

**BACTERIOLOGICAL QUALITY OF FRESH MEAT SOLD WITHIN
ELDORET TOWN**

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DECLARATION

Declaration by the Student

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DEDICATION

I dedicate this work to my parents Samwel Kosgei and Esther Kosgei in appreciation of their financial support, encouragement, and guidance. They have shown me that if I set my mind to anything, I can achieve it. I also dedicate it to my siblings John Koech, Miriam Shabati, Ruth Koech, Sarah Rotich, and Irene Tenai for their support and encouragement while I pursued my studies. Lastly, I give thanks to my Heavenly Father who made it all possible.

ABSTRACT

Meat is the primary source of proteins in many parts of Africa. In many cases however, it is major point of entry of many zoonotic diseases. Further, due to the long and many procedures involved in the meat industry, it is also implicated with many diseases associated with poor hygiene. The present study was carried out to assess the general microbiological quality of meat (beef, chevon and mutton) sold in Eldoret Town of Uasin Gishu County. The objectives of the study were; to investigate the physiological conditions of the meat, assessing the bacterial load of the meat, and biochemical characterization of selected bacterial pathogens found on the meat. Five major abattoirs were sampled for this study; Kaburwo in Langas, Maili Nne in Huruma, Cyrus in Munyaka, Eldoret main, and Teresia in Moiben representing all the major peri-urban centres of the Town. Five butchereries in the town were also selected for the study. The pH and temperatures of the carcasses were the physiological parameters measured and were recorded at the sampling sites. Meat samples were aseptically collected and taken in sterile bags and kept at 4°C. One gram of each meat sample was mixed with 0.4 ml of 0.1% of buffered peptone water for 2 minutes. Serial dilutions were carried out and followed by plating via pour plate technique. The plates were incubated for 24 hrs at 37 °C after which total plate count was done to determine the bacterial load of the meat samples. Biochemical typing was done on pure isolates to determine the presence of *Escherichia coli*, *Staphylococcus aureus*, and *Proteus vulgaris*. PH values ranged from 5.5 to 6.77 with the pH of the meat obtained from butchery consistently registering low values compared to the ones from the abattoirs. Temperatures of the carcasses ranged from 23 °C to 26.95 °C. The lowest temperatures were obtained from the carcasses from Teresia abattoir. Statistical analysis of variance was used to determine level of significance between the microbial load and factors; temperature and PH on meat sample collected. Correlation regression analysis was used to determine the influence of the physiological factors measured on the numbers of colony forming units(cfus). Statistical analysis was done using Ms Excel 2013. The meat in all the samples sites were found to be of poor bacteriological quality as the bacterial load was found to be higher than the recommended levels. The highest bacterial load was found in the carcasses in the butchery with an average of 19.8 cfus g/ml while the lowest was from the Maili Nne abattoir with an average of 4.9 cfus g/ml. Among the abattoirs, Teresia had the highest bacterial load of 9.2 cfus. Temperature was found to be positively correlated with cfu albeit weak (0.177) while pH was negatively correlated with the same (-0.478). *Staphylococcus aureus* was the major pathogenic bacteria isolated from all the carcasses being at 26% of the isolated bacteria. *Proteus vulgaris* was at 11% and *Escherichia coli* at 8%. These differed from one sampling site to another but the order of abundance remained uniform. More public health education on the appropriate hygienic practices ought to be rolled out to help achieve a better microbiological quality status of the meat sold in Eldoret Municipality.

TABLE OF CONTENTS

DECLARATION	ii
DEDICATION	iii
ABSTRACT.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF PLATES	x
LIST OF APPENDICES	xi
LIST OF ABBREVIATIONS AND ACRONYMS	xii
ACKNOWLEDGEMENTS	xiii
CHAPTER ONE	1
INTRODUCTION.....	1
1.1 Background.....	1
1.2 Statement of the Problem.....	5
1.2 Justification	5
1.3 Significance of the study.....	6
1.4 Objectives	6
1.4.1 Broad Objective	6
1.4.2 Specific Objectives	7
1.5 Research Questions.....	7
CHAPTER TWO	8
LITERATURE REVIEW	8
2.1 Food safety.....	8
2.2 Global status of Beef consumption	9
2.2.1 Status of Beef consumption in Kenya.....	9
2.3. Types of Slaughter Premises.....	10
2.3.1. Modern abattoirs	10
2.3.2 Old slaughter slabs	11
2.3.3 Makeshift	11
2.4 Hygiene of animals presented for slaughter.....	12
2.4.1. Hygienic dressing and handling carcass	12

2.5 The Humane Method and Conventional Techniques of Slaughter	13
2.5.1 Stunning	13
2.5.2 Bleeding	14
2.5.3 Skinning	15
2.5.4 Postmortem Inspection.....	17
2.5.5 Carcass Refrigeration.....	17
2.5.6 Transportation of meat.....	18
2.6 Microorganisms found in meat	20
2.6.1 <i>Staphylococcus</i>	22
2.6.2 <i>Salmonella</i>	22
2.6.3 <i>Escherichia</i>	23
2.6.4 <i>Proteus species</i>	24
2.7 The effect of pH on growth of micro-organisms	25
2.8 The effect of temperature on microbial growth	27
2.9 Factors affecting pH on Meat	28
2.9.1 Animal's age at the time of slaughter	29
2.9.2 Animal's diet.....	29
2.9.3 Stress	29
2.9.4 Processing techniques	29
2.10 Sources of food contamination	30
2.10.1 Primary contamination.....	30
2.10.2 Secondary contamination.....	31
2.11 Risk associated with informal slaughter	32
2.11.1 Impacts of contaminated slaughtered meat on human health	33
CHAPTER THREE	35
MATERIALS AND METHODS	35
3.1 Site of Study.....	35
3.2 Methods.....	36
3.2.1 Methods for sample collection.....	37
3.2.2 Sample collection.....	37
3.3 Sample Processing	38
3.3.1 The pH and temperature reading.....	38
3.3.2 Determination of Total plate count	38

3.3.3. Gram staining.....	39
3.4 Isolation of indicator bacteria	39
3.4.1 Detection of <i>E.coli</i>	39
3.4.2 Detection of <i>Staphylococcus aureus</i>	41
3.4.3 Detection of <i>Proteus vulgaris</i>	43
3.5 Characterization of Dominant Microorganism	44
3.5.1 Morphological Characterization of Dominant Bacteria.....	44
3.6 Data Analysis Methods	45
3.7 Research Permits.....	46
CHAPTER FOUR.....	47
RESULTS	47
4.1 Physiological Conditions of the carcasses	47
4.2 Bacterial Load.....	48
4.2.1 Influence of the physiological conditions on the Bacterial.....	50
4.3 Isolation of Selected Bacterial Pathogens.....	52
CHAPTER FIVE	53
DISCUSSION	53
5.1 Physiological Conditions of the Carcasses	53
5.2 Bacterial Load and the selected Pathogens.....	55
5.3 Selected Pathogens.....	56
CHAPTER SIX	58
CONCLUSION AND RECOMMENDATIONS	58
6.1 Conclusions	58
6.2 Recommendations	58
REFERENCES.....	59
APPENDICES	68

LIST OF TABLES

Table 3.1	Biochemical reactions of <i>E. coli</i> isolated from meat samples.....	40
Table 3.2	Acceptable ranges of bacterial counts on meat sample.....	45
Table 4.1	Means of pH, temperature, and cfu in the abattoirs.....	48
Table 4.2	Means of pH, temperature, and cfu in the butcheries.....	49

LIST OF FIGURES

Figure 1.1	Process of Slaughtering.....	13
Figure 2.1	Growth rate vs pH for 3 environmental type of prokaryotes.....	26
Figure 3.1	Uasin Gishu County Map.....	35
Figure 4.1	Temperature and pH obtained from the carcasses.....	47
Figure 4.2	Bacterial loads obtained from the different meat points within Eldoret Municipality.....	49
Figure 4.3	Relationship between the pH and bacterial load.....	50
Figure 4.4	Relationship between temperature and bacterial load.....	51
Figure 4.5	The selected bacterial pathogens from the different carcasses.....	52

LIST OF PLATES

Plate 3.1	Carcasses of the slaughtered animals.....	37
Plate 3.2	Indole and Eosin Methylene Blue positive for <i>E. coli</i>	41
Plate 3.3	Positive tests for Catalase and Lactose.....	42
Plate 3.4	Positive Simmons Citrate and Triple Sugar Iron test	44

LIST OF APPENDICES

Appendix I: One way ANOVA Table	68
Appendix II: Two-way ANOVA Table	68
Appendix III: Letter from the County Government of Uasin Gishu permitting the researcher to collect the samples	69
Appendix IV: Research License from the National Commission for Science, Technology & Innovation.....	70
Appendix V: Similarity Report.....	71

LIST OF ABBREVIATIONS AND ACRONYMS

CDCP	Centre for Disease Control and Prevention
CDC	Centre for Disease Control
CBD	Central Business District
CFU	Colony forming unit
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
TPC	Total Plate Count
WHO	World Health Organization
DFD	Dark Firm Dry
FDA	Food and Drug Administration
FSA	Food Standards Agency
PSE	Pale Soft Exudative
TSI	Triple Sugar Iron
PCA	Plate Count Agar
KMC	Kenya Meat Commission

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CHAPTER ONE

INTRODUCTION

1.1 Background

The term "red meat," which refers to meat from cattle, sheep, and goats, has been used since 8000 BC (McAfee *et al.*, 2010). Massive livestock businesses grew and output rose in order to meet the global demand for meat. Meat must go through specific processing steps in order to produce the finished product. Red meat is incredibly adaptable and has countless applications. The customer should adhere to certain buying guidelines, purchase red meat in accordance with its requirements and grades, and be knowledgeable about meat safety to ensure the highest quality product (Ekmekcioglu *et al.*, 2018).

Meat shelf life is extended by use of appropriate packaging and storage facilities (Ekmekcioglu *et al.*, 2018). Additionally, there are some significant misconceptions regarding its nutritional value of meat. Consumers should be informed about its health and nutritional highlights as well as certain medical problem alerts. However, with the support of some lifestyle eating recommendations meat might be incorporated in daily diets (Lew *et al.*, 2017).

The majority of people consume meat regularly, and its safety depends on the implementation of efficient control procedures at each level in production chain, or "farm to fork.". Cooperation between farmers, feed manufacturers, livestock market operators, livestock haulers, abattoir co-operatives, and workers in food processing plants is necessary to accomplish this crucial goal (Mazhangara *et al.*, 2019). On the other hand, those employed by meat inspection services and other regulatory authorities, veterinarians, food technologists, and specialists in occupational medicine,

public health, and epidemiology must also work together to minimize bacterial contamination of meat (Mazhangara et al., 2019).

Animal guts are the primary habitat of food-borne microorganisms, which are seldom seen in meat. However, during the slaughter process, bacteria may be spread to the surface of the meat by infected hands, equipment, or feces on the coats of unclean animals. Therefore, carcasses might get contaminated by microbes from a single diseased animal. Since contaminated carcasses cannot be seen, slaughterhouses are required to put in place mechanisms that lessen the chance of contamination. For instance, a Clean Livestock Policy was developed in 1997 in response to *E. coli* O157 outbreaks, and it mandates that any livestock that does not reach the necessary standard of cleanliness be rejected for slaughter (Davies *et al.*, 2000).

By using conventional meat inspection techniques, it is possible to remove meat from carcasses that have disease lesions that are apparent from the food chain (Alvseike *et al.*, 2018). It is easy to think that this will be enough to guarantee the safety of the carcasses that pass inspection, and that will be the end of the problem (Pal *et al.*, 2018). This is untrue, though, as significant risks to human health result from clinically healthy animals carrying dangerous microbial organisms like *Campylobacter* and *Salmonella* (Buncic *et al.*, 2019).

Only a fully integrated approach to food safety at all phases of production, processing, and distribution, which includes hazard analysis critical control point (HACCP) and comparable safety plans, can manage these hidden dangers (Minor *et al.*, 2020). It is crucial that every stake holder working in the meat business be aware of meat safety , since working together is the only way to make progress toward guaranteeing that all

meat and meat products marketed to the general public are healthy and safe (Yunusov & Achilov., 2022).

Meat and other foods that spoil quickly offer ideal circumstances for the development of dangerous germs. Fresh meat's quality can be affected by microbial infection, which can also reduce its shelf life and pose a health risk (Zdolec, & Kiš., 2021). The microbial population that comes into touch with fresh meat during slaughter, dressing, and processing is a difficult issue for the meat business. Therefore, to produce sanitary and wholesome meat and to protect the safety of the public's health, continuous monitoring and intermittent microbiological tests are required (Koffi-Nevry *et al.*, 2011)

In Kenya, the Food and Environmental Hygiene Department is responsible for monitoring of slaughter houses to ensure that their operations for the monitoring of slaughter houses to ensure that the operations meet the required hygiene and environmental standards and that only meat fit for human consumption is released for sale in the market. However, contamination by microbes frequently occurs post – inspection.

Meat production contributes significantly to household food security and domestic and export income in Kenya; thus, managing the safety of meat is essential to protect public health and ensure market access and economic benefit for the country. The management of microbiological risks associated with meat should be based on scientific assessment of health risks, the identification of the critical points through the chain where microbiological contamination occurs and quantitatively increases and at which prevention and control measures can be most effectively applied to protect public health (Cook EA *et al* 2017).

Slaughterhouses are defined as places where animals are slaughtered for foods (Stevenson., 2013). The development of the slaughter industry varies between countries due to cultural differences, the types of animals slaughtered and wealth. In developed countries such as the USA or the United Kingdom traditional slaughter facilities were small and local to town centres. In the 20th century they became centralized, large-scale, and mechanized. They are now predominantly meat packing plants where animals are slaughtered and the meat is packed ready for distribution. (Cook *et al.*, 2017). One of the factors contributing to this change was supermarkets replacing butchers as the primary suppliers and the increase in restaurants and fast food establishments requiring large amounts of standardized products (Cook *et al* 2017). Large slaughter facilities had the necessary capital to respond to these market demands and also to the increased government regulations aimed at improving public safety both of which required upgrading equipment.

Regulation of the slaughter industry aims to improve hygiene and reduce the contamination of meat and spread of disease, as well as protecting workers from occupational health hazards (Farmer *et al.*, 2012). The meat industry in Kenya is regulated by the Directorate of Veterinary Services under the State Department of Livestock in the Ministry of Agriculture, Livestock and Fisheries (Kenya Meat Control Act 2012). A revised Meat Control Act was introduced in 2012 to standardize the meat industry across the country. The revised Act provides information to reduce the risk of food borne disease and protect the consumer. The revised guidelines cover components of the slaughter process such as building structure and layout, equipment, personal hygiene, carcass handling, waste management, and meat inspection (Kenya Meat Act Control., 2012).

1.2 Statement of the Problem

The bacteriological quality of locally processed beef is poor despite increased hygienic regulations (Kimindu *et al.*, 2021). Due to its pH, temperature, water activity, and nutritional content, meat and meat products are especially prone to bacterial development (Jeffer *et al.*, 2021

According to FAO/WHO (2013), in Kenya, the majority of the time, carcasses that are delivered to informal settlements are transported in packed, unrefrigerated vehicles, or portions of meat and offal are transported at room temperature in non-insulated metal containers on taxis, motorcycles, and bicycles.

There are reported cases of consumption of contaminated meat; 80 people were hospitalized after consumption of contaminated meat in Tran-Nzoia County (Obare Osinde., 2019), one died and several others were hospitalized in Embu (George M 2023),

According to Omuruyi *et al.* (2011), butcheries and slaughterhouses, which may be sources of contamination, have a major impact on the meat's shelf life. Pathogens including *Salmonella*, *Vibrio cholera*, *Escherichia coli*, and *Listeria* species are examples of contaminants that might cause serious issues for consumers (Elmossadam, 2003).

1.2 Justification

The assurance of wholesomeness and the provision of high-quality meat marketed to consumers are the main goals of a meat hygiene and safety program (Kademi *et al.*, 2019). In the ante-mortem and post-mortem examinations, the presence of a meat inspection system looks for grossly obvious abnormalities but misses complex

microbial contamination, which could later result in serious risks to public health and financial loss due to food poisoning and meat spoilage (Ahmed *et al.*, 2002).

Meat carcasses remain on shelves for days before they are sold, it is important to determine the presence of pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* that are indicators of excessive human handling (Clarence *et al.*, 2009)

Finding harmful germs like *E. coli* and *Staphylococcus aureus*, are signs of excessive human handling, in the meat carcasses (Clarence *et al.*, 2009). There is therefore need to characterize the microbial contaminants in different abattoirs and slaughterhouses to identify specific potential pathogens in different sites.

1.3 Significance of the study

The results of this study may provide information on microbial loads and potential places for contamination that may be looked at in cases of meat poisoning. In order to prevent bacterial food contamination, data from this study will also be useful to hygiene officials and meat handlers in strengthening and enhancing meat seller sanitary practices. Additionally, the results will serve as the foundation for suggestions that, if carried out, will enhance sanitation initiatives. Furthermore, other researchers may utilize the results as a resource and a starting point for future research.

1.4 Objectives

1.4.1 Broad Objective

To investigate bacteriological quality and the impact of pH and temperature on the bacterial load on meat (beef, chevon and mutton) consumed in Eldoret Town.

1.4.2 Specific Objectives

- i. To determine pH and temperatures affecting bacterial contamination on meat obtained from selected sites within Eldoret Town.
- ii. To determine the bacterial load on meat (beef, chevon and mutton) on obtained from selected sites within Eldoret Town.
- iii. To characterize bacterial contaminants on meat (beef, chevon and mutton) obtained from selected sites within Eldoret Town.

1.5 Research Questions

- What is the effect of PH and temperature factors on bacterial contamination on meat obtained from selected sites within Eldoret Town?
- What is the bacterial load on meat obtained from selected sites within Eldoret Town?
- What is the identity of bacterial contaminants on meat sold in Eldoret Town?

CHAPTER TWO

LITERATURE REVIEW

2.1 Food safety

According to the World Health Organization (WHO) 2018, contaminated food causes two billion diseases annually, and the number is rising internationally. Each year, 700,000 people in Asia die from food-borne diseases. Diarrhea caused by contaminated food and water kills 2 million young children each year in the underdeveloped nations. A large portion of this issue is avoided with improved research, preventative measures, and appropriate food hygiene practices. Many episodes of food poisoning are caused by microbes, such as bacteria, viruses, molds, yeasts, or parasites, in the food or water we consume. Food poisoning can also be brought on by the harmful compounds that these microorganisms create or by naturally occurring pollutants in foods (Kačániová *et al.*, 2019).

According to Kačániová *et al.* (2019), the microbiological quality of fresh and preserved food items affects their safety, shelf-life and consumer acceptability. Food poisoning bacteria may multiply fast, particularly in warm, damp environments. A piece of food left out of the refrigerator overnight may have just microbe on it, but by morning, it could have millions of them, enough to get you sick if you eat it. Many cases of food poisoning are brought on by germs in the food we consume or the water we drink, such as bacteria, viruses, moulds, yeasts, or parasites. Additionally, these microorganisms' hazardous by-products or naturally occurring toxins in foods might result in food poisoning (Kačániová *et al.*, 2019).

Food poisoning bacteria may swiftly proliferate, especially in warm, damp environments (Kačániová *et al.*, 2019).

2.2 Global status of Beef consumption

Global production and consumption of beef are dominated by South America, Africa, Asia, and Australia (Frank, 2001). Because it is required by both individuals and owners of fast food restaurants, its production is consequently rising like that of many other commodities (Gill and Jones, 2005). Beef intake per capita and various countries' fortunes are related (Bett *et al.*, 2012). However, since the early 1980s, the global per capita consumption of beef has significantly grown (Omuruyi *et al.*, 2011). This is mostly explained by the rise in consumption per person that comes along with rising personal income (Featherstone, 2003; Omuruyi *et al.*, 2011; Bett *et al.*, 2012).

The socio-demographic patterns of consumers, the shifting cattle population structure, and changes in consumer tastes and preferences all influence the trend in red meat consumption (Boll, 2009). These developments have mostly affected the price of beef, which in turn affects both the rate of consumption and the level of output at slaughterhouses (Boll, 2009). Climate conditions, general economic expansion, private consumer spending, and the ongoing deregulation and liberalization of the agricultural sectors are among the other factors that influence beef processing (Norte & Noudic, 2009).

2.2.1 Status of Beef consumption in Kenya

The livestock industry in Kenya makes up 3.3 percent of the country's GDP and mostly consists of the production of dairy and meat as well as hides and skins from cows, sheep, and goats. Up to 80% of the meat consumed locally is red meat, which includes beef, mutton, goat, and camel meat. Over 9 million beef cattle are reared in Kenya, the majority of which are on rangelands (www.livestock.go.ke, accessed on February 20, 2012). Between 1997 and 2025, Africa's estimated meat consumption is expected to

more than double, from 5.5 to 13.3 million metric tonnes. What is known as the "Livestock Revolution" is partially responsible for this surge (Bett *et al.*, 2012). However, by the year 2050, it is anticipated that the emerging nations would consume a total of 326 million metric tonnes of meat, or an average of 44kg annually per person. Between 1991 and 2007, Kenya's domestic meat consumption more than doubled, from 361,115 tonnes to 606,169 tonnes. Meat intake per person increased from 14.90 kilograms in 1991 to 16 kilograms in 2007. By 2050, the FAO predicted that Sub-Saharan Africa will consume an average of 22 kg per person. According to these figures, beef has the greatest consumption, followed by chicken, fish, and pig meats, which have the lowest (Bett *et al.*, 2012). This is in accordance with consumption data gleaned from a cross-sectional survey conducted in a subset of six counties in Kenya, namely Kakamega, Siaya, West Pokot, Turkana, Bomet, and Narok. Respondents from 930 households in both urban and rural areas were interviewed using structured questionnaires.

Over the years, there have been various incidents of illness, even death caused by consumption of contaminated beef such as in the Baringo and Nakuru incidences of 2016 and many other cases occurring in small villages that go unreported.

2.3. Types of Slaughter Premises

Three types of slaughterhouses are typically found in developing nations: contemporary abattoirs, outdated slaughterhouses and slaughter slabs, and improvised facilities.

2.3.1. Modern abattoirs

When it comes to design, equipment, and services, modern abattoirs are the most innovative and perfect. These abattoirs are run on industrial lines with a wide variety

of services including cold storage, processing, by product usage, and waste recycling operations. They are frequently developed and administered by central governments with foreign technical aid and administration. Although occasionally some of their manufactured goods (and by-products) are channeled into local sale in substitute for imports, several of them have export aims notably in chilled and frozen meat. Few contemporary abattoirs in impoverished nations provide direct public consumption slaughter because they are for-profit businesses with little interest in providing low-value services (Shaibu *et al.*, 2021). A good example is the slaughter slabs run by Kenya Meat Commission (KMC).

2.3.2 Old slaughter slabs

The majorities of public slaughters take place at the slaughterhouses and slaughter slabs. These locations only provide facilities for licensed butchers and dealers to slaughter cattle in line with public health, inspection, and marketing laws and regulations for a charge. As a result, slaughterhouses and slaughter slabs are run as businesses by municipal and local administrations, and their areas of operation are frequently restricted to big towns and built-up regions (Shaibu *et al.*, 2021). This is the type of slaughter slabs found in Eldoret town whereby its operations is done by the County Government of Uasin Gishu, Department of Veterinary Services. Farmers pay a fee for the services at the slaughter slab. It is managed by County Governments'.

2.3.3 Makeshift

For lack of a better description, the third type of slaughter locations, the "makeshift," includes any location that a butcher or a community may deem practical for the procedure, including open, barren areas, shaded by trees, or converted houses or rooms. These facilities and their goods are not inspected, measured, or subject to trade

or health standards because they are largely privately held and operate without any formal authority or authorization. Village and rural settings are characterized by improvised slaughterhouses. But occasionally, they might be found at the outskirts of bigger towns or in the suburbs. They are occasionally thought to have ties to the illicit trade in cattle as well as the killing of injured and unhealthy animals in the latter. Their presence and operation is not recommended since they contravene evident standards for slaughterhouse architecture, equipment servicing, and cleanliness (Shaibu *et al.*, 2021).

2.4 Hygiene of animals presented for slaughter

Animals that are delivered for slaughter should be sufficiently clean to ensure sanitary dressing and killing. The holding circumstances of animals that are about to be put to death should reduce cross-contamination with food-borne diseases and enable quick slaughter and dressing. Ante-mortem examinations ought to be risk- and science-based as necessary, and they ought to consider all pertinent data from primary production levels. When possible, pertinent data from primary production and antemortem inspection findings should be included in the control process (Nkosi *et al.*, 2021).

2.4.1. Hygienic dressing and handling carcass

Care should be taken not to soil material from the hides, skins, and pelts as well as from the internal organ contents from contaminating the edible parts of the corpse. By good storage, microbial development on the outside of meat or carcasses is impeded. Any part of the carcasses judged unfit for ingestion by humans should be eliminated from the carcass process (Nkosi *et al.*, 2021).

2.5 The Humane Method and Conventional Techniques of Slaughter

The humane method and related techniques of slaughter are advised for usage since they allow for safer, more cost-effective, and sanitary operations and a desirable quality result, unless prohibited by rituals and established customs. Important steps in using the strategy include those listed below (Selvan et al., 2007)

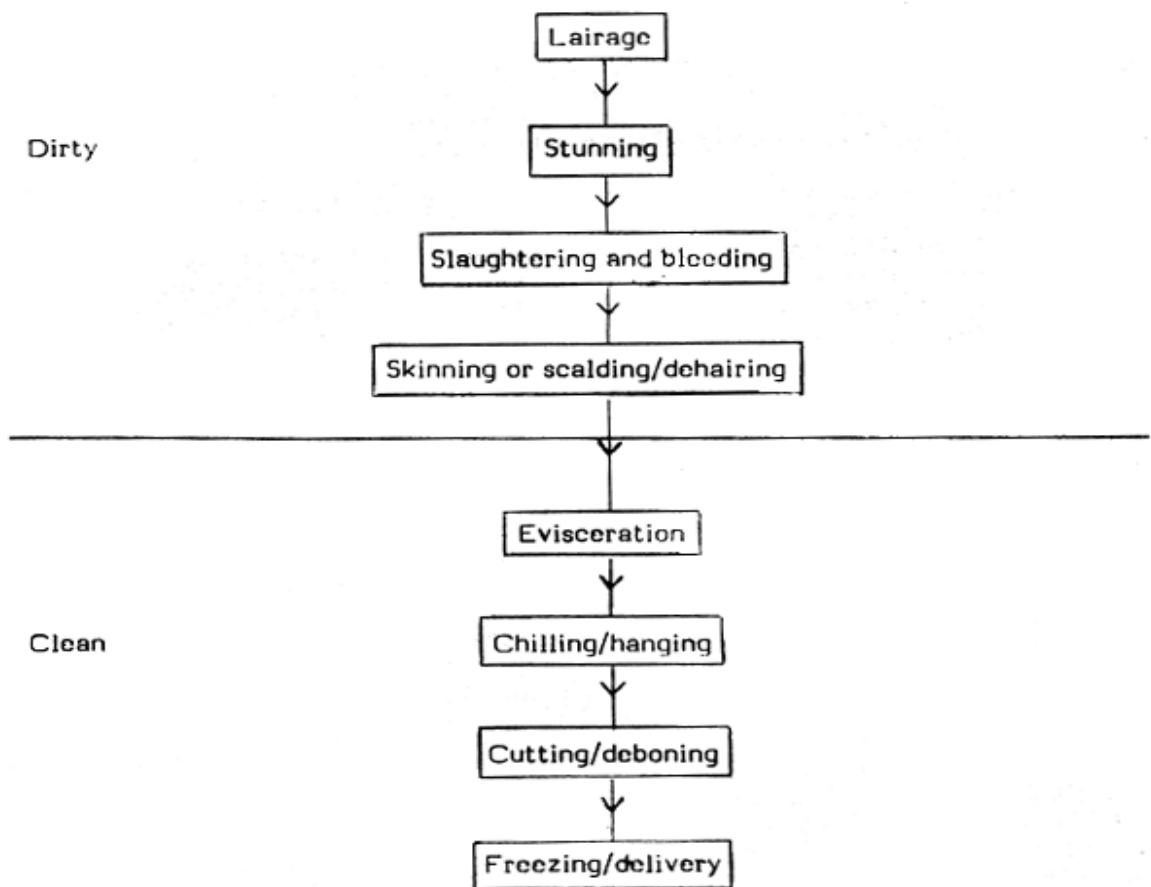


Figure 1.1: Process of slaughtering (Nkosi *et al.*, 2021).

2.5.1 Stunning

The current mechanical way of stunning animals involves firing and comes in two ways: using a captive bolt gun that concusses the animal's skull to render it unconscious. Using a weapon or free-bullet rifle that can penetrate. In order to

immobilize cattle, compression stunners with or without piercing heads that use air rather than cartridges are also used (Browning, & Veit, 2020).

In several nations, it is currently against the law to use a hammer to pound or strike an animal on the head, however in dire circumstances; a fast stroke to the back of the neck with the hammer can be used to paralyze tiny ruminants.

Electric stun guns are frequently employed on small animals, particularly pigs. The simplest approach uses electrodes or probes designed in the shape of tongs with insulated handles that are applied for 1-4 seconds between the animal's ear and eye. The animal must wait around 5–7 seconds before being bled. For sheep and goats, the voltage employed is between 60 and 70 volts/AC 50–60 cycles.

The term "chemical stunning" refers to the use of carbon dioxide to immobilize animals prior to bleeding. Similar to the electrical approach, CO₂ stunning is a pricey technique but is frequently applied to small livestock, such as sheep and goats. The animals are guided alone or in pairs into a pit, tunnel, or compartment where CO₂ is delivered for 60 seconds at a concentration of 65 to 75 percent (70 percent is ideal). The animals are not smothered, but they swiftly become asleep. They are then quickly removed and bled. The fact that startling just dulls consciousness is reinforced once more. Therefore, life is still there, and this includes the heart's pumping function, which forces blood out of the body and causes bleeding (Grandin. & Cockram, 2020).

2.5.2 Bleeding

Animals that are stunned need to be set up for bleeding first. By shackling an animal below the hock of one of its hind legs and raising it (head down) to a convenient height, a vertical or hanging posture is created. The animal can also be laid horizontally for bleeding on a concrete slab or a strong plastic pallet (Selvan *et al.*, 2007).

The real bleeding procedure is performed by inserting or sticking the sticking knife into the neck beneath the first neck bone and below the jaw bone. The goal is to cut the neck's blood arteries and allow blood out. The oesophagus might be slit and the viscera polluted if the sticking is done at a lower level than allowed (Selvan et al., 2007).

The bleeding should be as thorough as possible; for sheep and goats, this typically takes 2 minutes. Slow death and little bleeding may indicate that just the neck veins were severed during sticking, or more particularly, that the arteries leading to the head were not severed. However, the skill is improved via expertise and practice. Bleeding while being hoisted is more sanitary and advised. Additionally, it makes it easier to collect blood for later use.

2.5.3 Skinning

When removing the skin off sheep and goats, the skin is first removed around the leg to expose and release the hock tendon, which is then used to hang the carcass. Legging is the term for this procedure. The removal of the complete skin and preparing the animal body for evisceration constitute the second phase, which is referred to as "pelting" (after the term pelt typically given to the skins of lambs and other wool or fur-bearing animals). Because the bodies of tropical sheep and goats are covered with hair rather than wool, the word "skinning" is more applicable for them. Similar to stunning, skinning may be performed in either a horizontal or hanging posture, with the latter being more appropriate for larger facilities with larger orders and railing facilities or equipment for separate corpses one after another.

2.5.3.1 Eviscerating

The following stage is to cut open the animal body to release the contents and produce the carcass once the exterior structures, such as the skin, feet, and head, have been removed. Simple but well-planned precautions are taken to prevent infection of the carcass from unintentional wounds or punctures of the stomach and intestines. It is crucial for this that the corpse be left alone or is set up to hang. The knotted bung or rectum is sliced around, entirely freed from all attachments, and dropped into the pelvic cavity as the initial stage of evisceration (Bjørkdahl, & Syse, 2023).

The breastbone is sliced or chopped with a saw or cleaver along its midline and all the way to its tip. The skinning knife is used to make a second incision from the cod or udder, this time into the breast cut along the midline. According to custom (or the lower region of the abdomen), the pelvis is not severed (Selvan et al., 2007).

The ureter connections to the kidneys are severed as the intestines are further loosening, and the stomach and intestinal bulk (also known as the paunch) is then slightly pushed out of the midline hole. The spleen and kidneys of sheep are frequently left in the carcass in developed nations. At this point, the liver is held out and separated from its supporting tissues before being extracted together with the abdominal cavity's liberated contents and dumped into a paunch truck. When removing the gallbladder from the liver, care must be taken to prevent spilling its bitter contents over the corpse and tainting the meat (Selvan et al., 2007).

The evacuation of the chest cavity's contents completes the evisceration process. The pluck (heart, lungs, trachea, and oesophagus) can be removed as a group by severing the thin muscle layer or diaphragm dividing this chamber from the abdomen. To swell the shoulders, the foreshanks (i.e., the upper and lower arms) are joined by a tendon

or a thick rubber band. After being cleaned, the corpse is then railed to the examination bay (Gill, 2007).

2.5.4 Postmortem Inspection

The tongue, head, pluck, liver, and paunch are among the animal body parts gathered for examination in addition to the corpse. In the suspended state, the carcass is kept stationary. The stomach and intestines stay on the truck, while the visceral organs, such as the head and tongue, are hung in a separate area. The organs of each cadaver are used to identify it for examination. Although qualified public health inspectors are sometimes used, professional veterinarians often conduct inspections. It is their responsibility to inspect the slaughter products for signs of illness and irregularity and remove them from the public meat supply (Selvan et al., 2007).

2.5.5 Carcass Refrigeration

After the slaughtering procedure, carcasses are refrigerated to suppress the growth of spoilage and/or harmful bacteria that may still be on the carcasses and so lengthen their shelf life, in addition to meat maturation purposes (Dave & Ghaly, 2011). The quick chilling phase, which involves rapidly lowering the carcass temperature, is usually followed by a second phase of cold storage, which is meant to keep the carcasses' low temperature. Savell et al., (2005) thoroughly studied several carcass chilling techniques. Before being transferred to the boning hall in commercial slaughterhouses, carcasses are typically cooled for 48–72 hours. However, to enhance the quality of the meat, the carcass cooling period may be extended beyond 72 hours. At refrigeration temperatures, microorganisms experience low-temperature stress, which inhibits their ability to proliferate bacteria on corpses. In reality, the bacterial lag phase lengthens

as the temperature drops, while the growth rate slows and the total number of cells may decrease (Beales, 2004).

2.5.6 Transportation of meat

Carcasses and meat delivery vehicles should be seen as an extension of refrigerated storage. The goal must be to keep the temperature of the meat at or close to 0°C. Before loading, the meat needs to be refrigerated to 0°C. Stockinettes must be clean if they are to be placed on corpses. Meat trucks should only transport meat, according to Selvan *et al.* (2007). Typically, the refrigeration is created by blowing air over pieces of dry ice made of carbon dioxide or liquid nitrogen that have been injected into the compartment. These vans' temperatures may be regulated and managed to reduce temperature increase and prevent condensation on the surface of the meat (Selvan *et al.*, 2007).

Dry ice can be used to cool insulated vehicles that lack refrigeration. Although this is a passable substitute for a refrigerated vehicle, the temperature cannot be regulated (Selvan *et al.*, 2007). Particularly in warmer areas, uninsulated vans and open trucks shouldn't be thought of as viable modes of transportation for beef. Along with the temperature abuse, condensation will happen when the meat is put back into the refrigerator, and in open vehicles, it is vulnerable to insect assault. It is important to load and unload rapidly. Dry-ice blocks should be put in the partially filled vehicle if there are any unavoidable delays. Consequently, in accordance with the Meat Control Act of 2012 (www.kenyalaw.org. Accessed on July 26, 2013), the following requirements should be met by any vehicle used to carry meat in order to prevent meat contamination:

The freight compartment and the driving cab must be entirely separate and it's crucial that the freight compartment is in good condition. The freight compartment must be completely enclosed (dustproof), without joints, and continually lined with a surface material that is non-toxic, non-absorbent, simple to clean, rust free, and all of the above. It should also be insulated and/or mechanically chilled such that the meat's temperature does not increase by more than 5 °C each hour or by more than 2 °C while being transported locally (a distance of less than 200 km).

Under all circumstances, vehicle must be equipped with beams and stainless steel hooks suspended above the floor for the purpose of transporting sides or quarters. Upon examination, no square centimeter of the specified surface must have more than 100 live microorganisms present. According to the same regulation, the following transit methods are necessary to further avoid contamination:

Rough tripe, intestines, and other animal parts that might contaminate other meat or have an unpleasant odor must be transported in containers that are waterproof, simple to clean and disinfect, and have tight-fitting lids that won't come off during transit.

No individual is permitted to remain in the compartment where meat is kept during travel, and meat must be stored such that it is not directly in touch with the bottom of a carrier or container. Any person handling meat during any loading or unloading must wash their hands and other exposed body parts in clean disinfectants prior to doing so, and they must wear clean protective clothing that covers all body parts other than their hands that may come into contact with the meat. The said clothing must not be worn during the actual transport by either the driver or any attendant (Nastasijevic *et al.*, 2023).

2.6 Microorganisms found in meat

Freshly slaughtered meat should be largely free of germs, although tissues that were examined aseptically did contain some, typically fewer than 10 cfu kg⁻¹ (Osama and Gehan; 2011). However, there is proof that these numbers can rise in stressful situations and if the animal is ill, as is the case with animals that have *Brucella* infection. The majority of meat contamination comes from the animal's highly colonized parts, such the skin (fleece) and gastrointestinal system, and the kinds and quantities identified will depend on the animal's surroundings and native microflora (Steven *et al.*, 2019).

Micrococci, *staphylococci*, *pseudomonads*, *yeasts*, *molds*, as well as organisms derived from excrement and dirt, are among the many microbial populations present in animal hides. The surface microbial counts after dressing and chilling are normally in the range of 10²-10⁴ cfu cm², and they are typically greater in sheep carcasses than in beef, and much higher in pigs. Soon after the animal is killed, the fur on sheep, cattle, and pigs is completely burnt in several regions of the world. The technique has historically been used to taste the meat, but it should also be praised and considered as a first step in lowering the bacteria populations on the skin that may otherwise taint the flesh (Steven *et al.*, 2019).

Brucella abortus in cattle, *B. melitensis* in sheep and goats, *B. suis* in pigs, and *B. canis* in dogs are recognized human infections that can produce undulant fever and are linked to a specific animal host. Although the illnesses are spread by direct contact with diseased animals, they can also be consumed by humans who consume the animals' milk, dairy products, and meat (Steven *et al.*, 2019).

The way that meat animals are reared, butchered, and processed has a significant impact on the bacteriological state of the carcass meat (Osama & Gehan, 2011). Since it is very challenging to acquire clean meat from unclean animals, it is crucial that only relatively clean animals be given for slaughter. Therefore, animal husbandry, weather and temperature, modes of transportation, and storage at the slaughterhouse all affect how clean the animals are. According to Biswas et al. (2011), cattle from feedlots may transport more faecal bacteria than soil organisms. Food items, particularly meat, are a perfect source of substrate for microorganisms and often have pH levels in the range required for growth. During harvesting, processing, distribution, and preparation, food items, including meat, are contaminated with microorganisms that are found in the soil, air, and water (Enabulele & Uraih, 2009). In certain animals that appear to be healthy, the liver, kidneys, lymph nodes, and spleen may contain a variety of microorganisms. These germs, together with those from contamination during slaughtering, can move from the circulatory system to the skeletal muscles (Marriot, 2004). Some organisms enter the blood and lymph circulation of an animal over its lifetime, but a natural defense mechanism guarantees that there is a balance between bacterial assault and bacterial removal (Bekker, 1998). According to(Unc and Goss 2004), the micro flora of meat will typically be that of the feedlot or barnyard, which is present on the animal's exterior surfaces and can contaminate the meat by direct contact with air, water, dirt, manure, worker hands, and tools. Although microorganisms have been discovered in lymph nodes, bone marrow, and even flesh, it has been claimed that the healthy interior section of meats has little to no such creatures (Okonko et al., 2010). From the lymph nodes of red-meat animals, *Staphylococci*, *Streptococci*, *Clostridia*, and *Salmonella* have been identified. However, during bleeding, handling, and processing, external sources are where the

significant contamination occurs. The outside of the animal (hide, hooves, and hair) and the digestive tract serve as the primary sources of microbes during bleeding, skinning, and cutting (Selvan et al., 2007).

2.6.1 *Staphylococcus*.

A widespread Gram-positive bacteria called *Staphylococcus aureus* is present on the skin and nasal membranes of humans, other mammals, and farm animals (Pugazhendhi et al., 2020). It is an opportunistic pathogen that may cause a variety of diseases, such as toxic shock syndrome, food poisoning, and urinary tract infections. *Staphylococcal* food poisoning, a food-borne illness brought on by *staphylococcal* enterotoxins, is the biggest problem with food safety, though. Temperatures between 10°C and 48°C, a pH range of 4–9.6, and NaCl concentrations between 0–10% are the limitations for *staphylococcal* enterotoxin synthesis (Zeaki et al., 2019). As a result, enterotoxins are not formed when beef is matured at temperatures below 10°C.

2.6.2 *Salmonella*

With the exception of *S.typhi* and *S.gallinarum*, all *Salmonella* emit gas in glucose, however non-motile forms also often exist. *Salmonella* colonies injected on TSI agar butt and slants create an alkaline slant and hydrogen sulfide in the butt, respectively and are urease negative. Some species emit gas when placed on TSI agar. Richard (1970) The number of cases increased rapidly in the 1980s, reaching a peak of over 35,000 cases annually in 1992, but have subsequently gradually decreased. It continues to be the second most typical reason for food poisoning reports. Red meat, poultry, eggs, and meat products are the mainly implicated. *Salmonella* has more than 2,000 distinct subtypes, but *S. enteritidis* is the principal one that accounts for the majority of the rise in morbidity. Recently, another sub-type of *S. typhimurium* (StDT104),

which is resistant to a variety of widely used antibiotics, has appeared in both humans and animals.

Salmonella first appeared in cattle, but it has since been transferred to other species by sewage, excrement, or slurry. If animals are agitated and crowded, it can spread quickly. A little amount of the germs can make you sick. Although it can withstand freezing, cooking will destroy it. *Salmonella* is very contagious and frequently causes subsequent infections (Dean, 1980).

2.6.3 *Escherichia*.

The "poster child" of microbiologists and researchers, *E. coli* is the major member of the intestinal family. *Escherichia* are often easily recognizable and may be distinguished from other *enterobacteriaceae* members by their propensity to quickly ferment lactose with the generation of acid and gas. However, some bacteria either do not digest lactose or do so slowly. There are both motile and non-motile types. The most common bacteria found in clinical laboratories is *E. coli*. It is the leading cause of human urinary tract infections and has also been connected to illnesses in almost every other bodily system. Pathogenic strains of *E. coli* have been linked to a wide variety of ailments, including pneumonia, meningitis, and traveler's diarrhea. The natural flora of the human digestive tract includes *E. coli* but due to the production of a potent endotoxin, pathogenic strains of *E. coli* can cause severe bouts of diarrhea in people of all ages (Kyule, 2009). Numerous strains of the bacteria *Escherichia coli* (*E. coli*) can be found in the intestines of both humans and animals without harming them. But in the early 1980s, a new strain of *E. coli* O157 (commonly known as VTEC), which generates powerful, life-threatening toxins, was first discovered. Although it is

still very uncommon, there were over 1,100 occurrences (in England and Wales) in the 1996.

Additionally, there were an unusually high number of cases in Scotland, where *E. coli* O157 was the cause of a significant epidemic that claimed many lives in Lanarkshire, Scotland (Hussein, 2007). Compared to *Campylobacter* or *Salmonella*, it affects significantly fewer individuals, but it also causes more serious sickness, and it only takes a little amount of the bacterium to spread disease. Although the bacterium may be deadly to healthy adults, it is particularly dangerous to young children and the elderly. It is the primary cause of acute renal failure in children, and antibiotics cannot be used to treat it (the disease must be treated separately on each of its many fronts). It will be less likely for you to get *E. coli* if you follow the standard precautions to stay away from other types of food poisoning. But because cattle are the primary source of these germs, it is especially crucial to properly prepare goods made with minced beef, including burgers. Steaks and other meat cuts don't carry the same risk (Berry, Cutter, 2000).

2.6.4 *Proteus species*.

Proteus species is gram-negative aerobic bacteria. Their dimensions are 1.0-3.0 μ m in length and 0.4-0.8 μ m in diameter. Their capacity to go through morphological alterations of colonies is the basis for their names. *Proteus species* possess peritrichous flagella and is mobile. Swarming and an ammonia odor are two signs of a *Proteus* culture. The *Proteus* habitat is extensively dispersed throughout the natural world. *Proteus* is an opportunistic human pathogen that lives in the skin, oral mucosa, gastrointestinal tracts of both humans and animals, as well as in excrement, soil, water, and plants. Foods spoiled by *Proteus* includes canned food, uncooked meat, shellfish,

and vegetables. The presence of *Proteus species* shows that the meal in question was not cooked in sanitary conditions. The frequency of *Proteus* detection increases in the fall. *Proteus species* can thrive on the majority of culture medium as well as liquid gelatin and do not produce spores. In order for *Proteus* to develop, milk must first curdle before it can liquefy. Temperatures between 10 and 43 °C are suitable for growing some *Proteus* strains. *Proteus* prefers a temperature of 25 °C. Between 20 and 37 °C, swarming takes place. *Proteus species* break down organic materials, have proteolytic activity, hydrolyze urea, oxidative deaminate amino acids, and generate hemolysins and hemagglutinins.

2.7 The effect of pH on growth of micro-organisms

PH refers to the level of acidity or alkalinity in meat, and it plays a crucial role in determining the quality of the meat you eat. A proper pH level ensures that the meat is fresh, tender, and juicy. It also helps in preserving the meat for a longer period of time. When the pH level in meat is too high or too low, it can result in spoilage, discoloration, and even a sour taste. That's why it's crucial to maintain the right pH level in meat to ensure that it's safe and healthy for consumption.

Given that pH is expressed on a logarithmic scale, it is important to understand that the [H⁺] of natural settings ranges by over a billion-fold, with certain microbes existing at both extremes and every location in between. The majority of free-living prokaryotes may expand over a pH range of 3 units, or an approximately 1,000-fold shift in [H⁺]. The minimum pH, below which an organism cannot grow, the maximum pH, above which an organism cannot grow, and the optimal pH, at which an organism develops at its best, are the three cardinal points that define the pH range within which an organism may grow. To reflect the overall impact of changing [H⁺] on the rates of

enzymatic reaction, most bacteria exhibit an orderly increase in growth rate between the minimum and the optimum and a corresponding orderly decrease in growth rate between the optimum and the maximum pH (Polish Standard PN-ISO 4832).

Acidophiles are microorganisms that thrive at pH values that are significantly lower than neutrality 7.0 (Figure 2.1). Alkaliphiles are those that thrive in alkaline environments, whereas neutrophiles are those that thrive in neutral pH environments (Figure 2.1). Since their membranes break and their cells lyse at neutrality, obligatory acidophiles like some species of *Thiobacillus* really need a low pH for growth. *Sulfolobus* and *Thermoplasma* are two genera of *Archaea* that are acidophiles. (Polish Standard PN-ISO 4832), is the champion of low pH growth among eukaryotes.

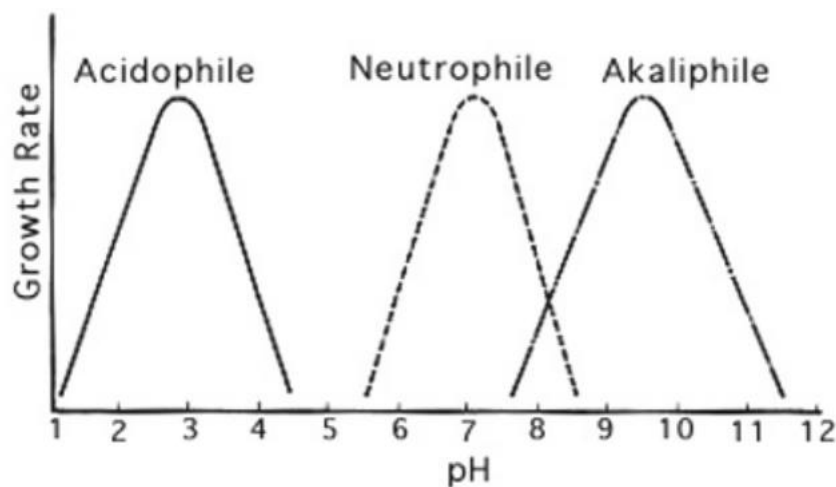


Figure 2.1. Growth rate vs pH for three environmental types of procaryotes is shown in graph 1. Over a pH range of around three units, the majority of free-living bacteria thrive. Keep in mind that the curves below and above the pH that promotes development are symmetrical. (Cooper, 2019)

Clostridium botulinum is ubiquitous and produces a toxin called botulinum with strong neurological effects. In anaerobically packaged foods with favourable pH,

temperature, water activity, and nutrients *C. botulinum* flourishes generating this toxin. Canned meals with low acidity provide this favourable atmosphere. The Good Manufacturing Practices state that *C. botulinum* development is inhibited when a product is acidified to a pH of 4.6 or below (FDA/CFSAN HFS-618).

2.8 The effect of temperature on microbial growth

Regardless of temperature, microorganisms have been discovered to flourish in almost every setting that contains liquid water. Professor Thomas D. Brock, then at Indiana University 1996, made the astounding finding that bacteria were not only thriving in Yellowstone National Park's boiling hot springs in 1966 (Malacca & Bruewicz, 2004). Procaryotes have now been shown to be growing close to hydrothermal vents and black smokers in the deep sea, at temperatures at least as high as 120 °C.

Additionally, microorganisms have been shown to flourish at very low temperatures. Some organisms may absorb water for development in super-cooled solutions of H₂O at temperatures as low as -20 degrees, and many types of life are able to thrive at 0 degrees in both domestic freezers and the seas of the Antarctic (Malacca & Bruewicz, 2004).

When considering the entire range of temperatures where liquid water exists, a specific microorganism will show a range of temperature over which it can grow, defined by three cardinal points similar to PH. The procaryotes may be divided into several sub-classes based on one or more of their cardinal points for growth. For instance, mesophiles are creatures whose ideal temperature is close to 37 degrees, which corresponds to the body temperature of warm-blooded vertebrates. Thermophiles are organisms having an ideal temperature between 45 and 70 degrees. Since they are generally carried in from their mesophilic homes and continue to thrive in the

refrigerator environment where they contaminate the food, psychrotrophs are the cause of food spoilage stored in refrigerators ((FDA/CFSAN HFS-618)

The majority of the fatty acids in the plasma membranes of psychrophilic bacteria are unsaturated, which helps them adapt to their chilly environment. Polyunsaturated fatty acids, which are often absent from prokaryotes, have been discovered in some psychrophiles, especially those from the Antarctic. Unsaturated fatty acids remain liquid at low temperatures but are also denatured at moderate temperatures; saturated fatty acids, like those found in the membranes of thermophilic bacteria, are stable at high temperatures but also solidify at relatively high temperatures. The degree of unsaturation of a fatty acid correlates with its solidification T or thermal transition stage (the temperature at which the lipid melts or solidifies). As a result, unsaturated fatty acids, such as sunflower oil, remain liquid in the refrigerator whereas saturated fatty acids, such as butter, are solid at room temperature.

The fluidity of a membrane, which directly influences its capacity to function, depends on whether the fatty acids within it are in a liquid or solid phase. Psychrophiles also contain enzymes that work at temperatures close to zero degrees, but at a slower rate. Typically, psychrophile proteins and/or membranes, which adapt them to low temperatures, do not work at warm-blooded animals' body temperatures (37 degrees), preventing them from growing at even mild temperatures (Sofos, 2003).

2.9 Factors affecting pH on Meat

The pH level of meat can be influenced by various factors, including the animal's age, diet, and breed, as well as the processing methods used.

2.9.1 Animal's age at the time of slaughter

Younger animals have a higher pH, while older animals have a lower pH. This is because younger animals have more glycogen stored in their muscles, which is converted to lactic acid after slaughter, increasing the PH. In contrast, older animals have less glycogen stored because with age, exercising muscle glycogenolysis is accelerated, resulting in a lower PH. Therefore, pH declines in meat are correlated with the enzymatic degradation of the animal's glycogen level. (Poveda-Arteaga *et al* 2023).

2.9.2 Animal's diet

Feeding animals a high-carbohydrate diet increases the amount of glycogen stored in their muscles, resulting in a higher pH after slaughter. On the other hand, feeding animals a low-carbohydrate diet decreases glycogen stores, resulting in a lower PH. Therefore, glycogen levels are greatly influenced by the amount and quality of the animal feed that it has eaten the month prior to slaughter(Poveda-Arteaga *et al.*, 2023).

2.9.3 Stress

When animals are stressed before slaughter, they release adrenaline, which depletes glycogen stores in their muscles due to muscle contraction. This leads to a lower pH, which can result in meat that is tough and dry, with a reduced shelf life. (Poveda-Arteaga *et al.*, 2023).

2.9.4 Processing techniques

These includes procedures like electrical stimulation and aging can also affect meat pH. Electrical stimulation can result in an early and rapid decrease in meat PH. This improves tenderness from a rapid loss of adenosine triphosphate (ATP), physical

disruption of the animal's muscle fiber, and a lowered PH. On the other hand, aging meat can increase meat pH and enhance flavor. For example, dry-aged meat has a higher pH. This is thought to be from the formation of nitrogen compounds in the meat. (Poveda-Arteaga, *et al* 2023).

2.10 Sources of food contamination

Food contamination can come from a primary source, such as an infected food animal or its excretions or secretions, or it can come from a secondary source, such as contaminated food handling (Marriot *et al.*, 2006).

2.10.1 Primary contamination

A food animal may be killed when it is either polluted with chemical or other residues or diseased with a microbial infection. This occasionally provides a risk to the employees in the stockyard or the slaughterhouse, but more frequently it endangers the customer. A very tiny portion of these instances are discovered during ante-mortem examination. The use of contaminated water, unhygiene handling techniques, the use of infected tables to display meat intended for sale, and the use of contaminated blades and other cutting tools during cutting operations cause meat contamination at abattoirs and retail meat shops (Fasanmi *et al.*, 2010). By increasing the likelihood of exposure and infections, the amount of time animals are kept in the abattoir before being slaughtered can have an impact on the pathogen load. The pathogen burden is influenced by the cleanliness of the walkways, pen floors, railings, feed, and water (Galland, 1997). In the later phases of the procedure, contamination of corpses is mostly caused by dirt, soil, bodily discharges, and animal excreta in holding pens or lairages. Whether the animals are healthy and have passed the ante-mortem check has no bearing on whether this occurs. According to Adzitey *et al.*, (2011), probable

sources of contamination include chopping boards, skins, meat handlers, containers, vehicles used to carry corpses, intestinal contents, cutting tools, and the area where meat is sold. According to Ali *et al.*, (2010), knives, wooden boards, and weighing scales from retail stores are bacterial contamination sources, notably for *Shigella* and *Staphylococcus species*. According to Akinro *et al.*, (2009), with insufficient slaughtering and disposal facilities, the slaughterhouse turns into a source of contamination and attracts domestic and wild predators, rats, and flies, which are disease vectors.

In order to keep meat fresh for a long time and avoid spoiling after lengthy room temperature storage, refrigerators and freezers are necessary storage equipment. Live calves intended for slaughter may get contaminated on farms, during meat transit to the butcher, or while the animals are aging at the abattoir. According to reports, the primary causes of *Salmonella* and virulent *E. coli* infections in cattle at the farm level are contaminated feed and water (Millemann., 2008).

2.10.2 Secondary contamination

Infected people or live animal carriers of pathogens, dirt, tools, hands, nasal secretions, contaminated wounds, polluted water, insects, or feed additives are all potential sources of secondary infection. However, the preparation of food for consumption is the stage of the food chain where infected persons are most usually blamed for contamination (Hubbert *et al.*, 1996). Employees are the main source of contamination, and those who don't practice good hygiene might spread dangerous bacteria and deterioration to the food they handle.

These microorganisms are spread to food during processing, packaging, preparation, and service by touching, breathing, coughing, or sneezing. Employees come into

contact with these microorganisms through their work and other aspects of the environment (Biswas *et al.*, 2011; Cohen *et al.*, 2006; Selvan *et al.*, 2007). They also carry microorganisms in their hands, hair, nose, and mouth. Personal hygiene is therefore crucial in the prevention of meat contamination since a healthy human body contains up to 200 different types of germs (Featherstone, 2003)

Retail cuts and ground beef in retail meat display cases may possibly lose some of their shelf life as a result of carcass contamination that was not eliminated by trimming or washing at the time of slaughter being distributed to newly exposed surfaces (Stivarius *et al.*, 2002; Marriot, 2004). Bacteria on the surface of the meat can spread throughout the product during the chopping and grinding process (Siriken., 2004; Salihu *et al.*, 2010). The final shelf life of ground beef is influenced by the bacterial content of the trimmings, sanitary processing conditions, processing duration and temperature, and storage temperature (Siriken, 2004; Salihu *et al.*, 2010). Because of the large surface area that grinding provides and the fact that these organisms are dispersed throughout the product, ground meat is an extremely favorable growth medium compared to uncut meat, where the bacteria would be nearly exclusively on the outer surfaces (Siriken, 2004; Salihu *et al.*, 2010)

2.11 Risk associated with informal slaughter

Cholera, botulism, shigellosis, and typhoid fever are just a few of the pathogens that may develop and spread easily through food. Due to poor cleanliness, the informal food trade and the informal killing of animals endanger the public's health. The environment is also negatively affected (Unc & Goss., 2004).

Costs associated with public health are also increased by informal marketing since items that don't adhere to standards for food safety pose significant dangers. The

absence of quality control and the ability to sell meat and by-products that ought to have been thrown away save money for butchers who choose the informal market. When it comes to the meat sector specifically, the utilization of animals that would have been rejected owing to poor quality is the main financial benefit for the butcher of selecting informal slaughter. But the financial savings that benefit the butcher can have an immediate negative impact on public health (Abu-Samra *et al.*, 2007).

2.11.1 Impacts of contaminated slaughtered meat on human health

Food-borne illnesses are a significant public health issue in both industrialized and developing nations, while the financial and health implications are sometimes hidden by a lack of data (Tauxe., 1997; WHO, 1995, WHO 2013). High rates of morbidity and death are caused by them in the general population, particularly in high-risk populations such babies, young children, the elderly, and those with impaired immune systems (WHO, 1995, WHO 2013). While some affluent nations have pretty good data on the effects of food-borne illnesses, poor countries seldom have access to comparable figures due to the absence of monitoring methods for gathering trustworthy data (Schneider, 2004). Since the majority of patients go to rural clinics where treatment is provided by nurses and little records are kept, it is impossible to establish what percentage of these disorders may be attributed to eating tainted meat. Due to cultural stigmas surrounding autopsy, the reasons of mortality in rural parts of developing nations are rarely probed (McCrinkle, 2004).

Accurate estimates of microbiological food-borne illnesses are difficult to come by, even globally. It is estimated that there are 76 million incidents of food-borne illness in the USA annually, leading to 325,000 hospitalizations and 5,000 fatalities. Food-

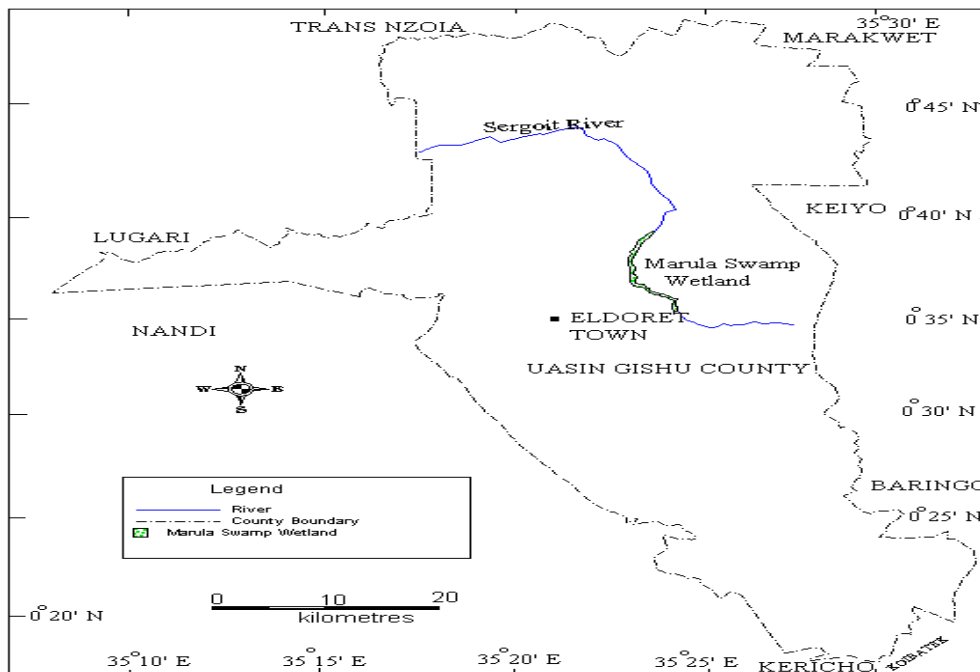
borne illnesses caused 2,366,000 cases, 21,138 hospitalizations, and 718 fatalities in England and Wales (Adak *et al.*, 2002; Mead *et al.*, 1999).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site of Study

The study was carried out in Eldoret town and its environs located at $0^{\circ} 31' N$ latitude and $35^{\circ} 17' E$ longitude, Uasin Gishu County (Figure 3.1). The governance of the County is divided into six constituencies and 27 wards. Major peri-urban areas around Eldoret town are Langas to the south, Munyaka to the east, and Huruma to the north. The slaughter houses selected for this study were Kaburwo in Langas ($0.47^{\circ} N 35.26^{\circ} E$), Maili nne around Huruma area ($0.57^{\circ} N 35.28^{\circ} E$), Cyrus in Munyaka ($0.52^{\circ} N 35.31^{\circ} E$), Eldoret Main very close to the town ($0.53^{\circ} N 35.2^{\circ} E$) and Teresia in Kuinet ($0.65^{\circ} N 35.3^{\circ} E$). All the three main peri-urban centres were represented by one selected slaughter house. The additional two, Eldoret main is the major one serving the town while Teresia is relatively an outskirts abattoir.



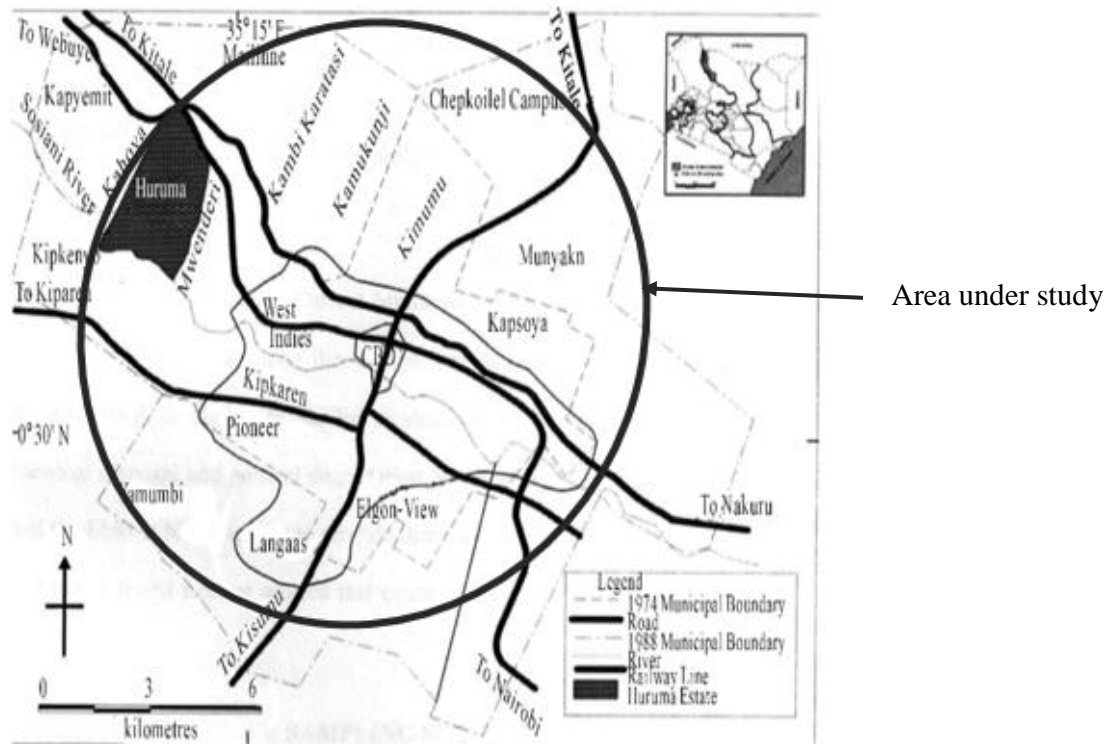


Figure 3.1. Uasin Gishu County Maps

(Source: Department of Geography, Moi University)

3.2 Methods

During the slaughter and dressing processes, all fresh meat becomes infected with some of these bacteria may include pathogens (these are microorganisms that cause food illness). Microbiological testing is a component of the Hazard Analysis Critical Control Points (HACCP) system. In order to confirm the approach utilized for managing microbiological contamination of goods, testing is employed to study the microbiological impacts of the operations inside or influencing any process (Brown *et al.*, 2000). The enumerator and indicator organism are required for HACCP microbiological testing.

3.2.1 Methods for sample collection

A destructive sample collection procedure was adopted as recommended by Hazard Analysis and Critical Control Point regulations (2002). A 5cm² portion of tissue from the cadaver. The sample was used as a template and placed in sterile tins that were labeled. Individual beef, chevon and mutton samples were gathered in each killing slab and butchery (Plate 3.1). The muscle taken .were from cattle i.e. neck, Sheep i.e. flank, goat i.e. thorax lateral.



Plate 3.1: Carcasses of the slaughtered animals as they hung in the slaughter house (a) and butcher's shop (b). (Source: Author, 2022)

3.2.2 Sample collection

At the slaughterhouse and butchers, meat samples were aseptically collected and placed in sterile plastic bags. These were then brought to the University of Eldoret laboratory for processing and examination. Appropriate tagging of the samples with the type, of sample, collecting locations and the date of collection was recorded.

3.3 Sample Processing

One gram of each of the meat samples was mixed for 2 minutes in 0.4ml of 0.1 percent buffered peptone water (diluent). Three replications was made per sample. Serial dilutions were made by adding 1ml of the preceding dilution to 9 ml of the sterile diluents was done. The pour plate method was used, and the samples were incubated at 37 °C for 24 hours. Total plate count (TPC) and the presence of *Escherichia coli*, *Staphylococcus species*, and *proteus species* were determined in the samples.

3.3.1 The pH and temperature reading

This was done out in accordance with (FAO) food and nutrition report (FAO, 2018)

Using a pH meter (Hanna Edge HI2020-01 USA), the pH and temperature of each sample were determined before the samples were cultured for bacterial counts using nutrient agar. Each tissue of the carcass was placed on a pH meter, and value readings were taken. PH and temperature were determined at the collection site.

3.3.2 Determination of Total plate count

This was done in accordance with (FAO), Food and Nutrition Report 14/4 rev 1, 1992 (14/4 rev, 2014). The pour plate method was used to get the total viable count of each sample dilution (10^{-4}) and was put onto pour plate agar. One ml of each sample dilutions was added to sterile petri dish and pour count agar added at mixed thoroughly. The preparation were then allowed to gel at incubated at 37°C. After incubating the plates, all growing colonies were observed and counted. Average counts obtained were multiplied by the dilution factor and expressed as Colony Forming Units (C.F.U/g) (Fawole and Oso, 2001). Conclusion of the bacteriological quality of meat was based on recommendations by Meat HACCP (Scotland) regulations 2002 N0.234 on acceptable and unacceptable total plate count on meat (Table 3.2).

3.3.3. Gram staining

This was done according American Society for Microbiology gram stain protocol (Gephardt et al, 1981, Feedback from ASMCUE participants, ASMCUE, 2005)

A suspension of loopful sample was smeared on glass slide. Then the slide was air dry and heat fixed. Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water. Then flooded with the gram's iodine for 1 minute and washed with water. Then, washed with 95% alcohol for about 10-20 seconds and rinsed with water. Safranin was added for about 1 minute and washed with water. Air and blot dry and observation were made under microscope. Results were interpreted as Gram Positive: Blue/Purple Color

Gram Negative: Red Color

3.4 Isolation of indicator bacteria

3.4.1 Detection of *E.coli*

This was done in accordance with FAO, Food and Nutrition Report 14/4 rev 1, 1992. (Review 2018). Ten grams of each meat sample was weighed and homogenized in 90mls of buffered peptone water. One milliliter of the portion was added to Lauryl tryptose (LT) broth and incubated at 35°C for 48 hours, with gas generation monitored at 24-hour intervals. The gassing LT tube was gently stirred, and a loopful of each suspension was transferred to Eosin C medium tubes and incubated at 45.5°C for 48 hours, with gas generation monitored at 24-hour intervals. Confirmation of *E coli* was accomplished by streaking a loopful of the suspension from the gassed EC media on Levine's Eosin-Methylene blue Agar (L-EMB) and incubating the plate at 35°C for 18-24 hours before looking for *E. coli* colonies. Two representative colonies from each L-EMB plate were selected and transferred to PCA slants for biochemical testing; the

plate count agar (PCA) slants were incubated at 35⁰ C for 18-24 hours. Other biochemical tests performed (Table 3.1)

Indole test: After inoculating Glucose Peptone broth with the test organism and incubating it at 37°C for 24-48 hours, 0.5mL of Kovac's reagent was added and gently stirred. A red ring on top denoted a positive test (Plate 3.2 a)

Table 3.1: Biochemical reactions of *E. coli* isolated from meat samples

Substrate	Positive	Negative	<i>E.coli</i>
Glucose	Yellow	Red	Negative
Hydrogen Sulphide	Blackening	No blackening	Negative
Urease	No colour change	Change to pink	Negative
Simmon citrate	Growth, Blue in colour	No growth, No colour change	Negative
Indole	Change colour red	No colour change	Positive
Gram stain			Negative
Oxidase	Colour change to blue	No colour change	Positive

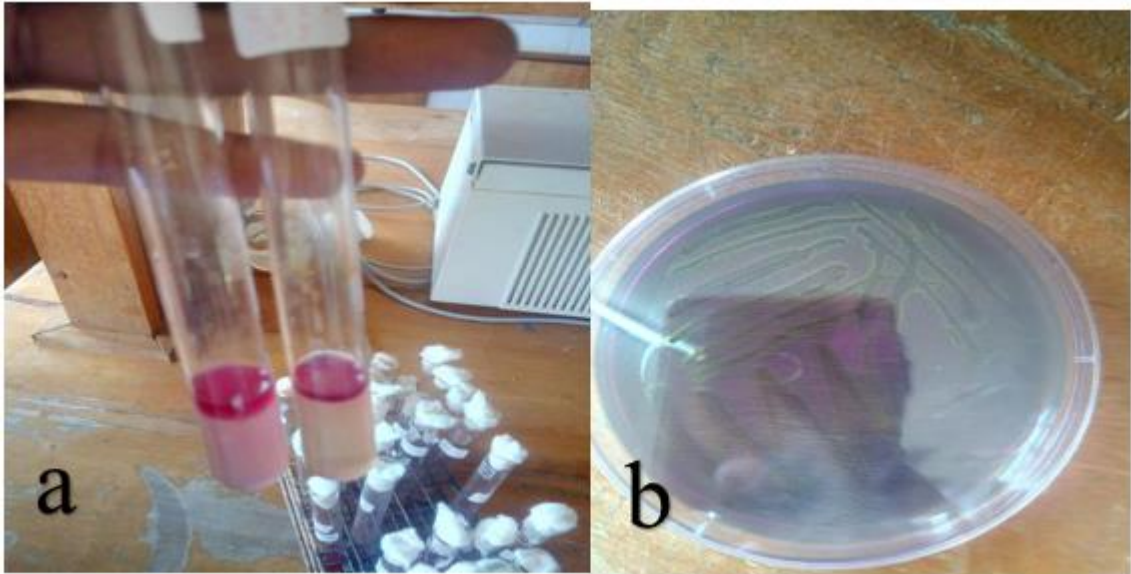


Plate 3.2. Plate 3.2 a; Indole Positive test, 3.2 b: The characteristic green sheen denoting a positive result for *E. coli* on Eosin Methylene Blue (EMB) agar. (Source: Author, 2022)

3.4.2 Detection of *Staphylococcus aureus*

This was done in accordance with the FDA bacteriological analytical protocol 2020. One ml of sample solution was aseptically transferred to 3 plates of Baird-Parker agar, spreading 1 ml of inoculum equally to 3 plates (e.g., 0.4 ml, 0.3 ml, and 0.3 ml) for each dilution to be plated. Using a sterile bent glass streaking rod, inoculum was spread over the surface of the agar plate. The plates were kept upright until the inoculum was absorbed by the agar (approximately 10 minutes on thoroughly dried plates). In cases where inoculum was not quickly absorbed, the plates were positioned upright in the incubator for 1 hour. The plates were inverted and incubated for 24 hours at 37°C. Below is a series of biochemical tests that were carried out to determine the presence of *S. aureus*.

Catalase test: A bacterial colony was picked up with a toothpick or a platinum loop and mixed with a drop of hydrogen peroxide (10% v/v in water) taken on a glass slide. The effervescence indicates the presence of the catalase enzyme. (Plate 3.3a)

Coagulase test. One or two colonies of organism was emulsify in one drop to make thick suspension of bacteria. A loopful of plasma was added to both the suspension and saline drop and mixed gently. Clumping of mixture was detected within 10-15 seconds.

Lactose test. An inoculum from a pure culture was transferred aseptically to a sterile tube of phenol red lactose broth. The inoculated tube was incubated at 35-37 C for 24 hours and the results are determined. A positive test consists of a color change from red to yellow, indicating a Ph change to acidic. (Plate 3.3b)



Plate 3.3. Positive tests for Catalase (a) and Lactose (b). (Source: Author, 2022)

3.4.3 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 slant and butt with hydrogen sulphide production on TSI were characterized as *Proteus vulgaris* and were distinguished from *Proteus mirabilis* as Test/substrate. The following is a detailed description of the tests.

Triple sugar iron agar test

Using sterile technique, each sample was inoculated into its appropriately labeled tube by means of a stab and streak inoculation. The last tube served as control. The tubes were incubated for 18 to 24hrs at 37°C. (Plate 3.4b)

Simmons citrate. Direct inoculum streak was used to slant the medium from the bottom up in a fish-tail motion. Inoculum was ensured it's not too heavy. Then it was incubated aerobically at 35°C. Examination was done between 24 and 48 hours. (Plate 3.4a)



Plate 3.4: Positive Simmons citrate test (a) and positive TSI test (b).

(Source: Author, 2022)

3.5 Characterization of Dominant Microorganism

After enumeration, from plate count agar (total aerobic mesophilic bacteria), about 5 colonies were picked randomly from countable plates and inoculated into tubes containing about 5ml nutrient broth. These were then incubated at 30°C overnight. The cultures were purified by repeated streak plating and characterized using morphological and biochemical tests (Bergey & Holt 2013)

3.5.1 Morphological Characterization of Dominant Bacteria

From overnight pure broth culture, the wet mount was prepared on a microscope slide and stained using methylene blue. The stained microbial cells were then observed under a light microscope using an oil immersion objective (x100). The morphological criteria considered during the observation were cell shape (spherical, rod, spiral, etc.) and cell arrangement (single, pair, chain, clusters, and tetrads) (P. Harley 2002).

Table 3.2: Acceptable ranges of bacterial counts on meat sample (Source: Meat HACCP (Scotland) Regulations 2002 No. 234)

<i>Daily log mean Values (cfu/cm²)</i>	<i>Acceptable Range</i>	<i>Marginal range (>M)</i>	<i>Unacceptable range (></i>
Total Viable Counts (TVC)	<i>Cattle/sheep/Goat</i> < 3.5 log	<i>Cattle/pig/sheep/goat</i> 3.5 log (pig: 4.0 log) log	<i>Cattle/pig/sheep/goat</i> > 5.0 log
Total Viable Counts (TVC)	< 3.5 log	1.5 log (pig: 2.0 log) log (pig:3.0 log)	> 2.5 log (pig > 3.0 log)

Values for the number of colonies for testing of surfaces (Source: Meat HACCP (Scotland) Regulations 2002 No. 234)

Acceptable range	Unacceptable range
Total viable Counts (TVC) 0 – 10/ cm ²	> 10/ cm ²
Enterobacteriaceae 0 – 1/ cm ²	> 1/ cm ²

3.6 Data Analysis Methods

Analysis of variance (ANOVA) was used to establish any significant differences in the values of pH and temperature among all the abattoirs and butcheries. Two way ANOVA was used to assess the differences in the colony forming units among the

different abattoirs and the different carcasses. Correlation analysis was done to assess the influence of the physiological conditions measured on the colony forming units. All the analysis was done using Ms. Excel 2013. Butcheries were coded as B1, B2, B3, B4 and B5 while slaughter houses were named in relation to the region.

3.7 Research Permits

Authority to conduct research was obtained from National Council for Research and Technology Institute Ref. No706179 in Nairobi dated 25 April 2022 to conduct research in Uasin Gishu County (Appendix I). Another research permit was obtained from County Director of Veterinary Services, Ref. No CDVS/UG/RESEARCH/VOL.I/94 (Appendix II).

CHAPTER FOUR

RESULTS

4.1 Physiological Conditions of the carcasses

The physiological conditions investigated were pH and Temperature of the carcasses. The pH ranged from 5.5 obtained from the goat meat in the butchery to 6.77 obtained from cow carcass at Maili 4 abattoir (Fig 4.1). There was a significant difference in terms of pH ($P < 0.05$) values obtained. The pH specifically from the butchery was consistently lower recording 5.50 from cow carcass, 5.73 from sheep carcass and 5.79 from goat carcass. The only other carcasses to record a pH lower than 6 were cow and sheep carcasses at Kaburwo abattoir. For the rest of the carcasses, the pH was above 6 with the lowest being 6.3 at Kaburwo.

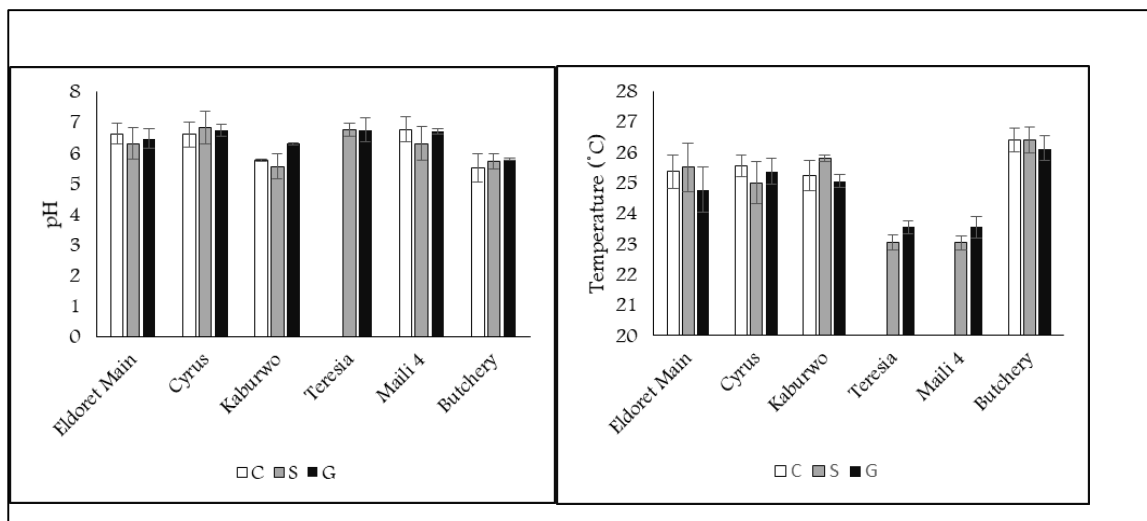


Figure 4.1: Temperature and pH obtained from the carcasses of Cow (C), Sheep (S), and Goat (G). There is no beef in Teresia abattoir.

Therefore, location (abattoirs, butchery) is the only factor influencing pH rather than the type of carcass.

Temperature ranged from a low of 23.0 °C from sheep carcasses in Teresia abattoir to 26.95 °C recorded from cow carcasses in Maili Nne abattoir. Here, recordings obtained

from Teresia were markedly different from the rest. Just as was the case for pH, location was the main factor determining the temperature of the carcass. Both the sheep and goat temperature recordings from Teresia were the lowest of all. Maili 4 on the other hand had an averagely higher temperature recordings.

4.2 Bacterial Load

From the study, the bacterial load obtained from the carcasses had higher number of Colony Forming Units (cfus) than the required threshold that permits meat's quality. The acceptable range is <3.5cfu/g for slaughter slab and <5cfu/g for butchery (Table 3.2). As reflected from the permitted ranges, the highest bacterial load was obtained from the Butchery carcasses ; (19.8cfus/g) (Fig 4.2). This was way above the rest. Maili Nne had the lowest count of 4.9 cfus/g while Teresia had the highest number in the second category with 9.2 cfus/g.

Table 4.1 Results of mean; pH,temperature (T) and colony forming unit (CFU) on selected abattoirs. Values represent means±SD.

		ABATTOIRS		
		Ph	T	CFU
ELD MAIN	C	6.62±0.3 ^a	25.37±0.6 ^a	6.67±1.5 ^{ad}
	S	6.32±0.5 ^a	25.50±0.8 ^{ad}	7±3.0 ^{ad}
	G	6.46±0.3 ^a	24.77±0.7 ^a	5.67±1.2 ^{ba}
CYRUS	C	6.6±0.4 ^a	25.57±0.4 ^a	6±4 ^{ad}
	S	6.83±0.5 ^a	25±0.7 ^a	8±2 ^{ad}
	G	6.74±0.2 ^a	25.37±0.4 ^a	12±8 ^{ac}
KABURWO	C	5.76±0.01 ^b	25.25±0.5 ^a	13.00±1.4 ^c
	S	5.56±0.4 ^b	25.8±0.1 ^a	9.33±2.1 ^d
	G	6.31±0.04 ^a	25.05±0.2 ^a	8.5±0.7 ^d
TERESIA	C			
	S	6.76±0.2 ^a	23.03±0.3 ^b	9.33±4 ^{adc}
	G	6.75±0.4 ^a	23.55±0.2 ^b	10.5±5 ^{adc}
MAILI 4	C	6.77±0.4 ^a	26.95±0.5 ^c	6.00±2 ^a
	S	6.30±0.6 ^{ab}	26.67±0.2 ^c	2.67±1 ^b
	G	6.7±0.1 ^a	26.45±0.4 ^{cd}	8.5±2 ^{ad}

Table 4.2 Results of mean; pH, temperature and colony forming unit on selected butcheries

BUTCHERIES				
	pH	T	CFU	
B1	5.76±0.1	24.23±1.6	20±2 ^{ac}	C
B2	5.62±0.2	24.2±0.7	18.67±5.5 ^{ac}	S
B3	5.76±0.3	24.3±1.8	20±3.6 ^{ac}	G
B4	5.88±0.1	23.87±0.6	16.67±1.5 ^b	S
B5	5.76±0.1	24.87±0.7	22.33±2.1 ^c	G

Key; B: Butchery, C: Cow, G: Goat, S: Sheep

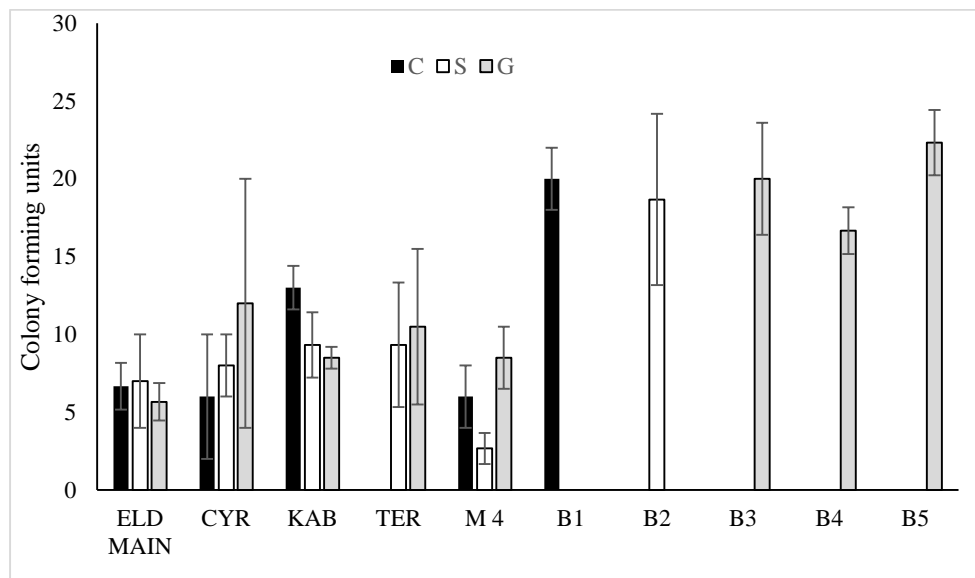


Figure 4.2: Bacterial loads obtained from the different meat points within Eldoret municipality. Butchery stands out with having significantly high number of Colony Forming Units (CFUs). Key: C; Cow, S; Sheep, and G; Goat.

However, because of the very large variance between the individual samples in each treatment as portrayed by very large standard deviations, there wasn't any significant differences among the types of carcasses in the abattoirs (Appendix II). Only the

bacterial load from the butchery was markedly different from the rest of the abattoirs ($P = 0.00$), (Appendix I).

4.2.1 Influence of the physiological conditions on the Bacterial

The influence of the physiological parameters investigated in the study towards the bacterial load was determined. A correlation analysis was done to establish this. pH showed a negative correlation with the bacterial load (-0.478). (Figure 4.3). On the other hand a positive correlation was observed between the CFUs and temperature albeit weak (0.177). (fig 4.4).

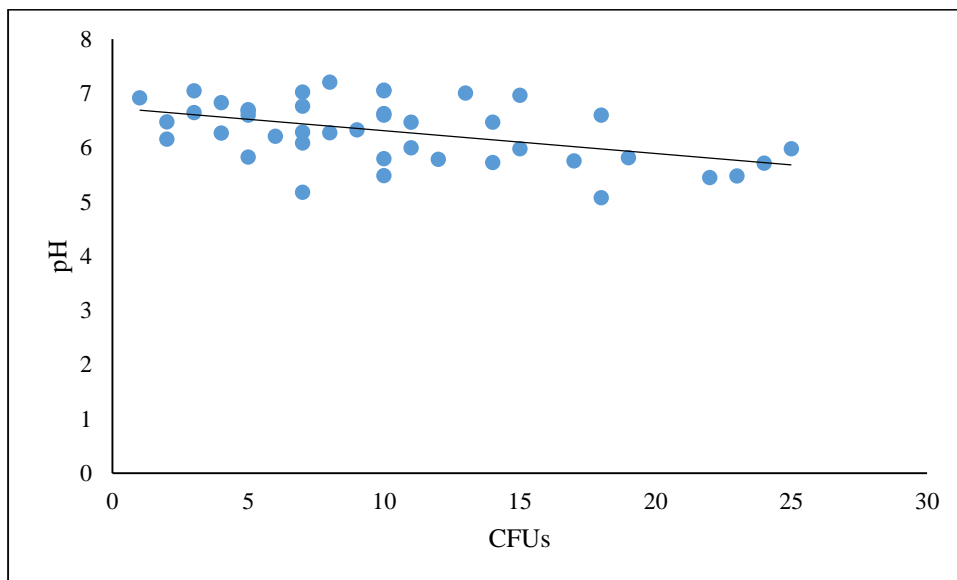


Figure 4.3. Relationship between the pH and bacterial load. The trend line depicts a negative correlation.

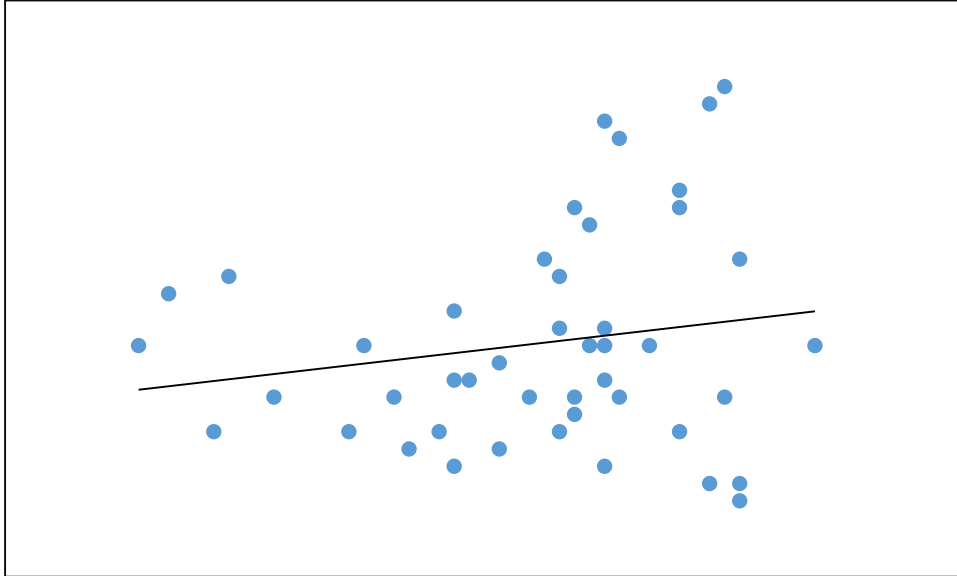


Figure 4.4: Relationship between Temperature and number of Colony Forming Units. A weak positive correlation between Temperature and the number of colony forming units can be observed.

4.3 Isolation of Selected Bacterial Pathogens

From this study the main contaminants in slaughter slab were *Staphylococcus aureus* 26(%) followed by *Proteus vulgaris* 11(%), *E. coli* 8(%). Butchery main contaminants were *Staphylococcus aureus* 18 (%) followed by *E. coli* 8 (%), *Proteus vulgaris* 8 (%).

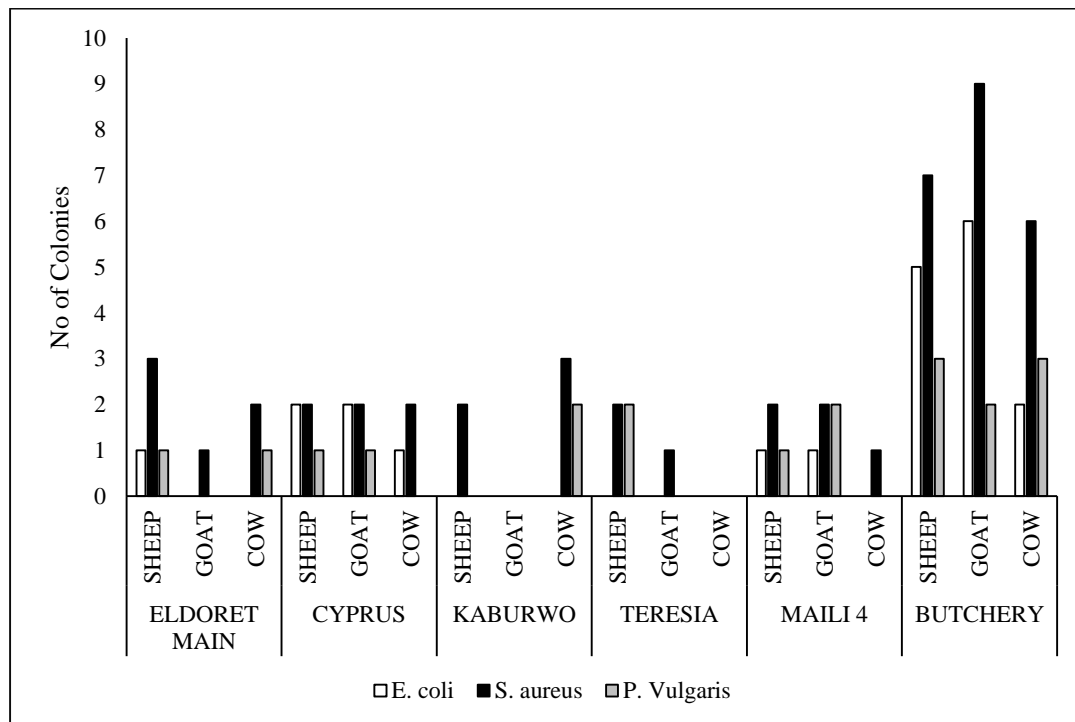


Figure 4.5. The selected bacterial pathogens from the different animal carcasses in the six sources.

Generally, carcasses from the butchery are the most contaminated (Fig 4.5). Beef was the least contaminated judging from the bacterial isolates obtained from the cow carcasses. With the exception of the butchery, there was no other source where the cow's carcass had all the three bacterial isolates.

CHAPTER FIVE

DISCUSSION

5.1 Physiological Conditions of the Carcasses

The study findings showed that PH from the selected butcheries had consistently lower recording of 5.50 from cow carcass, 5.73 from sheep carcass and 5.79 from goat carcass. These results are in line with a study where PH record was (5.65, 5.68 and 5.87) in cattle meat, sheep meat and goat meat respectively (Siham A, 2015). In this study goat meat had significantly higher pH values than beef at after slaughter. The pH of goat meat in this study agreed with values found (Cristofaneli, S 2004) and (Kadim, *et al* 2006) who reported values of pH in goat meat ranged from (5.7 to 6.0). The result in this study agreed to the result reported by (Siham, 2015) who reported that there was no significant ($P > 0.05$) different between beef and sheep meat and goat meat in pH measurement. The flesh of animals prior to slaughter usually has a pH value of 7.1. After slaughtering, some of the glycogen in the meat turns into lactic acid. As a result, the pH value is lowered. The increasing acidity of the maturing carcass varies in its speed, depending on a number of factors such as type of animal, breed, rearing characteristics and treatment of the animal prior to slaughter. Beef normally reaches its lowest pH value of 5.4 to 5.7 at 18-24 hours after slaughter. After the lowest pH level is reached, the pH starts to rise again slowly but steadily. By the time it reaches pH of 6.5 decomposition sets in. Pork already reaches its lowest pH value of 5.4 to 5.8 at 6-10 hours after slaughter.

pH is an equally relevant determinant of meat quality that affects meat water-holding capacity, colour, tenderness, and shelf life (Debrecéni *et al.*, 2018). The correlation between pH and meat colour indicates that the colour gets darker with increasing pH values.

A high percentage of meat (especially pork, but also beef) does not follow the normal pH value curve after slaughter. This is mainly PSE (Pale Soft Exudative) and DFD (Dark Firm Dry) meat. With PSE meat, weak watery pale pork, the lowest pH value of about 5.8 is reached within one hour of slaughter. (Khan et al., 2018).

This meat (beef) normally has poor water retention characteristics. Its use in the preparation of boiled sausages is therefore restricted. During boiling or roasting it loses its juices and becomes tough. For this reason many wholesalers and kitchens decline to buy PSE meat. (Khan et al., 2018).

DFD meat, another meat whose characteristics vary from the normal, can lead to losses if it is incorrectly used for processing (Khan et al., 2018). This meat has first class water retention properties. The glycogen degradation in this meat is delayed or shortened. It reaches a lowest pH value of about 6.2 to 6.5 (Khan et al., 2018). This meat is prone to decomposition from micro-organisms, and so is unsuitable for preparation of sausages from uncooked meat, for vacuum packaging of fresh meat, or for maturing. It is, however, ideal for the production of boiled sausages due to its water retention characteristics. (Khan *et al.*, 2018). These findings are in contrast with a previous study done in Greece by where average PH was 5.83 (Anestis Tsitsos, Vangelis Economou 2022).

Temperature of goat carcasses was significantly lower than that of sheep and cow carcasses (Table 4.1). Temperature decline between goat and sheep carcasses are probably due to the difference in fat coverage and size of goat and sheep carcasses. Leaner and small sized carcasses normally dissipate heat at a rapid rate during the immediate post mortem period whereas large carcasses with high fat cover are associated with a slower temperature decline (Kouakou *et al.*, 2005).

5.2 Bacterial Load and the selected Pathogens

This study revealed that fresh meat products available in local markets are seriously contaminated bacteria. The acceptable range is $<3.5\text{cfu/g}$ for slaughter slab and $<5\text{cfu/g}$ for butchery (Table 3.2). The presence of higher number of organisms makes meat more prone to spoilage and may serve as a tool for the transmission of pathogenic strains. The diseases of gastrointestinal tract are very common in this part of the world and they are mainly transmitted through contaminated food and water. It is largely due to improper handling, unhygienic conditions, and lack of awareness and ignorance of regulatory authorities. (Gitahi *et al.*, 2012).

Studies conducted in Pakistan to determine the microbiological quality of meat and meat products were done, 84% meat samples were found to be contaminated with variety of enteric organisms including some of the obligate pathogens (Ali, *et al.*, 2010). These microorganisms have been found to be associated with food handlers (Gitahi *et al.*, 2012). These microorganisms were similarly isolated from fresh meat samples from previous studies with similar patterns (Clarence *et al.*, 2009; Enabulele & Uraih, 2009; Sobukola *et al.*, 2009; Okonko *et al.*, 2010).

The presence of the bacteria can be traced back to the handling and processing methods of meat in the Eldoret Town. Animals are slaughtered in abattoirs and sometimes in backyards without observing strict hygienic practices. It is also a common practise to see people carrying carcasses just after dressing on their bare shoulders. Meats are normally transported to the markets either in meat vans (not be well maintained), taxi's, motorcycle and bicycles. Meats are sold in the open markets sometimes in sieves or without sieves, and on tables that are not well maintained or cleaned after

work. Butchers and meat sellers pay little attention to their personal hygiene and serve meats with dirty hands and clothing. (Frederick *et al.*, 2010)

Meat quality can be affected by various intrinsic and extrinsic factors, such as species, breed, sex, stress age and nutrition (Tsitsos *et al.*, 2021). Another reason for observed species variation in PH is the possible differences in response to pre-slaughter stress between sheep and goats (Santos *et al.*, 2008). Goats have been reported to be more prone to pre-slaughter stresses than sheep (Santos *et al.*, 2008). Depletion of muscular glycogen reserves because of pre-slaughter stress probably had a considerable influence on pH values in goat carcasses. The average pH of 5.73 and 5.79 for sheep and goats carcasses recorded after slaughter observed in the present study is <6.00 compared to other reported study (Safari *et al.*, 2010).

5.3 Selected Pathogens

Escherichia coli constitute an important factor of cattle contamination. Pathogens excreted in the faeces may contaminate the environment through which other cattle can acquire contamination and carry the bacteria in their digestive tract and/or on their hides (Rhoades *et al.*, 2009). In a study done in Denmark (Phillips *et al.*, 2012), reported that 0.1% of beef primates for dry-ageing were contaminated with shiga producing *E.coli* (STEC). These bacteria were not detected on dry-aged beef in a study by Ribeiro *et al.* (2021) but were present on wet-aged products. A study done in Algeria showed contamination of live cattle destined for slaughter occur at the farm level, during the transportation of meat to the slaughterhouse or during the lairage period in the abattoir. At the farm level, contaminated feed and water have been reported to be the main sources of Salmonella and pathogenic *E. coli* infections in cattle (Millemann, 2008).

Prevalence rates for *S. aureus* of 64%, 20% and 17% were reported in raw beef, sheep and lamb samples at retail in Pakistan, with an overall prevalence of 21% (Şanlıbaba, 2022). Similar studies in the United States of America, Japan and Poland isolated this bacterium from 28%, 33% and 68%, respectively (Hiroi et al., 2012; Krupa et al., 2014) Other studies reported different prevalence of *S.aureus*, including 71.6% in raw meat sampled in Awka, Anambra State(Akagha et al., 2015)

The presence of high *proteus* spp. counts in minced meat indicates poor sanitary conditions inside the butcher's shops especially for equipment which were used for meat cutting without periodical washing or cleaning and also workers hands which carry heavy contamination and contaminate meat by bad handling (Noha et al., 2014).

Proteus spp. is widely distributed in the environment with reservoirs. They are opportunistic pathogen; nevertheless, under favorable conditions they can cause urinary tract infection, which may lead to severe complications such as pyelonephritis or stone formation (JebaMercy et al., 2019). *Protues* spp. is one of the intestine's pathogens which are transmitted to human through food especially contaminated meats. The infected meat has no apparent symptoms of putrefaction and eating of such meat in raw and/or undercooked forms can lead to acute gastroenteritis, dysentery, mesenteric lymphadenitis and even septicemia (Razavilar., 2003; Gupta et al., 2015; Laura et al., 2019). In Iraq, it has been isolated from patients suffering from enteritis (Kanan and Abdulla, 2009). The presence of the pathogenic germs in the analyzed samples shows that it is important to determine the origin of contamination. This can be achieved only by the adoption and implementation of the quality approach in applying the guidelines of the Hazard Analysis Critical Control Point (HACCP) plan, as well as the mastery of the guide to good hygiene practices.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The pH of the carcasses is within the expected ranges in all the study sites. PH level of the carcasses from the butchery were low compared to the rest of the samples from the abattoirs. Temperature of the carcasses are heavily influenced by the environment. The bacterial load in the meat obtained from all the sampling sites were higher than the recommended levels. Highest contamination occurred in the butchery while on the other hand, the meat obtained from Maili 4 abattoir had the least bacterial load.

Staphylococcus aureus was the most common bacterial pathogen found on the carcasses, found from 26% of all the tested samples. *P. vulgaris* and *E. coli* were found in 11% and 8% respectively. The meat from the butchery had markedly higher numbers of these pathogens.

6.2 Recommendations

- i. Awareness creation to butcher shop workers regarding meat hygiene is essential
- ii. Meat inspections should be strengthened by veterinary professionals before and after slaughtering and before the meat is distributed to the public.
- iii. Good personal hygiene and meat handling practices should be followed strictly by butchers and personnel selling the meat.
- iv. Further research and studies are needed to assess knowledge, attitude and behaviors of worker in slaughters and meet butchers regarding food hygiene and safety. Policy makers should implement more safety and hygiene policy to promote healthy and safer food.

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APPENDICES


Appendix I: One way ANOVA Table

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	1252.815	5	250.563	19.83203	1.14E-10	2.408514
Within Groups	606.4444	48	12.63426			
Total	1859.259	53				

Appendix II: Two-way ANOVA Table

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Sample	16.93333	2	8.466667	0.649063	0.529717	3.31583
Columns	132.4444	4	33.11111	2.53833	0.060478	2.689628
Interaction	89.28889	8	11.16111	0.855622	0.563166	2.266163
Within	391.3333	30	13.04444			
Total	630	44				


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