RISK OF CONTAMINATION OF CATTLE CARCASSES WITH Escherichia coli O157 FROM SLAUGHTERHOUSES IN NAIROBI, KENYA.

A thesis submitted in partial fulfilment of the requirement for the degree of Master of Veterinary Public Health (MVPH) of the University of Nairobi

CAMELINE WANJIRU MWAI (BVM)

University of Nairobi

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY,
FACULTY OF VETERINARY MEDICINE,
UNIVERSITY OF NAIROBI

DECLARATION

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

Date 15.06.12.

CAMELINE WANJIRU MWAI (BVM.)

This thesis has been submitted for examination with our permission as University supervisors:

Date 18/06/2012

PROF. KANG'ETHE, E. K. (BVM, MSc., PhD)

Department of Public Health, Pharmacology and Toxicology, University of Nairobi

Date 15.06.2012

PROF. ARIMI, S.M. (BVM, MSc, PhD)

Department of Public Health, Pharmacology and Toxicology, University of Nairobi

Date 5-7-12

Dr. GRACE, D. (PhD, MVB, MSc. (Hons), Cert. Well., MRCVS)

International Livestock Research Institute, Nairobi

DEDICATION

To my Mothers Angela and Joyce, Father Elijah, Son Jeremy and Husband Gilbert.

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LIST OF ABBREVIATIONS

CAC	Codex Allimentarius Commission
CCPs	Critical Control Points
CFU	Colony Forming Unit
CI	Confidence Interval
E. coli	Escherichia coli
EAEC	Enteroaggretive *Escherichia coli*
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvasive Escherichia coli
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic Escherichia coli
FAO	Food and Agriculture Organization of the United Nations
GDP	Gross Domestic Product

GHP	Good Hygiene Practices
G.o.K.	Government of Kenya
HACCP	Hazard Analysis Critical Control Points
HC	Haemorrhagic Colitis
HUS	Haemorrhagic Uremic Syndrome
IMViC	Indole, Methyl Red, Voges-Proskaurer and Citrate
MRVP	Methyl Red Voges-Proskaurer
OIE	World Organization for Animal Health (Office Internationale de Epizooties)
PRA	Participatory Risk Analysis
SSOPS	Sanitation and Standard Operating Procedures
STEC	Shiga Toxin Escherichia coli
U.S.A	United States of America
ul	Micro litre

VT1 **Verotoxin 1**

VT2 **Verotoxin 2**

WHO **World Health Organization of the United Nations**

ABSTRACT

The study was carried out in three abattoirs supplying meat to butcheries in Nairobi and its environs. The objectives of the study were to assess the level of contamination of carcasses with *Escherichia coli* O157 in the slaughterhouses, determine the critical control points and train the slaughterhouse managers on practices that would reduce carcass contamination. Three slaughterhouses with different levels of hygiene control, classified as 'export', 'improved local' and 'typical local', were selected. Three hundred cattle were tracked along the slaughtering process to sample faeces and carcass. A rectal faecal sample was taken from each animal after stunning. Two carcass sites, flank and brisket were swabbed after flaying, evisceration and washing. Thus, in total seven samples were taken from each animal. *E. coli* O157 was isolated by culture and serotyped using card agglutination test. The isolates were further tested for verotoxin production. Monte Carlo simulation was run to determine the risk of carcass contamination. A Hazard Analysis Critical Control Point (HACCP) model was developed for one of the abattoirs. Interviews were done with slaughterhouse workers to test their knowledge, attitudes and practices towards slaughtering hygiene. Identified gaps on hygiene from slaughter personnel questionnaire were used to develop training materials for slaughterhouse managers and staff. *E. coli* O157:H7 was recovered from faecal and carcass samples at different stages of carcass dressing. Two hundred and eighty (280) out of 2,100 samples (13.3%) yielded sorbitol MacConkey negative *E. coli* isolates (IMViC (++--) which were presumptive *E. coli* O157. After serotyping with O157 antigen, 92 out of 280 (or 4.3% of the total 2,100 samples) isolates, were positive for *E. coli* O157. Forty-two isolates of the 92 were

tested for verotoxin production, eight were positive for VT1 only while two were positive for both VT1 and VT2. The risk of a carcass being contaminated with *E. coli* O157 on the abattoir was 29, 38 and 48 carcasses per 1000 slaughtered animals for the export, the typical local and the improved local abattoirs respectively at 90% confidence interval. There were significant differences in prior training received by the workers in the typical local abattoir and the improved local ($p=0.001$) with the typical local slaughterhouse having more trained workers than the improved local, but there was no significant difference between the export and the typical local slaughterhouse and between the export and the local improved slaughterhouse. More workers were significantly ($p=0.025$) washing their hands before, during and after slaughtering each animal in the typical local than the improved local slaughterhouse. The number of workers playing more than one role in the slaughter process was also significantly ($p= 0.027$) higher in the improved local than the typical local slaughterhouse. These factors may have contributed in the differences in carcass contamination in the three slaughterhouses. Slaughterhouse owners and staff were trained on good hygienic practices, food borne illnesses and risk of contamination of carcasses. Evaluation done one month after the training showed no change in the hygiene practices of the workers. This may have been the result of inadequate facilities like hot water, soap and disinfectants in typical and improved local slaughterhouses. Lack of motivation by the management and paying of the workers depending on the kill may affect the hygiene levels and workers attitude towards hygiene. This study shows that there is a risk of carcass contamination with *E. coli* O157 in all the different categories of slaughterhouses. Workers and operations hygiene are important factors contributing to this risk.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Livestock industry in Kenya

The livestock population in Kenya is estimated at 17.5 million cattle, 17.1 million sheep, 27.7 million goats 2.9 million camels 31.8 million poultry and 0.3 million pigs, (Kenya Bureau of Statistics 2009) . Rift Valley Province has the highest number of cattle (7.7 million), followed by North Eastern Province (2.7 million); Nairobi Province has least number (54 500). The livestock sector contributes 10.4% of the overall Gross Domestic Product (GDP) (Knips, 2004). Consumption of animal products in Kenya (milk excluded) is estimated at 15 kgs/person/year with beef estimated at 9kgs/person/year (FAO, 2007). Recheck again on FAO web recent figures

1.2 Beef slaughter process

As per the Meat Control Act in Kenya (G.o.K., 1977), food animals should be slaughtered and dressed in approved slaughter establishments where meat inspection is carried out. These are classified into two categories namely export slaughterhouses and local slaughterhouses. The local slaughterhouses classification has not been clear since they were all referred to as local slaughterhouses and slaughter slabs. However,

the local slaughter houses have further been classified as A (large), B (medium) and C (slabs) based on the land size, throughput, level of construction outlay operations and hygiene (Meat Control Act Cap 356 legal notice No: 110 2010).

The local slaughterhouses have to apply to the Director of Veterinary Services for them to be licensed under the new categorization. The category A local slaughterhouse will be the largest constructed on land size of at least 2.5 hectares, has a throughput exceeding forty units of bovine, and is allowed to sell meat to all parts of the country. Category B is the medium size slaughterhouse built on land size of not less than 1.5 hectares and has throughput of between 6- 30 units of bovine. It is allowed to supply meat to its locality, towns, and urban centres within fifty kilometres radius. Category C is the lowest category of slaughterhouses and has throughput not exceeding five bovine units, land size of not less than 0.5 hectares and it's allowed to supply meat to the urban area where it is located.

In all the categories of slaughterhouses, humane slaughter is a requirement. The stages of slaughter process include *ante- mortem* inspection of live animals, stunning, bleeding, flaying, evisceration, *post-mortem* inspection, washing and grading of the carcasses. In local slaughterhouses, carcasses are sold at the marketing hall attached to the slaughterhouse to willing customers and taken to butcheries, while in the export slaughterhouses, carcasses are chilled for 12 hours before processing starts thus adding value by making specific cuts and products.

All stages of slaughter can result in carcass contamination. The central aim of clean/ hygienic slaughter is to efficiently remove the skin/hide and viscera in a manner that will prevent contamination of the carcass with the hide or gastrointestinal contents. The hygiene of the operatives and implements used are crucial to attainment of process hygiene. An important concept for understanding the steps in the slaughtering process where contamination is likely to occur is that of Hazard analysis critical control point (HACCP). Critical Control Points (CCPs) in the slaughter process are points at which care and control are exercised in order to produce carcasses of acceptable hygienic quality in respect to the total bacterial load. For a slaughterhouse to achieve this, the slaughter management needs to adopt and implement good hygiene practices (GHP). These practices include personnel hygiene, sanitation and standard operating procedures (SSOPS), provision of potable water and waste disposal; they are a prerequisite to the adoption of HACCP, as recommended by Codex Allimentarius Commission (CAC) guidelines (2003).

HACCP is an internationally recognized system of managing food safety and protecting consumers. It provides a systematic way of identifying food safety hazards and making sure they are being controlled day in day out.

HACCP is based on the following seven (7) principles;

1. Hazard analysis and identification of any hazards that must be prevented, reduced or eliminated.

2. Identification of CCPs.

3. Establishment of critical limits thresholds, which must be met at each critical control point.

4. Establishment of procedure to monitor the CCPs.

5. Establishment of corrective actions to be taken,

6. Establishment of procedures to verify that the system is working effectively.

7. Establishment of an effective record keeping system that documents the HACCP system (CAC guidelines, (2003).

1.3 *Escherichia coli* and its significance in beef industry

A study done on slaughter hygiene by Kang'ethe (1993) showed that carcasses leaving slaughterhouses in Nairobi were highly contaminated with coliforms. This

observation has raised concerns on the hygienic levels in both local and export slaughterhouses and the probability of transferring enteric pathogens such as the enteropathogenic *E. coli* to the meat consumers.

1.4 Objectives of this study were:

1. To assess carcass contamination with *E. coli* O157:H7 in three categories of Kenyan slaughterhouses (export, improved local (best practice) and typical local).

2. To identify CCPs in the slaughter houses and measures to be taken to control carcass contamination

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Characteristics of the *E. coli* organism.

Escherichia coli (E. coli) is a member of the *Enterobacteriaceae* family, found normally in the intestinal tract of cattle, other animals and humans. This species can be differentiated from other members of the family by its ability to utilize sugars and to cause a range of other biochemical reactions such as indole production and formation of acid and gas from lactose and other carbohydrates, which takes place at 37°C. Most strains ferment lactose (Doyle and Schoeni, 1984) and grow over a wide range of temperature (15°C – 45°C). This species encompasses a variety of strains that cause disease in man and animals and some are haemolytic, a characteristic associated with pathogenicity. Pathogenic *E. coli* are placed into various groups based on the mode of pathogenicity. These groups include: enterotoxigenic *E. coli (ETEC)*, enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli (*EAEC*)*, enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC).

Strains of EHEC produce verocytotoxins and cause diarrhoea of varying severity as well as other life threatening conditions using strain specific pathogenic mechanisms. Among these is *E. Coli* serotype O157:H7 strain which has become important worldwide due to its public health importance. (Bleeme *et al.,* 1994). Cattle are the

main carriers of this serotype in their intestines although it is also found in the faeces of many other animals (Long *et al.*, 2004). It has caused serious disease outbreaks (Riley and Remiss, 1982, Watanabe *et al.*, 1996) in different parts of the world, which have resulted in human deaths, with consequential large economic losses in the food industries.

2.2 Pathophysiology of *E. coli* O157:H7 infections in humans

The human illnesses are characterised by mild diarrhoea, abdominal pain and vomiting, complication could lead to haemorrhagic colitis (HC), stroke, and haemorrhagic uremic syndrome (HUS) (Nataro and Kaper, 1998). HC is characterized by bloody diarrhoea, abdominal cramp, fever, and vomiting (Griffin and Tauxe, 1991). HUS is characterized by microangiopathic haemolytic anaemia and acute renal failure due to production of toxins that damage endothelial cells and trigger the clotting mechanism (Donnenberg, 2002). HUS is more common in infants, children, the elderly, and those with compromised immune function (Paton and Paton, 1998). Studies have shown that young children and females have an increased risk of HUS after infection (Gould *et al.*, 2009). Although most HUS patients recover, some die and some may develop strokes (Griffin and Tauxe, 1991), seizures, convulsions, coma, paralysis and chronic renal failure (Remuzzi, 1987; Siegler *et al.*, 1993). Other sequela of verotoxigenic *E. coli* infection include diabetes mellitus and necrotizing colitis (Paton and Paton, 1998).

While the majority of studies of foods linked to human outbreaks have not assessed the infective dose, some studies have indicated that it is low (<1000 cells) (American Gastroenterological Association, 1995). This puts the consumer at a higher risk compared to other food borne infections, highlighting the need for stringent control of contamination during food production.

2.3 Modes of transmission

Infection with *E. coli* O157:H7 occurs primarily through ingestion of contaminated food, especially ground beef. Other sources of infection include person-to-person transmission, which has been reported in nursing homes (Bell *et al.*, 1994).

Alfalfa sprouts, lettuce, un-pasteurised fruit juices, which may have been contaminated with cattle manure during harvesting or processing (Karch *et al.*, 1999), have also been implicated. White radish sprouts served during school lunches were implicated in school-going children in Sakai city in Japan (Watanabe *et al.*, 1996, Michino *et al.*, 1999) and raw milk was a vehicle in a school outbreak in Canada (Honish *et al.*, 2005).

Water-borne infection is also possible. The largest water-borne outbreak occurred in Canada in 2000 (Holmes, 2003), after people drank water contaminated with *E. coli* O157:H7; seven people died and over 2000 were ill. Four children in Netherlands

were infected with *E. coli* O157:H7 after visiting a recreational lake (Cransberg *et al.*, 1996).

2.4 Cattle as a reservoir of *E. coli* O157:H7

Cattle are a major reservoir for *E. coli* O157:H7 harbouring the pathogen in their intestinal tract. Contamination of the skin with dung due to unhygienic production systems can transfer these organisms onto the carcass (Elder *et al.*, 2000). Strict observance of good hygienic practices during slaughter is therefore necessary in order to reduce incidences of contamination of beef carcasses. Elder *et al.*, (2000) isolated *E. coli* O157:H7 from faeces on cattle hides and carcasses during slaughter: Ninety-one isolates were from faecal samples (91/327; 28%), 38 (11%) from pre-evisceration carcass samples and 148 (148/341, 43%) from post evisceration carcass samples taken from different cattle lots in Midwestern United States of America (U.S.A.).

E. coli O157:H7 cases have been reported in bovine products linked to human infections where identical strains of the microorganisms, have been isolated from both infected humans and cattle (Wells *et al.*, 1991, Renwick *et al.*, 1993). The microorganism is non-invasive in cattle and is not known to cause clinical signs. Preliminary evidence suggests that the shedding is transient and that the excretion period ranges from hours to weeks (Besser, 1999).

2.5 Occurrence and distribution of *E. coli* O157:H7

Reports of *E. coli* O157:H7 in cattle.

It has been estimated that 1 to 4% of cattle in the United Kingdom harbour the organism at slaughter (Chapman *et al.*, 1993; Richards *et al.*, 1998). Riddell and Korkaeal (1993) reported that the pre-slaughter faecal load in the live animal is an important determinant of carcass contamination levels. , Smith *et al.*, (2003) reported a 17% prevalence of EHEC from healthy animals in Lagos, Nigeria. Kang'ethe *et al.*, (2007) reported a prevalence of 5.2% and 2.2% of *E. coli* O157:H7 in faeces and milk respectively from urban dairy cattle herds in Nairobi, Kenya.

Reports of *E. coli* O157:H7 in humans.

In Kinshasa, Kelly *et al.*, (2004) isolated *E. coli* O157:H7 from children less than 15 years old, who had suffered bloody diarrhoea. In Kenya, Sang *et al.*, (1992) were unable to identify the cause of many diarrhoea cases in children in Kenyatta National hospital, some of these could have been due to *E. coli* O157: H7, which was not targeted for isolation in that particular study. Moreover, Said *et al*, (1997) isolated *E. coli* O157:H7 from a two-year-old boy suffering from haemorrhagic colitis in Malindi hospital. This was the first confirmed case of *E. coli* O157: H7 in Kenya.

Reports of *E. coli* OI57:H7 in food products

E. coli O157: H7 has emerged globally as an important human pathogen. The number of infections it causes has increased significantly since the first reported outbreak in the USA in 1982 that was traced to contaminated hamburgers (Riley and Remis, 1983). In Africa, cases of *E. coli* O157:H7 have been reported in various food products in different countries. Adjehi *et al.*, (2010) reported a prevalence of 2.4% from all dairy products sold in the streets of Abidjan. A study by Abong'o *et al.* (2009) in Amathole district, Eastern Cape Province of South Africa showed a prevalence of 2.8% of meat and meat products contaminated with *E. coli* O157:H7. In Kenya Arimi *et al,* (2005) also reported isolating *E. coli* O157:H7 from pooled raw marketed milk at the rate of 1.8%. Other countries that have reported isolation of *E. coli* O157:H7 *in* Africa include Swaziland, Uganda, and Tanzania (Raji *et al.*, 2003).

2.6 Slaughter process and hygiene

The slaughter process involves stunning of the animals, bleeding, removal of hooves, flaying, evisceration, cleaning, inspection and grading of carcasses before chilling or direct sale depending on the level of operations of the slaughterhouse. The main challenge in the process is to ensure that the enormous load of bacteria on the hide and the alimentary tract are not transferred to the carcass. In Kenya, the Meat Control Act 1977 governs slaughter process.

Omisakin *et al,* (2003) found a prevalence rate of E. *coli* O157:H7 at 7.5% at animal level and 40.4% at group level in cattle faeces, at slaughter in the United Kingdom. In Hong Kong Leung *et al.,* (2001) isolated VTEC from 409 (41.5%) faecal and 18(1.8%) carcass samples from 986 cattle, and from 10(2.1%) faecal and 1 (0.2%) carcass samples from 487 pigs from an abattoir. Only four (0.41%) cattle yielded VTEC from both faeces and carcasses. Schouten *et al.,* (2004) also reported a prevalence of 7.2% in pooled faecal samples from selected Dutch dairy farms. Mersha *et al.,* (2009) showed a prevalence of 8.1% and 8.6% in sheep and goat carcasses in Ethiopia before and after washing. In Canada, Gill *et al.,* (1996) reported that contamination of the brisket site with micro flora occurred after skinning and that trimming and washing achieved modest decontamination of the neck and brisket site, and extensive decontamination of the rump site. The presence of high shedding animals at the abattoir increases the potential risk of meat contamination during the slaughtering process and this call for thorough hazard analysis and implementation of control measures at identified critical control points. Although studies have been done on, ways to reduce pre-slaughter load of *E. coli* in cattle (Callaway *et al.,* 2003) these technologies (use of probiotics, antibiotics anti-pathogens, diet and management) have yet to be adopted in the developing world.

Kang'ethe (1993) evaluated hygienic slaughter of beef carcasses in Kenya and found that both export and local slaughterhouses were producing carcasses that were heavily contaminated with coliforms to the level above 10^5 colony-forming units (CFU) per

square centimetre. *E. coli* O157:H7 contamination of beef carcasses in Kenyan slaughterhouses has not been evaluated despite the high level of carcass contamination with coliforms arising from poor hygienic slaughtering processes.

2.7 Risk analysis

Risk analysis is a systematic approach recognized by the World Health Organization (WHO) and World Animal Health Organisation (OIE) aimed towards assessing the likelihood of occurrence of an adverse effect of a hazard (chemical, physical or biological) and suggesting intervention strategies. Risk analysis comprises three interlinked components: Risk Assessment; Risk Communication and Risk Management; the last two are now considered together. Risk analysis has been used in various fields including food hygiene and safety. (Maarten *et al.*, 2007)

Risk assessment studies carried out on tenderized steaks marketed in the U.S.A. (Schlosser *et. al.*, 2002) found that 0.000037% (i.e., 3.7 of every 1 million servings) contained one or more *E. Coli*O157:H7 bacteria. Grace *et al.*, (2007) did a quantitative model for *E. coli* O157:H7 in milk in East Africa and found that on any given day around 3 in 10,000 consumers would suffer clinical disease from drinking un-pasteurised milk bought from informal markets, as a result of the milk being contaminated with *E. coli* O157:H7

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Safe Food Fair Food (SFFF)

This study was supported by the Safe Food Fair Food (SFFF) project, which is led by the International Livestock Research Institute (ILRI) and funded by the German Federal Ministry for Economic Cooperation and Development (BMZ). The project collaborated with other partners namely, Promotion of Private Sector Development in Agriculture (PSDA) funded by BMZ and the University of Hohenheim.

Eight countries are involved in this project in East, West and South Africa. These are: Kenya, Tanzania, Ethiopia, Ghana, Mali, Côte d'Ivoire, Mozambique and Republic of South Africa. The Project was started to establish capacity for the sustainable promotion of risk-based approaches to improve food safety and participation of the poor in informal markets of livestock products in the region.

3.2 Study area

The study was carried out in selected export and commercial abattoirs in Nairobi and Kiambu regions. The abattoirs supply meat for export and local consumption in Nairobi and its environs.

3.3 Study design

The prevalence of *E. coli* O157:H7 on cattle carcases was assessed by a cross-sectional study conducted between 1st of August 2009 and 4th of October 2009. Knowledge, Attitude and Practice (KAP) of slaughterhouse workers was assessed by administering a KAP questionnaire between 1st of December 2010 and 14th December 2010. Training of the abattoir workers on gaps observed during the interview and changes in KAP were assessed by an observational study carried out one and a half months later.

3.4 Clearance to undertake the research

Authority to carry out the research in slaughterhouses in Nairobi and its environs was sought and granted by the Director of Veterinary Services, Ministry of Livestock Development.

3.5 Sampling and sample collection

Selection of abattoirs

Three abattoirs representing an export–quality abattoir, a best practice domestic market abattoir (municipal), here referred to as 'improved local', and a typical domestic market abattoir here referred to as 'typical local' were purposefully selected

for the study, depending on the nature of inputs, source of animals and output destination. There are three beef export slaughterhouses that supply meat to Nairobi city and its environs, two are privately owned while one is owned by the government. Local slaughterhouses are seven in Nairobi and its environs. All the local slaughterhouses are privately owned. The three slaughterhouses selected in this study slaughtered animals every day, facilitating obtaining of enough samples for the study. The criteria used for categorization of the local slaughterhouse has not been well defined in the law but the local slaughterhouses have been further classified as class A B and C (Meat control act- legal notice No.110 2010)

Animal sampling

Sample size was determined using the formula by Martin *et al.*, (1987)

$$n = \frac{(Z^2)(1-p)p}{M^2}$$

Where p = anticipated prevalence of *E. coli* O157:H7 in cattle faeces, which was estimated at 17% following Smith et al., (2003).

M = the required precision of 0.05

N = the expected sample size.

Z = z statistic for level of confidence

$$NZ^2P(1-P) \ / \ m^2 (N-1) + Z^2P(1-P)$$

N was adjusted according to Lavrakas, (2008)

Where N = total population

P = expected prevalence

M = Precision value

Z = z statistic for level of confidence

The export abattoir studied slaughtered 65 animals per day for five days in a week. Sampling was carried out over a period of two weeks in August 2009. Six hundred and fifty (650) cattle were slaughtered during the two weeks. Given this total population, the calculated sample size was 74 but 100 animals were sampled.

In the improved local abattoir, on average 20 cattle were slaughtered per day. Samples were collected for four weeks in August-September 2009. Total population N was 400 (20 cattle x 5 days x 4 weeks) which gave a required calculated sample size of 54

The typical local abattoir slaughtered 400 cattle (N) during the sampling month of September 2009. The calculated sample size was 54, but 100 samples were taken.

Faecal sampling

A rectal faecal sample was taken from each animal after stunning by inserting a hand covered with sterile latex glove into the rectum. About 100 grams of faeces was collected from each animal. The faecal material was put in sterile containers, labelled, placed on ice in a cool box and taken to the laboratory within one hour for culture and isolation of *E. coli* O157.

Carcass sampling

Two different sites of the carcass were sampled using the non-destructive method, wet and dry swabs, recommended in the European Commission decision (2001). A hundred animals from each slaughterhouse were sampled; the carcasses were followed during the whole slaughtering process from stunning to inspection stage. Samples were taken at four stages [stunning (faecal sample), flaying, evisceration and cleaning]. Seven samples were therefore taken from each animal (as shown in Figure 1) giving 2,100 samples. The carcass swabs were taken from the flank and the brisket sites. Kang'ethe, (1993), found these sites to be consistently contaminated with coliforms.

The sampling area (10 by 10 cm) was delineated with a sterile aluminium template, easily sterilized between samplings. For each sampling area, a swab moistened in bacteriological peptone (0.85% w/v sodium chloride, 0.1% w/v peptone), was rubbed

firmly across the carcass surface using 10 strokes in each of the horizontal, vertical and diagonal directions (European Commission, 2001). The procedure was repeated using a dry swab (Kang'ethe, 1993; Bunic *et al.*, 2004). The two swabs were put into one sterile universal bottle containing 20 *mls* of sterile bacteriological peptone. Samples were transported to the laboratory in a cool box within one hour of sampling.

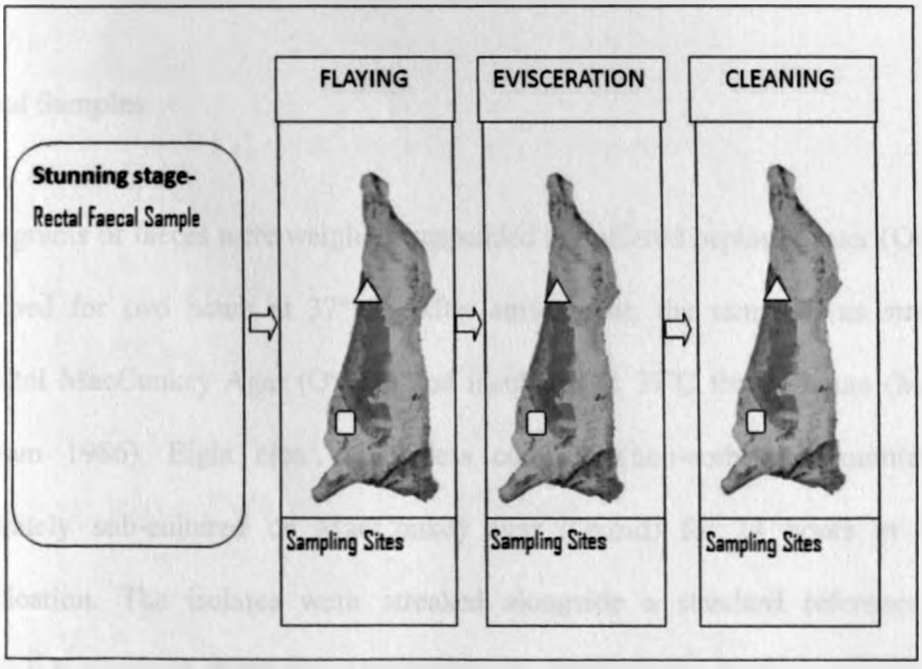

Figure 1: Sampling stages and sites in slaughter process.

Key: ⇨ *Carcass flow*

□ *Brisket*

△*Flank*

3.6 Preparation of media, diluents and test reagents

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

3.7 Culture and Isolation

Faecal Samples

Two grams of faeces were weighed, suspended in buffered peptone water (Oxoid) and enriched for two hours at 37° C. After enrichment, the sample was streaked on Sorbitol MacConkey Agar (Oxoid) and incubated at 37°C for 24 hours (March and Ratnam 1986). Eight clear, colourless colonies (non-sorbitol fermenters) were separately sub-cultured on MacConkey agar (Oxoid) for 24 hours at 37°C for purification. The isolates were streaked alongside a standard reference *E. coli* O157:H7 obtained from the University of Amsterdam, Department of Medical Microbiology. The purified, intensely red colonies with a pale periphery were tested for Indole, Methyl red reaction, Acetyl methyl–carbimol (Voges Proskaurer) and ability to use citrate as the sole carbon source. These tests are collectively abbreviated as IMViC.

Briefly, the test was carried out as follows. One colony was inoculated into four mls of Tryptone water (Oxoid) (Appendix 1), MRVP medium (Oxoid) (Appendix 1) and

streaked on Simons citrate agar slants (Oxoid) (Appendix 1) using a straight inoculation wire. Incubation was done for 48 hours at 37°C. After this seven drops of Indole reagent (Appendix 1) were added to the Tryptone water culture to test for Indole production (red positive) (Appendix 1). Methyl red pH indicator was added into one-half of the MRVP culture broth to test for acid production. Acetyl methyl carbimol was tested for by adding 0.1 ml of 5% alcoholic alpha-naphthol, 0.1 ml of 40% potassium hydroxide and a few creatinine crystals into the other half of the MRVP culture broth. The contents were well shaken and tubes sloped before taking the readings (Pink colour considered positive while yellow colour was considered negative). Growth on citrate slants was indicated by visible colonies and change of colour of the agar from green to blue (Oxoid). Isolates showing IMViC ++-- reaction were identified as *E. coli* and sub-cultured further onto Sorbitol Mac-Conkey agar to confirm that they were still non-sorbitol fermenters.

Carcass swabs

The bacterial swabs were sub-cultured in buffered peptone water overnight and subjected to similar tests for bacteriological analysis as faecal samples.

Serological test for *E. coli* O157

Sorbitol MacConkey negative and IMViC positive colonies were then serotyped using O157 group antisera in a card agglutination test (Oxoid, Basingstoke, and Hampshire,

England). *E. coli* O157 latex test employed latex particles sensitized with specific rabbit antibody reactive with the O157 somatic antigen. One drop of the test latex was dispensed onto one edge of the circle of the reaction card. Saline was placed on the circle away from the test latex.

Using a wire loop, a portion of the colony to be tested was picked and carefully emulsified in the saline drop until the suspension was smooth. This was then mixed with the latex beads to cover the reaction area using an applicator stick. The test card was rocked in a circular motion for one minute while observing for co-agglutination. To test if there was auto- agglutination a further portion of the colony was tested with the control latex reagent to ensure that the isolate was not an auto-agglutinating strain. Agglutination within one minute was an indication that the isolate belonged to the O157 serogroup, which was a potential verotoxin producer.

Positive and negative controls were used to check for the correct working of the latex reagents before the tests were carried out each day. The positive control used in this study was a suspension of inactivated *E. coli* O157 cells in a buffer and it caused visible agglutination with latex reagent in a minute. The negative control was a suspension of *E. coli* O116 cells in a buffer and this caused no agglutination with latex reagent.

Testing for Verotoxin production

The *E. coli* O157 isolates were tested for their potential to produce verotoxins (VT1 and VT2). The isolates -were inoculated onto Brain Heart Infusion agar (Oxoid CM375) slopes (10ml volumes) and incubated at 37 °C for 24 hours. A loopful of the growth was suspended in 1ml sterile physiological saline (0.85% NaCl) containing polymixin B (5,000 international units per ml) to facilitate the release of the toxin.

Extraction was continued for 30 minutes at 37°C shaking occasionally. After extraction, the culture was centrifuged at 4000 rpm for 20 minutes. The supernatant was retained for verotoxin assay using Oxoid test kit (Oxoid Unipart Limited, Basingstoke, Hampshire, England). The latex reagents were shaken thoroughly before use to ensure a homogeneous suspension. To reconstitute the control toxins, 0.5ml of test diluent was added to each vial. The contents were shaken gently until they were dissolved. The principle for testing for toxin was that the polymer latex particles sensitized with purified rabbit antiserum, reacts either with *E. coli* VT1 or VT2. Agglutination results in the formation of a lattice structure that on settling forms a diffuse layer at the base of the V- bottom micro titre well. If verocytotoxin is absent or at a concentration lower than the assay detection level, no such lattice structure forms. Instead, a tight button is observed.

The V-shaped micro-titre plate was arranged so that there were three columns each constituting eight wells for every sample tested. To start with, sample diluents (25*μl)*

were dispensed into each well followed by 25μl of test sample supernatant in the first well of each column. Starting with the first well of each column a micro titre pipette was used to mix the contents, pick 25 μl and perform doubling dilutions down each column up to and including the seventh column. Twenty-five μl of the mixture from the seventh well were discarded. The last well containing diluents only, acted as the control.

Test latex VT1 (25μl) was added to each well in the first column, test latex VT2 (25μl) in the second column and the latex control (25ul) in the third column for the purpose of detecting false agglutination reactions. The contents of each well were mixed by rotating the plate using a micro mixer taking care to avoid spillage. To avoid evaporation the plate was covered with a lid and left undisturbed on a vibration free surface at room temperature for 20 hours, after which each column was examined for agglutination against a black background using a magnifier. The agglutination tests and controls were judged in comparison with the illustrations given by the manufacturer.

3.8 Knowledge, Attitude and Practice (KAP) Assessment of Workers.

A questionnaire Appendix 3 was administered to the abattoir workers in the three slaughterhouses to assess their knowledge, attitudes and practices concerning slaughter hygiene. Fifty-two respondents were interviewed. They were distributed as follows: export slaughterhouse (11), improved local slaughterhouse (22) and typical

local slaughterhouse (19). All the workers involved in the slaughter process were targeted but only those who were willing to participate in the interview were interviewed. The number of those interviewed differed between slaughterhouses due to terms of employment (casual or permanent) and the throughput.

After the data analysis, key areas were identified for capacity building. This targeted training of the abattoir managers and workers in the three slaughterhouses. The main topics covered were food borne illnesses, importance of medical tests in food safety, sources of carcass contamination and ways to prevent contamination, personal hygiene and the roles of the workers and managers in keeping the hygiene in the abattoirs. An observation study was done one month after the training to check whether the workers were practicing what they were taught. A model HACCP was drawn for the typical local abattoir.

3.9 Data entry, cleaning and analysis

Data entry and cleaning

After completion of the field collection of data, both the laboratory and questionnaire data were entered into the computer using Microsoft Access® software database. Data coding and cleaning was carried out.

Data analysis

Both KAP and laboratory data were exported to Instat® statistical package for descriptive statistics. Digitized data were exported to Microsoft Excel® and a risk model was constructed in @ Risk (Palisade) using the laboratory data. Monte Carlo Simulation was run for 10,000 iterations using @ Risk. The KAP interview data were exported to R statistical package and a chi square was done for the significant findings.

3.10 Modelling for Risk Analysis in Monte Carlo

A carcass was sampled by tracking the same carcass (A) from faeces taken after stunning, (B) at flaying, (C) evisceration and (D) cleaning stages. Here, let the probabilities of carcasses contaminated with *E. coli* O157 at each stage be P (A), P (B), P(C) and P (D). Since the same carcass was traced and sampled, the probabilities at each stage are independent of the previous stage excluding P (A). Therefore, the risk of a carcass being contaminated with *E. coli* O157 after cleaning was modelled in sequence as below.

P (D) = P(D|C+)*P(C) + P(D|C-)*(1-P(C).When P(D|C+) is the probability of a carcass contaminated with *E. coli* O157 after cleaning given that a carcass was contaminated after evisceration. P (D|C-) is the probability of a carcass contaminated

with *E. coli* O157 after cleaning given that a carcass was not contaminated after evisceration. Likewise, P(C) was modelled as below using P (B).

P(C) = P (C|B+)*P (B) + P (C|B-)*(1-P (B)). When P (C|B+) is the probability of a carcass contaminated with *E. coli* O157 after, evisceration given that a carcass was contaminated after flaying, and P (C|B-) is the probability of a carcass contaminated given that a carcass was not contaminated after flaying. At the end of this tracing, P (B) was modelled as below using P (A).

P (B) = P (B|A+)*P (A) + P (B|A-)*(1-P (A)). Beta distribution was used to model all these probabilities with non-informative prior (1, 1).

Finally, the probability that *E. coli* O157 produces verotoxin (P (VT) was multiplied with P (D) to calculate the probability of a carcass contaminated with VTEC after cleaning. P (VT) was modelled with Beta distribution using the results of VT gene PCR using pooled E. *coli* O157 isolated from three abattoirs.

Monte Carlo Simulation was run for 10,000 iterations using @Risk (Palisade). Latin Hypercube was used for the sampling.

CHAPTER FOUR

4.0 RESULTS

4.1 Laboratory results

E. coli O157 isolation

A total of 2,100 samples were collected from 300 carcasses. Two hundred and eighty samples out of the 2100 (13.3%) were positive for *E. coli* (IMViC++--). These isolates were therefore tentative *E. coli* O157 since they were non - sorbitol fermenters. After serotyping, 92 out of the 280 presumptive isolates were positive for *E. coli* O157. This gave a prevalence of 4.3% (92/2100).Table 1 shows the isolation of *E. coli* O157 from the different slaughterhouses, various process stages and sampling sites.

Table 1: Isolation of *E. coli* O157 from export, improved local and typical local slaughterhouses at various slaughter stages and sites

Process / Stage		Stunning	Flaying		Evisceration		cleaning		Total
Slaughter House Type	Sampling Sites	Rectum N=100 isolates	Brisket N=100 isolates	Flank N=100 isolates	Brisket N=100 isolates	Flank N=100 isolates	Brisket N=100 isolates	Flank N=100 isolates	
Export		13	2	2	2	3	0	1	23
Improved local		9	4	4	1	0	2	2	22
Typical Local		12	2	0	1	3	0	2	20
Cumulative Total		34	8	6	4	6	2	5	65

Out of the 92 positive isolates, 42 were tested for VT1 and VT2. Of these 10 were positive, eight for VT1 only and two for both VTI and VT2.

4.2 Monte Carlo simulation models.

Tables 2-4 show the results from the probabilities that were used for the Monte Carlo simulation to model for the risks of carcass contamination. The results from the various stages are independent on the results from the previous stage. Presence of *E. coli* O157 in the faeces of the animals did not necessarily mean that it was present in all the stages of the slaughter process.

Table 2: The probability of positive (+) carcasses and negative (-) carcasses at each stage depending on the results of the previous stage in the export abattoir.

Stages	Carcasses contaminated with *E. coli* O157		Carcasses not contaminated with *E. coli* O157	
A. Stunning	13		87	
B. Flaying	A+, B+	A-, B+	A+, B-	A-, B-
	0	4	13	83
C. Evisceration	B+, C+	B-, C+	B+, C-	B-, C-
	2	2	2	94
D. Cleaning	C+, D+	C-, D+	C+, D-	C-, D-
	0	1	4	95

Table 3: The probability of positive carcasses and negative carcasses at each stage depending on the results of the previous stage in the improved local slaughterhouse

Stages	Carcasses contaminated with *E. coli* O157		Carcasses not contaminated with *E. coli* O157	
A. Stunning	9		91	
B. Flaying	A+, B+	A-, B+	A+, B-	A-, B-
	1	5	8	86
C. Evisceration	B+, C+	B-, C+	B+, C-	B-, C-
	0	1	6	93
D. Cleaning	C+, D+	C-, D+	C+, D-	C-, D-
	0	3	1	96

The isolation of *E. coli* O157 from one stage in the earlier stages of the slaughter process is not a guarantee that the organism will be isolated in later stages in the process. After stunning, the contamination of the carcasses varied between the various stages in the slaughter process. If contamination was found in the first stages, it did not necessarily mean that it was found in later stages, In some stages where there was no contamination at the first stages, the contamination was found later in the slaughter

process. It should be noted that the positive status at a stage does not influence the status at the next stage.

Table 4: The probability of positive carcassses and negative carcasses at each stage depending on the results of the previous stage in a typical local slaughter-house

Stages	Carcasses contaminated with *E. coli* O157		Carcasses not contaminated with *E. coli* O157	
A. Stunning	12		88	
B. Flaying	A+, B+	A-, B+	A+, B-	A-, B-
	0	2	12	86
C. Evisceration	B+, C+	B-, C+	B+, C-	B-, C-
	0	4	2	94
D. Cleaning	C+, D+	C-, D+	C+, D-	C-, D-
	0	2	4	94

4.3 Risk of a carcass being contaminated by E. coli O157

The risk of a carcass leaving the slaughterhouse being contaminated with *E. coli* O157 was 29, 48, and per 1000 carcasses slaughtered in the export, improved local and the typical local slaughter houses respectively, at 0.1 confidence interval. The risk that a carcass was contaminated with VTEC was seven, 12 and 10 per 1000 carcasses slaughtered in the export, improved local and typical local abattoirs respectively. The results are summarized in Table 5.

Table 5: Risk of a carcass contaminated with verotoxigenic *E. coli* O157 with respect to the slaughterhouses

Abattoir Type	Probability of a carcass being contaminated with *E. coli* O157 (90% CI)	Probability of a carcass being contaminated with VTEC (90% CI)
Export	0.0293 (0.0082 – 0.0612)	0.0074 (0.0018 – 0.0166)
Improved local	0.0481 (0.0197 – 0.0863)	0.0120 (0.0043 – 0.0237)
Typical local	0.0384 (0.0134 – 0.0728)	0.0096 (0.0029 – 0.0198)

4.4 Knowledge, Attitude and Practices of slaughterhouse Workers (KAP study)

Slaughter Staff Knowledge on Hygiene

Fifty-two staff members (11 from the export abattoir, 22 from the improved local and 19 from the typical local abattoir) were interviewed to assess their knowledge, attitudes and practices in the hygiene of the slaughter operatives. The workers were sampled from all the stages in the slaughter process i.e. stunning to the cleaning stage as summarized in Table 6.

There were significant differences in the training level of the workers in the typical local abattoir and the improved local abattoir with a p value of 0.001 but there was no significant difference between the export and the typical local slaughterhouse and between the export and the improved local slaughterhouses. The export and the typical local abattoirs had better training compared to the improved local. This was also noted in their hand washing during the slaughter process with a p value of 0.025 between the improved local and the typical local slaughterhouses. Number of workers playing more than one role in the slaughter process was also significant with a p value of 0.027 between the typical local and the improved local slaughterhouses. Most of the workers in the three slaughterhouses (37%) were flayers, while stunners were the least (2%). Other distributions of workers in the slaughter process for the three slaughterhouses are summarized in Table 6.

Table 6: Distribution of staff in the slaughter process sections in the three slaughter-houses

Stages in the slaughter process	% number of workers
Stunning	2.0
Bleeding	15.7
Flaying	37.3
Eviscerating	13.7
Splitting	7.7
Washing the carcasses	11.8
Others	11.8
Total	100

Slaughter Staff Knowledge Levels on Food Safety, Hygiene and Related Activities

All the workers had been trained on specific job but not all of them were trained on hygiene of the operatives. Most of the supervision was not hygiene based and not all the workers had undergone medical tests (about 30% were not tested) which is a requirement for someone to work in the slaughterhouse. More details on the hygiene knowledge of the workers are summarized in Table 7.

Table 7: Slaughter Staff Knowledge Levels on Food Safety, Hygiene and Related Activities

	Knowledge levels of workers in % in the slaughterhouses.		
Question	Export (n=11)	Improved local (n=22)	Typical local (n=19)
Specialization in work	36	14	53
Playing other roles in the slaughter process	63	32	16
Training on hygiene	82	73	100
Done medical tests	73	81	100
Supervision based on hygiene practices	18	9	5
Rewards for working well	27	13	11
Agree contamination poses a health risk	90	72	73
Number of registered flayers with the leather department	36	0	56

Slaughter Staff Attitude towards Food Safety and Hygiene

Majority of the workers (94%) felt that keeping hygiene is more important than working quickly while 98% felt health is more important than wealth . This shows that their attitude towards hygiene is good although 90% of them felt that if meat is well cooked it would not cause any harm. More results on the workers attitude on food safety and hygiene are summarised in Table 8.

Table 8: Summary of Results for Workers Attitude towards Hygiene in all Three Slaughter Houses (n=100)

Statement	Strongly Agree (%)	Agree (%)	Disagree (%)	Strongly Disagree (%)	Do not Know (%)
1. In this job, it is more important to work quickly than keep the carcases clean	0	6	40	54	0
2. People doing this job are more likely to get sick	6	50	23	21	0
3. In this type of working environment, keeping clean is easy	25	58	12	5	0
4. A small amount of dirt on clothing or utensils will not cause any harm	10	29	33	28	0
5. Health is more important than wealth	73	25	1	1	0
6. Ensuring hygiene is mainly the role of management	27	42	21	10	0
7. If meat is well-cooked then it is always safe to eat	46	44	4	4	2

Hygienic Practices at Slaughterhouses

The practices of the slaughterhouse staff during the slaughter process was assessed by an observation study that was done one and half months later after the training using a checklist. The results of this checklist are summarised in Table 9. More details on the checklist can be obtained from the questionnaire attached in Appendix 3

The majority of the workers had clean and short nails; veterinary meat inspectors were also present in all the slaughterhouses. However, some of the workers placed their equipment on dirty surfaces during their work and they washed them in bucket water instead of flowing water. Other attributes on their hygiene practices are summarized in Table 9.

Table 9: Summary of Observations on Abattoir Workers' Practices in the three Slaughterhouses

Practices and observations.	% number of workers in each slaughterhouse and observations made.		
	Export n=11	Improved local n=22	Typical local n=19
Workers with uncovered wounds	9	9	5
Clean clothes	9	0	26
Hand washing before, after and during cutting of meat	43	0	42
Use of hot water to wash hands	17	0	0
Soap present for hand washing	55	0	0
Clean and short finger nails	67	88	100
Clean and undamaged knives	71	44	90
Washing knives with bucket water	6	25	76
Washing knives with cold water	0	73	100
Equipment rested on dirty surfaces	18	27	0
Disinfectant	No	No	No
Latrine/toilet present	Yes	Yes	Yes
Water present in the latrine / toilet	Yes	Yes	Yes
Soap present in the latrine / toilet	Yes	No	Yes
Tissue present	No	No	No
Paper towel for hand drying	No	No	No
Separation between clean and dirty areas	Yes	No	Yes
Veterinary meat inspector present	Yes	Yes	Yes

4.5 Model HACCP for a Typical Local Slaughter House

A model HACCP (Fig. 2) was created for the typical local slaughterhouse. This entailed going through the whole slaughter process from the receiving of the animals at the lairages to the dispatch of the carcasses after slaughter.

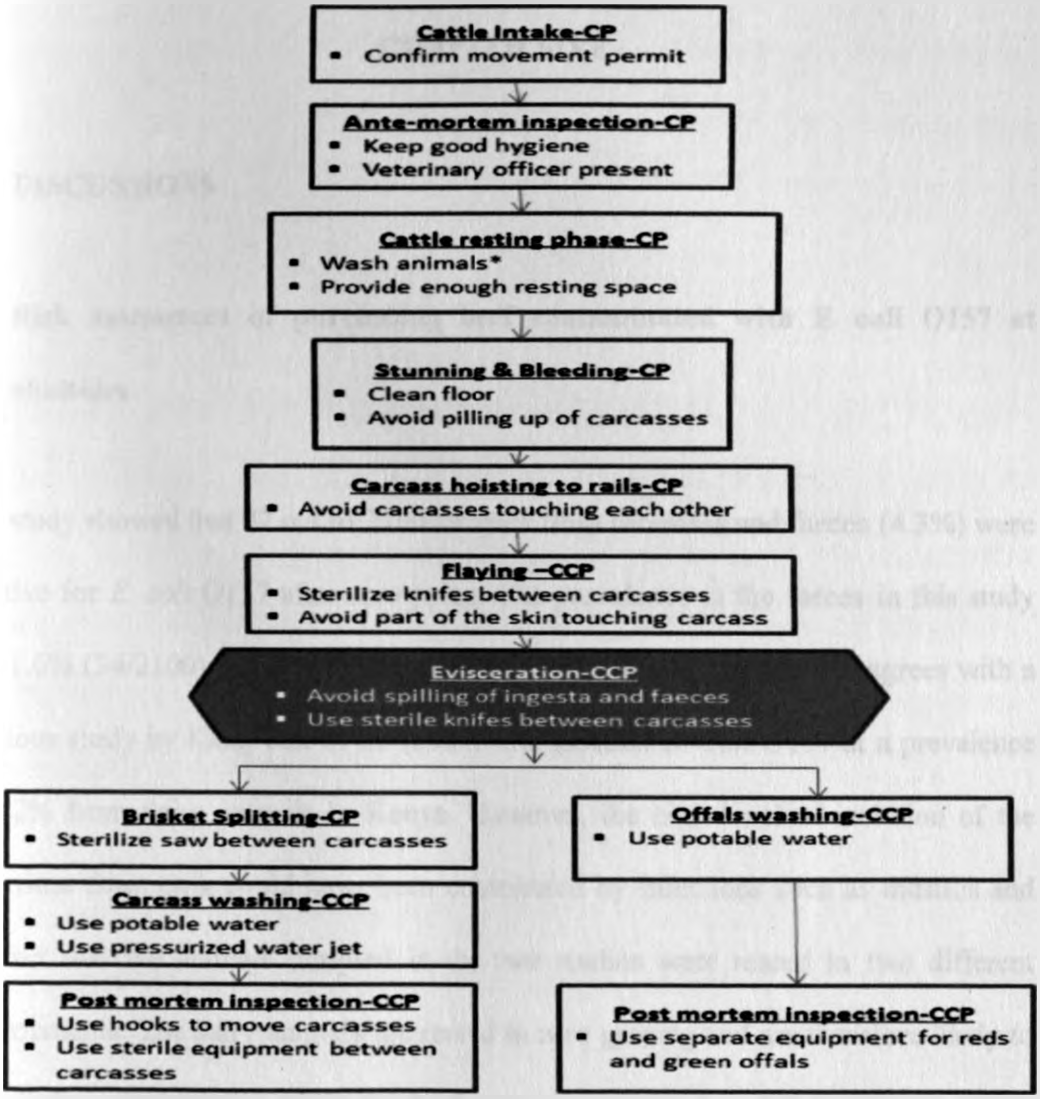

Figure 2: HACCP model for a typical local slaughterhouse.

Key: Critical control point (CCP); Critical Point (CP); Prerequisite (P)

*= Improvement on the lairages was required for ease of washing of animals.

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Risk assessment of purchasing beef contaminated with E coli O157 at abattoirs

The study showed that 92 out of 2100 samples from carcasses and faeces (4.3%) were positive for *E. coli* O157 after serotyping. The prevalence in the faeces in this study was 1.6% (34/2100) while that in the carcass was 1.7% (31/2100). This agrees with a previous study by Kang'ethe et *al.* (2007) who isolated *E. Coli* O157 at a prevalence of 5.2% from dairy animals in Kenya. However, the high level of isolation of the bacterium from milk could have been contributed by infections such as mastitis and the fact that the animals sampled in the two studies were reared in two different environments. The dairy animals are reared in zero grazing and are therefore likely to have high contamination while the beef animals are reared in the rangelands where faecal contamination is minimal.

The carcass prevalence in this study is lower than the findings of McEvoy *et al,* (2003) who found 3.2% prevalence in beef carcasses in a slaughterhouse in the UK. However, McEvoy *et al.* had no isolates positive for VT1 and VT2. In this study, 10/42, (23.8%) were positive for verotoxin production. Majority of these (8/42, 19%) tested positive for VT1 while 2/42 (4.8%) tested positive for both VT1 and VT2. The

samples testing positive for VT1 and VT2 could have been higher if all the isolates that were positive for O157 were screened; only half were tested due to the shortage of the reagents.

In France, Guyon *et al.* (2001) isolated *E. coli* OI57:H7 at the rate of 0.2% in bovine carcasses in a slaughterhouse in Normandy, while Omisakin *et al.* (2003) in UK, reported a prevalence of 7.5% in faecal samples of individual animals presented for slaughter. The faecal prevalence for individual animals in this study was higher with a prevalence of 11.3% (34/300). While the carcass prevalence was 31/300 (10.3%) Hussein (2006) reported a general prevalence of *E. coli* O157:H7 in whole carcasses to range from 0.01 to 43.4%.

Sixty-five carcasses out of 300 (21.6 %) were found to be positive for *E. coli* O157, 23 were from the export slaughterhouse, 22 from the improved local while 20 were from the typical local slaughterhouse. The highest number of the isolates were from the faecal samples, (34/65 53%) while the rest (31/65 48%) were from the carcass samples. This concurs with other studies where the prevalence of *E. coli* O157 in the faeces was found to be higher than the carcasses (Elder *et al.,* 2000, Bornadi *et al.,* 2001). This suggests that there was some level of hygiene observed so that the level of contamination of the carcasses with *E. coli* O157 was lower than the levels isolated in the faeces. In this study coliform counts for the slaughterhouses were not done. However, a previous study by Kang'ethe (1993) showed that all the three types of the

slaughterhouses were highly contaminated with coliforms exceeding 10^5 CFU per square centimetre.

E. coli O157 was isolated at the rate of 21.3% (14/65) from the brisket region and 26.2% (17/65) in the flank region; Ingham and Buege (2003) found the contamination to be higher in the flank, mean standard deviation of 5.0 log CFU than the brisket, which had a standard deviation of 4.5 log CFU. The higher recovery of bacteria in the flank than the brisket was also shown by Seager *et al.*, (2010) who recovered a mean of bacteria of 39.9% in the flank compared to 33.8% in the brisket in five beef abattoirs in Australia. This could presumably be attributed to the fact that carcasses were handled in the flank region with contaminated hands as the carcasses were manually pushed in the slaughter process by slaughterhouse workers who were not observing basic hygienic practices like washing hands with soap, and sterilisation of knives before handling the carcasses. The isolation rate of *E. Coli* O157 at individual animal level from the three slaughterhouses was 11.3% (34/300) at the faecal stage, 4.6% (14/300) at the flaying stage, 3.3% (10/300) at the evisceration stage and 2.3% (7/300) at the cleaning stage. This shows that there was a decline in contamination levels as the slaughter process continued. This could be as a result of washing and cleaning of the carcass or death of the bacteria.

The typical local slaughterhouse had less likelihood than the improved local slaughterhouses of isolating VTEC and *E. coli* O157. This suggests that there was a

higher risk of purchasing a carcass contaminated with *E. coli* O157 from the improved local slaughterhouse than from the typical local slaughterhouse. Despite being in a lower category, the typical local slaughterhouse had the same level of hygiene with the other two higher categories of slaughterhouses. This was shown by the overlapping confidence intervals in Table 5.

This means that food safety is not necessarily achieved by investing in many tools and equipment only but by also including basic hygiene measures.

The author of this thesis visited the typical local slaughterhouse where the owner had been working with the Promotion of Private Sector Development in Agriculture (PSDA). The workers had been trained on slaughter hygiene and the proprietor had been assisted in improving his slaughter facility by putting tiles on the wall and a biogas system to heat the water for use in the slaughterhouse. All the workers had undergone medical tests and they had been trained on slaughter hygiene by this organization. The biogas was however, not functional during the study period. The training of the workers and observing of good hygiene practice must have contributed to the good hygiene levels in this slaughterhouse compared with the improved local slaughterhouse.

From the KAP interview, workers in the export slaughterhouse were rarely motivated; there was therefore a high staff turnover. The slaughterhouse could thus have lost trained and experienced staff thus affecting the levels of hygiene. This may be the

reason why there was no difference in hygiene levels with the other two slaughterhouses as would be expected since they had better facilities. The export slaughterhouse had the same levels of *E. coli* O157contamination as the other two levels of slaughterhouses. The typical local slaughterhouse was small and therefore easy to manage the flow of people in and out of it. The owner also managed the slaughterhouse himself and he was likely to be more dedicated in his work.

There has not been a reported outbreak of *E. coli* O157 infection in Kenya, but this could be due to poor reporting, symptomatic treatment without laboratory support and lack of surveillance. Although the prevalences of *E. Coli* O157 infection are low it must be noted that the presence of this pathogen in food meant for human consumption is of great concern owing to the very low infective dose, less than 100 cells (Paton and Paton1998) and the seriousness of the disease in the infected person.

In this study, the prevalence of *E. coli* O157 is low in beef carcasses leaving the abattoirs; however, this should not be underrated since this bacterium has been a cause of large food borne infections in other parts of the world. The export slaughterhouse chills its carcasses for 24 hours before sale or processing but in the local slaughterhouses, the carcasses are sold to willing buyers immediately after slaughter. Given the high generation time of *E. Coli* (30 minutes at 37°C) (Doyle and Schoeni 1984) and with no measures (Chilling, organic acid solution rinsing, hot water carcass rinsing and steam vacuuming) to counter the multiplication, the

carcasses leaving these slaughterhouses could be highly contaminated by the time they reach the market. Ground beef is the one commonly associated with the infection due to poor cooking by meat consumers.

Most communities in the country cook meat before consumption but there are no set standards on the time and temperature for cooking. Eating of roasted meat (*Nyama choma*) is a common practice in Kenya and there are chances that this meat may not be well cooked. This increases the risk of people eating meat contaminated with *E. Coli*O157.

5.2 Behaviour and Perceptions of Slaughterhouse Workers

There were significant differences in the training levels of the workers in the typical local abattoir and the improved local abattoir (p of 0.001); the typical local slaughterhouse having more trained workers than the improved local, There was however, no significant difference between the export and the typical local slaughterhouse and between the export and the improved local slaughterhouses. This was also noted in their hand washing practices during the slaughter process (p 0.025) between the improved local and the typical local slaughterhouses. In the improved local slaughterhouse, majority of the workers (more than 90%) did not wash their hands before, during and after their work. This was because there was no hot water and soap in the two local slaughterhouses and even the few hand-washing sinks that

were there were far from the working stations and therefore the workers could not easily walk to wash their hands.

The improved local slaughterhouse was strained on the disposal of wastewater, a situation that led to the closure of the operations of the slaughterhouse by the National Environmental Management Authority in the year 2008 (Communication from the management). There was instruction to use minimum amount of water. This was evident from a poster on the wall that read, "Use limited water on the floor". The workers were therefore using water from the same bucket to wash their hands, knives and aprons. This water was used repeatedly and could have contributed to the higher levels of carcass contamination. (They also opted to squeeze the dirty from the floor instead of flushing it with water, this means the cleaning on the job was not done well and thus the working environment was dirty this may have contributed to the carcass contamination.

Number of workers playing more than one role in the slaughter process was also significant (p 0.027) between the typical local and the improved local slaughterhouses. This meant that a worker could be working in both the clean and dirty area (likely to be more contaminated) and this could have led to carcass contamination. In the export slaughterhouse, 63% of the workers were playing more than one role. These factors may have contributed in the differences of carcass contamination in the three slaughterhouses.

A gazette by the government dated July 2010 requires all local slaughterhouse owners to have their staff trained. If this law is enforced, the knowledge levels of the workers will be better and the managers will want to retain the people they have trained. It is hoped that this will in return improve the hygiene levels in these facilities, with better-trained workers.

Slaughterhouse workers also play a role in carcass contamination during the slaughter process. Of more importance is their knowledge attitude and practices towards hygiene. One of the workers said that *"Ng'ombe wanakula nyasi na kwa hivyo hawana magonjwa yoyote"* which means that cows eat grass and therefore they have no diseases an indication of low level of awareness of bacterial and other zoonotic diseases that can be acquired from diseased or contaminated animals. Majority of them (more than 70%) agreed that carcass contamination posed a health risk to the meat consumer and over 67% had their fingernails short and clean. This shows that some of the workers have some knowledge and good attitude toward their work but this can be improved through constant training and motivation.

Lack of motivation and poor working environment makes it difficult for the workers to keep clean during their work and thus affect the hygiene of the slaughter process at large. Majority of the workers (83%) were not receiving any motivation for working well. They were also paid depending on the daily kill while they were not provided with protective clothing and work tools like knives. The working environment was

also poor especially the lack of hot water, which was provided in the export slaughterhouse only and even then only 17% of the workers in the export slaughterhouse could access it. Since it was located on one working station only, very few people would wash their hands frequently with cold water especially in the morning when it is very cold and most of the slaughter takes place in the early morning hours. Hand drying paper towels, hand disinfectants and tissue paper were not provided in all the three slaughterhouses. These factors may have contributed in the differences of carcass contamination in the three slaughterhouses.

Majority of the abattoir workers were aware of the tests that are supposed to be done for one to work in the food industry, though few of them did the tests. Not all the workers did the sputum test for tuberculosis. This means there is a lapse in the system of how the workers get the tests done and in obtaining the health certificates. The workers also do the tests at their own cost and given the uncertainty of their remunerations, they may not give it a priority especially if the management and the law enforcers are not keen. This poses a high risk of spreading communicable diseases like tuberculosis and other food borne diseases.

Majority of the workers had a good attitude towards hygiene. However, in the typical local abattoir and the improved local abattoir some of the staff felt that ensuring hygiene was mainly the role of the management. They also felt that it was hard to keep clean in their working environment. A few of them also believed that working

quickly was more important than observing hygiene; this may be because in these two facilities some of the basic facilities like soap and hot water were absent.

Most of the supervision in the slaughterhouse was not geared towards hygiene. Only 20% of the workers said that the managers and supervisors checked on the hygiene of the slaughter process. Most of the time the managers were interested on how fast the work could be done to save on time and cost. This must have contributed to the carcass contamination.

HACCP model was done for the typical local slaughterhouse only. Those involved were: - The proprietor's son who was manager in the slaughterhouse and four slaughterhouse workers, representing a flayer an eviscerator, a splitter and a cleaner. (All the workers could not be involved in the process since this was done when work was going on). The whole exercise entailed going through the process from the receiving of the animals at the lairages up to the dispatch of the carcasses after slaughter. Key areas where carcass contamination was likely to occur were noted, preventive and corrective measures were also discussed. The export slaughterhouse was training for ISO 2200 evaluation and they felt that their standards were at a higher level, thus no need to establish HACCP, while the management in the improved local slaughterhouse was not co-operative because they felt their standards were very low. However, the preliminary results were shared with the managers of the three slaughterhouses and other stakeholders in the beef industry. This was going to

help the export slaughterhouse as they prepared for the ISO evaluation to know that there were hygiene issues to be addressed.

Despite the training on observed gaps during the interviews with the slaughterhouse workers, an evaluation one month later showed no improvements had been done in all the three facilities. This could presumably be due to lack of motivation, since the project did not offer monetary assistance or because the owners and the managers were not committed. Even if the workers were willing to change and implement the trainings lack of hot water soap and other basic hygiene facilities was still evident.

In the typical local abattoir, there is still a lot to be done though the proprietor is willing to improve. He is putting up a hot water system and he is improving on the floor of the lairages. He is also constructing his sewerage system to join the municipal council one and this will help in better effluent disposal.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Slaughter cattle are carriers of *E. coli* O157 and potential sources of contamination of carcass was present in the faeces of cattle in Nairobi slaughterhouses studied and can contaminate carcasses during the slaughter operations.

2. *E. coli* OI57 was high in prevalence in the faecal samples than the carcass samples.

3. More carcasses were found to be contaminated with *E. coli* OI57 in the export slaughterhouse compared to the two local slaughterhouses.

4. Size of abattoir and level of investment did not affect the hygiene but the sanitary measures exercised in the slaughter process did.

5. There was need for improvement in hygienic practices in the three slaughterhouses.

6. Risk of carcass contamination with *E. coli* O157 was highest in the improved local slaughterhouse followed by the typical local abattoir while the export had the least risk.

7. Lack of motivation and poor working environment made it hard for the workers to keep clean during their work and thus affected the hygiene of the slaughter process at large.

8. Lack of basic facilities like water and soap for hand washing and good manufacturing practices and SSOPs contributed to carcass contamination too.

9. The training given to the workers and managers was not implemented one and a half months later.

6.2 Recommendations

1. More studies should be done to evaluate the transport, handling and storage of meat leaving the abattoirs and chances of increasing or reducing the contamination.

2. The slaughterhouse workers should be trained on not only slaughter skills but also on hygiene of the operatives and its importance.

3. With some of the slaughterhouses having adopted SSOPs and HACCP system, another study should be done to check whether the hygiene levels vary.

4. The slaughterhouse workers should be motivated and rewarded for working well.

5. Workers should be employed on permanent basis and their welfare taken care of.

6. Slaughterhouses should invest in more tools and equipment like knives pouches, hot water, and soap and sterilization facilities to help in hygiene.

7. The slaughterhouses should do routine sampling of the workers hands, tools and implements and carcasses to assess their hygiene levels.

8. Control measures to reduce the public health risk arising from *E. coli O157:H7* in cattle needs to be addressed at abattoir level by reducing carcass contamination at various stages of the slaughter process.

CHAPTER SEVEN

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APPENDICES

Appendix 1: Preparation of media.

MacConkey agar (Oxoid CM7)

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

PH 7.4 (Approximately)

Fifty two grams of the powder were suspended in a litre of distilled water brought to boil to dissolve completely and dissolved completely and sterilised at (121C for 15 minutes).The molten agar was cooled to 50Oc and approximately 20ml poured into a Petri dish (90mm in diameter) and allowed to cool and solidify at room temperature.

MRVP Medium (Oxoid CM43)

Formula	grams per litre
Peptone P.	5.0
Dextrose	5.0
Phosphate buffer	5.0

PH 7.5 (approximately)

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

Simon citrate Agar (Oxoid CM155)

Formula	grams per litre
Magnesium sulphate	0.2
Ammonium dihydrogenphosphste	0.2
Sodium ammonium phosphate	0.8
Sodium citrate tribasic	2.0
Sodium chloride	5.0

Bromothymol blue	0.08
Agar	15.0

PH 7.0 (Approximately)

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

Tryptone soya broth (Oxoid CM129)

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

PH 7.3 (approximately)

Semi solid nutrient agar 1%

Tryptone soya powder (30g) and agar No.3 (Oxoid L 13) 10 grams were suspended in one litre distilled water and boiled to dissolve completely. The resulting solutions were dispensed in amounts of 1.8ml in to cry vials and sterilised (121°C for 15 minutes) the 1% agar was used for storing organisms at 4°C

Glycerine 10% nutrient broth (Mayer and baker)

Thirty grams of try tone soya broth powder and 100ml of glycerine were added to 900ml of distilled water and brought to boil to dissolve completely .The medium was dispensed in to cry vials in 1.8ml amounts These were used for storing culture at-20°C.

Brain Heart Infusion Agar Oxoid (m375)

Formula	Grams per litre.
Calf brain infusion solids	12.5
Beef heart infusion solids	5.0
Protease peptone	10.0
Dextrose	2.0
Disodium phosphate	2.5

P^H 7.4 (Approx.)

To one litre of distilled water, 37 grams of the powder were added, thoroughly mixed and distributed in universal bottles in10ml amounts. Sterilisation was by autoclaving at 121C for 15 minutes. The medium was then left to cool and dry while slanting.

Appendix 2: Preparation of reagents

IMVIC Reagents: Indole reagent (Ehrlichs reagent)

1-gram para-dimethylaminobenzaldehyde

95 ml absolute ethanol

20ml concentrated hydrochloric acid

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

Methyl red reagent.

0.04 grams methyl red

40 ml ethanol 100ml distilled water

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

Voges-Proskaurer test.

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

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

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

Appendix 3: Questionnaire for KAP analysis.

Administered to test knowledge, attitude and practice of meat hygiene and food safety in Slaughterhouses. It was administered to people who slaughter Cattle and prepare the carcass for human consumption (slaughterhouse workers)

Section A.

Date.....Questionnaire number .....
1. Name of Enumerator.....
2. Name of Slaughterhouse.....
3. Name of respondent: .....
4. Placement in slaughter process: a) Stunner b) cutting the throat c) Flayer d) Eviscerator) Splitter f) Carcass washer g) other (specify)

A. Knowledge.

A1. Do you play any other role in the slaughter process apart from the one mentioned above.

A) YES [ ] B) No [ ] (Tick appropriately)

A 2.If YES, which one(s)?

A3. If No, Why not?

A4. Did you receive any job related training? A) Yes [ ] B) No [ ] (Tick appropriately)

A5. If yes to A4, Where were you trained?

A 6-1 [*If there was no formal training*] Have you received informal training?
A6-2 Who trained you?.....
A6-3 For how long?.....
A6-4 what did you learn?

A7. Has the training been helpful? Yes [ ] No [ ] (tick appropriately.)

An 8.If YES In what ways?

1= I have become more efficient in my work

2= I have become more aware of hygienic practices e.g. Cleaning hands, wearing. Protective wear, cleaning of equipment

3= I have become more hygienic/ Cleaner

Four= others specify.....

A9-1) Have you undergone any job related medical tests to work in the slaughterhouse? Yes [ ] No [ ] (Tick appropriately)
A9-2) which medical evaluations should one undergo to work in the slaughterhouse?
I) II) III) IV) V)
A9-3) when was your last medical test done? One month [ ] Two months [ ] Three months [ ] Six months [ ] One year [ ]

10. Which of these evaluations have you undergone in the last six months? .....

A11. Are you a registered Stunner/ Bleeder/ Flayer/ Eviscerator/ Splitter/ Cleaner (*Tick as necessary*).....
A11-2 Can you show your certificate?.....
A11-3 How is your performance in the slaughter process monitored?.....
A11-4 How often,?.....
A11-5 By whom?
A11- 6what things do they check for?).....

A11-4 Are there any performance related incentives?.....
(Any rewards for working well?.....
What are you rewarded for?
How are you rewarded? .....
Any punishments?) .....

A12.What would cause carcass contamination? (Open question)
1= Faeces
2= Dirty Water
3= Handling with dirty equipment and hands
4= Other (Specify)?.....

A13. If a carcass was contaminated (by faeces), what would you do? (Open question)
1= Nothing
2= Wash the carcass
3= Call the Meat Inspector for advice
4= other (specify).....

A14. In your opinion, does contamination pose any health risk to meat consumers?
1=Yes [ ] 2= No [ ] (Tick appropriately)

A 15. If No, why? .....

A16. What is the risk?.....

A 17. How does a carcass get contaminated at?

Stage	How contamination occurs.
1=Stunning	
2=Bleeding	
3= Flaying	
4= Evisceration	
5= Splitting	
6= Inspection	
7= Washing	

A18. Propose a way to end carcass contamination?.....

Section B. Attitude.

B3. I will read you some statements about hygiene in the slaughter process. Please indicate whether you agree or disagree. KEY: SA= Strongly Agree, A = Agree, D= Disagree, DS=Strongly Disagree and DK=Don't Know

Question	SA	A	D	SD	DK
1. In this job, it is more important to work quickly than keep the carcases clean.					
2People doing this job are more likely to get sick					
3. In this type of working environment, keeping clean is easy					
4. A small amount of dirt on clothing or utensils will not cause any harm					
5. Health is more important than wealth					
6. Ensuring hygiene is mainly the role of management					
7. If meat is well-cooked then it is always safe to eat					

Section C. Practices. (Butcher observation checklist).

Cuts/wounds covered with an appropriate waterproof dressing.	Yes.....No.....N/A.....
Smoking or eating or chewing while working	Smoking.....Eating.....
Clothes clean and completely free from any dirt or blood.	Yes.....No.....
Hand washing: before after and during cutting meat	Before.....After..... During.....
How washed? Running water or bucket?	Running water.....Bucket.....Hot.....

Hot or cold? Brush or cloth? Soap?	Cold.....Brush.....Cloth.....Soap.....
Fingernails short and completely clean	Short.....Clean.....
All knives are completely clean and free from dirt and cracks and damages	Clean .....undamaged.....
knives are cleaned before after and during use	Before..... after..... during use.....
How cleaned tick as you think it should be used.	Running water.....bucket.....Hot.....cold....brush .....Cloth .....soap.....
Is any disinfectant used? Write name of disinfectant	Yes.....No.....
Latrine available nearby	Yes.....No.....
Latrine has water soap paper towels for hand washing(tick all that apply)	Water.....Soap.....Paper Towels.....Tissue paper.....
Equipment rested in dirty surface during working	Yes.....No.....
Strict separation between clean and dirty areas	Yes.....No.....
Veterinary inspectors present to examine the meat to be sold.	Yes.....No.....

Section D. Perceptions

D1. What constraints do you experience in your work? .....

D2. Do they affect your ability to achieve high levels of hygiene? 1=YES [ ] 2=NO [ ]

D3. If YES, in what way(s)? .....

D4. In your opinion, what role do you think the management should play in:

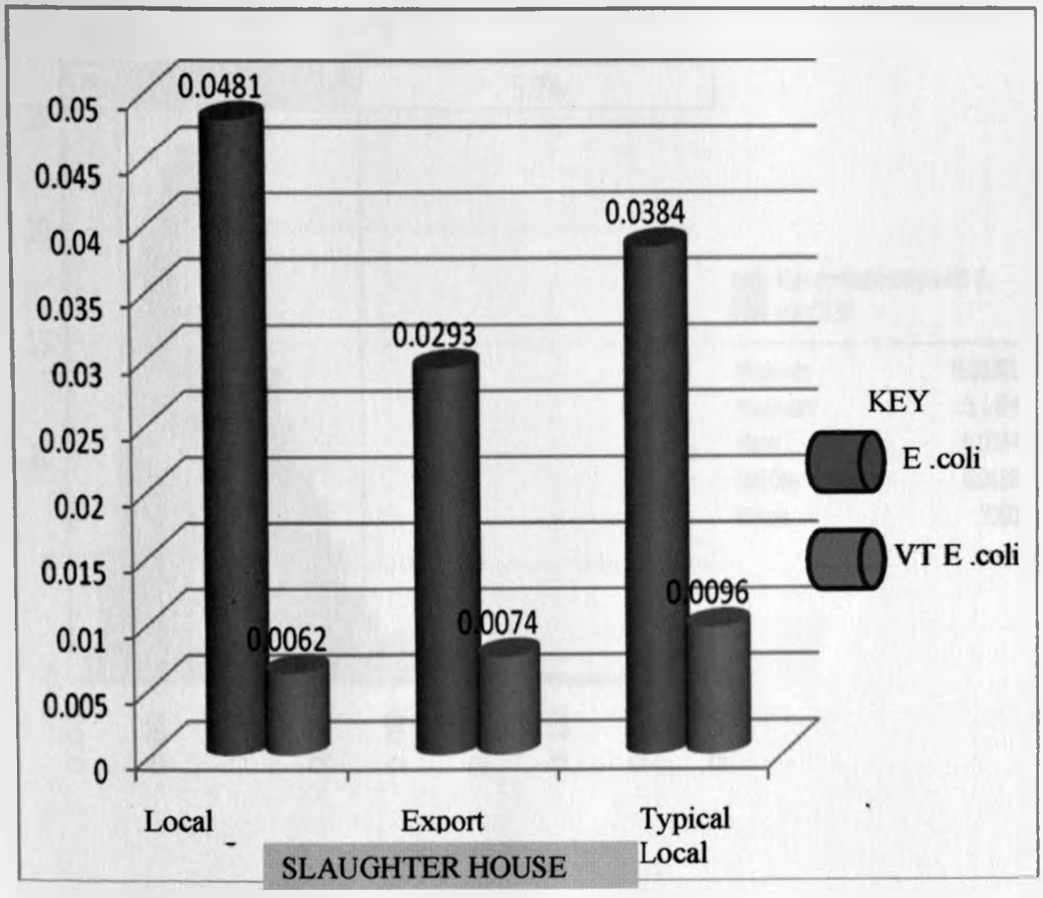
(a) Setting standards for hygiene in the slaughterhouse? .....

(b) Maintaining those standards? .....

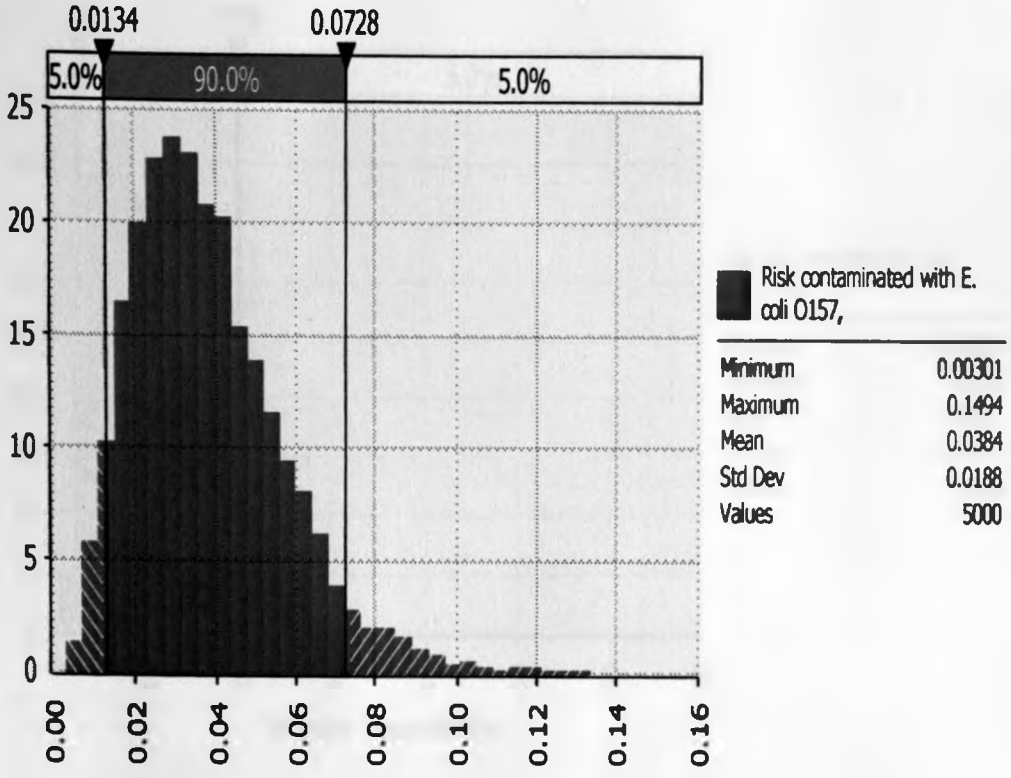
D5. In your opinion, what role do you think the workers should play in?

(a) Maintaining standards for hygiene in the slaughterhouse? .....

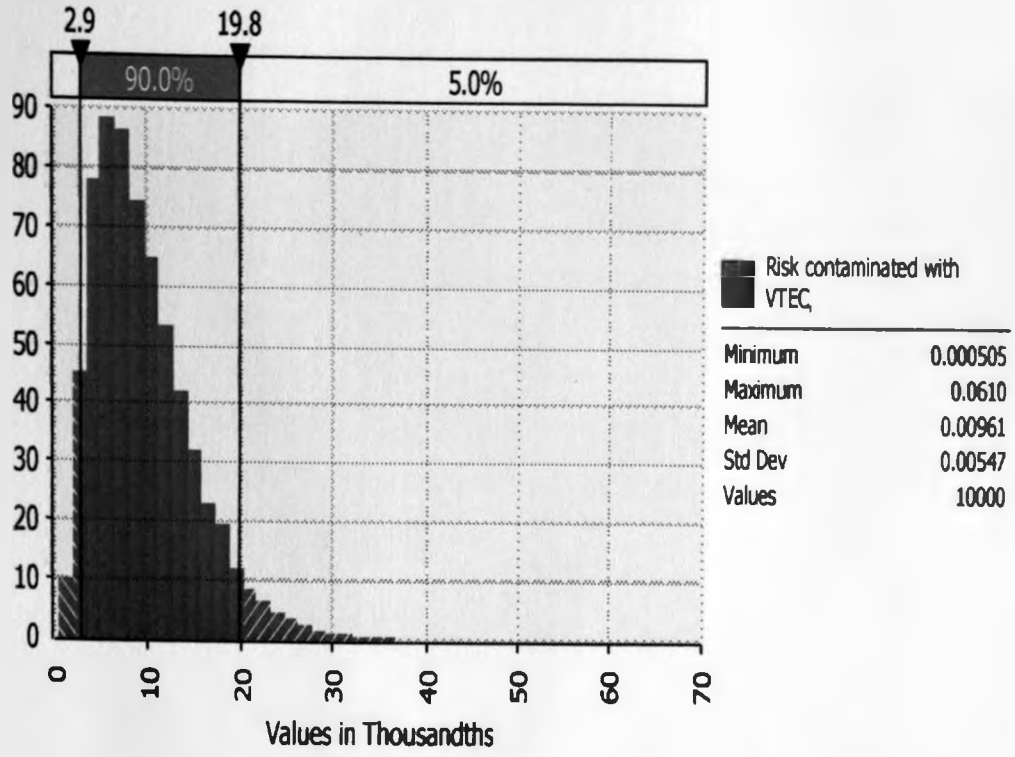
Appendix 4: Comparison of contamination with VT *E. coli* O157 and *E. coli* O157 in improved local, typical local and export slaughterhouses.



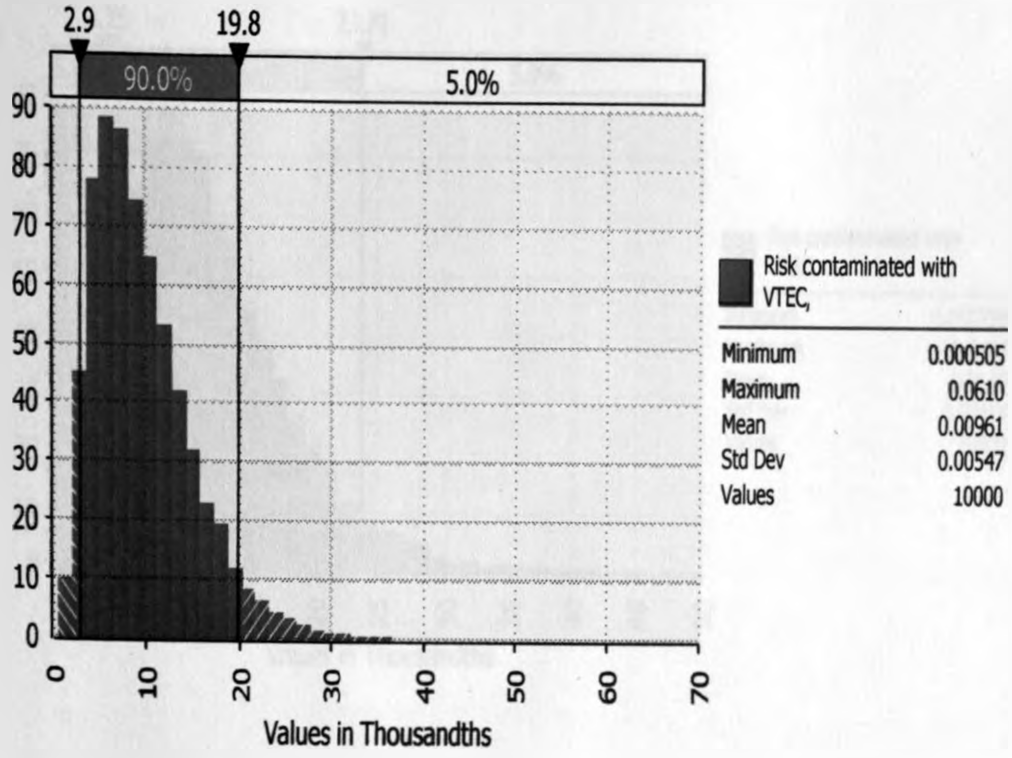
Appendix 5: Monte Carlo simulation of the risk of contamination with *E. coli* O157 in improved local slaughterhouse.



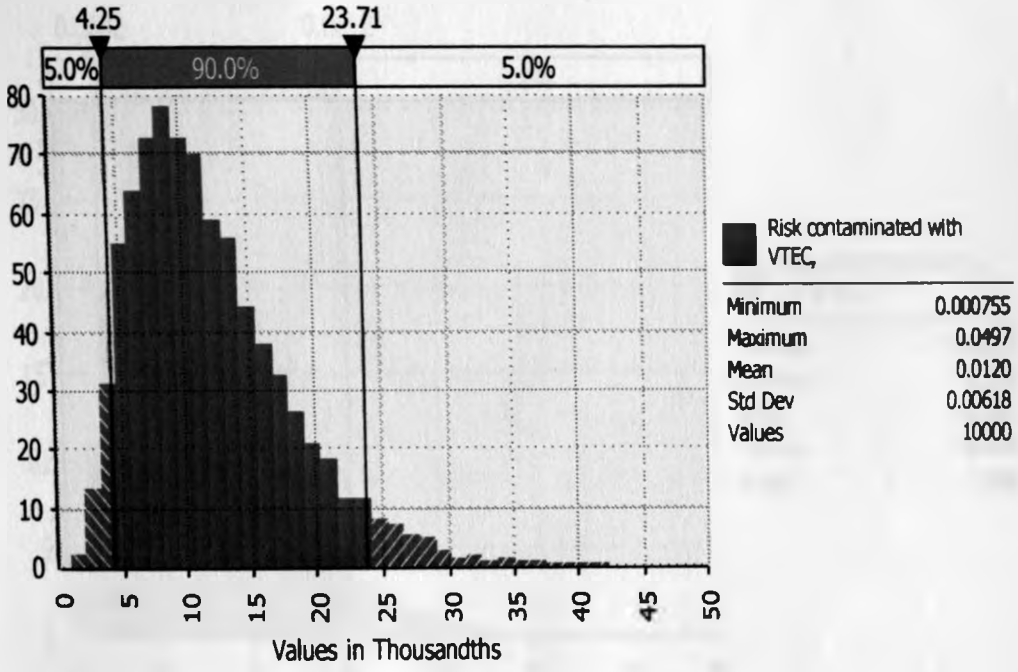
Appendix 6: Monte Carlo simulation of the risk of contamination with VT E. coli O157 in improved local slaughterhouse.



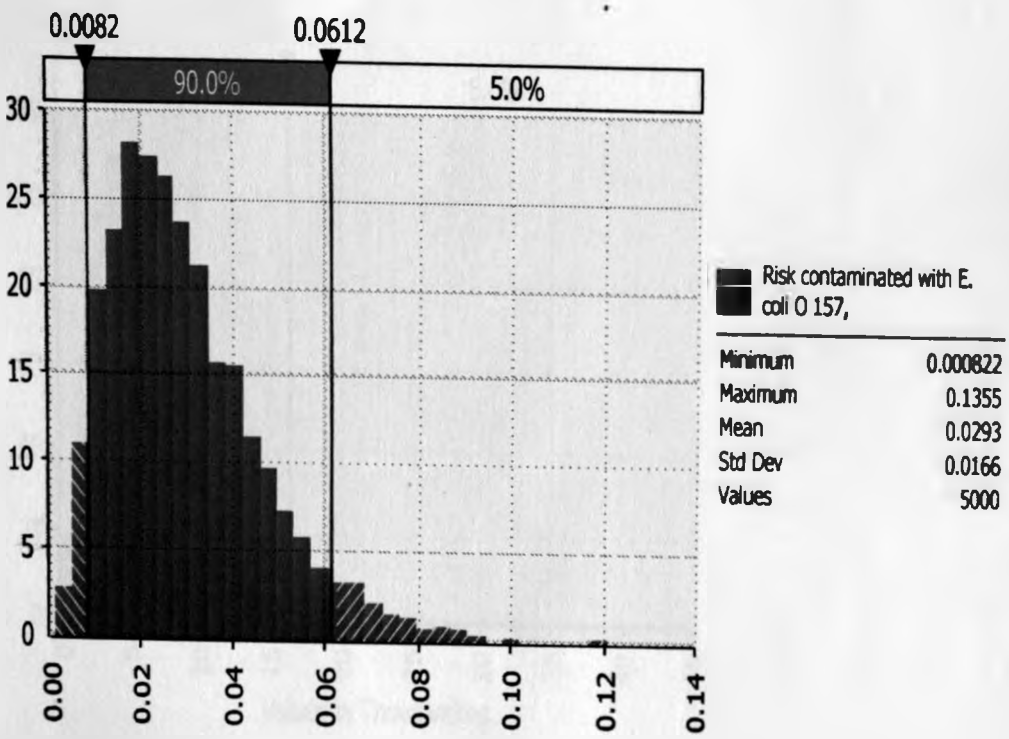
Appendix 7: Monte Carlo simulation of the risk of contamination with *E. coli* O157 in typical local slaughterhouse.



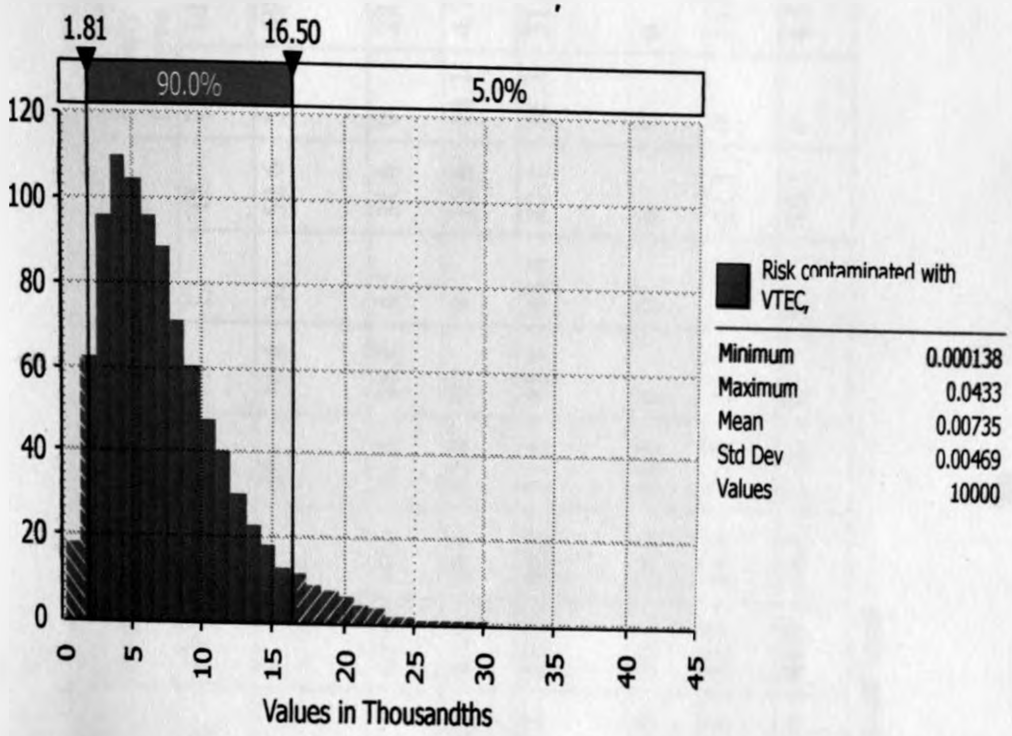
Appendix 8: Monte Carlo simulation of risk of contamination with VT E. coli O157 in improved local slaughterhouse.



Appendix 9: Monte Carlo simulation of the risk of contamination with VT E. coli O157 in improved local slaughterhouse.



Appendix 10: Monte Carlo simulation of risk of contamination with VT E. coli O157 in export slaughterhouse



Appendix 11: Summary of results for worker's attitude towards hygiene in slaughterhouses.

	Response %														
	Strongly Agree			Agree			Disagree			Strongly Disagree			Do not Know		
Questions asked about work	EP	LI	TL	EP	LI	TL	EP	LI	TL	EP	LI	TL	EP	LI	TL
It is more important to work quickly than keep carcases clean.	0	0	0	9	9	05	36.4	31	52.6	54.6	59	47.1	0	0	0
People doing this job are more likely to get sick	0	13	0	63.6	60.8	31.6	36.4	4.5	31.6	0	22.7	36.8	0	0	0
For this working set up, keeping clean is easy	9.1	31.8	26.3	63.6	54.5	57.9	9.1	9	15.8	18.2	4.7	0	0	0	0
A little dirt on cloths/ utensils won't cause any harm	0	9	15.8	18.2	40.9	21.1	45.5	18.3	42.1	36.3	31.8	21	0	0	0
Health is more important than wealth	63.6	71.4	78.9	36.4	28.6	15.8	0	0	0	0	0	5.3	0	0	0
Hygiene is mainly the role of management	0	36.4	31.6	72.7	31.8	36.8	27.3	18.2	21.1	0	13.6	10.5	0	0	0
Well-cooked meat is always safe to eat	18.2	54.5	52.6	81.8	36.5	31.6	0	0	10.5	0	4.5	5.3	0	4.5	0

KEY: EP-Export; LI- improved local; TL-Typical local