DETECTION AND PREVALENCE OF *SALMONELLA SPP* AND *SHIGELLA SPP* IN READY-TO-EAT GAME MEAT OBTAINED FROM DIFFERENT LOCATIONS IN NIGERIA

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCES IN MICROBIOLOGY

SEPTEMBER, 2022

DECLARATION

I hereby declare that this project report written under the supervision of Dr. O.E. Fayemi is a product of my research work, scientific information derived from various sources has been duly acknowledged in the text and a list of references provided. This research project report has not been previously presented anywhere for the award of any degree or certificate.

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OYEWOLE, TIJESULASE D.

_____

Date

CERTIFICATION

This is to certify that this research project titled '**DETECTION AND PREVALENCE OF *Salmonella spp.* AND *Shigella spp.* IN READY-TO-EAT GAME MEAT OBTAINED FROM DIFFERENT LOCATIONS IN NIGERIA**' was carried out by **OYEWOLE TIJESULASE DEBORAH** with matriculation **18010101002**. This project meets the requirement governing the award of **Bachelor of Science (B.Sc.)** degree in **MICROBIOLOGY** from the Department of Biological sciences of Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literacy presentation.

_____ (Signature and Date)

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Supervisor

(Signature and Date)

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Dr. (Mrs.) C.I. Ayolabi

Ag. Head of Department

DEDICATION

I dedicate this work to God the giver of life and also to my parents and sister for their unending support, prayers and care towards the successful completion of my project work.

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.

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ABBREVIATIONS

XLD - Xylose Lysine Deoxycholate Agar

RVS - Rappaport-Vassiliadis Medium

HEA - Hektoen Enteric Agar

BHI - Brain Heart Infusion Broth

BPW - Buffer Peptone Water

DNA - Deoxyribonucleic Acid

PCR – Polymerase Chain Reaction

SPP - Species

WHO - World Health Organization

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ABSTRACT

Game meat represents a primary source of animal protein. Concerns have been raised about the sustainability of hunting practices for wildlife populations where bushmeat harvest is common as well as the risk of exposing hunters and consumers to emerging, reemerging, and endemic zoonotic diseases as a result of the widespread reliance on game meat for nutritional and financial security. This study evaluated the prevalence of *Salmonella* and *Shigella* in varieties of bushmeat samples from Southwestern part of Nigeria. A total of 55 samples were tested for the presence of *Salmonella* and *Shigella* using Xylose Lysine Deoxycholate Agar and Hektoen Enteric Agar. All samples had presumptive *Salmonella* and *Shigella*, molecular identification of selected *Salmonella* (n=37) and *Shigella* (n=18) isolates using Simplex PCR was done which confirmed that *Shigella* and *Salmonella* were found to be present in the game meat samples. The total prevalence for positive *Salmonella* samples is 41.8% and for positive *Shigella* samples is 45.5%. The presence of a very high microbial load of *Salmonella* and *Shigella* poses a threat to public health which could lead to foodborne illnesses including gastroenteritis and bacterial diarrhea.

Key words: *Salmonella*, *Shigella*, Game meat, Foodborne disease, Gastroenteritis.

CHAPTER ONE

1 INTRODUCTION

1.1 Background to the study

Worldwide, *Salmonella* and *Shigella* continue to be of great concern for public health, putting a financial strain on both industrialized and developing nations due to the high expenses of disease surveillance, prevention, and treatment (Eng *et al*., 2015). The most typical *Salmonella* infection symptom worldwide is gastroenteritis, after which bacteremia and enteric fever occur (Chen et al., 2013). The genus *Salmonella* using the common Kauffman-White system, have been found to have over 2600 serotypes, and the bulk of these serotypes can adapt to a wide range of animal hosts, including people (Jayere & Saleh 2019). The most common foodborne pathogens to be isolated are *Salmonella*, which is mostly present in chicken, eggs, and dairy products (Eng *et al*., 2015). Fresh produce are among the other food items that contribute to the *Salmonella* outbreak (Fatica & Schneider, 2011). Generally speaking, Food animals like pigs, poultry, and cattle are one of the crucial infection sources for *Salmonella* (Hur & Lee, 2012). Pathogens are spread mostly through the trade in animals and raw animal food products (Eng *et al*., 2015). One of the major *Salmonella* infection sources of organs and carcasses in food animals is thought to be the slaughtering procedure at abattoirs (Kebede *et al*., 2016).

The third-most frequently reported food-borne bacterial pathogen was *Shigella* spp. according to the Center for Disease Control Emerging Infections Program (Molla *et al*., 2017). *Shigella* are Gram-negative, nonmotile, facultatively anaerobic, non-spore-forming rods (Madan *et al*., 2022). The annual number of all deaths attributable to shigellosis involving children episodes throughout the world is estimated to be 164·7 million, with 69% of all deaths attributable to shigellosis involving children <years of age 5 (Kotloff *et al*., 2017). However, foodborne outbreaks caused by *Shigella* mainly occur while foods are subjected to preparation or processing by hands and are expose to a limited heat treatment or are served raw to the consumer (Pakbin *et al*., 2021). Meat, salads, raw vegetables, chicken and raw milk are some of the foods linked to *Shigella* outbreak. Many of these foods are infected during preparation in homes or restaurants, and then mistreated before being consumed (Bintis & Thomas 2017).

Game meat in particular, serves as a significant factor in ensuring food security and livelihoods of millions of individuals throughout the developing world (Ripple *et al*., 2016). Game meat serves multiple roles and provides many benefits to those that use it (Cawthom *et al*., 2015). Most notably, this wild resource provides a crucial source of protein in places where domestic alternatives are scarce and expensive (Cawthom *et al*., 2015). Consuming game meat comes with additional risks due to the potential microbiological contaminant despite being perceived according to its natural and nutritional features as being healthy (Xie *et al*., 2020). Wild game meat may transport zoonotic infections that harm animals, people, and the environment (Smith *et al*., 2020). Game feeding, habitat, migration, and contact with farms may play a role in the spread of these zoonotic bacteria (Sauvala *et al*., 2021).

Salmonella spp and *Shigella spp* have been found from previous studies to be a part of the causative agents of infections of game meat (Dell *et al*., 2020).

1.2 Statement of Problem

Humans eating game meat come into contact with wildlife more frequently, which facilitates the spread of foodborne and viral diseases like Ebola and newly emerging infectious diseases. The spread of these diseases could be wider from the consumption of unsafe game meats that are potential reservoirs of causative pathogens.

1.3 Significance of the Study

It is important to investigate the microbiological safety and acceptability of game meat in order to ascertain the level of pathogens present in it. Samples of game meat from various markets across Nigeria would be examined for presence of *Salmonella spp* and *Shigella spp* in them which if present poses risks associated with consumption of contaminated game meats.

1.4 Aims and Objectives of the Study

- To isolate *Salmonella spp* and *Shigella spp* in ready-to-eat game meat sold in different markets across Nigeria.
- To assess the risk of consuming game meat contaminated with *Salmonella* and *Shigella.*

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Game Meat

The term “Game meat” refers to any wild animal that has been hunted for food (Demartini *et al*., 2018). Many communities around the world still rely solely on bushmeat as their only source of animal protein (Cawthom & Hoffman, 2015). Game meat can be an important source of micronutrients and macronutrients (Sarti *et al*., 2015). The general reliance of people on the use of bushmeat to provide food and income poses a concern for the risk and the long-term viability of hunting methods for wildlife populations where bushmeat harvest is common and exposure of hunters and consumers to endemic, reemerging, and emerging illnesses during hunting, preparation, and consumption (Dell *et al*., 2020).

Furthermore, zoonotic infections make for 62% of all newly emerging infectious diseases, more than 70% of those zoonoses involve wildlife reservoirs, making human interaction with wildlife a significant channel for endemic and emerging infectious diseases (Horspool, 2013). Game meat provides many opportunities for zoonotic diseases to spread, such as airborne and bloodborne during the killing and slaughtering of corpses, as well as foodborne concerns related to preparation (Ashby & Elizabeth 2021). After widespread and well reported outbreaks like the 2015 and current Ebola virus epidemics and the recent COVID-19 pandemic , have brought zoonotic illnesses into the public attention; each of these infectious agents originated from contact with wildlife species (Rothan & Byrareddy 2020).

The occurrence of endemic zoonotic bacterial diseases in hunted wildlife, including *Shigella, Campylobacter*, *Listeria*, *Pseudomonas*, *Staphylococcus*, *Salmonella*, *Shigella*, *E. coli*, *and Brucella* among others, is less widely known but arguably more ubiquitous in many local communities (Dell *et al*., 2020). Another issue is the possibility that viruses from harvested wildlife could come into contact with domestic animal species (Friant *et al*., 2015). Some of the most researched diseases that can spread from wildlife to cattle through interaction are African swine fever, avian influenza, rabies, anthrax, TB, Brucellosis, and Rift Valley Fever (Carpenter *et al*., 2022). Many of these multiple-host animal infections have the potential to spread from cattle to people, resulting in occasional cases or disease outbreaks (Kanoute *et al*., 2017). There are no legal limitations for microbial counts on game bird carcasses, and the microbial

contamination of corpses from game meat is not well reported (Sauvala *et al*., 2021). There is a risk of sporadic foodborne infections associated with the preparation and consumption of game bird meat (Sauvala *et al*., 2021). A limited number of reports exist on the prevalence of foodborne pathogens in game birds (Sauvala *et al*., 2021). *Shigella* and *Salmonella* have been found in other studies and these studies showed an abundance of *Salmonella* and *Shigella* in bush meat (Bachand *et al*., 2012).

2.1.1 Role of Game Meat in Spread of Diseases

Today, animal sources are blamed for the appearance of HIV-1, AIDS, the Ebola virus sickness, and Creutzfeldt-Jakob disease (Cook & Karesh, 2012). In the 1980s, it was discovered that Thomas' rope squirrels (*Funisciurus anerythrus*) and red-legged sun squirrels (*Heliosciurus rufobrachium*) were monkeypox viral reservoirs in the Democratic Republic of the Congo (MacNeill, 2022). Bushmeat hunters in Central Africa who had been exposed to wild monkeys in close proximity contracted the human T-lymphotropic virus (Mossoun *et al*., 2017). The most well-known virus to have evolved through the interaction between game meat and human is human immunodeficiency virus (HIV) (Cunningham *et al*., 2017). As a result of studies conducted on wild chimpanzees in Cameroon, it has been determined that these primates are naturally infected with the simian foamy virus and act as a reservoir for HIV-1, a virus that is a precursor to AIDS in humans (Li *et al*., 2012). There are numerous unique HIV strains, indicating that there have been numerous instances of this interspecies transfer (Peeters *et al*., 2014). It is likely that HIV was initially transferred to humans after having come into contact with infected bushmeat (Mwangi *et al*., 2016).

The natural reservoirs of ebolaviruses are unknown (Goldstein et al., 2019). Potential reservoirs include non-human primates, megabats, rodents, shrews, carnivores, and ungulates (Olivero *et al*., 2019). There has been a connection between the Ebola virus and bushmeat, and some experts believe that megabats are a main host for at least certain Ebola virus types (Ohimain, 2016). Gastrointestinal parasites in 15 different primate species in Cameroon were investigated (Pourrot *et al*., 2011). Pinworms, Bertiella, Trichuris, Entamoeba, Ascaris, Capillaria, and Endolimax nana infections were found in bushmeat primates (Pourrot *et al*., 2011).

2.2 Salmonella spp.

2.2.1 Background History

In 1855, Theobald Smith first discovered and isolated Salmonella in the intestines of pigs suffering from the common swine sickness (Eng *et al*., 2015). The bacteria was named after American pathologist Dr. Daniel Elmer Salmon, who worked with Smith (Mohammed *et al*., 2020). The Centers for Disease Control (CDC) and Prevention now uses the recommended nomenclatural scheme for Salmonella developed by the World Health Organization (WHO) Collaborating Centre (Eng *et al*., 2015). According to this nomenclatural technique, Salmonella enterica (S. enterica) and Salmonella bongori (S. bongori) are the two main species that make up the genus Salmonella (Jayere & Saleh 2019). Further classification divides *S. enterica* into 7 subspecies (subsp.) and more than 2600 serovars/serotypes till date (Van Vorst & Kira 2020). In addition to the classification system of subspecies on a phylogenetic level, serotyping is based on somatic (O), capsular (K) and flagella (H) antigens on the bacterial cell surface by means of the White-Kauffmann-Le Minor Scheme (Jayere & Saleh 2019).

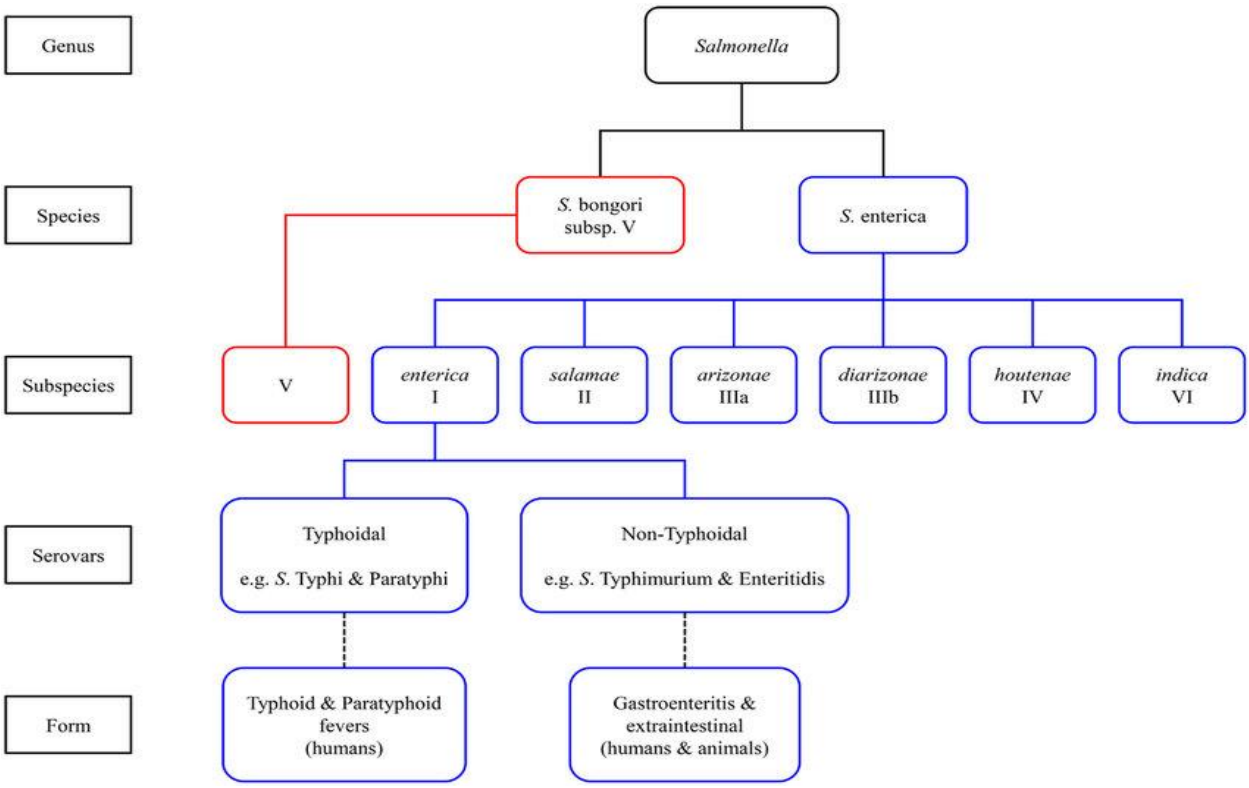

Figure 2.1: Classification of *Salmonella* species and subspecies adapted from Daniel *et al*., (2014).

2.2.2 Characteristics of *Salmonella* spp

Salmonella is a genus of nonspore-forming, Gram-negative, Oxidase-negative, and Catalase-positive rods belonging to the Enterobacteriaceae family (Majumdar *et al*., 2018). Salmonella species are facultative anaerobes and almost all of them are motile via Peritrichous flagella (Ryan et al., 2017). *Salmonella* grows best at temperature of 37°C, however growth has also been observed at 2°C, 4°C, and even 54°C (Catherine *et al*., 2017). *Salmonella* can live in a wide pH range from as low as ph 3.8 to as high as pH 9.5 with an optimum of pH 6.5-7.5 (Ryan *et al*., 2017). *Salmonella* are recognized for being non-fastidious since they may grow and live in a variety of environmental settings, even when they are not present in their living host (Jayere et al., 2019). *Salmonella* are extremely resilient to a variety of environmental stressors, including changes in temperature, acidity, dryness, and a lack of available nutrients (Li *et al*., 2012.

2.2.3 Serovars of *Salmonella* spp

S. enterica subsp. enterica serovars are subdivided into two distinct groups, typhoidal and non-typhoidal (Jacob *et al*., 2020). Typhoidal *Salmonella* cause typhoid fever from infection by *Salmonella typhi* and *Salmonella paratyphi* A and B (Saha *et al*., 2019). Typhoid fever is usually acquired following eating or drinking contaminated food or water (Deksissa & Gebremedhin 2019). The acute illness is characterized by fever, headache, nausea, loss of appetite, constipation and sometimes diarrhea (Siddiqui *et al*., 2019). Symptoms of typhoid fever may vary from a minor sickness characterized by low grade fever, headache, exhaustion, malaise, appetite loss, cough, constipation, skin rashes or rose spots (Habte *et al*., 2018). As typhoidal serovars are human restricted, carriers represent a key reservoir for *S*. Typhi, which contribute to the transmission and dissemination of typhoid (Johnson *et al*., 2018).

Non-Typhoidal *Salmonella* Serovars (NTS) such as *Salmonella typhimurium* are a global cause of self-limiting gastroenteritis (Smith *et al*., 2016). NTS are undoubtedly among the main causes of foodborne infections globally with an estimated 93.8 million cases annually, taking under-reporting and under-diagnosis into account. Additionally, 155 000 people die each year from complications originating from NTS from gastrointestinal tract infections (Van Vorst & Kira 2020).

Table 2.1: Classification of *Salmonella* Species and the current number of Serovars within each subspecies adapted from Oludapo *et al*., (2013).

Salmonella Species	**Salmonella subspecies**	**Number of Serovars**
Salmonella enterica	I enterica	1586
Salmonella enterica	II salamae	522
Salmonella enterica	IIIa arizonae	100
Salmonella enterica	IIIb diarizonae	341
Salmonella enterica	IV houtenae	76
Salmonella enterica	VI indica	13
Salmonella bongori	V bongori	22

2.2.4 Epidemiology of *Salmonella* spp

The epidemiology of illnesses caused by Salmonella spp. varies greatly depending on the specific Salmonella spp. Involved (Eng *et al.,* 2015). While NTS infections often are self-limiting and affect populations worldwide, enteric fever, which is caused by S. Typhi and S. Paratyphi, typically leads to a severe and life-threatening disease that largely affects communities in underdeveloped nations (Stepien, 2020). The changing epidemiology of these infections is provided in separate sections below.

2.2.4.1 Enteric Fever

Children and young adults are more likely than older patients to get enteric fever (John *et al*., 2016). Around the world, overcrowded, impoverished communities with limited access to sanitation are where enteric fever is most common (Radhakrishnan *et al*., 2018). South-central Asia, Southeast Asia, and southern Africa are regions having a high incidence of S. Typhi infection, according to incidence estimates (more than 100 cases per 100,000 persons yearly) (Harris & Brooks, 2020). The incidence ranges from 10 to 100 cases per 100,000 persons yearly in other countries of Asia and Africa, as well as in some portions of Latin America, the Caribbean, and Oceania (Kothari *et al*., 2008). Additionally, subsequent data from Africa have shown that there is a significant amount of country-to-country heterogeneity, with some Southern and Northern African countries having very low rates (5 cases per 100,000 persons yearly) and several Eastern and West African countries having rates >100 per 100,000 (Marks *et al*., 2017). S. Paratyphi A is still rare in Africa (Feasey & Gordon, 2013), but accounts for a substantial proportion of enteric fever cases in areas of South Asia (Arndt *et al*., 2014).

Typhoid fever caused 26.9 million illnesses and 200 000 fatalities by the year 2010 (Adesegun *et al*., 2020). However, typhoid and paratyphoid fever cases decreased globally in 2017 to roughly 14.3 million cases, with 12.1% (1.73 million) of those cases coming from sub-Saharan Africa (Adesegun *et al*., 2020). Data from Africa are, however, hard to come by due to poor epidemiological surveillance efforts and constrained laboratory capability (Dzinmarira *et al*., 2020). The current figures for enteric fever were consequently derived from studies that were published over time (Adesegun *et al*., 2020).

2.2.4.2 Non-Typhoidal Infections

Nontyphoidal *Salmonella* is one of the most common bacteria to cause bacterial diarrhea; it is thought that each year, it causes 153 million cases of gastroenteritis and 57,000 fatalities worldwide (Napiórkowska-Baran *et al*., 2021). The most frequent *Salmonella* infection in the world is gastroenteritis, which accounts for 93.8 million cases and 155,000 fatalities annually (Paswan & Park, 2020).

2.2.5 Transmission of *Salmonella* Infection

Salmonella can spread via fecal-oral route and can be transmitted by food and water, by direct touch with animals, and rarely from person-to-person (Magana-Arachchi & Wanigatunge 2020). An estimated 94% of salmonellosis is transmitted by food. Humans usually become infected by eating foods contaminated with feces from an infected animal (Mouttotou *et al*., 2020).

2.2.6 Pathogenesis of *Salmonella* spp

Salmonella displays a remarkable characteristic during its invasion of non-phagocytic human host cells (Hume *et al*., 2017) whereby it actually induces its own phagocytosis in order to gain access to the host cell (Mohammed *et al*., 2020). The epithelial cells of the intestinal wall are susceptible to invasion by bacteria when they enter the digestive tract through polluted water or food (Eng *et al*., 2015). *Salmonella* can inject its effectors over the membrane of the intestinal epithelial cell and into the cytoplasm using type III secretion systems, multi-channel proteins that are encoded by SPIs (Mohammed *et al*., 2020).The bacterial effectors then activate the signal transduction pathway and trigger reconstruction of the actin cytoskeleton of the host cell, resulting in the outward extension or ruffle of the epithelial cell membrane to engulf the bacteria (Eng *et al*., 2015).

Salmonella strains' capacity to endure in the host cell is essential for pathogenesis since strains lacking this capacity are not virulent (Eng *et al*., 2015). *Salmonella* is wrapped in a membrane compartment known as a vacuole, which is made of the membrane of the host cell, after being absorbed by the host cell (Bai *et al*., 2015). Under normal conditions, the presence of the bacterial foreign body would trigger the immunological response in the host cell, resulting in the fusion of the lysosomes and the secretion of digestive enzymes to destroy the intracellular bacteria (Ribert & Cossart, 2015). However, *Salmonella* modifies the compartment organization by introducing additional effector proteins into the vacuole via the type III secretion system

(Rolhion *et al*., 2016). The remodeled vacuole prevents the fusion of the lysosomes, allowing the bacteria to replicate and survive inside the host cells (Omotade & Roy, 2019). The reticuloendothelial (RES) system can carry the bacteria because of their capacity to live inside of macrophages (Liu *et al*., 2020).

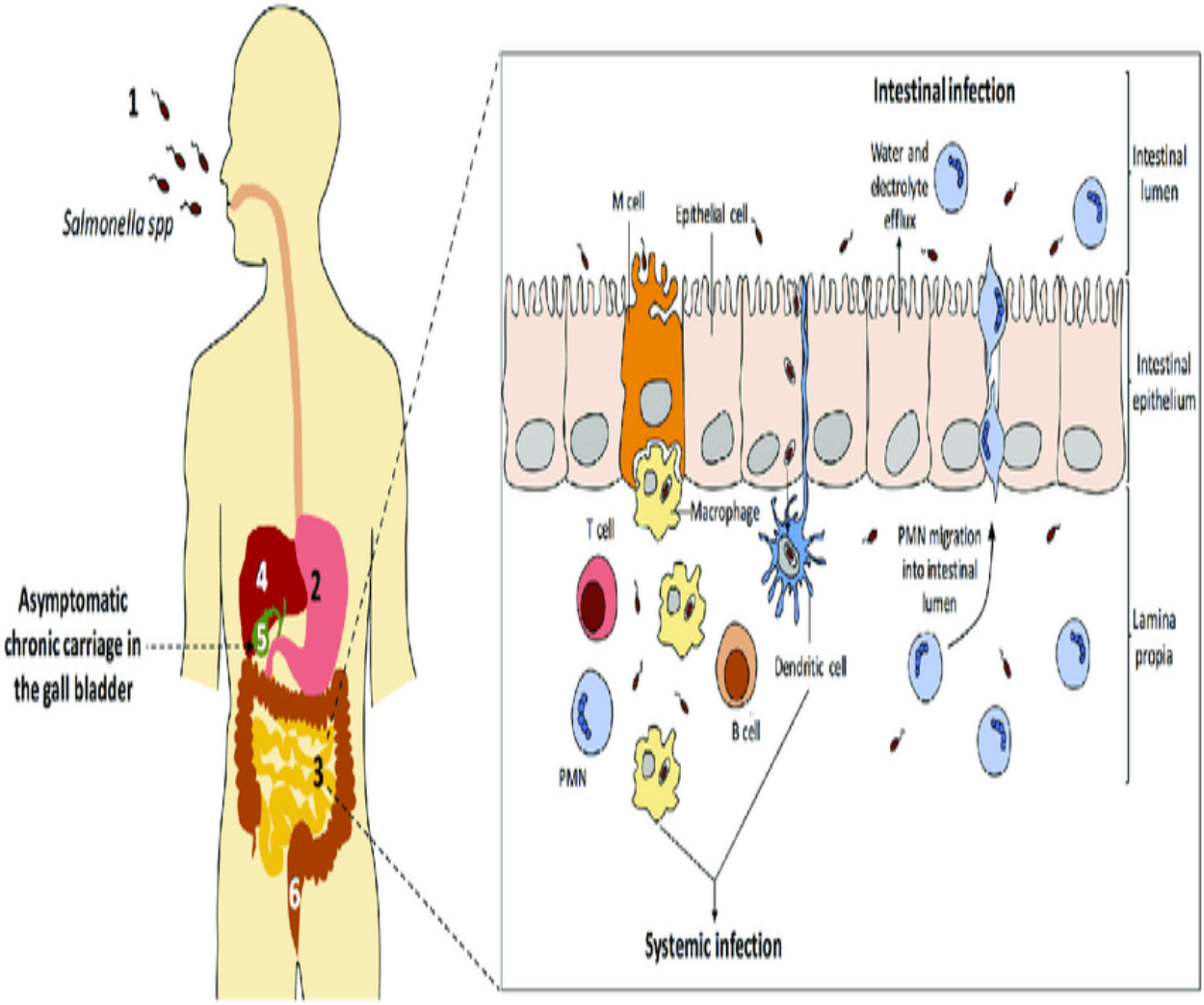

Figure 2.2: Salmonella Infection Pathogenesis adapted from Urdaneta *et al.*, (2017).

2.3 Shigella spp.

2.3.1 Background History

The genus *Shigella* was named after Japanese physician Kiyoshi Shiga, who researched the cause of dysentery (Lampel *et al*., 2018). Shiga enrolled in the Tokyo Imperial University School of Medicine in 1892, and in same year, he went to a lecture given by Dr. Shibasaburo Kitasato (Yokoyama & Fetters 2014). After graduating, Shiga joined Dr. Kitasato's team at the Institute for Infectious Diseases as a research assistant because of his intelligence and self-assurance. Shiga concentrated his efforts on what the Japanese called a "Sekiri" (dysentery) outbreak in 1897 (Uba *et al*., 2021). The Japanese population suffered from these diseases, which were common in the late 19th century (Uba *et al*., 2021). More than 91,000 people were affected by the 1897 sekiri epidemic, which had a mortality rate of more than 20% (Uba *et al*., 2021). Koch's Postulates were successfully employed by Shiga to isolate and identify the bacterium causing the disease after he analyzed 32 dysentery cases (Lampel *et al*., 2018). He worked relentlessly to develop a vaccine for the illness as he proceeded to research and define the bacterium, identifying its processes for producing the toxin known as Shiga toxin (Lampel *et al*., 2018).

2.3.2 Characteristics of *Shigella spp*

Shigella is a genus of bacteria that is Gram-negative, facultative anaerobic, non-spore-forming, nonmotile and rod-shaped (Hari *et al*., 2018). They belong to the family Enterobacteriaceae and do not collectively create gas from carbohydrates, yet they do exhibit traits that are extremely similar to those of Escherichia coli (Nataro et al., 2011). After a 24-hour incubation period, *Shigella* are non-lactose fermenting on MacConkey agar or desoxycholate citrate agar, unlike other members of the Enterobacteriaceae family (Juthi, 2016). Shigella are obligate anaerobic organisms that do not thrive in anaerobic environments. Shigella grows best at 37°C in meedia containing 1% peptone as a source of carbon and nitrogen. (Percival & Williams 2014). *Shigella* are destroyed in an hour at a temperature of 55 °C (Ahamed *et al*., 2019). *Shigella* can survive for a brief length of time in settings that are severely acidic (pH 2.5), but they prefer to grow in a neutral or slightly alkaline environments (pH 7.0-7.4) (Beckius & Schaar, 2016).

2.3.3 Serotypes of *Shigella* spp

There are at least 43 *Shigell*a serotypes known, according to the Centers for Disease Control and Prevention (CDC), although more recent research indicate that there may be far over 50 (Bliven & Lampel, 2017). S. *flexneri* serotypes 2a, 1b, 3a, 4a, and 6; *S. sonnei*, which has a single serotype; and *S. dysenteriae* type 1 are among those that frequently account for the bulk of cases globally (Qiu et al., 2015). Table 2.2 below shows the classification of *Shigella* species.

Table 2.2: Classification of Shigella species and number of serotypes adapted from Mattock and Blocker, (2017).

Species	Serogroups	Number of Serotypes
Shigella dysenteriae	A	15
Shigella flexneri	B	8
Shigella boydii	C	19
Shigella sonnei	D	1

2.3.4 Epidemiology of *Shigella spp*

Bacterial dysentery due to Shigella species is a major cause of morbidity and mortality (Saeed et al., 2015). Globally, there are 188 million incidents of dysentery or diarrhea each year, and 164,000 people die as a result (Herrera *et al*., 2022). Since intermediary bacterial replication is not necessary to get the low infectious dose, the minimal infectious dose can be transmitted immediately from contaminated fingers (Goldberg *et a*l., 2013). The majority of infections are transferred via fecal-oral transmission from individuals with symptomatic infection in nations with abundant resources (O'Ryan et al., 2020). In the United States, outbreaks occur mostly in institutions like daycare facilities, although they also happen less frequently when food or water is contaminated by a common source (Todd & Grieg, 2015). Outbreaks have also been associated with untreated recreational water; in a review of untreated recreational water outbreaks in the United States between 2000 and 2014, 14 of the 90 outbreaks with confirmed etiology (15 percent) were caused by *Shigella* (Goldberg *et a*l., 2013).

The global burden of Shigellosis is contributed by developing countries such as Nigeria with the dearth of basic social amenities (Ngoshe *et al*., 2017). Poor personal hygiene, overcrowding and suboptimal sanitation are risk factors for this disease (Ngoshe *et al*., 2017). Outbreaks among men who have sex with men are increasingly reported (Ingle *et al*., 2019). Our health facilities have been inundated with diarrhea and other diseases linked to poverty, war, internal displacement, inadequate sanitation, poor personal hygiene, and a lack of water supplies since the aftermath of insurgency attacks on communities in our region in 2009 (Ngoshe *et al*., 2017).

2.3.5 Transmission of *Shigella* spp

Human cases of *Shigella* are typically acquired through contact with infected persons or ingestion of food contaminated with *Shigella* bacteria (Ucar *et al*., 2016). *Shigella* species are spread by the fecal-oral route, and the majority of illnesses are passed from person to person, reflecting the low infectious dosage (Newell *et al*., 2010).

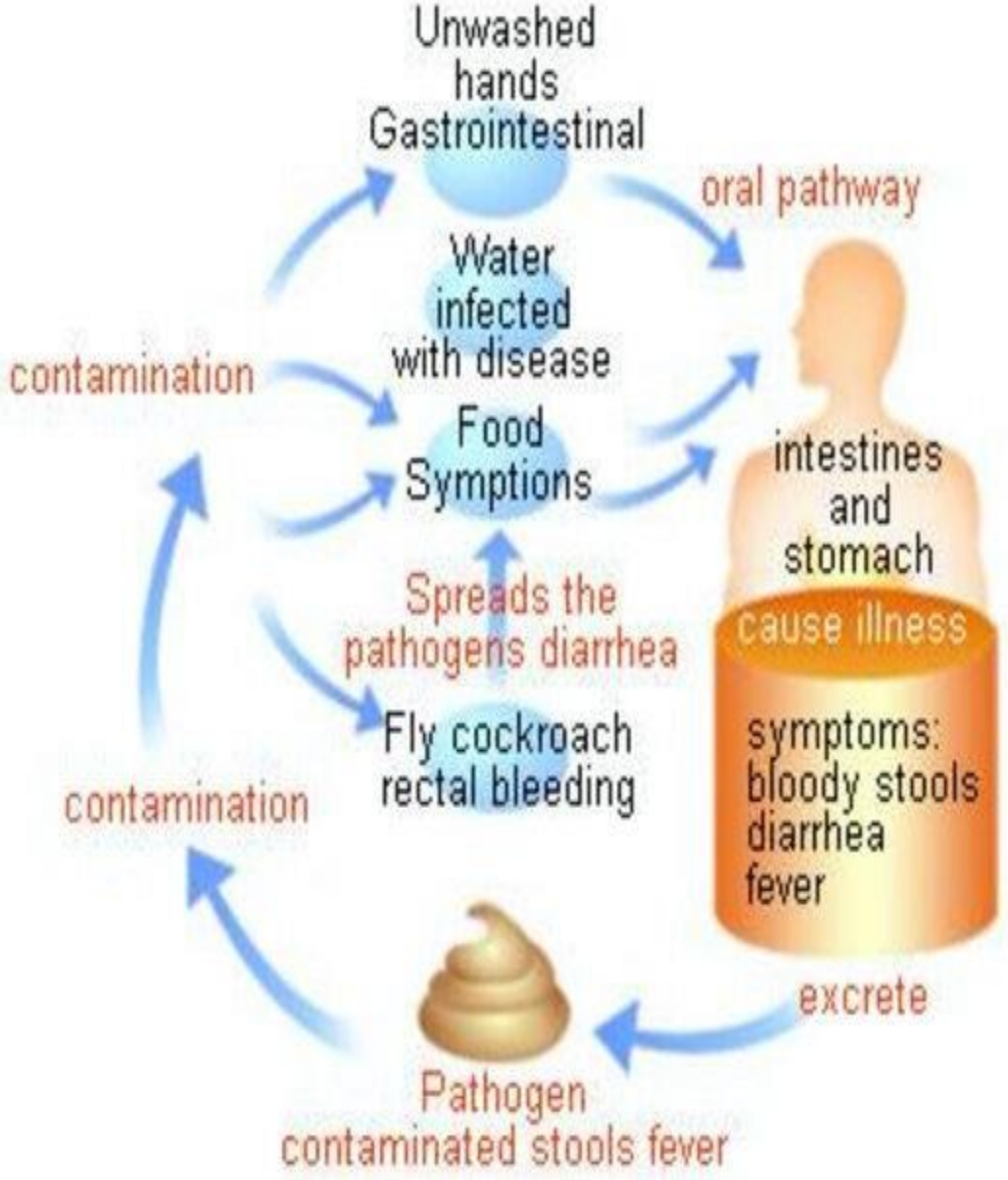

Figure 2.3: Transmission of *Shigella* Infection adapted from Aragon *et al*., (2007).

2.3.6 Pathogenesis of *Shigella spp*

Less than 100 bacterial cells may be sufficient to cause an infection, depending on the host. Invasion of the epithelial lining of the colon by Shigella species typically results in severe inflammation and mortality (Kotloff *et al*., 2018). Some Shigella strains release toxins that cause illness when they are infected. ShET1 and ShET2 are produced by S. flexneri strains, and they may be associated with diarrhea (Abbas *et al*., 2018). *S. dysenteriae* strains produce Shiga toxin, which is hemolytic similar to the verotoxin produced by enterohemorrhagic *E. coli* (Melton-Celsa, 2014). Both Shiga toxin and verotoxin are associated with causing potentially fatal hemolytic-uremic syndrome (Melton-Celsa, 2014). *Shigella* species invade the host through the M-cells interspersed in the gut epithelia of the small intestine, as they do not interact with the top of epithelial cells cells, preferring the basolateral side (Rrahman *et al*., 2015).

Shigella uses a type-III secretion system, it functions as a biological syringe to deliver harmful effector proteins to the intended human cell (Hajira *et al*., 2021). Effector proteins have the ability to change metabolism of the target cell, for instance leading to the lysis of vacuolar membranes or reorganization of actin polymerization to facilitate intracellular motility of *Shigella* bacteria inside the host cell (Sarantis & Grinstein, 2012). For instance, the IcsA effector (which is an autotransporter instead of type III secretion system effector) protein triggers actin reorganization by N-WASP recruitment of Arp2/3 complexes, helping cell-to-cell spread (Sarantis & Grinstein, 2012). *Shigella* cells grow intracellularly after infection and spread to nearby epithelial cells, causing tissue damage and the distinctive pathology of shigellosis (Schnupf & Sansonetti, 2019).

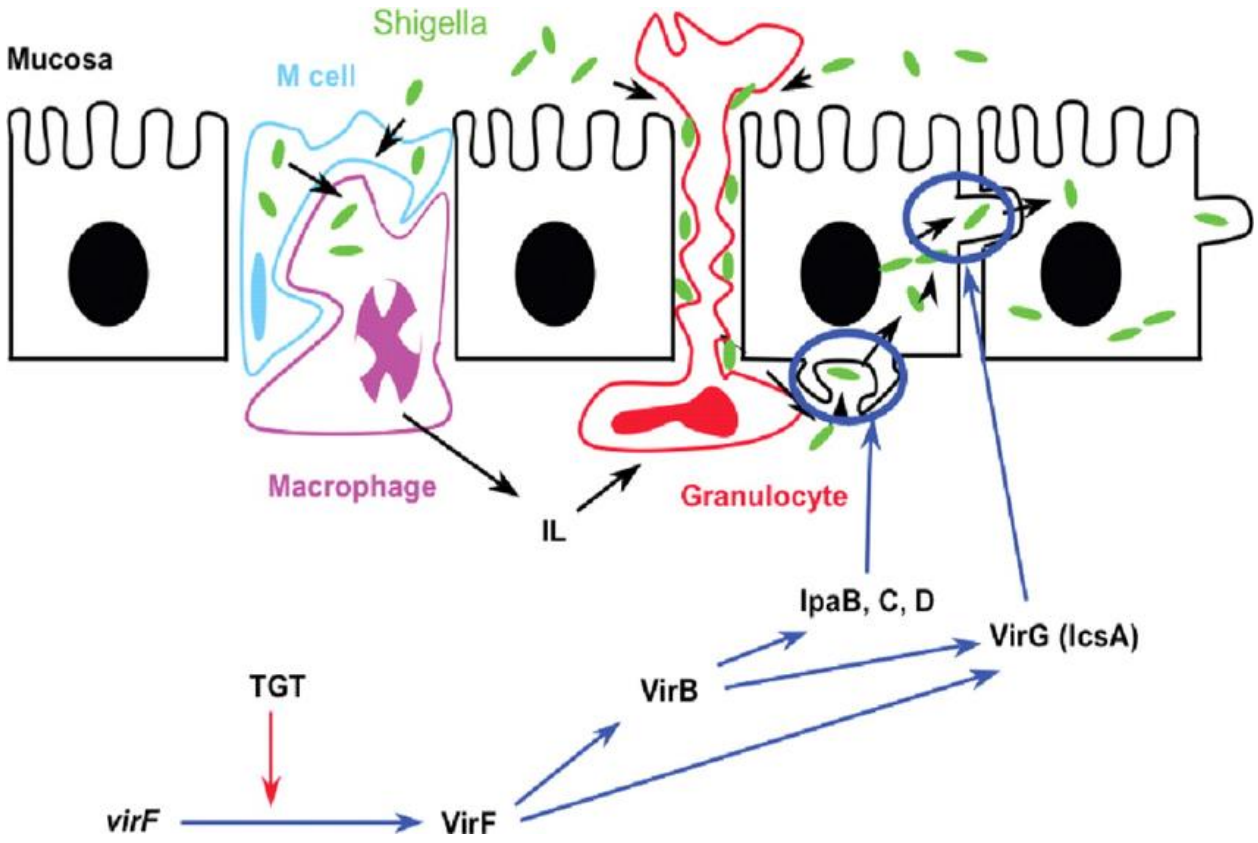

Figure 2.4: Pathogenesis of *Shigella spp* adapted from Hurt *et al*., (2007).

2.3.7 Treatment of *Salmonella* and *Shigella* Infection

Salmonella intestinal infection is treated with fluids given by mouth or, for severe infection, intravenously (Bakkeren et al., 2019). Antibiotics do not shorten recovery time for people with *Salmonella* intestinal infection and may result in bacteria being excreted in the stool longer (Chen *et al*., 2013). Therefore, antibiotics are usually not given (Chen *et al*., 2013). However, those who are at risk for bacteremia, such as babies, elderly nursing home residents, and HIV-positive individuals, as well as those who have implants such as replacement joints, heart valves, or blood vessel grafts, are given antibiotics. They may be given ciprofloxacin, azithromycin, or ceftriaxone for several days. Children are given trimethoprim/sulfamethoxazole (Bakkeren et al., 2019).

As long as the patient is not vomiting or experiencing severe dehydration-related shock, the oral rehydration therapy created by the World Health Organization has shown to be effective and safe in the treatment of acute diarrhea (Dekate et al., 2013). The choice to prescribe antibiotics is based on the severity of the condition because shigellosis typically resolves on its own with enough hydration (Guarino et al., 2014). In the latter case, intravenous fluid replacement is required until initial fluid and electrolyte losses are corrected (Guarino et al., 2014). Absorbable drugs such as ampicillin (2 g/day for 5 days) are likely to be effective when the isolate is sensitive (Nesbitt et al., 2020).

2.3.8 Antibiotic Resistance in *Salmonella* and *Shigella spp*

Emerging resistance in *Salmonella spp* has been described especially in Africa and Asia and the appearance of *Salmonella spp* DT104 in the late 1980s raised main public health concern thereby threatening the lives of infected individuals stated that multi-resistance occurred in *Salmonella* serotypes including albany, anatum, havana, london and typhimurium (Girma, 2015). Antimicrobial agents such as ampicillin, chloramphenicol and trimethoprim–sulfamethoxazole are used as the traditional first line treatments for *Salmonella* infections. *Salmonella spp*. resistant towards these agents are referred to as multi-drug resistant (MDR) (Eng et al., 2015). Fluoroquinolones and extended-spectrum cephalosporins have been introduced as the antimicrobial agents of choice for treating MDR *Salmonella spp* as a result of the establishment of resistance against conventional antibiotics (Ramanchadran *et al*., 2017). However, findings indicate a rise in the incidence of typhoid *Salmonella* cases that have fluoroquinolone resistance.

S. paratyphi exhibits higher levels of fluoroquinolone resistance than *S. typhi* does in nations where MDR isolates are more prevalent (Eng *et al*., 2015). This phenomenom has raised concern among public health authorities regarding both clinical management and prevention of the infection (Prestinaci *et al*., 2015).

The first drugs used to treat *Shigella* infections were sulphonamides, which was followed by tetracycline and then by chloramphenicol (Klontz & Singh, 2015). *Shigella* developed resistance to all of these and so accordingly treatment shifted to ampicillin and co-trimoxazole (Puzari *et al*., 2018). However, treatment recommendations were again changed to nalidixic acid because *Shigella* developed resistance to the former drugs (Klontz & Singh, 2015). Later resistance capacity to nalidixic acid developed and soon after that fluoroquinolones were introduced (Mohr, 2016). WHO recommends ceftriaxone, pivmecillinam and azithromycin for treatment of infection by fluoroquinolones resistant *Shigella* species (Williams & Berkley, 2018). However, ceftriaxone resistant and azithromycin resistant isolates have also been reported in some places (Puzari *et al*., 2018). This has now become a threat to mankind and a matter of huge concern (Williams & Berkley, 2018).

CHAPTER THREE

3 MATERIALS AND METHOD

3.1 Study Area

The sampling areas were 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 game-meat consumers.

3.2 Sample Collection

Various game meat was purchased from various open markets in Nigeria (Table 3.1). The purchased samples were placed in sterile food-grade bags and kept on ice packs in cooling boxes and then transported to the laboratory where they were immediately analyzed.

3.3 Apparatus and Equipments

Apparatus used include: stomacher bags, wash bottles, petri-dishes, measuring cylinder, glass pipettes, beakers, conical flasks, glass spreader, inoculating loop, wash bottles, eppendorf tubes, micro pipette (with their tips), test tubes and foil corks (with their racks), PCR tubes, glass slides, oxidase test disc.

Equipment used: Analytical balance, Hot plate stirrer, Autoclave, Vortex mixer, Water distiller, Water bath (set at 80°C), Incubator (37°C), Bunsen burner, Centrifuge, Heating block, Thermal cycler, Gel electrophoresis tanks, Gel documentation system, Microscope.

3.4 Media and Reagents

The work area was sterilized by using 70% ethanol to inactivate any microbial contaminants.

For isolation of *Salmonella species:*

1% Buffered peptone water (BPW), 0.1% BPW, Hektoen enteric Agar (HEA), Brain Hearth Infusion Broth (BHI), 20% Glycerol, Distilled water, Rappaport Vassiliadis Broth (RVS), Xylose Lysine Deoxycholate (XLD) Agar.

For isolation of *Shigella species*:

1% Buffered peptone water (BPW), Hektoen enteric Agar (HEA), Brain Hearth Infusion Broth (BHI), Hektoen Enteric Agar (HEA), 20% Glycerol, Distilled water, .

For molecular identification*:*

Agarose, 1x TAE buffer, master mix, specific primers, Nuclease free water, Ethidium Bromide.

For biochemical test:

Crystal Violet, Iodine, alcohol (95%), Safranin, 3% Hydrogen Peroxide.

Table 3.1: Study Area of Bushmeat Samples

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

3.5 Preparation of Culture Media Used for Isolation

For the isolation and identification of *Salmonella* and *Shigella* isolates, different selective and differential media were used for enhancement.

3.5.1 Buffered Peptone Water

Peptone water is a microbiological growth medium made of sodium chloride and peptic digest of animal tissue. The medium is rich in tryptophan and has a pH of 7.2±0.2 at 25 °C. Peptone water is a non-selective broth medium that is also useful for growing bacteria as a main enrichment media. It is also used for primary enrichment for the detection of *Salmonella* and *Shigella* strains.

Preparation

- In a conical flask, 10g of the dehydrated medium was well mixed with 1litre of distilled water. A foil cork was then used to seal the conical flask.
- The powder was then thoroughly dissolved by giving the mixture some time to stir using the magnetic stirrer hot plate.
- Then, 225ml of the 1% was poured into conical flasks.
- The media-containing conical flasks were then autoclaved at 121°C for 15 minutes.

3.5.2 Brain Heart Infusion Broth (BHI)

Brain heart infusion (BHI) is a growth medium for growing microorganisms. It is a nutrient-rich medium, making it useful to culture a variety of fastidious organisms. BHI is widely utilized in both clinical and research settings to cultivate a wide range of microorganisms. BHI provides a favorable environment for the growth of a variety of fastidious organism species, including certain bacteria, yeasts, and fungus.

Preparation

- According to the manufacturer's instructions, the dehydrated medium (37 grams) was dissolved in 1000 ml of distilled water and carefully mixed in a conical flask. A foil cork (made up of cotton wool wrapped in aluminium foil) was then used to seal the conical flask.
- Using the magnetic stirrer, the mixture was swirled for a while to completely dissolve the powder.

- After dispensing 5ml of the media into separate test tubes and covering them with foil cork, they were autoclaved at 121^0C for 15 minutes to sterilize them.

3.5.3 Rappaport Vassiliadis Soya Peptone Broth (RVS Broth)

Rappaport Vassiliadis Soya Peptone Broth serves as an enrichment growth medium for the isolation of *Salmonella* species. Salmonella is enriched as a result of their superior ability to withstand the high osmotic pressure in the medium as well as their ability to reproduce at higher temperatures and lower pH levels than other gut bacteria. The pH of RVS broth is about 5.2.

Preparation

- According to the manufacturer's instructions, the medium (26.6g) was dissolved in 1000ml of distilled water and carefully mixed in a conical flask. A foil cork (made up of cotton wool wrapped in aluminium foil) was then used to seal the conical flask.
- Using the magnetic stirrer, the mixture was swirled for a while to completely dissolve the powder.
- The media (9ml each) was then dispensed into various test tubes and covered with foil cork and was then sterilized by autoclaving at 121^0C for 15minutes.

3.5.4 Xylose Lysine Deoxycholate Agar (XLD)

Xylose Lysine Deoxycholate (XLD) Agar is a selective medium for the isolation of *Salmonella* and *Shigella* spp from clinical specimens and food samples. The USP microbiological limit test for screening samples for the presence or absence of Salmonella includes XLD Agar and is advised for the testing of foods, dairy products, and water.

Preparation

- The dehydrated medium (55 grams) was suspended in 1000 ml purified or distilled water.
- The medium was boiled with frequent agitation. Note: DO NOT AUTOCLAVE.
- The medium was transferred immediately to a water bath at 50°C.
- After cooling, It was poured into sterile Petri plates.

3.5.5 Hektoen Enteric Agar (HEA)

Hektoen Enteric Agar is a selective and differential medium designed to isolate and differentiate members of the species *Salmonella* and *Shigella* from other Enterobacteriaceae.

Preparation

- The medium of about 72.66 grams was suspended in 1000 ml purified/ distilled water.
- The medium was boiled to dissolve the medium completely. Note: DO NOT AUTOCLAVE.
- It was cooled to 45-50°C.
- Then it was mixed and poured into sterile Petri plates.

3.6 Isolation of *Salmonella spp.* and *Shigella spp.*

3.6.1 Primary Enrichment

Twenty-five grams of each game meat were placed in a sterile stomacher bag containing 225 milliliters of 1% buffered peptone water (enrichment broth) and homogenized for two minutes at 180 revolutions per minute using the stomacher. After homogenization, the material was transferred to conical flasks after which it was incubated at 37^{o}c for 24 hours to serve as primary enrichment for *Salmonella* and *Shigella*.

3.6.2 Secondary Enrichment

One milliliter of the primary enrichment was pipetted using the micro-pipette (set at 1000 µl) into test tubes containing 9 ml of RVS. The test tubes were labeled for proper identification and it was incubated at 42^{o}C for 18 – 24 hours.

3.6.3 Sub-Culturing

Sub-Culturing was done to purify the isolated bacterial colonies from a mixed culture to a new and single culture, the bacterial isolates sub-cultured were those which were differentiated on the basis of their colony morphology, shape, colour, elevation and other physical characteristics. A loopful of the isolate was taken using the inoculating loop (the inoculating loop was heated using the Bunsen burner and allowed to cool before taking the loop from the incubated RVS test tube and streaked onto the new XLD petri dish) using the streaking method procedure, the plates were inverted and incubated 37^{o}C for 18-24 hours.

The plates were checked after the duration for the growth a sub culturing needs to be done. Sub culturing was done to purify the isolated bacterial colonies to get a pure culture. Colonies differentiated based on their colour *Shigella* (Pink) and *Salmonella* (Black) were transferred onto fresh petri dishes containing HEA agar. A loopful of preferred isolates were taken using the inoculating loop (the inoculating loop is heated using the Bursen burner and allowed to cool for like 7 seconds before taking the loop from the original mixed culture and streaked onto the new petri dish).

3.6.4 Cryopreservation of Isolates

A loopful of pure cultured *Salmonella spp* and *Shigella spp* from the incubated Hektoen enteric agar (HEA) was inoculated into 5 ml of BHI broth and incubated at 37ºC for 18-24 hours a sterile. After incubating, 750µl of the inoculum was added into a sterile eppendorf tube containing 750µl of sterile 20% sterile glycerol (duplicated) which serves as cryoprotectant and it was stored in a -4^0C freezer.

3.7 Biochemical Tests

3.7.1 Gram Staining

A pure culture was smeared on a sterile slide and heated by quickly passing it through the flame with the smear facing up after the inoculating loop had been sterilized with a bunsen burner flame. The smear was covered in crystal violet stain and left for 1 minute before being carefully wiped off under running tap water. The smear was then flooded with Gram's iodine, which was allowed to sit for 1 minute before being drained off under a gentle running tap. The slide was then washed with a decolorizing chemical (70 percent alcohol) and let to stand for 10 seconds. After that, the slide was cleaned under running tap water, drained fully, and counterstained for 30 minutes with safranin. The slide was then blot dried with filter paper after being washed under gently running tap water until no color appeared in the effluent.

3.7.2 Oxidase Test

The pure culture was smeared on the filter paper, along with a few drops of the oxidase reagent, and the results were examined. Oxidase positive cultures produced a purple tint in less than 10 seconds. Oxidase negative cultures did not develop any purple colour (Archaya, 2012).

3.7.3 Catalase Test

Using a sterilized inoculating loop, the pure culture was smeared on a sterile slide. The smear was then treated with a drop of hydrogen peroxide. The outcome was then examined. The existence of oxygen bubbles indicated that catalase was present, while the absence of bubbles indicated that catalase was absent (Reiner, 2010).

3.7.4 Triple Sugar Iron Test

This test is conducted using an agar slant of a medium with various sugars that contains 1% lactose, 1% sucrose, 1% sucrose, 1% glucose, sodium thiosulphate, and ferrous sulphate, as well as a pH-sensitive dye (phenol red). Using a sterilized straight inoculation needle touch the top of a well-isolated colony. Insert a needle through the middle of the medium and all the way to the bottom of the tube, inoculate TSI agar, and then streak on the surface of the agar slant. Incubate the tube for 18 to 24 hours at 35°C with room air while leaving the cap unfastened. (Karki, 2018).

3.7.5 Motility Test

This is used to determine whether an organism is motile or non-motile. The Sulphide Indole Motility (SIM) medium was used. Using a sterile needle, pick a well-isolated colony and stab the medium within 1cm of the bottom of the tube. Remove the needle from the medium in the same line it entered the tube. Incubate at 35^{o} c for 18 hours or until growth is evident.

3.8 Molecular Identification

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 *Salmonella and Shigella* 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 *Salmonella* spp are summarized in Table 3.8.1. 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 30 s, 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: PCR Reaction Table for *Salmonella*

Reagent	**Initial concentration**	**Final concentration**	**Volume/Reaction**
Master Mix	5x	1x	2µl
Forward Primer	20µm	0.4µm	0.2µl
Reverse Primer	20µm	0.4µm	0.2µl
dH_2O			5.6µl
DNA			2µl
Total			10

Table 3.3: PCR Reaction components used for 16SRNA amplification

Reagent	**REACTION**
Mastermix	2 µl
16sRNAf	0.125 µl
16sRNAr	0.125 µl
H20	5.75 µl
DNA	2 µl
Total	10

Table 3.4: Protocol for thermal cycler

Analysis	**Step**	**Temperature**	**Time**
1x	Initial denaturation	95^0c	5 min
35x	Denaturation	95^0c	2 min
	Annealing	55^0c	30 sec
	Elongation	72^0c	4 min
1x	Final Elongation	72^0c	10 min

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.

3.9 Precautions

- Personal protective equipment, such as a covered shoe, a nasal cover, gloves, and a lab coat, were observed.
- At every stage of the project, aseptic practices were observed.
- There was no cross-contamination of the samples.
- Ensured that the samples were appropriately labeled at all times.
- To avoid killing the organism of interest, the inoculating loop was allowed to cool before making contact with the organism for sub-culturing.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Biochemical Test Results for Presumptive Salmonella isolates

The results of the biochemical tests indicated that all of *Salmonella* isolates were Gram negative while all appeared negative for oxidase and all were positive for motility test, positive result was also observed in all isolates for catalase and triple sugar iron tests shown in Table 4.1.

Table 4.1: Biochemical test for presumptive identification of *Salmonella* isolates

S/N	Sample	Gram reaction	Catalase	Oxidase	Motility	Triple Sugar Iron
1	AN_1S_1	-	+	-	+	+
2	RAS_1	-	+	-	+	+
3	IGS_1	-	+	-	+	+
4	RAA_1	-	+	-	+	+
5	GUA_1	-	+	-	+	+
6	CC_1O_1	-	+	-	+	+
7	RAO_1	-	+	-	+	+
8	AN_1O_1	-	+	-	+	+
9	GR_1O_1	-	+	-	+	+
10	PA_2L_1	-	+	-	+	+
11	BIL_1	-	+	-	+	+
12	D_2L_1	-	+	-	+	+
13	B_2L_1	-	+	-	+	+
14	G_1L_1	-	+	-	+	+
15	A_1L_1	-	+	-	+	+

4.1.2 Biochemical tests of Presumptive Shigella isolate

The results of the biochemical tests indicated that all *Shigella* isolates were Gram negative and also tested negative for oxidase and motility test and tested positive to catalase and negative to triple sugar iron tests shown in Table 4.2.

Table 4.2: Biochemical test for presumptive identification of *Shigella isolate*

S/N	Sample	Gram reaction	Catalase	Oxidase	Motility	Triple Sugar Iron
1	GRS_1	-	+	-	-	-
2	RAS_1	-	+	-	-	-
3	BUS_1	-	+	-	-	-
4	ANA_1	-	+	-	-	-
5	HEA_1	-	+	-	-	-
6	GUA_1	-	+	-	-	-
7	ALA_1	-	+	-	-	-
8	CC_1O_1	-	+	-	-	-
9	RAO_1	-	+	-	-	-
10	AN_1O_1	-	+	-	-	-
11	GR_1O_1	-	+	-	-	-
12	CC_2O_1	-	+	-	-	-
13	PA_1L_1	-	+	-	-	-
14	G_1L_1	-	+	-	-	-
15	A_1L_1	-	+	-	-	-

4.1.3 Molecular Identification of Results

The representative visualized result of gel electrophoresis for the detection of 16 rRNA gene from isolates obtained from game-meat using the PCR technique. *Salmonella* (n = 37), *Shigella* (n = 18) were randomly selected from 132 isolates for gene confirmation and the bands were positive indicating the presence of *Salmonella* and *Shigella* as shown in Figure 4.1 and 4.2.

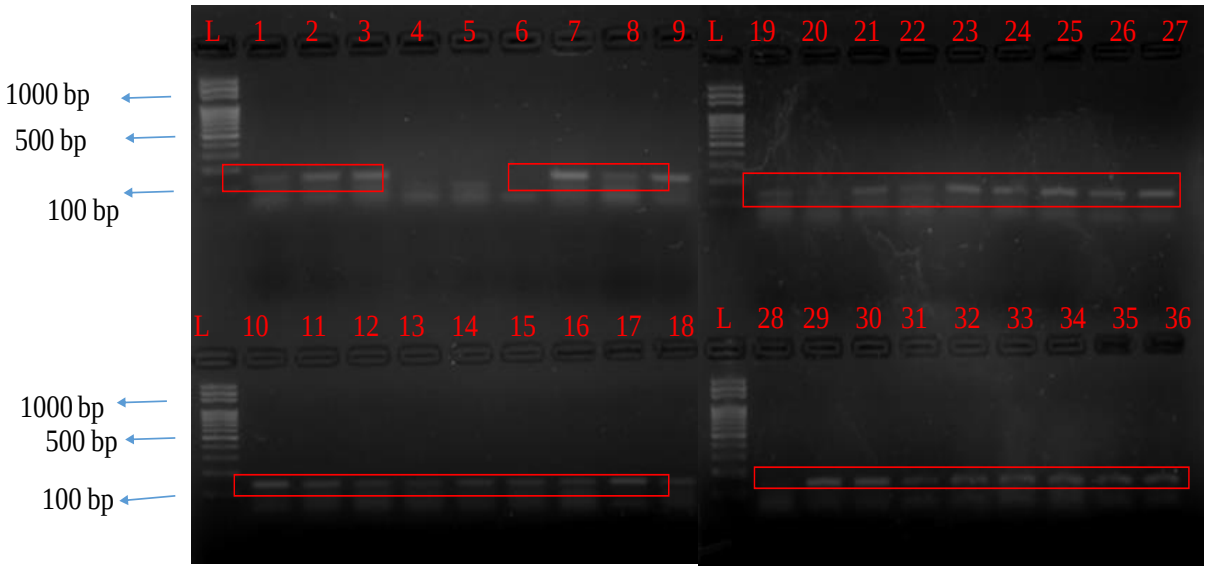

Plate 4.1: Agarose gel electrophoresis image of a Simplex PCR assay of bushmeat samples for detecting the *Salmonella enterica*subsp. I (SSI) gene(137 bp). Lane L: molecular weight marker 100 bp (Bio-Mark). Lane 1: positive control strain (*Salmonella Typhimurium*CCM 7205). Lanes 2-36: *Salmonella* spp. strain isolated from bushmeat.

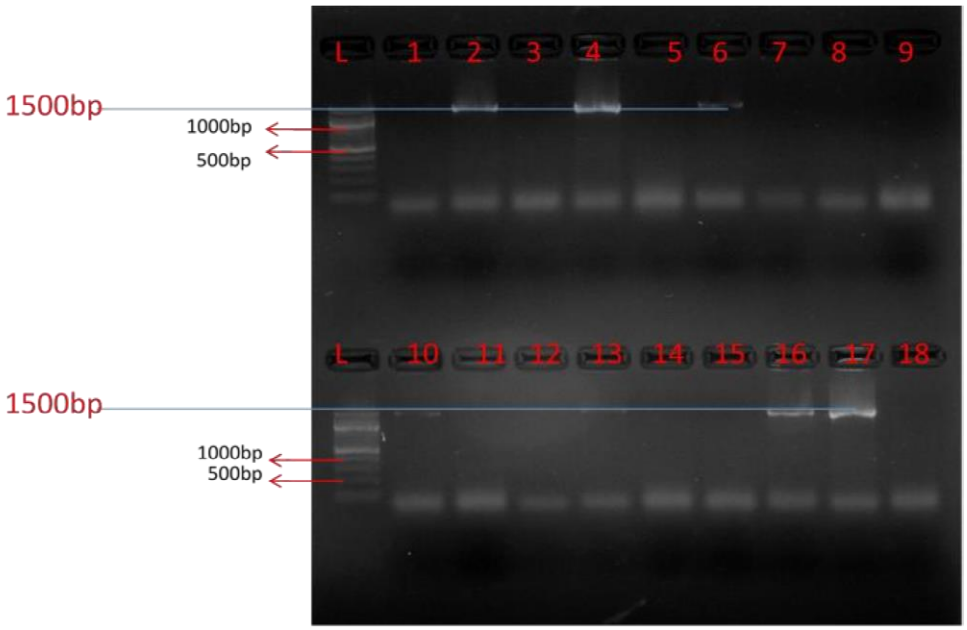

Plate 4.2: Agarose gel electrophoresis image of PCR amplified products of16 rRNA strains. Lane L: molecular weight marker 100 bp (Bio-Mark). Lane 1-10: *Shigella* spp. strain isolated from game meat.

4.1.4 Prevalence of Salmonella and Shigella in the Investigated Game meat

In this study, the total prevalence of positive *Salmonella* samples was 41.8% while for positive *Shigella* samples 45.5% as shown in Table 4.3.

Table 4.3: Prevalence of *Salmonella* and *Shigella* in Investigated game meat

Game meat Investigated	**Number of samples**	**Number of samples positive for *Salmonella*spp.**	**Prevalence (%)**	**Number of samples positive for *Shigella*spp.**	**Prevalence (%)**
Antelope	15	5	33.3	5	33.3
Grasscutter	7	3	42.9	5	71.4
Rabbit	6	3	50	3	50
Civet Cat	4	1	25	2	50
Guinea Fowl	4	2	50	1	25
Bushdog	3	2	66.7	1	33.3
Deer	3	2	66.7	1	33.3
Alligator	2	0	0	1	50
Pangolin	2	1	50	1	50
Hedgehog	2	0	0	1	50
Sparrow	2	1	50	1	50
Monkey	2	2	100	1	50
Hare	1	1	100	1	100
Porcupine	1	0	0	1	100
Quail	1	0	0	0	0
Total	55	23	41.8	25	45.5

4.1.5 Chart representation of prevalence of Salmonella in Investigated Game meat

Figure 4.1 represents the total number of samples obtained (n= 55) and the number of samples that tested positive for Salmonella which is (n = 23). For the game meat samples, 55 isolates were obtained for Salmonella (Monkey and Hare being the most prevalent; 100%) in the Game meat samples.

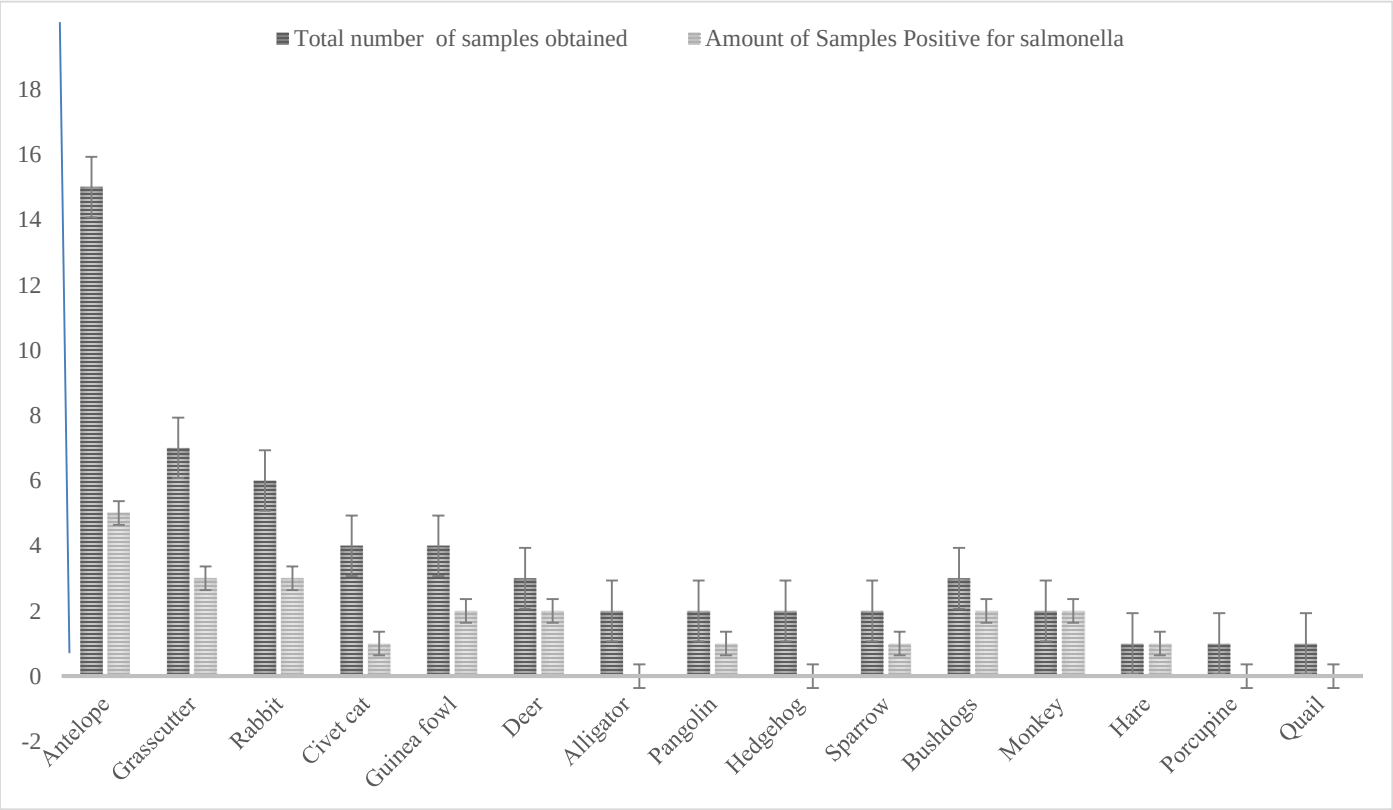

Figure 4.1: Prevalence of *Salmonella* spp in the game meat samples

4.1.6 Chart representation of the prevalence of Shigella spp in Investigated Game meat

Figure 4.2 represents the total number of samples obtained (n= 55) and the number of samples that tested positive for Shigella which is (n = 25). For the game meat samples, 55 isolates were obtained for *Shigella* (Hare and Porcupine) being the most prevalent; 100%).

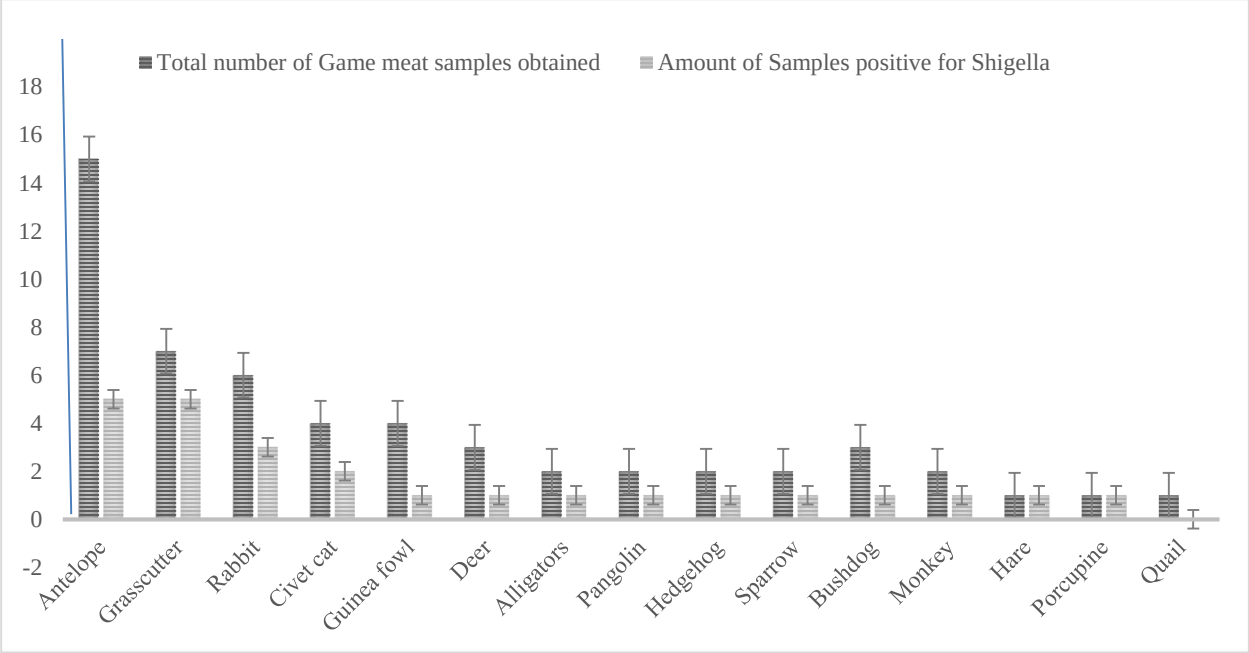

Figure 4.2: Prevalence of *Shigella spp* in the Game meat samples

4.2 Discussion

Worldwide, *Salmonella* and *Shigella* continue to be of great concern for public health putting a financial strain on both industrialized and developing nations due to the high expenses of disease surveillance, prevention, and treatment (Eng *et al*., 2015). The morphological characteristics of individual microrganisms serve as preliminary criteria for identification. The morphological properties, colonial characteristics, biochemical tests, PCR amplification were employed in the identification of isolates. In this study, colonies of distinct *Salmonella* and *Shigella* spp morphological features obtained with Xylose Lysine Deoxycholate (XLD) Agar and Hektoen Enteric Agar (HEA) were isolated and selected for further characterization. Each isolate was subjected to various tests for easy identification.

Biochemical tests carried out in this study indicates presumptive *Salmonella* and *Shigella* spp. These organisms are pathogenic bacteria and one of the important sources of contamination is the slaughtering process of game meat (Kebede *et al*., 2016). Isolation of these pathogens in the bushmeat samples examined is a major problem in game meat safety practices. *Salmonella* and *Shigella* have been found in other studies and these studies showed the prevalence of *Salmonella* and *Shigella* in bush meat (Bachand *et al*., 2012). In this study there was also a high prevalence of *Salmonella* and *Shigella*. The total prevalence of positive *Salmonella* samples is 41.8% while for positive *Shigella* samples 45.5%. This is probably due to unhygienic procedures from the game meat sellers.

According to all results, all samples had presumptive *Salmonella* and *Shigellla* isolates in them. This shows the probability of an unhygienic handling, slaughtering and marketing of gamemeat. *Salmonella* and *Shigella* presence in these samples suggests faecal-oral route contamination from the gamemeat handler, as well as coming in contact with the infected animal which leads to spread of zoonotic diseases. From the samples, 37 isolates were picked randomly for *Salmonella* gene identification and 18 isolates were picked for *Shigella* and the bands confirmed that the isolates were *Salmonella* and *Shigella.* The presence of *Salmonella* and *Shigella* indicates that there has been a recent contamination by faecal matter and there is a high tendency of it causing food borne gastroenteritis and bacterial dysentery (Facciala et al., 2017).

Almost all *Salmonella* strains are harmful because they can enter, multiply, and survive in human host cells, where they can cause potentially fatal disease. Some strains of *Shigella* produce toxins

which contribute to disease during infection (Abbas *et al*., 2018). The game meat in these locations is displayed carelessly on top of surfaces and most times the surfaces are not covered to protect them from getting contaminated from the surrounding environments thus making them to be easily contaminated by airborne pathogens. However there are consequences, Game meat offers numerous potential for zoonotic infections to spread, including airborne and bloodborne during hunting and the butchering of carcasses, as well as foodborne issues associated with preparation (Ashby & Elizabeth 2021). Sixty-two percent of all newly emerging infectious diseases are zoonotic, and more than seventy percent of those zoonoses involve wildlife reservoirs, making human interaction with wildlife a significant channel for endemic and emerging infectious diseases (Horspool, 2013). It is therefore important that the government should implement rules concerning general hygiene and proper game meat handling and also creates public awareness.

CHAPTER FIVE

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The study showed possible public health hazard related with consuming Ready-To-Eat game meat gotten from Southwestern Nigeria. A high microbial load and the presence of *Salmonella* and *Shigella* in fresh produce is a risk to public health and can cause life- threatening foodborne illnesses.

5.2 Recommendations

The government should implement control measures aimed at preventing any practice that potentially contaminate game meat to prevent public health hazard. Consumers should be educated about the potential risk of consuming contaminated game meat. Regulatory and educational efforts from the government officials and academic community also are needed to improve the safety of that are game meat that intended for use as ready-to-eat meat product in Nigeria. More precautions are needed for the processing and handling of game meat. Therefore, efforts should be made to control this bacterium in Nigerian game meats in order to avoid illness or death as a result of eating *Salmonella* and *Shigella* contaminated food.

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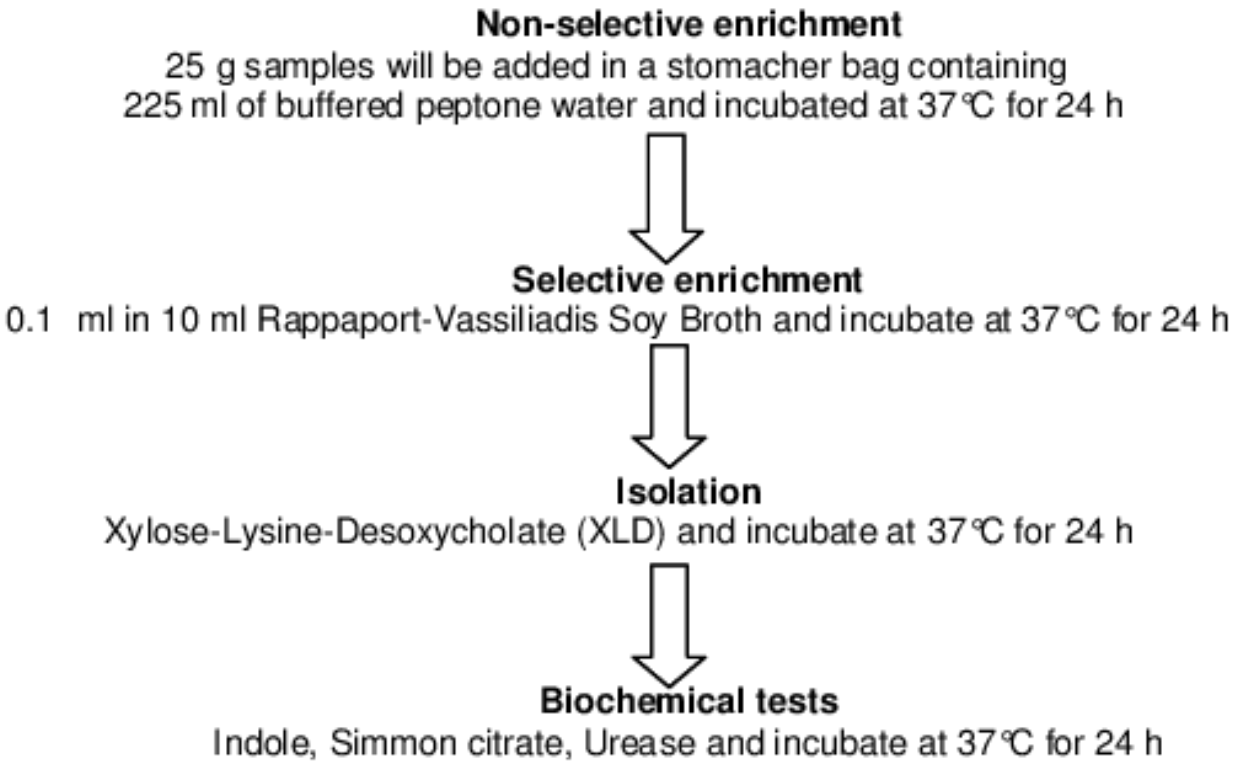
APPENDIX



Appendix 1: Flow diagram showing ISO 6579: 2002method for detection of Salmonella and Shigella.

Appendix 2: Composition and preparation of culture media and reagents

A) Buffered peptone water (BIOMARK)

Composition (g/Litre):

Proteose peptone	10.0 g
Sodium chloride	5.0 g
Disodium phosphate	3.5.0 g
Monopotassium phosphate	1.50 g
Water	1000ml

Final pH (at 25°C): 7.2 ± 0.2

Preparation: Add 20 gram of the components in the 1000 ml of distilled water, Mix well and distribute into universal bottle of suitable capacity to obtain the portions necessary for the test. Sterilize by autoclaving for 15 min in the autoclave set at 121 °C.

B) Rappaport -Vassiliadis (RV) Soya enrichment broth of 500 g (Merck KGaA,Germany)

Composition (g/Litre):

Enzymatic digest of Soya……...4.5 g

Soya peptone …….…….5.0 g

Sodium chloride……..7.2 g

Potassium dihydrogen phosphate ….1.26 g

Magnesium chloride anhydrous …...13.4 g

Malachite green……...0.036 g

Preparation: Weigh 30 g (the equivalent weight of dehydrated medium per Litre) and add to 1 Litre of distilled water. Heat gently until completely dissolved. Dispense 10 ml volumes into screw capped bottles or tubes and sterilize by autoclaving at 115ºC for 15 minutes.

C) Xylose lysine deoxycholate agar (XLD agar) 500 g (Merck KGaA,Germany)

Composition (g/Litre):

Yeast extract…….. 3.0

Lactose monohydrate…….7.5

D (+)- Xylose…….3.5

L(+)- Lysine……..5.0

Sucrose……7.5

Sodium deoxycholate……..2.5

Sodium chloride……..5.0

Sodium thiosulphate……6.8

Iron (III) ammonium citrate……0.8

Phenol red……...0.08

Agar-agar..……..13.5

pH 7.4 ± 0.2 at 25ºC

Preparation Suspend 55.2gm in 1000 (1 Litre) of distilled water by heating, with frequent agitation, until the medium starts to boil. Avoid overheating. Adjust the pH, if necessary, so that after sterilization it is 7.4 at 25 °C. Heat with frequent agitation until the medium boils and the agar dissolves. Do not overheat. Transfer immediately to a water bath at 50 °C, agitate and pour into plates. Allow to solidify. Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in the oven set between 37 °C and 55 °C until

the surface of the agar is dry. It is advisable not to prepare large volumes which will require prolonged heating.