DETERMINATION OF BACTERIOLOGICAL QUALITY OF FRESH BEEF POST- HARVESTING IN NYAGACHO SLUM, KERICHO, KENYA

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B. Ed. (Science)

I56/23798/2011

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (MICROBIOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

September, 2013

DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other university or for any other award

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APPROVAL BY SUPERVISORS

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DEDICATION

This work is dedicated to my parents Samwel Cheruiyot and Esther Cheruiyot for their support, encouragement and financial assistance. They have taught me that I can achieve anything if I put my mind to it. I also dedicate it to my siblings Dominic Ronoh and Winny Cheruiyot and my late grandmother for their love and encouragement to pursue further studies. Finally, I thank my Father in heaven who made it all possible.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following individuals and institutions for their contributions to the successful completion of the study;

My supervisors, Dr. Anthony Kebira, and Dr. John Maingi, of the Department of microbiology for their positive attitude, guidance, planning and technical assistance in executing this study. Officer in charge Regional Veterinary Investigation Laboratory Kericho, Dr. David Wamalwa, his assistant Dr. David Keitany, their work mates, Dr. Wilson Kusiru , Dr. Jared Omondi and Dr. Caren Okonga for their advice, training and assistance in my research. The senior chief technician, Regional Veterinary Investigation Laboratory Kericho, Mr Fredrick Odhiambo and entire lab mates for being supportive during my study. The Regional Veterinary Investigation Laboratory Kericho, for permission to utilize their facilities in conducting the entire research. Public Health Office, Kericho for the assistance they offered during my study. Kenyatta University, for the assistance in providing relevant literature sources through the library and information services for the completion of my study.

LIST OF ABBREVIATIONS AND ACRONYMS

CDCP Centre for Disease Control and Prevention

CDC Centre for Disease Control

CBD Central Business District

FAO Food and Agricultural Organization

HACCP Hazard Analysis Critical Control Point

MRSA Multi-drug Resistance *Staphylococcus aureus*

NIAID National Institute of Allergy and Infectious Diseases

RVIL Regional Veterinary Investigation Laboratory

TVC Total Viable Count

TPC Total Plate Count

WHO World Health Organization

CAC Codex Alimentarius Commission

GMP Good Manufacturing Practices

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

While food borne diseases remain an important public health problem worldwide, one of the most significant food safety hazards is associated with those from animals. Food borne infections and illnesses are a major international health problem with consequent economic reduction and deaths. Meat is considered the most important source of proteins consumed by humans, yet most perishable. For highly perishable foodstuffs such as fresh red meat, the threat of food poisoning is particularly high since it contains all the nutrients that support bacterial growth. Inappropriate slaughtering and retail operation can compromise food safety and more so, in densely populated areas like informal settlements. In the present study, the microbial quality of meat from slaughterhouse and butcheries supplying residents of Nyagacho, Kericho County was assessed to determine their safety for human consumption and to highlight the potential contamination points. The bacteriological quality of the meat samples were performed using the Total Plate Count (TPC) method, while standard culture methods were used for bacteria isolation and identification. Equipment, walls, floors, hands and clothing of meat personnel were swabbed and analyzed by means of Rodac plates. The results obtained indicated that the meat quality from the butcheries exceeded acceptable range over the study period. The bacteriological load obtained from the study in the slaughterhouse however, was within the acceptable range <3.5 log (HACCP 2002). During the study, the meat sampled from the slaughterhouse was therefore fit for human consumption (ranged between ± 3.20 - ±3.50 log). However, butchery isolates recorded high counts exceeding the acceptable maximum limits prescribed by Meat HACCP (Scotland) regulations 2002 No. 234. TPC yielded counts as high as ±6.49 - ±7.50 and exceeded the accepted range > 5.0 cfu/g and hence all butchery meat sampled during the study was not fit for consumption. It was established that there was a high significant difference ($p=0.000$ at $p<0.05$) of the means from the two sites (slaughterhouse and butcheries). From 27 samples cultured from the slaughterhouse, 15 tested positive for pathogens. Of the 15, 4 (27%) were positive for *Staphylococcus aureus*, 6 (40%) for *Proteus vulgaris* and 5 (33%) for *Proteus rettgeri*. *Pseudomonas aeruginosa* was not isolated in all the samples of the slaughterhouse. From the 27 samples collected from butcheries, 24 were positive for pathogens. Of the 24, 7(29%) were positive for *Staphylococcus aureus*, 6 (25%) for *Proteus rettgeri*, 7 (29%) for *Proteus vulgaris* and 4 (17%) for *Pseudomonas aeruginosa*. During the study period, meat from the sampled butcheries was not suitable for human consumption since the counts exceeded the acceptable limits and hence a serious threat to the consumers' and calls for urgent intervention.

CHAPTER ONE

INTRODUCTION

1.1 Background

Food security is a complex issue, where animal proteins such as meats, meat products, fish and fishery products are generally regarded as a high risk commodity to infection and toxication (Yousef *et al.,* 2008). These food borne infections and the consequent illnesses are some of the major international challenges that lead to high mortality and economic loss (Adak *et al.,* 2005). In the industrialized world, food borne infection cause considerable illnesses that heavily affect healthcare systems (Adak *et al.,* 2005; Clarence *et al.,* 2005).

Food borne diseases are diseases resulting from ingestion of bacteria, toxins and also cells produced by microorganisms present in food (Clarence *et al.,* 2009). The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin. Meat and meat products are sometimes contaminated with germs after leaving the manufacture plant and during handling (Stagniitta *et al.,* 2006). Hygiene conditions are poor when foods are produced in non-industrial establishments, mainly due to insufficient monitoring during processing. These contaminated food ends up infecting or intoxicating children, elderly and immunosupressed individuals who are highly susceptible (Stagnitta *et al*., 2006). Meat from bovine (including the species *Bubalus bubalis* and *Bison bison*), donkey, duck, farmed deer, fowl, goat, goose, guinea fowl, horse, kangaroo, mule, ostrich and other related *ratite* species, partridge, pheasant, pig, pigeon, quail, rabbit, sheep, turkey from

which meat is derived is regarded as a domesticated slaughter animal (Meat Control Act, 2012). The muscle tissue of healthy living animals is usually free from micro-organisms. However, during the slaughtering process, this meat gets contamination on external surface, such as hair and skin, the gastrointestinal and respiratory tract (Unc and Goss, 2004; Biswas *et al.,* 2011). Based on research, the equipment used in the slaughtering and dressing operations (knives, saws, cleavers and hooks) make significant contributions to the overall contamination through direct contact with hides and hair as well as by contact with steels, knife scabbards and the clothing of operatives (Marriot, 2004; Biswas *et al.,* 2011; Omuruyi *et al.,* 2011).

Research have shown that when carcasses and cuts are subsequently handled through the food distribution channels, they increasingly get more contaminated (Adzitey *et al.,* 2011; Biswas *et al.,* 2011). Though no foodborne outbreak has been reported in Kenya of late, a recent study conducted to evaluate meat associated pathogens showed a high microbial contamination and hence warrants a research (FAO/WHO, 2013). An estimated 9 million cases of food-borne illnesses occur in the United States every year, amounting to a cost of around $8.4 billion (Klontz *et al.,* 1995). In developing countries, diseases caused by contaminated food constitute one of the most widespread health problems as well as a major cause of reduced economic activity (Clayton *et al.,* 2002; Clarence *et al.,* 2009). In the past decades, the epidemiology of foodborne diseases has changed with several new microorganisms re-emerging (Angelillo *et al.,* 2000, FAO/WHO, 2013).

Public concern has risen due to numerous food borne infections such as those surrounding bovine spongiform encephalopathy and foot and mouth disease epidermic

(Pickrell and Enserink, 2001; Tauxe, 2002). For highly perishable foodstuffs such as fresh red meat, the threat of food poisoning is particularly high (Nel *et al.,* 2004; Yousuf *et al.,* 2008). However, despite physical inspection of the carcass, complex microbial contamination are sometimes not captured hence precipitating major public health hazards leading to economic loss (Ahmed *et al.,* 2002; FAO/WHO, 2013). Therefore this system requires an understanding of the many risk factors between the point of production and the point of consumption and the ability to systematically target Sintervention efforts along the production-consumption chain (Batz *et al.,* 2005; FAO/WHO 2013).

1.2 Problem statement and justification

Meat is an important source of protein and a valuable commodity in resource-poor communities (Datt *et al.,* 2003; Garcia, 2007). However, while meat is a rich nutrient source, it can also be a potential vehicle of human foodborne illnesses (FAO/WHO, 2013). Inappropriate slaughtering and retail operation can compromise food safety and more so, in densely populated areas like informal settlements (Garcia, 2007). Slaughtering process is frequently unhygienic and this makes meat to be easily contaminated. Meat products from such condition often deteriorate rapidly and pose a health hazard (Datt *et al.,* 2003; FAO/WHO, 2013).

In Kenya, carcasses supplied to informal settlements are normally transported in crowded, unrefrigerated trucks or portioned meat and offal transported at ambient

temperatures in non-insulated metal bins on taxis, motorcycle and bicycles (FAO/WHO, 2013). External contamination of meat constitutes a major problem in most developing countries. Slaughterhouse and butcheries, where there are potential sources of contamination reportedly have significant effect on the meat shelf life (Omuruyi *et al.,* 2011). Contamination may include pathogens such as *Salmonella, Vibrio cholera, E. coli, and Listeria* spp, thereby causing severe problems to consumers (Elmossadam, 2003).

Since the beef carcasses can even remain on shelves for days before they are sold, it was important to determine the presence of pathogenic bacteria such as *E. coli* and *Staphylococcus aureus* that are indicators of excessive human handling (Clarence *et al.,* 2009). In addition, bacteriological quality of meat was also evaluated. Despite the recorded risk and diseases being reported due to meat, there is still limited surveillance of presence of pathogens hence infections by these pathogens end up undetected (Ombui *et al.,* 2001; Kelly *et al.,* 2004). In Kenya, there is lack of quantitative data to assess hygiene and pathogen presence along the food continuum and risk factors (FAO/WHO, 2013). In developing countries, food borne infection leads to death of many children and the resulting diarrheal disease can have a long-term effect on children's growth as well as on their physical and cognitive development (Adak *et al.,* 2005; Clarence *et al.,* 2009). This therefore warrants the need to determine the meat quality and hence necessary intervention recommended.

1.3 Research questions

i. Does bacteriological load of fresh beef meat increase from slaughterhouse to butcheries?

ii. What are the bacterial counts in the sampled fresh beef meat, contact surfaces and equipments from the slaughterhouse and butcheries in Nyagacho?

iii. Which bacterial pathogens are present in the fresh beef meat offered for sale in Nyagacho?

1.4 Research hypotheses

i. There is no significant difference in bacteriological load on fresh beef offered for sale in Nyagacho at slaughterhouse and butcheries.

ii. The meat, contact surfaces and equipments from the slaughterhouse and butcheries in Nyagacho are not contaminated with pathogenic bacteria.

iii. The bacteriological load of the contact surfaces and equipments from the butcheries and slaughterhouse in Nyagacho are within the acceptable range.

1.5 Objectives of the study

1.5.1 General objective

To determine whether meat consumed in Nyagacho is fit for human consumption.

1.5.2 Specific Objectives

i. To determine the bacteriological load on beef carcasses from slaughterhouse and butcheries.

ii. To isolate and identify pathogenic strains of bacteria on meat samples, equipments and contact surfaces in the food chain to establish possible source of contamination.

iii. To determine the level of bacteriological contamination of the contact surfaces and equipments from the slaughterhouse and butcheries.

1.6 Significance of the study

The finding of this study may give an insight on the possible contamination areas that could be investigated in cases of food poisoning. Data from this study will also be of use to hygiene officers and food handlers in improving and strengthening hygienic production of retail meat to avoid bacterial food contamination. In addition, the findings will form the basis of recommendations which if implemented will improve sanitation programmes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Meat as food

Meat is those parts of a slaughtered animal which are ordinarily intended for human and animal consumption and which have not undergone any processing other than de-boning, cutting up, mincing, cooling or freezing. This also includes meat that has been treated with a substance that does not substantially alter the original characteristics thereof. Meat is defined by the Codex Alimentarius as all parts of an animal that are intended for, or have been judged as safe and suitable for human consumption from the nutritional point of view. The primary unit of meat is called carcass. It represents the ideal meat after removal of head; hide, intestine and blood (Rao *et al.,* 2009). The edible parts of a carcass include lean flesh, fat flesh and edible glands or organs such as heart, liver, kidney, tongue and brain. Meat is considered to be spoiled when it is unfit for human consumption. Meat is subjected to change by its own enzymes, by microbial action and its fat may be oxidized chemically. Microorganisms grow on meat causing visual, textural and organoleptic change when they release metabolites (Jackson and Mcgowan, 2001).

Most meat for human consumption comes from domestic animals, including cattle, pigs, sheep, chickens, turkeys, ducks and rabbits. Meat is a nutritious food as the protein provides all essential amino acids in the proportionate amounts required by man and is also an excellent source of iron, thiamine and niacin, phosphorus, potassium and sodium

(Rao *et al.,* 2009). Butcher meat is a valuable part of the human diet because (a) it is the most concentrated and is a good source of first class protein, that is, it contains those amino acids which are essential for human life; (b) it is stimulating to metabolism due to its high protein content, that is to say, it assists the body in the production of heat and energy; (c) it is satisfying, for the presence of fat in the diet delays emptying of the stomach (Ercolini *et al.,* 2006). Lean beef comprises of more than fifty percent heart-healthy monounsaturated fat variety, with forty-six percent saturated and four percent polyunsaturated fat (Ercolini *et al.,* 2006). Furthermore, thirty percent of the saturated fat in beef is made up of cell-repairing stearic acid (Ercolini *et al.,* 2006). Although dieticians normally recommend a diet with lower saturated fat content, higher amount of saturated fat is found in sirloin for example than in T-bone (Ercolini *et al.,* 2006).

It is estimated that more than 2 billion people in the world are deficient in key vitamins and minerals particularly vitamin A, iodine, iron, and zinc (Bett *et al.,* 2012). Deficiencies occur when people have limited access to micronutrient-rich foods such meat, fish, fruits and vegetables (Bett *et al.,* 2012). Most people with micronutrient deficiencies live in low income countries and are typically deficient in more than one micronutrient. Highly nutritious foods such as meat are particularly required for HIV AIDS infected communities and also children and women (FAO, 2004).

2.2 Beef consumption per capita

Beef is produced and consumed worldwide with South America, Africa, Asia and Australia being the higher consumers of the product (Frank, 2001). Its production therefore is increasing like that of many other commodities, as it is needed by both individual and operators of fast food centres (Gill and Jones, 2005). There is a link between per capita consumption of beef and the fortune of different nations (Bett *et al.,* 2012). However, per capital consumption of beef has increased tremendously worldwide since the early 1980s (Omuruyi *et al.,* 2011). This is mainly attributed to the increase in consumption per person with increasing personal income (Featherstone, 2003; Omuruyi *et al.,* 2011; Bett *et al.,* 2012).

Red meat consumption trend is determined by changes in tastes and preferences associated with socio-demographic trends of consumers, and the changing livestock population system (Boll, 2009). The price of beef has mainly been influenced by these trends, hence determining rate of consumption as well as that of production in slaughterhouses (Boll, 2009). Processing of beef is also driven by a number of factors; climate conditions, overall economic growth, private consumption expenditure and the continued deregulation and liberalization of the agricultural sectors (Norte and Noudic, 2009)

In Kenya, the livestock sector contributes 3.3 percent of the Gross Domestic Production and comprises mainly dairy and meat production, hides and skins from cows, sheep and goats. Red meat comprised of beef, mutton, goat, and camel meat accounts for up to 80

percent of all the meat consumed locally. Kenya's beef cattle population stands at over 9 million where most are kept on rangelands (www.livestock.go.ke, accessed on 20 February, 2012). The projected consumption of meat is expected to be more than double between 1997 and 2025 from 5.5 to 13.3 million metric tonnes in Africa. This increase is partly linked to what is referred to as the "Livestock Revolution" (Bett *et al.,* 2012). However, the overall annual per capita meat consumption is expected at an average of 44kg or a total consumption of 326 million metric tonnes of meat in the developing countries by the year 2050.

In Kenya, the domestic consumption of meat has increased tremendously from 361,115 tonnes in 1991 to 606,169 tonnes in 2007. The per capita consumption of meat was 14.90 Kg in 1991 and rose to 16 Kg in 2007. FAO projected a per capita consumption of 22 kg by the year 2050 on average for the Sub-Saharan Africa. Following these statistics, beef is the highest followed by poultry, fish, and pig meats as the least (Bett *et al.,* 2012). This is according to consumption data obtained from the cross-sectional survey done in selected six counties of Kenya namely, Kakamega, Siaya, West Pokot, Turkana, Bomet, and Narok, which is a result of an interview of respondents from 930 households in the urban and rural areas using structured questionnairess.

According to statistics obtained from the District Veterinary Office Kericho, on average, 3561 cattle were slaughtered per year for the period 2004 to 2012 in Kericho municipal main slaughterhouse hence constituting 45% of total slaughter (Table 2.1). According to the researcher's calculation, the average beef carcass yield is ± 200kg of the more

important cuts including the tongue and tail, but excluding the other offal. Thus the average consumption of beef from Kericho municipal main slaughterhouse per year is approximately 3561 cattle x 200 kg = 712,200 kg of beef per year, plus the offal.

Table 2.1 The annual slaughter of animals in the main slaughterhouse (source: District Veterinary Office- Kericho)

Year	**Bovines**	**Caprines**	**Ovines**
2004	3083	2645	2522
2005	3763	2522	2522
2006	4224	3385	2793
2007	3997	3234	2701
2008	3307	1587	825
2009	3992	2855	979
2010	3061	2950	726
2011	3058	2890	764
Totals	**28485**	**22068**	**13832**

2.3. Categories of abattoirs

Beef is produced in slaughterhouses sometimes called abattoir and the designs varies from state to state including ownership but they are principally a place where livestock are slaughtered (Marriot, 2004). Slaughter premises normally seen in developing countries are of three kinds; modern abattoirs, old slaughterhouses and slaughter slabs

and finally, makeshift premises. Of the three, modern abattoir represent the most progressive and ideal in the conventional abattoir design, equipping and services, often built and controlled by the central government with foreign technical assistance and management. These abattoirs are operated on industrial lines with a wide range of services featuring cold storage, processing, by-product utilization and waste recycling activities. Some of them have export objectives primarily in chilled and frozen meat although at times, some of their manufactured products (and by products) are channeled into local sale in substitution for imports. Few modern abattoirs in developing countries slaughter directly for public consumption, as they are commercial or profit-motivated establishments with little inclination for low revenue services (FAO, 2004).

The old slaughterhouses and slaughter slabs handle the bulk of public slaughters. These premises merely make facilities available for use by licensed butchers and traders for the slaughter of livestock at stipulated fee and in accordance with public health, inspection and marketing regulations. Slaughterhouses and slaughter slabs thus operate as service establishments under the management of municipal and local authorities, their field of activities often being limited to the larger towns and built up areas. The third category of slaughter premises, the makeshift that include all kinds of places such as converted buildings or rooms, shade of trees or bare grounds, that a butcher or a community may find convenient for the operation. They are characteristic of village and rural locations (FAO, 2004). Kericho municipal main slaughterhouse, from which the meat supplying Nyagacho is slaughtered, falls under the second category.

2.4 Quality control in the supply chain of meat

To enable risks involved to be estimated and appropriate measures to be taken, analysis of the slaughtering process has to be implemented by collection of abattoir - specific microbiological monitoring data, in accordance with Hazard Analysis Critical Control Point, (HACCP) principles (Zweifel *et al.,* 2005). A regular microbiological examination of carcasses allows reliable conclusions to be drawn with regard to long term hygienic conditions in slaughterhouses and butcheries. The microbiological control includes testing of the whole production chain; with samples taken from production and consumption (Hatakka, 2000).

The United States Department of Agriculture has been on the fore in efforts to improve the microbiological safety of meat in general, through the implementation of HACCP systems during the slaughtering process (Katchayanad *et al.,* 2007). In Autralia and New Zealand, the microbiological testing programmes have been jointly developed by the industry and regulatory agencies, where the primary objective of testing is to verify the control of the processes. Microbiological data are required to correlate microbiological contamination with visible soiling to identify the microbiological effects of individual operations processes and to confirm or reject suspected sources of microbiological contamination on products. Failure to meet microbiological standards precipitates investigative activities aimed at improving control over processes (Brown *et al.,* 2000).

In Kenya, more than 22 acts of parliament legislated within different government organizations governing food safety have been enacted. However, the implementation is poor and lack a thorough chain approach (FAO/WHO, 2013). At retail, the presence of

informal food outlets and markets provide challenges for effectiveness of surveillance and enforcement of existing food safety standards (FAO/WHO, 2013).

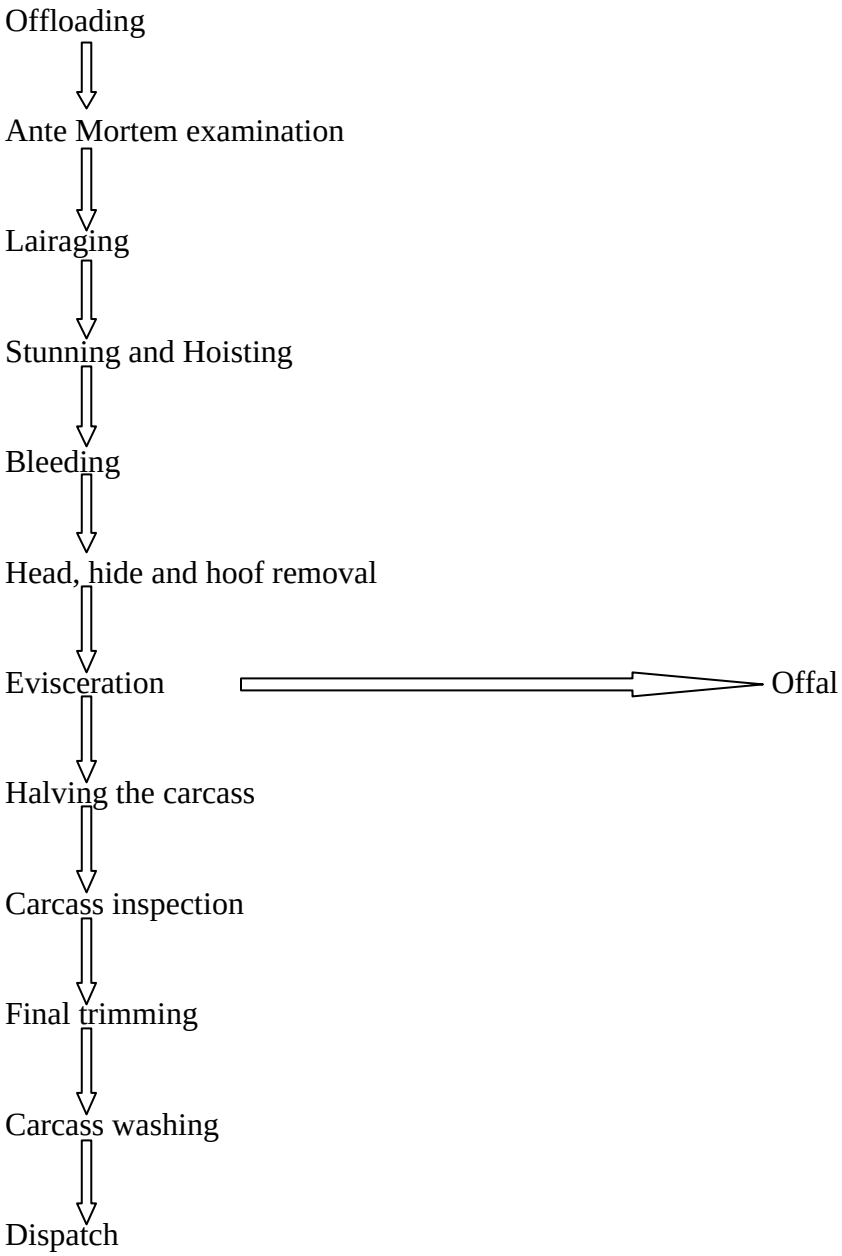

Figure 2.1: Sequence of slaughtering process (Bekker, 1998)

2.5 Contamination on live slaughter animal

The source of bacteria is likely to be from the skin of the animal from which the meat was obtained (Adzitey *et al.,* 2011). The exterior surfaces (hide, hair, skin) of healthy live

animals are naturally contaminated with large numbers (10^7 organisms per cm^2 of hide) of a variety of organisms (Featherstone, 2003). Slaughter stock themselves are therefore a major source of carcass contamination. The hide or intestinal tracts of slaughtered animals are the main areas where potentially pathogenic and spoilage bacteria reside (Okonko *et al.*, 2010).

The soil (ground) is also a major source of micro-organisms and has comparable numbers (10^7) of bacteria per gram of soil (Featherstone, 2003). Faeces are about 100 times more contaminated and have an aerobic plate count and coliforms of about 10^9 and 10^8 per gram of faeces, respectively (Unc and Goss, 2004). It can therefore be said that all of these can serve as sources of microbial contaminants of the meat. Dressing procedures currently available cannot be relied upon to prevent or remove all of the bacterial contamination on the carcass surface. What is also important is that the skinning and evisceration steps are major sites of contamination. If these procedures are conducted carefully, the degree of contamination can be reduced (Marriot, 2004; Unc *et al.*, 2004).

2.5.1 Contamination during slaughter

The instruments used in dressing and killing e.g. knives, saws, cleavers and direct contact with hair, the vessels, receptacles and the personnel may all act as sources of contamination during slaughter (Biswas *et al.*, 2011).

2.5.2 Method used for killing and bleeding

All animals have dirty skins and contain large numbers of bacteria that lead to the knife becoming contaminated during cutting of the skin. As a result, bacteria may enter the blood stream and spread throughout the body (Adzitey *et al.*, 2011). It is therefore important to sterilize the knife at 82 °C for 2 minutes in between cuts of different animals (Meat Control Act, 2012).

2.5.3 Skinning and de-hiding

Contamination of beef products processed in the slaughterhouse and manipulations such as skinning, evisceration, storage and distribution can be a major source of contamination (Selvan *et al.*, 2007). Due to high numbers of bacteria on the hide, dehairing hides before carcasses are skinned or dressed with the skin on, or by performing skinning and eviscerating operation in manners that avoid the transfer of microbes from hide to the meat surface is highly recommended (Gill, 2007). The available data appear to indicate that, of the various actions that can be taken to obtain carcasses that are free of contamination, only minimizing contamination of meat during skinning and evisceration can ensure a degree of control over the microbiological contamination of meat (Gill, 2007).

2.5.4 Bacterial load in the gut

Withholding feed for 24 hours before slaughter is highly recommended before slaughtering the animal in order to empty the digestive tract since they harbor the heaviest and potentially dangerous load of bacteria (Marriot, 2004). The Food and

Agricultural Organization stresses the importance of not puncturing the viscera at this stage, failure to adhere to this may results into bacterial contamination (www.fao.org, 15 February, 2005). If the intestinal tract is not ruptured or punctured, evisceration can be carried out with minimal contamination of the carcass (Cohen *et al.,* 2006). Fecal matter is a major source of contamination and could reach carcasses through direct deposition as well as by indirect contact through contaminated and unclean equipments, contact surfaces, workers, installations and air (Omuruyi *et al.,* 2011).

2.5.5 Halving the carcass

The operator must ensure that the saw is sterilized at 82°C for at least 2 minutes after each carcass and the sterilizing cabinet always maintained in good functioning condition (Meat Control Act, 2012). Cross contamination occurs majorly on the saw used for halving the carcass since it is continuosly used to half most, if not all of the carcasses in the slaughter house (Biswas *et al.,* 2011).

2.5.6 Carcass washing

This step comes in after the final inspection point. The carcass is sprayed with cold water to remove all blood, visible soil, slight blood marks, bone dust and marrow before going to the cold room for chilling (www.fao.org, 18 February, 2012). It is generally recommended that only approved, uncontaminated carcasses should be washed with running water in order to remove from the carcass any bone splinters and blood which might be present thus, improving the appearance of the carcass (Okonko *et al.,* 2010). Excess moisture in the cold room must however be eliminated by means of providing

adequate time and rail length so that the carcasses can drip-dry to inhibit bacterial growth (www.fao.org, 18 February, 2012). Washing of carcasses however, does not significantly influence ($p>0.05$) the microbiological load on beef carcasses (Bekker, 1998).

2.6 Carcass division

A beef carcass is divided into four main sections, two forequarters and two hindquarters and is normally divided between the tenth and eleven-rib bone (www.karanbeef.co.za, 15 February, 2013). For the purpose of this study, only the primary cuts of the fore and hindquarter relevant to the study are discussed. The sections which are normally cut for mincing are the brisket, flank, neck and the rump. These are therefore the regions which can be utilized to attest the microbiological quality of meat. The reason is because they pose a great risk to consumers as it is further reduced to small cuts and hence allowing cross-contamination and finally the contamination of the whole lot (Mc Nally *et al.,* 2005).

2.7 Dispatch and transport

Maintaining the cold chain as well as hygiene during the transport of meat is of utmost importance. Unnecessary contamination and microbiological growth will be the result if there is a breakdown of the above-mentioned and will have a direct impact on the shelf-life quality of the meat (Adzitey *et al.,* 2011). Therefore, according to Kenyan laws of Meat Control Act, 2012 (*www.kenyalaw.org*. Accessed on 26 July, 2013), the vehicle used for the transport of meat should comply with the following in order to prevent contamination of the meat:

i. The driving cab shall be completely separated from the freight compartment.

ii. It is important that the freight compartment is in a good state of repair. The freight compartment shall be of the fully enclosed type (dustproof), continuously lined with a smooth (free from joints), easy to clean, rust free, non- toxic and non-absorbent interior surface material.

iii. Insulated and/ or mechanically refrigerated in such a way that the temperature of the meat shall not rise more than 5 °C per hour more than 2 °C during the duration of local transport (less than 200 km).

iv. For the purpose of carrying, sides or quarters, the vehicle shall be fitted with beams and stainless steel hooks in a suspended position, clear of the floor.

v. No square centimeter of the said surface shall upon analysis contain more than 100 viable micro-organisms. To further prevent contamination, the following transport practices are required in terms of the same legislation:

vi. "Rough tripe, intestines or other parts of an animal which may contaminate other meat or may have an offensive odour shall be transported in receptacles which are watertight and are easily cleaned and disinfected and which have close-fitting lids so secured as not to fall off during transport.

vii. Meat shall be placed in such a manner that it shall not be in direct contact with the floor of a carrier or container and no person shall be allowed to stay in the compartment where meat is kept during transport.

viii. Any person who, during any loading or un-loading of meat, handles meat, shall before such handling, wash his hands and other exposed parts of his body in clean disinfectants and shall, during loading or un-loading, be wearing clean protective clothing

which covers the parts of his body, with the exception of his hands, which may come into contact with the meat, and the said clothing must not be worn during the actual transport by either the driver or any attendant".

Special care should be taken in order to prevent contamination due to the nakedness of carcasses during the unloading and transportation of meat. This area may be a major source of contamination through handling during loading, unloading, and contact with vehicle surfaces (Raji, 2006). After chilling, the amount of contamination increases slightly with further increase during transportation from the packing plant to the retail store (Bekker, 1998). The high levels of contamination may be attributed to more contamination through handling and changes in meat temperature during transportation. Vehicles for the transportation of meat and carcasses should be considered as an extension of the refrigeration process (Sulley, 2006).

2.8 Physiological changes

Evidence suggests that when animals are excited or fatigued, bacteria enter the tissues easier since they use up muscle glycogen, which forms lactic acid and changes the pH of the meat tissue (Rao, 2009). Therefore, the number of microbes found on the surface of the meat immediately following slaughter would depend on how hygienically the work in the abattoir has been done (Osama and Gehan, 2011). Immediately after slaughtering, carcasses contain high levels of microbial contamination, and moist carcass areas are highly contaminated (Biswas *et al.,* 2011). Contamination by contact with unhygienic surfaces, by personnel and airborne organisms, will remain a possibility in all operations

during the subsequent history of the meat (Adzitey *et al.,* 2011). These will include chilling, freezing, processing, cutting, packaging, transport, sale and domestic handling, although some sources of contamination are obviously removed when the carcasses leave the slaughter floor (Biswas *et al.,* 2011). It is therefore important to exercise hygiene in slaughterhouses, meat stores, during transport, in wholesale and retail distribution and in the home, to control exogenous contamination (Kirkpatrick, 2002).

2.9 Contamination during handling and processing

Bacteriological quality of meat products is strongly influenced by the prevailing hygiene condition during their production and handling (Osama and Gehan, 2011). The carcass of a healthy animal slaughtered for meat and held in a refrigerated room is likely to have only minimal surface bacteriological contamination while the inner tissues are sterile. After chilling, further processing of beef carcasses can result in product contamination. When carcasses and cuts are subsequently handled through the food distribution channels where they are reduced to retail cuts, they are subjected to an increasing number of micro-organisms from the cut surfaces (Okonko *et al.,* 2010).

Contamination subsequently occurs by the introduction of micro-organisms on the meat surfaces in operations performed during cutting, processing, storage, and distribution of meat (Clarence *et al.,* 2009). However, if the meat is kept clean by preventing contamination through dirty hands, clothing, equipment and facilities and the meat is kept cold and covered, there will be little or no contamination by micro-organisms whether bacteria, yeasts, moulds, viruses or protozoa (Osama and Gehan, 2011). Fresh meat cut

from the chilled carcasses has its surface contaminated with micro-organisms characteristic of the environment and the implements used to cut the meat (Biswas *et al.,* 2011). Employees are the largest contamination source and employees who do not follow sanitary practices, contaminate food that they touch with spoilage and pathogenic micro-organisms. Employees come in contact with these micro-organisms through work and other parts of the environment while their hands, hair, nose and mouth, harbor micro-organisms that can be transferred to food during processing, packaging, preparation and service by touching, breathing, coughing or sneezing (Biswas *et al.,* 2011; Cohen *et al.,* 2006; Selvan *et al.,* 2007). Therefore, in the prevention of meat contamination, personal hygiene plays an important role as there are as many as 200 different species of micro-organisms on a healthy human body (Featherstone, 2003).

Carcass contamination not removed by trimming or washing at slaughter is spread to newly exposed surfaces, which in turn can potentially decrease the shelf life of retail cuts and ground beef in retail meat display cases (Stivarius *et al.,* 2002; Marriot, 2004). The process of chopping and grinding enables bacteria present on the meat surface, to be distributed throughout the product (Siriken, 2004; Salihu *et al.,* 2010). The ultimate shelf life of ground beef depends on the bacterial level of the trimmings, sanitary conditions during processing, time and temperature of processing and storage (Siriken, 2004; Salihu *et al.,* 2010). Ground meat is especially good growth medium because of the extensive surface area provided by the grinding and because these organisms are distributed throughout the product, whereas on the uncut meat the bacteria would be present almost entirely on the outer surfaces (Siriken, 2004; Salihu *et al.,* 2010). Freshly minced meat

constitutes one of the most challenging of meat products for quality assurance and public health protection (Osama and Gehan, 2011). If retail mince samples show microbiological counts well in excess of 10^6 per gram it is an indication of poor quality and a potential hazard, which can markedly increase if the mince is held in ambient temperature and for these reasons, the storage of unfrozen minced meat is prohibited in many countries (Marriot, 2004). The storage life of ground beef that contains 1 million bacteria per gram is approximately 28 hours at 15.5 °C. At a normal refrigerated storage temperature of approximately -1 to 3 °C, the storage life exceeds 8 days (Marriot, 2004).

Shelf life is therefore obviously influenced by the initial load of contaminating micro-organisms and there is evidence that poorly cleaned mincing equipment can contribute to a lot of contamination (Enabulele and Uraih, 2009). Minced meat, unless maintained under refrigerated conditions, rapidly deteriorates. Strict sanitary fabrication practices of beef carcasses can (a) reduce total bacterial counts of beefsteaks, (b) reduce the percentage of typical Gram-negative spoilage bacteria of steaks, and (c) reduce off-odour development of refrigerated vacuum-packaged steaks (Marriot, 2004).

2.10 Microorganisms found in meat

The bacteriological condition of carcass meat is highly dependent on the manner, in which meat animals are reared, slaughtered and processed (Osama and Gehan, 2011). It is important that only relatively clean animals be presented for slaughtering, since it is extremely difficult to obtain clean meat from dirty animals. Therefore, the cleanliness of livestock depends on husbandry, weather and climate, methods of transport and holding

conditions at the abattoir. Cattle from feedlots may carry more faecal bacteria and less soil organisms (Biswas *et al.,* 2011). Food products (including meat) provide an ideal nutrition source for micro-organisms and generally have pH values in the range needed for proliferation. Food products (including meat) are contaminated with soil, air and waterborne micro-organisms during harvesting, processing, distribution and preparation (Enabulele and Uraih, 2009). Some apparently healthy animals may harbour various micro-organisms in the liver, kidneys, lymph nodes and spleen. These microorganisms and those from contamination through slaughtering can migrate to the skeletal muscles via the circulatory system (Marriot, 2004).

During the life of an animal, some organisms enter the blood and lymph circulation, but a natural defense system ensures that there is a state of equilibrium between bacterial attack and bacterial elimination (Bekker, 1998). In general, the micro-flora of meat will be that of the barnyard or feedlot which are on the external surfaces of the animal contaminating the meat by direct contact through air, water, soil, manure and the hands and tools of the workers (Unc and Goss, 2004). The healthy inner part of meats has been reported to contain few or no micro-organisms, although they have been found in lymph nodes, bone marrow, and even flesh (Okonko *et al.,* 2010). *Staphylococci, Streptococci, Clostridia* and *Salmonella*, have been isolated from the lymph nodes of red-meat animals. The important contamination, however, comes from external sources during bleeding, handling and processing. During bleeding, skinning and cutting, the main sources of micro-organisms are the exterior of the animal (hide, hooves and hair) and the intestinal tract (Selvan *et al.,* 2007). Approved "humane" methods of slaughter mechanical,

chemical and electrical have little effect on contamination, but each method is followed by sticking and bleeding, which can introduce contamination (Bekker, 1998).

Comminuted meats such as ground beef invariably have higher numbers of micro-organisms than non-comminuted meats such as steaks (Siriken, 2004; Salihu *et al.*, 2010). Commercial ground meats generally consist of trimmings from various cuts. These pieces have been handled excessively and consequently normally contain more micro-organisms than meat cuts such as steaks. Ground meat also provides a greater surface area, which itself accounts in part for the increased flora. This greater surface area of ground meat favours the growth of aerobic bacteria, the usual low-temperature spoilage flora.

One heavily contaminated piece of meat is sufficient to contaminate others, as well as the entire lot, as they pass through the grinder. This heavily contaminated portion is often in the form of lymph nodes, which are generally embedded in fat. These organs have been shown to contain high numbers of micro-organisms and account in part for hamburger meats having a generally higher total count than ground beef (Siriken, 2004; Salihu *et al.*, 2010).

2.10 Consequences of food-borne diseases from microorganisms

Infectious diseases spread through food or beverages are a common, distressing and sometimes life-threatening problem for millions of people around the world (NIAID, 2002). The U.S. Centre for Disease Control and Prevention (CDCP) estimated that, 76 million people suffer from food-borne illnesses each year in the United States (CDCP, 2008). This accounts for the 325,000 hospitalizations and more than 5,000 deaths. Kenya

recorded one of the highest ever numbers of food poisoning cases in 1990 (Kimani, 2001). A total number of more than 200,000 outpatients were treated at government hospitals. Food-borne diseases especially those caused by pathogenic organisms, remain a serious problem in all countries and are extremely costly to treat (Duff *et al.*, 2003). Health experts estimates that the yearly cost of all food-borne diseases in the United States is $5 to $6 billion in direct medical expenses and lost productivity (NIAID, 2002). In developing countries, where the problem of diarrheal disease is far greater, the effect on economical activity and development can only be far more severe (Adams *et al.*, 1999).

Diarrhea is a feature of most of the food-borne diseases and up to 70 percent of all episodes of diarrhea may result from ingestion of contaminated food and water (Adams *et al.*, 1999). World Health Organization (WHO) reported that 50 million children under 5 years get diarrheal diseases each year due to contaminated water and foodstuff (Tavakoli and Riazipour, 2008). In most cases, food is not contaminated intentionally, but rather due to carelessness or insufficient education or training in food safety. The problem of informal slaughter is not restricted to Africa alone. In Brazil it has been reported that 40% of the meat originates from informal slaughtering, a fact that constitutes a major problem for food safety. Meat consumption without sanitary care may cause diseases such as tuberculosis, salmonellosis and cysticercosis in consumers (Azevedo and Bankuti, 2003).

2.12 Bacterial pathogens associated with food poisoning

2.12.1 *Staphylococcus aureus*

S. aureus is a normal flora in human and animals, their presence in foods are indications of excessive human handling (Clarence *et al.,* 2009). *Staphylococcus aureus* is a Gram positive coccus, resistant to heat, drying and radiation. Its strains can be pathogenic and relatively non pathogenic. They produce disease when the bacteria contaminate food. They produce some enzymes which are implicated in staphylococcal invasiveness and many extracellular substances some of which are heat stable enterotoxins that render the foods dangerous even though it appears normal. Once the bacteria have produced toxin, the food can be extensively and properly cooked, killing the bacteria without destroying the toxin. Many of their toxins are gene–based that is carried on plasmids. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin. Some signs and symptoms of staphylococcal food poisoning include: Nausea, vomiting, abdominal cramp, prostration and diarrhea.

Since *Staphylococcus aureus* can colonize on various sites of food animals asymptomatically, such as pig or cow, these animals may serve as reservoir and/or a transmission vehicle of spreading *S. aureus* and Multidrug Resistant *Staphylococcus aureus* (MRSA). Food products derived from the animals may be contaminated with *S. aureus* or MRSA during slaughtering and processing. MRSA has been isolated from meat or dairy products in several countries including Netherlands, Italy, Australia, Japan and United States (Dinges *et al.,* 2000).

2.12.2 *Salmonella* spp.

Salmonella species such as *Salmonella typhi* is a bacterium that causes typhoid fever (enteric fever), an acute, life-threatening febrile illness (CDC, 2008). The disease is a cause for concern and a major public health problem in developing countries (Asia, Africa); especially in Kenya due to poor sanitary conditions and lack of or inadequate potable water. It is mainly transmitted through food, drink, or water, contaminated with urine or faeces of infected people or a chronic carrier. Since 1987, *Salmonella enteritidis* has been one of the most frequently isolated salmonellae associated with food borne outbreaks, which have been linked to consumption of chickens, eggs, and foods that contain eggs and it presents an interesting challenge from an epidemiologic perspective (Zheng *et al.*, 2007). Infections with nontyphoidal *Salmonella* have increased during the last 3–4 decades, and although a decrease has been reported over the last decade, *Salmonella* infections continue to be a major public health concern in many countries. These salmonellae are zoonotic, and the infections are generally food borne (Helms *et al.*, 2005).

The main reservoir of zoonotic *Salmonella* is food animals, and the main sources of infections in industrialized countries are animal-derived products, notably fresh meat products and eggs (Helms *et al.*, 2005). Rapid spread of a limited number of successful *Salmonella* clones in different sectors of food animal production (swine, broiler chickens, and particularly layer hens) has been suggested as the most important cause of this increase. Salmonellosis may occur in small, contained outbreaks in the general

population or in the large outbreaks in hospitals, restaurants, or institutions for the children or the elderly (NIAID, 2002).

2.12.3 *Escherichia coli* serotypes

Certain types of *Escherichia coli* can cause food borne illness (NIAID, 2002). *Escherichia coli* O157: H7 outbreaks due to plants and animal produce have become increasingly common (Schroeder *et al.,* 2005). While half of produce associated outbreaks were due to kitchen-level cross-contamination, which calls for further prevention efforts targeting food preparers, the other half were due to produce already contaminated with *Escherichia coli* O157: H7 before purchase (Schroeder *et al.,* 2005). *Escherichia coli,* which are normal flora of the human and animal intestine, have been identified as a leading cause of food borne illness all over the world. *Escherichia coli* and *Escherichia coli* 0157: H7 strain has previously been isolated from meat samples (Hussein, 2007). However, diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC) is highly prevalent in young children in developing countries as well as in travelers. It spreads through contaminated water and food (Qadri *et al.,* 2005). The potentially high mortality associated with *Escherichia coli* and *Escherichia coli* 0157: H7 strain infection, therefore make its presence in any food material worrisome and of serious public health sconcern as most of the outbreaks recorded has been traced to consumption of beef contaminated with the *Escherichia coli* 0157:H7 strain (Hussein, 2007).

In spite of the wide knowledge of the organism and its interaction, there seem to be no report on the prevalence of the organism in Africa and particularly Kenya. An *E. coli*

outbreak infection in the United States of America in 1997 resulted in the recall of 11 million kilograms of ground beef (NIAID, 2002). In Kenya, most incidents of food-borne diseases are due to the *E. coli* bacteria (Kimani, 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out in Kericho Municipal main slaughterhouse which supplies selected butcheries of Nyagacho, an informal settlement in Kericho County. The settlement is located to the northwest of Kericho (latitude 00º 22ˈS longitude 35º 15ˈE and 2096m above the sea level), 6 km from CBD. The slaughterhouse handles approximately 3,561 cattle annually and supplies butcheries located in the town centre, Brook and Nyagacho (Source: District Veterinary Offlce- Kericho). Nyagacho has an estimated population of 3,000 most of them working in the town centre. A report by the Ministry of Health on outpatient morbidity rated diarrhoeal diseases among the first top ten frequently treated diseases in the district and hence the need for the study.

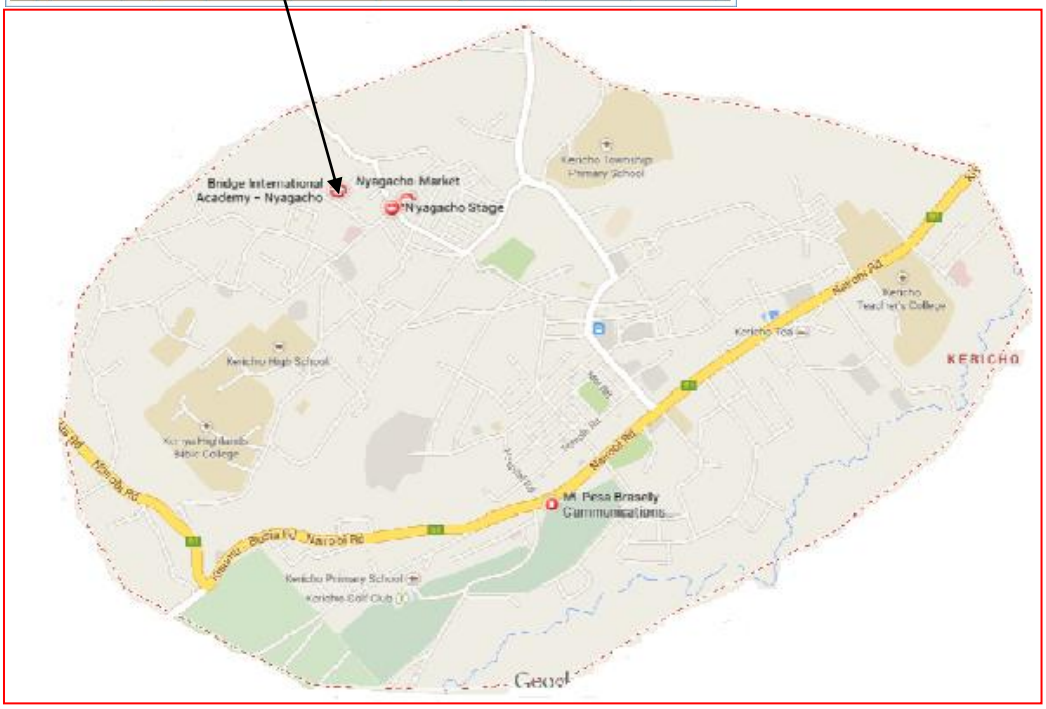

Figure 3: Map of Nyagacho

3.2 Sampling design and carcass sampling for bioassay from the slaughterhouse

Simple random design was applied during the study. The day's bovine carcasses slaughter were numbered on the left hind shank with a non-toxic pen, sequentially arranged and corresponding numbers marked on separate tabs, put into a closed container and tossed until they were thoroughly mixed. One tab bearing a number was selected from the container, recorded and tossed back to the pool again. If the same number was drawn twice, the second drawing was ignored. The number was returned to the pool and second drawing made. Drawings and mixing the numbers went on until three (3) carcasses from the day's bovine carcasses slaughter were selected from a total of sixteen.

3.3 Butchery selection

The three selected carcasses should not be from the same butchery. If the same number selected was from the same butchery, the number was returned to the pool and second drawing made. This was done until three carcasses from three different butcheries were selected. In order to ensure traceability to the butcheries the selected carcasses at the abattoir were marked by means of a tag indicating the date of slaughter, butchery that the carcasses will be sent and carcass number e.g AC1 represents butchery A, carcass 1. From the three selected carcasses, the lateral surfaces of the rump (n=3), the loin (n=3) and the proximal part of the neck area (n=3) (Strydom and Buys, 1995) were sampled.

Three tissue discs were removed from the three regions (loin, rump and proximal part of the neck) by means of the excision technique, using a sterile cork borer (2.52 cm^2), scalpel and forceps to a depth of approximately 5 mm from the surface (Snijders *et al.*,

1984; Gill and Jones, 2000). Sterile plastic bags were labeled to identify the date of sampling, abattoir, butchery that the carcass was sent to, carcass number and the sampling area; for example, AAC1R represents abattoir, butcher A, carcass one, rump. Samples were placed in the labeled bags and kept at 4°C in an insulated cooler box while transported to the Regional Veterinary investigation laboratory (RVIL) – Kericho where it was further stored at 0-2 °C until analysed (Gill and Jones, 2005). The proximal part of the neck, the loin and the rump of the sampled carcasses in the slaughterhouse were considered appropriate for bacteriological analysis. The sampling regions were based on ones recommended by the Meat (Hazard Analysis and Critical Control Point) (Scotland) Regulations 2002 No. 234 (Table 3.1).

Table 3.1 Recommended sites for analysis in different animals (source: Meat (Hazard Analysis and Critical Control Point) (Scotland) Regulations 2002 No. 234).

Animal	**Region**
Cattle	Proximal part of the neck, loin, flank and rump
Sheep and goat	Flank, thorax, brisket and breast
Pig	Back, jowl (or cheek), hind limb medial (ham), and belly
Horse	Flank, brisket, back, and rump

3.4 Sample collection from the butcheries

The three selected carcasses from the slaughterhouse were visited on the respective butcheries for sample collection and sampled approximate again on the same spot. The samples were handled in the same manner as previously indicated. Samples were labeled

with the date of sampling, butchery, carcass number and sampling area; AC1L represents butchery A, carcass 1, loin. The butcheries were visited after a period of 12 h on receiving the carcasses at an interval of 10 min from one butchery to the other, located almost 100 M apart. They were revisited three times at an interval of 1 month for sample collection (CAC, 2005).

3.5 Sampling of contact surfaces, equipments and clothing from the slaughter house and butcheries

The Replicate Organism Detecting and Counting (Rodac) agar plate method was used to sample the areas. These were done by pressing the agar plate on meat sawing machines which were sampled three times on each visits (n=9), chopping blocks (n=9), knives (n=9), scales (n=9), containers (n=9), butchery floor (n=9), clothing (9), hands (9) and walls (n=9). The agar plates were transported under 4 °C in an insulated cooler to Regional Veterinary investigation laboratory (RVIL) – Kericho, where samples were then incubated (25°C), for two days in the inverted position to remove excess moisture and to check for contamination (Andrews and Hammack, 2003). After incubation, the colonies were counted with the aid of a colony counter and recorded as CFU cm^{-2}. This was done to determine the bacterial load for the contact surfaces, equipments and clothing in slaughterhouse and butcheries.

For isolation of bacterial pathogens, a sterile, cotton-tipped swab moistened with sterile physiological saline was used to swab the contact surfaces, equipments, clothing and hands (100 cm^2) of the personnel in the food chain. The swabs were enriched and

streaked on MacConkey sorbitol, MacConkey, Salmonella - Shigella and Mannitol salt agar and incubated. The colonies were then identified based on morphology and colony characterization. Further biochemical tests including; citrate, urease, TSI, indole and oxidase test were carried out.

3.6 Enumeration, isolation and identification of organisms from the meat samples, contact surfaces and equipments from the slaughterhouse and butcheries

3.6.1 Sample preparation

Twenty- five grams of the meat samples were put into 225ml of 0.1 percent buffered peptone water (diluent) and blended for 2 min. Serial dilutions were prepared by adding 1ml of the previous dilution to 9 ml of the sterile diluents and homogenized in a stomacher for 2 min.

3.6.2 Determination of Total Plate Counts (TPC)

One ml of each dilution was added to a sterile Petri dish and Plate Count Agar (kept at 45°C in a water bath) added and mixed thoroughly. The preparation were then allowed to gel and finally incubated at 37° C for 24h and isolated distinct colonies counted and recorded. However, exact number of colonies between 30-300 colonies was counted. Average counts obtained were multiplied by the dilution factor and expressed as Colony Forming Unit per gram (C.F.U/g) (Fawole and Oso, 2001). Conclusion of the bacteriological quality of the meat was based on recommendation by Meat HACCP (Scotland) regulations 2002 No. 234 on acceptable and unacceptable total plate count on meat.

3.6.3 Isolation of bacterial pathogens

This was done by streaking on selective media for the most common meat bacterial pathogens. These include *Salmonella* sp., enteropathogenic *E. coli,* and coagulase positive *Staphylococcus aureus.*

3.6.3.1 Detection of *Salmonella*

A 10 ml of the homogenate was enriched by adding onto 100ml Tetrathionate brilliant green broth and incubated at 37° C for 18 h. The enrichment was sub-cultured onto Brilliant green agar, MacConkey agar and Salmonella-Shigella agar. Pink colonies on Brilliant green, colourless on MacConkey and pale colonies with black centres on Salmonella-Shigella agar were taken into consideration (Andrews and Hammack, 2003). The colonies were confirmed by biochemical tests; TSI, Simmon's citrate agar and urea (Fawole and Oso, 2001). The TSI was examined for characteristic reactions that included alkaline slant/acid butt with hydrogen sulphide production. Typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90% of the cases) formation of hydrogen sulfide (blackening of the agar) (Yah *et al.*, 2007).

Table 3.2 Biochemical reactions of *Salmonella* sp isolated from the meat samples and contact surfaces from various sampling sites

	Results		
Test/substrate			
	Positive	**Negative**	***Salmonella* sp. Reaction**
Glucose	Yellow butt	Red butt	+
Hydrogen sulphide	Blackening	No blackening	+
Urease	No color change	Change to pink	-
Simmon citrate	Growth;Blue color	No growth;No color change	+
Motility	Turbidity	No turbidity	+
Indole	Color change	No color change	+
Oxidase	Color change to blue	No color change	-

KEY: + Positive

- Negative

3.6.3.2 Detection of Enteropathogenic *Echerichia coli*

For isolation of *E. coli,* 10 ml of the homogenate were inoculated on 1.5 % peptone water and incubated at 37°C for 18-24 h before being inoculated on MacConkey Sorbitol agar and MacConkey agar. Pathogenic *E. coli* produces colourless colonies (Alam *et al.,* 2010). All isolates were identified as *E. coli* based on morphological and confirmed by

biochemical characteristics (March and Ratnam, 1986; Bopp *et al.,* 1999). Enteropathogenic *E. coli* was characterized by colourless colonies on MacConkey Sorbitol agar and indole positive test.

Table 3.3 Biochemical reactions of *Echerichia coli* isolated from the meat samples and contact surfaces from various sampling sites

	Results		
Test/substrate			
	Positive	**Negative**	***E.coli* Reaction**
Glucose	Yellow butt	Red butt	-
Hydrogen sulphide	Blackening	No blackening	-
Urease	No color change	Change to pink	-
Simmon citrate	Growth;Blue color	No growth; No color change	-
Motility	Turbidity	No turbidity	+
Indole	Color change	No color change	+
Oxidase	Color change to blue	No color change	+

KEY: + Positive
- Negative

3.6.3.3 Detection of *Staphylococcus aureus*

A 10 ml diluents of the samples were inoculated on Mannitol salt agar and incubated at 37° C for 18-24 hrs (Okonko *et al.,* 2010). Typical large, round, creamy and smooth colonies with golden yellow halo were isolated and sub-cultured. Presumptive diagnosis involved mannitol fermentation with an accumulation of acid production indicated by

phenol red indicator turning yellow. These colonies were further confirmed by Gram staining, catalase test, and coagulase test (Fawole and Oso, 2001).

Table 3.4 Biochemical reactions of *Staphylococcus aureus* isolated from the meat samples and contact surfaces from various sampling sites

Substrate/ Test	Results
Gram stain	Positive
Catalase	Positive
Coagulase	Positive
Mannitol sugar	Positive

3.6.3.4 Detection of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa produced colourless colonies with characteristic greenish colour on MacConkey agar. The colonies were confirmed by biochemical tests; TSI, Simmon's citrate agar, urea and oxidase test. *Pseudomonas aeruginosa* was characterizied by alkaline slunt and butt and the colonies were oxidase positive.

Table 3.5 Biochemical reactions of *Pseudomonas aeruginosa* isolated from the meat samples and contact surfaces collected from the various sampling sites

Test/substrate	Results		
	Positive	**Negative**	***P.aeruginosa* Reaction**
Glucose	Yellow butt	Red butt	-
Hydrogen sulphide	Blackening	No blackening	-
Urease	No color change	Change to pink	+
Simmon citrate	Growth;Blue color	No growth;No color change	+
Motility	Turbidity	No turbidity	+
Indole	Color change	No color change	+
Oxidase	Color change to blue	No color change	+

KEY: + Positive

- Negative

3.6.3.5 Detection of *Proteus vulgaris*

Proteus vulgaris produced characteristic pale dark centred colonies with swarming ends on Salmonella-Shigella agar, and colourless colonies on MacConkey agar. Indole, Citrate, urease test, reaction on TSI and Gram stain were further done to confirm the pathogen. Colonies with acidic slunt and butt with hydrogen sulphide production on TSI were characterized as *Proteus vulgaris* and were distinguished from *Proteus mirabilis* as

they are indole positive. The land mark characteristic of *Proteus* sp., is the utilization of urea noted by the change of media to pink within a period of 2 hours.

Table 3.6 Biochemical reactions of *Proteus vulgaris* isolated from the meat samples and contact surfaces from various sampling sites

Substrate/ Test	**Results**
TSI- Slunt	Yellow
-Butt	Yellow
Hyrogen sulphide	Positive
Urease test	Positive
Citrate test	Positive
Gram stain	Negative

3.6.3.6 Detection of *Proteus rettgeri*

Both *Proteus vulgaris and Proteus rettgeri* are indole positive and colonies were differentiated from *Proteus vulgaris* by examining for characteristic reactions that included alkaline slant/acid butt with no hydrogen sulphide production on TSI. The colonies were confirmed by biochemical tests; indole, TSI, Simmon's citrate agar, urea and salicin test. *Proteus morganii* cultures are citrate negative and salicin negative.

Table 3.7 Biochemical reactions of *Proteus rettgeri* isolated from the meat samples from various sampling sites

Substrate/ Test	Results
TSI- Slunt	Yellow
-Butt	Yellow
Hyrogen sulphide	Negative
Urease test	Positive
Citrate test	Positive
Gram stain	Negative

3.7 Data analysis

Data on bacteriological quality of meat was summarized using descriptive statistics; means, frequencies and percentages. Microbial counts (CFU/g) were represented as $\log_{10}$ CFU/g and means were calculated and presented in graphs and tabular form. To examine any statistical significant difference of the means between and within the three regions from the three butcheries, one way ANOVA was used. Statistical significance was set at $p<0.05$ using a computer package, MINITAB version 13.0.

CHAPTER 4

RESULTS

4.1 Bacteriological counts of the loin, neck and rump from the slaughterhouse

4.1.1 Bacteriological counts of the loin for slaughterhouse isolates

The collected meat samples of the loin from the three butcheries showed considerably high numbers of colonies (Plate 4.1).

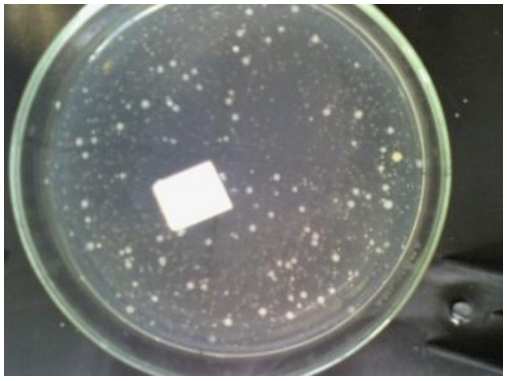

Plate 4.1: Plate count for dilution 10^{-3} of the loin from butchery A in the slaughterhouse.

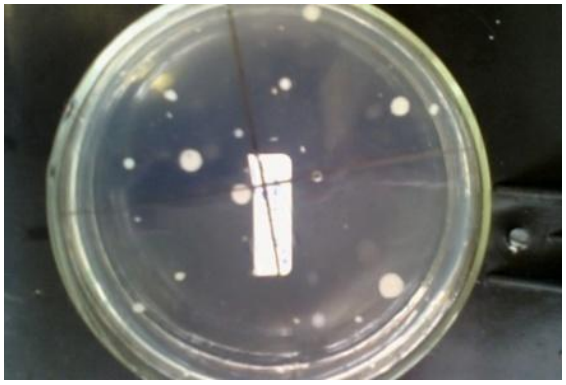

Plate 4.2: Plate count for dilution 10^{-7} of the loin from butchery A in the slaughterhouse.

Butchery A had the highest bacterial count, (log_{10}) 3.56 cfu/g followed by butchery B, (log_{10}) 3.55 cfu/g with butchery C recording the lowest count (log_{10}) of 3.22 cfu/g (Table 4.1). Although the mean counts recorded differed, there was no significant difference in the mean bacterial count from the loin ($p=0.440$ at $p>0.05$) from the three butcheries sampled.

4.1.2 Bacteriological counts of the neck for slaughterhouse isolates

Butchery B recorded the highest bacterial counts, (log_{10}) 3.58 cfu/g followed by butchery A, (log_{10}) 3.33 cfu/g while butchery C recorded the lowest, 3.26 cfu/g (Table 4.1). It was established that there was no significant difference in the total mean counts isolated from the neck ($p=0.670$ at $p>0.05$).

4.1.3 Bacteriological counts of the rump for slaughterhouse isolates

For the rump, the TPC recorded from the samples of the three butcheries also showed considerably high CFU counts. Butchery B recorded the highest count, (log_{10}) 3.59 cfu/g while butchery C recorded the lowest count, (log_{10}) 3.27 cfu/g (Table 4.1). On comparison of TPC mean count of the rump from the three butcheries, there was no significant difference ($p=0.606$ at $p>0.05$) that was established.

Table 4.1 Mean bacteriological counts (log_{10}) of the regions (neck, loin and the rump) from the three butcheries (A, B and C) in the slaughterhouse

Butchery	Neck	Loin	Rump
A	3.33	3.56	3.38
B	3.58	3.55	3.59
C	3.26	3.22	3.27

During the study, the mean bacterial counts of the three regions (neck, loin and rump) from individual butchery were compared to establish any statistical significant difference. However, there were no significant differences; butchery A, ($p=0.690$ at $p>0.05$), butchery B, ($p=0.554$ at $p>0.05$) and butchery C, ($p=0.994$ at $p>0.05$).

4.1.4 Bacteriological counts of the loin (butchery isolates)

The meat samples of the loin collected from the butcheries 12 hours after transportation showed considerably high CFU counts (Plate 4.3). The count seemed to have doubled the ones from the slaughterhouse.

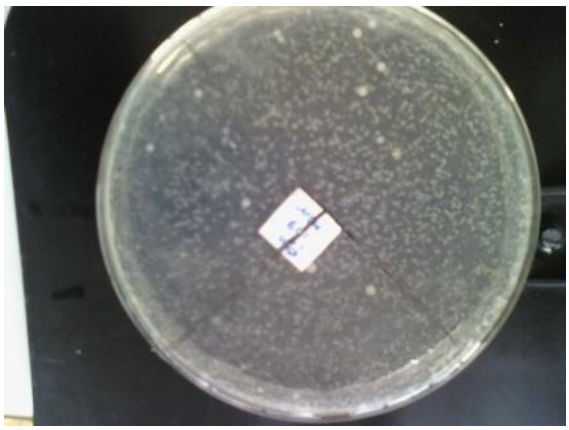

Plate 4.3: Plate count for dilution 10^{-4} of the loin in butchery B for meat sampled from the butcheries

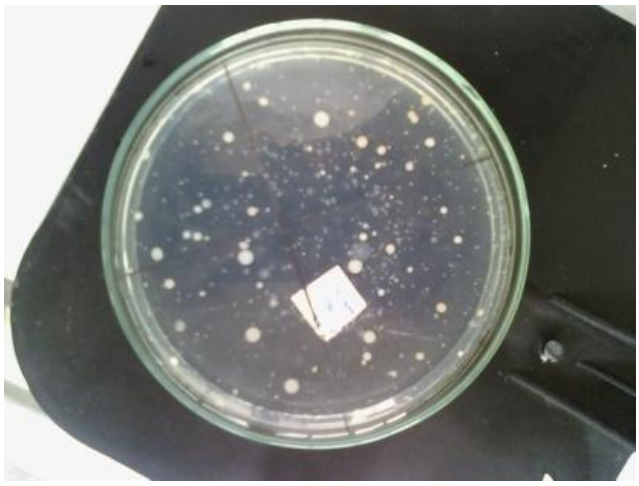

Plate 4.4: Plate count for dilution 10^{-9} of the loin in butchery B for the meat sampled from the butcheries

All the three butcheries had significantly high CFU counts which ranged from mean counts as high as ($\log_{10}$) 6.98 cfu/g of the meat sample of the loin recorded at butchery B to as low as ($\log_{10}$) 6.62 cfu/g of the meat sample recorded in butchery C (Table 4.2). A comparative study was carried out to establish any statistical difference in the mean

bacterial counts. No significant difference of the mean total counts of the loin (p=0.573 at p>0.05) from the three butcheries was established.

4.1.5 Bacteriological counts of the neck (butchery isolates)

The meat sampled from the neck after 12 hours of collection showed considerably high CFU counts. In butchery A, it had the highest count (log_{10}) 7.31 cfu/g on meat while butchery C was the lowest (log_{10}) 6.65 cfu/g (Table 4.2). However, There was no significant difference in levels in total bacteria count across the butcheries (p=0.183 at p>0.05).

4.1.6 Bacteriological counts of the rump (butchery isolates)

The meat samples of the rump collected from the butcheries also showed considerably high CFU counts in butchery B, (log_{10}) 7.00 cfu/g and as low as (log_{10}) 6.65 cfu/g at butchery C (Table 4.2). There was no significant difference in the mean bacterial count of the rump from the three butcheries (p=0.713 at p>0.05).

Table 4.2 Mean bacteriological counts (log_{10}) of the regions (neck, loin and the rump) from the three butcheries (A, B and C) in the butcheries

Butchery	Neck	Loin	Rump
A	7.34	6.90	6.66
B	7.01	6.98	7.00
C	6.65	6.62	6.65

During the study, the mean bacterial counts of the three regions (neck, loin and rump) from individual butchery were compared to establish any statistical significant difference. There was no significant difference in either sampled butcheries; A ($p=0.153$ at $p>0.05$), B, ($p=0.997$ at $p>0.05$) and C, ($p=0.873$ at $p>0.05$).

4.2 Bacterial pathogens isolated from slaughterhouse and butcheries

A total of 54 meat samples were collected and analyzed; (27) each from slaughterhouse and butcheries (Appendix I). The isolated bacteria varied within butcheries and sites sampled (the neck, loin and rump).

4.3 Detection of pathogens in meat samples from different sources

4.3.1. Slaughterhouse isolates

From the 27 samples cultured from the slaughterhouse, 15 tested positive for pathogens. Of the 15, 4 were positive for *Staphylococcus aureus* (Butchery A-7%, Butchery B-20% Butchery C-0%). Six were positive for *Proteus vulgaris* (Butchery A-20%, Butchery B-13%, Butchery C-7%), 5 for *Proteus rettgeri* (Butchery A-7%, Butchery B-13%, and Butchery C-13%). *Pseudomonas aeruginosa* was not isolated in all the samples of the slaughterhouse (Fig. 4.1).

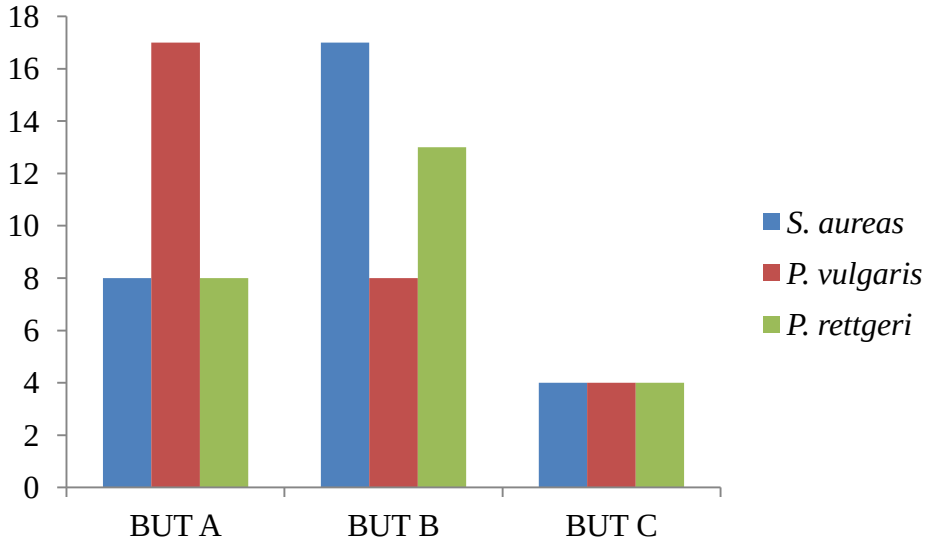

Figure 4.1: Graph of frequency of *Proteus* sp., *Staphylococcus aureus and Pseudomonas aeruginosa* isolated from the slaughterhouse

4.3.2 Butchery isolates

From the 27 samples collected from butcheries, 24 were positive for pathogens (Fig. 4.2). Of the 24, 7 were positive for *Staphylococcus aureus* (Butchery A-8%, Butchery B-17% Butchery C-4%). Six were positive for *Proteus rettgeri* (Butchery A-8%, Butchery B-13%, Butchery C-4%), 7 for *Proteus vulgaris* (Butchery A-17%, Butchery B-8%, Butchery C-4%) and 4 for *Pseudomonas aeruginosa* (Butchery A-8%, Butchery B-4%, Butchery C-4%)

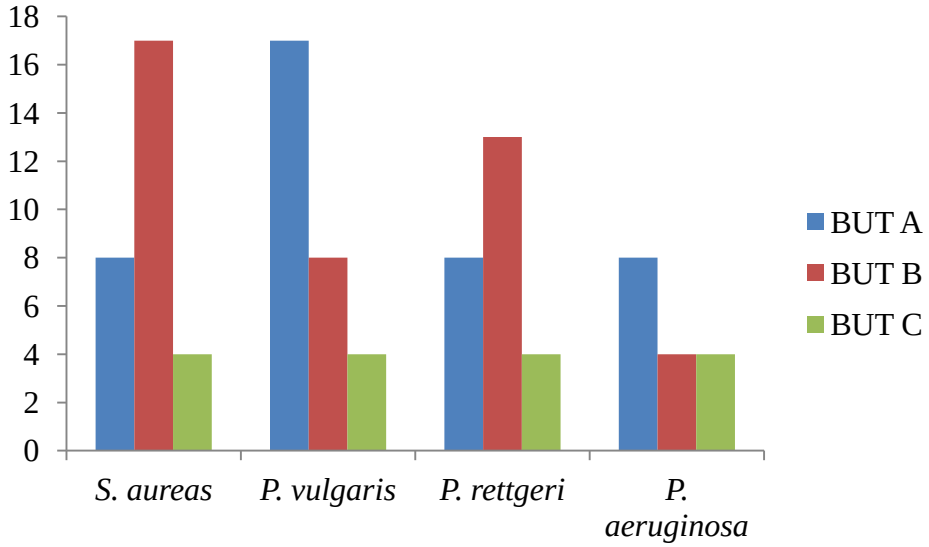

Figure 4.2: Graph of frequency of *Proteus* sp., *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates from the butcheries

4.4 Bacteriological analysis of the equipment and contact surfaces

The results for the samples taken from equipment (saws, knives, chopping block, scales, containers, vacuum sealer, hands, clothing and contact surfaces (floors and walls) at the three different butcheries were all found to be too numerous to count (Table 4.3).

Table 4.3 Bacteriological analysis of the equipment and contact surfaces sampled on slaughterhouse and from the three butcheries (A, B and C)

Equipment and contact surfaces of butcheries	**Results**
Butchery A	TNTC for all equipment and surfaces
Butchery B	TNTC for all equipment and surfaces
Butchery C	TNTC for all equipment and surfaces

Key:
TNTC- Too Numerous To Count

4.5 Sources of contamination

The bacteria isolated from the contact surfaces, equipments, clothing and hands of the personell in the food chain were; *Pseudomonas aeruginosa, Staphylococcus aureus Proteus* sp. and normal flora *Echerichia coli*.

4.5.1 *Pseudomonas aeruginosa*

Though *Pseudomonas aeruginosa* was not isolated from the slaughterhouse meat samples, the pathogen was isolated from the clothing (apron) of one slaughter man and the chopping board of a butchery (Table 4.4).

4.5.2 *Staphylococcus aureus*

Staphylococcus aureus was isolated from knives and hands of slaughter men taken at random in the slaughterhouse. In the butcheries, the pathogen was isolated from; knives, saws, clothing and hands of the butcher men (Table 4.4).

4.5.3 *Proteus vulgaris*

Proteus vulgaris was the most prevalent pathogen and was isolated from; knives and transport containers (transport box) used for transportation of the carcasses to the subsequent butcheries. This also raises eyebrows since cross contamination occurs and finally all the carcasses transported on the same box become contaminated. In the butcheries, the pathogen was isolated from; knives, chopping board and the scales (Table 4.4).

4.5.4 *Proteus rettgeri*

The pathogen was isolated from transport box in the slaughterhouse and scales from the butcheries (Table 4.4).

Table 4.4 Bacterial pathogens isolated from equipments, contact surfaces, clothing and hands of the personnel in the slaughterhouse and butcheries

Bacterial isolates from the slaughterhouse

Bacterial pathogens	cloths	knives	hands	Saws	Transport box	floor	Wall
S. aureus	-	+	+	-	-	-	-
P. vulgaris	-	+	-	-	+	-	-
P. rettgeri	-	-	-	-	+	-	-
P. aeruginosa	+	-	-	-	-	-	-
E. coli	+	+	+	+	+	+	+

Bacterial isolates from the butchery

Bacterial pathogens	Cloths	Board	Knives	Hands	saws	Transport box	Scale	floor	Wall
S. aureus	+	-	+	+	-	-	-	-	-
P. vulgaris	-	+	+	-	-	+	+	-	-
P. rettgeri	-	-	-	-	-	+	+	-	-
P. aeruginosa	-	+	-	-	-	-	-	-	-
E. coli	+	+	+	+	+	+	+	+	+

Key:
+ Present

- Absent

CHAPTER 5

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Bacteriological load in the slaughter house

The bacteriological load obtained from the study in the slaughterhouse was within the acceptable range <3.5 log and hence during the study, meat supplied to the slaughterhouse was fit for human consumption (ranged between ± 3.20 - ±3.50 $\log_{10}$). These could be attributed to minimal flies during the early morning hours and also due to strict adherence of rules enforced by the inspection officers (CAC, 2005). Low temperatures of the morning hours also suppress growth and multiplication of mesophilic bacteria and hence may have accounted for the low counts in the slaughterhouse during this study. Also the actual animal slaughter may have contributed since there are differences from animal to animal (CAC, 2005). Nevertheless, the meat contained pathogenic bacteria.

The study agrees with previous reports by Raji, 2006 who reported a total bacterial count of 3.5 cfu/g on dried sliced beef, locally called 'kilishi' in Nigeria but lower than those reported by Ahmad *et al.* (2013) (5.35 cfu/g) in Tanzania. The results were however slightly higher than those reported by Philips *et al.* (2001) and Scanga *et al.* (2000) (2.52 cfu/g and 1.4±0.6 log CFU/g respectively) in Australia. Total Plate Count (TPC) in the slaughterhouse were found to be higher than those reported by FAO/WHO, 2013 in four selected counties in Kenya ($\log_{10}$ 2 cfu/g). Variations in microbial counts among studies

are contributed by factors such as the differences in numbers of collected samples, the manner in which they were collected, the season in which the samples were collected and the same might have applied in the study (Li *et al.,* 2004).

5.1.2 Bacteriological load in the butcheries

The total plate count obtained from the butcheries during the study exceeded the accepted range of (> 5.0 cfu/g) and hence no meat sampled from the butcheries during the study was fit for consumption. The high TPC recorded in this study was attributed to poor handling and hygienic practices leading to high cross contamination and recontamination of meat (FAO, 2004). Cutting of meat presents an increase in surface area resulting into greater potential for exposure to microorganisms. Ambient room temperature in the butcheries also favours fast growth and multiplication of the microorganisms and hence these facts could account for the increased load of microorganisms found in the butcheries during this study (Gill *et al.,* 2000). The study agrees with previous report from Ghana (±5.57cfu/g) (Adzitey *et al.,* 2011) and those from Tanzania (7.15 cfu/g) (Ahmad *et al.,* 2013).

Okonko *et al.* (2010) found that retail cuts could result in greater microbial load because of the large amount of exposed surface area, more readily available water, nutrients and greater oxygen concentration available hence provides conditions that favour microbial growth and proliferation, which leads to spoilage of meat in Nigeria.

5.1.3 Bacterial pathogens isolated

From this study the main contaminant were *Proteus vulgaris* 13(33%) followed by *Proteus rettgeri* 11(28%), *S. auereus* 11(28%) and *P. aeruginosa* 4(10%) as the lowest. These organisms have been found to be associated with food handlers (Gitahi *et al.,* 2012). They were isolated from the clothing, equipments and hands of the personnel indicating poor sanitation and non observance on health check ups. These findings were slightly higher than those previously reported in Kenya in aircraft food (Maina *et al.,* 2013). Variations in bacterial load in the study may have been attributed to diverse types of samples, the improvement of enrichment and isolation procedures (Li *et al.,* 2004). These isolated microorganisms were similarly isolated from fresh meat samples from previous studies with similar patterns (Clarence *et al.,* 2009; Enabulele and Uraih, 2009; Sobukola *et al.,* 2009; Okonko *et al*., 2010).

5.1.4 Sources of origin of the bacteria

The sources of origin of all the isolated organisms were identified. The main source of *Staphylococcus aureus* in the slaughterhouse was identified to be, the knives and hands while saws, clothing and hands of the butcher men were identified to be the source in the butcheries. *Staphylococci* are ubiquitously distributed in the man's environment and strains present in the nose often contaminate hands, fingers, and face, hence the isolation of the pathogen from the personnel coming in contact with equipments during this study (Gill, 2007). Shaking of hands, sneezing and handling money while in food production and processing area may be attributed to the propagation of the bacteria to the equipments and clothings of the butchermen during this study (Bolder, 2007; Gill, 2007).

Though *Pseudomonas aeruginosa* was not isolated in any of the slaughterhouse isolates, the pathogen was isolated on the clothing (apron) of one of the slaughter men. Reduction of refrigeration temperature not only affects bacterial growth, but also the composition of the bacterial flora and may have accounted for the absence of pathogen in the slaughterhouse (Nychas *et al.,* 2008). The absence therefore from the meat samples collected is not guaranteeing complete absence of the pathogen since it was isolated from the chopping board on the butchery and the presence of the pathogen on the region poses a great hazard to consumers (Crowley *et al.,* 2010). The presence of the pathogen on the meat during this study may be accounted for by its association with water, soil, and vegetation that the personnel use or come in contact with during the processing or retailing of the product and more so human beings reported to act as carriers of the pathogen (Rodríguez-Calleja *et al.,* 2005). This on itself becomes a risk factor since it allows for cross contamination on the entire meat cut on the chopping board.

Proteus sp. was isolated from the knives, transport box and scales in slaughterhouse and butcheries. The pathogen may have come from the offal since they were transported together with the carcass leading to cross-contamination (CAC, 2005). It was also noted that the knives and scales used to cut and weigh the offal were interchangeably used on the meat in the butcheries and hence the offal introduced the pathogen on the carcass. The carcass and the offal should be transported in different containers and should not come in contact by all means (CAC, 2005). Omuruyi *et al.* (2011) isolated *Pseudomonas aruginosa, Proteus vulgaris,* and *E. coli* from the clothing, hands and equipments used by the personnel during a study on bacteriological quality of beef contact-surfaces in

Nigeria. In Ghana, Sulley (2006) and Ansah *et al.* (2009) reported that vehicles and transport boxes are usually the main contaminants and that the transport box is usually not cleaned properly and thus contains high microbial loads. In Tanzania, Ahmad *et al.* (2013) isolated *E. coli, S. aureus and salmonella* sp. from abattoir and meat shops.

5.1.5 Level of bacteriological contamination of contact surfaces and equipments

The level of bacteriological contamination of the contact surfaces and equipments during this study was above the acceptable range (Table 3.2). This could be attributed to poor hygiene practices by the food handlers in the entire meat production and supply chain (CAC, 2005). Hands of food handlers as well as their protective clothing should be kept clean when handling food. The presence of pathogenic strains of bacteria from contact surfaces and equipment may have contributed to the high counts recorded on the meat during this study (Martinez- Tome *et al.,* 2000). The study agrees with previous reports by Van der Walt, (2005) in South Africa and those by Neel (2012) in Tanzania.

5.2 Conclusions

The bacteriological load obtained from the study in the slaughterhouse was within the acceptable range <3.5 log and hence during the study, meat supplied to the slaughterhouse was fit for human consumption (ranged between ± 3.20 - ±3.50 log (Table 4.1). However, the high bacteria count and diversity of bacteria isolated from the

butchery samples indicated that the retailed meat and meat products were not fit for human consumption and hence possible source of infection.

The pathogenic bacterium isolated was *Staphylococcus aureus.* The presence of the pathogen is largely as a result of human contact and this suggests poor hygiene practices of the operators since this organism is a normal flora of the skin and nasal passage.

The general sanitary conditions at the meat shops and the poor hygienic practices by the butchers were found to be probable contributors to high unacceptable counts on contact surfaces and equipments. Food borne illness can be prevented by good hygiene practices such as the use of Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Point (HACCP) application in the chain of food production and processing.

5.3 Recommendations

The following recommendations are suggested based on the findings obtained in this study:

i) Standard hygienic practices be enforced at both pre- and post-production stages.
ii) Establishment of cleaning programmes with subsequent monitoring and microbiological verification to determine effectiveness should be encouraged both in slaughterhouse and in butcheries.

5.4 Future research

The study also recommends further research in the following area:

i) Knowledge, attitude and practices of food handlers throughout the chain from the slaughterhouse to butcheries be carried out in Nyagacho.

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APPENDICES

Appendix I: Bacterial species isolated from the meat samples of the loin, neck and rump during the entire study in the slaughterhouse and butcheries

Samples	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Proteus vulgaris***	***Proteus rettgeri***
1	-	-	-	-
2	+	-	-	-
3	-	-	-	-
4	-	-	+	+
5	-	-	-	-
6	-	-	-	-
7	-	-	+	-
8	-	-	-	-
9	+	-	-	+
10	+	-	-	-
11	-	-	-	-
12	-	-	-	-
13	-	-	+	-
14	-	-	-	+
15	-	-	-	-
16	-	-	-	-
17	-	-	-	-
18	-	-	+	+
19	-	-	-	-
20	-	-	-	-
21	-	-	-	-
22	-	-	-	+
23	+	-	+	-
24	-	-	-	-
25	-	-	-	-
26	-	-	+	-
27	-	-	-	-
28	+	-	-	-
29	-	-	-	-
30	-	-	-	+
31	-	+	+	-
32	-	-	-	-
33	+	-	-	-
34	-	-	-	-
35	-	-	-	-
36	-	-	-	-
37	+	+	+	+

38	-	-	+	-
39	-	-	-	-
40	+	-	-	-
41	-	-	-	+
42	-	-	+	-
43	-	-	-	-
44	+	-	-	-
45	-	+	-	+
46	-	-	+	-
47	-	-	-	-
48	+	-	-	-
49	-	-	-	+
50	-	-	-	-
51	+	+	+	-
52	-	-	-	-
53	-	-	-	-
54	-	-	+	+
TOTALS	**11**	**4**	**13**	**11**

Key:

+ Present

- Absent