PREVALENCE OF SHIGA-TOXIGENIC *Escherichia coli* IN READY-TO-EAT GAME MEAT SOLD IN DIFFERENT CITIES IN SOUTH-WESTERN, NIGERIA

By

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, MAKOGI, IBAFO, OGUN STATE, NIGERIA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE (B.Sc.) IN MICROBIOLOGY.

SEPTEMBER, 2022

DECLARATION

I hereby declare that this project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other University. All citations and sources of information are clearly acknowledged by means of reference.

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AMAECHI, CHINENYENWA VICTORIA

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Date

CERTIFICATION

This is to certify that the content of this project entitled **"Prevalence of Shiga-Toxigenic *Escherichia coli* in Ready-To-Eat Game Meat Sold in Different Cities in South-Western, Nigeria"** was prepared and submitted by **AMAECHI, VICTORIA CHINENYENWA** in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN MICROBIOLOGY**. The original work was carried out by her under my supervision and is hereby accepted.

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DEDICATION

This project is dedicated to my mother, Prof. Blessing N. Igwesi and my sisters, Joy and Blessing Amaechi without whom my educational journey would have suffered some setbacks.

ACKNOWLEDGEMENT

All glory to God Almighty for his unending love and faithfulness throughout my journey in Mountain Top University. I am sincerely grateful for the Grace to start well and finish this degree program successfully.

My profound gratitude goes to my wonderful mother, Prof. Blessing N. Igwesi and my sisters, Joy and Blessing Amaechi for their endless moral, financial and spiritual supports.

I also appreciate the Head of Department, Biological Sciences Dr. (Mrs) C. I. Ayolabi.

Special thanks to my wonderful supervisor Dr. O. E. Fayemi whose constructive criticism and mentorship and guidance have helped in putting me on the right path during my research work.

My special thanks also to Dr. G. B Akanni who showed support and put me through with my project, I am very grateful sir.

I acknowledge Dr. Adebami and Dr. Moses Abiala for their warm love, support and assistance towards the success of this research.

I am also grateful to Miss Joy Anyasi and Mr. Favour Okunbi for their constant help and sacrifices during these research work. God shall greatly reward you in Jesus Name.

My heartfelt gratitude goes to Robert Abimbola who believes in my success.

Finally, my course mates and friends who have made this journey memorable and worthwhile especially Ajayi Ayoola, Oladele Precious, Ashibogwu Chuks, Adebayo Fisayo, Ajide Esther, Adeboye Oluwatofunmi, Oruh Victoria, Adediran Israel, Adediran Taiwo, Oluwatoyin Joshua. God bless you all.

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ABBREVIATION

STEC – Shiga-Toxigenic *Escherichia coli*

ETEC - Enterotoxigenic *E. coli*

EPEC - Enteropathogenic *E. coli*)

EHEC - Enterohemorrhagic *E. coli*

EIEC - Entero-invasive *E. coli*

EAEC - Enteroaggregative *E. coli* (and

DAEC - Diffusely adherent *E. coli*

UPEC - Uro-pathogenic *E. coli*

MAC – MacConkey Agar

SMAC - Sorbitol MacConkey agar

HC - Hemorrhagic Colitis

HUS – Hemolytic Uremic Syndrome

PAH- Polycyclic Aromatic Hydrocarbons

SIV - Simian Immunodeficiency Virus

HTLV – Human T-Lymphotropic Virus

HIV - Human Immunodeficiency Virus

UTI - Urinary Tract Infection

CFU – Colony Forming Unit

TVC- Total Viable Count

DNA - Deoxyribonucleic Acid

PCR – Polymerase Chain Reaction

TAE – Tris Acetic Ethylene Diamine Tetraacetic Acid

Stx/Vtx - Shiga toxin

ABSTRACT

Game meat constitutes a necessary part of the human diet, it provides essential vitamins, minerals, and protein. However, the rising consumption of Ready-To-Eat Game meat has probably elevated the number of foodborne illness outbreaks around the world. Game meat are often consumed smoked without cooking, therefore; which potentially increase the risk of contracting foodborne illness and in extreme cases death especially in children. In Nigeria, Game meat is purchased in road side open kiosk and on most occasions are not processed hygienically. This study evaluated the prevalence of Shiga toxin producing *E. coli* in varieties of Ready-To-Eat Game meat (Monkey, Civet cat, Antelope, Grasscutter, Guinea fowl, Porcupine, Rabbit) from various roadside vendors at different locations around the South-west region of Nigeria using cultural method and molecular techniques. A total of 55 samples were tested for presence of *E. coli* using Sorbitol MacConkey Agar and MacConkey Agar plates. All samples had presumptive STEC, molecular identification of isolates (n = 55) for STEC virulence genes (stx_1 stx_2 and eae) using multiplex PCR was done. The total viable counts (TVC) for presumptive STEC in the samples was in the range of 4.1 to 9.5log_{10}cfu/g with the highest recorded in samples from Lagos State. Using Multiplex PCR, stx1 gene was detected in some of the isolates which confirmed the presence of STEC in the Game meat. The presence of STEC and a very high microbial counts of presumptive STEC poses a threat to public health which could lead to foodborne illnesses including hemorrhagic colitis (HC) or hemolytic uremic syndrome (HUS).

Keywords: Microbial count load, Game meat, STEC, E. coli, foodborne illness

CHAPTER ONE

1 INTRODUCTION AND PROBLEM STATEMENT

1.1 Background to the Study

The edible portion of domestic animals is known as meat; but, more recently, the term has come to refer to all mammalian species as well as fish, shellfish, poultry, and more unusual species like frogs and alligators (Nakai and Moddler, 2000).Similar to this, the term "Game meat" also refers to animal tissue utilized as food, primarily the skeletal muscles and related fat, but it can also refer to organs like the lungs, livers, skin, brains, bone marrow, kidneys, and a range of other internal organs as well as blood (Hammer GF, 1987).The rising demand for Game meat is the result of a number of developments and changes, including population growth, more effective hunting with more advanced tools, and easier access to formerly inaccessible remote forest areas (Fa, J.E *et al*., 2002; Wilkie, D.S *et al*., 1999).

In Nigeria's largest cities, Game meat is sold in outdoor markets without packaging where it is exposed to the elements and microbial infection results (BCTF. 2003). The demand for Game meat has sparked a thriving industry, and as a result, hunters are using outdated hunting techniques like bush burning, threatening species, and ruining forests and grasslands. About 25 percent of the population in Nigeria only consumes animal protein from Game meat. Up to 60% of the people in Ghana use Game meat as their primary source of animal protein. 60% and 35% of the populations of Tanzania and Kenya, respectively, rely primarily on Game meat as their only source of animal protein. For most cultures, the main source of animal protein has been "Game meat." As a result, Game meat has become more popular and is now more expensive than beef and other types of meat. For many rural residents who live in poverty and trade in it, Game meat is essential to their life (ACET; The African Centre for Economic Transformation., 2014).

Due to the indiscriminate and unsustainable usage of wild animals for food, which has a significant negative impact on their population, game meat has replaced conventional sources of animal protein. However, as human populations increase, interactions between people and wildlife will intensify, increasing the likelihood of zoonotic disease transmission from animal hosts (Hogenboom, Melissa., 2014).

Additional studies in Nigeria and Ghana analyzed the presence of toxic elements in bushmeat. Adei and Forson (2008) examined the livers of grass cutters in Ghana and concluded that livers can be a significant source of heavy metals (Cd, Hg, Pb) in the diet. Igene *et al.* (2015) analyzed metal contamination on fresh and dried grass cutter (*Thryonomys swinderianus)* in Nigeria and found the meat unsafe for consumption given their high concentration of nickel and chromium. The authors hypothesize that these concentration levels might come from contaminated water and soils due to mining or smelting waste-water production,

cooking with nickel–steel alloy utensils, and eating from nickel-pigmented dishes. Yemi, (2015) found a high concentration of heavy metals in *Cephalophus spp.* hunted in an agricultural landscape from Nigeria that may be caused by acute or chronic contamination of their diet and habitat. Abdul *et al*. (2014) analyzed the Polycyclic Aromatic Hydrocarbons (PAH) in smoked bushmeat in Ghana and found that bushmeat smoked with gas produced smaller PAH values compared to bushmeat smoked using wood mixed with spent oil, plastics mixed with refuse, and discarded car tires. Kurpiers *et al*. (2016) gave a detailed description of pathogens discovered in bushmeat species from Africa. This provided records for nine main types of viruses (SIV; HTLVs, Foamy viruses, Monkey pox, Marburg virus, Lassa virus, Ebola, Nipah virus and Herpes) that can be transmitted to humans (Wolfe *et al*, 2004) and is hosted mainly by small primates, apes and other Game animal. Bacterial pathogens were also identified and the most common bacteria were *Escherichia coli, Salmonella spp., and Campylobacter spp*.

This study's objective is to determine how often *Escherichia coli* is in the smoked game meat sold in South-Western, Nigeria.

1.2 Statement of the Problem

Foodborne and viral illnesses like Ebola and diarrhea, as well as newly emerging infectious diseases, can spread more easily because game meat puts people in close contact with wildlife. Due to the consumption of contaminated game meats, which may act as reservoirs for the bacteria responsible for the disease, the spread of these illnesses could be more extensive.

1.3 Significance of the Study

To determine the number of pathogens, present in game meat and to identify the most common organisms, it is crucial to look at the microbiological safety of game meat. The presence of *E. coli*, which, if present, raises the risk of illness and mortality linked with the ingestion of contaminated game meat, will be checked in samples of diverse game meat from various areas in Nigeria.

1.4 Aims and Objectives of the Study

- To isolate *E. coli* in ready-to-eat game meat sold in the various market areas of Nigeria using cultural methods.
- To identify isolated Shiga-Toxigenic *E. coli* using molecular techniques.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Game Meat

Game meat refers to meat obtained from wild animals and meant for human consumption. It was formerly only used to refer to African meat, but it is now also used to refer to "wild" meat from Asia or South America (Emelue and Idaewor 2018). Game meat can be eaten fresh, smoked, salted, or sun-dried and comes from wild creatures such as pangolin, grasscutter, snake, and many others.

Smoked Game meat, on the other hand, is the most common and widely available final product in most African rural, suburban, and metropolitan markets (Ntiamoa, 1997 and Onadeko, 2004)

There are different types of Game meat (Onadeko, 2004):

- Non-human primates (Monkey (*Chlorocebus pygerythrus);* Ape (*Hominoidea sp*.); Chimpanzee (*Pan troglodytes);* Gorilla *(Gorilla gorilla*)
- Large herbivores (Antelope (*Antilocapra americana*); Deer (*Cervus elapus*); Duiker (*Cephalophus grimmia)*
- Rodents (Grasscutter (*Thryonomis swiderianus*); Porcupine (*Hystrix cristata)*
- Reptiles (Python (*Python sebae*); Monitor lizard (*Varanus sp.)*
- Carnivores (Tree Pangolin (*Manis tricuspis*); Bush dog (*Speothos venaticus);* Bush cat (*Felis nigripes*)

Game meat has featured prominently as a source of foodborne disease and a public health concern. Lack of properly processed bushmeat results. An increase in the outbreak of foodborne diseases causes microorganisms which can consequently become an epidemic or even a pandemic.

Some of these microorganisms include: *Staphylococcus aureus, Bacillus spp., Shigella sp.* And *Escherichia coli.* There is a focus on *Shiga-Toxin producing Escherichia coli* as a major causative agent of foodborne diseases associated with game meat.

2.2 Disease Outbreak Associated with Game Meat

In the case of bushmeat, it is now evident that many viral variants are prevalent in hunted nonhuman monkey species, which have had the most study attention, and that these variants have crossed between nonhuman primates and humans on multiple occasions (Peeters and Delaporte 2012).

Recent viral outbreaks that have led to a high mortality rate among adults and children across the world include:

- HIV/AIDS
- Ebola virus
- Lassa fever virus
- Human monkey pox virus
- Covid-19 (Corona virus)

All of these and other viral outbreaks have led to issues of mental health, susceptibility to other disease and an increase in mortality rate leading to a decline in the number of people living in that area.

2.2.1 HIV/AIDS

Human Immunodeficiency Virus is the most notable virus to emerge from the bushmeat interface (HIV). While the origin of HIV was long obscured, Human HIV-1 and HIV-2 are believed to have evolved from strains of Simian Immunodeficiency Virus (SIV) (Hahn *et al.* 2000; Kazanji *et al*. 2015; Lemey *et al*. 2003; Peeters and Delaporte 2012). Evidence suggests that SIV crossed over to humans by blood contact when hunters had an exposed open wound or injured themselves during the butchering of nonhuman primates (Karesh and Noble 2009; Wolfe *et al*. 2004a,b). SIVcpz and SIVgor, from common chimps and western gorillas in west central Africa, are the closest relatives of HIV-1 found among nonhuman primates (Keele *et al* 2006; Sharp *et al.* 2005; 2006, 2007; Takehisa *et al*. 2009), and at least four separate spillovers have occurred (Peeters *et al*. 2013). HIV-2 is derived from SIVsmm in West African sooty mangabeys (*Cercocebus atys)* (Apetrei *et al.* 2005; Ayouba *et al*. 2013), where viral genetic diversity is high and transmission is thought to have occurred at least eight times.

SIVs have a high potential for future and ongoing spillovers, and multiple species-specific variants exist. Peeters *et al*. (2002), for example, estimated that more than 20% of non-human primates hunted for food are infected with a SIV variant; At least 45 species-specific SIV variants from at least 45 monkey species are currently characterized, according to Locatelli and Peeters (2012) and Peeters *et al*. (2013). In Cameroon bushmeat markets, Aghokeng *et al*. (2010) collected 1,856 nonhuman primates' carcasses from 11 species. They discovered a low overall frequency of SIV (only 2.93 percent of corpses), with the lowest prevalence among the most often sold species. They did, however, detect SIV variations in roughly 70% of the monkey species studied. At least 40 primate species have been found to have SIV infection (Aghokeng *et al.* 2010). Cross-species transmission of strains and co-infection with multiple strains have been documented, with genetic recombination sometimes occurring (Aghokeng *et al.* 2007; Bibollet-Ruche

et al. 2004; Gogarten *et al.* 2014; Hahn *et al.* 2000), paving the way for future spillovers into humans (Aghokeng *et al.* 2007; Bibollet-Ruche *et al.* 2004; Locatelli and Peeters 2012).

2.2.2 Ebola and Marburg Viruses

There are currently seven identified species of filoviruses, five of which are found in Sub-Saharan Africa (Olival and Hayman 2014)

- Tai forest ebolavirus (TAFV)
- Sudan ebolavirus (SUDV)
- Zaire ebolavirus (EBOV),
- Bundibugyo virus (BDBV);
- Genus Marburgvirus: Marburg virus (MARV).

These pathogens are viruses that emerge on a regular basis, usually as a result of a single spillover event, and cause hemorrhagic fevers (reviewed by Olival and Hayman 2014; Rougeron *et al*. 2015). From 1976 to 1979, three initial Ebola virus outbreaks in the Democratic Republic of the Congo involved victims who were reported to have handled western gorilla or common chimp carcasses or to have had physical contact with people who touched the animals (Leroy *et al.* 2004a, b).

The Marburg virus was discovered in laboratory workers who had dissected imported grivet (*Chlorocebus aethiops*). Following a rash of common chimp deaths in the Tai National Park in Côte d'Ivoire, a single case of TAFV was discovered in an ethnologist who was likely infected while performing a necropsy on a dead common chimp (Le Guenno *et al*. 1995; Wyers *et al.* 1999). Other than primates, other inadvertent hosts in the wild are feasible, as proved by Duikers (*Cephalophus spp*). Rouquet *et al*. 2005; Leroy *et al.* 2004a). According to Weingartl *et al.*, (2013), both dogs (naturally) and pigs (at least experimentally) can be infected During the 2001–2002 EBOV outbreak in Gabon, Allela *et al*. (2005) reported more than 30% seroprevalence in dogs residing in villages with EBOV human and animal cases. Those canines appeared unaffected and were presumed to have been exposed by wild animals foraging for food. Although incidental hosts are likely significant in the ecology of these viruses, especially when moribund or dead animals are consumed, strong evidence suggests that at least Marburgvirus and EBOV are natural reservoir hosts (Allela *et al*, 2005).

2.2.3 Rabies and other Lyssaviruses

Rabies is the oldest known zoonotic disease, dating back to the 23rd century BC. Each year, an estimated 25,000 people die in Africa from rabies (Dodet *et al*. 2015), some of whom may have been exposed during bushmeat-related activities, though the majority of human cases can be attributed to domestic dogs. The

Lyssavirus genus includes the Rabies virus (RABV). In Africa, it is joined by at least five other species (Dodet *et al*. 2015):

- Lagos bat virus (LBV)
- Mokola virus (MOKV)
- Duvenhage virus (DUVV)
- Shimoni bat virus (SHIBV) and the newly proposed
- Ikoma lyssavirus (IKOV).

With two exceptions, bats serve as reservoir hosts for these viruses (Banyard *et al.* 2014). The Mokola virus is carried by shrews (*Crocidura spp*.), rusty-bellied brush-furred rats (*Lophuromys sikapusi*; Saluzzo *et al.* 1984), and companion animals (Kgaladi *et al.* 2013). The Ikoma virus has only been detected in African civets (*Civettictis civetta*) (Marston *et al.* 2012). Rabies has been found in a variety of nonhuman primate species, including those used in the bushmeat trade (e.g., in Botswana) and a variety of wildlife species (Moagabo *et al.* 2009; Gautret *et al*. 2014).

2.2.4 Lyssavirus

Africa has the largest genetic variety of lyssaviruses, and the Lagos bat virus could be a mix of different species. While the rabies virus is responsible for the vast majority of human cases, the Duvenhage virus has been connected to human mortality caused by bat bites. Two human cases of the Mokolo virus have been discovered, however neither has ended in death (Kgaladi *et al.*, 2013). Ikoma virus was recently found in an African civet in Tanzania's Serengeti National Park.

2.2.5 Lassa and other Arenaviruses

Arenaviruses are a zoonotic virus family that spreads from rodents to humans. With outbreaks in Guinea, Sierra Leone, Nigeria, and Liberia, the Lassa virus is the most well-known of the viral hemorrhagic arenaviruses in Africa (Ter Meulen *et al,* 1996). The principal risk is peri-domestic exposure to the rodent host, the natal mastomys (*Mastomys natalensis*), through urine or feces (L.A. Kurpiers *et al,* 2020). Meulen *et al.* 1996) observed a strong correlation between peridomestic rodent hunting and Lassa virus antibodies and symptoms, linking bushmeat-related activities to the virus's spread to humans.

2.2.6 Human Monkeypox Virus

Rodents, not monkeys or humans, serve as reservoir hosts for the human monkeypox virus. Squirrels (e.g., Thomas' rope squirrel, *Funisciurus anerythrus*; Khodakevich *et al.* 2000); African ground squirrels; *Xerus*

sp.) are among the infected. Recent MPX rises in the Democratic Republic of the Congo and elsewhere have been attributed to the end of the human smallpox vaccine.

2.2.7 Prions

"Mad cow disease" and "chronic wasting disease" are examples of acquired prion disorders in ungulates from Europe and North America. Only humans and captive monkeys have been discovered to have BSE, most likely as a result of consuming contaminated meat. Prions can be present in nearly all tissues and are resistant to breakdown, making them a disease worth keeping an eye on in regard to bushmeat-related behaviors (Bons *et al* 1999).

2.2.8 Processing Methods of Game Meat

Processing techniques are essentially the procedures used to prepare bushmeat n order to prevent meat contamination. These consist of post-process handling and packaging conditions that prevent the reintroduction of vegetative pathogens onto or into the product before packaging. It also involves the use of packaging materials that, while they do not provide a hermetic seal, do prevent the reintroduction of vegetative pathogens into the product. This is possible when the product formulation is capable of inhibiting spore germination (Scallan *et al.*, 2011).

For the processing of game meat, the following processes are used: (Gideon and Joseph, 2018).

- **Stunning and Bleeding:** A technical method of rendering an animal immobile or unconscious, with or without killing them, at or at the beginning of the slaughtering process so that the animal is not afraid or in agony when it is killed later on (Nakyinsige *et al*., 2013).
- **Skinning:** This is the act of skin removal. During this process, equipment used can be sources of *Bacillus, salmonellae and E. coli* contamination unless properly cleaned.
- **Evisceration:** This is the procedure of removing the internal organs from carcasses while processing meat.
- **Trimming and Washing:** To make the carcass seem better and get blood, bone dust, hair, and mud off of it, trimming and washing are done. Some bacterial contamination is removed during trimming. But some bacteria such as *E. coli* still thrive.
- **Flaying (removal of hairs):** The action of stripping an animal's carcass of its hide and skin.
- **Brining (soaking in Salt solution for preservation):** Traditional brining involves soaking things, primarily meat or fish, in a salt solution called a brine before they are cooked.
- **Smoking:** To do this, place the meat over a controlled flame until it is fully dried and edible.

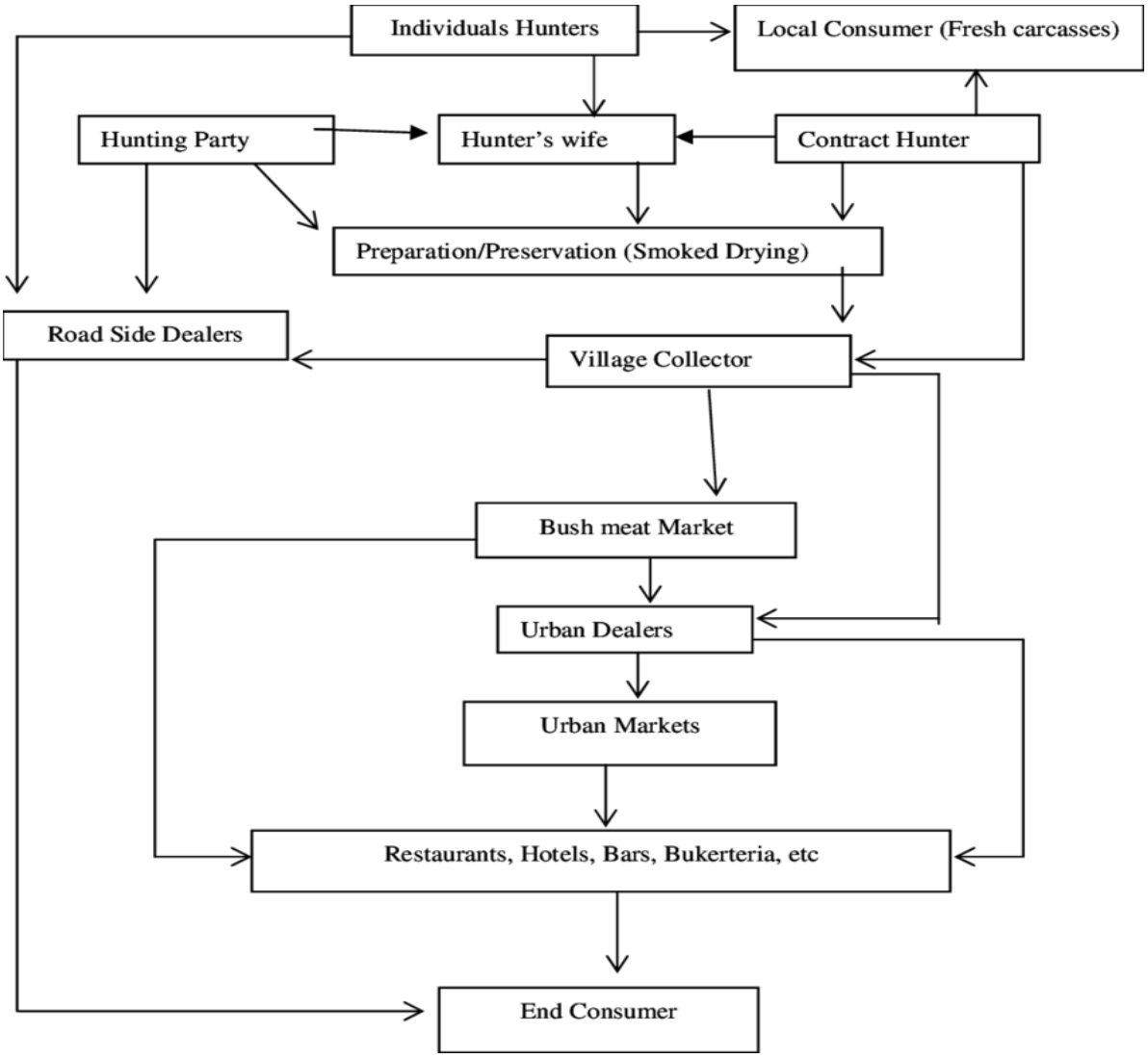

FIGURE 2. 1 Sequence of Game meat trade and consumption in Oban Hills region, Adapted from Eniang, *et al* (2008)

2.3 Background History of *Escherichia coli*.

In 1885, Theodor Escherich, a German–Austrian pediatrician and a professor at universities in Graz and Vienna, Austria, reported the isolation of a bacterium called *Bacterium coli* from a fecal sample of children. Later, in 1919, the bacterium was renamed *Escherichia coli* belongs to the family Enterobacteriaceae. It is a Gram-negative, short motile rod that inhabits the intestinal tract of animals and humans from birth. They are ubiquitous, commonly found in water, soil, and as commensal in normal intestinal microflora (also are opportunistic pathogens). It has been used extensively as a model organism to study bacterial physiology, metabolism, genetic regulation, signal transduction, and cell wall structure and function. The bacterium is one of the natural micro-flora of the human and animal gut microbial community. Hence, fecal shedding and contamination of water and food with *E. coli* or coliforms are common. (Lim *et al., 2007)*

A majority of *E. coli* strains are non-pathogenic; however, only a small subset is pathogenic and causes a variety of diseases in humans and animals. The diseases include gastroenteritis, dysentery, Hemorrhagic Colitis (HC), Hemolytic Uremic Syndrome (HUS), Urinary Tract Infection (UTI), septicemia, pneumonia, and meningitis. In recent years, however, the major concern has been the increasing numbers of foodborne outbreaks, caused by pathogenic *E. coli* in industrialized countries due to consumption of contaminated meat.

2.4 Sources of *E. coli*

Escherichia coli is a member of the intestinal microflora of humans, animals, and birds. The bacterium is routinely shed into the environment through feces, and it can contaminate drinking water, irrigation water, and soil, consequently, bushmeat, especially if processed poorly can harbor this microorganism and become a source of transmission of foodborne disease. (Nataro *et al., 2007)*

Some *E. coli* pathotypes such as Enterohemorrhagic *E. coli* (EHEC) can be transferred through game meat, which may be acquired during slaughter through fecal and hide contact.

2.5 Characteristics of *Escherichia coli*

Escherichia coli (EPEC) are in the family Enterobacteriaceae. The bacteria are gram negative, rod shaped, non-spore forming, motile with peritrichous flagella or nonmotile, and grow on MacConkey agar (colonies are 2 to 3 mm in diameter and red or colorless). They can grow under aerobic and anaerobic conditions and do not produce enterotoxins (Farmer *et al*., 2007)

2.6 Pathotypes of *Escherichia coli*

The most common facultative organisms in humans' guts are commensal *E. coli* strains. Despite being outnumbered by anaerobic organisms, *E. coli* is important to human health because it participates in biofilm communities and subsequent oligosaccharide and polysaccharide digestion (Conway and Cohen, 2015).

Several pathogenic *E. coli* strains exist, unfortunately. Although the names of these strains change depending on the source, we'll use the ones mentioned below for the purposes of this chapter.

- Enterotoxigenic *E. coli* (ETEC)
- Enteropathogenic *E. coli* (EPEC)
- Enterohemorrhagic *E. coli* (EHEC),
- Entero-invasive *E. coli* (EIEC)
- Enteroaggregative *E. coli* (EAEC) and
- Diffusely adherent *E. coli* (DAEC) are the six strains of potentially pathogenic intestinal-based diarrhea-causing *E. coli*

In addition, Uro-pathogenic *E. coli* (UPEC), an extraintestinal pathogenic strain, causes urinary tract infections.

Figure 2.2 gives a schematic illustration of the pathotypes of *E. coli*

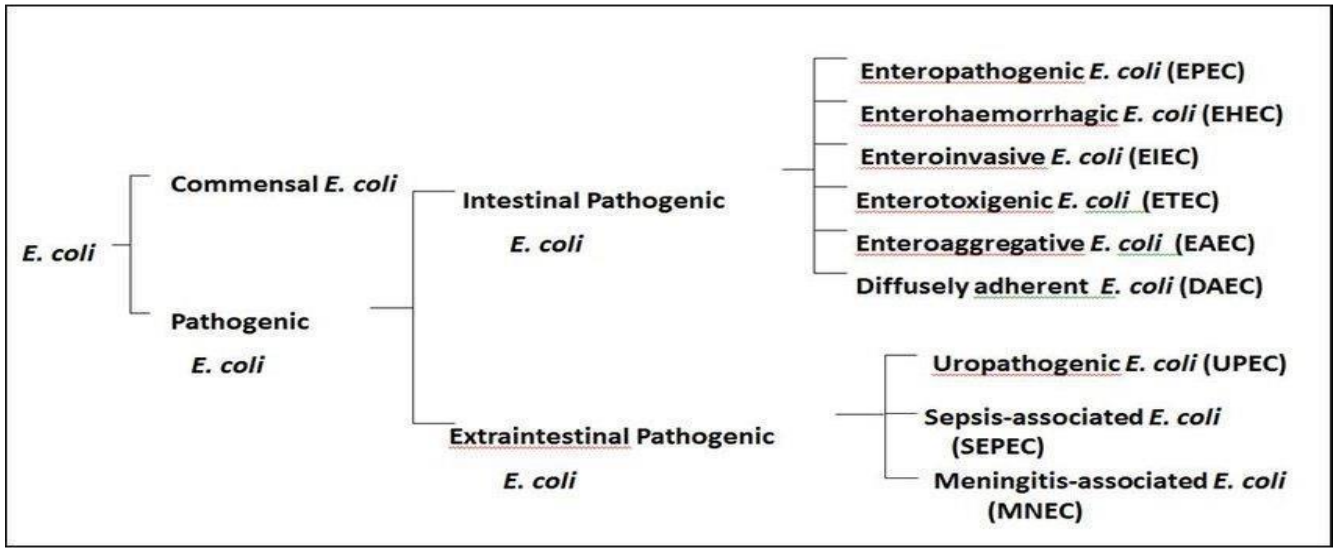

FIGURE 2. 2 *Escherichia coli* species and its subspecies classification. Adapted from Kaper *et.al*., (2004)

2.7 Enterotoxigenic *E. coli* (ETEC)

The bacteria Enterotoxigenic *Escherichia coli* (ETEC) is one of the most common causes of bacterial diarrhea (Fleckenstein and Kuhlmann, 2019). ETEC can be spread through food or water contaminated with animal or human faeces. Estimates from the Institute for Health Metrics and Evaluation (IHME) and maternal child epidemiology, ETEC is thought to cause approximately 220 million diarrhea episodes worldwide in 2021. Around 75 million episodes in children under the age of five, resulting in between 18 700 and 42 000 deaths (Reygaert, 2017).

2.8 Enteropathogenic *E. coli* (EPEC)

EPEC causes acute, prolonged, and chronic diarrhea in children under the age of two. Watery, non-bloody diarrhea with mucous, vomiting, and low-grade fever are some of the symptoms (Matthew *et al*, 2021). EPEC strains do not invade enterocytes right away. Instead, these bacteria attach to microvilli and deliver effector proteins into the cell via a type III secretion system (Manu *et al.,* 2011).

2.9 Enterohemorrhagic *E. coli* (EHEC)

The E. coli O157:H7 strain causes severe abdominal cramps, bloody diarrhea, and potentially fatal Hemolytic Uremic Syndrome (HUS). The most prevalent products connected to the spread of this infection are raw meat (especially ground beef), raw milk, and raw vegetables (Fatima and Aziz, 2021). The cost of illness caused by this bacterium is estimated to be $405 million per year, which includes medical bills, death, and lost productivity.

2.10 Entero-Invasive *E. coli* (EIEC)

Enter invasive E. coli (EIEC) are non-motile and slow to ferment lactose. They are the cause of a disease similar to bacillary dysentery caused by Shigella. EIEC is endemic in developing countries and accounts for 1% to 5% of all patients with diarrhea who see a doctor. Illnesses as a result of this pathogen occur within 8 to 24 hours after ingestion of food or water containing this organism (DuPont *et al*., 1971; Ira *et al*., 1982).

2.11 Enteroaggregative *E. coli* (EAEC)

Enteroaggregative *E. coli* was given that name because of their proclivity for adhering to enterocytes in dense clusters (David *et al*., 2007). The EAEC is a heterogeneous collection of microorganisms that produce non-bloody diarrhea and share a common pathogenesis. Infection begins when bacteria attach themselves to microvilli via fimbriae, resulting in increased mucus production, biofilm development, inflammatory response, and toxin release (Reygaert, 2017; Jensen *et al*., 2014).

2.12 Diffusely Adherent *Escherichia coli* (DAEC)

Diarrhea in the presence of DAEC has also been demonstrated to stimulate the production of inflammatory cytokines such as IL-8 (Le Bougu'enec and Servin, 2006). DAEC is suspected to be responsible for some types of persistent diarrhea in newborns, even though it is not known to cause severe diarrhea (Reygaert, 2016).

2.13 Uro-Pathogenic *Escherichia coli* (UPEC)

Every year, approximately 150 million people globally are affected by urinary tract infections (UTIs), which have major socioeconomic effects. According to Micali *et al.*, (2014), 40% of women will have at least one UTI in their lifetime, and 11% of women over the age of 18 will have a UTI episode every year. Germs' capacity to cling to the mucosa of the urinary tract is a key pathogenicity trait (Croxen and Finlay, 2010).

2.14 Shiga-Toxin producing *E. Coli*

Shiga toxins are named after Kiyoshi Shiga, a Japanese microbiologist who first described the bacteria *S. dysenteriae* in 1898. This bacterium releases a toxin that is structurally and antigenically similar to Shiga toxin 1 (Stx1) generated by E. coli. Shiga toxins belong to the family of ribosome-inactivating proteins. The presence of any of these STEC serogroups in food has been believed to indicate the possibility of serious illness.

2.15 Transmission of *E. coli*

E. coli is a bacterium that can live in the human body for weeks or even years if they are exposed to the right environment (pH, humidity, temperature, and moisture). *E. coli* transmission is most prevalent in immunocompromised populations, including children, pregnant women, and those with HIV/AIDS (less than 5 years old). Eating contaminated food and person-to-person distribution are both ways it is transmitted (Srisawat and Panbangred, 2015).

2.16 Resistance and Susceptibility of *Escherichia coli*

Most strains of *E. coli* are susceptible to most antibacterial agents such as Gentamicin, amikacin, Fluoroquinolones and cephalosporins that are active against gram-negative bacteria. Resistance can be acquired via plasmids and drug efflux systems, also the resistance of amoxicillin, cotrimoxazole and trimethoprim have increased over the years (Yu *et al*., 2004).

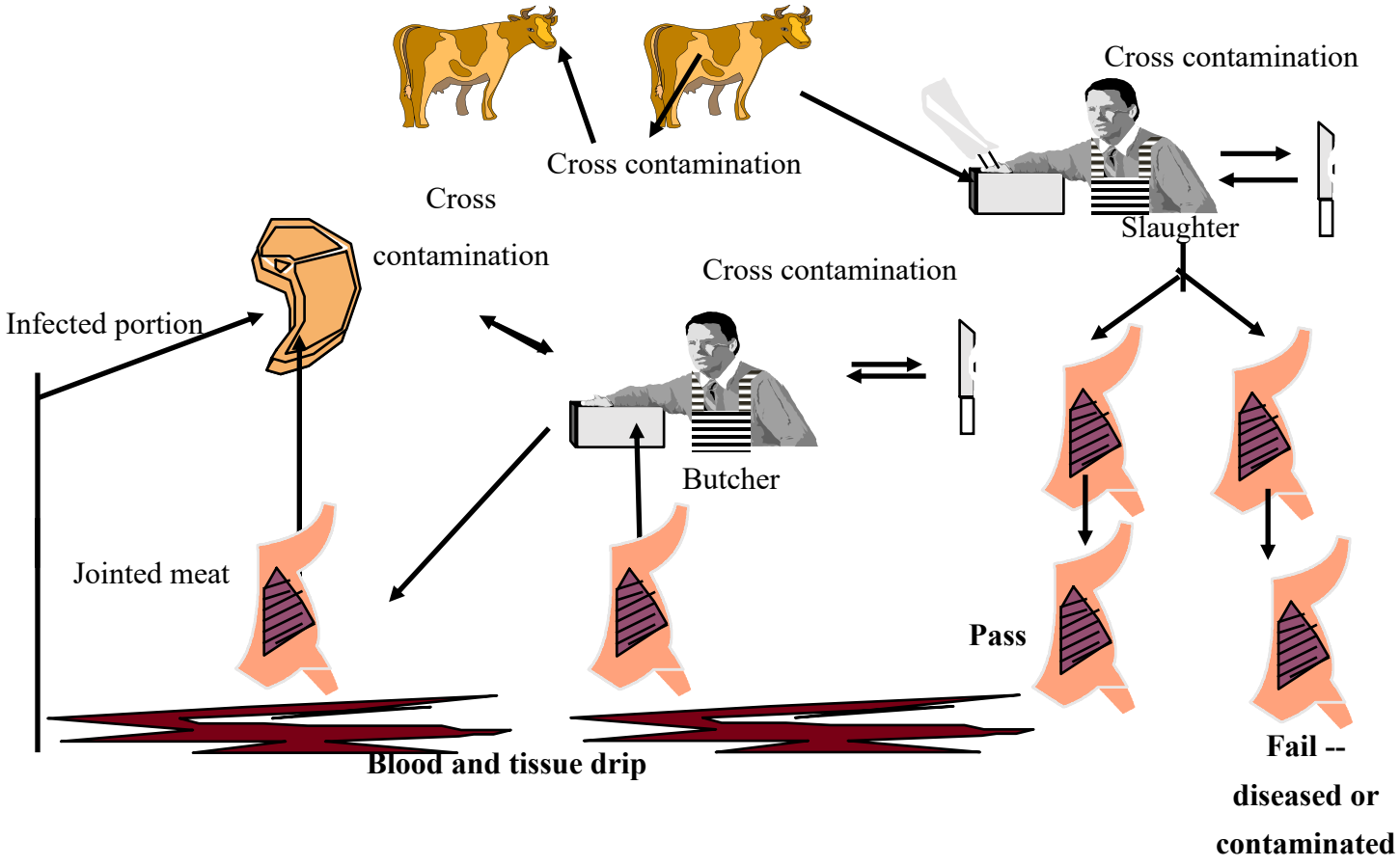

FIGURE 2. 3 Contamination chain of *E. coli* in bushmeat from farm to point of consumption. Adapted from Eniang *et al*., (2008)

CHAPTER THREE

3 MATERIALS AND METHODOLOGY

3.1 Study Area/Location

The sampling areas were Ibadan, Sabo, Akure, Ore, Sango-ota, Abeokuta, Festac and Epe which are located in Oyo, Osun, Ondo, Ogun and Lagos states. The Southwestern part of Nigeria has been identified as some of the states with the highest numbers of bushmeat consumers, hence the reason for study. In Lagos state, Epe markets is one central market. This market was used for consecutive samplings due to its size and also the high prevalence of different types of bushmeat and unsafe processing safety practices.

3.2 Sampling of Game Meat

Smoked bushmeat samples were collected from different markets. After buying from the vendors, the samples were correctly identified and packed in airtight bags. The samples were put in airtight bags to ensure aseptic conditions and to preserve spoilage. The bags containing samples were then transferred to the laboratory (within 6-10 hours) for further analyses.

TABLE 3.1 Various game-meat and sampling locations in Nigeria

LOCATION	GAME-MEAT	NUMBER OF SAMPLES
	Pangolin	
	Bird	
	Deer	
Lagos State	Bush dog	25
	Grasscutter	
	Etu	
	Wild Cat	
	Atika	
	Agbonrin	
	Antelope	
	Monkey	
	Rabbit	
	Porcupine	
	Antelope	
	Grasscutter	
Ogun State	Rabbit	
	Bush rat	12
	Igala	
	Hedgehog	
	Guinea fowl	
	Alligator	
	Civet Cat	
	Rabbit	
Ondo State	Antelope	9
	Grasscutter	
	Grasscutter	
	Guinea Fowl	
	Hare	
Osun State	Sese	5
	Antelope	
	Aparo	
Oyo State	Eta	4
	Esii Tuku	
	Guinea Fowl	
Total		55

3.3 Reagents and Equipment Used

Materials used include: test tubes (with their racks), micro pipette (with their tips), petri-dishes, beakers, scotch bottles, flasks, Eppendorf tubes, spatula, filter paper, glass spreader

Equipment used are: incubator (set at 37°C), Autoclave, weighing balance, distiller, wash bottles, water bath (set at 50°C and 100°C), oven, inoculating loop, Bunsen burner.

3.4 Preparation Of Culture Media used for isolation

For the enumeration, isolation and identification of *Escherichia* isolates different media were used for enhancement of their viability and isolation.

They include the following:

3.4.1 Buffered Peptone Water (BPW)

A non-selective broth medium, composed of peptic digest of animal tissue and sodium chloride, rich in tryptophan with pH of 7.2±0.2 at 25 °C. It was used as a primary enrichment medium to detect *Escherichia* strains. 0.1% of this medium was used for serial dilution of samples for isolation.

Preparation

- Based on manufacturer instructions (Titan Biotech Limited, India), the medium was dissolved in the appropriate volume of distilled water to make up 0.1% peptone water in a conical flask and mixed thoroughly.
- The conical flask was corked using a cotton wool tightly wrapped in aluminum foil to prevent contamination of the medium
- The mixture was heated for few minutes to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15 minutes.
- Finally, the autoclaved medium was then dispensed by pipetting 5 ml of the medium into various test tubes for serial dilution.

3.4.2 MacConkey Agar

A selective media made up of ingredients that allow the growth of target microorganisms and inhibit the growth of unwanted microorganisms, they contain sugars, salts, antibiotics and dyes that only the selected microorganism can utilize because of the way it changes the metabolic systems of microorganisms, these ingredients could be the only carbon or nitrogen sources and this results in the inhibition of other unwanted or screened out microorganisms due to their inability to assimilate these sources of nutrients used for gram-negative enteric bacteria isolation and lactose fermentation

differentiation from non-lactose fermenting bacteria and lactose fermenting bacteria but provides pink colonies on MacConkey Agar as *Escherichia coli.*

Preparation

- The dehydrated medium was dissolved in the appropriate volume of distilled water i.e., 48.5g of MacConkey in 1000 ml distilled water based on manufacturers' instructions (Titan Biotech Limited, India) in a conical flask and mixed thoroughly. The conical flask is then closed using a cork.
- The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15 minutes. Avoid overheating.
- The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify. The medium is neutral red in colour.

3.4.3 Sorbitol-MacConkey Agar (SMAC)

Sorbitol MacConkey agar was prepared according to the manufacturer's instruction (Titan Biotech Limited, India) for isolation and detection of *E. coli* O157:H7.

Preparation

a. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e., 51.5g of SMAC in 1000 ml distilled water based on manufacturers instruction's instructions in a conical
b. flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
c. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15 minutes.
d. The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify. This medium is reddish-purple in color.

3.4.4 Nutrient Agar

Nutrient agar was prepared according to the manufacturer's instruction (Titan Biotech Limited, India) for isolation and detection of total count of mesophilic organism.

Preparation

a. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e., 28g of Nutrient agar in 1000 ml distilled water based on manufacturers instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.

b. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15 minutes.
c. The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify. The medium appears opalescent and is light amber in colour.

3.5 Isolation of STEC from Game Meat

3.5.1 Sample Preparation

25g of each sample are weighed and soaked in 1⁄ of buffered peptone water.

3.5.2 Primary Enrichment

Another (Twenty-five) 25grams of sample was weighed and kept into 225ml of peptone water which was subjected to 7 different dilution factors starting with the original 10^1 - 10^7 using serial dilution method.

3.5.3 Serial Dilution

One milliliter (1ml) of the samples were pipetted using the micro-pipette (set at 1000 ul) into test tubes containing 9ml of BPW (0.1%) to obtain 10^2, followed by the transfer of 1ml from 10^2 into a new test tube (containing 9ml of BPW) to create 10^3 dilution, the test tubes are then put in the vortex mixer for even mixing. The dilution factor was repeated for 10^2, 10^3 and 10^5. The test tubes were labelled for easy identification.

3.5.4 Plating (Spread Plate Technique)

For the Nutrient agar and MacConkey agar plates, spread plate technique was used for plating of inoculum (samples). About 15-20ml of agar were poured into sterilized petri dishes (observing aseptic methods and conditions), then allowed to cool, set and solidify. 0.1ml of the inoculum directly from dilutions 10^2, 10^3 and 10^5 were plated (using pipettes) onto appropriately labelled agar-containing petri-dishes for Nutrient agar and MacConkey agar, this will suffice for the enumeration, identification and isolation of Total Viable Counts and *Escherichia coli* strains respectively. After the dispensing, the glass rod is used to spread or distribute the inoculum all around the agar (the glass rod was dipped into alcohol and then flamed in the Bunsen burner before spreading so as to maintain aseptic conditions). Replicates were made for each dilution for each sample.

3.5.5 Subculturing

The plates were checked after the required duration for the growth a sub-culturing needs to done. Subculturing was done to purify the isolated bacterial colonies from a mixed culture to a new and single culture, the bacterial isolates transferred or sub-cultured were those were differentiated on the basis of their colony morphology, shape, colour, elevation and other physical characteristics. Colonies differentiated by morphological characteristics are transferred onto fresh petri dishes containing Nutrient agar. A loopful of

preferred isolate was taken using the inoculating loop (the inoculating loop is heated using the Bunsen burner and allowed to cool for like 5 seconds before taking the loop from the original mixed culture and streaked onto the new petri-dish). For subculturing, the isolate containing loop is transferred to the new petri-dish using the streaking method procedure.

3.6 Preservation of Isolates

A loopful of each isolate was inoculated into 5 ml peptone buffered water (BPS) containing 10% glucose for long-term conservation and then incubated for 18 hours. The suspension is then added to the Eppendorf tubes containing sterile 20% glycerol as cryoprotectant and homogenized. It was then stored in a -4℃ freezer at -20°C.

Preparation of broth.

Prepare 0.1% of Buffer Peptone Water (i.e., 0.5g in 500ml distilled water).

Prepare 2% glucose in BPW (i.e., Weigh 10g of D-Glucose and add to the 0.1% BPW), allow to dissolve.

Dispense 5ml each of 2% of glucose into test-tubes, then autoclave.

After autoclaving, allow to cool. Inoculate a loopful of your isolates/pure culture into the test tubes.

Incubate at 37°C for 18hours.

After 18hrs

Prepare 75% glycerol (i.e., 225 glycerol in 500ml of distilled water), then autoclave.

Get Eppendorf tube, autoclave them.

Allow to cool.

Add 0.5ml of cultured broth + 0.5ml of glycerol into Eppendorf tube.

Store in - 20°C.

3.7 Biochemical Tests

3.7.1 Gram Staining

A smear of the isolate was prepared on a glass slide, it was placed on a staining rack then flooded with crystal for a minute which was washed under tap water and then it was flooded again with Lugol's iodine for a minute and then washed under water and flooded with a mixture of ethanol and water(70% alcohol) for 10 minutes and also washed under water and then lastly, was flooded with Safranin for 30seconds and then washed under water, then it was then dried using absorbent paper and examined under the microscope the oil immersion objective.

3.7.2 Catalase Test

On a clean microscopic glass slide a small amount of the culture from the nutrient agar slant with a sterilized and cooled inoculating loop was mixed and emulsified. With a Pasteur pipette, a drop of hydrogen peroxide was placed over the test smear. The fluid was observed over the smears for the appearance of gas bubbles, if bubbles were formed then the organism is catalase positive and if there were no bubbles formed then the organism cannot produce catalase enzyme.

3.7.3 Oxidase Test

A filter paper soaked with oxidase reagent and then moistened the paper with a sterile distilled water and picked the colony to be tested with an inoculating loop and smear in the filter paper.

The inoculated area of filter paper was observed for a color change to deep blue or purple within 10-30 seconds.

3.7.4 Motility Test

A semisolid agar medium was prepared in a test tube and inoculated the isolate with a straight wire loop, making a single stab down the center of the tube to about half the depth of the medium. It was inoculated under normal conditions which favors motility. Then the test tube was incubated at 37°C

and examined at intervals (depending on generation time of bacterium). The tube was held to the light and looked at the stab line to determine motility.

3.8 Molecular Characterization

3.8.1 Activation of Isolates

- Isolates were taken out of the freezer and allowed to thaw at room temperature.
- 1ml of BHI was added to Eppendorf tubes and autoclaved.
- 100µl of *E. coli* isolates were added to the Eppendorf tubes containing the BHI and incubated at 37°C for 24 hours bringing about the activation of the isolates.

3.8.2 DNA Extraction

- The isolates were centrifuged for 5 minutes at 10,000RPM, then the supernatant was decanted.
- The Eppendorf tube was filled with 1ml of sterile distilled water, vortexed, and centrifuged at 10,000 RPM for 5 minutes, the supernatant was discarded, and the process was repeated.
- After which, 200 µl of sterile nuclease-free water was pipetted into the Eppendorf tube containing the pellets, vortexed, and then placed in the heating block to boil at 100◦C for 15 minutes, the solution was then placed in ice to cool, and the contents of the Eppendorf tube were centrifuged for 5 minutes at 14,000RPM.

- A new set of Eppendorf tubes were labelled with the corresponding codes of the isolates and 150μl supernatant containing the extracted DNA was then transferred into the new Eppendorf tubes and stored in the freezer at -20^{o}C for further analysis.

3.8.3 Polymerase Chain Reaction

The components of the PCR and the constituents' mixes used to identify *E. coli* are summarized in table 3.2 below. The PCR cocktail was made and then transferred to a PCR tube before being placed in the thermocycler. Initial denaturation was performed at 95°C for 5 minutes, followed by 35 cycles of 95°C for 2 minutes, 42°C for 30secs, and 72°C for 4 minutes, followed by a final elongation step at 72°C for 10 minutes. There were also negative control reactions provided. The template DNA was replaced with sterile water for the negative controls. Electrophoresis was used to confirm the PCR products, and a Gel Documentation system was used to visualize them under UV light.

TABLE 3.2 Gene targets, primer sequences, primer concentrations and amplicon sizes for the multiplex PCR. Adapted from Persson *et al, (*2007)

Gene Target	**Virulence factor/gene**	**Sequence (5´-)**	**Final concentration(µm)**	**Amplicon size(bp)**
Human estA	STIh	TTTCGCTCAGGATGCTAAACCAG CAGGATTACAACACAATTCACAGCAG TA	0.4	151
Porcine estA	STIp	CTTTCCCCTCTTTTAGTCAGTCAACTG CAGGATTACAACAAAGTTCACAGCAG	0.4	160
vtx1	VT1	GTTTGCAGTTGATGTCAGAGGGA CAACGAATGGCGATTTATCTGC	0.25	260
Eae	Infimin	GGYCAGCGTTTTTTCCTTCCTG TCGTCACCARAGGAATCGGAG	0.15	377
vtx2	VT2	GCCTGTCGCCAGTTATCTGACA GGAATGCAAATCAGTCGTCACTC	0.5	420
EltA	LTI	AAACCGGCTTTGTCAGATATGATGA TGTGCTCAGATTCTGGGTCTCCT	0.45	497
IpaH	IPaH	TTGACCGCCTTTCCGATACC ATCCGCATCACCGCTCAGAC	0.1	647
16SrDNA	16SrDNA	GGAGGCAGCAGTGGGGAATA TGACGGGCGGTGTGTACAAG	0.25	1062

3.8.4 Agarose Gel Electrophoresis

Dry Agarose powder was used to make the agarose, and 1.8g of it was dissolved in 100ml of 1x TAE buffer. The mixture was heated until a clear solution was obtained. A micropipette was used to add 3 µl of ethidium bromide to the mixture. It was then spun, allowed to cool and then transferred to the gel tray which results in a slab with the combs in place, that create wells in the slab. It was then allowed to solidify before carefully removing the combs. The gel tray containing the slab was placed into the gel tank and 1x TAE buffer was added to the gel tank. 3 µl of DNA ladder was added to the first well, and 4 µl of amplicon (one sample per well) was pipetted into each well that was produced. The tank was attached to the power pack and the gel was ran at 100 volts for 45 minutes. The resulting gel was inspected using the gel documentation system.

3.8.5 Precautions

• Aseptic techniques were observed at every stage of work.

• Personal protective technique was also observed, such as wearing of covered shoe, nose cover, gloves, lab coat, etc.

• The inoculating loop cooled before picking the organism when sub-culturing in order not to kill organism of interest.

• The petri-dishes were incubated inverted.

• Proper timing, most especially during autoclaving was ensured.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

The microbial analyses of the bushmeat samples gotten from Oyo, Ondo, Osun, Lagos and Ogun state were carried out. All samples had pink (non-O157) and white (O157) raised, circular and smooth colonies on SMAC and MAC agar which indicates the presence of *E. coli* in the samples.

The results of the findings were summarized in Table 4.1 shows that the Total Viable Count of isolates on Sorbitol MacConkey agar with bacterial isolates collected from bushmeat sampling from 9 locations in Lagos, Osun, Ondo, Ogun and Oyo states. While Table 4.2 shows the results of the biochemical characterization of fifteen (15) isolates using catalase, motility and oxidase tests alongside gram stain results from isolates obtained.

4.1 Microbial Count of *E. coli*

According to Table 4.1, Monkey had the highest TVC range of 9.5 $\log_{10}$ cfu/g followed by Antelope with a TVC range of 8.6- 8.8 $\log_{10}$ cfu/g. The TVC in grasscutter had ranged between 8.3- 8.5 $\log_{10}$ cfu/g; lower TVC was recorded in Quail, Agbonrin, ETA, Hare, and Guinea Fowl which ranged between 4.1- 4.8 $\log_{10}$ cfu/g.

In Lagos state, monkey had the highest count (9.5 $\log_{10}$cfu/g) while Quail had the lowest count of 4.1$\log_{10}$cfu/g. In Ogun state, Antelope had the highest TVC (8.6 log10cfu/g) with Guinea fowl having the lowest (4.8log10cfu/g). In Ondo state, Antelope had the highest TVC (8.8 log10cfu/g) with Guinea fowl as the lowest (4.4log10cfu/g). Antelope had the highest TVC (8.6 log10cfu/g) with Hare as the lowest (4.8 log10cfu/g) in Osun State. In Oyo state, Esii Tuku had the highest TVC (7.1log10cfu/g) with Eta as the lowest (4.3 log10cfu/g).

TABLE 4. 1 Various game-meat sampling locations in Nigeria showing Total Viable Count of the *E. coli* colonies

LOCATION	GAME-MEAT	NUMBER OF SAMPLES	TOTAL VIABLE COUNT (cfu/ml)
	Pangolin		8.6×10^6
	Quail		4.1×10^6
	Deer		8.1×10^6
Lagos State	Bush dog	25	6.4×10^6
	Grasscutter		8.5×10^6
	Etu		5.5×10^6
	Wild Cat		7.3×10^6
	Atika		6.3×10^6
	Agbonrin		4.5×10^6
	Antelope		8.7×10^6
	Monkey		9.5×10^6
	Rabbit		7.5×10^6
	Porcupine		8.3×10^6
	Antelope		8.6×10^6
	Grasscutter		8.4×10^6
Ogun State	Rabbit		7.8×10^6
	Bush rat	12	6.2×10^6
	Igala		6.7×10^6
	Hedgehog		5.2×10^6
	Guinea fowl		4.8×10^6
	Alligator		7.3×10^6
	Civet Cat		7.2×10^6
	Rabbit		7.4×10^6
Ondo State	Antelope	9	8.8×10^6
	Grasscutter		8.3×10^6
	Guinea Fowl		4.4×10^6
	Hare		4.8×10^6
Osun State	Sese	5	6.8×10^6
	Antelope		8.6×10^6
	Aparo		5.5×10^6
Oyo State	Eta	4	4.3×10^6
	Esii Tuku		7.1×10^6
	Guinea Fowl		5.0×10^6
Total		55	

4.2 Biochemical results of presumptive isolates

The results of the biochemical tests indicated that all *E. coli* isolates were Gram and oxidase negative; and all were positive for motility test, positive result was also observed in all isolates for catalase as shown in Table 4.2

TABLE 4. 2 The results of Biochemical test for presumptive identification of *E. coli* in all isolates

S/N	Sample	Gram reaction	Catalase	Oxidase	Motility	Probable organism
1	**SS_1**	-	+	-	+	*E. coli*
2	RAS_1	-	+	-	+	*E. coli*
3	IGS_1	-	+	-	+	*E. coli*
4	HEA_1	-	+	-	+	*E. coli*
5	G_1L_1	-	+	-	+	*E. coli*
6	CC_1O_1	-	+	-	+	*E. coli*
7	ESI_1	-	+	-	+	*E. coli*
8	A_1L_1	-	+	-	+	*E. coli*
9	ETI_1	-	+	-	+	*E. coli*
10	PA_2L_1	-	+	-	+	*E. coli*
11	SPL_1	-	+	-	+	*E. coli*
12	M_1L_1	-	+	-	+	*E. coli*
13	D_2L_1	-	+	-	+	*E. coli*
14	GUA_1	-	+	-	+	*E. coli*
15	HS_1	-	+	-	+	*E. coli*

4.3 Distribution of positive *E. coli* isolates among the bushmeat samples

E. coli were isolated from all the game meat sampled. Antelope had the highest prevalence (21.81%) and Etu, Wild Cat, Atika, Quail, Aparo, Eta, Esii Tuku, hedgehog, sese, Sparrow, Bush dog, hare, alligator had the lowest prevalence (1.82%)

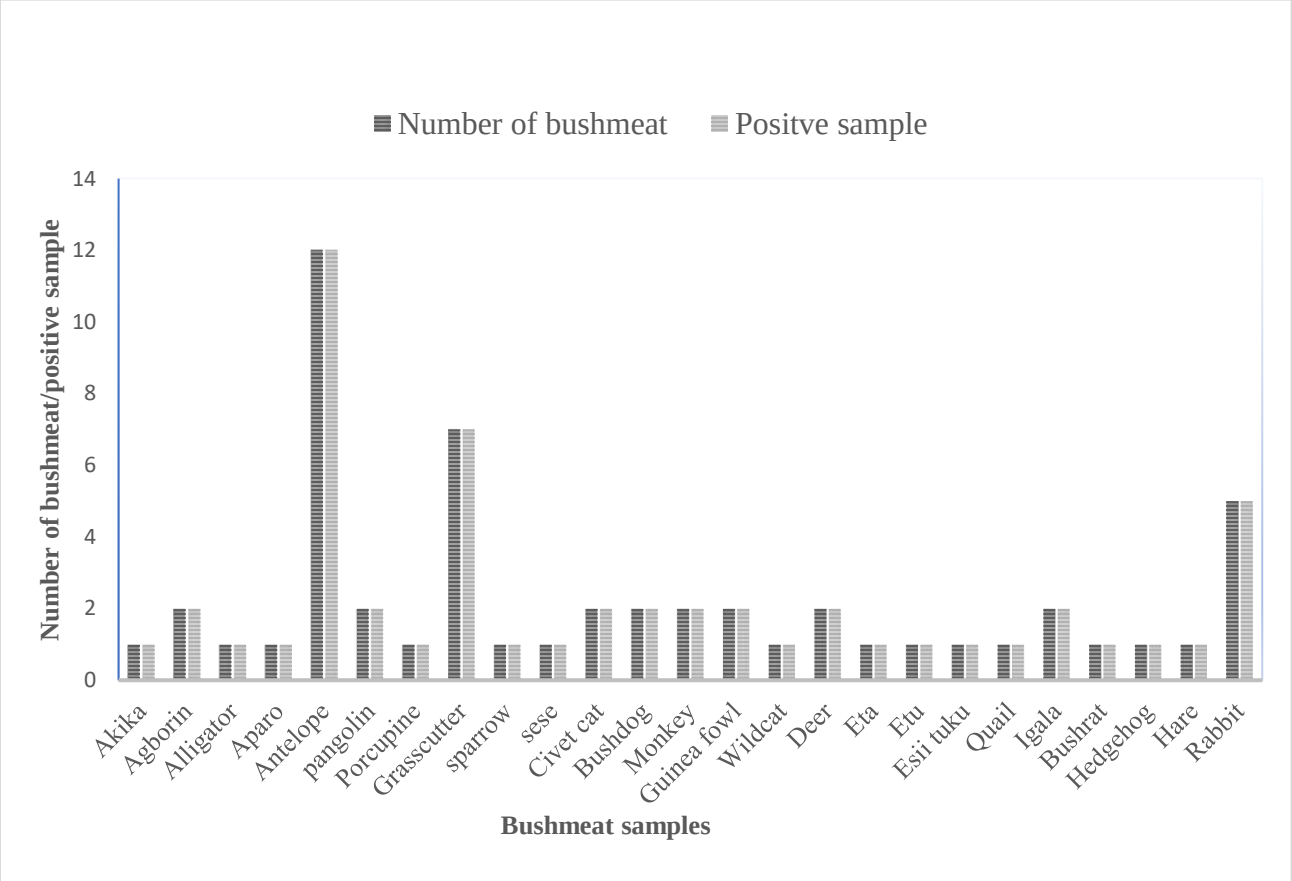

FIGURE 4. 2 The prevalence of pathogenic *E. coli* in the bushmeat samples

4.4 Polymerase Chain Reaction Amplification Image

The representative visualized result of gel electrophoresis for the detection of Vtx1 gene from isolates obtained from different game meat samples using the PCR technique confirm the presence of Shiga-Toxigenic *E. coli* (Figure 4.2). Overall, Vtx1 was detected in 7.27% of *E. coli* - positive game meat samples,

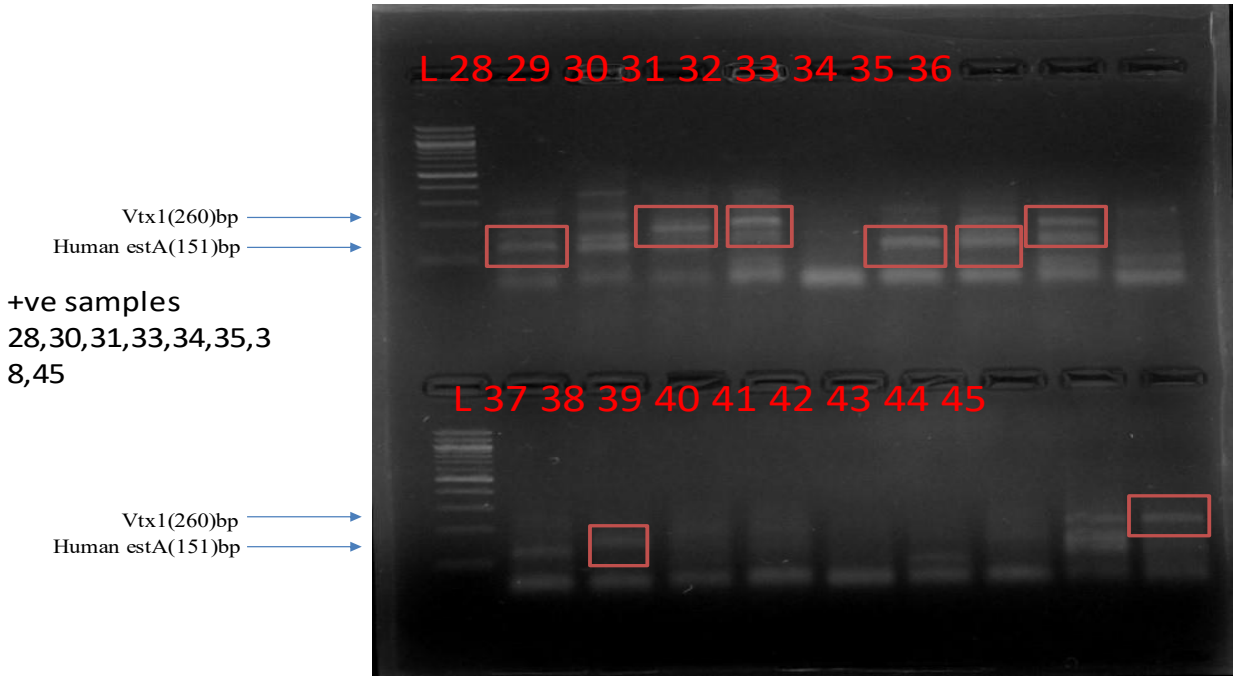

FIGURE 4.2 Illustrative agarose gel electrophoresis image of multiplex-PCR products (Human *estA*, *Porcine estA*, *vtx1*, *vtx2*, *ipaH*, *eae*, *eltA*). Lane L: marker (100-bp ladder), lane 28: *E. coli* isolate (Human *estA*), lane 30:(*vtx1*).

4.5 Discussion

Based on the findings of this study and the deductions derived, it could be concluded that bushmeat samples vended in Osun, Oyo, Ondo, Ogun market and Lagos State are highly contaminated with strains of pathogenic *E. coli*. The results show that the bush meats samples had significantly high microbial viable counts. Bacteria counts ranged from 4.1×10^6 to 9.5×10^6 cfu/g.

Similar study conducted in Nigeria on the microbiological assessment of game meat also confirmed the presence of *E. coli* O157:H7 and other Enterobacteriaceae (Eniang *et al*., 2008). The Bacteria counts obtained from the research ranged from 1.72×10^6 to 9.46×10^6 cfu/g.

The prevalence of STEC obtained in the sampled bush meat falls within the recommended limit for consumption of meat. The premise for such deductions is indicated in the microbial load requirements in meat product. Relatively, the total viable microbial count of less than half a million is satisfactory. Microbial count of 500,000 < 10,000,000 in meat is acceptable and passable. Microbial count of ten million and more is considered as unsatisfactory (Illés *et al., 2018*).

The results of the biochemical tests indicated that all the isolates were presumptive *E. coli*.

The four isolates that were positives for vtx, further confirmed the presence of pathogenic Shiga toxin producing *E. coli* (STEC) in Antelope, Pangolin, Monkey, and Grasscutter sold in Ondo, Lagos and Ogun. Consumption of these game meat will create a public health hazard.

The finding of this study demonstrated that consumption of game animals could be considered as the major risk due to the associated STEC. This is an indication that these animals may contribute to the prevalence of foodborne illnesses in South-Western, Nigeria.

CHAPTER FIVE

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The findings of this study revealed that game meat sold by the road sides have significant microbial loads (aerobic plate counts) and poor meat quality. This makes the game meat a possible source of foodborne illness. The lack of safety of the game meat is due to a variety of circumstances, including the workers' low educational levels, lack of training, and inadequate hygiene and sanitation practices at the abattoir and butcher shops. The high prevalence of pathogenic *E. coli* also shows that contamination existed from the slaughter houses or killing locations to the vendor's shops.

5.2 Recommendation

Therefore, it is crucial to raise awareness about game meat hygiene and sanitation, and suitable problem-solving techniques should be developed and put into practice. Control measures should be aimed at preventing any practice that potentially contaminates game meat and should be implemented to prevent any form of contamination. Consumers need to be informed about the potential risk of consuming not properly cooked game meat.

Regulatory and educational efforts from the government officials are needed to improve the safety of fresh meat that are intended for use as ready to eat products in Nigeria.

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