ASSESSMENT OF BEEF CARCASS CONTAMINATION WITH *ESCHERICHIA COLI* 0157:H7 POST SLAUGHTER IN KENYA

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Dedication

To my wife Josephine, son Samuel and daughter Terry Anne.

Acknowledgement

I am grateful to God for his blessings and favour to me and my family; that He has enabled me to carry out this research work to completion. I thank all who have supported and encouraged me during this period.

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Abbreviations

BPW	Buffered Peptone Water
CCFH	Codex Committee on Food Hygiene
CDC	Centre for Disease Control
CFSPH	Centre for Food Security and Public Health
E. coli	*Escherichia coli*
EHEC	Entero-haemorrhagic *Escherichia coli*
EPZ	Export Processing Zone
ESR	Environmental Science and Research Limited
FAO	Food and Agriculture Organisation of the United Nations
GOK	Government of Kenya
HACCP	Hazard Analysis of Critical Control Points
HUS	Hemorrhagic Uremic Syndrome
IMViC	Indole, Methyl red, Voges-Proskauer and Citrate
IOMC	Institute of Medicine Committee
MRVP	Methyl Red Voges Proskaurer
RKI	Robert Koch Institute
SMaC	Sorbital MaConkey
STEC	Shiga Toxigenic *Escherichia coli*
Stx	Shiga toxin
VT1	Verotoxin 1
VT2	Verotoxin 2
WHO	World Health Organisation

Abstract

A cross sectional study was carried out on meat sourced from three slaughterhouses: Dagoretti in Nairobi, Limuru in Limuru and Eldoret Township in Eldoret. The objectives of the study were to assess the probability of obtaining an *Escherichia coli* O157 serotype contaminated carcass at loading, off loading and butchery stages of the transportation value chain, the prevalence of *E. coli* O157 serotype contamination of the carcass at the three stages and at the butchery equipment and highlight the unhygienic practices that could lead to the contamination and cross-contamination of the carcasses at each stage of transportation.

The three slaughterhouses were selected on the basis of they being the main sources of meat consumed in Nairobi and Eldoret; the former being the capital city of Kenya and the most populated and the latter a stopover town in transit to the western side of Kenya. A total of 250 carcasses were randomly selected for non destructive sampling. Swab samples from a single carcass were obtained from three sites of the carcass, including the rump, the flank and the brisket at the three stages of the transportation value chain; the loading, the off loading and follow up after a day at the butchery. Swab samples were also obtained from four butchery equipment that was constantly in contact with the meat during sale: the cutting/chopping board, the knives/saw, the hooks and the weighing balance. A single carcass delivered to the butchery gave rise to eleven samples giving a total of 2750 samples.

E. coli O157 serotype was isolated through culturing in sorbital MacConkey agar, further purification in MacConkey agar and nutrient agar and then serotyping using card agglutination test. The confirmed serotype were then tested for verotoxin production (both VT1and VT2). The prevalence was determined through running data using Statistical Package for Social Sciences (SPSS) ver17. The prevalence data was then modelled to determine the probability of carcass contamination at each stage of sampling. Monte Carlo simulation using winBUGS® software was used to determine the risk of obtaining contaminated carcass at each stage. The meat carrier temperature and humidity were taken using a sling hygrometer. A semi structured questionnaire was used to assess the knowledge, attitude and practice of the meat transporters and butchery attendants. Observations were made on some of the practices by these key players.

The presumptive *E. coli* O157 isolates (non sorbital fermentors and with IMViC reaction of ++-) recovery from 2750 samples was 217 (7.89%). Only 20 (9.21%) of the presumptive isolates were positive for *E. coli* O157 serotype. The *E. coli* O157 serotype isolates that tested positive for

verotoxin production were; one for VT1, two for VT2 and one for both VT1 and VT2. These were distributed along the transportation value chain of meat from the three slaughterhouses. The prevalence of *E. coli* O157 serotype contaminated carcasses was 2.4% along the transportation value chain and that of contaminated equipment at the butchery was found to be 0.6%. The contamination prevalence at offloading was significantly higher compared to loading (P=0.05). The probability of obtaining an *E. coli* O157 serotype contaminated carcass at Dagoretti, Limuru and Eldoret respectively was 14, 16 and 19 at loading and 31, 39 and 66 at offloading per 1000 carcasses handled. The temperature in the meat carrier significantly increased (p=0.004) during transportation between loading and offloading. The average time taken to transport the meat from the slaughterhouses to the butchery was found to be 65 minutes.

The respondents interviewed for the knowledge, attitude and practices were 119 where 87 were butchery attendants and 32 meat transporters. Of those interviewed, 83 (69.75%) had worked in the meat industry for at least 5 years but only 19 (16%) had had formal training on meat hygiene. Most of the butchery attendants interviewed (97%) said they washed their hands frequently although only 9% of the butcheries had functional water taps in their premises. The meat transporters did not wash their hands during the transportation although 53% had said that they did so regularly. Cleaning of the butchery surfaces was done using cold water and soap only and no disinfectant was used.

During meat transportation, it was observed that the carcasses were loaded on the shoulders of the transporters and placed on the floor of the carriers or heaped on top of other carcasses. Offloading at the butchery was done by the same person without any change over of clothes. Kraft papers were used to separate the carcasses and avoid staining those beneath with blood. Bacteriological quality of the papers was unknown. These unhygienic practices in combination with the temperature increase during transportation could have led to the significant increase in contaminated carcasses at offloading as compared to loading. Therefore this significant increase was mainly due to cross contamination and bacterial multiplication.

There was risk of obtaining contaminated carcasses at the three stages of sampling. The risk increased along the transportation value chain. This was due to poor hygiene practices by both the transporters and butchery attendants who had little information on prevention of carcass contamination. Increase in temperature due to lack of the observation of the cold chain also led to the increase in the prevalence and probability of obtaining a contaminated carcass. *E. coli* O157 serotype contamination in the carcass could persist from loading to offloading.

CHAPTER ONE: Introduction

1.1Background Information

Beef is the major source of red meat with an estimated value of KShs 34.4 billion constituting 79.6% of the red meat earnings in Kenya while white meat accounts for only 19% of the total meat produced in the country (EPZ, 2005). Most of the meat produced in the country is consumed locally. The abattoirs that are used in the slaughter of the animals are either owned by individuals, private companies or the municipal council. The level of hygiene in some of the abattoirs is below the standards due to poor hygiene as most of the employees are not be well trained on meat handling practices (Kang'ethe, 1993). Training on meat handling includes but is not limited to Good Manufacturing Practices (GMPs), Good Hygiene Practices (GHPs) and Hazard Analysis of Critical Control Point (HACCP). The training and implementation of HACCP and other quality management procedures in the developing countries is constrained (Jirathana, 1998)

Meat handling during transportation and in the retail shops further increases the possibility of contamination. The mode of transportation is in vehicles or in a box mounted on a pickup, motorcycle or a bicycle. There are few vehicles with rails available for the transportation of whole carcasses as recommended (FAO, 1991). The level of hygiene kept by most transporters and retailers in Kenya is not well known.

Most consumers get their meat from the butcheries (retail shops) to be cooked in the house and taken with other servings. Some of the meat finds its way to roasting places especially in entertainment places popularly known as "Nyama Choma Joints". The mode and duration of cooking is different depending on individuals, the place and preference of the consumers. However, there are private processing companies like Farmer's Choice who make sausages and other processed meat products to be sold in the local formal and informal market. The effect of the mode of distribution on these meat and meat products and the Kenyan consumer cooking preferences have not been investigated.

The probability of the meat being contaminated with pathogenic micro-organisms might be high. Reduction of the same at the cooking stage is not guaranteed especially when a consumer prefers medium done meat. Re-contamination of the cooked food by personnel and equipments can easily occur due to lack of keen observation of good hygiene practices (FAO, 2006; Kaspar

et al 2010). Some of the probable micro-organisms that contaminate meat are coliforms which include *Escherichia coli* O157:H7 and other entero-haemorrhagic *Escherichia Coli* strains. (Buchanan and Doyle, 1997; ESR, 2002)

There have been reported cases of food poisoning associated with *E. coli*, especially *E. coli* 0157:H7, in various parts of the world. The food implicated in these outbreaks has been varied. A recent case of *E. coli* outbreak was reported in May 2011 in Germany with the food source suspected to be bean sprouts from an organic farm and by 16th August 2011, Robert Koch Institute reported 3842 people being sick, of whom 2987 had EHEC gastroenteritis (without Hemorrhagic Uremic Syndrome-HUS) and 852 developed HUS. A total of 53 people died (RKI, 2011). Reuters on July 13, 2009 reported a recall of 219 pounds of ground meat suspected to be contaminated with *E. coli* 0157:H7 by E.S Miller Packing Company in USA (Burgdorfer, 2009). Trickett (2001) has cited a case in Scotland linked to meat from a butcher's shop that resulted to 500 cases with 21 deaths. FAO/WHO, 2006 has shown a continued increase in the number of reported cases of *E. coli* O157in three countries over seven years (Figure 2.0).

In Kenya, there have been very few reported cases of *E. coli* from the hospitals probably because of the complexity of the testing of the *E. coli* infections in the laboratory and therefore not routinely screened for, the failure of the physician to ask for the sample to be investigated or underreporting of symptomatic cases (WHO, 1994). In Ontario, USA, underreporting of the symptomatic cases of *E-coli* has been reported as between 78% and 88% **(**Michel et al, 2000). Presence and prevalence of *E. coli* 0157:H7 in cattle milk, faeces and in meat in Kenya has been reported by Arimi *et al,* (2000), Kang'ethe *et al,* (2007) and Mwai, (2012) respectively. The study sought to determine the extent to which beef carcasses after slaughter and the butchery surfaces were contaminated with *E. coli* O157 serotype. The factors that lead to the contamination were also highlighted.

1.2 Problem Statement

E. coli 0157:H7 is one of the most pathogenic strains of Shiga toxin producing *Escherichia coli* (STEC). It was first recognized as a pathogen in 1982 during investigations of an outbreak of hemorrhagic colitis in Michigan and Oregon, USA (CCFH, 2003). It has continued to be of concern as it has been associated with many food borne illnesses worldwide. Data obtained in

United States of America from 1982 to 2002 showed that it causes approximately 73,000 illnesses annually (Rangel et al., 2005). It also showed that 17% of the patients were hospitalized, 4% developed Haemorrhagic Uremic Syndrome (HUS) and 0.4% died. Approximately 70% of the symptomatic individuals have been known to develop bloody diarrhoea (CCFH, 2003).

Cases of diseases caused by *E .coli* O157:H7 have been reported in many parts of the world. Many places in Africa; including South Africa, Swaziland, and Malawi, Central African Republic, Cameroon , Nigeria and Ivory Coast have also reported cases associated with enterohemorrhagic *E. coli* (Koyange et al., 2004)**.** There have been isolated reported cases on Shiga toxin producing *Escherichia coli* in Kenya from animal products (Arimi et al., 2000; Kang'ethe et al., 2007) and from human faecal samples (Sang et al., 1997). There has been no recorded reported severe case of disease caused by this bacterium in Kenya.

Many sources of enterohemorrhagic *E. coli* (EHEC) infection in humans have been identified but red meat especially from ruminants is still considered the principal source (Lake et al, 2002). Beef is the major red meat source in Kenya and therefore widely available to the population in the country. The slaughter, distribution and retailing of beef and meat in general are mainly in the hands of private entrepreneurs (EPZ, 2002; Muthee, 2006). Training of the meat and food handlers has been found inadequate (Abdul-Mutalib et al., 2012; Adzitey et al., 2011). The regulation of the traders who are mainly informal and usually acting as middle men is a hard task. Unhygienic practices are rampant in such unregulated setting. Pursue for higher profits override the necessity for good hygiene practices especially if the demand for higher quality is not consumer driven (Mayes and Mortimore, 2001). Contamination and cross contamination of the carcasses at the slaughterhouses, during transportation and at the retail shops are high (Kang'ethe, 1993; Kariuki et al., 2013).

Unhygienic handling of the carcasses during slaughter and distribution will lead to contamination with microorganisms from the handlers, equipment and other carcasses (FAO, 1991). *E. coli* is one of the microorganisms likely to contaminate the carcasses and its principal source is the faecal matter of the warm blooded animals (Tarr et al., 1994). The strain *E. coli* O157:H7 cause disease to human beings if ingested at low levels and multiplication rate is high at temperature of 25 ^{o}C (Doyle and Shoeni, 1984) and this can be attained in enclosed carrier box with no refrigeration. Cross contamination of carcasses occur from poor handling and will lead to

unacceptable high prevalence. The level of carcasses contamination in Kenya is high and the hygiene during slaughter is poor (Kang'ethe 1993). This poses a risk to meat consumers.

1.3 Justification

In Kenya, there have been reports of *E. coli* O157 serotype contamination in faecal and milk samples obtained from the same cattle in dairy households, milk at the collection point, and recently in beef carcasses at the abattoir during slaughter (Kang'ethe *et al*, 2007; Arimi *et al*, 2000; Mwai, 2012). The prevalence and the possible paths of contamination of beef with *Escherichia coli* along the distribution value chain in Kenya have not been conducted.

Escherichia coli could contaminate meat at any stage of the value chain: during slaughtering, transportation to the retailers, at the retailers' shops or/and during handling by the consumer (FAO, 2006). The mode of transport of meat could be a major contributor to the contamination especially if good hygiene practices are not observed (Reilly, 1998). It has been shown that the level of *E. coli* increase during transportation of live animals (Arthur et al, 2007). Proliferation of the *E. coli* during transportation may be high especially if the meat is not refrigerated (Doyle and Schoeni, 1984; CCFH, 2003). Strict observation of the hygienic practices along the meat value chain is also in question. The research sought to answer the question; how does the handling of meat from the abattoir to the butchery affect the meat safety especially on *E. coli* 0157:H7 contamination? The research work will help highlight the areas that need improvement during the distribution of beef carcasses to avoid and reduce contamination with pathogens. The results will inform the policy makers on areas that need reinforcement with regulations and an appropriate action plan.

1.4 Objectives

1.4.1 Main Objective

The main aim of the study is to assess the likelihood of beef contamination with *Escherichia coli* 0157:H7 from the slaughterhouse to the retail shops and identify the risk factors that contribute to this contamination.

1.4.2 Specific Objectives

(i) To determine the likelihood of meat contamination with *E. coli* O157:H7 at the loading point of the abattoirs, during transportation and at the butcher's shops.
(ii)To assess the prevalence of *E. coli* O157:H7 in beef carcasses from Dagoreti, Limuru and Eldoret slaughterhouses at loading, offloading and surfaces of retail shops.
(iii) To determine the risk factors that may contribute to the increase in the cross contamination during handling and transportation
(iv) To assess the possibility of persistence of *E. coli* O157:H7 contamination in beef carcasses from the abattoir to the butchery.

1.6 Hypotheses

1. There is no risk of meat contamination with *E. coli* O157:H7 at the abattoirs, along the transportation chain and the butchers' shops.
2. The prevalence of *E. coli* O157:H7in beef carcasses obtained from Dagoretti, Limuru and Eldoret is low at the three points of sampling.
3. The hygiene practices observed along the beef transportation value chain and at the butchery are adequate to prevent contamination and cross contamination of meat with *E. coli* 0157:H7.
4. Carcass contamination with *E. coli* O157:H7 at abattoir is effectively controlled to avoid persistence to the butchery/retail shops.

CHAPTER TWO: Literature Review

2.1 Beef Industry

The beef industry is important as it is the third largest meat source in the world after pork and poultry (de Haan, 2009; FAO, 2009). FAO indicated that by the end of 20th Century, meat from cattle would be in the tune of 50 million tonnes (Wilson et al., 2005). An upward trend in meat production in the world and especially in the developing countries has been reported (de Haan, 2009; Rae and Nayga, 2010). In Kenya, livestock industry contributes 3.3% to the national GDP (EPZ, 2005).

The meat industry experiences different challenges, among them being transmission of zoonotic diseases. This has adverse effects including loss of food, spread of diseases, death of people and other economic losses related to loss of consumer confidence. For example, one of the micro-organism that has emerged to be of importance to the meat and the food industry at large is the *E. coli* O157:H7 because of the epidemiology and economic implications associated with it. Frenzen et al (2005) estimated the annual cost of illnesses due to *E. coli* O157:H7 in United States to be $405 million which included $370 million for premature deaths, $30 million for medical care, and $5 million for lost productivity.

The cattle have been identified as the main reservoir of the *E. coli* O157:H7 in their gastrointestinal tracts. The pathogen gets to the environment when it is shed through faeces. The slaughtering process, unless done hygienically contaminates the meat and this lower the shelf life of the meat (Elder et al, 2000; Mwai, 2012). The beef meat has therefore been identified as the major source of food borne *E. coli* O157:H7 related illnesses (CCFH, 2003). Application of the hazard analysis of critical control principles and strict observation of good hygiene practices have been noted as the most effective way of controlling EHEC infections (WHO, 1998). In most developing countries, the Sanitation and Standard operation procedures (SSOPs), hygiene standards and hazard analysis of critical control points (HACCP) programme may be well observed as various institutions have proposed their adoption and implementation (USDA, 1996; van Schothorst and Jongeneel, 1993). In the developing countries, this may only apply to the large scale slaughterhouses mainly for export purposes but not to the small scale abattoirs targeting the domestic market. Blackburn and McClure (2002) have indicated that the small and medium scale enterprises (SMEs) have low perception of the severity of hazards arising from

daily operations and therefore find the cost of HACCP implementation high compared to the benefits gained. They further suggest that consumers in developing countries may not regard food safety as a major issue and thus, the workforce used may be untrained and ignorant of any repercussions emanating from unhygienic practices because the risk of prosecution is low.

The transportation process from the abattoirs to the butchery may compound the problem if done under high temperatures. Most of the butcheries are small scale and therefore do not own vans for ferrying the meat from the abattoirs but hire the available vans (Aklilu et al., 2002). Too much human handling may complicate the problem further as most of the personnel in direct contact with the meat do not maintain high hygiene standards (Mwai, 2012-unpublished). An assessment of the meat chain may be done through an audit of the HACCP system. HACCP system in the informal chains may be nonexistent and the identification of the critical control points may be hard due to lack of proper training on implementation (Mayes and Mortimore, 2001). It is therefore recommended that a risk assessment of a certain hazard be carried out in order to identify contamination and decontamination stages and the appropriate mitigation strategies that may have the greatest impact if implemented. It is a cost effective way to comparing options of managing risks before they are implemented (Cassin *et al*, 1998).

2.2 Risk Assessment.

Risk assessment is part of the larger framework for risk analysis of the food products from farm to fork. Risk management and risk communication form the rest of the framework as defined by Codex Alimentarius commission (CAC, 1999). It also defines risk assessment as a scientifically based process consisting of the following steps: hazard identification, hazard characterization, exposure assessment, and risk characterization.

2.2.1 Hazard Identification

2.2.1.1 Escherichia Coli

E. coli is a species of Gram negative, facultative anaerobic, rod shaped bacteria commonly found in the lower part of the intestine of warm blooded animals (Tarr et al, 1994). In the laboratory, *E. coli* are differentiated from other *Enterobacteriaceae* (family of Gram-negative, catalase-positive, oxidase-negative, facultative anaerobic rods) on the basis of their ability to grow and

produce gas in EC broth at 44.5°C. There are several known serotypes of *E. coli* and they are distinguished by their "O", "H" and "K"antigens on their body, flagella and capsule respectively (Aslani and Alikhani, 2009). Some serotypes cause diseases to human while others are not harmful. The harmful serotypes , EHEC , produce Shiga toxin (Stx), either Stx1 or Stx2, that cause sudden onset of abdominal pain, severe cramps followed by bloody diarrhoea, and then hemorrhagic colitis (Griffin and Tauxe, 1991; Buchanan and Doyle ,1997; Boyce et al, 1995). *Escherichia coli* O157:H7 is classified in the group of shiga toxigenic *Escherichia coli* because of its ability to produce shiga-like toxins and as enterohaemorrhagic *Escherichia coli* as it causes haemorrhagic colitis in human (ESR, 2002). *Escherichia coli* O157:H7 grow at temperature between 8-44^{o}C. Growth of *E. coli* O157H7 at the temperature between 44-45^{0}C is very slow if any unlike other *E. coli* strains (Buchanan and Doyle, 1997). The generation time for *E. coli* O157:H7 at optimum temperature of 37^{0}C is 0.49 hours and it has been found to survive at very low storage temperatures of -20 ^{0}C for 9 months. (Doyle and Schoeni, 1984). *E. coli* O157:H7 is a slow fermentor of sorbital MaConkey agar media where they appear as colourless colonies in 18-24hr at 37 ^{0}C. (CCFH, 2003; Wells *et al*, 1983)

2.2.2 Hazard Characterisation

Various strains of EHEC have been implicated in sporadic illnesses arising from contamination of various foods by faecal matter (CCFH, 2003). The onset of hemorrhagic colitis (HC); mild, non-bloody diarrhea that may be followed by a period of "crampy" abdominal pain and short-lived fever in 1–2 (sometimes 3–5 days) days after eating contaminated food, characterize their infection. The initial diarrhea increases in intensity in 24–48 hr and in 4- to 10 days of bloody diarrhea accompanied by severe abdominal pain and moderate dehydration is experienced. Although not all EHEC strains are associated with bloody stools, *E. coli* O157:H7 related cases usually involve bloody diarrhea (Buchanan and Doyle, 1997). Some of the infection cases proceed to hemorrhagic uremic syndrome (HUS) leading to kidney damage and/or death FAO/WHO, 2006). The percentage per illness for the cases that proceed to HUS and that of HUS to death in children has been estimated at 10% and 5% respectively (Cassin *et al*, 1998).Ingestion of as few as 10 cells of *E. coli* O157:H7 can result in illness (CFSPH, 2009; Paton and Paton., 1998). This microorganism has been reported to cause sporadic illnesses in many parts of the world. Surveillance data indicate that children under the age of 5 and immune-

compromised individuals are more susceptible to infection and are highly likely to develop HUS (FAO/WHO, 2006). There have been various data showing the trends of the cases arising from *E. coli* O157:H7 over the years. In some countries, there has been increased surveillance since the pathogen was known to cause food borne diseases and death. Figure 2.0 shows the number of reported cases in three different countries in a span of seven years.

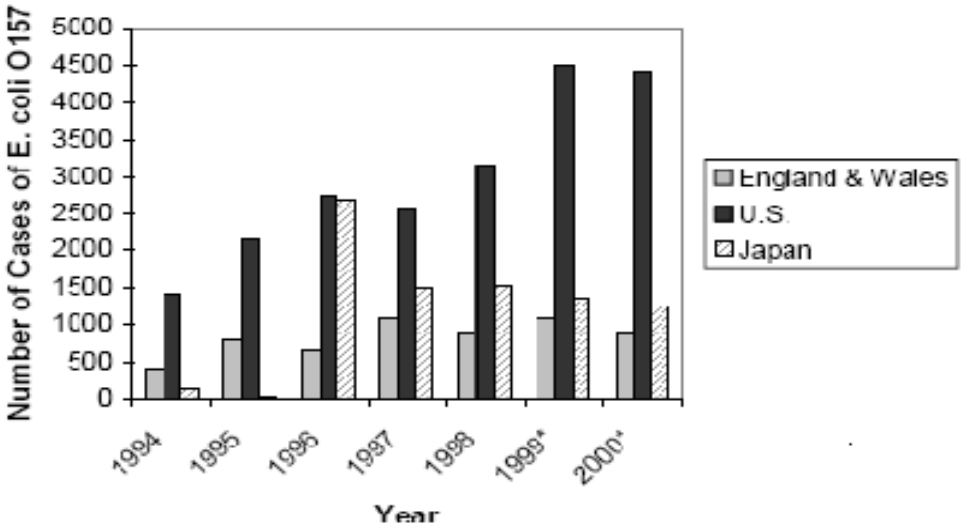

Figure 2.1: **Number of reported *E. coli* O157 cases in England, U.S. and Japan.** Source: FAO/WHO 2006.

An estimated 62,000 cases of symptomatic *Escherichia coli* O157:H7 infections occur every year in the United States due to the consumption of contaminated foods, resulting in an estimated 1,800 hospitalizations and 52 deaths. Mead et al (1999) approximated that 3,000 of these cases may result in haemolytic uremic syndrome. There have been several reported cases in different countries in Africa including South Africa, Swaziland, Central African Republic, Kenya, Gabon, Nigeria and Ivory Coast as cited Raji et al (2006).

A study conducted in Kenya by Kenya Medical Research Institute in conjunction with Osaka University, Japan, reported 13.8% (119) *E-coli* prevalence in 862 children with diarrhoea (Sang et al, 1997). Arimi et al (2000) reported *E. coli* O157:H7 serotype in two samples (<1%) of milk collected in Nairobi. One of the two isolates produced verocytoxins. Kang'ethe et al (2007) reported a prevalence of 5.2% and 2.2% in milk and faecal samples respectively in samples collected in Nairobi. Recent studies done in luxurious hotels have detected pathogenic

Escherichia coli among 39 (4.4%) subjects of the total 885 food handlers in the study (Onyango et al, 2009). WHO, (1997) has reported the prevalence of *E. coli* O157:H7 to range from 0.1-5% in meat and 1.5-28% in cattle; the variance being brought about by different methods used in sampling and reporting by different researchers.

The Shiga-toxin producing *Escherichia Coli* (STEC), which include *E. coli* 0157:H7 are mainly found in the guts of ruminant animals, including cattle, goats, sheep, deer, and elk (CCFH, 2003; CDC, 2008)

E. coli O157:H7 is transferred from the carrier cattle faeces to the meat through contamination during slaughtering and handling. According to CDC website on general information on *E. coli*, other kinds of animals, including pigs and birds, sometimes pick up STEC from the environment and may spread it. STEC that cause human illness generally do not make animals sick. Epidemiological evidence from outbreak and sporadic cases of infection with *E. coli* O157:H7 indicates that ground beef is a major food borne source of exposure (Slutsker et al, 1998). In recent years, however, non intact beef products other than ground beef have also been suspected to cause *E. coli* O157:H7 related outbreaks due to tenderization process where needles are used and may transfer the microorganism from the surface and equipment into the muscle (Sporing, 1999) . Faecal contamination of water and other foods and cross-contamination during food preparation are important routes of infection (Armstrong et al, 1996). Examples of foods implicated in outbreaks of E. coli 0157 infection include hamburgers, roast beef, raw milk, unpasteurized apple juice, yoghurt, cheese, fermented sausage, cooked maize, mayonnaise-containing dressings, lettuce, and seed sprouts (WHO, 1998). Faecal contamination in meat arises from unhygienic handling at the slaughter process (Armstrong, 1996).

2.2.3 Exposure Assessment

Exposure assessment has been described as the evaluation of the degree of intake likely to occur (Cassin et al, 1998). USDA-FSIS (2001) looked at exposure assessment in consideration of the factors that may lead to consumption of contaminated beef serving. They noted that the risk of contamination of meat up to the consumer is dependent on many factors as per the handling along the meat value chain. These include and not limited to herd or feedlot prevalence, cross contamination of animal hides during transportation, cross contamination and dilution factors

during the slaughter process, temperatures during storage and handling and the preparation step prior to consumption.

The status of the incoming cattle and outgoing processed meat at the abattoir has been noted as an important step in exposure assessment (CCFH, 2003). Likewise the other steps that may include dilution or multiplication of the bacteria could be deemed as important. Cross contamination and multiplication of bacteria during transportation of meat from the slaughterhouse to the butchery and at the retail shops is certainly an important step especially if the cold chain is not observed (FAO, 1991; USDA: FSIS, 2001; Ali et al, 2010). The conditions encountered at the butchery will also determine the likelihood of consumer obtaining contaminated meat and final level of contamination. The factors that could lead to the increase or decrease in the prevalence and level of carcass contamination with *E. coli* 0157:H7 at slaughter, distribution, sale and preparation are discussed hereafter.

2.2.3.1 Cattle Slaughter

It is considered that initial *E. coli* contamination in meat is from the faeces of animals shedding *E. coli* or from a contaminated skin of cattle which is either shedding or not shedding *E. coli*. This is usually at the stage of slaughter (Elder et al., 2000). The slaughter process is therefore a key step in the control of meat contamination with *E. coli.*

Poor meat hygiene and slaughter practices therefore contribute largely to the prevalence and concentration of *E. coli* on the surface of meat. Meat hygiene would encompass personal hygiene, slaughter and meat processing hygiene and hygiene of slaughter and meat processing premises and equipment (GOK, 1977). Enabulele and Uraih (2009) have reported 6.94% prevalence in fresh meat from the abattoirs and noted poor hygiene practices from the slaughterhouses where the isolates were obtained from. Meat control (local slaughterhouse) regulations, 2010 in the Kenyan meat control act cap.356 legal notice No: 110 2010 provides that each slaughterhouse and slaughter slab should employ workers trained on food safety. It also stipulates that training on food safety for the employees should be done at least twice annually.

The slaughter process has the following steps: stunning, bleeding, hide removal, evisceration, splitting, washing, inspection, weighing and cold storage. Along the process chain, contamination from the skin of the animal to the personnel hands and the equipment and then to the carcass and cross-contamination from the other carcasses may occur (Gallard, 1997). Good Hygiene Practices and HACCP may be applied to help reduce the probability contamination

(CCFH, 2003). Buchanan and Doyle (1997) conclude that the most effective way of reducing the risk associated with *E. coli* O157: H7 and other pathogens is through implementation of the HACCP system in food industries.

Mwai (2012) have studied carcass contamination across slaughterhouses in Nairobi with an aim to determine *E. coli* O157serotype contamination during slaughter. She found the average prevalence of carcass contamination with *E. coli* O157 as 11.3% and the probability of obtaining a carcass contaminated with the same serotype to be 29, 38 and 48 per 1000 slaughtered carcasses at export, typical local and improved local slaughterhouses. She reported poor manufacturing and hygiene practices among slaughterhouses workers in Kenya. Kang'ethe (1993) also found out that carcasses in a slaughterhouse sampled were highly contaminated and noted the poor hygiene practices. The safety of meat from Kenyan slaughterhouses has raised questions in the past leading to loss of markets (Muthee, 2006). The poor disposal of the effluent from one of the major slaughterhouses supplying Nairobi led to its closure by National environment management authority (NEMA) in August 12, 2008.

2.2.3.2 Transportation

Transportation of the carcasses is recommended to be done in vehicles with rails and under lowered temperature, preferably -3^0C to 0^0C (USDA/FSIS, 2005). The Kenya Meat Control Act (G.O.K., 1977) provides guidelines for the transportation of meat under no refrigeration and within 50km radius from the abattoir. The carriers are to be fitted with rooftop rotating ventilators.

Multiplication of *E. coli* at high temperatures as found in the tropics can be very fast. The ambient temperature in Nairobi is on average 25^0C, a temperature at which *E. coli* generation time is 1.46h (Doyle and Schoeni, 1984). The time and temperature combination in which the transportation takes place may lead to the increase in microorganism concentration in many folds especially in unrefrigerated conditions (FAO, 1991). The prevalence levels may also be higher than at the slaughterhouse due to the possibility of cross-contamination of the carcasses and contamination by personnel during loading.

2.2.3.3 Butchery

This refers to the retail shops that sell small portions of meat to the consumers after obtaining the carcasses from the abattoirs. The shops are widely distributed in the towns to the convenience of

the customers. They are privately owned. The butcheries have to meet regulations as spelt out by the Kenya Public Health Act cap 254 (GOK, 1986).

Marketing of uncooked meat and meat products is recommended to be done under refrigeration and if under room temperature to be sold within a day (FAO, 1991). The temperature at which the beef carcass is stored may vary as the number of market players increase. It may vary from room temperature (usually 18-26^{0}C) to refrigeration temperatures. The different temperatures will affect the *E-coli* O157:H7 growth differently. Meat spoilage at 20 ^{0}C has been shown to occur after one day if the initial bacterial load was 10^3 (FAO, 1991).

2.2.3.4 Preparation for Consumption

This is the critical stage before consumer exposure to microorganism. Most of the preparation methods used for meat before consumption involve a heat treatment step. However, some people consume raw meat (WHO, 1995). The heat treatment step differs in terms of the temperatures achieved and the time regime used. This is mainly dictated by the individual cooking preferences and method used during preparation. McIntosh et al. (1994) identified that some people preferred their hamburgers cooked rare, medium rare, medium well and well done. Cassin et al. (1998) has matched different preference of meat doneness to a corresponding internal temperature. This may correspond to a certain internal temperature which may be less than the recommended. FAO/WHO (2006) recommended internal time-temperature regime for the comminute meat to be 66^{0}C for 1 minute or 68^{0}C for 15 seconds or 70^{0}C for less than 1 second.

The different preparation methods used and the hygiene practices observed determine the presence or absence of *Escherichia coli* O157:H7 in the cooked meat. Although no data is available to show the effects of hygiene practices during roasting, steaming, boiling or frying have on the presence or absence of *Escherichia coli* O157:H7 in the final product, there are possibilities of cross contamination especially during roasting in places where raw meat is in close proximity to preparation area or staff are in contact with both raw and cooked meat (WHO, 1997). Cross contamination from beef has been identified as a likely route of *E. coli* contamination during food preparation (IOMC, 2002). To control the cross contamination and the eventual *E. coli* infection to consumers, separation of storage, processing, sale, and display at the meat retail and preparation points has to be observed (CFSPH, 2009). It may involve separate refrigeration, working areas, equipment, utensils and staff as well as strictly following the hygiene practices (Gallard, 1997; IOMC, 2002). The second key in World Health Organization

(WHO) document “Five Keys to Safer Food Manual” (WHO, 2006) is about separation of raw and cooked food to avoid cross contamination.The different methods used by different people during preparation bring about large and varied effects on micro-organisms reduction.

2.2.4 Risk Characterisation

Risk characterization is defined as the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment (USDA:FSIS, 2001). The results of risk characterization show the likelihood of the hazard occurrence and its impact (Cassin et al, 1998). In qualitative estimation, it is given in descriptive terms like high, moderate and low. In quantitative estimation, numerical figures are the output. It is either deterministic (point estimate) or stochastic. The point estimate usually obtained from the measurements obtained is usually expanded by feeding it to Monte Carlo simulation model. In Monte Carlo methods, the computer uses random number simulation techniques to mimic a statistical population (Anon., 2012).
For each Monte Carlo replication, the computer simulates a random sample from the population, analyzes the sample, and stores the result. After many replications, the stored results will mimic the sampling distribution of the statistic (FAO/WHO, 2001).
This step also involves a summary of source of uncertainties and the assumptions made during the assessment. Uncertainties arise from lack of available data to be used in the assessment while variability comes from the different pathways that the food may follow before it reaches the consumer. The uncertainties and variability encountered during risk assessment can be reduced through research and process intervention respectively (Cassin et al, 1998).

CHAPTER THREE: Materials and Methods

The materials used in the execution of the project are shown in annex 1.

3.1 Study Sites

The areas under study were Nairobi, Limuru and Eldoret. One Slaughterhouse was chosen from each of the study sites. Nairobi is the capital city and the most populous in Kenya. Slaughterhouses in Limuru have some of the butcheries they supply in Nairobi while Eldoret is a major stopover for people travelling to the western part of Kenya. Nairobi is supplied with beef meat by eight slaughterhouses with Dagoretti slaughterhouse supplying about 55.8% (Aklilu et al., 2002). There are two major slaughterhouses in Limuru and the rest are slaughter slabs. The two slaughterhouses supply meat to Nairobi and Limuru. Eldoret has one major slaughterhouse and three other slaughter slabs. The slaughterhouse is at the periphery of Eldoret town and supplies Eldoret town and the suburbs.

3.2 Research Design

This was a cross sectional study. The carcasses were randomly selected at the slaughterhouse as they were loaded to the transportation vehicles where the first sampling was done. Carcasses on fifth, tenth and fifteenth position, in that order were chosen for sampling. The carcass was then followed up at the butchery where more samples were taken. A questionnaire was administered to the meat transporters and the butchery attendants to asses on the knowledge, attitude and practices (Annex 2).

According to Aklilu et al (2002), there were 65 operational slaughterhouses in Kenya in the year 2000, most of which were in the major cities and towns. In Nairobi, the cattle slaughtered in the same year was 38 998. The number of middlemen operating in Dagoretti alone per day was estimated to be 100 to 120. They number of licensed butcheries in Nairobi in 2002 was 65. This was estimated to be an eighth of all the operating butcheries.

3.3 sampling

3.3.1 Sample Size Calculation

The sample size for carcasses was calculated according to the formula cited by Daniel, (1999):

$$n = \frac{z^2 p(1-p)}{d^2}$$

Description:

n = required sample size

z = z statistic for level of confidence at 95% (standard value of 1.96).

p = estimated prevalence of *E. coli* O157:H7 in beef meat-5% (WHO, 1997).

d = adjusted margin of error, a value of 0.027 calculated as suggested by Naing et al, 2006 for low prevalence of disease and limited available resources.

The calculated sample size was: $n=1.96^2*0.05*0.95/0.027^2=250$ carcasses.
These carcasses were randomly identified immediately after slaughter before loading into meat carriers. The number was distributed to the three slaughterhouses proportionately to the throughput of each (55%, 25% and 20% for Dagoretti, Limuru and Eldoret respectively).
A sample of 250 transporters and butchery attendants each was to be interviewed. However, the number of transporters and butchery attendants was dictated by their willingness to participate and the selected carcasses, that is, the butchery they were delivered to. The researcher had no prior knowledge on where the carcasses were to be delivered. Carcasses that were to be subdivided among a number of butcheries were not included in the research.

3.3.2 Sampling procedure.

Slaughterhouses supplying Nairobi with meat from cattle include, Dandora, Nyonjoro, Hurlingham, Dagoretti, Kayole, Ngong, Olekesasi and Keekonyokie. The largest proportion (55.8%) is obtained from Dagoretti (Aklilu et al., 2002). In Limuru, there are two major slaughterhouses, Bahati and Limuru township slaughterhouse while the others are slaughter slabs. In Eldoret, one major slaughterhouse and three other slaughter slabs are available.

Abattoirs sampled from were purposefully chosen as large capacity Municipal controlled, serving a large consumer base in the city of Nairobi, Limuru and Eldoret town. The selected slaughterhouses were Dagoretti, Bahati in Limuru and Eldoret township slaughterhouse in Eldoret town.

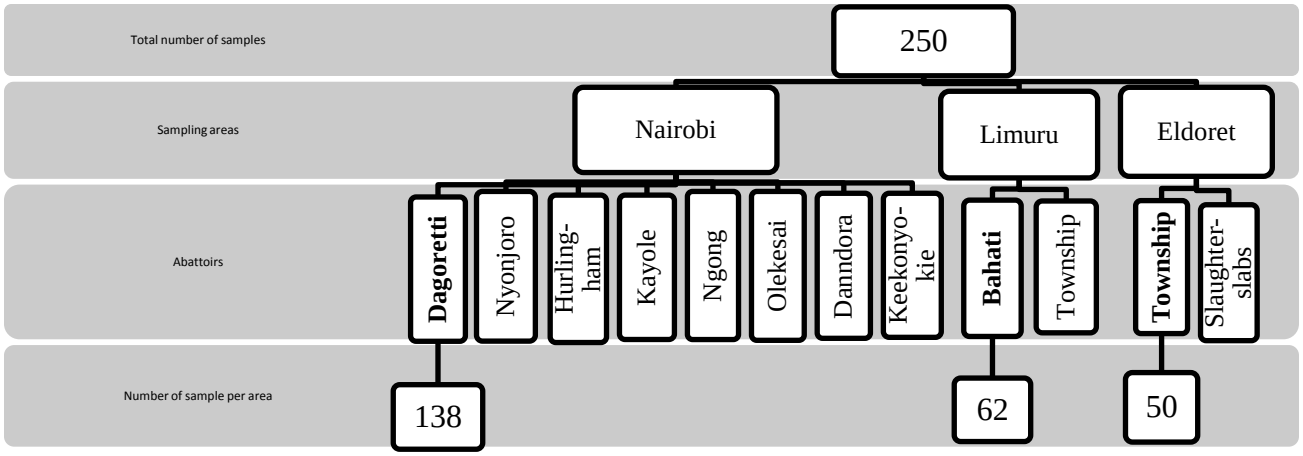

Fig. 3.1: Sampling frame for Nairobi, Limuru and Eldoret

Samples for microbial analysis were obtained from the carcass as explained in section 3.5. The temperature and the humidity of the carrier boxes were also monitored (section 3.11). A semi structured questionnaire (Annex 2) was used to assess the knowledge, attitude and practices of the transporters.

The meat transporters preferred were those using vehicles mounted with veterinary approved boxes, collecting more than one carcass from the abattoir and supplying two or more butcheries. The transporters who collected meat daily were preferred to those who collected occasionally. The transporters were briefed on the objective of the research and those were willing to cooperate were chosen to avoid prejudiced fears on interfering with the study.

The butcheries included in the study were pre- determined as per the transporter chosen.

3.4 Data Collection.

3.4.1 Carcass samples collection

A fault tree (Annex 3) was used to decide on the possible sources of *E. coli* and therefore the points to sample at. This was identified as at loading, offloading and the butchery surfaces. The rump, the flank and the brisket constituted the sampling sites as used in other studies (Mwai, 2012; Arthur *et al*, 2003; Jericho *et al*, 1994) and recommended by the European commission (Commission Decision, 2001) and USDA/FSIS, 1996.

Carcass sampling involved wet and dry swabs on an area of 100cm^2. A sterile non-absorbent swab was rubbed 10 times vertically then horizontally and finally diagonally on each of the three sites (Commission Decision, 2001 and Buncic *et al*, 2004). The area was alienated using an easily sterilized aluminum template. (The aluminum plate was sterilized by flaming after wiping with cotton wool soaked in alcohol). The swabs from each site of the carcass were combined in a universal bottle containing 10ml buffered peptone water (BPW-0.85% w/v sodium chloride 0.1% w/v peptone). The carcass was marked using sterile labels and followed to the respective butcheries where it was off loaded and swabbed for the second time at the three sites as previously indicated. A follow up sample was taken the following day at the butchery by swabbing the remainder of the identified carcass delivered the previous day.

The knives, hooks, chopping board and the weighing balance used at the butchery were swabbed on the first day of delivering the carcass. The surface area in contact with meat for each of the equipment was swabbed using the same criteria as on the carcass. Swabs from the same surfaces were combined in the same bottle containing 10ml BPW. The universal bottles were placed in a cooler box and transported to the laboratory for analysis.

3.4.2 Identification of *E. coli* O157:H7

3.4.2.1 Isolation of *E. coli* O157:H7

The collected samples from the carcasses and the butchery equipment were pre-enriched by storing them in the BPW under ambient temperature (20-25^{0}C) for about 12 hours. The pre-enriched sample was plated onto sorbitol MacConkey (SMaC) agar. The inoculated SMaC plates were incubated at 37°C for 18-24 hrs. Eight of the non sorbitol fermenters (NSF) clear colonies

were selected from SMaC plates. The selected colonies were streaked onto plates containing MacConkey agar alongside standard reference *E. coli* O157:H7 obtained from University of Amsterdam, Department of Medical Microbiology and incubated at 37°C for 24 hours for purification. The isolates, with intensely red colour and pale periphery, indicating their ability to ferment lactose were characterized further by biochemical test (IMViC); tests for indole, acid production by use of methyl red indicator, acetyl methyl-carbomyl (Voges Proskaurer test) and ability to utilize citrate as carbon source.

3.4.2.2 Indole Test

The test for indole production was done by sub-culturing a single colony into a culture tube (pyrex) containing four milliliters of Tryptone water (Oxoid) for 24 hours at 37^0C. Seven drops of Indole reagent were then added and results read immediately. In the tubes where a pink ring was formed was considered positive.

3.4.2.3 Methyl red Test

Acid formation was tested by incubating culture tubes (pyrex) containing four milliliters MRVP medium (Oxoid) at 37 ^{0}C for 24 hours. Two drops of methyl red indicator were added and the results read immediately. A positive culture had a red ring at the top.

3.4.2.4 Voges Proskaurer Test

This is a test for the ability of the isolates to produce alcohol from simple sugars like sucrose. The isolate to be tested was cultured in MRVP medium at 37 0C for 24 hours. A mixture (0.1ml) containing few crystals of creatinine dissolved in 5% alcoholic alpha-naphthol was added followed by 0.1ml of 40% potassium hydroxide. Voges Proskaurer was read after one hour. Tubes with a pink ring at the top of the culture were considered positive.

3.4.2.5 Ability to Utilize Citrate

Ability to utilize citrate as carbon source was tested by incubating bijou bottles (pyrex) containing Simons citrate agar (Oxoid) slants for 48 hours at 37^0C. Positive slants had visible growth and colour changed from green to blue.

3.4.2.5 Confirmation and Storage

Colonies that gave ++ − − results respectively from the IMViC test were identified as exhibiting typical characteristics of *E. coli* species. All sorbitol non-fermenting, lactose fermenting and IMViC positive colonies were sub-cultured in sorbitol MacConkey agar for 24 h at 37 °C for confirmation of their inability to ferment sorbitol. The confirmed NSF colonies were then sub-cultured in Nutrient agar (Oxoid) for purification and stored in trypticase soy broth with 10% glycerol at -20 ^{0}C awaiting serotyping.

3.4.3 Serotyping for *E. coli* 0157:H7

Serotyping was performed according to the kit manufacturer (Oxoid). The stored samples were then serotyped using *E. coli* O157 antisera in a card agglutination test (Oxoid, Basingstoke and Hampshire, England) after sub-culturing them in trypticase soy agar (Oxoid). The test uses latex particles sensitized with specific rabbit antibody reactive with O157 somatic antigen.
A drop of sterile normal saline was placed at the edge of each circle. A portion of a single colony was picked with a wire loop and carefully emulsified in the normal saline until the suspension was smooth in circle number one and two. A drop of the test latex was then placed at the other end of circle number one and carefully mixed using an applicator stick to cover the circular reaction area only. In circle number two, control latex was used to check for auto-agglutination in the isolate. In the other circles, different colonies were placed and mixed as the first colony. The card was then rocked in a circular motion for one minute while observing for agglutination. Agglutination positive colonies were regarded as *E. coli* O157.
Positive and negative controls were run each day to check on the correct working of the Latex reagents. The positive control and negative control were suspensions of inactivated *E. coli* O157 and *E. coli* O116 cells in a buffer respectively.

3.4.4 Testing for Verotoxin Production

3.4.4.1 Preparation of Isolate for Verotoxin Assay.

The confirmed *Escherichia coli* O157 isolates were tested for verotoxin production, VT1 and VT2 as per the procedure in the Oxoid test kit (Oxoid Unipart Limited, Basingstoke, Hampshire, England). The *E. coli* O157 isolates were first inoculated onto Brain Heart Infusion agar (Oxoid

CM375) slants of 10ml and incubated for 24 hours at 37^0C. A loopful of the cells was suspended in 1ml sterile physiological saline solution (0.85% NaCl) containing polymixin B (5,000 international units per ml) to facilitate the release of the toxin. Extraction was done by incubating for 30 minutes at 37^0C with occasional shaking. The culture was then centrifuged at 4000 rpm for 20 minutes. The supernatant was retained for serotoxin assay using the Oxoid test kit.

3.4.4.2 Test and Control Latex Preparation

Latex reagents were brought to ambient temperature and shaken thoroughly to ensure they were homogeneous. The control toxins were first reconstituted by adding 0.5ml of test diluents to each vial and shaking gently until all contents were dissolved.

The test latex contains particles sensitized with rabbit antiserum which reacts with either *E. coli* VT1 or VT2. This result to agglutination forming a lattice structure that settles to the base of a V-shaped micro titre well. It appears as a diffuse layer when observed. In the absence of verotoxin or at concentration lower than detectable limits, a tight button is seen.

3.4.4.3 Sample Dilution

The V-shaped micro-titre plate was arranged so that there were three columns; each with eight wells for every sample tested. The diluents (25μl) were dispensed in the first row of wells followed by 25μl of test sample in the first well of each column to make 50μl. A micro-pipette was used to mix the contents. Using the same pipette, double dilution was performed by picking 25μl from the first well and dispensing into the second well. Mixing by use of micro-pipette was then done. This was sequentially repeated up to and including the seventh well of each column where 25μl were discarded after thorough mixing. The eighth well contained diluents only to act as the control.

3.4.4.4 Test and Observation for Agglutination

Twenty five micro-litres test latex VT1, latex VT2 and latex control were added to each well in the first, second and third columns respectively. The control latex was used to check for false agglutination.

The contents of each well were mixed by rotating the plate gently using a micro mixer to avoid spillage. The plate was covered with a lid to avoid evaporation and left at ambient temperature for 20 hours on a vibration free surface. Each column was then observed for agglutination

against a black background in aid of a magnifier. The agglutination tests and controls were judged in comparison with the manufacturer's instructions.

3.4.5 Hygiene Practices Data Collection

Data on hygiene knowledge and common practices by meat transporters and butchers was obtained through administration of a semi-structured questionnaire (Annex 2) to the meat transporters and the butchery attendants and by observing the way meat is handled. Only the butchers and carcass transporters in direct contact with the meat and those who consented were incorporated in the study. The butchers were predetermined as those to whose butchery a sampled carcass was delivered. A conceptual frame (figure 3.2) for the possible contamination and decontamination practices to guide in the formulation of the questionnaire and in making observations was used.

3.4.6 Temperature and Humidity Monitoring

The temperature and the relative humidity in the meat carriers were monitored by taking dry bulb and wet bulb temperature readings of the sling psychrometer as the vehicle left the slaughterhouse and before the first carcass was offloaded at the respective butcheries. The temperatures were read after putting the sling psychrometer in the box; closing the doors and waiting for about 5 minutes till the readings stabilized. Relative humidity was obtained from the tables provided with the sling psychrometer.

3.5 Data Management

3.5.1 Modeling for the Probability of Contamination at Various Stages.

The prevlence of *E .coli* O157:H7 in meat along the transportation value chain was determined by tracking the carcasses from loading (A), to offloading (B) and then to follow-up (C) after overnight stay at the butchery. The following was considered during modelling: Let the probabilities of the carcasses contamination with *E. coli* O157:H7 at loading, offloading and follow-up stages be P(A), P(B) and P(C) respectively. Since the carcasses were traced and sampled at each stage independently, the probabilities at each stage would be independent of the

previous stage excluding P(A). The risk of the carcasses being contaminated at each sampling stage was modelled as follows.

After one day at the butchery: This stage modelling was done using probability of contamination at the offloading to be; P(C) = P(C/B+)*P(B) +P(C/B-)*[1-P(B)].

Where P(C/B+) is the probability that the carcass was found contaminated at the follow-up given that it was still contaminated at the offloading. P(C/B-) is the probability that the carcass was found contaminated at the follow-up stage but not at the offloading.

Offloading stage: This was modelled using probability of contamination at the loading as shown. P(B) = P(B/A+)*P(A) + P(B/A-)*[1-P(A)].

Where P(B/A+) is the probability that the carcass was found contaminated at offloading given that it was contaminated at loading while P(B/A-) is the probability the carcass was found contaminated at the offloading but not at the loading stage.

3.5.2 Data Analysis.

Data obtained from the laboratory tests for *E. coli* and *E. coli* O157:H7 was entered in the access data base and then imported to excel spreadsheet where cleaning was done. Descriptive statistics such as mean and percentages were performed using Statistical Package for Social Sciences (SPSS) *version* 17. A t-test to assess whether there were differences in the means of the temperature and humidity before transportation and after transportation was performed using the *R* statistical package. The probability of carcass contamination with *E. coli* O157:H7 was modelled as explained in section 3.9 and a Monte Carlo simulation run for 10,000 iterations using winBUGS® software.

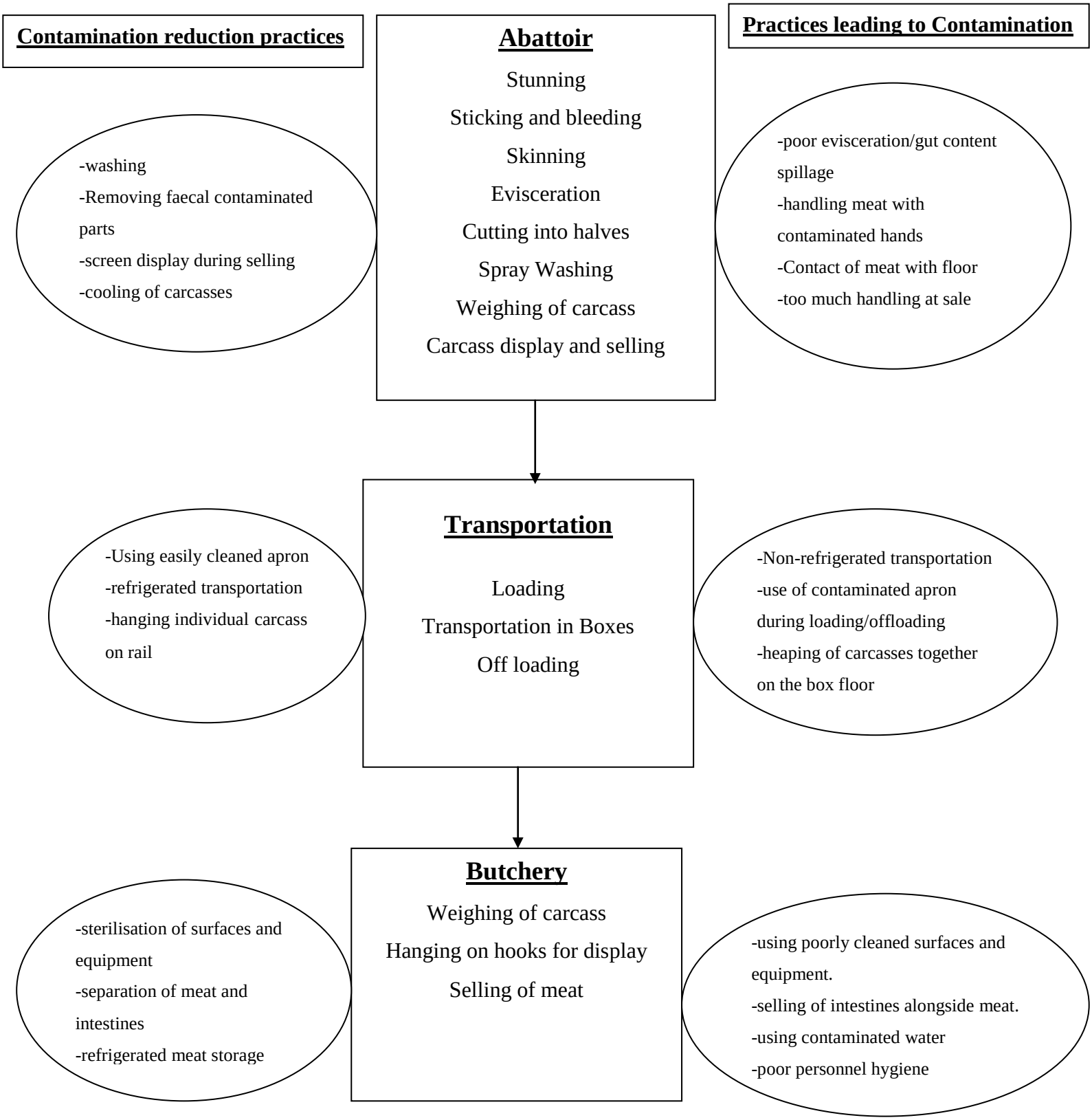

Figure 3.2: **Conceptual framework for practices leading to contamination and decontamination of carcasses.**

CHAPTER FOUR: Results

4.1 Meat Contamination with *Escherichia coli* O157 Serotype

4.1.1 Prevalence of Presumptive *E. coli* O157:H7

There were four stages of sampling; at loading, offloading, butchery surfaces and follow-up (after one day at the butchery). The samples obtained at loading, offloading and follow-up were 750 for each stage. The butchery surfaces samples were1000 giving a total of 3250 samples.

A total of 217 isolates, an average prevalence of 6.68% presumptive for *E. coli* O157:H7 (after being non sorbitol fermenters and *E. coli* positive after IMVIC biochemical tests) were obtained. The prevalence at loading was 5.87% (44 isolates), 9.33% (70 isolates) at offloading, 8.5% (85 isolates) from the butchery surfaces and 7.2% (18 isolates) from the the follow-up samples. Table 4.1 summarises the results for presumptive *E. coli* O157:H7.

Table 4.1: **Percentage of presumptive *E. coli* O157:H7 contamination at different stages of the transportation value chain from different slaughterhouses**

	Sampling stage			
Abattoir	Loading	offloading	Buchery surfaces	Follow-up
Dagoretti	8.28	31.88	37.68	9.42
Limuru	16.13	14.52	17.74	6.45
Eldoret	2	8	8	2

Presumptive *E. coli* O157:H7 at offloading were significantly higher as compared to loading ($p<0.05$). On comparing presumptive *E .coli* O157 contamination between slaughterhouses at different stages, the following was obtained: At loading, the level of presumptive E. coli contamination was significantly higher ($p<0.05$) in Dagoretti and Limuru as compared to Eldoret but there was no significant difference between Dagoretti and Limuru . Dagoretti slaughterhouse had significantly higher ($p<0.05$) contamination levels at offloading when compared to Eldoret and Limuru but there was no significant difference between Limuru and Eldoret at the same sampling point.

The butchery surfaces at Dagoretti had a significantly higher contamination ($p<0.05$) as compared to Limuru and Eldoret but there was no significant difference between Limuru and Eldoret. The level of contamination from loading to offloading at Dagoretti and Eldoret increased significantly ($p<0.05$) but no significant increase was noted at Limuru.

4.1.2 Prevalence of *E. coli* O157 Serotype

Only 14 isolates obtained from 6 carcasses (2.4% prevalence) tested positive for *E. coli* O157 after serotyping. *E. coli* O157 isolates were obtained from all stages: One from loading stage, 6 from offloading, 6 from butchery surfaces and equipment and 1 from follow-up samples (Table 4.2)

Table 4.2: **Prevalence of *E. coli* O157 serotype at each stage**

SOURCE	LOADING	OFFLOADING	BUTCHERY	FOLLOW-UP
Dagoreti (n=138)	1	3	5	0
Limuru (n=62)	0	1	0	0
Eldoret (n=50)	0	2	1	1
Total (n=250)	1	6	6	1

The carcass from which the *E. coli* O157 was isolated at the loading from Dagoretti slaughterhouse was noted to persist in contamination at offloading. Therefore, only three carcasses from Dagoreti were contaminated with *E. coli* O157:H7 serotype giving a prevalence of 2.17% at offloading and 0.7% at loading. In Limuru slaughterhouse, only one carcass was contaminated with *E. coli* O157:H7. This was isolated at offloading from the brisket giving a prevalence of 1.6%. None of the surfaces at the butchery supplied from Limuru slaughterhouse was found contaminated at the time of the sampling. In Eldoret, 4 *E. coli* O157 serotype isolates were obtained. Three of the isolates were from two carcasses (a prevalence of 4%) where one carcass was found to persist in *E. coli* O157 serotype contamination even at the follow-up stage. Only one positive sample was obtained from the butchery equipment (cutting board) (Table 4.2). Contamination level at offloading was significantly higher ($p < 0.05$) than at loading. However, neither was there a significant difference on carcasses contamination with *E. coli* O157 serotype at loading nor at offloading among the three slaughterhouses.

Table 4.3: **Number of butchers' shops with *E. coli* O157 serotype contaminated equipment/surfaces**

Butchery Surface/equipment	**Slaughterhouse supplying the carcasses**		
	Dagoretti (%) n=138	**Eldoret (%) n=62**	**Limuru n=50**
Weighing Balance	2 (1.4)	0	0
Cutting Board	2 (1.4)	1 (1.6)	0
Hook	0	0	0
Knife and Saw	1 (0.7)	0	0
Total	5 (3.6)	1 (1.6)	0

n = number of carcasses from each slaughterhouse

The isolates in Table 4.3 were obtained from different butcheries. Butcheries supplied from Dagoretti had a significantly high number of *E. coli* O157 serotype contaminated surfaces giving a prevalence of 3.6% as compared to butcheries supplied from Eldoret slaughterhouse where only 1.6% surfaces were contaminated. None of the surfaces from butcheries supplied from Limuru slaughterhouses was found contaminated with *E. coli* O157:H7.

4.1.3 Probability of Carcass Contamination with *E. coli* O157 Serotype

During the modelling for the likelihood of carcass contamination with *E. coli* O157 serotype at each stage along the transportation value chain from Dagoretti, Limuru and Eldoret abattoirs, tables shown in Annex 4 were derived. The figures show the number of carcasses found either contaminated or not contaminated at each stage given the previous stage results.
The data was used for Monte Carlo simulation and yielded the results as shown on Tables 4.4, 4.5 and 4.6. The distribution curves for the same are shown in Annex 5,6,7 and 8.

Table 4.4: **Probability of a carcass being contaminated with *E. coli* O157 serotype at each stage along Dagoretti Slaughterhouse transportation value chain.**

Sampling Stage	Mean (95 CI)	Standard Deviation (sd)
Loading	0.014 (0.002-0.039)	0.01
Offloading	0.031 (0.01-0.066)	0.015
Follow-up	0.013 (0.002-0.036)	0.009

Table 4.5: **Probability of a carcass being contaminated with *E. coli* O157 serotype at each stage at Limuru Slaughterhouse transportation value chain.**

Sampling Stage	Mean (95 CI)	Standard Deviation (sd)
Loading	0.016 (0.00012-0.0575)	0.015
Offloading	0.039 (0.0071-0.0947)	0.023
Follow-up	0.028 (0.0032-0.079)	0.02

Table 4.6: **Probability of a carcass being contaminated with *E. coli* O157 serotype at each stage at Eldoret Slaughterhouse transportation value chain.**

Sampling Stage	Mean (95 CI)	Standard Deviation (sd)
Loading	0.019 (0.00024-0.069)	0.019
Offloading	0.066 (0.016-0.146)	0.034
Follow-up	0.052 (0.046-0.123)	0.03

4.1.4 Verotoxin Production

One of the *E. coli* O157 serotype isolate obtained from equipment (weighing balance) in a butchery supplied from Dagoreti was positive for VT2. The isolate obtained at offloading during supply from Limuru abattoir was positive for both VT1 and VT2. At Eldoret, two isolates were found positive for verotoxin: one from offloading was positive for VT2 and the second from the cutting board (butchery equipment) was positive for VT1 as shown in Table 4.7.

Table 4.7: **The number of verotoxin producing *E. coli* O157 serotypes per sampling site.**

Carcass source	**Loading (N=1)**	**Offloading (N=6)**	**Follow-up (N=2)**	**Butchery surfaces (N=6)**
Dagoreti	0	0	0	1*
Limuru	0	1***	0	0
Eldoret	0	1*	0	1**

* Positive for VT1. **Positive for VT2. ***Positive for both VT1 and VT2.
N = number of *E. coli* 0157 serotype isolates per stage

The verotoxin producing O157 serotypes were obtained from different carcasses. The *E. coli* O157 serotypes obtained on carcasses that persisted with contamination from Dagoretti and Limuru slaughterhouses did not produce verotoxin.

4.2 Factors Leading to Carcass Contamination with *E. coli* O157 Serotype Contamination

4.2.1 Transporters and Butchers Characteristics

A total number of 119 respondents were interviewed, 87 of whom were butchery attendants and 32 meat transporters. Most of the butchery attendants (92%) were employees. The respondents having 5 or more years of experience in the meat industry were 83 (69.75%) while 116 (97%) had at least one year experience.

Meat transportation and selling at the butchery in these areas is male dominated with only 2 (0.02%) of the respondents being female (Table 4.8).

Only 16% of the respondent had been formally trained on meat hygiene practices by the meat inspectors or by visiting private organisation. Meat handlers trained on meat hygiene were 6.90% of butchery attendants and 40.63% of all meat transporters. Most of those trained on meat hygiene (53%) had acquired primary school education only. A few (3%) had acquired post secondary education.

Table 4.8: Background characteristics of the butchery attendants and meat transporters.

Characteristics	Interviewee	Designation		Total (%)
		No. of Butchers	No. of Transporters	
Gender	Male	85	32	117 (99.98)
	Female	2	0	2 (0.02)
Year of experience	≥5 years	69	14	83 (69.75)
	1-4 years	17	16	33 (27.73)
	< 1 year	1	2	3 (2.52)
Meat hygiene training	Trained	6	13	19 (15.97)
	Untrained	81	19	100 (84.03)
Education level	Primary	25	16	41 (34.45)
	Secondary	52	11	63 (52.94)
	Post secondary	10	5	15 (12.61)
Sub-Total	Total interviewed	87	32	119

4.2.2 Knowledge, Attitude and Practices at the Butchery

Some of the butchery attendants (87%) knew that meat handled with dirty hands and equipment (unhygienic) could cause diseases. Although 13% of those aware didn't know the kind of diseases it could cause, 75% mentioned diseases caused by poor hygiene.

Half of the butchery attendants had an accumulated experience of ≥1 year but none had undergone formal training on meat handling.

None of the attendant was permanently employed except where the owners coupled as butchery attendants. They were either casuals or contracted employees. Prevention of meat contamination was cited (83%) as the main reason for use of the carrier.

Although majority of the butchery attendants (Table 4.9) reported washing hands regularly only 9% of the butcheries were noted to have taps with running water nearby. The others could have other facilities like basins with water for hand washing. The white coats were mainly worn for the whole day without changeover. Other practices in the butchery are shown in Table 4.9.

Cold water and soap were mainly used in cleaning butchery surfaces and equipment in 66% of the butcheries while hot water and soap were used in 32% of the butcheries. The rest (2%) used cold water only. None of the butchers used a disinfectant while washing the surfaces in contact with meat because they claimed it was expensive. The source of water was municipal tapped water (94%), borehole (5%) and hawkers (1%).

Table 4.9: **Butchery characteristics and practices**

Characteristics	**Activity**	**Description/frequencies**	**% Butchery**
Peronal hygiene	Coat changeover	No changeover	90
		Once daily	8
		once after two days	2
	Handwashing	Frequent	97
		≤twice per day	3
	Medical check-up	≤ 6 months	28
		≥ 6months	72
Equipment hygiene	sterilisation	Hot water available	0
		No hot water	100
	Cutting board make	wooden	91
		Hard plastic	9
Meat preservation	Refrigeration	Available	52
		Unavailable	48
	meat-offal separation	seperation done	69
		No seperation	31

There was no set time or frequencies for cleaning the equipment used at the butcheries. The methodology of cleaning also differed with the type of equipment. The knives and the cutting board were cleaned more frequently in most butchery as compared to the hooks (Table 4.10). Other frequencies of cleaning included lower frequencies of once in three days to once per week. The cutting boards in butcheries were either wooden (91%) or of hard to flake plastic (9%). In between work, they were usually cleaned by wiping them using a dumpy cloth and especially in butcheries selling intestines and stomachs alongside meat.

None of the butcheries had a hot water bath (approximately 82 ^{0}C) for sterilisation of knives cutting boards and other equipments. They were unaware of the need to sterilize with a hot water bath.

Table 4.10: Cleaning frequencies of various equipment and surfaces in butcheries.

Frequency of Washing	Items/Surface					
	Knife (%)	Saw (%)	Cutting board (%)	Hooks* (%)	Floor (%)	Chopping** board (%)
Once daily	52	68	62	52	78	77
Twice daily	14	12	10	2	8	7
>twice daily	31	7	26	3	12	10
Once in two days	1	5	1	6	0	5
Others	2	8	1	37	2	1

*The hooks were rarely cleaned. Some attendants wiped them with a dry cloth once in a week.
**The chopping board (Figure 4.1) refers to a tree stump where bony meat was placed when being cut into smaller pieces. The cleaning regime for the board in 98% of the butcheries was by scrapping off the wooden chips and the accumulated fat and applying a layer of fat from the freshly delivered meat.

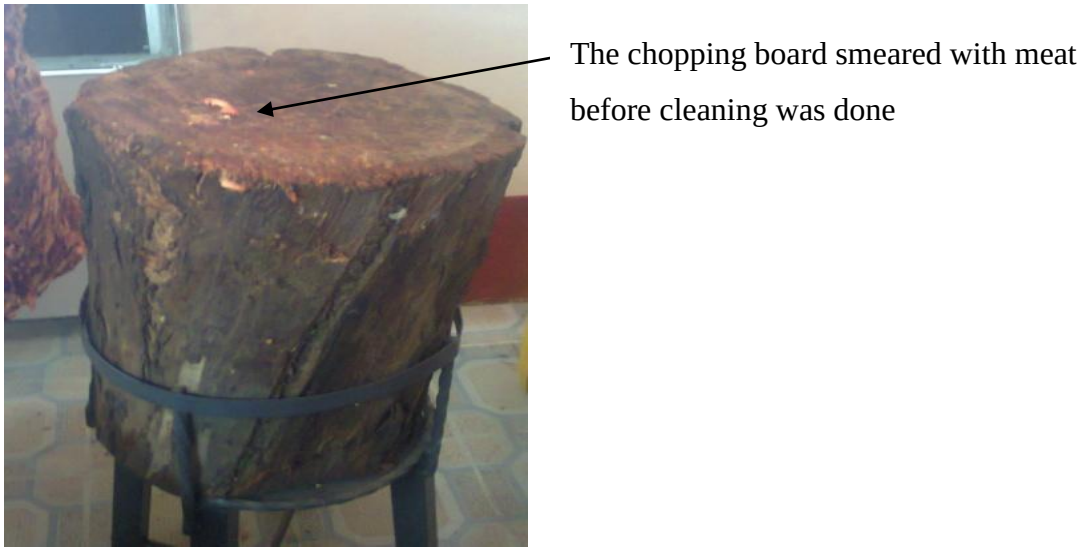

Figure 4.1: The chopping board used by most of the butcheries for reducing the size of bone meat.

A quarter of the butchery attendants said it was good practice to hang or keep meat and intestines at close proximity and majority (64%) sold meat alongside offal. The same equipment (knife, weighing balance and cutting board) were used while handling both. The equipment were cleaned in between by wiping with a dry cloth. Although majority (75%) of the butchery attendants knew of the need to separate meat and intestines, 31% of them still kept them at close proximity to each other.

About half of the butcheries (48%) did not have refrigeration chambers. In most cases meat could stay for up to 2 or 3 days in butcheries with and without refrigeration respectively before the carcasses were sold out (Figure 4.2). One fifth 19.5% of the butchery attendants confirmed to have received complaints on foul smell in meat from their customers. It was also observed that the old stock of meat was sold alongside fresh meat and sometimes used to top up to the desired weight. Visible faecal contamination of carcasses was observed in 14% of the butcheries.

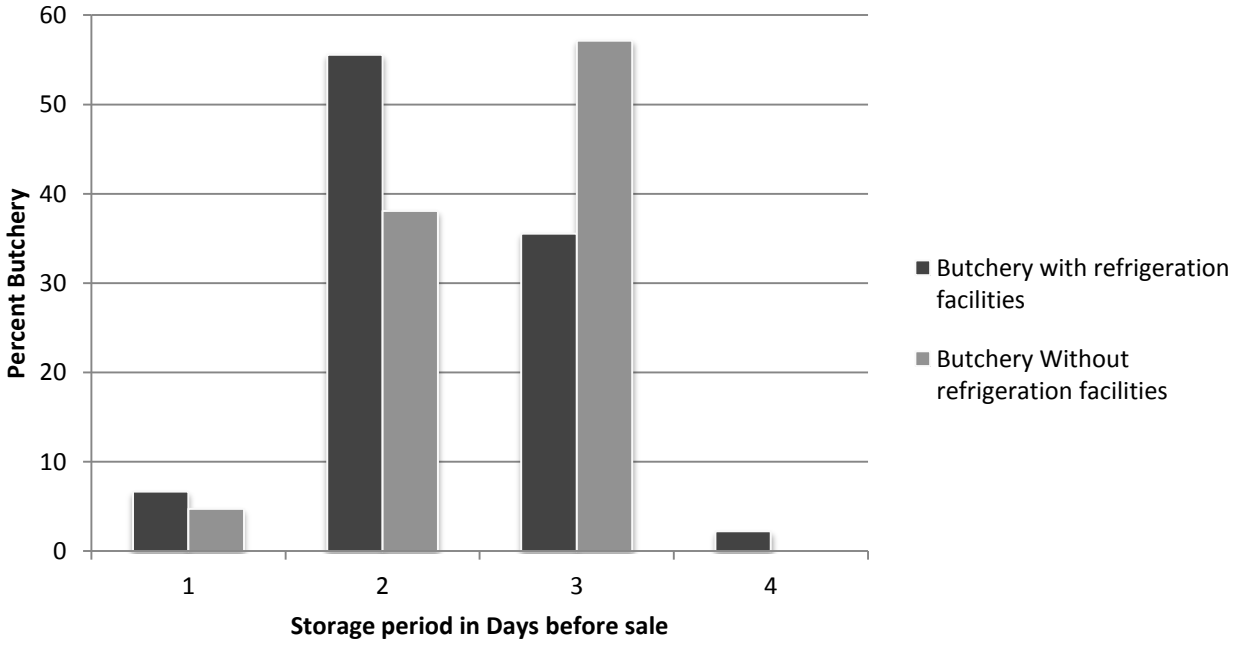

Figure 4.2: Carcass turnover in the butcheries with and without refrigeration facilities

Display cabinets whose make varied from wooden to stainless steel were found in 44 (51%) of the butcheries visited. Only 16% of the display cabinet had a working cooling system. However, 59% of the display cabinets had working lighting systems.

In twenty one (24%) of the butcheries visited, minced meat was sold. Some (71%) did their own mincing; 10% took elsewhere for mincing while 19% bought the already minced meat from other dealers whose work was mincing meat for sale. A high percent (85%) of the butcheries' premises had a ready to eat section under the same roof. Although most (93%) had exclusive personnel for working in food preparation places, in some of the butcheries (19%) the staff employed in the food preparation sections helped in attending customers at the butcheries.

4.2.3 Knowledge, Attitude and Practices of Meat Transporters.

Most of the meat transporters (91%) interviewed knew it was important to wash their hands frequently and especially after handling other items and surfaces. They knew that poor hygiene caused diseases. However, only 53% washed their hands regularly (Table 4.11) but they did not wash hands every time they offloaded the carcasses at the butchery. The rest washed before and after work. All transporters loaded and offloaded the carcasses during the transportation with no change over of the clothing.

All carcasses were carried on a personnel shoulder and were in contact with the white coats or overall during loading and off-loading. The first carcass was also placed on the carrier floor (Figure 4.3) and not hung on rails as recommended. Kraft paper (Figure 4.4) was used to separate carcass and prevent dripping of blood from one carcass to the other.

Figure 4.3.

Figure 4.4.

Kraft paper placed on top of a quartered carcass during transportation

Figure 4.3: A quarter of beef carcass just loaded and placed in contact with the transportation container floor.

Figure 4.4: Two quarters in contact with each other inside the transportation container and kraft paper placed on top ready to be loaded with the third quarter.

Table 4.11: **Characteristics of meat transporters**

ACTIVITY	**DESCRIPTION**		**%TRANSPORTERS N=32**
Washing hands	Frequency	Regularly	53
		Twice per day	47
Protective clothing	Items used in washing	Cold water, soap and bleach	56
		Cold water and soap	38
		Cold water only	3
	Reason for Wearing	To avoid soiling own clothes	25
		To reduce contamination of meat	72
		To gain access to slaughterhouse	3
	Change-over frequency	At least once	0
		No change over	100
Meat Transportation box	Method of arranging carcasses	Heaped in box with kraft paper in between	53
		Heaped in a box with no separating material	47
	Reason for its use	To control meat contamination	75
		To abide by regulations	25
	Items used in washing	Used hot water and soap	28
		Used cold water and soap	72
	Frequency of washing	Washed once per day	63
		Twice per day	25
		Frequently	12

The transportation carriers had no refrigeration facilities. Some transporters carried offal alongside meat but in separate chambers where 75% used plastic bags while 25% used separate chambers attached to the main boxes. Visible faecal contamination on carcasses was noted on 22% of the inspected boxes. The reason for use of the carrier, the items used for its washing and the frequency of washing are shown in Table 4.11. Water used for its washing is municipal council supplied.

Some of the transporters said they got wounded by the bones as they worked. A small percentage (7%) of the wounded did not cover the wound at all and 83% used water proof bands or gloves. However, none of the transporters was seen wearing gloves during the time of research.

The mean time taken to transport the meat was 65 ±44 minutes and a range of 20-240 minutes. The average time taken for transportation of meat from Dagoretti, Eldoret and Limuru slaughterhouses was 105, 60 and 30 minutes respectively.

4.4 Temperature and Relative Humidity in the Meat Carrier Box.

The carrier boxes were mainly made of aluminium or coated iron sheets were painted white to the outside, as way to reflect heat. They were opened for loading from the top or rear of the vehicle carrying it (Figure 4.4 and 4.5). They remained closed during transportation and they had no refrigeration facilities fixed on them. There was no other means of regulating the temperature and relative humidity of the box . The prevailing weather conditions and the period of transportation determined the final temperature and humidity inside the box. This increased during the transportation as shown in Table 4.12.

Table 4.12: **Mean of temperature and relative humidity at loading and offloading.**

Stage	**Temperature (^{0}C) N = 47**	**Relative Humidity (%RH) N = 47**
Loading	22.11	82.34
Offloading	23.72	80.94

The temperature rose significantly between loading and offloading (p= 0.004) at 95% confidence interval. The relative humidity however significantly dropped during transportation between loading and offloading at 95% confidence interval (p= 0.37).

CHAPTER FIVE: DISCUSSION

5.1 Carcass Contamination with *Escherichia coli* O157 Serotype

5.1.1 General *E. coli* O157:H7 Prevalence

Escherichia coli O157:H7 contamination of beef carcasses in this research revealed a prevalence of 2.4%. The prevalence rate falls within the range of 0.1-5% as reported by the World Health Organization (WHO, 1997). Kang'ethe *et al,* (2007) has reported an almost equal prevalence (2.2%) in faecal samples obtained from cattle reared in urban household farms. However, Mwai (2012) in her thesis reported a prevalence of 11.3% based on the carcass studied in three abattoirs in Nairobi and equal prevalence in the faecal samples in the same study (unpublished data). These results show high prevalence in both meat and faecal samples suggesting that a lot of contamination and cross-contamination at the abattoirs in Kenya occur. This is supported by Kang'ethe (1993) who found that total viable counts in carcasses from three slaughterhouses studied exceeded 10^5 per cm^2 and noted the unhygienic conditions under which the slaughter process took place. Although carcass washing is done after slaughter before loading to transportation vehicles, this research confirms that some carcasses leave the slaughterhouses contaminated with *E. coli* O157 albeit at lower prevalence.

5.1.2 *E. coli* O157 serotype during transportation

The highest prevalence of carcass contamination with *E. coli* O157 serotype along the transportation value chain appears to be at offloading. The increase in the prevalence (0.4% to 2.4%) of contaminated carcasses from loading to offloading was found to be significant ($p<0.05$). This suggests cross contamination or/and bacterial proliferation during transportation. The average temperature in the transportation container and time of transportation time were found to be approximately 24 ^{0}C and 1.08 hours (range 0.33 to 4 hours), respectively. This is higher than the range of -3 to 0 ^{0}C as recommended by FAO (1991). The significant increase in presumptive *E .coli* O157 at offloading as compared to loading at Dagoretti and Eldoret could also be as a result of cross contamination and growth of microorganisms. The short transportation time (30 minutes) and low average temperature increase (1^0C) at Limuru, could have caused the insignificant increase of presumptive *E. coli* O157 at the two sampling stages.

The probability of obtaining a carcass contaminated at loading was 14, 16 and 19 per 1000 carcasses at Dagoretti, Limuru and Eldoret slaughterhouses respectively. This was lower than what was reported by Mwai (2012) of 29, 48 and 38 per 1000 carcasses at export, local and local improved slaughterhouses in Nairobi, Kenya respectively; where she sampled along the slaughter process. This may be because carcasses' washing at the last stage before dispatch may have reduced the contamination levels. The probability increased at the offloading to 31, 39 and 66 for meat obtained from Dagoretti, Limuru and Eldoret abattoirs, respectively. This could be due to the cross contamination and proliferation of the microorganisms during transportation at ambient temperature of 24 ^{0}C as good hygienic and handling practices were not strictly observed (Table 4.11). Kang'ethe (1993) indicated the bacterial load of carcasses to be high at slaughterhouses in Kenya and related this to the unhygienic conditions under which the carcasses were subjected to during slaughter. Kariuki et al, (2013) observed the mean bacterial counts (log CFU) in beef in Kenya to be higher at retail outlets than at post slaughter and reported that carcasses were transported in crowded, unrefrigerated trucks.

5.1.3 *E. coli* O157 serotype at the Butchery

Surfaces, personnel and equipments along the meat value chain have been shown to harbour microorganism and therefore they are possible sources of contamination to the meat (Gill et al., 1999； Schlegelova´ et al, 2004; Kaspar et al, 2010; Ali et al 2010 and Adetunji and Isola, 2011). The surfaces sampled at the butchery (the knife and the saw, the weighing balance and the cutting board) were found to be contaminated with *E. coli* O157, but none was found on the hooks. The prevalence at the meat retail shops supplied from Dagoretti were 1.4%, 0.7% and 1.4% for weighing balance, knife/saw and cutting board respectively. Only the cutting board (prevalence 1.6%) was found contaminated in retail shops supplied from Eldoret while none of the surfaces were contaminated in those supplied from Limuru slaughterhouses. Although the samples from the surfaces were obtained before the freshly delivered carcasses got into contact with the equipments, there appear to be a similar trend in the level of contamination where retail houses supplied from Eldoret and Dagoretti slaughterhouses were found to have high levels of *E. coli* O157 contaminated surfaces. The isolation of *E. coli* O157 suggests that the equipments

were sources of contamination of the freshly delivered meat at the retail shops from a carry-over of contamination from previous carcasses.

The average prevalence of presumptive *E. coli* O157 n carcasses during follow-up at the butchery stage was 7.2%. This was not significantly different ($p>0.05$) from contamination levels at offloading but it was higher than at loading. Cattle carcasses have been shown to be highly contaminated at the abattoirs as well as at the retail shops in developing countries, above the acceptable limits of log mean 3.5 and 1.5 for total plate count and *Enterobacteriaceae* as set by the European Commission, (Commission Decision, 2001). Ali et al, (2010) found high contamination in meat carcasses obtained from Karachi, Pakistan where total viable counts ranged from 10^6 –10^{10} CFU/g and 38% of the retail shops were contaminated with potential pathogenic bacteria. The mean count for *Enterobacteriaceae* and coliform in wooden tables used in sale of meat in Nigeria were found to be (8.81-11.47log10CFU/cm2) and (8.35-10.86log10CFU/cm2) respectively (Adetunji and Isola, 2011).

E. coli O157:H7 has been found to be resistant to acidic, fermented and dry environments (Baker et al, 1999). Once the carcasses were taken to the retail shops, they were hung hooked from the hind leg from an overhead rail at the ambient temperature of 18-24^{o}C. They developed a thin skin on the surface, a sign of desiccation. The freshly cut parts had no such appearance. One carcass from Eldoret slaughterhouse was found to persist in *E. coli* O157 serotype from the offloading to the follow-up stages after a day at the retail shop. The *E. coli* O157 isolated at this stage could have been from persistence or new contamination from the tools and equipments. Carcasses from Dagoretti persisted at offloading with additional contaminated carcasses but there was no further carcass contamination at follow-up stage. The carcasses obtained from Limuru had contamination at offloading and none was detected at the loading stage. This could have been because of cross contamination from other carcasses or proliferation of the *E. coli* O157 to detectable limits. The low detection rate at follow-up stage may be explained by the fact that some part of the carcass previously sampled or the whole carcass could have been sold by the time we sampled at this stage.

The butcheries that obtained meat from Dagoretti had significantly high *E. coli* O157:H7 compared to those that sourced meat from Limuru and Eldoret, with 83% of the isolates at the

butchery level being from there. Significantly high level of the isolates (50%) at offloading was obtained from the meat sourced from Dagoretti slaughterhouse. This could be an indication of high contamination level of the meat from this slaughterhouse. Mwai (2012) in her work also indicated high contamination at the abattoir level in this slaughterhouse (local improved) as compared to the other two slaughterhouses she studied.

Despite the reported cases in faecal, milk and meat samples in this research and others in Kenya (Mwai, 2012; Arimi et al., 2000: Kangethe et al., 2007), pathogenic *Escherichia coli* prevalence and reported cases in human from the health centres and the sporadic outbreaks is minimal. Onyango *et al*., (2009) was able to isolate pathogenic *E. coli* from stools of food handlers in tourist hotels: 2(5.1%) of the isolates were serotype O157. Sang *et al*. (2012) has reported 24.1% STEC prevalence from loose stool in Maasai in Kenya, one of the highest reported prevalence in the world. Brooks *et al* (2003) was not able to isolate *E. coli* O157:H7 or any other STEC from bloody diarrhoea investigated from Nyanza, Kenya. The low level of reporting is possibly because the disease surveillance system for *E. coli* O157 serotypes related illnesses is not well developed. It may also suggest that the consumers are able to cook meat and meat products to reach an internal temperature $\geq$ 68 ^{0}C, an assumption that could not be true.

5.2 Factors leading to carcass contamination

Information to the food handlers and other market players along a certain food value chain is important in curbing the zoonoses arising from such a food. Martins et al., 2012 concluded that, to ensure food handlers practise the correct way of handling food, knowledge and training are essential as part of their job. Knowledge is obtained through observation, education and training. Abdul Mutalib et al, (2012) found a significant relationship between Educational and knowledge level of a worker in food premises and attitude level. The workers with higher level of education and knowledge had a positive attitude and good practice measures. Although the butchery attendants were not permanently employed and therefore the turnover rate was very high, half of them had an accumulated experience of $\geq$1 year in the meat industry. However, none of them had received formal training on meat handling. Most of them had had formal education up to the primary level (basic education).The lack of formal training and lack of knowledge on the effect of their actions to customers' health could have been the reason for the low level of hygiene

practices. Most of the personnel handling meat were not aware of the possible microbial cross contamination from faecal matter or intestines. Although 97% said that they washed their hands regularly, only 9% had taps or hand washing facility nearby. It was also observed that the butchery attendant handled money and meat simultaneously. The use of same equipment while handling both meat and stripes is evidence of poor practice.

5.2.1 Transporters: Knowledge, attitude and practices

Although the Kenya Meat Control Act provides for the transportation of meat in unrefrigerated carriers or containers, the design available did not conform to what it recommends; that they should be double walled and have a roof-top rotating ventilator. For some carcasses that took a long time to transport, there could have been multiple replications as the generation time of *E. coli* at 25 ^{0}C is 1.46. (Doyle and Schoeni, 1984). It was also observed that some of the practices by the loaders and transporters were not as per the good manufacturing and handling practices and personal hygiene as outlined in the Kenya Meat control Act Cap 356 (GOK, 1977). These discrepancies include lack of frequent changeover of the soiled clothes and wash of hands during loading and offloading. FAO (2006) noted that beyond application of good hygienic practices, adoption of multiple interventions at slaughter, and strict temperature controls throughout the food chain, there are no practical risk management options available today that would entirely eliminate the pathogen from live animals, from carcasses, or in raw ground product, with the exception of irradiation. The latter is not commonly used in the developing countries meat value chain as the process calls for heavy investment in plant equipment and the suspicion some consumers have on irradiated products (Bender ,1992).Therefore, the former three interventions remain core to control of carcass and meat bacterial contamination.

A number of guidelines indicate the appropriate method of transporting carcasses or raw meat to curb on the likelihood of contamination and microbial growth. USDA/FSIS (2005), FAO (1991) and Kenya Meat Control act Cap 356 for instance recommend transportation of carcasses when hang on rails and in such a manner that they are not in contact with the wall, the floor and each other. The transportation containers are recommended to be constructed such that meat is protected from contaminants from outside. However, the transporters heaped carcasses one on top of another and lain on the floor of the transportation container and in some cases kraft paper

used to separate them. Enquiry on to the purpose of the kraft papers determined that they were to protect the carcasses underneath from blood dripping from carcasses above them. The source and microbial quality of the kraft papers was not determined. Physical appearance of the carcasses when hung at the butchery was seen as of outmost importance. The transporters using these papers claimed that the butchery attendants complained that consumers perceived the blood soiled carcasses (that appeared dark after surface desiccation) to be of low quality. Microbial contamination of the carcasses seemed to be of less importance to the butchery owners and transporters than the physical appearance. This may be because of lack of knowledge and the fact that microbial contamination cannot be determined by sight.

Hygiene during handling of meat is paramount. The personnel handling the meat and surfaces in contact with meat have been found to be sources of contamination of carcasses (Gill et al.,1999; Schlegelova´ et al 2004). FAO (1991) outlines the hygienic requirements during meat handling to increase on its shelf life. Kenya Meat Control Act Cap 356 also states that protective clothes worn by personnel should be clean and personnel hands be cleaned before loading and offloading of meat. This research found that the transporters rarely washed their hands especially during offloading of carcasses. Their clothes were stained with blood by the time they finished loading from the carcasses they placed on their shoulders during loading and offloading and there was no changeover until meat distribution to the butcheries was over (Table 4.9). This could take long and several carcasses could have been handled by this time.

5.2.2 Butchers: Knowledge, attitude and practices

Retail sale of fresh meat in Kenya is usually done at the small scale retail shops (butcheries) to the consumers. Bacterial load of the meat at these retail shops is dependent on the initial contamination from the slaughterhouse and conditions favouring growth and practices leading to cross contamination during transportation because there is no dilution step in between. Strict observation of hygienic practices is therefore critical in control of zoonoses emanating from food contamination (CFSPH 2009). Kenya has formulated regulations to guide the control of food contamination during processing, transportation or sale. The Kenyan Food, Drugs and Chemical Substances (Food Hygiene) Regulations (GOK, 1978), for example states “storage and transportation of food shall be under such conditions as shall prevent contamination, including

development of pathogenic or toxigenic micro-organisms or both". The Public Health Act Cap242 also prohibits the preparation, manufacture, storage or exposure of any food without taking enough precaution to prevent contamination.

However, it was observed that offal and meat were hanged in the same room at the retail shops and sometimes at close proximity. The equipments used in their handling (the knives, the cutting board and the weighing balance) were shared between the stripes and the meat. This practice could lead to contamination of the meat with faecal coliforms like *E. coli* O157 serotypes that could cause illness to humans. Frequent and proper cleaning of equipment like the knives, weighing balance and cutting board was not observed. At some retail shops, cleaning of these surfaces during the working hours was through wiping with a cloth which could also be contaminated and washing with water and detergent was done once daily. There was no use of disinfectant in their cleaning regime. Strict and proper cleaning and hygiene was not observed

Ali *et al* (2010) has found surfaces at the retail shops (including meat mincing equipments) contaminated with aerobic mesophiles. Armstrong *et al* (1996) noted that the use of meat from different sources to make minced meat products and meat grinders on different lots without cleaning in between increased the possibility of cross contamination. He estimated that *E. coli* O157:H7 contamination from a single carcass could be passed on to 8 tonnes of ground meat .In this research, some of those who sold minced meat either obtained already minced meat or took meat chunks to a third party for grinding. This is a practice that could lead to wide spread cross contamination of meat from a single source. Other surfaces have been shown to contain *E. coli* O157 and similarly, the meat mincer could be contaminated. According to this research, up to 66 carcasses in 1000 could be contaminated with pathogenic *E. coli* which may theoretically translate to equal probability of the meat mincing equipment being contaminated. Further research on the effect of this practice could give rise to interesting results towards understanding exposure assessment keeping in mind that meat products containing ground meat has been the common vehicle for *E. coli* O157:H7 in food borne infections since the 1982 incidence in the US (Rangel et al, 2005).

The popularity of wood as a food contact surface has reduced as it is a porous and absorbent material. Organic matter along with bacteria become entrapped and cross-contamination is a

main concern (Lauzon, 1998), It is therefore capable of aiding in bio-film build up and has been discouraged for use in food preparation or processing institution (Costerton *et al*, 1999). Wooden tables used during the sale of carcasses in Ibadan slaughterhouse, Nigeria were highly contaminated with *Enterobacteriaceae*, *Listeria* and *Salmonella* sp. (Adetunji and Isola, 2011). In Kenyan meat retail shops, use of wooden surfaces is common during the size reduction of the bony meat at the low turnover retail shops is a challenge. The equipment used is either axes or modified knives (with weights welded on the blunt side) to chop on the bones. The meat is also placed on a tree stump-wooden chopping board (Figure 4.2) and hacked to obtain the desired weight of the meat cuts. This process creates crevices on the chopping board and loose wooden chips may attach themselves on the meat. A better equipment than the chopping board as observed in some of the retail shops for sizing down the bony meat was the electric saw. Some of the butcheries used a hand held bow saw for the same purpose.

Although it has been noted that large investments do not necessarily translate to improved food safety (Mwai, 2012), some basic tools and training is necessary. Jirathana (1998) has noted the lack of funds at small scale companies for training their employees as a major constraint in adoption and implementation of HACCP in the developing countries. This is manifested in the cleaning regime applied by the butchery attendants on the chopping boards where fat from fresh meat is applied after scrapping using knives. This is neither aimed at having a bactericidal nor a bacterial static effect. The main purpose for the cleaning regime is to reduce on the meat contamination with the chips from the wooden board. The fat applied is obtained from the fresh carcasses and may be contaminated with faecal matter and microorganisms.

Some of the consumers prefer ready to eat meat prepared at the butchery. The consumers who buy fresh meat thereafter prepare the meat at home for family consumption. Raw meat consumption is not a popular habit in Kenya. Cooking is a common step during meat preparation. The extent to which consumers heat treat meat before eating appears not to have been studied in Kenya. A temperature of 66^0C for 1 minute or 68^0C for 15 seconds or 70^0C for less than 1 second has to be achieved at the thermo centre to ensure complete destruction of *E. coli* O157:H7 (FAO/WHO, 2006). Consumers who prefer ready to eat meat at the butchery may be at a risk of taking *E. coli* O157:H7 contaminated meat due to habits that may lead to cross contamination from fresh meat. WHO, (1997) recommends that food hygiene practices be

carried out by all prayers along the chain including consumers for effective control of EHEC. In the 19% of the butcheries that prepared meat for their consumers, workers confirmed handling both fresh and cooked meat constantly. The workers at the butchery had no training on hygiene practices. They sometimes could not wash hands in between handling the fresh and cooked meat. The equipment and surfaces used in handling fresh meat could be used for cooked meat too. Most of the meat handlers (81%) confirmed to have no formal training on meat handling and therefore the workers could be oblivious of the risks for such behaviour. Therefore education to transporters, butchers and consumer on good handling practices would be an important intervention step in *E. coli* O157 serotype control in meat value chain.

5.2.3 Transportation and storage conditions:

Temperature in the meat carrier box increased significantly ($p<0.05$) during transportation from loading to offloading but the relative humidity reduced. Temperature control to reduce multiplication of bacteria during distribution and storage has been identified as an important factor towards controlling the growth of *E. coli* O157:H7 (CCFH 2003). Keeping meat at low temperatures extends the shelf life to 7 days at 1 ^{0}C unlike 1 day at 16 ^{0}C (FAO, 1991). Meat Control Act Cap 356 specifies that the meat carrier box should have refrigeration facilities or a roof top ventilator for proper ventilation. This was disregarded by the meat transporters.

At the butchery, the carcasses were hung at ambient temperatures from where the cuts for the customers were obtained. Only 48% of the retail shops had refrigeration facilities. A carcass could take up to 4 days in some retail shops before it was over. This meat was used to top up the fresh meat for the consumers. Some of the butchery attendants admitted to have received complaints from their customers of off-odours and off-tastes from meat they had sold to them. This is in disregard to the Food, Drugs and Chemical Substances Act Cap 254 of Kenyan Laws that prohibits the sale of unwholesome food that may be injurious to human health.

CHAPTER SIX: Conclusions and Recommendations

6.1 Conclusions

1. The probability of obtaining *E. coli* O157 serotype contamination carcass increased along the supply chain. This suggests contamination of the carcasses and multiplication of bacteria to detectable levels. There were no dilution stages during supply of carcasses. Limited butcheries used bacterial growth control measures like cold storage.
2. The prevalence of *E. coli* O157 serotype in beef carcasses increased from loading to offloading in the three sampling sites. The increase at Dagoretti was from 0.7% to 2.17% and from 0% to 1.6% and 4% for Limuru and Eldoret respectively. The increase could pose a risk to consumer who bought the meat immediately after delivery of the carcass to the butchery. The consumer was more likely to obtain an *E. coli* O157 serotype contaminated carcass immediately after delivery than after a day at the butchery.
3. The butchery surfaces at Limuru were well cleaned to eliminate the risk of contaminating the newly delivered carcasses. Some of the surfaces in the butchery at Eldoret and dagoretti spread *E. coli* O157 serotype during sale to the consumer. The number of the consumer buying contaminated meat at these butcheries could be high due to the fact that all meat was handled by this contaminated equipment.
4. There was an increase in the prevalence and probabilities of obtaining *E. coli* O157 serotype contaminated carcasses from loading to offloading. The factors that lead to the increase include :
    - Poor hygiene condition of the personnel, equipment and surfaces used in carcass handling.
    - Lack of observation of the cold chain during transportation and storage at some butcheries
    - Bad manufacturing practices.

6.2 Recommendations

1. The contamination of carcass with E. coli O157 serotype should be aimed at being reduced to below detectable limits by the time loading for transportation is done. This can only be achieved by observing good manufacturing and hygiene practices during slaughter.
2. Training of the meat handlers along the transportation value chain should be carried out frequently; at least twice per year as recommended by the Meat Control Act cap.356. The hygiene practices should be monitored regularly to ensure conformance.
3. The material for and design of meat contact surfaces and area should be aimed at reducing levels of contamination of carcasses during transportation and storage.
4. Research on exposure assessment and risk characterisation should be carried out to help assess the risk posed by beef.

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ANNEXES

Annex 1: Material and Reagents used in carrying out research

Reagents	**Manufacturer**	**Equipment and apparatus**	**Make/Manufacturer**
Buffered Peptone Water	Oxoid CM0509	*E. coli* O157:H7serotyping kit	Oxoid
Sorbital MacConkey agar	Oxoid CM0813	Verotoxin production test kit	Oxoid
MacConkey agar	Oxoid CM0007	Micro-titre pipette	pyrex
Tryptone water	OxoidCM0009	V-shaped micro-titre plate	pyrex
MRVP medium	Oxoid CM0043	Incubator	Carbolite S/N 7/98/1490
Simons citrate	Oxoid CM0155	Eppendorf Centrifuge	Model 5413
Methyl red Indicator	BDH Chemical ltd	Inoculation Wire loops	
Indole reagent	HiMidea Laboratory	Bunsen burner	
Creatinine	HiMidea Laboratory	Culture tubes	pyrex
5% alcoholic alpha-naphthol	HiMidea Laboratory	Disposable petri dish	pyrex
40% potassium hydroxide	Kobian Kenya Ltd	Pipette filler	pyrex
Nutrient agar	Oxoid	5 and 10ml Pipettes	pyrex
Tryptone soya broth	Oxoid CM0129	Conical flasks	pyrex
Glycerol	Kobian Kenya Ltd	Blow lamp	
Normal (physiological) saline	BDH Chemical ltd	Cotton wool	
Polymixin B (5000 international units/ml)	Laboratory and allied limited	Applicator sticks	
75% alcohol	Kobian Limited	Aluminum foil	
Brain heart infusion agar	Oxoid CM0375	Sling psychrometer	

Annex 2

Sample Questionnaire/observation guidelines

Demographic characteristics

1.Name …….

2.Age (Yrs) 3.Gender: tick as appropriate Male ☐ Female ☐

4. Residential area…….

5. Religion: tick where appropriate

Christian ☐ Muslim ☐ Hindu ☐ Traditional ☐ Others ☐

6. Place of work: tick as appropriate. Slaughterhouse ☐ Transport ☐

butchery ☐

7. Level of education primary sch ☐ secondary sch ☐ college ☐

others ☐

(if others, specify)……..

8. Have you ever received any formal training in meat handling? Yes [ ] No [ ]

9. If Yes which area?

10. Are you trained on any other area ☐Yes ☐ No

11. If yes, specify…….

12. Work experience in the meat industry (yrs)…….

13. Have you worked in any other field Yes ☐ No ☐

14. If yes, specify……..

Knowledge

1. How frequent would you advice a person in constant contact with meat to wash his/her hands.

Once daily ☐ Thrice (morning, afternoon and evening) ☐ Any time they touch surfaces(including money) ☐ Only after visiting the toilet ☐

2. If Meat handled wrongly may pass diseases from one person to another
   True ☐ false ☐

3. If yes, name a few of these diseases?
   .....
   .....

4. Which of the following is the best to use while you are cleaning the surface that comes into contact with meat?
   Cold water and soap ☐ Hot water and soap ☐ Cold water, soap and sanitizer ☐
   Hot water, soap and sanitizer ☐ Cold water and ash ☐ others (specify) ☐

5. Mixing of meat and offal is ok as long as they all are from same species. True ☐
   false ☐

Attitude

1. The government is unfair to businessmen on insisting on certificate of good health.

True ☐ False ☐

2. Give a reason for your answer ……

……

……

3. In your opinion, do you feel that the boxes you use to transport the meat should only be used for that purpose and not any other? Yes [ ] No [ ]

4. Please give a reason for your answer.

.....

.....

Annex 3: QUESTIONNAIRE FOR THE TRANSPORTERS

1(a) Do you wear protective clothing while loading and offloading the meat? Yes [ ] No [ ]

1(b) Give a reason for your answer in 1 above

.....

.....

2(a) Do you own protective clothing? Yes [ ] No [ ]

2(b) If yes, how many? One [ ] Two [ ] Three or more [ ]

2(c) If no to 2(a) above, and yes to 1(a), where do you obtain your coat from? .....

.....

3 Do you ever find the need to change the protective clothing in a single day as you work?

Yes [ ] No [ ]

4 How often do you wash your protective clothing? once per day [ ] twice per day [ ] once it gets soiled with blood [ ] once per every two days [ ] others(specify) [ ]

5 What do you use to wash your protective clothing?

Cold water only [ ] cold water and soap [ ]

Cold water, soap and disinfectant [ ] Cold water, soap and bleaching agent [ ]

6(a) Do you wash hands while handling meat? Yes [ ] No [ ].

6(b). If yes, how often? Once in the morning as I start loading [ ] At beginning and end of every loading [ ] In the morning and after work [ ] others(specify) [ ]

7(a) Are there times when you get wounds as you work? Yes [ ] No [ ]

7(b) If yes, how do you take care of the wound? By tying a cloth around it [ ] By leaving it open [ ] By use of waterproof band [ ].

8 What do you use for carrying meat? Vet officer approved box on a vehicle [ ]
specifically designed vehicle for carrying meat only [ ] polythene bags [ ]
Others (specify) [ ].

9 How do you pack meat in the carrier?
Hang on a rail [ ] Heaped on each other separated by craft paper [ ]
Heaped on each other without any separating material [ ] others [ ]

10 Indicate how often you wash the meat carrier. Once per day after use [ ] twice per day, before use and after use [ ] once in every two days [ ] others(specify) [ ]

11 What is the source of the water used in washing of the carrier? River [ ]
City council tap water [ ] Collected rain water [ ]

12 Do you have refrigeration facilities in your carrier? Yes [ ] No [ ]

13 How long does it take you to supply the meat after loading?.the shortest distance from slaughterhouse [hh, mm ------].....the longest distance from slaughterhouse [hh mm--------]

14(a) Is loading and off loading done by the same person? Yes [ ] No [ ]

14(b) If yes, is there change of the protective clothing between loading and off loading? Yes [ ]
No [ ]

15. Do you observe any carcass contaminated with faecal matter as loading into the respective vehicle continues? Please indicate how many.....

Annex 4: QUESTIONNAIRE FOR THE MEAT HANDLERS AT THE BUTCHERY

1 Do you have a display cabinet? Yes [ ] No [ ]

2 How is the ventilation at the cabinet and the butchery? Good [ ] fair [ ] poor[ ]

Guidelines

Good-ventilation allows air flow into the butchery but sieves off dust and other particles

Fair-ventilation allows air flow but do not sieve dust or other particles or allows very little air flow

Poor-ventilation does not allow air flow at all.

3 Is there use of bulbs at the display cabinet (observe) yes [ ] No [ ]

4 Are there refrigeration at the display cabinet? Yes [ ] No [ ]

5 Do you have a refrigerator for storage of the meat that remains? Yes [ ] No [ ]

6 How many protective coats do you have? Nil [ ] One [ ] Two [ ]
more than two [ ]

7 How frequent do you wash the protective coat? Once per day in the evening [ ] Twice per day, morning and evening [ ] once after every two days [ ] once per week [ ] others [ ]

7 Do you have a cutting board? Yes [ ] No [ ]

8 If yes, what material is it made of? (Observe) Wood [ ] Easy to flake plastic [ ]
Hard to flake plastic [ ] Metal [ ]

9 What is your source of water for use in the butchery? City/Municipal council [ ] borehole [ ] rain collected water [ ] River [ ] others (specify) [ ]

10. How often do you wash the following butchery surfaces and equipment?

(a) Once per day in the morning
(b) Once per day in the evening
(c) Twice per day
(d) More than twice
(e) Once in every two days
(f) Others (specify)

Knife [ ] saw [ ] cutting board [ ]

hooks [ ] floor [ ]

Describe the cleanliness status as observed.....

.....

.....

11. Do you have any hot water baths (approx. 82^0C) for dipping of knives at the premises?

Yes [ ] No [ ]

12How long does the meat stay in your butchery before it is over? Less than 12 hours [ ] one day [ ] Two days [ ]

13 Do you sell minced meat? Yes [ ] No [ ]

14 If yes, where do you get it from? Have my mincer [ ] Take elsewhere for mincing [ ] buy already minced meat [ ]

15 Do you prepare meat/other food for consumption at the premises? Yes [ ] No [ ]

16 If yes, do you have other specific people, apart from the butchery attendant to perform that duty? Yes [ ] No [ ]

17If yes, what other duties do they perform?

.....

.....

18 Do you ever receive complaints from the consumers on the quality of the meat you sell?

Yes [ ] No [ ]

19. If yes, what kind of complaint? Abdominal upsets [ ] Tough meat [ ] Dirty meat [ ] Others [ ]

20 Have your workers gone for medical checkups in the last 6 months? Yes [ ] No [ ]

21. Do you have different storage and display cabinets for the stripes/offal and meat? (Observe)

Yes [ ] No [ ]

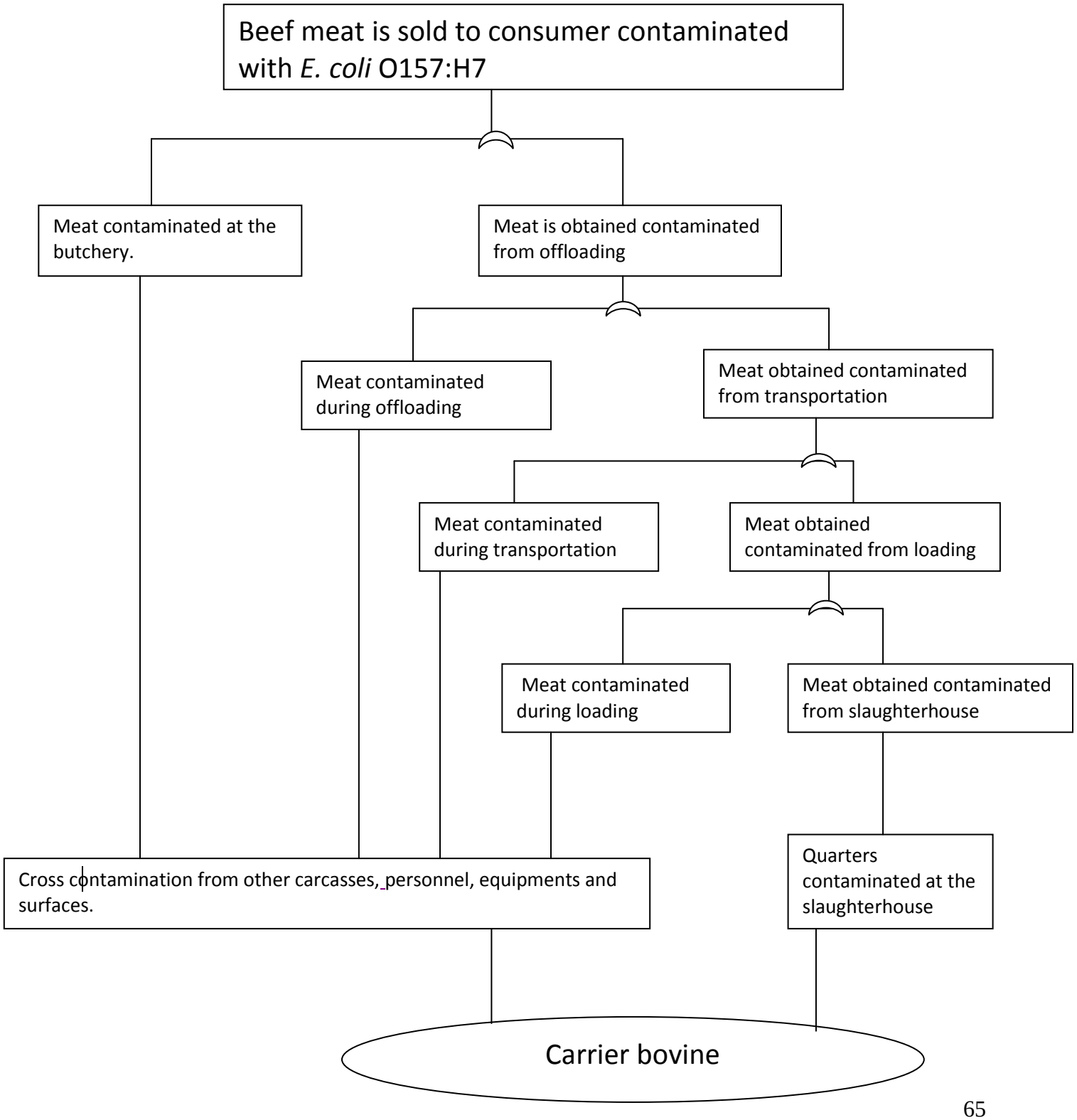
22. Do you use the same equipment while handling meat versus the offal and stripes?

Yes [ ] No [ ]

23. How many carcasses do you observe as contaminated with faecal matter? .....

Annex 5:

Fault tree showing possible points of beef meat contamination with E. coli O157:H7 during transportation from slaughterhouse to the butchery.



Annex 6:

Modelling for carcass contamination with *E. coli* O157 serotype from three slaughterhouses at Loading, Offloading and Follow-up stages during transportation chain

a) Dagoretti slaughterhouse

Stages	Carcass contaminated with *E. coli* O157:H7		Carcass not contaminated with *E. coli* O157:H7	
Loading(A)	1		137	
Offloading(B)	A+B+	A-B+	A+B-	A-B-
	1	2	0	135
Follow-up(C)	B+C+	B-C+	B+C-	B-C-
	0	0	3	135

b) **Limuru slaughterhouse**

Stages	Carcass contaminated with *E. coli* O157:H7		Carcass not contaminated with *E. coli* O157:H7	
Loading(A)	0		62	
Offloading(B)	A+B+	A-B+	A+B-	A-B-
	0	1	0	61
Follow-up(C)	B+C+	B-C+	B+C-	B-C-
	0	0	1	61

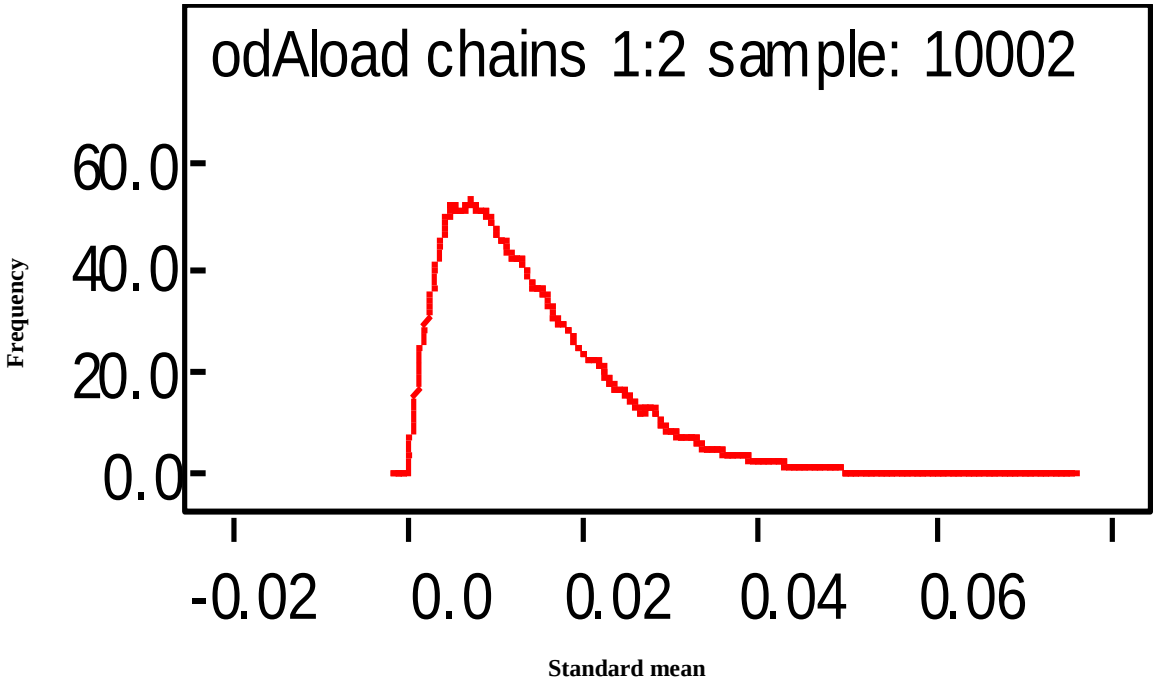
c) **Eldoret slaughterhouse**

Stages	Carcass contaminated with *E. coli* O157:H7		Carcass not contaminated with *E. coli* O157:H7	
Loading(A)	0		50	
Offloading(B)	A+B+	A-B+	A+B-	A-B-
	0	2	0	48
Follow-up(C)	B+C+	B-C+	B+C-	B-C-
	1	0	1	48

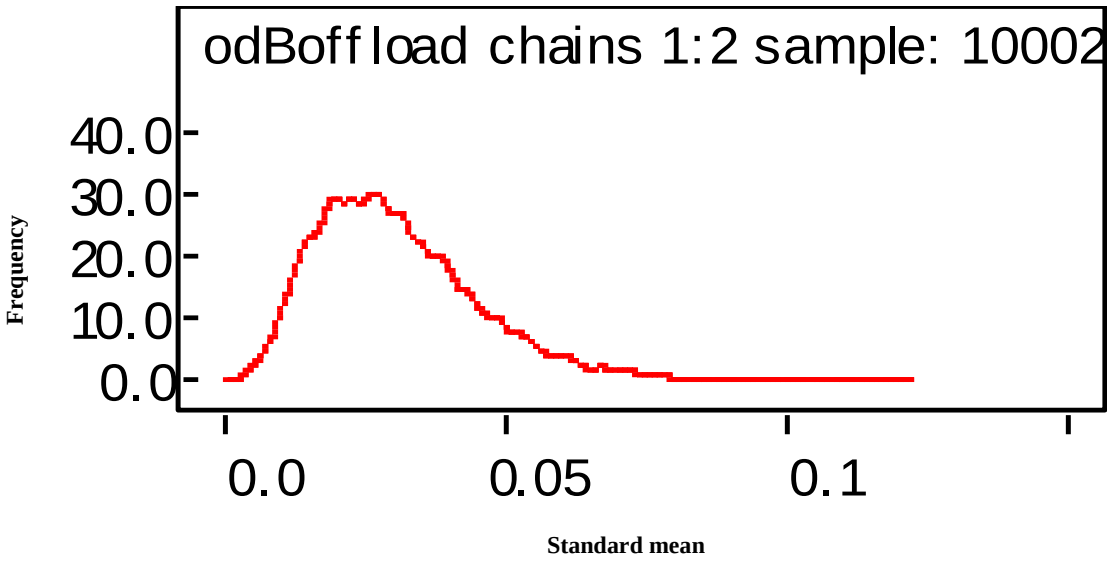
Annex 7

The probability of a carcass from Dagoretti slaughterhouse being contaminated with *E. coli* O157:H7 during transportation

a) Loading



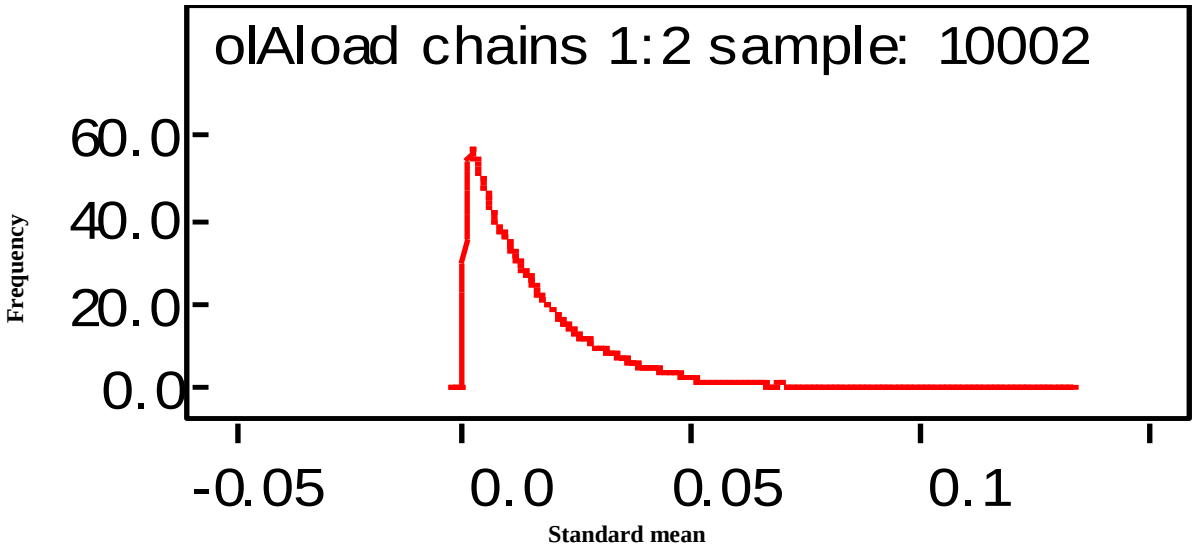
b) Off-loading



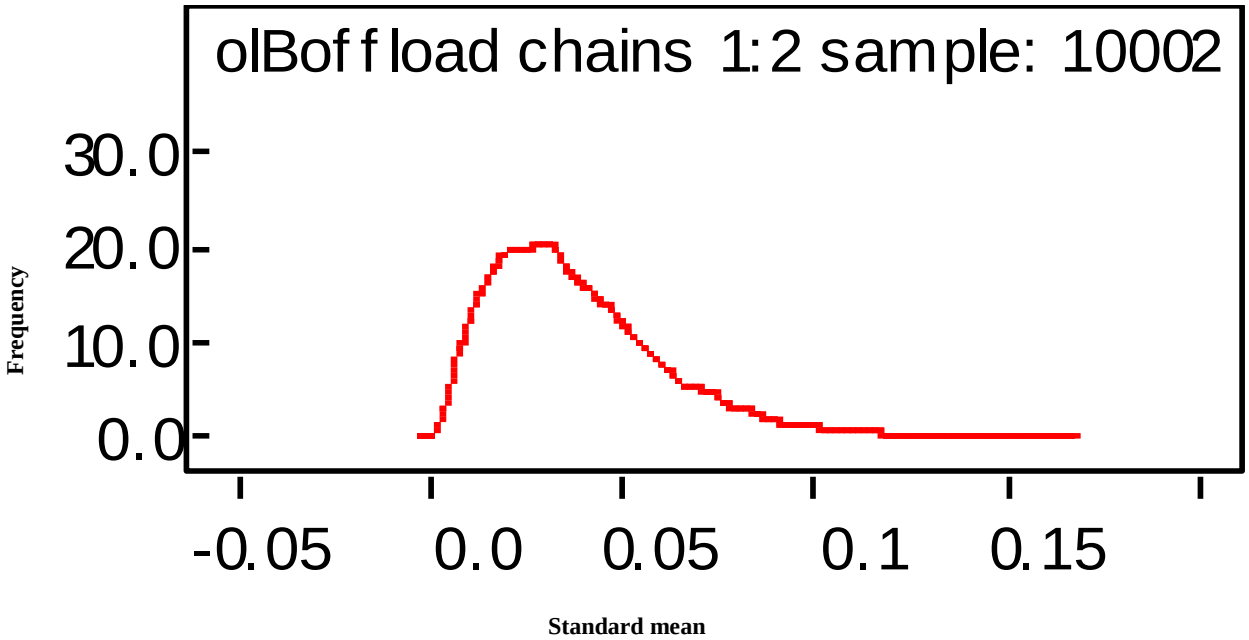
Annex 8

The probability of a carcass from Limuru slaughterhouse being contaminated with *E. coli* O157:H7 during transportation

a) Loading



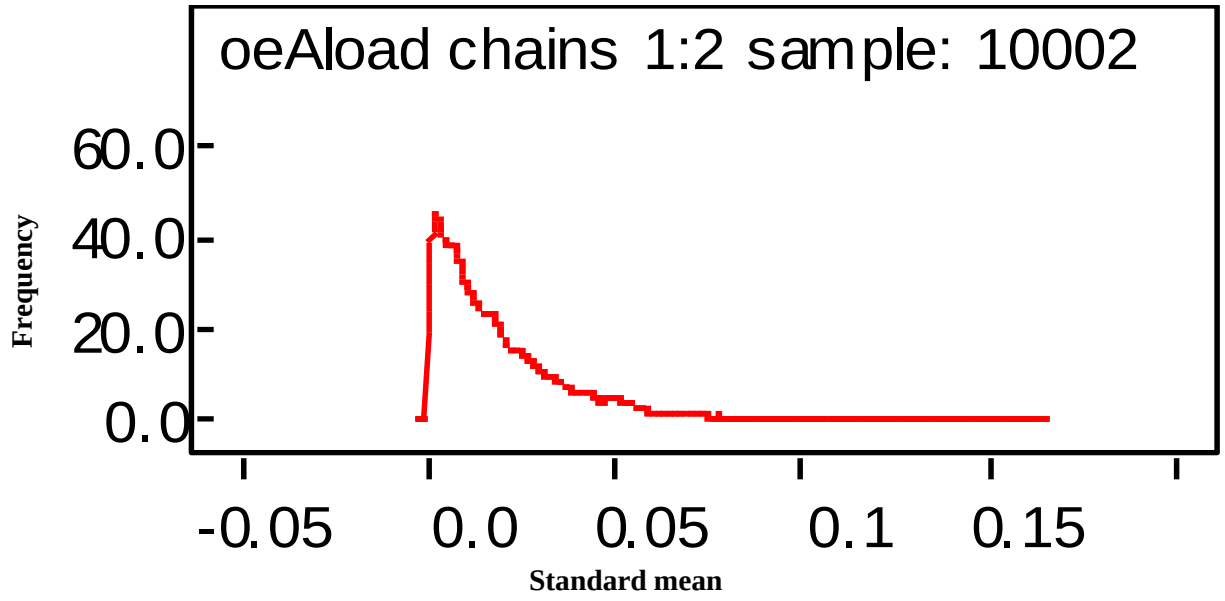
b) Off-loading



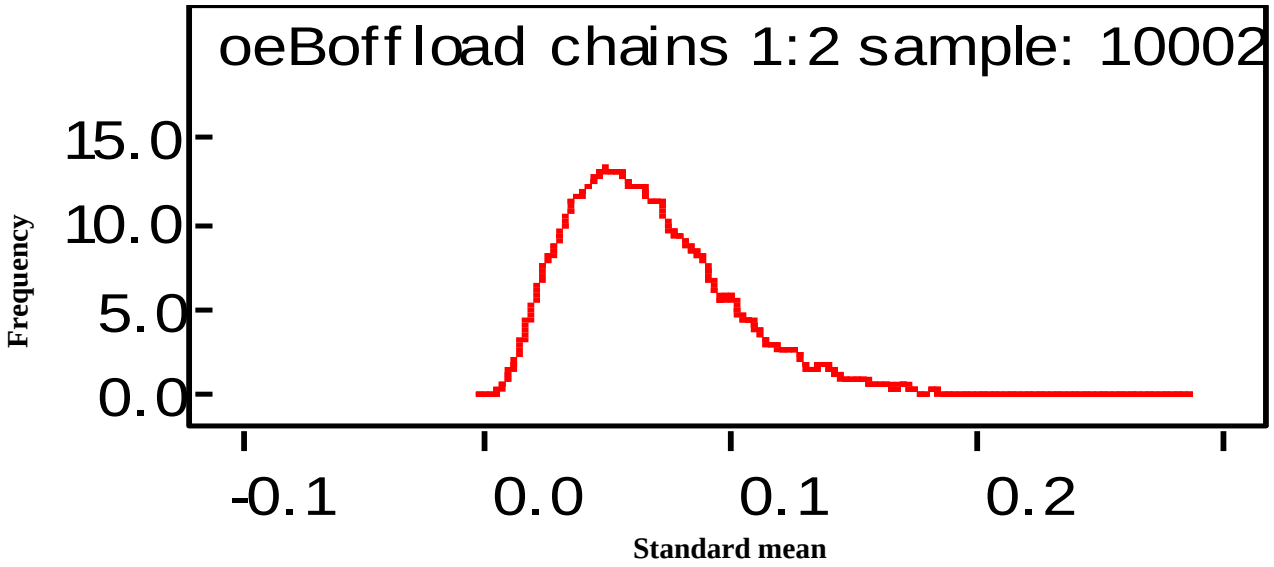
Annex 9

The probability of a carcass from Eldoret slaughterhouse being contaminated with *E. coli* O157:H7 during transportation

a) Loading



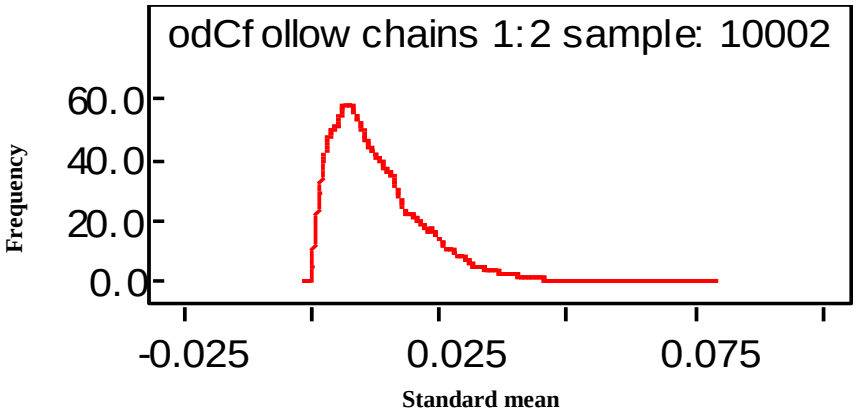
b) Off-loading



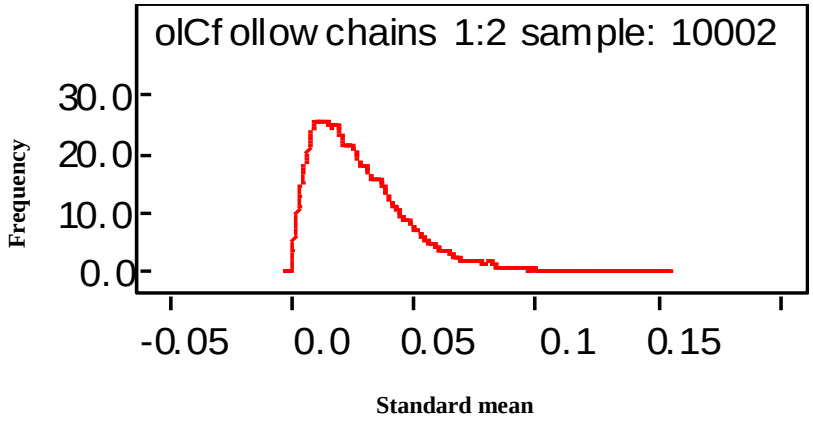
Annex 10

The probability of obtaining a carcass contaminated with *E. coli* O157:H7 from butcheries supplied from Dagoretti, Limuru and Eldoret slaughterhouses, one day after supply

a) Dagoretti



b) Limuru



c) Eldoret