PREVALENCE AND ASSOCIATED HEALTH RISKS OF *ESCHERICHIA COLI* O157:H7 IN URBAN SMALLHOLDER DAIRY FARMING AND NON-DAIRY FARMING HOUSEHOLDS IN DAGORETTI, NAIROBI.

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DECLARATION.

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

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DEDICATION.

I dedicate this work to my beloved mother Rose Onono.

ACKNOWLEDGEMENT.

TABLE OF CONTENTS.

LIST OF TABLES.

LIST OF FIGURES.

LIST OF APPENDICES.

LIST OF ABBREVIATIONS.

APHIS................................American Public Health Inspectorate Services.

CDC...Centre for Disease Control

CI...Confidence interval.

EHEC...Enterohaemorrhagic *E.coli*

EIEC... Entero-invasive *E.coli*

EPEC...Enteropathogenic *E.coli*

ESBLs...Extended spectrum beta-lactamases

ETEC...Enterotoxigenic *E.coli*

FAO...Food and Agriculture Organisation

H...Flagella antigen

HUS...Haemolytic Ureamic Syndrome

O...Somatic antigen

PCR...Polymerase Chain Reaction

SMAC...Sorbitol MacConkey agar

STEC...Shiga toxin *E.coli*

TTP... Thrombotic Purpura

USDA......... ....United States Department of Agriculture.

VS..Veterinary Services

VT 2...Verotoxin 2

VT I..Verotoxin 1

VTEC...Verotoxigenic *E.coli*

ABSTRACT.

A prevalence study of *E.coli* O157:H7 was done amongst smallholder dairy farming and neighbouring non-dairy households in Dagoretti Division of Nairobi city, to determine the herd prevalence of *E.coli* O157:H7, and to assess potential exposure pathways to household members.

Two hundred and ninety smallholder dairy farmers and 136 neighbouring non-dairy farmers were interviewed. Several exposure pathways were assessed: (a) use of cattle faeces as manure (fertilizer) on farms, (b) consumption of raw vegetable salads (c) consumption of fermented (soured) raw milk, (d) Consumption of raw milk, (e) Household water source (f) water treatment (g) Handling of cattle faeces without protective gloves. The households were also interviewed on basic household characteristics such as (a) level of education for the household head (b) household land size (c) number of years lived in the location (d) number of members in a household. Community workshops and gender disaggregated focus group discussions were organised within the division. Proportional scoring was done by men and women on daily activities that are potential exposure factors in a dairy farming household.

Cattle faecal and milk samples were used for culture and isolation of *E.coli* O157: H7. Colonies of *E.coli* were isolated using standard microbiological methods. *E.coli* positive isolates were serotyped with O157 antiserum and polymerase chain reaction done to detect genes coding for Verotoxin production.

Hired male worker spends over 50% of his daily time doing dairy related activities. Seventy three percent of the dairy farming households were using cattle faeces as manure on vegetable farms, 85% were not using protective gloves while handling m anure. F ifty t hree p ercent w ere consuming r aw v egetable s alads n ot washed in clean water. Twenty one percent were consuming fermented raw milk and 4% of both the dairy farming and non-dairy farming households were not boiling the milk and were therefore at risk of infection.

Ninety six percent of both dairy and non-dairy households mitigated the risk of infection by boiling the raw milk before consumption, while household drinking water was boiled by 41% of the dairy farming and 47% of the non-dairy households. Other households also added chlorine to the drinking water (22.7% of dairy households and 6% of the non dairy households). Most of the households obtained water from the city council (78% dairy farming and 94.5% of non dairy households) which was considered to be chlorinated.

Women had a lower level of education in both dairy and non-dairy households than men with an estimated proportions of (0.32; CI 0.219-0.436) for women having informal education compared to (0.11; CI; 0.07-0.16) for men in dairy households and a proportion of (0.04, 0.02) for women and men, respectively, in non-dairy households. Male headed dairy households also had a higher estimated proportion of secondary level of education (0.30; CI; 0.24-0.36; P<0.0001).

There were 15 faecal *E.coli* O157 sample isolates from dairy farming households a nd 3 m ilk *E.coli* O 157 s ample i solates f rom n on-dairy h ouseholds

that agglutinated with the antiserum against O157. Only one faecal *E.coli* O157 isolate had genes for verotoxin 1 production. Of the 18 *E.coli* O157, sample isolates 27.7% were resistant to Sulphamethoxazole and 11.1% to Tetracycline. The isolate that was amplified by primers of *E.coli* O157:H7 on polymerase chain reaction was however sensitive to all the antimicrobials. The apparent prevalence of *E.coli* O157 in cattle faeces and milk was determined as 5% (CI; 3-8) and 2% respectively. However, the apparent herd prevalence of *E.coli* O157:H7 was less than 2%.

The study concluded that the risk of infection by *E.coli* O157:H7 in urban dairy farming households was low, however, the presence of the *E.coli* O157 at a prevalence of 5% indicates a potential health hazard because the *E.coli* O157 can acquire the verotoxin producing gene by bacteriophage through conjugation. Therefore there is a need for continued surveillance to prevent any future outbreak in case the prevalence increases. The findings from this study and other studies on benefits of urban agriculture can be used by the policy makers to legalise urban dairy farming activities in Kenya.

CHAPTER 1

1.0 INTRODUCTION.

Mougeot, (1999) described urban agriculture as an industry located within or on the fringes of a town, a city or metropolis, which grows or raises, processes and distributes a diversity of food and non-food products using largely human and material resources, products and services found in and around the urban area, and in turn supplying human and material resources, products and services largely to the urban area. Urban agriculture refers to a wide range of agricultural activities (crop and livestock farming occurring within the city limits). This may range from small plots where households can tend and use the land to produce crops for household consumption to entrepreneurial gardens where vegetables, flowers and animals are raised for retail and wholesale marketing.

In 2000, 1.9 billion people lived in cities of the developing world; by 2030, the number will swell to nearly 3.9 billion (FAO, 2000). As cities grow in population and area, they require more extensive structures to bring food to consumers, including distribution systems as wholesale and retail markets (FAO, 2000).

According to Lee- Smith *et al.* (1987), two thirds of urban households in Kenya grow part of their own food supplies, with 29% doing so on urban land. The food produced in one season in urban areas was estimated at US$ 4 million and total value of livestock at the time of the survey was estimated at US$ 17 million. Most of the crops and livestock produced were not for subsistence but for

sale although livestock products such as eggs and milk were both sold and consumed by the households.

Despite lack of legislation legalizing urban agriculture, councils in Kenyan towns of Isiolo, Kitui and Kitale have been pro-active in supporting urban agriculture, unlike Nairobi and Kisumu (Lee-Smith and Memon, 1994).

Urban households are engaged in urban agriculture because of two main reasons, (a) they moved from rural to urban areas and brought along their rural practices; (b) some households have an urban background and got involved in agriculture by choice or by need. Female gender is more involved in urban agricultural activities than the male gender in many regions including Kenya, Tanzania, Uganda, Mozambique, Senegal, Poland and Thailand (Harvoka, 1998; Wilbers, 2004). The predominance of women is due to two factors. (a) The women bear the first responsibility for household sustenance and well-being, (b) women tend to have lower educational status than men and therefore have more difficulties in finding formal wage employment. In many situations, men can be found to be active on the sideline of urban agriculture. An example from Kampala shows that men are more involved in helping to provide cash for the purchase of inputs, and in obtaining land for farming than in the actual urban farming (Wilbers , 2004).

Food s ecurity c oncerns a re e specially i mportant i n t he c ities o f d eveloping world where poverty rates often exceed 50%, for example Guatemala City (80%) and Kampala, Uganda (77%) (FAO, 2000). The poor urban consumers spend as

much as 60 to 80 percent of their income on food, making them more vulnerable to higher food prices, such as those caused by transport costs or monopolistic practices by powerful traders. They are also the last link in a long food chain, and have little choice of where to buy, increasing the risk that they will consume food of poor quality (FAO, 2000). Food safety is therefore a serious concern in urban areas, w here poor handling, refrigeration and unscrupulous vendors can l ead to contaminated or adulterated food.

Food safety hazards arise principally from: bacteria and other microbial agents resulting from improper food handling, environmental contaminants, and residues of substances used in agricultural production and processing such as pesticides. Other emerging food safety concerns include antibiotic resistance by certain pathogens (including some strains of *Salmonella* and *Escherichia coli)* making them hard to treat using most antibiotics prescribed for patients infected with these organisms.

This study focused on one potential hazard, *E. coli* O157:H7 as part of a study to characterize benefits and health risks associated with urban smallholder dairy production in Dagoretti division. *E. coli* O157:H7 has become a significant health problem worldwide. One of the most important factors influencing the pathogenesis of *E.coli* O157:H7 is the low infectious dose, estimated at 10 organisms (US.FDA, 1982; Bachrouri *et al.*, 2002). The organism has been isolated from cattle faeces (Chapman *et al.*, 1997; Kaddu- Mulindw *et al.*, 2001; Smith *et al.*, 2003). It can survive in cattle faeces for over 12 weeks and in soil for

over 20 weeks (Maule, 1999). The risk pathways are direct from animal to animal keeper, farm children and farm visitors (Charmers *et al.*, 1997; Parry *et al.*, 1998; Milne *et al.*, 1999); drinking unpasteurised milk (Chapman *et al.*, 1997; Bachrouri *et al.*, 2002); consuming contaminated vegetables (Morgan *et al.*, 1988) and person to person spread (Armstrong *et al.*, 1996). Young children under five years and the elderly are more at risk than adults. They develop severe, even life-threatening symptoms. *E. coli* O157:H7 from cattle faeces can also contaminate water used directly or indirectly by communities (Jones and Roworth., 1996; Aloysio *et al.*, 1999; Muller *et al.*, 2001), thus posing a health risk to these communities.

In Kenya, Sang *et al.* (1992) was unable to explain the causes of many diarrhoea cases in children in Kenyatta National hospital, some of which could have been *E. coli* O157:H7, which was not targeted for isolation in their study. In a different study in Malindi, Sang and Saidi, (1996) isolated *E. coli* O157:H7 from a two year old boy suffering from hemorrhagic colitis, a disease associated with *E.coli* O157:H7. According to Besser *at al.* (1993) *E. coli* O157:H7 is associated with haemolytic uremic syndrome in humans, which causes kidney damage and renal failure. Arimi *et al.* (2000) reported isolation of *E. coli* O157:H7 from pooled raw milk samples from households and market agents in Nairobi and Nakuru with a prevalence of less than 2%.

1.1 Study Goal

The study aimed at improving human health by investigating the prevalence of *E.coli* O157:H7 and associated health risks in an urban dairy farming and neighbouring non-dairy households.

1.2 Study objective

Determination of the prevalence of *E.coli* O157:H7 in urban dairy farming and non-dairy households, assessment of the risk of exposure and identification of mitigation strategies.

1.3 Specific objectives

i) To determine the occurrence, ability to produce verocytotoxins and antibiotic sensitivity of *E.coli* O157:H7 in cattle faeces and milk obtained from smallholder dairy farming households and milk obtained from neighbouring non-dairy households.

ii) To assess, based on the findings in (i) the risk of exposure to *E.coli* O157:H7 by household members from dairy farming and neighbouring non-dairy households.

iii) Use the generated information to identify mitigation strategies to reduce the probability of exposure to this health hazard.

CHAPTER 2

2.0 LITERATURE REVIEW

Acute diarrhoea is common in less developed countries and is the most important symptom associated with poor hygiene, faecal contamination of food, water and environment. The first worldwide study of morbidity and mortality from diarrhoea diseases, based on population estimates in 1980, showed that there were 744 to 1000 million episodes of diarrhoea and 4 to 6 million related deaths from diarrhoea in children below five years in Africa, Asia and Latin America each year from 1970 -1980 (Raji *et al.*, 2003). Ten years later, improved management reduced worldwide mortality to 3.3 million deaths per year (estimated range: 1.5- 5.1 million), but the incidence of diarrhoea (2.6 episodes per child per year) remained virtually unchanged (Raji *et al.*, 2003). In Brazil, infants less than one year of age represent the age group with a higher than average risk of death due to diarrhoea, with a death rate of 5.5 deaths per 1000 inhabitants (Neto *and* Scaletsky, 2000). The main bacterial enteric pathogens in less developed countries, and particularly among infants two months to five years of age, are entero-pathogenic *E.coli (EPEC)*, enterotoxigenic *E.coli* (ETEC), entero- invasive *E.coli* (EIEC) and enterohaemorrhagic *E.coli* (EHEC) (Neto *and* Scaletsky, 2000).

E. coli O157:H7 that belongs to enterotoxigenic group was first recognized in outbreaks that occurred in 1982 in Oregon and Michigan and was associated with

eating hamburgers from a particular fast food chain (Riley *et al.*, 1983). All strains of EHEC produces shiga toxin 1 (*stx 1*) and or shiga toxin 2 (*stx 2*). Also referred to as verotoxin 1 (*vt1*) and verotoxin 2 (*vt2*). The ability to produce toxins was acquired from a bacteriophage, presumably directly or indirectly from *shigella* (Buchanan and Doyle, 1997; Sharma *et al.*, 2003). The toxin is 70,000 dalton protein composed of a single A subunit (32 kDal) and five B subunits (7.7kDal). The B subunit provides tissue specificity by binding to globotriaosylceramide (G_b 3) receptors on the surface of eukaryotic cells. The A subunits h as a n N -glycosidase t hat i nactivates t he 2 8S r ibosome, t hus b locking protein synthesis. Endothelial cells that have high G_b 3 receptors are the primary target, accounting for the colon and renal glomerulli associated with haemolytic uraemic syndrome and haemorrhagic colitis. The toxins can also damage cells by realising cytokines, such as tumour necrosis factor. Toxins alone are not sufficient to make *E.coli* pathogenic, it requires the presence of other virulence markers. The *eae* chromosomal gene encoding for the outer membrane protein associated with attachment and the presence of a plasmid encoded *enterohaemolysin* is characteristic for EHEC (Buchanan and Doyle, 1997).

Evidence indicating a rare sporadic infection occurred prior to 1982. This came from a retrospective review by the Centres for Disease Control and Prevention (CDC) of over 3,000 *E. coli* serotypes identified from 1973-1983 in which serotype O157:H7 was detected among isolates from a 50 year old Californian woman (Riley *et al.*, 1983). The subsequent occurrence of large

outbreaks and the widespread distribution of cases have lead to the designation of *E.coli* O157:H7 as a new emerging pathogen.

E.coli O157: H7 causes hemorrhagic colitis, which is characterized by severe cramping (abdominal pain) and diarrhoea (watery and/ or bloody). Other symptoms may include vomiting and/ or low-grade fever. The illness lasts for an average of eight days. Treatment of the infection is primarily supportive, including management of dehydration and complications such as anaemia and renal failure. Anti-gut motility agents do not appear to diminish the severity of illness o r p revent d evelopment o f h aemolytic u remic sy ndrome (HUS) (Su a nd Brandt, 1995). Potential explanation for the lack of benefit for antibiotic treatment is the elimination of the competing bowel flora by antibiotic giving a competitive advantage to *E.coli* O157:H7, and secondly lyses and or death of *E.coli* O157:H7 leads to increased release of verotoxin (Su and Brandt, 1995). It's also believed that certain antibiotics, such as fluoroquinolones can induce shiga toxin encoding bacteriophages in vivo and thus lead to increased expression of shiga toxin genes (Galland *et al.*, 2001). The proportion of all cases of diarrhoea estimated to be associated with *E.coli* O157:H7 is 0.6% to 2.4% (Su and Brandt, 1995). Serious complications of *E.coli* O157:H7 infection occurs in 0 to 15% of cases and is experienced more frequently by the very young and elderly. These complications are haemolytic uraemic syndrome (HUS) and thrombotic purpura (TTP) (Bachrouri *et al.*, 2002). The HUS primarily affects infants and young children and is characterized by renal failure and haemolytic anaemia. Haemolytic

uraemic syndrome is the most common cause of acute renal failure in children and has a mortality rate of 5% to 10% (Su and Brandt, 1995). Thrombotic purpura primarily affects the elderly and is characterized by HUS plus two other symptoms namely fever and neurological syndromes. Other potential complications are erroneous surgical intervention, coma or seizures, pancreatitis and diabetes mellitus.

2.1 Prevalence of verotoxigenic *E.coli* in animals.

2.1.1 Cattle

The early associations between *E.coli* O157:H7 and cattle products quickly led to identification of cattle as natural hosts of verotoxigenic *E.coli* O157:H7 (Kudva *et al.*, 1996). Subsequent investigations have confirmed that a variety of animals, especially ruminants may carry numerous serotypes of VTEC in their intestinal tract (Kudva *et al.*, 1996; Chapman *et al.*, 1997). Animals and herds prevalence estimates varies with study designs, numbers of herds and cattle sampled, type and age of cattle, methodology and season (Raji *et al.*, 2003). In three surveys, involving up to nine selected dairy herds in Wisconsin, *E.coli* O157:H7 was isolated from faeces of 1.2-2.2% of cattle on 27.3-100% of farms (Wells *et al.*, 1991). Where higher numbers of farms were investigated on a simple sampling, the prevalence rates were lower both in individual animals (0-0.7%) and in farms (0-16%) (Wilson *et al.*, 1996). Prevalence rates were higher in growing cattle, especially amongst newly weaned calves, and during the summer (Wilson *et al.*, 1996; Hancock *et al.*, 1997). In a point prevalence study of 100

feedlots in the USA, *E.coli* O157:H7 was isolated from 63% of the feedlots and overall, from 1.8% of 11,881 faecal samples (Hancock *et al.*, 1997). Prevalence rates between feedlots and between pens within feedlots were highly variable, with the highest rates (32-53%) for pens holding cattle recently entering the feedlots.

According to Wilson *et al.* (1996) the peak rates of shedding verotoxigenic *E. coli* in UK ranged from 40-68%. Infection was not associated with disease in either study. These findings are generally consistent with the fact that *E. coli* O157:H7 is not a bovine pathogen in naturally reared cattle (Cray and Moon, 1995) and is shed for 1-2 months following natural exposure (Cray and Moon, 1995). There are however, herds in which *E. coli* O157:H7 was isolated more frequently overtime (Hancock *et al.*, 1997), possibly due to continued exposure to the organism in water, feed or other environmental sources or to management factors.

Serological studies provide further evidence that *E.coli* O157:H7 is widespread in cattle. In a study of 80 dairy farms (Wilson *et al.*, 1996), over 85% of 885 adult dairy cattle and 49% of 589 calves less than three months old had antibodies reactive with the O157:H7 lipopolysaccharide antigen (LPS). Calves aged 9-13 weeks had the lowest rate of seropositivity (37%) to this antigen (Johnson *et al.*, 1999). However, the high rates of seropositivity to the O157:H7 lipopolysaccharide should be interpreted with awareness of the occurrence of non

verotoxigenic *E.coli* of serogroup O157:H 7 in cattle, as well as potential cross-reactions due to antibodies to other organisms (Johnson *et al.*, 1999).

Feeding grain to cattle has a significant effect on the ruminal microbial ecosystem and overall animal health (Callaway *et al.*, 2003). Some dietary starch bypasses ruminal fermentation and goes through to the caecum and colon where it undergoes microbial fermentation (Huntington, 1997). Some early studies indicated that reducing hay, overfeeding grain, or switching from a better to poorer quality forage increased generic *E.coli* and/or O157:H7 population (Brownlie and Grau, 1967; Allison *et al.*, 1975; Kudva *et al.*, 1995; 1996; Hovde *et al.*, 1999; Buchko *et al.*, 2000a, 2000b). In a recent research, cattle fed a feedlot type ration had generic *E.coli* population 1000 fold higher than cattle fed only hay (Diez-Gonzalez *et a*l., 1998). When cattle were switched from a finishing ration to 1 00% h ay diet, f aecal *E .coli* p opulation d eclined 1 000 f old. In addition, t he population of *E.coli* resistant to acid shock declined by 100,000 fold within 5 days (Diez-Gonzalez *e t a l.*, 1 998). In another s tudy, cattle k ept o n a h igh grain d iet were screened for natural *E.coli* shedding, the cattle were divided into two groups; one g roup was maintained on f eedlot r ation and the other abruptly s witched to hay. Of the grain fed cattle 52% were positive for *E.coli* O157:H7 compared with 18% of the hay fed cattle (Keen *et al.*, 1999). Additional research with experimentally inoculated calves indicated that animals fed high concentrates diet consistently shed more *E.coli* O157:H7, and that isolates grown in ruminal fluid from grain fed animals were more resistant to an acid shock than those grown in

hay fed ruminal fluid (Callaway *et al.*, 2003). The induction of acid resistance in *E. coli* O157:H7 could increase the risk of human food-borne illness. Normally, stomach acid is an effective barrier to infection by food-borne pathogens because the organisms die in an acid environment. Acid resistant bacteria are able to survive this defence mechanism, reproduce, and produce the toxins that cause disease.

2.1.2 Sheep and goats

Domestic animals other than cattle also harbour verotoxigenic *E. coli* (Kudva *et al.*, 1996). In Germany, sero-prevalence rates of all VTEC were higher in sheep (66%) and goats (56%) than in cattle (21%) (Beutin *et al.*, 1996). Also 43% out of 400 sheep and 51.1% of 262 goats tested in Italy had antibodies to verotoxin I (vt I) (Conederal *et al.*, 1994). While the serotype of VTEC isolated from these species differed somewhat from those in cattle, several were common to cattle, sheep and goats and included serotypes associated with bloody diarrhoea and/ or HUS in human (Beutin *et al*; 1996). *E. coli* O157:H7 was present in 1-4% of sheep surveyed at abattoir in Australia, UK, the Netherlands and USA (Chapman *et al.*, 1997).

2.1.3 Pigs.

Many *E.coli* O157 outbreaks have been traced to eating contaminated beef or milk products. However, evidence is appearing now that pigs might occasionally also be a source of infection. A survey of a thousand pigs at a British abattoir and a study in Germany revealed that pigs can also be infected

(Fairbrother and Nadeau, 2006). In Japan an on-farm survey of pigs has recently shown that more than one percent are infected, an infection rate only slightly lower than in cattle (Muneo *et al.*, 1999).

2.1.4 Chicken.

Doyle and Schoeni, (1987) isolated *E. coli* O157:H7 from 1.5% of chicken meat sample while Abdul-Raouf *et al.* (1996) isolated the organism from 4% of chicken meat samples. In both instances, cross contamination from other meat products may have occurred. Other surveys have failed to isolate the organisms from poultry product indicating a prevalence of less than 0.25 % (Chapman *et al.*, 1997).

2.1.5 Other animals.

Some animals have been associated with *E.coli* O157:H7 as reservoir or epidemiologically implicated in infections, these include dogs, deers, goats, pigs, orang-utans and wild birds (Raji *et al.,* 2003; Fairbrother and Nadeau, 2006).

2.2 Mode of transmission.

The earliest reported outbreaks of *E. coli* O157:H7 infections were associated with consumption of ground beef (Riley *et al.*, 1983). Carcass can become contaminated with verotoxigenic *E.coli* during the slaughtering process. Faecal contamination and leakage of the visceral contents has been shown to be the most likely source of contamination (Riley *et al.*, 1983). Processing steps such as washing of the carcass can potentially lead to the redistribution of contaminants on the surface of individual carcass and to cross- contamination of other carcasses.

Some of the unexpected food borne vehicles of transmission are acidic foods, vegetable salads, turkey roll, lettuce and venison (Hancock *et al.*, 1997). The acidic foods confirmed as sources of outbreaks include unpasteurised apple and apple cider, mayonnaise and yoghurt (Morgan *et al.*, 1988). Fresh pressed, unpasteurised apple cider was first identified as a vehicle for *E.coli* O157:H7 in an outbreak in Massachusetts in 1991, although HUS was first linked to apple juice in 1982 (Besser *et al.*, 1993). In October 1996, two separate outbreaks associated with drinking unpasteurised apple cider occurred, one in Connecticut and the other in the western USA. The Connecticut outbreak involved 14 cases and was associated with drinking a specific brand of cider (Morgan *et al.*, 1988). The second outbreak involved 66 persons in multiple states in the Western USA and was associated with drinking a specific brand of apple juice or brand's juice mixtures containing apple juice (USDHHS/CDC, 1996). Vegetable salad has also been implicated as a vehicle, populations of viable *E.coli* O157:H7 inoculated on vegetables declined when vegetables were stored at $5^{O}C$ and increased on vegetables stored at $12^{O}C$ and $21^{O}C$ for up to 14 days (Abdoul-Raouf *et al.*, 1996).

Dry cured salami was implicated as the vehicle in an outbreak in the state of Washington (Alexander *et al.*, 1995) and venison jerk was reported as the likely vehicle for an outbreak in Oregon outbreak (USDA/APHIS/VS, 1997). Consumption of deer steak was being investigated as the cause of *E.coli* O157:H7 illness in two individuals in Illinois in early 1997.

Raw milk can be a vehicle of transmission for *E.coli* O157:H7 but confirmed outbreaks have been few. The presumed mechanism of contamination was during milking. Two outbreaks associated with raw milk have been documented by the CDC, one in 1992 with nine cases and the other in 1993 with six cases. Both outbreaks occurred in Oregon and were traced to two specific dairies, which were licensed to sell raw milk (Armstrong *et al.*, 1996). The estimated number of raw milk consumers in the USA is only (1 - 2) percent (Armstrong *et al.,* 1996). This small population at risk may partly explain the small number of outbreaks due to raw milk consumption.

Drinking recreational water has been linked to outbreaks of *E.coli* O157:H7 infection (Swerdlow *et al.*, 1992). The only known outbreak in the USA, associated with drinking water occurred in 1989 in Missouri. An unchlorinated municipal water source and deficiencies in the water distribution system were implicated as the probable source of contamination (Swerdlow *et al.,* 1992). Outbreaks associated with swimming/ the recreational areas have been more frequent. During 1982-1992 period, only 2.8% of outbreaks associated with swimming were identified (Swerdlow *et al.*, 1992). During 1994-1995, however, there were (eight) 1.3% outbreaks associated with swimming water.

The importance of person-to-person spread should not be overlooked. During 1994-1995 in the USA, person-to-person spread was identified as the likely vehicle in seven (11%) outbreaks (Armstrong *et al.*, 1996). In 1996 there were nine (31%) outbreaks attributed to person-to-person spread. The most frequent

setting for person-to-person spread is a day care facility, but person-to-person spread has occurred in other institutional settings such as nursing homes and mental health facilities, and is common among family members. A small outbreak involving five cases of *E.coli* O157:H7 in Florida involved two cousins and three siblings. The two cousins contacted *E.coli* O157:H7 during international travel, and upon return to the USA, had contact with three siblings who became infected. Person to person transmission from asymptomatic cases also occurs (Armstrong *et al.*, 1996).

2.3 Diagnosis of *E.coli* O157:H7 infections.

Isolation of *E.coli* O157:H7 from faeces in most laboratories is done by use of a selective differential agar with or without an enrichment phase. Commonly used media is sorbitol Mac Conkey agar (SMAC), cefixime tellurite SMAC (CT-SMAC) and CT-SMAC supplemented with rhamnose (CTR-SMAC).

This relies on phenotypic characteristic of *E.coli* O157:H7, such as inability to ferment sorbitol and rhamnose and tolerance to tellurite. This method has a disadvantage that its designed to detect only serogroup O157 strains and not strains of other serotypes, however, it is inexpensive and the most technically straight forward method. But there are *E.coli* O157 strain that ferments sorbitol and they are susceptible to tellurite (Sernowski and Ingham, 1992; Ojeda *et al.*, 1995). Sensitivity of cultures can be improved by using immunomagnetic separation (IMS) (Karch *et al.*, 1999). The major disadvantage of this method is the serotype specificity. However, IMS followed by culture on CT-SMAC is

twice as sensitive as direct culture (Karch *et al.*, 1999). Presumptive colonies of *E.coli* O157:H7 can further be characterized by agglutination in commercially available O157 or H7 antisera. Because of cross reaction between the O157 antigen and other *E.coli* O157 serotypes, *Escherichia* species and other members of the *Enterobacteriaceae*, biochemical confirmation of isolates is mandatory (Karch *et al.*, 1999).

Identification of *E.coli* O157:H7 can also be done rapidly, specifically and sensitively using DNA based polymerase chain reaction methods (Pass *et al.*, 2000). One multiplex PCR method amplifies simultaneously three different DNA sequences of *E.coli* O157:H7, a specific fragment of the *eae* A gene, conserved sequence of verotoxin 1 (*vt1*) and verotoxin 2 (*vt2*) and a fragment of 60mda plasmid. Since this test detects other virulence markers besides verotoxin, it's more specific than tests that only identify verotoxin genes. PCR methods however are affected by many laboratory variables and are less reproducible between laboratories than other methods and are often less sensitive than direct culture.

Molecular methods of interstrain differentiation of *E.coli* O157:H7 are also done. The most commonly used method is DNA fingerprinting tests that are based on restriction length polymorphism (RFLP) methodology where restriction enzymes are used to cut genomic DNA into fragments that are separated by agarose gel electrophoresis. Several RFLP methods have been developed; one uses pulsed field gel electrophoresis (PFGE), while others uses conventional gel electrophoresis (Ojeda *et al.*, 1995; Jeffrey *et al.*, 1997).

2.3 Situation of verotoxigenic *E. coli* O157:H7 in Africa

2.3.1 South Africa.

The first reported *E. coli* O157:H7 haemorrhagic colitis case was in 1990 (Browning *et al.*, 1990). According to Effler *et al.* (2001) a large outbreak of bloody diarrhoea caused by *E.coli* O157:H7 infections occurred in Swaziland, Southern Africa. As many as 40,912 patients less than five years of age visited physicians for diarrhoea treatment during October through November 1992. The attack rate was 42% among 778 residents screened. Female gender and consumption of beef and untreated water were significant risks of illness. Galane and Le Roux, (2001) used molecular techniques for studying the epidemiology of diarrhoeal infections due to *E.coli* in Guateng region in South Africa. A total of 151 *E.coli* isolates from stool of patients with diarrhoea and 30 strains isolated from stool of healthy individuals were collected from March 1996 to May 1997. Forty eight (26.5%) strains belonged to enteropathogenic *E.coli* (EPEC) O groups and 14 (7.7%) to verotoxigenic *E.coli* (VTEC) O157:H7 serotype. A high percentage (28.2%) of a typical strain (EPEC) possessing the *eae* A genes were isolated. Muller *et al.* (2001) also investigated the occurrence of *E.coli* O157:H7 using chromogenic rainbow O157-agar medium. They took 204 water samples from 15 different watering sites where water was used for direct or indirect human consumption. None of the suspected colonies contained the virulence factors found in *E.coli* O157:H7.

2.3.2 East Africa

In Uganda, faecal samples were collected from 237 diarrheic infants in Kampala and from 159 healthy cattle from a ranch in the central region of Uganda. These were investigated for the presence of *E.coli* O157:H7 and other serotypes of Shiga toxin producing *E.coli* (STEC) (Kaddu *et al.*, 2001). *E.coli* O157:H7 was not detected in 150 stool samples on sorbitol Mac-Conkey agar cefixime- tellurite media (SMAC-CT). Eighty-seven additional human stool samples were tested with an enzyme- immuno assay for Shiga toxins (premier EHEC) and were negative. Forty-two stool samples from infants were also investigated for enteropathogenic *E.coli* (EPEC) by hybridization with *eae* specific gene probe. Compared to STEC, EPEC were frequent and isolated in six (14.31%) of the 42 randomly selected stool samples. In the same study by Kaddu *et al.* (2001) STEC were isolated from 45 out of 159 cattle from a herd in the central region of Uganda. STEC strains from cattle belonged to different O and nine different H types. Only one bovine STEC strain was positive for *eae*-gene and O group associated with Enterohaemorrhagic *E.coli* (EHEC) types (O26, O103, O111, O145 and O157) was not isolated. Their reports demonstrated that STEC are not frequent in urban children in Uganda but domestic cattle were identified as an important natural reservoir for these organisms.

In Tanzania a study was conducted by Gaswn *et al.* (2000) by matched case-control study in the maternal and child health clinic in Ifakara region during the rainy season in order to elucidate the risk factors for and aetiology of diarrhoeal

diseases in children under five years of age. Enterohaemorrhagic, enteropathogenic, enterotoxigenic and enteroaggregative strains of *E.coli* were not related with diarrhoea and neither were *Giardia lamblia* or *Salmonella species*. But studies in beef animals by Hayghaimo *et al.* (2001) showed that beef carcasses were contaminated with VTEC organisms. The authors concluded that this might pose a health hazard especially with undercooked meat and meat products in the region.

In Kenya, a study done by Saidi *et al.* (1997) isolated diarrhoeagenic *E.coli*, including EPEC, ETEC and EHEC strains from 13.8% of the patients. These authors didn't show the percentage of each type of *E.coli* isolated. Sang and Saidi, (1996) isolated *E coli* O157: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* O157:H7 in Kenya. In another study to assess the risks of zoonotic *E.coli* O157:H7 and brucellosis in informally marketed and unpasteurised milk in Nairobi and Nakuru districts, Arimi *et al.* (2000) isolated *E.coli* O157:H7 from one milk sample from Nakuru urban area. The strain from Nakuru urban area was shown to have the verotoxin-producing gene *VT* 1.This gave a prevalence of less than 2% in milk that is consumed by households. This study (Arimi *et al.*, 2000) showed that the organism was present in dairy farming systems but no farm level study had been done to determine the prevalence in the urban dairy farming systems.

2.3.3 West Africa

In Nigeria, Akinyemi *et al.* (1998) studied *E.coli* infection for over a period of 12months. A total of 852 stool samples from patients (both children and adults) with acute diarrhoea diseases attending some public and some government recognized health institutions in Lagos metropolis, were screened for diarrhoeagenic bacterial agents. Of all 83 isolates of *E.coli* group (59%) 49 were EPEC, 17 (20.5%) ETEC, 10 (12.1%) EIEC and seven (8.4%) EHEC. The EPEC strain was mostly encountered in children aged over five years. On the other hand, EIEC and ETEC were mainly found in adults while EHEC O157:H7 strains occurred in all the age groups studied. Their study further stressed the important role EIEC and ETEC play in acute diarrhoeal diseases and the possible implication of EHEC in acute gastroenteritis especially in children in Lagos. Olorunshola *et al.* (2000) examined the presence of sorbitol non-fermenting *E.coli* O157:H7 EHEC in 100 patients with diarrhoea by stool culture on sorbitol Mac-Conkey agar. The detection rate of *E.coli* O157:H7 was 6%. Five of the six were from children below five years of age and one was a teenager. All strains induced a cytotoxic effect on the vero cell assay. All isolates were susceptible to most of the antimicrobials tested. In another study by Smith *et al.* (2003), seventeen out of a hundred faecal samples collected from healthy animals yielded *E.coli* O157:H7. Antibiotic susceptibility pattern showed the isolates were highly susceptible to the various antibiotics screened with a few showing multiple antibiotic resistances,

18% of the faecal *E.coli* O157:H7 isolates showed multiple antibiotic resistances, with tetracycline alone constituting 35% of the resistance.

2.3.4 North Africa

Abdoul-Raouf *et al.* (1996), conducted a survey in Egypt to determine if *E.coli* O157:H7 was present in 175 samples of raw ground beef, chicken, lamb and unpasteurised milk. *E.coli* O157:H7 was detected in three out of 50 (6%) beef samples, two out of 50 (4%) chicken samples, one out of 25 (4%) lamb samples obtained from slaughterhouses and three out of 50 (6%) milk samples obtained from supermarkets and farmers homes.

Surveillance of VTEC O157: H7 infection is well established in many developed countries and it is now apparent that there are geographical differences in the incidences of infection. Although cattle and other ruminants are regarded as the main reservoirs of VTEC, these bacteria have also been isolated from a number of non-ruminant animal species. The review also shows that verotoxigenic producing *E.coli* are also present in man and animals in Africa and specifically in Kenya and this poses a health risk to the public. There is a need for more studies on the distribution of VTEC in these reservoirs, and for investigation of the pathogenic potential of the many non-O157 VTEC in animals and foods.

2.4 Antimicrobial Resistance.

There are several factors that contributes to the uncertainty in the future dissemination and control of antimicrobial resistance, these include (a) molecular characteristics of the pathogen such as virulence, susceptibility and survival fitness of the organism (b) prescribers of the antimicrobial drugs, physicians who

may change their prescription patterns (c) characteristics of the patient populations and host related factors (d) macro level factors related to the health care environment, such as regulatory policies that influence the use of antimicrobial drugs, drug discovery, infection control practises and technology development (Harbarth and Samore, 2005; Metlay *et al.*, 2006).

Bacteria become resistant to antimicrobial agents by one or more of the four mechanisms; (a) altering the target site of the antibiotic, (b) modifying the antibiotic so that it's no longer active, (c) preventing the antibiotic from entering the cell and (d) specifying an enzyme which provides a substitute for the bacteria specified enzyme which is the target for the antibiotic (Senerwa, 1988; Paterson, 2006). Genes determining resistance to antimicrobial agents are located on the chromosome, p lasmids o r b oth. A cquired resistance i n b acteria r esults from R - plasmid transfer by conjugation, transduction and transformation or by chromosomal DNA mutation.

The emergence and spread of resistance in *Enterobacteriaceae* are complicating the treatment of serious nasocomial infections and threatens to create species resistance to all currently available microbial agents. Most resistance in *Klebsiella pneumoniae* and *Enterobacter* species in the United States to third generation cephalosporins are caused by acquisition of plasmids containing genes that encodes for extended- spectrum beta lactamases (ESBLs), and these plasmids often carry other resistance genes (Paterson, 2006). ESBLs-producing *K. pneumoniae* and *E.coli* are common in health care facilities and

exhibits multi-drug resistance. Resistance to *Enterobacter* species to third generation cephalosporins is mostly caused by overproduction of AmpC beta-lactamases and treatment with third generation cephalosporins may select for AmpC overproducing mutants. Some *Enterobacter* cloacae are now ESBL and *AmpC* producers conferring resistance to both third and fourth generation cephalosporins (Paterson, 2006). Quinolone resistance in *Enterobacteriaceae* is usually the result of chromosomal mutations leading to alterations in target enzymes or drug accumulation. Most recently plasmid mediated resistance has been reported in *K. pneumoniae* and *E.coli* associated with the acquisition of *qnr* gene (Paterson, 2006).

There is no distinction between plasmid encoded and chromosomally encoded resistance because of the dynamic movement of transposons between plasmids and the chromosome (Senerwa, 1988; Sharma *et al.*, 2003).

Most of the clinical antibiotic resistance is mediated by plasmids (Senerwa, 1988). Plasmids also contribute to chromosomal resistance by transposition and transduction. Insertion elements and transposons make plasmids a major mechanism of gene reassortment (Sharma *et al.*, 2003).

2.4.1 Antimicrobial susceptibility testing methodology.

Diffusion plate assay methods were developed in 1940s and depended on the diffusion of antibiotics from reservoirs on or in the agar plate. Measurement of the amount of diffusion is made by comparing the sizes of the zone of growth inhibition, using standard strains of susceptible organisms and known amounts of reference antibiotics (Bauer *et al.*, 1966; Wheat, 2001). The result depends on the critical rate of diffusion of the antibiotic, critical growth rates of the standard organisms and the critical minimal inhibitory coefficient levels of each organism. The reservoirs may be holes cut into the agar and filled with known concentrations of antibiotics, or metal cylinders placed on the surface of the agar and similarly filled, or paper discs charged with antibiotics (Wheat, 2001; Oxoid, 2005). Plates of 150 x 15 mm dimension are normally used, and agar poured to 4 mm depth and then allowed dry at $37^{O}C$ for 30minutes. Upto 5 colonies of the test organism are then transferred into 4 ml of Tryptone Soya broth. The plates are then incubated at $37^{O}C$ for 2-5 hours and turbidity of the broth adjusted to match the opacity of the tube containing 0.5 ml of 1% barium chloride in 1% sulphuric acid (N/36), or any other method can be used to standardize the amount of innoculum used for the test (Bauer *et al.*, 1966).

Tube dilution methods are alternatives with the advantage of short incubation period (3-4 hours) but they lack the accuracy of the diffusion assays (Oxoid, 2005). The samples must be clear and not absorb the wavelength of the light used in the spectrophotometer. Dilution of samples and dilution of the antibiotics are

made in parallel in 1ml volumes and 9 ml volumes of seeded broth added to each tube. The tubes are then incubated in water bath for 3-4 hours at the appropriate temperature and at the end of the incubation the growth is stopped by the addition of formaldehyde to each tube. The amount of growth that has taken place within the incubation period is measured by light transmission in a spectrophotometer. Comparison of the readings of the unknown samples and those of the known dilutions of the antibiotic will establish the level of antibiotic in the sample. The tube with the lowest concentration of antibiotics to show no growth indicates the minimum inhibitory concentration of the organism (Bauer *et al.*, 1966; Wheat, 2001; Oxoid, 2005).

2.5 Treatment and management of *E.coli* O157:H7 infections.

According to Su and Brandt, (1995) no specific treatment currently exists for *E. coli* O157:H7 infection other than supportive therapy and management of complications such as anaemia and renal failure. A significantly longer duration of bloody diarrhoea in persons treated with antibiotics than in untreated persons has been reported (Ostroff *et al.*, 1989: Pavia *et al.*, 1990). They suggested that the use of antibiotics is a risk factor for infection and that an association exists between the use of antibiotics and increased mortality. They attributed the increased virulence to two mechanisms: (a) the elimination of competing bowel flora by antibiotics, leading to an overgrowth of *E. coli* O157:H7. (b) Lyses of or sub lethal damage to the infecting organisms, with the subsequent liberation of Shiga-like toxins.

Isolates of *E. coli* O157:H7 have been found to be uniformly susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, norfloxacin, sulfisoxazole, tetracycline, ticarcillin, tobramycin, trimethoprim, and trimethoprim-sulfamethoxazole (Pai *et al.*, 1984; Griffin *et al.*, 1988; Tarr *et al.*, 1988). Isolates have been found to be resistant to erythromycin, metronidazole, and vancomycin, and some resistant to tetracycline (Remis *et al.*, 1984; Swerdlow *et al.*, 1992; Kim *et al.*, 1994). A study of antibiotic-resistant *E. coli* O157:H7 in Washington State showed an emergence of antibiotic resistance to streptomycin, sulfisoxazole, and tetracycline, from zero isolation (0 of 56) between 1984 and 1987 to 7.4% isolation (13 of 176) between 1989 and 1991 (Kim *et al.*, 1994).

CHAPTER 3

3.0 MATERIALS AND METHODS.

3.1 Study area

The investigation of the prevalence of *E.coli* O157:H7 was done as part of a larger project on the characterization of benefits and health risks associated with urban smallholder dairy production in Dagoretti Division of Nairobi province. The division has six locations; Uthiru, Waithaka, Riruta, Kawangware, Mutuini and Ruthimitu (Figure 1). The area lies between an altitude of 1760 and 1940 meters above sea level (Jaetzold and Schmidt, 1983). It has an annual bimodal rainfall pattern, with the long rains coming between March and May and the short rains coming between October and November. The annual mean rainfall is about 1200mm and varies from 653 mm to 1632 mm. The annual mean temperature ranges between $11.8^{O}C$ and $23.4^{O}C$ (source: Meteorological Department: *Nairobi AWR*, 2005).
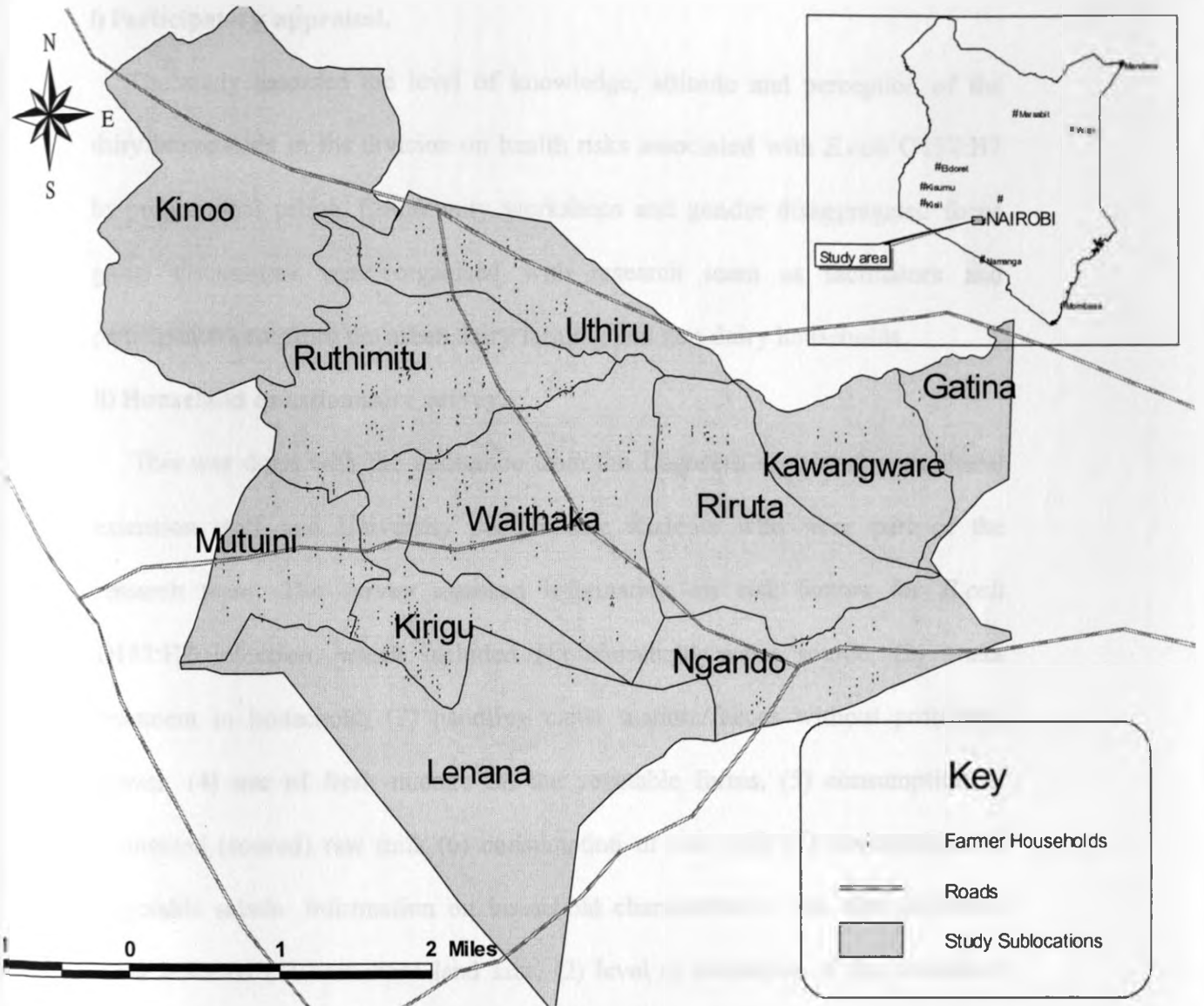
Figure 1: Map of the study area showing urban dairy households.

3.2 Study methods.

i) Participatory appraisal.

The study assessed the level of knowledge, attitude and perception of the dairy households in the division on health risks associated with *E.coli* O157:H7 by proportional piling. Community workshops and gender disaggregated focus group discussions were organised with research team as facilitators and participants were from the urban dairy farming and non dairy households.

ii) Household questionnaire survey.

This was done with the assistance from the Dagoretti divisional agricultural extension staff and University postgraduate students who were part of the research team. The survey assessed information on risk factors for *E.coli* O157:H7 infection, which included (1) household water source, (2) water treatment in household, (3) handling cattle manure/faeces without protective gloves, (4) use of fresh manure on the vegetable farms, (5) consumption of fermented (soured) raw milk (6) consumption of raw milk (7) consumption of vegetable salads. Information on household characteristics was also collected. This included (1) household land size, (2) level of education of the household head, (3) number of members in a household, (4) how long the family had lived in the location.

iii) Isolation and characterization of *E.coli.*

The organism was isolated from milk and faecal samples that were collected from dairy farming and non-dairy households. The isolation was done by sub-

culturing samples in sorbitol Mac-Conkey agar and Eosin Methylene blue agar. The isolates were confirmed to be *E.coli* by biochemical tests (Indole production, Methyl red test, Voges proskaeur test, Citrate utilization test). The isolates were also characterized by serotyping using O157 antiserum and by antimicrobial sensitivity tests. Polymerase chain reaction was done to detect genes coding for verotoxin 1 and 2 production.

3.3 Selections of study households.

3.3.1 Dairy farming households.

The unit of investigation was the dairy smallholder household. A simple random sample was taken based on a sampling frame constructed by the extension personnel of Dagoretti division and updated during the stakeholder meetings held in each of the six locations. The households were assigned a random computer number and a sample was drawn in proportion to the number of listed dairy households in the location.

The sample size calculations were based on the government agricultural extension team's estimate of the dairy household population of 1200 households. Two main calculations were done, one was the sample size needed to estimate the prevalence of brucellosis based on the tests of household milk samples with a precision of +/- 5 percentage points. In Uganda some preliminary testing had detected *Brucella* antibodies in about 40% of the milk samples (Nasinyama and Randolph, 2005). Therefore with a "guessed" prevalence of 0.4 and desired

precision of +/- 5 percentage points using the formula by (Martin *et al*. 1987), the sample size was calculated as shown below.

$$n = \frac{(Z_{(1-\frac{\alpha}{2})})^2 \; p(1-p)}{L^2}$$

$n=(1.96)^2 \, (.4) \, (.6) / (0.05)^2 = 369$.

Where p is "guessed at" population prevalence and L is one half the width of the desired confidence interval the estimated sample size was about 370 households. Since this was a large fraction of the population of dairy households as estimated by the extension team, a finite population correction was applied. $1-n/N =$ approximately 1-0.3.Therefore, the corrected sample size =256 households.

A second calculation was made based on sampling to detect *E.coli* O157:H7 from faecal samples. It was not known if this pathogen existed and it was desirable to take sufficient samples so that if all the tested households turned out to be negative the prevalence of the pathogen could be estimated to be very small (1%) with confidence. This was taken so that hypothesised value for P would not be rejected if the probability of observing 0 infected animals (out of the total tested), i.e. $(1-p)^n$, exceeded 0.05 (Martin *et al.,* 1987). This was setting an upper confidence limit for proportion for different values of sample sizes. Therefore if 300 household samples tested negative for the pathogen then the prevalence of the pathogen would be less than 0.001 (<1%) with 95% confidence.

3.3.2 Non-dairy farming household.

A sample size was chosen for the investigation of hazards in the milk samples of non-dairy households who were neighbours to sampled urban dairy farming households. The difference in prevalence between dairy farming and non-dairy households was estimated with a precision of +/- 0.15 at 95% confidence with a power of 0.8 given that the sample size of the dairy farming households was fixed at 300.

$$n = \frac{[Z_\alpha \{2\bar{p}(1-\bar{p})\}^{1/2} - Z_\beta \{p_E(1-p_E) + p_C(1-p_C)\}^{1/2}]^2}{(p_E - p_C)^2}$$

where

Z_α is the value of Z which provides an $\alpha/2$ in each tail of a standardized normal curve if the test is two-tailed or α if a one tailed test where α is the type I error =1.96

Z_β is the value of Z which provides β in the lower tail of the standardized normal curve

   β is the type II error = (-0.84)

p_E = Estimated proportion in the exposed group (first population), estimated at 0.35

p_C Estimated proportion in control group (alternate population) estimated at 0.2

$$\bar{p} = \frac{(p_E + p_C)}{2} \quad = (0.35 + 0.2)/2 = 0.275$$

The sample size for non-dairy farming neighbours was calculated to be 137.

This estimate was rounded up to 150 neighbour households to account for losses and error in the estimation. The 150 non-dairy were randomly sampled around the 300 r andomly s ampled dairy farming h ouseholds. In s ome c ases t here were n o non-dairy neighbours near a sampled dairy farming household and in such situations another dairy farming household was identified and neighbouring non-dairy household sampled.

The dairy farming household sampling was done in two phases. The first phase included 201 households drawn randomly from the 300. The second phase sampling constituted the remaining 99 households. This was done to accommodate uncertainty about the presence of *E.coli* O157 from the first round sampling so that if all samples in the first phase were negative for non-sorbitol fermenters then 20 of the dairy farming households would be sampled longitudinally every month for three months. However, since many non-sorbitol fermenters were isolated in the first round of sampling the remaining 99 dairy farming households were sampled cross sectionally. The targeted number of dairy households was 300. during questionnaires survey and biological sampling 41 dairy households scheduled for sampling were replaced due to refusal to participate or because owners had recently sold their animals (14% replacement rate). At the end of the study there were 290 sampled dairy households for which questionnaires and laboratory sampling were complete.

3.4 Participatory research.

Community workshops were organized in the six locations within the division with participants from the dairy farming, non dairy farming households and key informants from the division, the discussions were facilitated by the research team. Gender disaggregated focus group discussions were conducted with male dairy farmers, female dairy farmers and key informants. The knowledge, attitudes and perception of the dairy farmers of *E.coli* O157:H7 as a health hazard was sought using a check list (appendix 7). Using the Harvard analytical framework for gender analysis the participants in the respective groups prepared a daily activity calendar for a dairy farming household indicating potential exposure and /or contamination pathways to *E.coli* O157:H7 : cleaning cattle shed of manure piles/faecal material, disposal to manure pit and milking of the cow. Individual participants did scoring by distributing ten stone pebbles amongst household members disaggregated as: man, woman, hired male and female worker, boy and girl, depending on the amount of time taken while doing the dairy activity.

3.5 Questionnaire survey.

A baseline questionnaire was administered to 290 dairy farming households and 136 non-dairy households who consumed milk bought from the dairy households. This was done with the assistance from the Dagoretti Division agricultural extension staff who were part of the research team (appendix 1 and 2). Information was collected on risk factors such as (a) consumption of raw

vegetables salads (b) consumption of raw (unpasteurised) milk (c) consumption of fermented raw milk (d) use of cattle faeces as manure on farms (e) handling manure without protective gloves (f) household water source (g) treatment of water in households and (h) supplementation of the dairy cow with concentrate. The household characteristics was also collected in the survey; this included (a) level of education to the household head, (b) household land size, (c) number of household members,(d) how long the family had stayed in the location. The questionnaire survey data was triangulated with the community workshop data on potential exposure pathways to *E.coli* O157:H7.

3.6   Isolation of *E.coli* O157.

3.6.1   Collection of faecal and milk samples.

Faecal material was collected from up to three cattle per dairy farming household by thrusting a hand covered with sterile latex gloves into the rectum. The faecal material was put in a sterile container and placed into a cool box. All the collected samples were taken to the laboratory for culture and isolation of *E.coli*. A quarter litre of milk was purchased from both dairy farming and neighbouring non-dairy households. The household members were advised to keep the milk in the vessels/containers that they always used for storage of milk and were instructed not to boil the milk before storage.

3.6.2   Culture and isolation of *E.coli*.

NOTE: Procedures for media preparation are as described in appendix 4.

Faecal samples from a household were pooled together and 0.2 grams weighed. This was suspended in Mac-Conkey broth (Oxoid Ltd) and enriched for

two hours at 37°C. After enrichment, using a flamed wire loop, the broth was streaked on sorbitol Mac-Conkey agar (Oxoid Ltd) and incubated at 37°C for twenty four hours. The sample size of 0.2 grams was used because 1 gram of faecal material produced overgrowth on the sorbitol Mac-Conkey agar plate with no individual colonies after enrichment. Each milk sample was vortexed and a loopful streaked on sorbitol Mac-Conkey agar and then incubated at 37°C for twenty four hours.

Eight clear/colourless colonies (non-sorbitol fermenters) were picked from a sorbitol Mac-Conkey plate using a wire loop after the twenty-four hour incubation period. The eight colonies were used to increase the chance of isolating *E. coli* O157:H7. The individual colonies were separately sub-cultured on Eosin Methylene blue agar (Oxoid limited) for twenty four hours at 37°C. Colonies that were medium in size, raised and smooth with dark centres and with or without a greenish metallic sheen were subjected to biochemical tests, to confirm that they were *E. coli*.

3.6.3 Confirmation of *E.coli* isolates.

3.6.3.1 Biochemical tests.
i) Indole test.

This was done according to the standard microbiological procedure (Oxoid Manual, 2005).

Principle.

Tryptone water (Oxoid limited), which is a good substrate for indole production, was used because of its high content of tryptophan amino acid. The ability of *E.coli* to break down tryptophan with the formation of indole is an important property used for its classification and identification.

A tube of tryptone water was inoculated with colonies from Eosin-Methylene blue agar and incubated for 24-48 hours at 37^OC, after which 7 drops of Kovac's reagent were added and allowed to stand for 10 minutes. A dark red colour in the amyl alcohol surface layer constituted a positive indole test, whereas no colour change from the original colour of the reagent was a negative test.

ii) Methyl Red and Voges Proskauer (MVi) test.

This was done according to the standard microbiological procedure (Oxoid Manual, 2005). The Methyl Red and Voges Proskauer medium (Oxoid Ltd) is recommended for the differentiation of the coli-aerogenes group. It contains glucose, phosphates and peptone. Organisms capable of forming large amounts of acid from glucose would result in a pH fall below 4.4 at which level Methyl Red, which is a pH indicator remains red when added. Methyl Red changes colour depending on the pH levels as follows.

pH below 4.4   …………...red colour

pH between 5.0-5.8……..orange colour

pH above 6.0   ……….yellow colour

In a positive Voges Proskauer test, a red fluorescent coloration appears after the addition of potassium hydroxide and creatine to the broth culture. Under alkaline conditions and exposure to the air, the acetoin produced from the fermentation of the glucose is oxidized to diacetyl which forms a pink compound with the creatine.

Technique.

Colonies were inoculated into a 10 ml broth of Methyl Red and Voges Proskauer medium in a tube and incubated at $37^{O}C$ for 48 hours. After incubation, the broth was divided into two portions.

a) Methyl Red Test.

To one portion of broth 5 drops of 0.4% w/v Methyl Red solution was added and the colour change on the surface of the medium read immediately. Positive cultures turned red in colour while the negative ones remained yellow.

b) Voges Proskauer Test.

To the second portion of the broth two drops of (1%w/v) creatine solution and 5 ml of 40% potassium hydroxide solution were added and the tube shaken gentle for 30 seconds. Appearance of a bright pink or eosin red colour was considered positive while no colour change was considered negative.

iii) Simon citrate test

This was done according to the standard microbiological procedure (Oxoid manual, 2005). Simmon citrate (Oxoid limited) is a medium recommended for the

differentiation of the enterobacteriaceae based on whether or not they utilize citrate as a sole source of carbon.

Technique

The medium was used as a slant. The surface of the medium was lightly inoculated by streaking and the butt inoculated by stubbing. The tubes were then incubated for 120 hours (5 days) at 37^OC. Positive utilization of citrate was seen by growth of colonies on the slant and production of an alkaline reaction that changed the colour of the medium from green to bright blue while in a negative test (no citrate utilization) there was no colonial growth on the slant and the colour of the medium remained unchanged.

E.coli isolates were positive on Indole and Methyl Red tests but were negative on Voges Proskauer and Citrate utilization tests (Table 1).

Table 1: Identification of coliforms and related organisms.

(adapted from; National mastitis council inc, 1990).

Test	E.coli	Klebsiella	Enterobacter	Citrobacter	Proteus
Indole	+	-/+	-	-/+	+
Methyl red	+	-	-	+	+
Voges Proskauer	-	+	+	-	-
Citrate	-	+	+	+	+

Colonies that were confirmed as isolates of *E.coli* were sub cultured again on sorbitol Mac-Conkey agar to confirm their inability to ferment sorbitol, showing clear/colourless colonies. Non-sorbitol fermenters were stored in sterile 50% glycerol mixed with tryptone Soya agar at -20°C.

3.6.4 Serological identification of *E.coli* O157.

The colonies of non-sorbitol fermenting *E.coli* that were stored under a medium made of 50% sterile glycerol and tryptone Soya agar were subjected to serogrouping u sing s lide a gglutination t est (Oxoid d iagnostic r eagent f or *E .coli* test) employing latex beads sensitized with specific rabbit antibody reactive with the O157 somatic antigen (Oxoid limited, Basingstoke, England). This was done to determine whether the *E.coli* isolates belonged to the O157 serogroup and therefore a potential verocytotoxin producer.

Procedure:

One drop of the test latex was dispensed onto a circle on the reaction card; a loopful of sterile physiological saline was placed separately 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.

The test latex and the suspension were then mixed and spread to cover the reaction area on the card using an applicator stick. The card was then rocked and co- agglutination observed within one minute. A further portion of the colony was simultaneously tested with control latex that had blue latex particles sensitized with pre immune rabbit globulins to ensure that the test sample was not an auto agglutinating strain.

Agglutination within one minute was an indicator that the *E.coli* isolate belonged to the O157 serogroup, which is a potential verocytotoxin producer. Positive and negative controls (Oxoid Ltd) were also tested alongside the *E.coli* samples isolates to check for the correct working of the latex reagents with the test samples. Positive control used in the study was a suspension of inactivated *E.coli* O157 cells (Oxoid Ltd) in a buffer and this caused visible agglutination within one minute. Negative control was a suspension of *E.coli* O116 cells (Oxoid Ltd) in a buffer and this caused no agglutination with latex reagents.

3.6.5   Characterization of *E.coli* O157.

3.6.5.1   Antimicrobial sensitivity test.

This was done according to (Bauer *et al.*, 1966) using the plate agar diffusion method. The number of colonies that produced confluent growth on Mueller Hinton agar plate was determined by measuring the diameter of zone of inhibition around the antibiotic disc (Abtek Biological Ltd, Liverpool, United Kingdom) for the standard organism (*E.coli* 29053) in millimetres using a vernier calliper. These were compared to the diameters of the control organism *E.coli* 25922. Briefly, 4ml of Mueller-Hinton broths were inoculated with a standard *E.coli* (29053*)* colony and incubated at 37$^{\mathrm{O}}$C for 3 hours. The broth was poured to the Mueller Hinton agar plates (4mm depth) and left to dry at room temperature for five minutes. Multi antibiotic discs (Abtek Biological Ltd) were applied to the Mueller Hinton agar plate using a sterile forceps and plates incubated at 37$^{\mathrm{O}}$C for 24 hours. The diameters of the zones of inhibition were measured in millimetres and compared with that of *E.coli* ATCC 25922. The Mueller Hinton agar plate to which 3 colonies of standard organism were sub cultured had comparable diameters to the accepted range with *E.coli* ATCC 25922.

Three colonies of *E.coli* O157 from household samples were used for sensitivity testing. The colonies were enriched in 4 ml of Mueller- Hinton broth and incubated at 37$^{\mathrm{O}}$C for 3 hours. Sterile Mueller Hinton agar (Oxoid Ltd) was poured in plates to a depth of 4mm; the plates were dried at 37$^{\mathrm{O}}$C for 30 minutes. Then a sterile cotton swab attached to an applicator stick was dipped into the

cultured broth, squeezed against the inner surface of the tube containing the broth

to remove excess broth and then used to swab over the surface of Mueller Hinton

agar evenly. The plates were then left to dry at room temperature for five minutes,

before the multi antibiotic discs (Abtek Biological Ltd) were applied using a

sterile forceps. The plates were then incubated at 37^OC for twenty-four hours after

which the diameter of the zone of inhibition for each antibiotic (including the

diameter of the antibiotic disc) was measured in millimetres using a vernier

calliper. The breakpoints were then determined by using the current National

Committee for Clinical Laboratory Standards (NCCLS, 2002). The sensitivity of

the isolates were tested against antibiotics that are commonly prescribed for

patients in health clinics and whose chemical ingredients are also contained in

drugs used in animal production activities: Ampicillin, 25µg; Tetracycline,

100µg; Nitrofurantoin, 200µg; Nalidixic acid, 30µg; Sulphamethoxazole, 200µg;

Gentamicin, 10µg.

Table 2: Standards for antimicrobial sensitivity testing for Enterobacteriaceae; adapted from NCCLS (2002) Vol.22 No.1.

Test /report group	Antimicrobial agent	Disk content	Zone diameter nearest whole numbers			Equivalent MIC Breakpoint µg/ml	
			R	I	S	R	S
A	Ampicillin	10µg	≤13	14-16	≥17	≥32	≤8
C	Tetracycline	30µg	≤14	15-18	≥19	≥16	≤4
O	Nalidixic acid	30µg	≤13	14-18	≥19	≥32	≤8
B	Sulfamethoxazole	1.25/23.75µg	≤10	11-15	≥16	≥8/152	≤2/38
U	Nitrofurantoin	300µg	≤14	15-16	≥17	≥128	≤32
A	Gentamicin	10µg	≤12	13-14	≥15	≥8	≤4

KEY:

I=Intermediate, R=Resistant, S=Susceptible.

3.6.5.2   Detection of the presence of verocytotoxin genes.

Polymerase chain reaction (PCR) was done on isolates of *E.coli* that had agglutinated with O157 antiserum to determine the gene that codes for verotoxin production. Colonies of *E.coli* O157 were suspended in 100μl of deionised water in eppendorf tubes. The tubes were arranged on a metal rack, immersed in water pre-heated to 65$^{\mathrm{O}}$C. The water was then heated to boiling for 30 minutes to lyse the bacterial cells and expose the DNA material. The tubes were then centrifuged at 12,000 $X g$ for 5 minutes and the supernatant decanted into another sterile tube. The supernatant having the DNA material was stored at -20$^{\mathrm{O}}$C.

Polymerase chain reaction was performed in 0.5ml Eppendorf tubes on a Techne PHC-3 thermal cycler with a reaction volume of 50μl. DNA template (5μl) (extracted DNA) was added to 45μl of the reaction mixture containing 8μl of 0.1 mM of each dNTPs (dATP, dCTP, dGTP and dUTP); 5μl of 10X buffer consisting of $(NH_4)_2SO_4$,67mM Tris HCL (pH8.8 at 25$^{\mathrm{O}}$C) and 2μl of 10% bovine serum albumin; 6μl of 1.5mM $MgCl_2$ and 2μl of each PCR primer set (30.4μg/ml diluted 1:300 in the Tris-Borate EDTA buffer -From The International Livestock Research Institute (ILRI) Standard operating protocol: segoli P007); 21.75μl of PCR water and 0.25μl of Taq polymerase enzyme. The PCR reagents were purchased from Sigma limited, Saint Louis, Missouri, USA.

The Polymerase chain reaction program was 94 $^{\mathrm{O}}$C for 1 minute, 50 $^{\mathrm{O}}$C for 1 minute, and then 72 $^{\mathrm{O}}$C for 3 minutes for 40 cycles and 72 $^{\mathrm{O}}$C for 10 minutes when the isolated DNA was amplified.

The reaction products were separated by agarose gel electrophoresis by adding 7 µl o f t he l oading d ye containing 1 .5µl o f 0 .25% b romophenol b lue i n 40% sucrose to a 20 µl of the reaction mixture and loaded to 1.5% agarose gel. The buffer in the electrophoresis chamber was x 1buffer: 20ml of x 5 buffer (Tris –Borate EDTA) +80 ml of dH$_2$0 and contained Ethidium bromide (1µg/ml) and 8µl of 100kb ladder DNA marker. The gel was run at 125 volts for 1 hour. DNA in the gel was visualised by exposing the gel to UV light and photographed on a Polaroid film. The DNA pattern was produced with a computer graphic program after computer scanning of the Polaroid photographs.

Table3; Primer sequence for verocytotoxin.

Gene(Nucleotides)	Primers sequence
O157-AF	AAG ATT GCG CTG AAG CCT TTG
O157-AR	CAT TGG CAT CGT GTG GAC AG
O157-FB	TCT GCG CTG CTA TAG GAT TAG C
O157-RB	CTT GTT TCG ATG AGT TTA TCT GCA
O157-PF	CGG ACT CCA TGT GAT ATG G
O157-PR	TTG CCT ATG TAC AGC TAA TCC

3.7 Data analysis:

A database was created in Microsoft Access for the questionnaire and laboratory data analysis. Selected queries where exported to Microsoft Excel and Instat version 3.029 (statistical services centre, 2005) statistical programme for analysis. Summary descriptive statistics were then calculated using Instat version 3.029 (statistical services centre, 2005) giving proportions of every group of risk pathway investigated. The participatory data was entered into Microsoft Excel and average scores calculated from the proportional piling scores obtained from the focus group discussions. Prevalence of *E.coli* O157:H7 in dairy farming and non-dairy farming households was determined from seropositive and vt1 positive isolates. Confidence limits were constructed around the proportions at 95% confidence level.

CHAPTER 4.

4.0 RESULTS.

4.1 Perception on risk factors to *E.coli* O157:H7.

The hired male worker spends over 50% of their daily time clearing cattle shed of manure piles and on disposal of cattle faeces to manure pit (Table 4 a, b). The woman in the dairy households also spends more time doing the dairy activities that are potential exposure factors to *E.coli* O157:H7 (Table 4 a). The men had a lower proportional score over women on these exposure factors (Table 4 b). However, the assessment of attitudes and practices on dairy farming households revealed that the woman was more involved in dairy related activities (unpublished data). Other members of the household were also involved in these dairy farming activities and were therefore at risk of exposure. During the community workshops, the interviewees responded that they were drinking fermented (soured) raw milk, consuming raw vegetable salads, raw carrots and tomatoes without washing in clean water (unpublished data). Hancock *et al.* (1997) had reported exposure to *E.coli* O157:H7 through these routes.

Table 4 (a). Proportional scoring on daily activities by women.

location	N	Range	Cleaning cattle shed						Disposal of manure						milking					
			M	W	H/M	H/W	B	G	M	W	H/M	H/W	B	G	M	W	H/M	H/W	B	G
Ruthimitu	20	0-10	1.3	2.5	3.8	0	0	0	0	2.5	7.5	0	0	0	1.3	3.5	3	2.3	0	0
Uthiru	9	0-10	2.8	0.5	6.8	0	0	0	1.3	0	6.8	2	0	0	0.5	2.5	5.3	0.5	1.3	0
Kawangware	9	0-10	0	0.7	5.3	0	4	0	0	9.3	0	0	0.7	0	0	1	5.3	2.3	1.3	0
Mutuini	9	0-10	0	6	4	0	0	0	0	2	8	0	0	0	0	7	3	0	0	0
Riruta	9	0-10	0	0	10	0	0	0	0	0	10	0	0	0	0	0.8	9.2	0	0	0
Waithaka	10	0-10	1	4	5	0	0	0	3.3	0	6.7	0	0	0	0.7	7.7	1	0.7	0	0
			1	2	6	0	1	0	1	2	7	0	0	0	0	4	5	1	1	0
% score			10	20	60	0	10	0	10	20	70	0	0	0	0	40	50	10	10	0

KEY:

M=Man , **W**=Woman, **H/M**=Hired male worker, **H/W**=Hired female worker,

B=Boy, **G**=Girl , N=Number of participants, **Range**=range of scores.

Table 4 (b). Proportional scoring on daily activities by men.

location	N	Range	Cleaning cattle shed						Disposal of manure						milking					
			M	W	H/M	H/W	G	B	M	W	H/M	H/W	G	B	M	W	H/M	H/W	G	B
Ruthimitu	17	0-10	2	0	8	0	0	0	0	4	6	0	0	0	1	3	6	0	0	0
Uthiru	11	0-10	2.3	1	6.7	0	0	0	3.7	1.3	5	0	0	0	0.7	0	9.3	0	0	0
Kawangware	4	0-10	3	2.3	4.7	0	0	0	5.3	3	1.7	0	0	0	4.3	4.3	1.3	0	0	0
Riruta	7	0-10	1.7	1.7	1.7	0	0	0	5	0.7	4.3	0	0	0	0.7	0	9.3	0	0	0
Waithaka	8	0-10	5.3	0.3	0.3	4.3	0	0	1.3	2	6.7	0	0	0	3.3	1.7	5	0	0	0
			3	2	5	1	0	0	3	2	5	0	0	0	2	2	6	0	0	0
		% score	30	20	50	10	0	0	30	20	50	0	0	0	20	20	60	0	0	0

KEY:

M=Man,   **W**=Woman,   **H/M**=Hired male worker,

H/W=Hired female worker,   **B**=Boy,   **G**=Girl,

N=Number of participants,   **Range**=range of scores.

4.2 Characteristics of dairy and non-dairy households.

Majority of the dairy households lived on 2 acres of land (appendix 4). The number of people who lived in male headed households were slightly larger (1.4, p<0.01) than those in female headed households but there was no significant difference in land size holding (p=0.3). The average age of dairy households was 49±16.2 years; while that of non-dairy household was 36.3±13 years. The dairy household members had lived longer in the area (27.9±13.5 years) compared to the non-dairy households who had lived in the neighbourhood for a period averaging 12.4±15.5 years. The non-dairy households were probably new immigrants who were tenants.

Educational level for the dairy and non-dairy household heads is given in table 5 below. Educational level tended to be lower for women than for men with an estimated proportions of women having only informal education (0.32; CI 0.219-0.436) compared to men (0.11; CI; 0.07-0.16) in dairy households. Male headed dairy household heads also had a higher estimated proportion of secondary school level education (0.30; CI; 0.24-0.36; P<0.0001).

The educational levels for the non-dairy households did not differ significantly between the gender, but informal education proportion were lower (0.04, 0.02) for females and males respectively, than those of dairy households heads (0.32, 0.11). The proportions of secondary level of education were also significantly higher (0.385, 0.47) (p<0.0001) than those of dairy household heads (0.15, 0.30). The difference is reflected by the older mean age of dairy household head respondents 49.8 years compared to that of non-dairy respondents 36.3 years(p<0.001). This is also shown by the smaller

proportion of dairy respondents who had not lived at their present home all their lives (0.45, 0.56) compared to non-dairy respondents who had not lived at their present homes (0.68, 0.84) and the longer time that dairy respondents had lived at their present home 27.9±13.5 years as compared to a mean of 12.4±15.53 years for non dairy respondents (p<0.001) (appendix 4).

Table 5: Educational level of household head by gender.

Level of education	Urban dairy household				Non-dairy household			
	Female		Male		Female		Male	
	N	%	N	%	N	%	N	%
Informal	26	32	23	11	1	4	2	2
Lower primary	15	19	17	8	4	15	4	4
Upper primary	20	25	75	35.5	7	27	44	40
Secondary	12	15	64	30	10	38.5	52	47
Tertiary	6	7.5	29	14	3	11.5	8	7
University	0	0	3	1.5	1	4	0	0
Total	79	100	211	100	26	100	110	100

4.3 Survey risk assessment.

i) Water source.

Seventy-eight percent of the dairy farming and 94.5 % of the non-dairy households were using water from the city council. However, other dairy farming households were using water from other sources: hand dug wells (15%) and boreholes (6%). The non-dairy households were using well water for drinking more than the dairy farming households (P < 0.0455). These water wells were covered using wooden materials and/or iron sheets while others were open and therefore faecal contaminated runoff water could contaminate the well water. Fourty three percent of dairy farming and 34.3 % of the non-dairy households were drinking untreated water (P < 0.0436) and were at risk of infection. Seventy-seven percent of the dairy farming and 94% of the non-dairy households were not adding chlorine while 59% of dairy farming and 53% of non-dairy households were not boiling drinking water and were at risk of infection.

ii) Consumption of fermented (soured) raw milk.

The non-dairy households (64% CI; 56-72) got their milk from dairy farming neighbours while the rest purchased milk from neighbouring kiosks. Four percent of both dairy and non-dairy households who consumed raw milk were at risk of infection. The non-dairy households (31% CI; 23.2-38.8) were at a significantly higher risk of infection to *E.coli* O157:H7 than the dairy farming households (21% CI; 16.3-25.6) from consumption of fermented raw milk (P < 0.0132) (Table 6).

iii) **Manure handling.**

Seventy three percent (CI; 68-78) of the dairy farming and 59.7% (CI; 51.5-67.9) of non-dairy farming households used cattle faeces /manure as fertilizer on their vegetable farms. However, there was a significant difference in the rate of manure usage between the two groups (P < 0.0029). The dairy farming households were more exposed than the non-dairy households. Eighty seven percent (CI; 81-89) of dairy farming households and 86% (CI; 80.2-91.8) of the non-dairy households did not use protective gloves when handling cattle faeces. Eighty seven percent (CI; 83-90.8) of the dairy farming households considered protective gloves unnecessary and expensive; this increases the risk of infection because cattle faeces is the main source of the environmental contamination.

iv) **Consumption of raw vegetable salads.**

Fifty three percent (CI; 47.1-58.7) of the dairy farming households and 52% (CI; 44.2-60.4) non-dairy households were consuming raw vegetable salads. The rate of raw salad consumption between the households was not statistically different (P < 0.4247), but the high consumption rates in the households was a more likely route of exposure (Table 6).

v) **Mitigation on risk by dairy and non-dairy households.**

The various exposure pathways in households were however mitigated for by both dairy and non-dairy households. Raw milk was boiled by ninety six percent of both households. Water for drinking was boiled before drinking by 41% and 47% of the dairy farming and non-dairy households respectively, while other household's added chlorine to their drinking water (22.7 % of dairy farmers and 6% of non-dairy farmers). Most

households also used water from the city council, which is regarded as chlorinated (94.5% of non-dairy and 78% of dairy households). The urban dairy household members who handled cattle faeces with bare hands would wash their hands (46%), wash the whole body (36%) or change their cloths (17%) after the farm activities. The interviwees at the workshops also responded that before milking a dairy animal, they would clean the udder with warm water and dry it up with a towel, and after milking they apply teat dips (mastrite) to control any form of infection.

Table 6: Proportion of risk factors in households.

Risk factor	Dairy hhd	Non-dairy hhd	S.E	z-value	p-value
Consumption of raw vegetable salads.	0.53	0.52	0.052	0.194	0.4247
Consumption of maziwa lala (raw soured milk).	0.21	0.31	0.045	2.222	0.0132
Consumption of raw milk.	0.04	0.04	0.021	0	0.05
Drinking of well water.	0.15	0.216	0.0389	1.687	0.0455
Drinking of untreated water.	0.43	0.343	0.051	1.706	0.0436
Handling cattle faeces without gloves	0.85	0.86	0.037	0.2728	0.3936
Manure/faeces use on vegetable farms.	0.73	0.597	0.0478	2.7625	0.0029

KEY:

Dairy hhd   =dairy households

Non-dairy hhd =non-dairy households

S.E   =standard error

4.4 Prevalence of *E.coli* O157 in households.

Seventy seven faecal samples and 31 milk samples from the dairy farming households and 13 milk samples from the non-dairy households had non-sorbitol fermenting (colourless and /or transparent) colonies of *E. coli* (Table7). Fifteen isolates from faecal samples and 3 isolates from milk samples from non-dairy households agglutinated with O157 antiserum. The apparent herd prevalence of *E.coli* O157 in cattle faeces and milk was determined as 5 % (CI; 3-8) and 2% respectively. Only one faecal isolate had the gene coding for VT 1 of 420 basepairs (Figure 2).

Table 7: Prevalence of *E.coli* O157 in household samples.

Household	sample		Sorbitol -ve	Seropositive	P	S.E	CI 0.95
	Type	N					
Dairy	Faeces	290	77	15	0.05	0.014	0.03-0.08
Dairy	Milk	260	31	0	0	0	
Non-dairy	Milk	136	13	3	0.02	0.013	0-0.045

KEY:

P=Prevalence,   0.95 **CI**=95% confidence level,

Seropositive= O157 antiserum positive,   **Sorbitol –ve** =non-sorbitol fermenters.

Type= Types of samples.   **N** = Number of samples.

S.E=Standard error
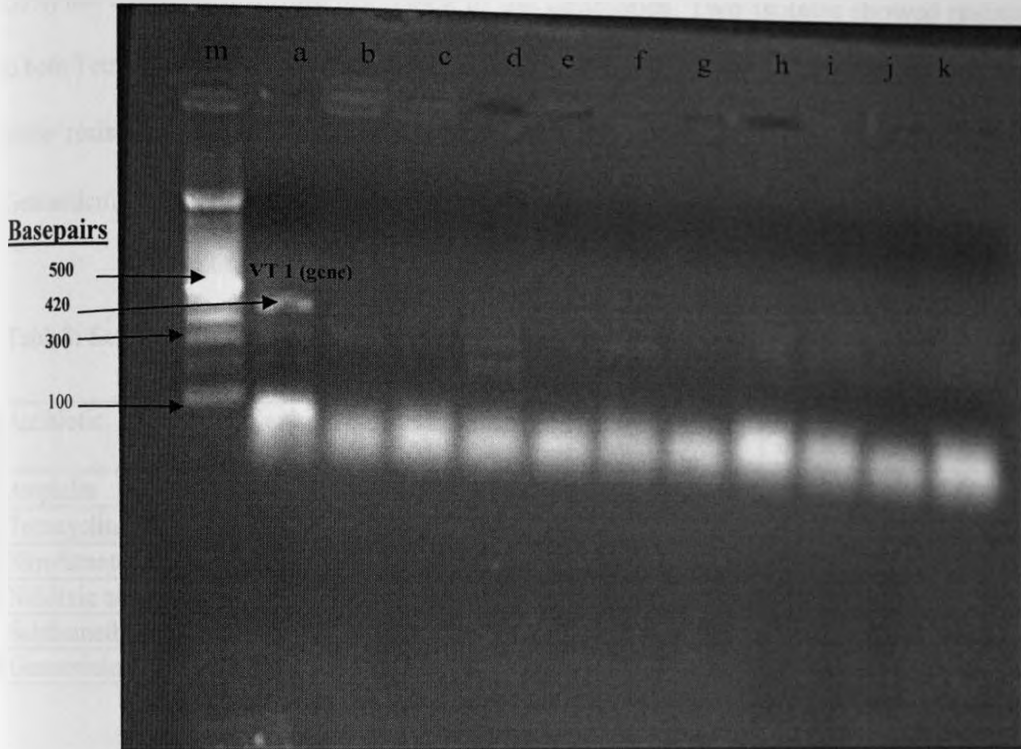
Figure 2: PCR gel output showing band for Verotoxin 1 gene.

KEY:

Ladder (m) =100kb, a =isolate 419, b=isolate 811, c =isolate 865, d=isolate775,

e=isolate 497, f =isolate 898, g =isolate 214, h =isolate 623, i=isolate 785,

j =isolate 861, k =isolate 588.

4.5 Antimicrobial sensitivity of the isolates.

All the fifteen faecal *E.coli* O157 isolates and the 3 milk *E.coli* O157 isolates were highly sensitive to the various antibiotics (Table 8). Out of the 18 positive isolates, 6 (33%) had single to multiple resistance to the antibiotics. Two isolates showed resistance to both Tetracycline and Sulphamethoxazole. Resistance to Tetracycline alone was 11.1% while resistance to Sulphamethoxazole was 27.7%. All isolates were sensitive to Gentamicin. The sensitivity patterns of the isolates are attached in Appendix 6.

Table8: Sensitivity results.

Antibiotic	Sensitive	Resistant	Intermediate	% Sensitive	% Resistant
Ampiclin	17	1	0	94.4	5.5
Tetracycline	15	2	1	83.3	11.1
Nitrofurantoin	17	1	0	94.4	5.5
Nalidixic acid	17	0	1	94.4	0
Sulphamethoxazole	13	5	0	72.2	27.7
Gentamicin	18	0	0	100	0

CHAPTER 5

5.0 DISCUSSION.

The apparent herd prevalence of *E.coli* O157: H7 in dairy farming households was less than 2%. However, the apparent herd prevalence of *E.coli* O157 in cattle faeces and milk was 5% and 2% respectively. One faecal isolate had genes coding for verotoxin 1 production but verotoxin 2 gene was not detected by the primer set. However, there were 15 faecal isolates and 3 milk isolates that were positive for O157 antigen reactive with O157 antiserum; these isolates are potential verotoxin producers. The *E.coli* O157 strains acquires the verotoxin producing gene either by conjugative plasmids, transposon like elements, bacteriophages or integrans, and hence become pathogenic by producing the verotoxin which would then lead to serious health problems like haemorrhagic colitis and haemolytic ureamic syndrome to the vulnerable.

Cattle faecal material is the main reservoir for environmental contamination, and thus a higher isolation rate of potential verotoxin producing *E.coli* O157 (Wells *et al.*, 1991; Kudva *et al.*, 1996). The milk samples from the dairy farming household did not yield any *E.coli* O157 possibly due to the appropriate hygiene measures practised at the household level during milking, such as cleaning of the udder with warm water before milking. The non-dairy households purchased milk from kiosks, neighbouring dairy farming households and milk bought from areas away from the division. The contamination of the non-dairy household milk was probably due to poor handling by use of contaminated containers or from milk that are traded in the area from other areas away from the division. The Raw milk was consumed by 4% of both dairy farming and non-

dairy households, this would be a likely route of exposure to *E.coli* O157 to the vulnerable household members.

The shedding pattern of *E.coli* O157:H7 is affected by season (Wilson *et al.*, 1996; Hancock *et al.*, 1997; Smith *et al.*, 2001), type of ration fed to the animals (Callaway *et al.*, 2003) and age of cattle sampled (Wilson *et al.*, 1996). According to Wilson *et al.* (1996) the rate of shedding of *E.coli* O157:H7 was higher in growing cattle, especially newly weaned calves and during the summer. The study was done in a wet season between the months of April and June and therefore do not represent the status in dry season. The study area lies within the tropics and therefore do not experience the effects of temperate seasonal variation, but the rate of shedding of the pathogen by cattle could be influenced by the dry or wet season.

In a cross sectional study to assess the risks of zoonotic *E.coli* O157:H7 and brucellosis in informally marketed and unpasteurised milk in Nairobi and Nakuru districts, Arimi *et al.* (2000) isolated *E.coli* O157:H7 from one milk sample. However, a longitudinal study done in USA to describe the ecology of *E.coli* O157:H7 in pens of commercial feedlots by seasons (summer and winter), detected a herd prevalence of 42% from pooled faecal samples and ropes that were tied in pens for cattle to rub on and chew (Smith *et al.*, 2001; 2003). These studies also concluded that using immunomagnetic separation method in isolating *E.coli* O157:H7 from samples has higher sensitivity than sub culturing in sorbitol Mac Conkey medium. According to the previous studies (Wilson *et al.*, 1996; Arimi *et al.*, 2000; Koroti, 2002; Kaddu *et al.*, 2001), cross sectional

mpling detects lower prevalence of *E.coli* O157:H7. Therefore, a longitudinal study is equired to find out seasonal shedding of this organism by cattle.

The antibiotic sensitivity of the isolates was consistent with results from other studies hat had shown an increase in resistance to Sulphamethoxazole and Tetracycline's (Remis et al., 1984; Swerdlow et al., 1992; Kim et al., 1994). The organism's resistance to Tetracycline (11.1%) and Sulphamethoxazole (27.7%) would increase *E.coli* O157:H7 virulence. This occurs either by eliminating all competing bowel flora by the antibiotics leading to an overgrowth of *E.coli* O157:H7 or by lyses of /or sub lethal damage to the infecting organisms, with the subsequent liberation of shiga-like toxins (Su and Brandt, 1995). This would result in prolonged duration of diarrhoea in patients (Ostroff et al., 1989), and complications like haemolytic uraemic syndrome or thrombotic purpura (Su and Brandt, 1995). Certain antibiotic such as fluoroquinolone induces shiga toxin encoding bacteriophages in vivo and these leads to increased expression of shiga toxin genes (Galland et al., 2001). However, the *VT* 1 positive isolate was sensitive to all the antimicrobial agents.

Ninety two percent of the dairy farming households supplemented dairy cattle with grain rations. This study obtained 77 non-sorbitol fermenting faecal *E.coli* isolates on sorbitol Mac-Conkey Agar. Other studies had shown that animals fed feedlot type rations (high grain diet) shed 1000 fold higher generic *E.coli* population as compared to those animals fed on hay ration (Kudva et al., 1995; 1996; Diez-Gonzalez et al., 1998; Keen et al., 1999; Callaway et al., 2003). The increased rate of shedding of the generic *E.coli* population and /or *E.coli* O157:H7 would pose a great health risk to the 86 % of the dairy

farming households and 85% of the non-dairy households who handled cattle manure/faeces without protective gloves. According to Maule, (1999) the organism can survive in cattle faeces for over 12 weeks and in the soil for over 20 weeks and therefore would contaminate the environment.

The participatory research and the survey risk assessment showed that there were daily activities in the household that are potential exposure pathways for the pathogen, both in the urban dairy farming and non-dairy households. Dairy farming households (73%) and non-dairy households (59.7%) used cattle faeces on vegetables and other crops farms. Fifty three percent of the dairy farming households and 52% of the non-dairy households were consuming raw vegetable salads from fresh vegetables and eating raw carrots and potatoes that are potential exposure factors (Su and Brandt, 1995; Abdoul Raouf et al., 1996; Hancock et al., 1997). These households should take precautions to avoid infection by this pathogen. All raw vegetables should be washed in clean water before preparing salads and household members should avoid eating raw carrots and potatoes.

The findings from this study on the members of the household who are involved in urban agriculture activities was in agreement with others who had reported that women are more involved in urban agriculture because of their lower levels of education with majority of women having only informal education (Harvoka et al., 2006; Wilbers, 2004). The low level of education amongst the women leaves them a disadvantaged group because they can not effectively compete for formal employment opportunities with the men who have higher level of education. Urban agricultural activities, provides

practical and strategic needs of urban households who engages in the activities. Evidence from this study (unpublished data) together with other studies show that women uses the urban agricultural activities to support their households on daily basis, while others uses it as an avenue for social and economic empowerment over a long term (Lee-smith *et al.*, 1987; Wilbers, 2004; Harvoka *et al.,*2006). The local authority, therefore should consider reviewing the illegal status of urban agricultural activities because of its enormous economic contribution to the households and also as an alternative employment to the women who are not highly educated.

Twenty-one percent of the dairy farming households and 31% of the non-dairy households were consuming raw fermented (soured) milk which is an acidic food that had been associated with infection (Morgan *et al.*, 1988). A study done in Ethiopian to determine the survival of *E.coli* O157:H7 in traditionally soured milk products, reported that the number of *E.coli* O157:H7 organisms multiplied from $\log_{10}3$ cfu/ml to 9.4 cfu/ml after 72 hours, thus confirming the hypothesis that *E.coli* O157:H7 is acid resistant (Tsegaye and Ashenafi, 2005). Acid resistance plays a major role in bacterial enteric infections. Food borne pathogens must survive in the stomach (pH<3) for 2 upto hours before passage to the intestinal tract, where colonization occurs. Cellular mechanisms governing the ability of some pathogenic organisms to adapt to environmental stressing conditions, such as pH, have been related to the expression of some virulence factors. A regulatory gene *RpoS* is involved in acid resistance in *E.coli* (Bachrouri *et al.*, 2002). Consumption of raw milk that has been associated with infection was by 4% of both dairy and non-dairy households, this is a potential route of exposure to infection,

however, ninety six percent (CI; 94-98) of the dairy households were boiling raw milk before consumption. Raw milk had been linked to two *E.coli* O157:H7 outbreaks in Oregon, USA (Armstrong *et al.*, 1996). In addition to boiling fresh milk, the dairy farming households reported using warm water to clean the udder of the cow (unpublished data). This practice would reduce the probability of milk contamination with cattle faeces, which is a reservoir of the *E.coli* O157 pathogen (Kudva *et al.*, 1996; Chapman *et al.*, 1997).

Seventy eight percent (CI; 73-82) of dairy farming households were using water from the city council of Nairobi, 59% were not boiling their water and 77.3 percent were not adding chlorine to the water. These households would be at risk of infection from untreated water if the city council fails to chlorinate water in their distribution system. Untreated water had been associated with infection in Missouri, USA and in South Africa (Swerdlow *et al.*, 1992; Effler *et al.*, 2001). Fifteen percent of dairy farming households and 21.6% of non-dairy households were using water from open wells, these water wells were covered by wooden materials, iron sheets while some were open. These could be contaminated by faecal contaminated runoff water, thus posing a heath risk to users. Other studies had reported infection through contaminated well water (Sonja *et al.*, 2002; Dianna *et al.*, 2003).

5.1 Conclusion and recommendations.

Conclusion.

The apparent herd prevalence of *E.coli* O157:H7 in the urban dairy farming households was less than 2%. Although several potential exposure pathways were

present in the urban dairy farming and non-dairy households, these study concluded that there was minimal risk of infection by *E.coli* O157:H7 because of the low apparent herd prevalence. However, because *E.coli* O157 organisms can acquire the verocytotoxin gene by conjugative plasmids or bacteriophages, the health authorities should establish a continuous surveillance system to prevent any future outbreaks.

Recommendation.

1) Open wells should be covered with cemented slabs to reduce chances of seepage of faecal contaminated runoff water into the well and urban dairy and non dairy households should continue either boiling or chlorination of drinking water.

2) Household members who spend most of their daily time handling manure/cattle faeces should use protective gloves to reduce chances of hand contamination.

3) The household members should avoid consuming fermented (soured) raw milk and raw vegetable salads and carrots that are risk factors of infection.

4) Exposure factors as raw vegetable salads, soured raw milk, meat and meat products and water sources should be investigated for the presence of the pathogen in urban smallholder dairy farming system.

5) Given the low prevalence of verotoxigenic *E.coli* in the urban smallholder dairy households, policy makers should consider reviewing the illegal status of urban agricultural activities because of the numerous benefits that urban households get from it.

CHAPTER 6.
6.0 REFERENCES.

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APPENDIX

1. Non Farmer Questionnaire.

Serial No of farmer neighbours ☐☐☐

Neighbour number _____

Name of Household Head_____

Date...................................

Name of interviewer...

Location

Ruthimitu ☐

Kawangware ☐

Uthiru ☐

Waithaka ☐

Mutuini ☐

Riruta ☐

Sublocation.................Geographical location

Latitude: **S** ☐☐ . ☐☐☐☐

Longitude **E** ☐☐☐ . ☐☐☐☐☐

1. How do you treat your water before drinking?

i) boiling ☐

ii) Chlorinate ☐

iii) Filtrate ☐

Other (specify)____

2. What are your sources of water?

Tap water ☐

River/ stream ☐

Hand dug well ☐

Rain water ☐

Bore hole ☐

Other (specify)_____

3. Do you use milk in this household even if occasionally?

1. Yes ☐ 2. No ☐ If yes for above, where do you get this milk from?

Buy from neighbours with dairy cattle ☐

Buy unpacked milk from milk kiosks ☐

From relative/s who keep/s dairy cattle nearby ☐

Buy packed milk from shops ☐

Other ☐ Specify _____

4. Do you boil milk before consuming it?

1. Yes ☐ 2. No ☐ 3. Sometimes ☐

5. Do you prepare maziwa lala?

1. Yes ☐   2. No ☐   3. Sometimes ☐

6. Do you prepare salad in this household?

1. Yes ☐   2. No ☐   3. Sometimes ☐

7. Do you or any member of the household think there are any health hazards associated with consumption of unpacked fresh milk?

1. Yes ☐   2. No ☐   3. I don't know   ☐

8. Has anyone in the household had any health problems associated with drinking unpacked fresh milk?

1. Yes ☐   2. No ☐

9. If yes for Q8. above what problems / Symptoms?...

10. Do you handle cattle waste

1. Yes ☐   2. No ☐

11. If yes Q10. above, do you use gloves to handle cattle wastes?

1. Yes ☐   2. No ☐

12. If you don't not use gloves why? Don't you

Was not aware of them   ☐

Are expensive   ☐

Uncomfortable to wear   ☐

Think are not necessary   ☐

Others   ☐   Specify_____

13. Has anybody in the household suffered from bloody diarrhoea?

1. Yes ☐   2. No ☐

14. Has anybody in the household suffered from kidney failure?

1. Yes ☐   2. No ☐

2. Farmer Questionnaire on Risk factors.

Serial No ☐☐☐

Name of household owner_____ (as per sample

selected)

Date……………………………....

Name of interviewer……………..

Location

Ruthimitu   ☐

Kawangware   ☐

Uthiru   ☐

Waithaka   ☐

Mutuini   ☐

Riruta   ☐

Sublocation…………………….

Geographical location

Latitude: **S** ☐☐ . ☐☐☐☐☐

Longitude: **E** ☐☐☐ : ☐☐☐☐☐

1. What are your sources of water?

	For cows	Humans
a)Tap water	☐	☐
b)River/ stream	☐	☐
c)Hand dug well	☐	☐
d)Rain water	☐	☐
e)Bore hole	☐	☐

f)Other (specify)_____

2. Do you treat your water before drinking?

1. Yes ☐   2. No ☐

3. If yes, how do you treat it? Multiple response

Boil   ☐ 2. Chlorinate   ☐ 3. Filtrate   ☐ 4. Other (specify)_____

4. Do you use protective clothing when milking? 1. (Yes) 2. (No) 3.

(Sometimes)

5. Do you boil milk before consuming it? 1. (Yes) 2. (No) 3. (Sometimes)

a) Do you prepare maziwa lala? 1. (Yes) 2. (No) 3. (Sometimes)

b) Do you prepare salads at home? 1. (Yes) 2. (No) 3. (Sometimes)

6. Do you clean the udder with warm water before milking?

1. Yes ☐   2. No ☐

7. How do you pack milk for sale?   1. (Plastic paper bags) 2. (Plastic jars/ bottle)   3. (Plastic containers) 4. (Glass jar/bottle) 5. (Any other_____).

8. How do you clean milking utensils? 1. (Warm water alone) 2. (Detergent and warm water) 3. (Detergent and cold water) 4. (Cold water alone) 5. (Other_____).

9.Who cleans cattle shed/stalls the most (the main person) 1. (Woman) 2. (Man)   3. (Girl) 4. (Boy) 5. (Male worker) 6. (Female worker).

10. Who is the second back up person who cleans the cattle shed? 1. (Woman) 2. (Man)   3. (Girl) 4. (Boy) 5. (Male worker) 6. (Female worker).

11. How often is it cleaned? _____

12. Where do you dispose cattle wastes?

a)Sell to neighbours   ☐

b)Fertilize vegetable gardens   ☐

Any other   _____

13. Do you use gloves to handle cattle wastes? 1. (Yes)   2. (No)

14. If you don't use gloves why Don't you

a)Was not aware of them   ☐

b)Are expensive   ☐

c)Uncomfortable to wear   ☐

d)Think are not necessary   ☐

e)Others   Specify _____

15. What do you do after handling wastes?

a) Wash the whole body and change clothes

b) Wash hands only

c) Take a shower after removal of the clothes, which are kept for this work

d) Others specify

16. How do you carry the dung to the manure pit? 1. (Wheelbarrows) 2. (Gunny bags) 3. (Any other_____)

17. Who milks the animal/cattle most? 1. (Woman) 2. (Man)   3. (Girl) 4. (Boy) 5. (Male worker) 6. (Female worker).

18. Do you or any member of the household think there are any health hazards associated with consumption of unpacked fresh milk?

1. (Yes)   2. (No)

19. Has anyone in the household had any health problems associated with drinking unpacked fresh milk?

1. Yes ☐   2. No ☐   3. I don't know   ☐

20. If 'Yes'(for Q 19) what problems / Symptoms?..

21. Has anybody in the household suffered from bloody diarrhoea?

1. Yes ☐   2. No ☐

22.Has anybody in this household suffered from kidney failure

1. Yes ☐   2. No ☐

23.Do you feed concentrates to your cows

1. Yes ☐   2. No ☐

3. Confidence intervals for the risk factors.

Dairy Households				Non-Diary Households		
Factor	Prop.	S.E	0.95%CI	Prop.	S.E	0.95 CI
Kidney failure	0.027	0.0094	0.008-0.045	0.034	0.016	0.004-0.0645
Bloody diarrhoea	0.05	0.013	0.025-0.0747	0.1	0.026	0.05-0.15
Ferment milk	0.21	0.024	0.163-0.256	0.31	0.04	0.232-0.388
Salads	0.53	0.03	0.471-0.587	0.52	0.04	0.442-0.604
Water source						
Tap	0.78	0.024	0.73-0.82	0.945	0.02	0.91-0.98
Well	0.15	0.021	0.11-0.19	0.22	0.04	0.147-0.285
Borehole	0.1	0.017	0.066-0.1	0.061	0.02	0.021-0.101
Water treatment	0.57	0.03	0.51-0.629	0.657	0.041	0.577-0.737
Chlorination	0.23	0.024	0.18-0.275	0.061	0.021	0.021-0.101
Boiling water	0.41	0.03	0.35-0.47	0.47	0.043	0.39-0.55
Manure usage	0.73	0.026	0.68-0.78	0.597	0.042	0.515-0.679
Non usage of gloves on manure	0.85	0.021	0.81-0.89	0.86	0.03	0.802-0.918
Gloves unnecessary	0.87	0.02	0.83-0.908	0.3	0.039	0.22-0.38
Gloves expensive	0.38	0.028	0.33-0.44	0.09	0.024	0.042-0.138
Farmers wash hands only	0.46	0.029	0.404-0.516			
Wash whole body	0.36	0.028	0.306-0.414			
Change cloths	0.17	0.022	0.127-0.213		'	
Source of milk						
From farmer neighbour				0.64	0.041	0.56-0.72
Boiling milk	0.96	0.011	0.94-0.98	0.96	0.017	0.927-0.993
Concentrate feeding	0.92	0.016	0.889-0.951			

4: characteristics of dairy and non-dairy households.

	Dairy household		Non dairy household	
Household characteristics	Mean	SD	Mean	SD
Land size (acres)				
Dairy household	2.1	2.9		
Female headed	2.2	2.7		
Male headed	2.0	3.0		
Household size				
Overall	5.28	2.7	4.9	5.2
Female headed	4.23	2.4	4.0	1.9
Male headed	5.67	2.7	5.1	5.8
Age of respondent				
Overall	49.5	16.2	36.41	12.9
Female headed	57.04	15.61	37.66	13.02
Male headed	46.94	15.81	33.79	12.02
Years lived in area	26.39	13.7	11.85	15.2

5. Media preparation.

Eosin Methylene Blue Agar.

Typical formular.

Peptone 10.0,lactose 10.0,Di-pottasium hydrogen phosphate 2.0,Eosin Y 0.4,Methylene blue 0.06,Agar 15.0.

Suspend 37.5 grams in 1 litre of distilled water, boil until its dissolved completely, sterilize by autoclaving at $121^{O}C$ for 15 minutes. cool at $60^{O}C$ and shake the medium in order to oxidize the Methylene blue(i.e. to restore the blue colour)and to suspend the precipitate which is an essential part of this medium.

Tryptone Soya agar(TSA).

Typical Formular.

Tryptone 15.0-,soya peptone 5.0,cloruro sodico 5.0,agar 15.0.

Suspend 40 grams in 1 litre of distilled water, boil until its dissolved completely, sterilize by autoclaving at $121\ ^{O}C$ for 15 minutes.

Sorbitol MacConkey

Typical Formular.

Peptone 20.0,Sorbitol 10.0,Bile Salts NO. 31.5,Sodium Chloride 5.0,Neutral Red 0.03,Crystal Violet 0.001 Agar 15.0.

Suspend 15.5 grams in 1 litre of distilled water, boil to dissolve completely ,sterilize by autoclaving at 121°C for 15 minutes.

Muller Hinton Agar.

Typical Formular.

Beef, dehydrated infusion from 300 casein hydrolysate 17.5, starch 1.5 ,agar 17.0.

Suspend 38 grams in 1 litre of d stilled water, boil to dissolve completely, sterilize by autoclaving at 121 ^{O}C for 15 minutes.

Indole Test

Kovac's reagent

Paradimethylaminobenzaldehyde...............................5 grams

Amyl alcohol 75ml

Concentrated hydrochloric acid 25ml

Formular	grams/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+-0.2

Direction

23 grams of the media was suspended in 1 litre of distilled water, boiled to dissolve completely and then sterilized by autoclaving at $121^{O}C$ for 15minutes.

 Tryptone water

Formula	grams /litre
Tryptone	10.0 grams
Sodium chloride	5.0 grams

PH 7.5+-0.2

Direction .

15 grams was dissolved in 1 litre of distilled water and distributed into final containers, then sterilized by autoclaving at $121^{O}C$ for 15 minutes.

Methyl red and voges proskauer medium

formula	grams/litre
peptone	5.0
glucose	5.0
phosphate buffer	5.0

pH 7.5+-0.2

direction

15 gram was added to 1 litre of distilled water and mixed well, this was then distributed into final containers and sterilized by autoclaving at $121^{O}C$ for 15 minutes.

Simmon Citrate Test

Formular	grams/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+-0.2

Direction

23 grams was suspended in 1 litre of distilled water, boiled to dissolve completely and then sterilized by autoclaving at $121^{O}C$ for 15minutes.

6. Antimicrobial resistance pattern.

Household	Antibiotic zone of inhibition in mm.					
Antibiotics	Ampicilin	Tetracycline	Nitrofurantoin	Nalidixic acid	Sulfamethoxazole	Gentamicin
F130	S	S	S	S	S	S
F651	S	S	S	S	S	S
F811	S	S	S	S	S	S
M865	S	S	R	S	S	S
F775	S	R	S	S	R	S
F422	S	S	S	S	S	S
F497	S	S	S	S	S	S
F898	S	S	S	S	S	S
F214	S	S	S	S	S	S
F419	S	S	S	S	S	S
F623	S	S	S	S	S	S
F785	S	S	S	S	R	S
F861	R	S	S	S	R	S
F588	S	S	S	S	R	S
M640	S	R	S	S	R	S
F58	S	S	S	S	S	S
M14	S	S	S	S	S	S
F97	S	I	S	I	S	S

7. Daily activity profile of dairy farming households

(Indicate who does the activity, at what time of the day and how long they take).

Activity	Person doing the activity				Time of the day	Time taken
	Man	woman	Hired man	Hired woman		
1. Preparing utensils and water for milking						
2. Milking						
3. distributing the milk						
4. Getting the fodder						
5. Watering the animals						
6. Cleaning the shed						
7. Disposing off manure						

Attitude and perceptions to infection by *E.coli*

O157:H7

(i)   Do you consider yourself to be at risk of acquiring the disease?

(ii)   If yes, what do you think are the risk factors?

(iii)   If no, why don't you consider yourself to be at risk?

(iv)   How can the disease be prevented?

Milking

(i)   Do you use protective clothing when milking?

(ii)   Do you wash you hands before milking?

(iii)   During milking, do you clean the udder with warm water? Dry the udder using a clean towel? Strip the teats? Teat dip?

(iv)   How is the milk stored after milking?

(v)   How is the milk used? Boiled milk consumed raw and /or fresh milk or fermented raw milk.

(vi)   How are the utensils cleaned and kept?

Handling of Manure

(i)   Do you clear cattle sheds of manure? How often? If some don't, what do they do?

(ii)   Do you use protective clothing when cleaning the shed and disposing off manure?

(iii)   How do you carry the dung/cattle faeces to the manure pit?

(iv)   How far are the cow shed and the manure pit from the house?