Nairobi and Nakuru districts.
- Estimate the occurrence of brucellosis and enterohaemorrhagic *E.coli* 0157:H7 in raw milk market pathways.
- Assess the health risks that consumers of raw milk might be exposed to with regards to the hazards above.
- Recommend ways of preventing / minimising the health risks without discouraging raw milk markets.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Enterohaemorhagic *Escherichia coli* 0157:H7

2.1.1 Characteristics of the organism.

Enterohaemorrhagic *E. coli 0157:H7* infection has emerged as an important foodborne zoonosis in the recent years (Bleeme, 1994). The causative *E.coli 0157:H7* cerotype (the 0 refers to the somatic and the H to the flagella antigen) does not ferment sorbitol rapidly (Farmer and Davis, 1985) and does not produce β-glucoronidase (Thompson and Borczy, 1990). In addition it does not grow well above 41°C, so it may not be detected by standard procedures for enumerating faecal coliforms in food or water (Raghubeer and Mathes, 1990). Almost all *E. coli* 0157:H7 strains are uniformly susceptible to antimicrobial agents effective against gram-negative organisms (Ratnam *et al.*, 1988).

2.1.2 Pathophysiology of *E. coli* 0157:H7 infections

In humans, *E. coli* 0157:H7 causes haemorrhagic colitis characterised by severe abdominal cramps, bloody stools and little or no fever. Up to 10% of the cases may progress to haemolytic uremic syndrome (HUS), which has a case fatality rate of 3-5% (Neill, 1989; CDC, 1993). Infection with *E. coli 0157:H7* occurs primarily through the ingestion of contaminated food. Other documented sources of infection are *E. coli 0157:H7* contaminated water, person-to-person transmission and cow to human (Bleeme, 1994). Children under 5 years of age and the elderly have the highest risk of developing HUS.

Cattle have been indicated as a reservoir for *E. coli* 0157 as a result of the linkage of bovine products to human outbreaks and isolation of identical strains of the micro-organism from both infected humans and cattle (Wells *et al.*, 1991; Renwick *et al.*, 1993). The micro-organism is non-invasive in cattle and is known not to cause clinical disease. The *E. coli* 0157 strains are believed to inhabit the lower intestinal tract of cattle and are shed in the faeces. Preliminary evidence suggests that the shedding is transient and that the excretion period ranges from hours to weeks. On-farm studies have shown that generally, less than 1% of the U.S. cattle shed *E. coli* 0157, whereas the herd prevalence is higher (Wells *et al.*, 1991; Hancock *et al.*, 1994).

2.1.3 Pathogenesis of *E.coli* 0157:H7 infections

The pathogenesis of infection with *E.coli 0157:H7* is incompletely understood. The virulence properties involved are distinct from those of the enteropathogenic (EPEC), enterotoxigenic (ETEC) and enteroinvasive (EIEC) classes of *E. coli* (Sang *et al.*, 1992). The EIEC causes dysentery-like diarrhoea due to the invasion of epithelial cells and intracellular multiplication in the large bowels leading to inflammation and ulceration (Tzipori *et al.*, 1986; Toth *et al.*, 1990). The ETEC pathogenesis involves elaboration of enterotoxins (heat labile and heat stable). EPEC were the first group of diarrhoea causing *E.coli* to be identified and remain an important cause of disease worldwide (Gross, 1983). EPEC is different from the above two groups of *E.coli* because it belongs to a restricted set of serotype. They do not produce enterotoxins or induce keratoconjuctivitis on inoculation

into the conjunctival sac of the guinea pigs (Sereny test) (Gross, 1983). Adherence to intestinal mucosal cells and production of shiga-like toxin appear to be the major virulence properties (Griffin and Tauxe 1991). The *E. coli* 0157:H7 target follicle associated epithelium in humans where it causes attaching/effacing lesions. The same human isolate can cause attaching/effacing lesions in cattle indicating that similar pathogenic mechanisms operate across human and bovine species (Phillips *et al.*, 2000). Enterohaemorrhagic *E. coli* (EHEC) is associated with haemorrhagic enterocolitis and also haemolytic uremic syndrome (HUS). The vero/shiga toxins produced by the bacteria play a role in the occurrence of blood in the faeces and in the HUS by their action on the endothelial cells of the intestinal submucosa and the renal glomeruli respectively after resorption through the intestinal walls (Mainil, 1999).

2.1.4 Occurrence and distribution of *E.coli 0157:H7*

There is evidence that many causes of diarrhoea in Kenya are unexplained as was shown by the study of (Sang *et.al.*1992) on children with diarrhoea and measles from Kenyatta National Hospital. They reported on another group of enteropathogenic *E. coli* referred to as enteroadherent *E. coli* (EAEC). In a study carried out by Ombui *et al.* (1995) on raw commercial milk in Kiambu District there was no *Escherichia coli 0157:H7* among five non-sorbitol fermenting *E. coli* isolates (latex agglutination test for identification of *E.coli* serogroup 0157 was used). In an epidemiological study on infectious diarrhoerial diseases in children in a coastal rural area of Kenya, Saidi *et al.* (1997) isolated diarrhoeagenic *E.coli*

including EPEC, ETEC, and EHEC strains from 13.8% of the patients. These authors did not show the percentage of each type of *E.coli* isolated. Sang and Saidi (1996) isolated *Escherichia coli* 0157:H7 from a two year old boy with haemorrhagic colitis at Malindi hospital. This was the first confirmed case of haemorrhagic colitis due to *E. coli* 0157:H7 in Kenya. In a study on the Maasai in Southern Kenya, Sang and Davis found out that *E. coli* caused 21.2% (35 out of 165) of the diarrhoea cases. Of these 35 *E. coli*, 8 isolates were shiga toxin producing. Two isolates produced VT1 while 3 produced both VT1 and VT2 and 3 isolates produced VT2 (Sang personal communication). In Lagos, Nigeria, Akinyemi *et al.* (1998) found out that *Escherichia coli* accounted for 46% (83 out 182) of the bacteria causing acute gastroenteritis. Of these 83 *E.coli*, 49 (59%) were EPEC, 17 (20%) ETEC, 10 (12.1%) EIEC and 7 (8.4%) EHEC.

In the temperate countries, the reporting on this emerging zoonosis is quite extensive compared to developing countries. In 1982, the *Escherichia coli* serotype 0157:H7 was first identified as a result of two outbreaks of bloody diarrhoea in Michigan and Oregon, U.S.A. They were associated with the consumption of fast food hamburgers (Riley *et al.*, 1983). The public was generally unaware of *E. coli* 0157 existence until a decade later, when more than 500 laboratory-confirmed infections and four associated deaths in children occurred in four western states, also as a result of hamburger consumption (Centers for Disease Control (CDC), 1993). Recently, the number of reported *E. coli* 0157 cases associated with raw milk consumption has increased (Bleeme, 1994). In April 1986, a group of 60 kindergarten

children visited a dairy farm in Ontario, Canada and consumed raw milk (Borczyk *et al*., 1987). The 0157- infection was confirmed in 43 of 48 symptomatic cases, and the micro-organism was isolated from one of 67 healthy cattle from which faecal samples were collected. In August 1986, two Wisconsin dairies were associated with one HUS case, each in young children who drank raw milk (Martin *et al*., 1986). The *E. coli* 0157 was isolated from one of the cases and healthy heifers from both herds were found to be asymptomatically carrying the micro-organisms.

In 1996, Japan experienced nation-wide *E. coli 0157:H7* food poisoning outbreaks, which accounted for 700 confirmed cases with 9 deaths; many were of school children. The probable source of outbreak was eventually traced back to white radish sprouts which are common in boxed lunches for school children and office workers throughout Japan (Michino *et al*., 1999). As recently as November 1996, *E. coli 0157* was reported in two separate food poisoning outbreaks, this time on the West Coast of the United States and also in Lanarkshire, Scotland. In the states, the source of the infection was traced back to un-pasteurised apple juice, resulting in a nation-wide recall of the offending range of products. As a result of the outbreak in Scotland, 17 people died and more were hospitalised, demonstrating that this particular microrganism continues to cause serious health problems worldwide.

In the less developed areas of the western countries, no *E.coli 0157:H7* has been detected in

the studies (Griffin and Tauxe, 1991). In Central Australia, none were detected in diarrhoeal stools from 1443 aboriginal patients living in poor hygienic conditions (Albert and Bettelheim, 1989). In Korea, no *E.coli 0157:H7* were isolated from 231 children with diarrhoea (Kim *et al.*, 1989). In Sao Paulo, Brazil, no *E. coli 0157:H7* was isolated from stools of 95 children under one year of age with diarrhoea (Giraldi *et al.*, 1990). In China, stool blots of 15 out of 221 (7%) children with diarrhoea, and six out of 108 (6%) controls hybridised with the enterohaemorrhagic *E. coli* probe (Levin *et al.*, 1987). Of these, one strain from a case was *E. coli* 0157:H7, while no *E. coli* 0157:H7 was isolated from the other hybridising strains (Giraldi *et al.*, 1990). In India no *E. coli* was isolated from stool samples of 240 children less than 3 years of age whose stool blots hybridised with the haemorrhagic *E .coli* probe (Bhan *et al.*, 1989).

The majority of the outbreaks of *E. coli* 0157:H7 have been the result of transmission via foods of bovine origin. Among the eight outbreaks with an identified food vehicle in the United States, six were traced to ground beef and two to roasted beef. A Canadian outbreak was associated with drinking raw milk. Compared to other foods, raw milk is a relatively important vehicle for *E. coli 0157:H7*. In sporadic cases, the sources of infection were similar to those that caused outbreaks, particularly ground beef, raw milk, untreated water and person to person spread (Griffin and Tauxe, 1991).

2.1.5 Recovery of *E.coli 0157:H7* from milk

Although association of *E. coli* 0157 with consumption of milk has been well established, the organism is rarely recovered from milk. One reason may be that unlike other *E.coli*, *E. coli* 0157 does not multiply well at 44°C to 45°C (111 to 113°F), which is the temperature most often used to recover *E.coli* from foods (Riley, 1987). Secondly, *E. coli* 0157 is present in milk in very low numbers and thus requires enrichment medium to enhance growth prior to its isolation (Hill *et al.*, 1985). A chromogenic selective/differential plating medium called BCM^{TM}0157:H7 (+)(Biosynth International Inc.,1997) has been developed. This is a selective and differential plating agar for *E. coli 0157:H7*, which in identifying positive biochemical characteristics of the organism, utilises a more direct means of selecting and differentiating strain *0157:H7* from other *E. coli* strains. The selective/differential medium utilises chromogenic substances, biochemical indicators, selective carbohydrates fermentation scheme and a unique inhibitory system that recognises positive reactions for *E. coli 0157:H7* strain. This organism forms blue-black colonies that are dome shaped and measure 1.5-2.5 mm in diameter. A black precipitate surrounds the colony, colonies of other *E. coli* appear green on the medium (Biosynth International Inc.,1997).

We used two serological tests to type *E. coli 0157:H7* and these are: a) the latex agglutination test for the identification of *E .coli* serogroup 0157 therefore a potential verocytotoxin producing strain and b) reverse passive latex agglutination test (VTEC-RPLA) for detection of verocytotoxin (VT1 and VT2) produced by *Escherichia coli*

isolated from food and faecal samples (Oxoid Limited). Multiplex Polymerase Chain Reaction (PCR) is also available for identification of *Escherichia coli* virulence genes (Pass *et al*., 2000). The virulence mechanisms that characterise *E. coli* are genetically coded for by chromosomal, plasmid and bacteriophage DNAs. These include heat-labile (LT1, LT2a and LT2b) and heat-stable (ST1 and ST2) toxins, verotoxin types 1, 2, 2e (VT1, VT2 and VT2e respectively), cytotoxic necrotising factors (CNF1, CNF2), attaching and effacing mechanisms (eaeA), enteroaggregative mechanisms (Eagg) and enteroinvasive mechanisms (Einv). With the advent of PCR, it has become possible to identify these genes in bacterial isolates, offering the possibility of rapid diagnosis of the mechanisms operating in specific *E. coli* infections (Pass *et al*., 2000).

2.2 Brucellosis

2.2.1 Aetiology

Brucellosis is primarily an animal disease of economic importance in most countries of the world (McDermott *et al*., 1987). The disease affects human beings and domestic animals as well as wildlife. *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, and *Brucella canis* infect both animals and human beings but *Brucella ovis* affects only animals. *Brucella abortus* is the most widespread due to the universal distribution of cattle. However, *Br. melitensis* is the most frequently isolated as a cause of human illness (Wright *et al*., 1953; Manson-Bahr, 1956; Cox, 1966, 1968). *Br. melitensis* is common wherever goats are raised and is prevalent in developing countries. The 1978 Food and Agriculture Organisation

(FAO) animal health yearbook indicated that there is a high prevalence of bovine brucellosis in Africa. Similarly, *Br. abortus* and *Br. suis* are prevalent in cattle and pig raising areas, respectively.

Human infection with brucellosis depends upon contact with infected animals, their products or materials contaminated with animal discharges (Muriuki *et al*., 1994). Consumption of un-pasteurised milk and dairy products is the common method of transmission (Young, 1983). Raw, semi-cooked or pickled meat is also a source of human infections (Saddler, 1960). The main risk groups for human brucellosis are people who consume un-pasteurised animal products and all those in contact (directly or indirectly) with animals and their products e.g. veterinarians, butchers, abattoir workers and also laboratory workers handling brucella cultures. The widespread adoption of pasteurisation of milk and heat treatment of meats have reduced the morbidity of brucellosis in developed countries.

2.2.2 Clinical disease in man and animals

In man, infection may be abrupt and accompanied by chills and fever or it may be insidious with mild clinical symptoms (Flores-Castro and Baer, 1979). Diurnal fever is the most common symptom of human brucellosis. In most cases, chills and fatigue, weakness, profuse nocturnal sweating, anorexia with rapid weight loss accompany the fever (Young, 1983). Depending on where the organisms localise, other symptoms may be observed and this may be polyarthritis, pharyngitis, abdominal pain from splenomegally, hepatomegally

or mesenteric adenitis (Peery and Belter, 1960). Renal damage, orchitis and epididymitis are rare occurrences.

In cattle, after brucella infection is introduced in a herd, numerous abortions and a low fertility rate follows. Abortions and mastitis are accompanied by arthritis, weight loss, lameness and bronchitis with a short hacking cough. Abortion storm with retention of foetal membranes due to an inflammatory enlargement of the maternal villi are initial signs of introduction of brucellosis in a herd (Muriuki *et al.*, 1994). Abortion may occur at any stage of pregnancy but mostly occur in the third trimester. Most affected animals usually abort only once but subsequent infertility develops in some animals due to chronic endometritis and caruncular fibrosis. In male animals, organisms localise in the reproductive organs and associated lymphnodes. This leads to orchitis, epididymitis, seminal vesiculitis and inflammation of the ampullae of the ductus differens. These lesions lead to impaired sperm production and deformities leading to reduced fertility. Young animals infected in utero or post natally tend to recover spontaneously before reaching sexual maturity. Some, however may, remain infected until maturity when the disease manifests during pregnancy. The presence of brucellosis in animals is a potential threat to the health of people in direct or indirect contact with them (Muriuki *et al.*, 1994).

2.2.3 Occurrence

Brucellosis in Kenya has been documented in both animals and human beings (Wright *et*

al., 1953; Cox, 1966; Oomen, 1975; Kagunya, 1977; Paling et *al.*, 1988). The first report of brucellosis in animals dates back to 1914 (Anon. 1914). This was followed by widespread abortions in 1923, which were suspected to have been due to brucellosis. A milk ring test (MRT) survey at Kenya Co-operative Creameries (KCC) branches gave an overall reactor rate of 19% with a range of 12-38.3% (Anon, 1955). Just before this finding, W right *et al.* (1953) had described the first cases of human brucellosis. Manson -Bahr (1956) then described the clinical aspects of brucellosis while Cox (1966) studied pastoral communities in Karamoja in North- East Uganda and found a high prevalence of human brucellosis. Heisch *et al.* (1963) isolated *Br. suis* from rodents at the coast, while Waghela and Gathuma (1975) reported a serological evidence of porc ine brucellosis in Kenya. Waghela (1976) examined cattle sera at the Kenya Meat Commission (KMC), Athi River and found the highest reactor rate in cattle from Nyanza Province (15.78%) and the lowest in cattle from Central Province (1.67%). Kagunya (1977) reported the presence of anti-*Brucella abortus* agglutinins in cattle and camels and also anti-*Br. melitensis* agglutinins in sheep and goats. Wildlife species have similarly been reported to have anti-brucella agglutinins although no isolation has been made in Kenya so far (Paling *et al.*, 1988).

The presence of brucellosis in the major domestic animal species in Kenya (Philpot t and Auko, 1972; Waghela and Gathuma, 1975; Kagunya, 1977; Waghela *et al.*, 1978) and in some wildlife species (FAO\WHO, 1971; Paling *et al.*, 1988) indicates an abundant source of infection for man. The prevalence of brucellosis in animals and man is undoubtedly higher in the pastoral areas of Kenya where large numbers of livestock are kept in close

contact with people (Oomen and Wegener 1982; Muriuki *et.al.*, 1994). Kagumba and Nandokha (1978) reported higher prevalence in cattle from Maasai land and other semi-arid areas in East Africa than in the intensive livestock farming areas.

Human and animal brucellosis has also been reported in Zimbabwe, Botswana (Cooper and Carmichael, 1974), Sudan (Ibrahim, 1975), Sierra Leone (Opitz, 1969), Tunisia (El Fourgi, 1973) and Zambia (D'cruz, 1976). In Nigeria, human brucellosis has been documented (Collard, 1962). Gidel *et al.* (1976) found a 10% apparent prevalence among pastoralists' people of Upper Volta (Burkina Fasso). In 1962, Collard found that 26.4% of human serum samples from the Fulani of Nigeria contained *Br. abortus* agglutinins. Ovine and caprine brucellosis (Anon. 1985) and human brucellosis (Boargob and Muhammed, 1989) occur in Libya.

There is a direct relationship between the level of brucellosis in animals and the incidence of human infection (Oomen and Waghela, 1974), which has been shown to be influenced by methods of animal husbandry, standards of hygiene and food customs (Escalante and Held, 1969). Milk from cattle, sheep, goats, camels, water buffaloes and other domesticated animals is a common source of infection to humans. *Br. abortus* is, however, less likely to be transmitted this way, compared with *Br. melitensis*, which is more infective (Flores-Castro and Baer, 1979). Dairy products like cheese, cream, butter, chocolate and yoghurt can be a source of infection if they are prepared from un-pasteurised milk (CDC, 1976). The

isolation of brucella organisms from goat milk in Kenya (Philpott and Auko, 1972) suggests that raw milk may be a medium of transmission. The cream function of milk is more heavily laden with organism than the skimmed fraction (FAO\WHO, 1971) and requires more heating to kill the organism.

Application of serological diagnostic tests for bovine brucellosis has been achieved in diverse areas using the Rose Bengal Plate Test (RBPT) (Kagumba and Nandokha, 1978). and complement fixation test (CFT) (Cargill *et al.*, 1985 and Sutherland *et al.*, 1986). Most milk testing for antibody to *B. abortus* is currently done using MRT , which has a sensitivity of about 89% (Nicoletti, 1969; Hunter and Allen, 1972). The MRT is a good screening test for cattle, however, milk samples taken shortly after parturition, near the end of the lactat ion cycle and from mastitic quarters may give false positive reactions and MRT is of limited use in beef cattle (Macmillan, 1990).

A more accurate indirect enzyme immunosorbent assay (ELISA) (sensitivity=95% and specificity=99%) for testing brucella antibodies in milk has also been recently improved and validated (Kerkhofs *et al.*, 1990; Nielsen *et al.*, 1996). The milk ELISA is more sensitive than MRT, CFT and RBPT (Sutherland *et al.*, 1986; Kerby *et al* 1997) and reportedly is able to detect antibodies in dilutions up to 1:100 (Forschner and Bue nger, 1986). The indirect ELISA is used to detect *B. abortus* antibodies in bovine milk and serum samples. The assay uses *B. abortus* smooth lipopolysaccharide as antigen, immobilised on a polystyrene matrix; milk diluted 1:2 or serum diluted 1:50, in a buffer contain ing divalent

cation chelating agents EDTA and EGTA (ethyleneglycol-bis-aminoether-N,N,N,N-tetraacetic acid) to reduce non-specific reactions; and a mouse monoclonal anti body specific for an epitope of bovine IgG, conjugated with horseradish peroxidase (Vanzini *et al.*, 1998).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Areas of study

Study sites were selected within production systems (extensive and intensive) and human population density (urban, peri-urban and rural) strata. The geographical units selected from each site also covered a variation from low to high income classes. Nakuru district represented extensive production systems and low population density (also medium market access). Nairobi represented intensive production systems and high population density (also high market access)(Table 1).

3.2 Marketing Channels and Milk Targeted for Sampling

Only about 12% of marketed milk originating from smallholder dairy farmers in Kenya is pasteurised while the rest is sold raw through informal market channels that handle small quantities of milk, generally less than 100 litres/day per unit (Omore *et al.*, 1999). The latter was targeted for sampling (Figure 1).

Table 1: Study population: estimates of consumer concentration, market agents and cattle density at each site[a] and stratification criteria.

Acronym	District	Consumers			Market agents		Cattle Production system			
		Market access	Human density/km^2	No. of small traders	No. Coops/SHG[b]	No. processor	AEZ[c] potential	Density/ KM^2	Main breed	Main feeding system
Site1 (IHMA)	Nairobi	High	>500	>1000	16	8	High	100	Exotic	Intensive
Site2 (EMMA)	Nakuru	Med	150	>300	6	>20	High	48	Exotic	Extensive
		Low	28	<100	0	0	Med	52	Zebu	Extensive

[a]Estimates derived from Peeler and Omore (1997) and SDP systems and sub systems studies.

[b]SHG - Self Help Groups

[c]AEZ - Agroecological Zone potential

IHMA - Intensive High Market Access

EMMA - Extensive Medium Market Access

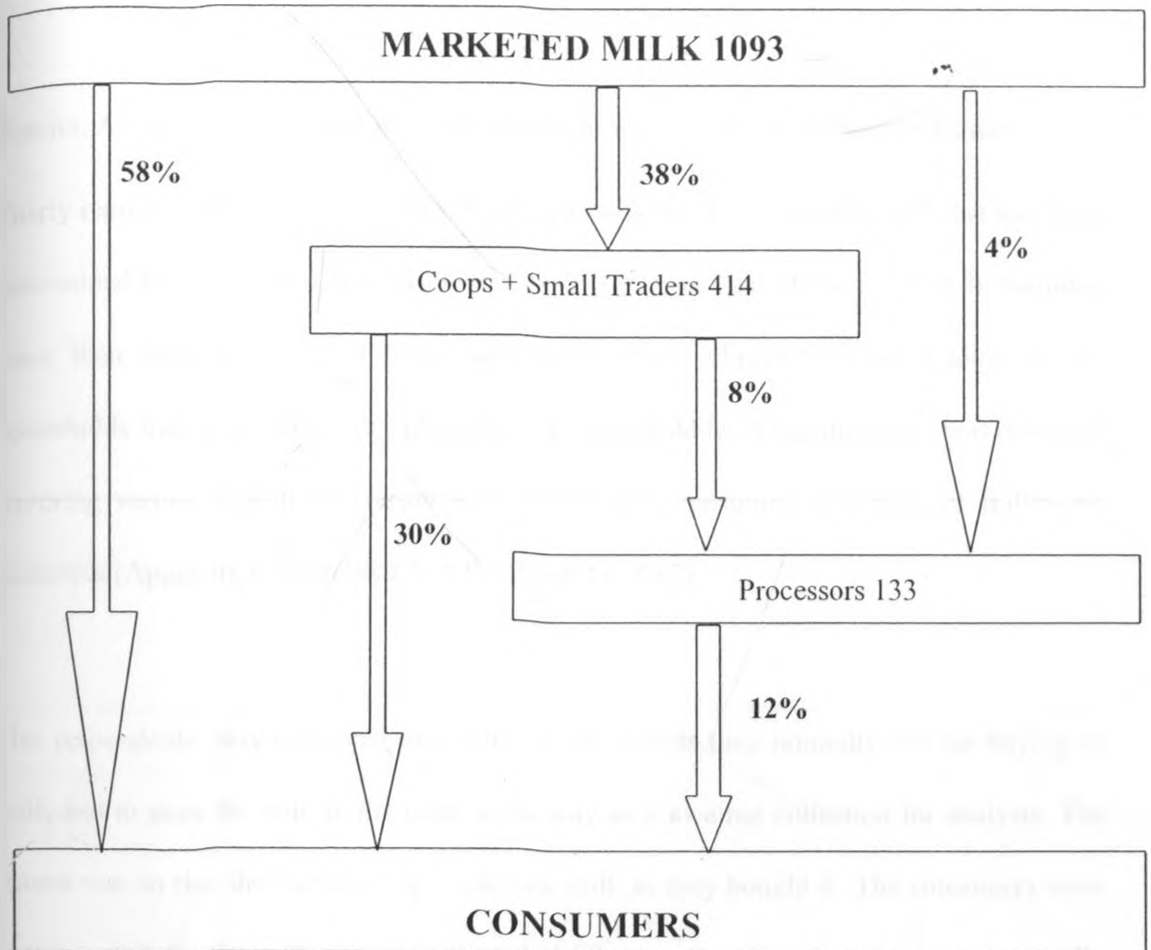

Figure 1: Marketed National Milk Flow from Smallholders in Kenya ('000 MT)

(Adapted from Omore et al., 1999).

3.3 Sampling

Between November 1998 and August 1999, a total of 264 households purchasing un-pasteurised milk were surveyed, 211 in the dry and 53 in the wet season. Of the 211 surveyed in the dry season, 49 were from Nairobi district and 162 were from Nakuru

district. All the 53 households surveyed in the wet season were from Nairobi district.

Thirty representative clusters were randomly selected out of the available 120 that had been determined by the Central Bureau of Statistics (CBS) in each district. Seven households were then randomly selected from each of the thirty clusters, giving a total of 210 households that were studied in each district. A household level questionnaire was designed covering various aspects of consumption and to test consumer awareness of milkborne zoonoses (Appendix 6, Sections 2.3, 3.1-3.9 and 4.0-4.12).

The respondents were requested to use the usual vessels they normally use for buying in milk and to store the milk in the usual same way as it awaited collection for analysis. The reason was so that the consumers provide raw milk as they bought it. The consumers were compensated for the milk sample collected. Milk samples (50 ml each) were aseptically collected in sterile plastic tubes in the mornings and transported in ice cooled boxes to the laboratory for analysis. For the purpose of analysis in the laboratory, the consumer was asked if the milk given was raw or boiled. Analysis commenced immediately the samples arrived in the laboratory and within six hours of collection.

Those households reported to be purchasing un-pasteurised milk were noted on their questionnaire. In Nairobi, out of the 210 households selected for the survey, only 52 were found to be purchasing un-pasteurised milk and out of these it was possible to collect milk samples from only 49 households (94.4%) in the dry season. In the wet season, 53 milk

samples were collected from 56 households surveyed in Nairobi. In Nakuru district, 199 households were identified during the dry season but only 162 households (81.4%) were sampled. In Nairobi, effort was made to administer questionnaire to and collect milk samples from the same households during the two seasons. Where it was not possible to interview the same respondent during the second season, because the family had moved residence or for any other reason, a replacement was selected within the same locality. For the purpose of analysis in the laboratory, the consumer was asked if the milk given was raw or boiled and this was indicated on the sample bottle. This information was checked in the laboratory by the peroxidase test and the agreement was good between the consumers response and the peroxi dase results (kappa 0.7).

3.4 Preparation of media, diluent and test reagents

Unless otherwise stated, details of media, diluents and reagents preparation are as given in appendices 1-4.

3.5 Laboratory Analysis of the milk samples

3.5.1 Assessment of the bacteriological quality of milk

The bacteriological quality of the sampled raw milk was assessed using direct culture methods. Each sample was thoroughly mixed and tenfold serial dilutions ranging from 10^{-1} to 10^{-8} made in potassium hydrogen phosphate buffer pH 7.2 (appendix 3).

Appropriate dilutions were then cultured for total bacterial count and coliform count.

3.5.1.1 Total bacterial count

Because the milk was suspected to have heavy bacterial load, as many as 10^{-4} to 10^{-8} dilutions were chosen to culture for total counts. One millilitre of diluted sample was dispensed in a well-labelled 90mm diameter petri dish (sample number and dilution factor) and immediately mixed with approximately 20ml of cooled molten standard plate count agar (SPCA) (Oxoid, APHA, appendix; 1.1). After cooling and solidification of agar, the plates were inverted and then incubated at 32^0C for 48 hours. Plates with countable colonies (25-250 c.f.u/plate) were selected and the number of colony forming units determined with the aid of a colony counter (Gerber). Total counts per millilitre of milk were computed following guidelines by Speck (1984) and the Kenya Bureau of Standards (KEBS, 1996) (Table 2). Total counts were transformed to the log base 10 before calculating the summary statistics and analysis of variance. Dummies were generated for district, season setting and purchase points and total counts were compared between the different districts, seasons, settings and purchase points.

3.5.1.2 Coliform counts

Milk dilutions 10^{-1} to 10^{-4} were chosen for coliform counts. One millilitre of each dilution was dispensed into a well-labelled 90mm petri dish and 15ml of cooled molten Violet Red Bile (VRB) agar (appendix1.2) immediately added and mixed thoroughly. The mixture was then allowed to cool and solidify after which, all the plates were overlaid with a thin layer of the same VRB agar to prevent spreading of surface colonies. All the plates were incubated

at 37^0C for 24 hours. Plates showing typical red coliform colonies in the countable range of 15-150 per plate were chosen and the colonies counted. Computation was done following guideline by Speck, (1984) and KEBS (1996)(Table 2). Coliform counts were transformed to the log base 10 before calculating the summary statistics and analysis of variance. Dummies were generated for district, season setting and purchase points and coliform counts were compared between the different districts, seasons, settings and purchase points.

Table 2: Standard specification s of unprocessed whole milk KEBS, 1976 revised 1996

Quality judgement	Total bacterial counts/ml SPCA 32°C for 48 hours	Coliform count/ml VRBA 37°C for 24 hours
Very good	<1,000,000	<1,000
Good	1,000,000-2,000,000	1,000-50,000
Bad	2,000,000-5,000,000	50,000-500,000
Very bad	>5,000,000	>500,000

Key:

SPCA- Standard Plate Count Agar

VRBA- Violet Red Bile Agar

3.6 Further studies on coliforms

After counting, the coliform colonies were subjected to further studies to determine the types and proportion of the contaminating coliforms and to screen for the presence of zoonotic *E.coli 0157:H7*.

3.6.1 Confirmation of the coliform isolates

Four to six pink colonies were picked from VRBA and streaked on Mac Conkey agar (Oxoid) (appendix 1.3). They were then each inoculated into 4ml brilliant green broth (Oxoid)(appendix1.6) in a fermentation tube fitte d with inverted Durham tube. Incubation was done at 37^0C for 48 hours. Production of gas and acid confirmed the presence of coliforms. The confirmed coliforms were purified by streaking a loopful of culture broth from the positive tube onto eosin methylene blue agar (Oxoid)(appendix 1.5). Further purification was done by subculturing the dark centred colonies, with or without a metallic sheen, onto tryptone Soy agar (Oxoid)(appendix 1.4). Purity of the coliforms was ascertained by gram staining and examining slide smears under the microscope. Two reference strains, *E. coli* K-12 and *E. coli* V517 were used. K-12 is a non-pathogenic laboratory strain used in genetic and molecular studies.

3.6.2 Characterisation of the coliform isolates base d on the IMViC pattern

The purified coliforms were tested for the production of indole, acid (methyl red react ion), acetylmethyl-carbinol (Voges proskauer test) and growth on citrate agar slants as the sole source of carbon, (collectively abbreviated as IMViC). Briefly, the test was carried out as

follows; one colony was inoculated into 4ml of tryptone water (Oxoid)(appendix 1.7), MRVP medium (Oxoid)(appendix 1.8) and Simmons citrate (Oxoid)(appendix1.9) agar slants (a straight inoculating wire was used). After incubation at $37^{0}C$ for 48 hours, seven drops of indole reagent (appendix 2.1.1) were added to the tryptone water culture to test for indole production (red-positive). Methyl red (appendix 2.1.2) pH indicator was added into one half of the MRVP culture broth to test for acid production. To the other half of the MRVP culture broth, 0.1 ml of 5% alcoholic alpha-naphthol, 0.1 ml of 40% potassium hydroxide and a few creatine crystals were added into the other half of the MRVP culture broth to test for acetyl-methyl carbinol; the contents were well shaken and tubes sloped before taking the readings (pink colour- positive while yellow colour- negative). Growth on citrate slants was indicated by visible colonies and colour change of agar from green to blue. The results were interpreted and organisms identified as in Table 3. Citrate positive cultures were tested for urease by streaking on urea slants and colour change to pink noted as positive.

Table 3: Identification of coliforms and related organisms isolated from milk samples (adopted from: National Mastitis Council, Inc.,1990)

Characteristics	Strong lactose (+)		Weak lactose (+)		Lactose negative (-)		
	E. coli[1]	Kleb[2*]	Ent[3*]	Citro[4]	Prot[5*]	Ser[6]	Peudo[7]
Indole	+	-/+	-	-/+	+	-	-
Methyl red	+	-	-	+	+	-	-
VP	-	+	+	-	-	+	-
Citrate	-	+	+	+	+	+	-
Urease	-	+	-	d	+	-	-

-/+ most strains are negative

d different biochemical types

* some species of the genus will show different reactions for some of the tests

Key:

1. Escherchia coli

2. Klebsiella pneumonia

3. Enterobacter aerogenes

4. Citrobacter freundii

5. Proteus vulgaris

6. *Serratia* spp.

7. *Pseudomonas* spp.

3.6.3 Test for the origin of the coliforms

Confirmed coliforms were inoculated into lactose broth (Oxoid)(appendix 1.10) in culture tubes fitted with inverted Durham tubes and incubated in a water bath at 44.5^{0}C for 48 hours. Faecal coliforms were identified by show of growth and production of gas. All the *E.coli* were checked for purity by gram staining and most of the isolates showed uniform gram-negative rods. Identified *E.coli* cultures were stored in duplicates, one set in 10% glycerol at –20°C and the other set in semi solid agar (1%) at 4°C (Appendix 1.13 & 1.12 respectively).

3.6.4 Identification of *E.coli 0157:H7* by culturing

This was done by streaking confirmed *E.coli* isolates onto Biosynth selective indicator medium for *E.coli 0157:H7* BCM^{TM}0157:H7(+) medium (Biosynth Biochemica, Biosynth International Inc., U.S.A)(appendix1.14). The stored isolates were resuscitated by leaving them overnight at room temperature then streaking on TSA slants. The BCM ^{TM} 0157:H7(+) medium agar plates were prepared according to the instructions of the manufacturer (appendix 1.14). After streaking the organism on this medium the plates were incubated at 35°C for 24 hours. The isolates were streaked alongside a standard *E. coli 0157:H7* obtained from the University of Amsterdam, Department of Medical Microbiology. A positive *E. coli* 0157:H7 strain showed blue – black colonies with a black precipitate surrounding the colony, which was dome shaped and approximately

1.5-2.5 mm in diameter. Ordinary *E.coli* (negative) showed green colour or clear to light

blue colour.

3.7 Serological tests for E.coli 0157:H7

3.7.1 Serogrouping (Latex agglutination test)

The blue black colonies from BCM™0157:H7(+) medium were cultured on non-selective tryptone soy agar and serogrouped using slide agglutination test (Oxoid, Diagnostic Reagents, *E. coli 0157* Test) employing latex beads coated with specific rabbit antibody reactive with the 0157 somatic antigen. The kit for this test was provided by Oxoid Limited, Basingstoke, England. For details of the kit see appendix 5.1. Briefly, one drop of the test latex was dispensed onto a circle on the reaction card. A loopful of saline was dropped on the circle and then a portion of the colony to be tested was carefully emulsified in the saline drop to form a smooth suspension. One drop of the test latex and one drop of the suspension were then mixed and spread to cover the reaction area by using an applicator stick. The card was rocked to and fro and co-agglutination observed within one minute. If co-agglutination with the test reagent occurred, a further portion of the colony was tested with the control latex reagent to ensure that the isolate was not an auto-agglutinating strain. Co-agglutination within one minute was an indication that the isolate belonged to the 0157 serogroup, which is a potential verocytotoxin producer.

Positive and negative controls were used to check for the correct working of the latex reagents before tests were carried out each day. The positive control used in this study was a

suspension of inactivated *E.coli 0157:H7* cells in a buffer and this caused visible agglutination with latex reagent within one minute. The negative control was a suspension of *E.coli 0116* cells in a buffer and this caused no agglutination with latex reagent.

3.7.2 Test for Verocytotoxin Production

The test kit was obtained from Oxoid Unipath Limited, Basingstoke, England. Details on the components of the kit are in appendix 5.2. Briefly, the *E.coli 0157:H7* organisms isolated in this study were tested for their potential to produce verocytotoxin 1 (VT1) and verocytotoxin 2 (VT2). The organisms were cultured on brain heart infusion agar at 37 °C for 24 hours. After incubation, the growth was removed using a microbiological loop and suspended in 1ml of physiological saline (0.85% Nacl) containing polymixin B at a concentration of 5,000 units/ml to facilitate the release of verocytotoxin. Extraction was continued for 30 minutes at 37°C and shaking occasionally. After extraction, the culture was centrifuged at 4,000 rpm for 20 minutes at 4°C. The use of polymyxin B facilitates the release of verocytotoxin. The supernant was used in the assay for toxins. The principle involved in the testing for toxin was that, the polymer latex particles sensitised with purified rabbit anti-serum that is reactive either with *E.coli* verocytotoxin 1 or verocytotoxin 2. Co-agglutination results in the formation of a lattice structure that on settling forms a diffuse layer on the base of the V-bottom micro titre well. If *E .coli* verocytotoxin is absent or at a concentration lower than the assay detection level, no such lattice structure will form. Instead a tight button will be observed. Multiplex Polymerase

Chain Reactions (PCRs) were run on the two serologically confirmed and one highly suspect *E. coli* 0157:H7 isolates for identification of the virulent genes. Briefly, a 24-hour nutrient agar growth was inoculated into Tryptic Soy Broth and incubated overnight. The growth was then centrifuged at 1200 rpm for 5 minutes and the supernatant discarded. To the sediment, 500μl of deionised water was added and heated at 95°C for 5 minutes to lyse the bacteria. This was then stored at -80°C until ready to use. The PCRs were run according to Pass *et al.* (2000) with the following minor modifications. Total volume for one reaction was 20μl which consisted of, DH_2O 11.4μl, Buffer 2.0μl , $Mgcl_2$ 1.2μl, dNTPs 0.8μl, Primers 0.2μl (the primers were obtained from PE-Applied Biosystems, Warrington, United Kingdom), Template 4.0 μl and Taq 0.4μl.

3.8 Tests for Brucellosis

The possibility of the milk containing brucella bacteria that cause the disease brucellosis in man was indirectly assessed by testing for the presence of *Br.abortus* antibodies in the milk using brucella milk ring test (MRT) and indirect enzyme linked immunosorbent assay (ELISA). The results were recorded as either 1 for positive or 0 for negative reaction. Dummies were generated for both the MRT and ELISA. Logistic regression of ELISA was done on the season, district, setting and on the purchase point.

3.8.1 Brucella Milk Ring Test

The MRT was carried out as described by Alton *et al.* (1977). Briefly, 1 millilitre of non-homogenised milk was pipetted in a skatron tube (Skatronas, LIER, Norway). Then a drop

of haematoxylin stained *Br. abortus* antigen (IFFA-Merieux, Lyon) was added and mixed well by inverting the tube several times. This was then incubated in a water bath at 37^{0}C for one hour. A positive clear result consisted of a blue coloured cream layer and a white milk column while a negative result consisted of a blue milk column and white cream layer.

The mechanism of a positive reaction was thought to dependent on two reactio ns (Brinley Morgan, 1967). (I) The milk fat globules are aggregated by a fat globule agglutinin (an antibody), (ii) the added stained brucella cells are agglutinated by their antibodies and these aggregates. The antibody-antigen complexes adhere to the milk fat globules and rise with them to the surface to form a coloured cream layer.

3.8.2 Indirect Milk ELISA

This is a highly sensitive and specific test (sensitivity 95% and specificity 99%) for milk antibodies to *Br. abortus* described by Nielsen *et al.*, 1996. Briefly, polysterene 96-well flat bottomed plates were coated with 100μl of 0.5mg/well of the *Br.abortus* smooth lipopolysaccharide antigen in coating buffer (0.06M carbonate buffer pH 9.6) and kept overnight in a humid box. The plates were thereafter washed five times with phosphate buffer (0.01M phosphate buffer of pH 7.2 containing 0.05% Tween-20 and 0.15M NaCl), dried and blocked using 200μl/well of 0.1% gelatin and incubated at 25°C for 30 minutes. The plates were washed again, dried and milk samples added at 100ul/well diluted 1:2 in milk diluent (0.01M phosphate buffer, pH 6.3 containing 0.015M NaCl, 0.05% Tween-20, 15mM EGTA). The plates were then shaken for 2 minutes in an orbital shaker and

incubated for 28 minutes at 25°C. The plates were then washed and 100 μl/well of monoclonal antibody conjugated to horseradish peroxidase (dilution 1:1600) added and incubated for one hour at 25°C. The plates were washed again, dried and the substrate (0.05M citrate buffer, pH 4.5 containing 1mM hydrogen peroxide and 4mM 2,2-azino-bis 3-ethylbenthiazoline-6-sulfonic acid (ABTS) added at 100μl/well. The plates were incubated for a maximum of 15 minutes and the absorbance read at 414nm. Brucella positive and negative serum and milk controls were included. The control serum samples were diluted 1:50, while milk samples were diluted 1:2 in the milk diluent. Each milk sample was tested in duplicate. The modification of this procedure was that the cut-off value was determined by using twice the mean of the negative control samples (Savingy and Voller, 1980) and not by the targeted reading described by Wright *et al.* (1985).

3.9 Statistical Analysis

Data were entered in Stata program (STATA[tm] 7.0 Statistics/ Data Analysis copyright 1984-2001, Stata corporation). Descriptive statistics for continuous variables (Total and coliform counts) was done after the counts were normalised through logarithmic (base 10) transformation. Comparison of milk quality was done between the two districts (Nairobi and Nakuru), the two seasons, wet and dry (Nairobi consumer season 1 and Nairobi consumer season 2, respectively), between urban and rural milk (Nakuru urban and Nakuru rural) and between main purchase points (producer gate, street vendor, kiosk and corner shop) using one-way ANOVA (Appendices 5.1-5.4). Kappa test statistics in stata was used

to assess level of agreement between ELISA and MRT tests for brucellosis. Logistic regression of MRT and ELISA was done on district, season, setting (urban vs. rural) and purchase points.

CHAPTER FOUR

4.0 RESULTS

4.1 Consumer survey

Most of the households purchasing raw milk actually boil it prior to consumption. In Nairobi and Nakuru urban, 100% of the households reported boiling milk prior to consumption while in Nakuru rural, 96% reported boiling milk . Pasteurised milk is also frequently boiled indicating that boiling of milk is routine mainly to lengthen shelf-life. Those "not boiling" consume it heat treated in foods such as tea and po rridge.

The main purchase point of raw milk in Nakuru is the producer gate (66%) and milk bars/shops (29%) while in Nairobi it is mainly shops/ milk bars (59%) and street vendors (40%). There was no significant difference in milk quality between the different purchase points ($p>0.05$). Consumers preferred raw milk to pasteurised milk based on better taste, affordable price and high butterfat content. The main constraints on the purchase of raw milk were listed as (I) health risks involved (42% households reported) and (ii) bad quality due to adulteration (27% households reported). Only one and four households in Nairobi and Nakuru, respectively, reported having had a member of their household diagnosed with brucellosis.

4.2 Bacteriological analysis

Generally Nairobi dry season milk was of poorest quality (85% unacceptable) base d on the total counts while the wet season milk had the poorest quality of milk based on the coliform counts (over 70% unacceptable). Milk from Nakuru rural was of better quality based on both coliforms (70% good) and total counts (90% good) (Figure 2 &Table 4). Total and coliform counts were normalised through logarithmic (base 10) transformation before analysis.

Table 4: Proportion of Milk samples with unacceptably high bacterial counts

District/season	Total counts >2 million cfu/ml		Coliform counts > 50,000cfu/ml	
	N	%	N	%
Nairobi (dry)	41/49	84%	22/46	48%
Nairobi (wet)	43/53	81%	38 /53	71%
Nakuru (urban)	47 /58	81%	23/58	40%
Nakuru (rural)	35/104	34%	11/104	11%

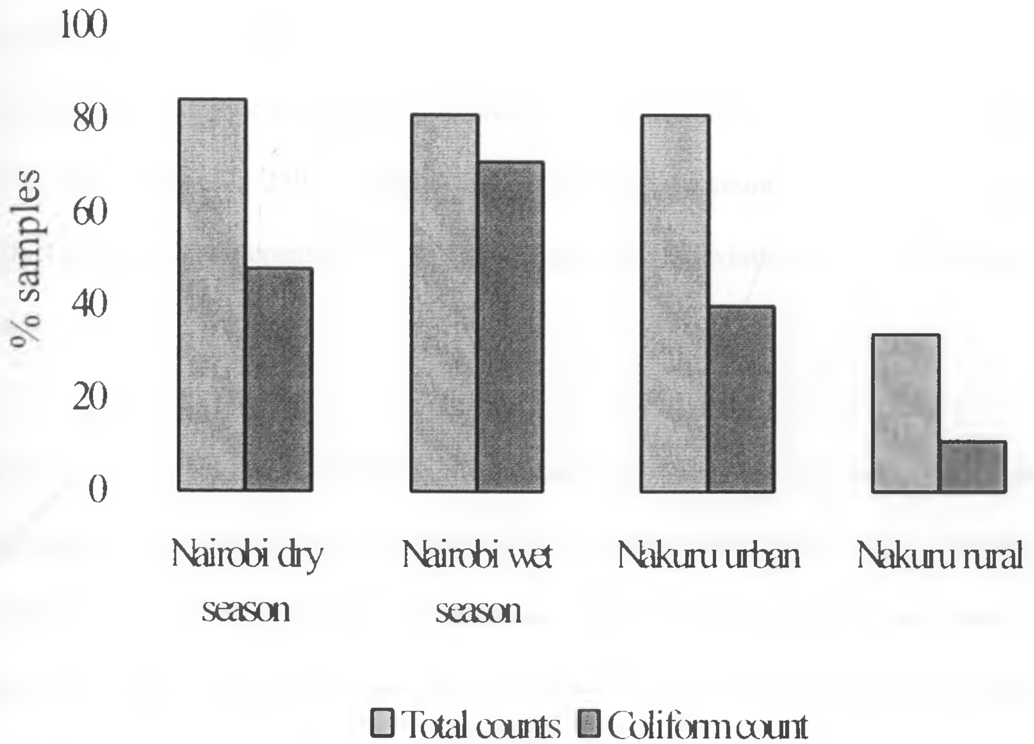

Figure 2: Comparison of bacterial counts in milk among consumers of Nairobi and Nakuru.

4.2.1 Total bacterial count

For the dry season survey in Nairobi, 16.3% of the milk was of good quality as per the Kenya Bureau of Standards. Most of the milk, 41 out of 49 samples (83.7%), was of unacceptable quality i.e. it had total counts of over 2,000,000 per ml (KEBS, Table 2). Two samples had less than 25 c.f.u/plate on the plate count agar while 15 samples had colonies in the countable range (25-250 c.f.u/plate) and 29 samples un accountable colonies per plate (> 250 c.f.u/plate). Mean count of 1.7 x 10^8/ml and standard deviation of 1.3 x 10^8/ml was recorded.

Ten out of 53 (18.9%) samples from the wet season in the Nairobi survey were found to be of good quality. The rest of the milk samples (81.1%) were of bad quality. Only 8 samples showed no growth on PCA, while 31 samples had colonies in the countable range and 22 samples had uncountable colonies. The mean count was 2.1 x 10^8 with a standard deviation of 1.9 x 10^8.

Of the 58 samples from Nakuru urban (dry season), 11 (18.9%) qualified as good milk while the rest (81.1%) had unacceptably high counts. Nine samples had no growth while 44 samples had colonies in the countable range and 5 samples had overgrowth. A mean of 1.8 x 10^8 standard deviation of 2.1 x 10^8 was recorded.

Nakuru rural (dry) had by far the best quality of milk with over 60% samples being of

acceptable quality (69 out of 104). Thirty six samples had no growth while 62 had growth in the countable range and six samples had uncountable colonies. Mean count of 5.2×10^7 and standard deviation of 1.4×10^8 (Table 5).

Based on total counts, the milk from rural settings was of significantly better quality than that from urban settings (p=0.0000) and milk consumed in Nakuru district as a whole was of better quality compared to that in Nairobi district. However, there was no significant difference in quality based on total counts in the milk consumed during the dry and wet seasons neither was there any difference in milk quality between the different purchase points ($p > 0.05$) (Appendices 5.1-5.3).

4.2.2 Coliform counts

In Nairobi dry survey, only 46 samples were processed for coliform counts while three samples got spoiled during the processing. Out of the 46 samples, 24 were of good quality. Seven samples showed no growth while 25 samples had colonies in the countable range (15-150 c.f.u/plate) and 14 had uncountable colonies (>150 c.f.u/plate). A mean count of the coliforms was 4.5×10^5 and standard deviation of 6.2×10^5.

About 28.3% of the samples from Nairobi wet season had coliforms less than 50,000/ml while 71.7% (38 out of 53) had unacceptable high counts. Seven samples had no growth while 24 had growth in the countable range and 22 had overgrowth. The mean count was 8.5×10^5 with standard deviation of 6.2×10^5.

Samples from Nakuru urban area, had 60.3% qualifying as good while 39.7% were bad milk samples (23 out of 58). Four samples had no growth while 46 had countable colonies and eight had overgrowth. Mean count of 2.8×10^5 with a standard deviation of 5.4×10^5 were recorded.

Ninety-three out of 104 (89.4%) samples collected from Nakuru rural (dry season) were of good quality and only a few (10.6%) were of unacceptably bad quality. Forty-four samples had less than 15 colonies per plate while 57 plates had between 15 and 150 colonies and three samples had over 150 colonies per plate colonies. A mean of 9.5×10^4 and standard deviation of 3.4×10^5 was recorded (Table 5).

Quality of milk based on coliform counts varied significantly between urban and rural settings, Nairobi and Nakuru districts and between the two seasons, wet and dry ($p < 0.05$). Milk consumed in rural setting, and that of Nakuru district was of better quality than that consumed in urban centres and in Nairobi district respectively. Also milk consumed during the wet season was of better quality than that consumed during the dry season. However there was no difference in quality of milk based on coliform counts between the different purchase points ($p > 0.05$). (Computer outputs 5.4-5.6).

Table 5: Description of bacterial counts by location of consumer households

Parameter	Nairobi (dry)			Nairobi (wet)			Nakuru (urban)			Nakuru (Rural)		
	n	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd
Total plate counts/ml $x10^6$	49	1700	1300	53	2100	1900	58	1800	2100	104	520	1400
Coliform plate counts/ml $x 10^6$	46	0.45	0.62	53	0.85	0.75	58	0.28	0.54	104	0.09	0.34

4.2.3. Proportion of the different coliforms

Out of 264 milk samples, three were lost during processing and therefore not included in the analysis for coliforms. Of the remaining 261 samples, 210 (79.5%) yielded coliforms distributed as follows: 91 (43.3%) were *E coli*, 28 (13%) were *Enterobacter aerogenes*, 16 (7.7%) *Klebsiella spp* and 10 (4.8%) C*itrobacter spp.* Sixty-five isolates could not be classified in any of the above groups (Table 6).

4.2.4 . Isolation of *E coli* and Identification of *E.coli* 0157: H7

Faecal *E. coli* is a widely accepted indicator of food contamination with faecal matter. Of the 91 *E.coli* positive samples, 57 (63%) yielded *E. coli* of faecal origin. All the *E. coli* isolates were subjected to the tests for identifying *E. coli 0157:H7.* Of the 91 isolates, 3 (3.3%) showed blue-black colonies reminiscent of *E. coli 0157:H7* on BCM^{TM}0157:H7(+) medium. Two of these isolates were serologically confirmed to be *E. coli 0157:H7* and one of them, from Nakuru, produced VT1. The three positive isolates on the BCM medium were from three different samples out of the total 261 examined for *E.coli* which gives an overall recovery rate of 1.2%. The three isolates that showed blue/black colonies on BCM^{TM}0157:H7(+) medium were screened for genes coding for virulence using PCR and one isolate from Nakuru, VT1 positive, had genes coding for verocytotoxin production (Table 7).

Table 6: Distribution of different coliforms in milk by district and season

	SAMPLES POSITIVE FOR:											
District/season	Coliform		E. coli		Kleb.		Entero.		Citro.		Atypical spp.	
	n	%	n	%	n	%	n	%	n	%	n	%
Nairobi dry	29/46	63	8/46	28	1/46	3	5/46	17	3/46	10	12/46	41
Nairobi wet	45/53	85	29/53	64	3/53	7	7/53	16	2/53	4	4/53	9
Nakuru urban	55/58)	95	21/58	38	6/58	11	5/58	9	1/58	2	22/58	40
Nakuru rural	81/104	78	33/104	5	6/104	7	11/104	14	4/104	9	27/104	33
TOTAL	210/261	80	91/261	43	16/261	8	28/261	13	10/261	5	65/261	

Key:

E. coil- *Esherichia coli*

Kleb.- *Klebsiella pneumonia*

Entero.- *Enterobacter* spp

Citro.- *Citrobacter* spp

Table 7: Isolation of *E.coli* and Identification of strain 0157:H7

Milk sample and test details	Nairobi urban	Nakuru urban	Nakuru rural	Total
Examined for coliforms	99	58	104	261
Positive for *E. coli*	37	21	33	91
Faecal *E. coli*	18	15	24	57
Suspect *E. coli 0157:H7* on BCM™(+) medium	1	2	0	3
Serologically confirmed *E. coli 0157:H7*	1	1	0	2
Verocytotoxin producing *E. coli 0157:H7*	0	1	0	1
PCR Positive for verocytotoxin genes	0	1	0	1

4.3. Brucellosis

The presence of brucella antibodies in milk indicates that the animal was at one time exposed to brucella bacteria either by vaccination or infection.

4.3.1. Milk ring test

During the dry season consumer survey in Nairobi, four out of the 49 milk samples (8%) were positive for *Brucella abortus* antibodies but during the wet season, all the milk from Nairobi were negative for *Br.abortus* antibodies. In Nakuru consumer survey, 10.3% (6 out of 58) milk samples from urban area and 5.8% (6 out of 104) from the rural area were positive for Br. abortus antibodies. Urban areas had more positive samples than the rural areas but this difference was not significant ($p>0.05$). In the entire study, MRT classified 6.1% (16 out of 264) samples as positive (Table 8 & figure 3).

4.3.2 ELISA

Indirect ELISA classified 9.7% (11out of 107) of the milk from the urban area (Nairobi and Nakuru) and 10.4% (10 out of 104) of the milk from Nakuru rural as positive for brucella antibodies. Compared to MRT, ELISA detected more samples 21 out of 264 (7.9%) as positive for brucella antibodies than those detected by MRT (Table 8). There was moderate agreement between the two tests (*Kappa*=0.4). The ELISA positive samples varied between the two districts

(p = 0.021) with more positives in Nakuru district than in Nairobi district. There was no significant difference in ELISA between the seasons, urban and rural settings and between the different purchase points (Computer output 5.7).

Table 8: Summary results of milk ring test and ELISA

Source of milk samples	Antibody prevalence			
	MRT		ELISA	
	n	%	n	%
Urban consumers (Nairobi & Nakuru)	10	6.2	11	6.9
Rural consumers (Nakuru)	6	5.8	10	9.6
TOTAL	16	6.1	21	8.0

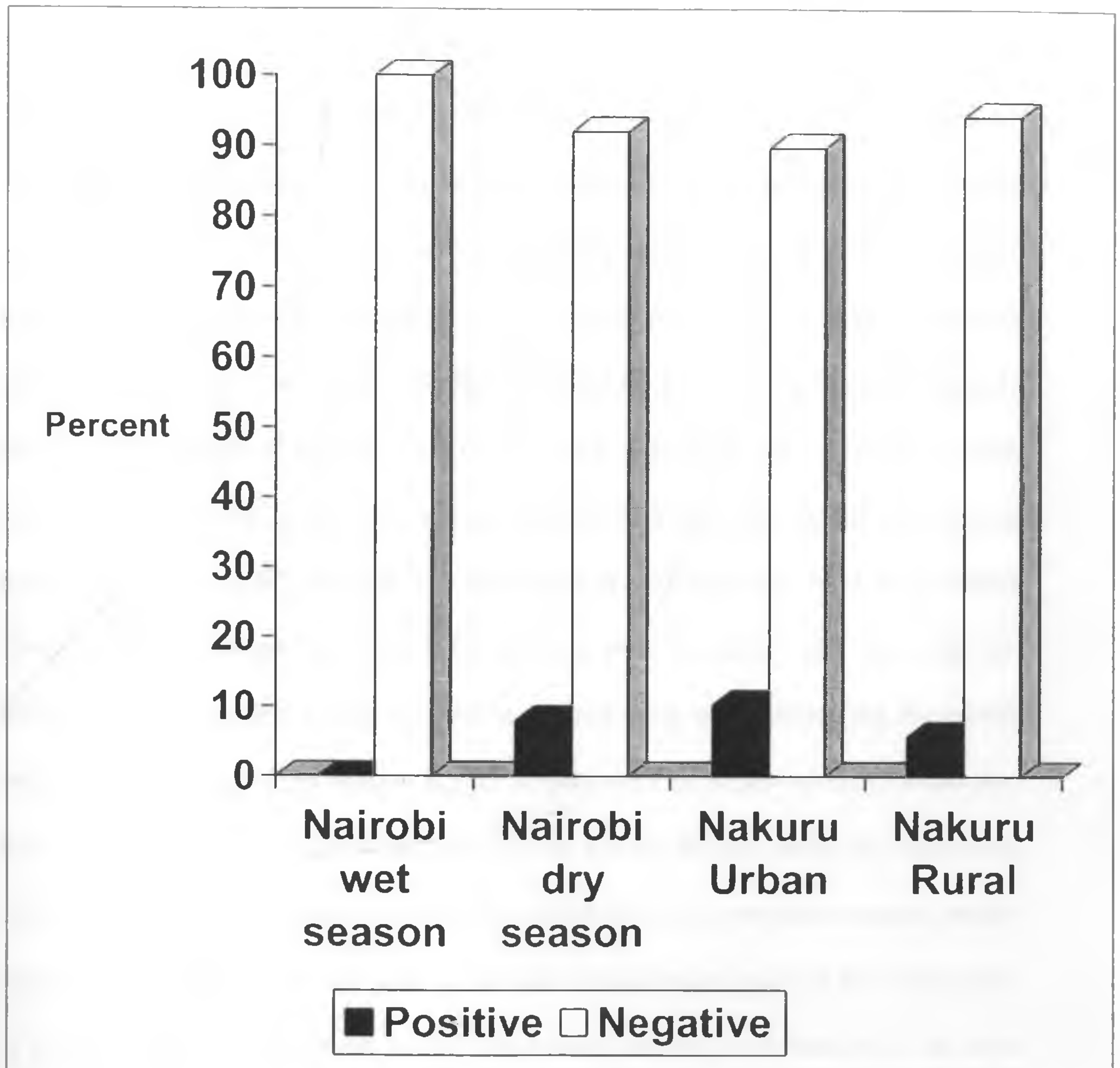

Figure 3: Proportional detection of brucella antibodies in consumer milk using MRT

CHAPTER FIVE

5.0 DISCUSSION

Quality of milk based on coliform counts in this study varied between the two seasons (wet and dry) with the milk consumed in the wet season being of better quality than that of the dry season ($p<0.05$). During the dry season, the ambient temperatures are high and if milk is kept under such temperatures, bacteria multiply rapidly to large numbers as opposed to the cooler wet season. The final bacterial load in the milk of course depends on the initial number of bacteria. Coliforms in milk indicate faecal contamination and during the dry season, milk could be contaminated with dust that carries along faecal matter. Another cause of poor quality of milk in the dry season is milk handling problems whereby poor water quality is used for washing milk containers and thus ends up contaminating the milk. Last but not least, milk production is low during the dry season and there is a tendency for market agents to bulk milk from several sources and this increases the bacterial load of milk. Also, due to low production during the dry season and given the constant demand for milk throughout the year, some unscrupulous market agents would be tempted to add water in the milk to make more profit. If the water added is of poor quality it may result in the high counts that we were finding in the milk samples.

There was a significant difference in milk quality ($p<0.05$)) between Nakuru urban and Nakuru rural with the milk being consumed in the rural area being of better quality than

that of the urban areas. This could be due to the fact that, in the rural setting, people buy milk from their neighbours and some of the households were producing their own milk. Milk was not transported over long distances thus minimising contamination and excessive multiplication of bacteria enroute. Possible adulteration by the middlemen was also greatly reduced. Similarly, quality of milk from Nakuru district as a whole (urban and rural) was significantly different from that from Nairobi district ($p < 0.05$), with milk in Nakuru being of better quality than that in Nairobi. This could be due to the fact that most (66%) of the milk consumed by Nakuru households was bought from the producer. The producer in this case was usually a neighbour. The quality of milk from a neighbour is likely to be better because the initial bacterial load is expectedly low and transportation distance from the producer to the consumer is short which limits bacterial multiplication time. However, the quality of the milk consumed in the two urban centres, Nairobi and Nakuru, was not significantly different from each other ($p=0.1353$) because the two towns have similar settings whereby milk passes from the producer to the market agents before reaching the consumer in the urban centre.

Generally the quality of milk as judged by the total counts and coliforms I this study was of poorer quality than that reported by Ombui *et al.* (1995) in Kiambu; 89.5% of the milk samples, which they analysed from the farmers cans, was of good quality.

The isolation of high coliforms counts from the milk in this study, especially the faecal coliforms implies that the raw milk being marketed in Kenya can be a potential risk to the

consumer. Presence of unacceptable coliform counts (over 50,00 c.f.u/ml) indicates that milk was drawn under unhygienic conditions or handled unhygienic ally either by the consumers or those who sell milk. Common sources of bacterial contamination especially coliforms are faeces (of animal or human origin), personnel, water and containers. Generally high counts of coliforms and total bacteria may indicate that potential human pathogens are present and should warrant further screening (Bleeme, 1994). In the study it was also found out that the highest percentage of the coliforms belonged to *E.coli*. They constituted 63% of the coliforms analysed. *E.coli* has since the 1950s been established to be among the etiological agents of enteritis and several extra-gastrointestinal diseases such as urogenital infection, mastitis, septicaemia and meningitis. Enteropathogenic strains of *E.coli* have a potential of causing diseases in the gastrointestinal tract. In the present study, 91 out of 261 samples were positive for *E .coli* from which three suspect *E .coli 0157:H7* strains were isolated but only 2 confirmed to be *E. coli 0157:H7* by serology. The *E. coli* 0157:H7 is a zoonotic organism and thus its presence in milk poses danger to the consumer. PCR revealed that one of these isolates contained genes for verocytotoxin production. The verocytotoxin produced by *E. coli* 0157:H7 causes haemorrhagic colitis (HC) leading to bloody diarrhoea and haemolytic uremic syndrome (HUS) in humans. HUS is associated with serious kidney damage and renal failure (Jay, 1992; Besser *et al.*, 1993).

In a study carried out by Ombui *et.al.*, 1994 on raw commercial milk in Kiambu district,

there was no *E. coli 0157:H7* identified among the five *E. coli* strains that were sorbitol non-fermentors (Latex agglutination test for identification of *Escherichia coli* serogroup 0157 was used). In an epidemiological study on infectious diarrhoeal diseases in children in a Coast rural area of Kenya, Saidi *et al.* (1997) isolated diarrhoeagenic *E. coli* including EPEC, ETEC and EHEC strains from 13.8% of the patients. These authors did not show the percentage of each type of *E. coli* isolated. Sang and Saidi (1996) isolated *E. coli* 0157:H7 from bloody diarrhoea of a two year old boy in Malindi District hospital.

In the western countries, isolation of the *E. coli 0157:H7* from foods and faeces have often followed outbreaks of bloody diarrhoea. In this study, isolation of the organism from milk was prompted by public health concerns regarding possible spread of the disease through the informally marketed raw milk. This could explain why the recovery rate was low (1.2%). Secondly although association of *E. coli* 0157:H7 with consumption of raw milk is well established, the organism is rarely recovered from milk (USDA: APHIS: VS, 1994) and the reason is that, unlike other *E. coli*, strain 0157 does not multiply well at 44 to 45°C (111 to 113°F) which is the temperature most often used to recover *E. coli* from foods. *E. coli 0157:H7* is a rare strain among *E. coli* organisms. It therefore requires the screening of large numbers of samples and *E. coli* colonies in order to detect it. In this study, over 800 coliform colonies isolates (4-6 colonies/sample) were analysed for *E. coli 0157:H7*. The larger the sample and the higher the proportion of faecal *E. coli* in any set of milk samples, the greater the chance of isolating the strain. The

culture results showed that many samples had high total counts and coliform counts and faecal *E. coli* thus providing a fair chance of recovering strain 0157:H7. The pathogen was recovered from three out of 261 samples which translates to a recovery rate of 1.2%. This rate is very low but significant for a number of reasons. Currently, the strain is highly acknowledged as an important foodborne zoonosis. Secondly, the haemolytic uremic syndrome caused by potent verocytotoxin produced by this organism often leads to permanent kidney damage necessitating a transplant (Riley *et al.*, 1983; Jay, 1992). In this study, VT1 was produced by one isolate. The role of *E. coli 0157:H7* in causing the aforementioned diseases here in Kenya is not clear. What the findings in this study show, however, is that those who consume un-pasteurised/unboiled milk, are at risk of getting infected. Fortunately, parallel studies have shown that over 95% of the households boil milk before consumption, which destroys *E. coli*. As far as we know, this is the first time *E. coli 0157*:H7 has been recovered from marketed raw bovine milk in Kenya. Its origin could be from dairy herds or milk handlers. The fact that isolations were made from two towns, which are far apart (150Km) may indicate that its occurrence is widespread. If its origin is dairy cows, which contribute milk that is sold in the urban centres, then the spread could be even bigger considering the spatial distribution of the dairy herds. Farm level studies would help ascertain the actual origin.

Other coliforms were also isolated in the milk samples in this study and these were *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp. which were isolated from 8%,

13% and 5% of the milk samples respectively. These organisms are important as they cause majority of extra intestinal infections such as urinary tract infections, especially cystitis. They also cause respiratory, wound, bloodstream and central nervous system infections. Many of these infections are life threatening especially sepsis and meningitis. Thus consumers of raw milk are at risk of infection with these coliforms but, as mentioned above, most households boil their milk before consumption and the risk is eliminated.

Brucella abortus antibodies were demonstrated in 16 samples out of 264 (6%). Four of the positive samples were from Nairobi dry season while Nakuru urban and Nakuru rural had six positive samples each. The MRT classified 6% positive samples while ELISA classified 8% as positive. The test results generally reflect previous findings by Kerkhofs *et al.* (1990) , which showed that ELISA was on average 22 times more sensitive than MRT. In our study, there was moderate agreement between ELISA and MRT (kappa 0.40, 95% confidence interval=0.19-0.60). Kagumba and Nandokha (1978) reported a prevalence of 10% bovine brucellosis in extensive production system in Nakuru and Kadohira *et al.* (1997) reported a 2% prevalence of bovine brucellosis in the smallholder farms in Kiambu. In our study, 4.7% of the milk samples from Nairobi and Nakuru urban (urban setting) were positive for brucella antibodies compared to Nakuru rural , which had 3.2%, samples positive but this difference was not significant. However, there was a significant difference in samples that tested positive on ELISA between the two districts

($p<0.05$) with Nakuru district as a whole having more positives than Nairobi district. This could be explained from the fact that Nakuru district has the extensive cattle production systems and so more prevalent to cattle brucellosis cases in cattle. Human brucellosis is also common where extensive cattle production systems predominate. Muriuki *et al.* (1994) found out that as high as 21% of the human flu -like cases reported in health facilities in Narok (area of extensive production) were diagnosed as brucellosis (tests were done using Rose Bengal Plate Test). However, most of the milk consumed in Nairobi comes from intensively zero grazed animals and it has been shown that the brucellosis prevalence from such herds is as low as 2% (Kadohira *et al.*, 1997).

The presence of brucella antibodies in milk is an indication of exposure of the dairy cows to brucellosis and a possible presence of the organisms in unpasteurised milk which poses a risk to consumers. Consumers at risk are those who consume traditionally fermented milk "maziwa lala" and they constituted 6% of the households mainly in rural areas. This milk is not normally boiled before fermentation yet the fermentation process only lowers the pH of milk from 6.8 to 4.5 and *Br. abortus* is only mildly affected by this acidity (Farrel, 1996).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

Most of the milk samples (63%) in this study were of unacceptable quality. Therefore good hygiene and heat treatment of milk before consumption sh ould be encouraged and everybody should be educated on these practices. Milk handlers (processors, hawkers and co-operatives) should also be trained on proper handling techniques a nd use of proper milk containers

Appropriate standards for acceptable and u nacceptable milk in Kenya should be put in place considering the prevailing conditions i.e., lack of the cold chain.

E.coli 0157:H& organisms were isolated, proof enough that this cause of an emerging zoonosis is here with us, only that there has been not been a reported foodborne outbreak so far. I suggest further farm level studies to ascertain the actual origin of this organism.

Brucella antibodies were detected in 8% of the milk samples indicating that the animals were at one time in their life exposed to the brucella bacteria. The presence of brucellosis in cattle poses a risk to the consumers of raw milk. Boiling of milk practised by over 90% of the consumers minimises the risk . Consumers of "maziwa lala" should be encouraged to boil milk before fermenting it ,while for those already boiling their milk, proper temperatures and boiling times should be re -emphasised.

CHAPTER SEVEN

7.0 LIST OF REFERENCES

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LIST OF APPENDICES

Appendix 1: Preparation Of Media

1.1 Standard Plate Count Agar (Oxoid CM463)

Formula	Gram per litre
Yeast extract powder	5.0
Pancreatic digest of casein	5.0
Glucose	1.0
Agar	5.0

pH 7.0 (approx.).

PCA powder (23.5 grams) was suspended in one litre of distilled water, and brought to boil to dissolve completely. This was then sterilised by autoclaving at 121 ^{0}C for 15 minutes. The medium was cooled in a water bath and maintained at 45-50^{0}C until used.

1.2 Violet Red Bile Agar (Oxoid CM107)

Formula	Gram per litre
Yeast extract powder	3.0
Peptone	7.0
Sodium chloride	5.0
Bile salts No.3	1.5
Lactose	10.0

Neutral red	0.03
Crystal violet	0.002
Agar	12.0

pH 7.4 (approximately)

The powder, 38.5 grams, was suspended in one litre of distilled water and boiled to dissolve. The molten agar was cooled to between 45-50^{0}C in a water bath until used in pour plating (coliform counts) or streaking (poured plates).

1.3 MacConkey Agar (Oxoid CM7)

Formula	Gram per litre
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0

pH 7.4 (approximately)

Fifty two grams of the powder were suspended in a litre of distilled water, brought to boil to dissolve completely and sterilised (121^{0}C for 15 minutes). The molten agar was cooled to 50^{0}C and approximately 20 ml poured into a petridish (90m m diameter)) and allowed to cool and solidify at room temperature.

1.4 Tryptone Soya Agar (Oxoid CM131)

Formula	Gram per litre
Tryptone	15.0
Soya peptone	5.0
Sodium chloride	5.0
Agar	15.0

pH 7.3 (approximately)

To one litre of distilled water, 40 grams of the powder were added and boiled to dissolve completely. Three millilitres were then dispensed into Bijou bottles and sterilised by autoclaving at 121^{0}C for 15 minutes. The sterilised medium was allowed to cool and solidify in slanting position in bottles. Plates were prepared by pouring approximately 20ml of the sterilised medium into 90mm diameter petri dishes.

1.5 Eosin Methylene Blue Agar (Oxoid CM69)

Formula	Grams per litre
Peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.065

Agar	15.0

In a litre of distilled water, 37.5 grams of powder was suspended and brought to the boil to dissolve completely. Sterilisation was done by autoclaving at 121 ^{0}C for 15 minutes. The medium was then cooled to 60^{0}C in a water bath. This was then shaken to oxidise the methylene blue to restore its blue colour and to resuspend the precipitate, which is an essential part of the medium. The medium was then poured into petridishes allowed to cool, solidify and dry.

1.6 Brilliant Green Bile (2%) broth (Oxoid CM31)

Formula	Grams per litre
Peptone	10.0
Lactose	10.0
Ox bile (purified)	20.0
Brilliant green	0.0133

pH 7.4 (approximately)

To one litre of distilled water, 40.0 grams of the powder was a dded. This was mixed well and distributed into culture tubes (4ml) containing inverted Durham tubes and then sterilised at 121^{0}C for 15 minutes.

1.7 Tryptone water (Oxoid CM87)

Formula	Grams per litre
Tryptone	10.0
Sodium chloride	5.0

pH 7.5 (approx.)

To one litre of distilled water, 15 grams of the powder were added, mixed well to dissolve and distributed into culture tubes in 4ml amounts. These were then autoclaved at 121 ^{0}C for 15 minutes.

1.8 MRVP Medium (Oxoid CM43)

Formula	Grams per litre
Peptone P	5.0
Dextrose	5.0
Phosphate buffer	5.0

pH 7.5 (approx.)

Fifteen grams were added to a litre of distilled water mixed to dissolve and then distributed in 4ml amounts into culture tubes.

1.9 Simmons Citrate Agar (Oxoid CM155)

Formula	Grams per litre
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	0.2
Sodium ammonium phosphate	0.8
Sodium citrate tribasic	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0

pH 7.0 (approx.)

Twenty three grams of powder were suspended in one litre of distilled water and brought to boil to dissolve completely. The medium was then dispensed in B ijou bottles in 4ml amounts and sterilised by autoclaving at $121^{0}C$ for 15 minutes. The bottles were placed in slanting position for the agar to solidify forming a slope.

1.10 Lactose Broth (Oxoid CM 137)

Formula	Grams per litre
Lab-lemco powder	3.0
Peptone	5.0
Lactose	5.0

pH 6.9 (approx.)

To one litre of distilled water, 13 grams of the powder were added mixed well to dissolve and then distributed into culture tubes (4ml) fitted with inverted Durham tubes. Sterilisation was by autoclaving at 121^{0}C for 15 minutes.

1.11.Urea agar base (OxoidCM53)

Formula	Grams per litre
Peptone	1.0
Dextrose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Phenol red	0.012
Agar	15.0

To 95 ml of distilled water, 2.4 grams of urea base were added, mixed and boiled to dissolve completely. Sterilisation was by autoclaving at 115^{0}C for 20 minutes. The medium was cooled to 50^{0}C in a water bath and 5ml of sterile 40% urea solution (Oxoid SR20) aseptically added. After thorough mixing, the medium was distributed into sterile Bijou bottles in 4ml amounts and allowed to set in slope position.

1.12 Tryptone Soya Broth (Oxoid CM129)

Formula	Grams per litre
Pancreatic digest of casein	17.0
Papaic digest of soybean meal	3.0
Sodium chloride	5.0
Dibasic potassium phosphate	2.5
Dextrose	2.5

pH 7.3 (approx.)

Semi Solid Nutrient Agar 1%

Tryptone Soya broth powder (30g) and agar No.3 (Oxoid L13) (10 grams) were suspended in one litre distilled water and boiled to dissolve completely. The resulting solution was dispensed in amounts of 1.8ml in to cryovials and sterilised (121^{0}C for 15 minutes). The 1% nutrient agar was used for storing cultures at 4^{0}C.

1.13 Glycerine 10% Nutrient Broth (MAYER AND BAKER)

Thirty grams of tryptone Soya broth powder and 100ml of glycerine were added to 900ml of distilled water and brought to boil to dissolve completely. The medium was dispensed in to cryovials in 1.8ml amounts. These were used for storing cultures at -20^{0}C.

1.14 BCM^{TM} 0157:H7(+) medium (Biosynth Biochemica, Biosynth International Inc., U.S.A)

Formula	Grams per litre
BCM^{TM} 0157:H7(+)	80.0
N, N-Dimethylformamide	5ml (Sigma product No. D4254)
Sodium novobiocin	0.01 (Sigma product No. N1628)
Potassium tellurite	0.001 (Sigma product No. P0677)

Eighty grams of the BCM^{TM} 0157:H7(+) medium powder were added to 1000ml of distilled water containing 5ml of N, N-Dimethylformamide. The final pH was 6.9 at 25^{0}C. The mixture was boiled to dissolve completely while avoiding overheating. After cooling in a water bath to 55^{0}C, filter sterilised (Type HA 0.45μm diameter membrane filters), 5ml of 0.2% (w/v) sodium novobiocin (Sigma) and 0.2ml of 0.1%(w/v) potassium tellurite (Sigma) were aseptically added and mixed. Medium was poured into petri dishes and allowed to dry at room temperature ready for streaking. For better results, precaution in handling the medium to avoid excessive exposure to the atmosphere was followed according to the instructions of the manufacturer.

1.15 Brain Heart Infusion Agar (Oxoid M375)

Formula	Grams per litre
Calf brain infusion solids	12.5
Beef heart infusion solids	5.0

Proteose peptone	10.0
Dextrose	2.0
Sodium chloride	5.0
Disodium phosphate	2.5

pH 7.4 (approx.)

To one litre of distilled water, 37 grams of the powder were added, thoroughly mixed and distributed in universal bottles in 10ml amounts. Sterilisation was by autoclaving at 121^0C for 15 minutes. The medium was then left to cool and solidify while slanting.

Appendix 2: Reagent Preparation

2.1 IMViC Reagents

2.1.1 Indole Reagent (Ehrlichs reagent)

1 gram Para-dimethylaminobenzaldehyde

95 ml absolute ethanol

20 ml concentrated hydrochloric acid

One gram of P-dimethylaminobenzaldehyde was dissolved in 95ml absolute ethanol before adding 20ml of concentrated hydrochloric acid. The solution was stored in an amber bottle. For testing presence of indole 7 drops were added to the culture medium; and a red colour indicates a positive result and no change in colour a negative result

2.1.2 Methyl Red Reagent

0.04 grams methyl red

40 ml ethanol

100 ml distilled water

The methyl red powder was dissolved in the ethanol and then the 100ml distilled water added and mixed. To the culture medium, 4 drops of methyl red were added without shaking. A red colour at the top layer indicates a positive while orange is +/- and yellow colour is negative.

2.1.3 Voges Proskauer Test

1% creatine (1-gram creatine dissolved in 100 ml 0.1 hydrochloric acid)

40% potassium hydroxide (40 grams potassium hydroxide dissolved in 100 ml distilled water)

To the test culture, 2 drops (50 μl of 1% creatine) was added followed by 1 ml of 40% potassium hydroxide. This was then well shaken and sloped. Results were read after 2 hours. A pink colour indicated positive result while yellow or colourl ess a negative result.

Appendix 3: Diluents

3.1 Potassium Dihydrogen Phosphate Buffer

Formula	Grams per 500 grams
Assay	Not less than 97%
Chloride	Not more than 0.02% (CL)
Sulphate	Not more than 0.05% (SO_4)
Dipotassium phosphate	Not more than 2%
Lead	Not more than 0.0005% (Pb)

34 grams of Potassium dihydrogen phosphate (34 grams) was dissolved in a litre distilled water to make the stock solution; pH was adjusted to 7.2 using 1N sodium hydroxide 1N. To make the working solution, 1.25 ml of the stock was added in to a conical flask and made up to 1 litre. This solution was then dispensed in 10 ml test tubes, capped and sterilised by autoclaving at 121 ^{0}C for 15 minutes.

3.2 0.85% Saline

Physiological saline solution was made by dissolving 0.85 grams of sodium chloride (BDH, AnalaR) crystals in 100 ml of distilled water. Sterilisation was by autoclaving at 121 ^{0}C for 15 minutes after distributing it in amounts of 10 ml.

Appendix 4: Disinfectants

4.1 70% Alcohol

To a one litre measuring cylinder 700 ml of ethanol was added followed by distilled water to make up to a litre. This alcohol was used for disinfecting incubators and washing hands.

4.1 5% Savlon

Fifty millilitres of neat savlon was measured into the measuring cylinder and 950 ml of distilled water added to make up to a litre. This savlon was used for wiping the working benches, incubators, the bacteriological hood and washing hands.

Appendix 5: Serological tests for *E coli* 0157:H7

5.1 *E. coli* 0157 Test (Oxoid Diagnostic Reagents)

Components of the kit

DR 621M Test Latex which consists of blue latex particles sensitised with specific rabbit antibody reactive with the 0157 somatic antigen.

DR622M Control Latex, which consists of latex particles, sensitised with pre-immune rabbit globulin.

DR 623M Positive Control suspension of inactivated *E. coli* 0157 cells in buffer.

DR 624M Negative control suspension of *E. coli* 0116 cells in a buffer.

DR 500G reaction cards

Other materials used in the test but not provided in this kit were, microbiological loop , Bunsen burner, 0.85% saline and disinfectant.

5.2 VTEC-RPLA (Oxoid, Unipath Limited)

Components of the kit

TD 961 Sensitised latex VT1 that is latex suspension sensitised with specific antibodies (rabbit IgG) against *E. coli* verocytotoxin type 1.

TD 962 Sensitised latex VT2 that is latex suspension sensitised with specific antibodies (rabbit IgG) against *E. coli* verocytotoxin type 2

TD 963 Latex control, which is a suspension, sensitised with non-immune rabbit globulins.

TD 964 Verotoxin control (VT1), which was, dried *E. coli* verocytotoxin type 1.

TD 965 Verotoxin control (VT2), which was, dried *E. coli* verocytotoxin type 2

TD 966 Phosphate buffered saline.

Appendix 6: Computer outputs

6.1 One way ANOVA for total counts by setting (Urban Vs Rural)

Source	DF	SS	MS	F	P
Between	1	12.6276369	12.6276369	66.53	0.0000
Within	262	49.7322115	0.189817601		
Total	263	62.3598485	0.23710969		

Bartlett's test for equal variances : chi2(1) = 3.3985 Prob> chi2 = 0.065

6.2 One way ANOVA for total counts by season (Dry Vs Wet)

Source	DF	SS	MS	F	P
Between	1	0.43174335	0.43174335	1.83	0.1777
Within	262	61.9281052	0.236366814		
Total	263	62.3598485	0.237100969		

Bartlett's test for equal variances : chi2(1) = 0.0617 Prob> chi2 = 0.804

6.3 One way ANOVA for total counts by District (Nairobi Vs Nakuru)

Source	DF	SS	MS	F	P
Between	1	4.62963288	4.62963288	21.01	0.0000
Within	262	57.7302106	0.220344315		
Total	263	62.3598485	0.23710969		

Bartlett's test for equal variances : chi2(1) = 4.4541 Prob> chi2 = 0.035

6.4 One way ANOVA for coliform by season (wet Vs dry)

Source	DF	SS	MS	F	P
Between	1	8.63814529	8.63814529	43.61	0.0000
Within	262	51.8921577	0.198061671		
Total	263	60.5303303	0.230153243		

Barlett's test for equal variances: chi2 (1) =0.067 prob> chi2 =0.804

6.5 One way ANOVA for coliforms by setting (urban Vs rural)

Source	DF	SS	MS	F	P
Between	1	10.7500146	10.7500146	56.58	0.0000
Within	262	49.7802885	0.190001101		
Total	263	60.530303	0.230153243		

Barlett's test for equal variances: chi2 (1) = 26.3391 prob> chi2 =0. 000

6.6 One way ANOVA for coliform by District (Nairobi Vs Nakuru)

Source	DF	SS	MS	F	P
Between	1	8.21948241	8.21948241	41.17	0.000
Within	262	52.3108206	0.199659621		
Total	263	60.530303	0.230153243		

Barlett's test for equal variances: chi2 (1) = 4.2843 prob> chi2 =0.0 38

5.7 Logistic regression of ELISA on district, se ason,urban, and purchase points

ELISA	Coef	Std Err	z	P > z
Nairobi vs Nakuru	-2.362326	1.023889	-2.31	0.021
Dry vs wet season	0.1448437	1.293354	0.11	0.911
Urban vs rural	-0.0847118	0.6043378	0.14	0.889
Producer gate[a]	-2.017685	1.549777	-1.30	0.193
Home delivery[a]	-1.909156	1.511068	-1.26	0.26

[a] Compared to own production

SMALLHOLDER DAIRY PROJECT
MOA/KARI/ILRI

ANALYSIS OF THE DEMAND AND CONSUMPTION OF DAIRY IN KENYA, AND THE PUBLIC HEALTH RISKS TO CONSUMERS FROM INFORMALLY MARKETED MILK

(Collaborative Research Project of the Ministry of Agriculture (MoA), The Kenya Agricultural Research Institute (KARI), University of Nairobi, Central Bureau of Statistics and The International Livestock Research Institute (ILRI)

DATE __ __/__ __/__ __ TIME STARTED _____ TIME ENDED ______

NAME OF ENUMERATOR ________________________________

CODE OF ENUMERATOR [_______]

CLUSTER NAME ______________________ CLUSTER CODE [_______]

HOUSEHOLD CODE [________]

DISTRICTS

1 = NAIROBI
2 = NAKURU

DIVISIONS

1 = CENTRAL	8 = WESTLANDS
2 = EMBAKASI	9 = MUNICIPALITY
3 = DAGORETTI	10 = NAIVASHA
4 = PUMWANI	11 = RONGAI
5 = KASARANI	12 = BAHATI
6 = KIBERA	13 = MOLO
7 = MAKADARA	14 = NJORO

SUB-LOCATIONS

24=Gilgil	31=Ngata	38=Nyandundo	45=Nyamamithi
25=Central	32=Rongai	39=Kabazi	
26=Nakuru-West	33=Naishi	40=Dundori	
27=Baruti	34=Likia/Teret	41=Gilgil	
28=Cheptuech	35=Kabatini	42= Sokoni	
29=Banita	36=Kiamaina	43=Maella	
30=Nessiut	37=Ndungiri	44=Subukia East	

RELATIONSHIP TO THE HH HEAD

1 = Household head
2 = Spouse
3 = Son
4 = Daughter
5 = Other (specify)________________

ETHNIC AFFILIATION

1 = Kikuyu
2 = Embu
3 = Meru
4 = Luhya
5 = Luo
6 = Kisii
7 = Kalenjin
8 = Maasai
9 = Somali
10 = Kamba
11 = Mijikenda
11 = Asian
13 = European
14 = Other (specify)__________

RELIGION

1 = Catholic
2 = Protestant
3 = Seventh day Adventists (SDA)
3 = Muslim
4 = Hindu
5 = Traditional
6 = Others (specify) ________________

HOUSEHOLD CLASSIFICATION

1 = Rural
2 = Urban

ENUMERATOR CODE | | HOUSEHOLD CODE | | DATE (DD MM YY) |

DAIRY DEMAND AND CONSUMPTION ANALYSIS IN KENYA

SCHEDULE I: GENERAL INFORMATION REGARDING THE HOUSEHOLD

DISTRICT [_____]

DIVISION [_____]

SUB-LOCATION [_____]

CLUSTER CODE [_____]

RESPONDENT'S NAME ____________________________________

RESPONDENT'S RELATIONSHIP TO THE HH HEAD [______]

COMPLETE ADDRESS ____________________________________

____

HOUSEHOLD CLASSIFICATION [_____]

ETHNIC AFFILIATION [_____]

RELIGION OF HH HEAD [_____]

ENUMERATOR CODE [ ] [HOUSEHOLD CODE [ ] [DATE (DD MM YY) [ ]

RELATIONSHIP TO THE HEAD
1 = Household head
2 = Spouse
3 = Son
4 = Daughter
5 = Daughter-in-law
6 = Son-in-law
7 = Grandchild
8 = Niece
9 = Nephew
10 = House maid/ house boy
11 = Grandparent
12 = Other (specify)________________

EDUCATION LEVEL
0 = No formal education
1 = Primary school
2 = Secondary school ('o' level)
3 = Post secondary school ('A' level)
4 = Technical college (Agric, Teacher's etc)
5 = Adult literacy education
6 = University
7 = Others (specify)

PRIMARY ACTIVITY
0 = None
1 = Farm management
2 = Salaried employment
3 = Businessman
4 = Labourer
5 = Retired with pension
6 = retired without pension
7 = Pupil/student
8 = Other (specify)

1.1/. Give details of household members (including HH head) living permanently on the compound

Name	Sex	Age (yr)	Relationship To hh head	Education Level	Primary activity
Household head			1		

* Person is in permanent residence if he/she sleeps in the house a majority of nights per week

UNITS

0 = Count
1 = Litre
2 = Cup
3 = Kg
4 = Grams
5 = Sack
6 = Heap (fungu)
7 = Bundle
8 = Bunch
9 = Crates
10 = Fingers
11 = 300 ml bottles
12 = 500 ml bottles
13 = 750 ml bottles
14 = Packet
15 = Tin (debe)
16 = 2 kg Kasuku or Kimbo tin
17 = Pieces
18 = Basket
19 = Gas cylinders
20 = Kilowatts
21 = Others (specify) ______________________

SCHEDULE 2: INFORMATION ON CONSUMPTION/EXPENDITURE ON FOOD AND NON-FOOD ITEMS

2.1/. Consumption/expenditure on home production and purchased food items

	Item	Home production last season (Since the 1st of May '98)			Quantity purchased in the last 30 days			
		Unit	Quantity produced	Quantity Sold or given away	Unit	Quantity	Price/unit (Ksh)	Total expenditure in the last 30 days (Ksh)
A)	**Cereals**							
1	Maize							
2	Maize cobs							
3	Wheat							
4	Rice							
5	Millet & sorghum							
6	Others (specify)							
B)	**Roots and tubers**							
1	Sweet potatoes							
2	Irish potatoes							
3	Cassava							
4	Yams							
5	Others (specify)							
C)	**Vegetables**							
1	Sukuma wiki							
2	Cabbages							
3	Cowpeas leaves (Kunde)							
4	Mnafu							
5	Tomatoes							
6	Onions							
7	Carrots							
8	Pumpkins							
9	Ladies finger (Binda)							
10	Cauliflower							
11	Spinach							
12	Others (specify)							
D)	**Beans and other pulses**							
1	Beans							
2	Green grams (Ndengu)							
3	Cow peas (Kunde)							
4	Pigeon peas (Baazi)							
5	Garden peas (Mbinzi)							
6	Dolicos (Njahe)							
7	Other pulse							
E)	**Fruits**							
1	Bananas							
2	Orange							
3	Coconut/copra (Nazi)							
4	Apple							
5	Grapes (Zabibu)							
6	Pineapple							

UNITS

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20 = Kilowatts
21 = Others (specify) ______________________________

ENUMERATOR CODE | | HOUSEHOLD CODE | | DATE (DD MM YY) | |

2.1/. Continued

	Item	Home production last season (Since the 1st of May '98)			Quantity purchased in the last 30 days			
		Unit	Quantity produced	Quantity Sold or given away	Unit	Quantity	Price/unit (Ksh)	Total expenditure in the last 30 days (Ksh)
7	Passion fruit							
8	Avocado							
9	Watermelon							
10	Papaw							
11	Quava							
12	Plum							
13	Sugarcane							
14	Others (specify)							
F)	**Oil seeds**							
1	Sunflower							
2	Groundnuts							
3	Cashewnuts							
4	Cottonseed							
5	Simsim (ufuta)							
6	Castor bean							
G)	**Eggs**							
1	Eggs							
H)	**Live animals**							
1	Live goat							
2	Live sheep							
3	Chicken							
4	Ducks							
5	Rabbits							
6	Other (specify)							
					Quantity purchased last season (since 1st of May '98)			
	Item				**Unit**	**Quantity**	**Price/unit (Ksh)**	**Total expenditure (Ksh)**
I)	**Farm inputs**							
1	Animal feeds/concentrates/ forage							
2	Seeds, fertiliser, agro-chemicals							
3	Veterinary services and drugs							
4	Farm labour							
5	Other (specify)							

ENUMERATOR CODE [ ] HOUSEHOLD CODE [ ] DATE (DD MM YY) [ ]

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16 = 2 kg Kasuku or Kimbo tin
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18 = Basket
19 = Gas cylinders
20 = Kilowatts
21 = Others (specify) ______________________________

2.2/. Consumption/expenditure on purchased food and non-food items

	Item	Quantity purchased in the last 30 days				Remarks
		Unit	Quantity	Price/unit (Ksh)	Total expenditure in the last 30 day (Ksh)	
J)	**Cereals**					
1	Maize meal					
2	Wheat flour					
3	Loaves of bread					
4	Millet & Sorghum flour					
5	Others (specify)					
K)	**Meat, fish and eggs**					
1	Beef					
2	Goat meat					
3	Mutton					
4	Chicken					
5	Duck					
6	Fish					
7	Pork					
8	Others (specify)					
L)	**Edible oils and fats**					
1	Kimbo. Kasuku, cowboy					
2	Blueband margarine					
3	Elianto,					
4	Mazola, corn oil					
5	Palm oil					
6	Other edible oil and fats					
M)	**Sugar and accessories**					
1	Sugar					
2	Tea leaves					
3	Other beverages (coffee, milo, cocoa etc)					
N)	**Salt and spices**					
1	Salt					
2	Spices					
O)	**Soaps, detergents and other sanitary goods**					
1	Laundry (Bar) soaps					
2	Bathing soap					
3	Detergents (Omo, Toss etc)					
4	Disinfectants					
5	Toilet paper					
6	Tooth paste					
7	Sanitary towels					
8	Other toiletries					
P)	**Refreshment**					
1	Biscuits, and snacks					
2	Carbonated drinks (Coca cola, Fanta, Pepsi)					
3	Fruit juices					
4	Tea with milk					
5	Tea without milk					
6	(coffee, cocoa, milo) with milk					
7	(coffee, cocoa, milo) without milk					

ENUMERATOR CODE [ ] HOUSEHOLD CODE [ ] DATE (DD/MM/YY) [ ]

UNITS

0 = Count
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9 = Crates
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14 = Packet
15 = Tin (debe)
16 = 2 kg Kasuku or Kimbo tin
17 = Pieces
18 = Basket
19 = Gas cylinders
20 = Kilowatts
21 = Others (specify) ______________________

2.2/. Continued

	Item	Quantity purchased in the last 30 days				Remarks
		Unit	Quantity	Price/unit (Ksh)	Total expenditure in the last 30 day (Ksh)	
Q)	**Alcoholic beverages, tobacco and other intoxicants:**					
1	Bottled/ canned beer					
2	Local beer					
3	Wines and spirits					
4	Cigarette					
5	Leaf tobacco, snuff					
6	Others (specify)					
R)	**Fuel, light and water**					
1	Firewood					
2	Coal/charcoal					
3	Cooking gas					
4	Biogas					
5	Kerosene					
6	Electricity					
7	Solar					
8	Water charges					

2.3/. Expenditure on meals and milk taken away from home

S)	Meals taken by household members away from home in the last 30 days		
	Frequency	Total expenditure in the last 30 days (Ksh)	Remarks

T)	Milk and milk products consumed away from home in the last 30 days			
	Milk or milk product consumed	Units consumed	Price/unit (Ksh)	Total expenditure in the last 30 days (Ksh)
1	Tea with milk			
2	Raw fresh milk			
3	Packed fresh milk			
4	UHT milk			
5	Home made fermented milk			
6	Fermented packed milk (mala)			
7	Yoghurt			
8	Cheese			
9	Butter/Ghee			
10	Tinned condensed milk			
11	Milk powder			
12	Ice cream			
13	Cream			
14	Skimmed milk			

UNITS

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17 = Pieces
18 = Basket
19 = Gas cylinders
20 = Kilowatts
21 = Others (specify) ______________________________

2.4/. Expenditure on miscellaneous goods and services

U)	Miscellaneous goods and services	Total expenditure in the last 30 days (Ksh)	Remarks
1	Postage		
2	Telephone		
3	Transport and petrol expenses		
4	Dry cleaning		
5	Hair dressing		
6	Domestic servants		
7	Cosmetics		
8	Pets		
9	Others (specify)		
	Item	**Total expenditure in the last 12 months (Ksh)**	**Remarks**
V)	**Clothing and footwear**		
1	Clothing		
2	Footwear		
W)	**Education and other miscellaneous goods and services:**		
1	Literary activities (newspaper, books, etc)		
2	Educational fees (school fees, tuition & other fees in colleges)		
3	House rent		
4	Land rent		
5	Life insurance		
6	Insurance for other goods		
7	Security		
8	Medical expenses		
9	Social customs and festivals (gifts to relatives, religious offerings), recreation and entertainment		

2.5 /. Expenditure on durable goods

	Item	Total expenditure in the last 12 months (Ksh)	Date purchased (dd/mm/yy)
X)	**Durable goods**		
1	Furniture		
2	Carpets and other floor mating		
3	Souvenirs (paintings, wood carvings etc)		
4	Suitcase, trunks handbags etc		
5	Cooking appliances		
6	Radio		
7	Television		
8	Record player		
9	Radio Cassette		
10	Refrigerator		
11	Washing machine		
12	Video player		
13	Video recorder		
14	Transport equipment (car, motorcycle, bicycle)		
15	Other durables (specify)		

POINT OF PURCHASE
1 = Own production
2 = Producer gate
3 = Home delivery
4 = Street vendor
5 = Kiosk
6 = Corner shop
7 = Local market
8 = Supermarket
9 = Not consumed

COMPANY/BRAND NAME
1 = KCC
2 = Limuru dairies
3 = Express dairies
4 = Brookside dairies
5 = Palm house dairies
6 = Aberdare creameries
7 = Dalamere dairies
8 = Ilara dairies
9 = Egerton University dairies
10 = Meru central dairies
11 = Eldairy products
12 = Premiere
13 = Kitinda dairies
14 = SpinKnit (Everfresh, Tuzo)
15 = Nyota Dairies
16 = Everfresh (UHT from Uganda)
17 = Machakos milk processors
18 = Echuka Dairies
19 = Eldoville Dairies
20 = Bio-foods ltd.
21 = Kigwa
22 = Green valley Dairies
23 = Farm best
24 = Bubayi Dairies
25 = Other (specify)____________

NATURE OF PAYMENT/CONTRACT
1 = Cash sale – single sale
2 = Cash sale – informal contract
3 = On credit sale – single sale
4 = On credit sale – informal contract
5 = On credit sale – formal contract
6 = Other (specify)

BUYER TYPE
1 = Neighbour
2 = Middlemen
3 = Farmer group
4 = Co-op
5 = Other (specify)

UNIT
1= Litre
2 = Kg
3 = Treetop bottle (750 ml)
4 = Large cup (500 ml)
5 = Small cup (350 ml)
6 = Other (specify)

FORM CONSUMED
1 = Taken alone
2 = Ingredient (e.g. in tea, porridge etc)
3 = Processed into other dairy products (specify)
4 = Compliment with other meals (e.g. Ugali)
5 = Other

MILK AND MILK PRODUCTS
1 = Raw fresh milk
2 = Packed fresh milk
3 = UHT milk
4 = Home made fermented milk
5 = Fermented packed milk (mala)
6 = Yoghurt
7 = Cheese
8 = Butter/Ghee
9 = Tinned condensed milk
10 = Milk powder
11 = Ice cream
12 = Cream
13 = Skimmed milk

Demand Analysis Kenya

SCHEDULE 3: CONSUMPTION OF DAIRY PRODUCTS

3.1/. Point of purchase of the various dairy products

Where did you get the following dairy products when you purchased them last?

Milk and milk products	Date when last purchased (dd/mm/yy)	Point of purchase on the last date of purchase	Distance from homestead (Km)	Brand Name	Unit	Price/ unit (Ksh)	Nature of payment/contract
Raw fresh milk							
Packed fresh milk							
UHT milk							
Home made fermented milk							
Fermented packed milk (mala)							
Yoghurt							
Cheese							
Butter/Ghee							
Tinned condensed milk							
Milk powder							
Ice cream							
Cream							
Skimmed milk							

3.2/. Home production and sale of raw milk yesterday

unit	Quantity Produced	Quantity Sold or given away	Sale price/unit (Ksh)	Remarks

3.3/. Milk and milk products consumed by the household in the last 30 days

Product type	Unit	Quantity consumed	Price/unit (Ksh)	Total expenditure in the last 30 days	Form consumed	Remarks
Raw fresh milk						
Packed fresh milk						
UHT milk						
Home made fermented milk						
Fermented packed milk (mala)						
Yoghurt						
Cheese						
Butter/Ghee						
Tinned condensed milk						
Milk powder						
Ice cream						
Cream						
Skimmed milk						

3.4/. Which milk or milk products are consumed now but not consumed 10 years ago?

|___||___||___||___|

3.5/. Which milk or milk products were consumed 10 years ago but are not consumed now?

|___||___||___||___|

ENUMERATOR CODE | | HOUSEHOLD CODE | | DATE (DD MM YY)

REASONS FOR NOT CONSUMING	**ADVANTAGE/DISADVANTAGE**
1 = Can not afford	1 = Taste
2 = Not available at purchase spot	2 = Price
3 = Point of purchase too far away	3 = Shelf life
4 = Bad quality	4 = Butterfat
5 = Too expensive	5 = Availability (convenience)
6 = Other (specify)____________	6 = Other (specify)

ENUMERATOR CODE | HOUSEHOLD CODE | DATE (DD MM YY) |

3.6/. Do you boil raw fresh milk before you consume it?
0) No
1) Yes
2) Sometimes

3.7/. Do you refrigerate milk that you consume?
1) yes
2) 2)No
3) 3) sometimes

3.8/. What are the reasons for not consuming particular dairy products that you would like to consume?

Milk type	Reason for not Consuming
Raw fresh milk	
Packed fresh milk	
UHT milk	
Home made fermented milk	
Fermented packed milk (mala)	
Yoghurt	
Cheese	
Butter/Ghee	
Tinned condensed milk	
Milk powder	
Ice cream	

3.9/. Dairy product preferences
Rank the following milk types according to the criteria given (please rank separately for fresh and fermented milk)

	Fresh milk (1= best, 2 = medium, 3 = least preferred)			**Fermented milk** (1= best, 2 = least)	
Criteria for preference	Raw fresh milk	Packed fresh milk	UHT milk	Home made fermented milk	Packed fermented milk (mala)
1. Taste					
2. Price					
3. Shelf life					
4. Butterfat					
5. Availability					
Overall rank					

3.10/. State the main Advantage and main disadvantage for each milk type listed below

Milk type	Main advantage	Main disadvantage
Raw fresh milk		
Packed fresh milk		
UHT milk		
Home made fermented milk		
Packed fermented milk (mala)		

Schedule 4: Assessment of milk-borne public health hazards

4.1/. Do you or any member of your household consume unpacked fresh raw milk ?
0) No
1) Yes
2) Sometimes

4.2/. Do you or any member of your household think there are any health risks associated with drinking unPacked fresh milk?
0) No
1) Yes ________________
If yes, why?____
____
2) Do not know

4.3/. Has anyone in the household had any health problems associated with drinking unPacked fresh milk?
0) No (if No skip to 4.5)
1) Yes (specify confirmed diagnosis) _________________
if yes, what problems?____
____
2) Do not know

4.4/. If yes, where was the diagnosis done?
0) Not diagnosed
1) Private Hospital
2) Public Hospital
3) Private medical practitioner
4) Other (specify) __________________

4.5/. Has any member of the household had malaria- or flu-like symptoms in the last one year?
0) No (if No skip to 4.8)
1) Yes

4.6/. If yes, was this diagnosed?
0) No
1) Yes (specify confirmed diagnosis) _________________

4.7/. If either malaria- or flu-like symptoms has been diagnosed, specify who diagnosed it?
0) Not diagnosed
1) Private Hospital
2) Public Hospital
3) Private medical practitioner
4) Other (specify) __________________

4.8/. Has any member of your household been diagnosed with Brucellosis in the past one year?
0) No
1) Yes

4.9/. Has any member of your household been diagnosed with Tuberculosis in the past one year?
0) No (if no skip to 4.11)
1) Yes

ENUMERATOR CODE | | HOUSEHOLD CODE | | DATE (DD/MM/YY) | |

4.10/. If either Brucellosis or Tuberculosis has been diagnosed, specify who diagnosed it?
0) Not diagnosed
1) Private Hospital
2) Public Hospital
3) Private medical practitioner
4) Other (specify) ___________________

4.11/. Where do you access water?
1) Piped/tap
2) River/Stream
3) Private ground pump
4) Community ground pump
5) Collected rain water
6) Other, specify ___________

4.12/. If fresh raw milk is consumed, which container was used to purchase it?
0) not consumed
1) plastic
2) polythene paper bags
3) glass (e.g., glass bottles)
4) metallic
5) other (specify)_______________________

4.13/. In which of the following groups do you estimate **your total household income**, from all working members, business income, pensions and remittances from elsewhere? [_______]

1 = less than 2500 Ksh per month
2 = 2,500 – 5,000
3 = 5,000 – 10,000
4 = 10,000 – 20,000
5 = 20,000 – 30,000
6 = More than 30,000