COMPARATIVE STUDIES OF *ESCHERICHIA COLI* AND *SALMONELLA* SEROVARS ISOLATED FROM KAFUE LECHWE AND PASTORAL CATTLE IN THE INTERFACE AREAS OF THE KAFUE FLATS OF ZAMBIA

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DECLARATION

I, **CHARLES MIYANDA MUBITA,** do hereby declare that this thesis represents my own work and that it has never been submitted before for the award of a degree or any other qualification at this or indeed any other university.

Signature: ..... Date: 10/08/2011

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CERTIFICATE OF APPROVAL

This thesis submitted by **CHARLES MIYANDA MUBITA**, is approved as fulfilling the requirements for the award of the degree of **MASTER OF SCIENCE IN VETERINARY MICROBIOLGY** at the University of Zambia.

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ABSTRACT

This study was conducted at Lochinvar and Blue Lagoon National Parks in the interface areas of wildlife and livestock of the Kafue Flats of Zambia to establish the prevalence and relatedness of *Escherichia coli* and *Salmonella* serogroups isolated from Kafue Lechwe (*Kobus leche kafuensis*) and pastoral cattle. A total of 593 faecal samples were processed of which 232 (39.1%) were from Kafue lechwe while 361 (60.8%) were from pastoral cattle. Seventy-seven (33.2%) of 232 samples were compound faecal contents from rectum (R), ceacum (C), small intestine (SI) and the ileo-ceacal junction(ICJ) of each carcass, while 155 (66.8%) were faecal droppings conveniently picked from the grazing pastures. Altogether, there were 1283 presumptive *E. coli* isolates from Kafue lechwe and pastoral cattle out of which 248 (19.3%) had growth characteristics as Shiga toxin-producing *E. coli* O157 (STEC) on sorbitol MacConkey agar and CHROMagar O157. Of 248 suspected STEC isolates, 104 (41.9%) were from Kafue lechwe while 144 (58.1%) were from pastoral cattle. Only 18 out of 104 (17.3%) *E. coli* isolates from Kafue lechwe were serogrouped into 8 serogroups while *E. coli* isolates from pastoral cattle were typed into 16 serogroups.

In case of *Salmonella,* 59 isolates from Kafue lechwe were identified on biochemical and physiological characterization, of which 39 were from Lochinvar NP, while 20 were from Blue-lagoon NP. Nineteen (32.2%) were confirmed on serological typing with *Salmonella* somatic polyvalent antisera, while 40 (67.8%) were non-typeable. Furthermore, of the 19 *Salmonella* isolates, 5 (26.3%) were identified to belong to serogroup 8 serovars while the rest did not react to the available *Salmonella* antisera groups. Thirty suspected *Salmonella* isolates were isolated and biochemically identified from faecal samples of the pastoral cattle, out of which 13 (43.3%) tested positive on agglutination test with *Salmonella* polyvalent antisera while the rest of the isolates were non-typeable. Only one (3.3%) isolates was found to belong to *Salmonella* group 8.

Most *E. coli* isolates from Kafue lechwe were resistant to metronidazole (98.8%) a drug for protozoan and anaerobic infections, followed by penicillin G (97.6%) and amoxicillin (29%) drugs targeting gram positive bacteria. Resistance to co-trimoxazole was also

observed (15.1%). All the *E. coli* isolates from pastoral cattle were resistant to metronidazole, while resistance to other antimicrobials tested (co-trimoxazole, oxacillin, tetracycline, amoxicillin, cephalexin and penicillin G) showed values below 7.1%.

The antibiotics to which *Salmonella* isolates from Kafue lechwe were most resistant to were metronidazole (100%), followed by penicillin G (89.2 %), co-trimoxazole (67.7%), cephalexin (59.5%) and gentamycin (18.9%). *Salmonella* isolates from pastoral cattle were most resistant to metronidazole (90.9%), followed by penicillin G (72.7 %), and gentamycin (54.5%). None of the isolates were resistant to amoxicillin, co-trimoxazole, oxacillin and tetracycline. Similarly, the study showed that *Salmonella* serovars from Kafue lechwe and pastoral cattle had an identical pattern of antimicrobial resistance, suggesting they were related.

Escherichia coli and *Salmonella* isolates from Kafue lechwe and pastoral cattle in the wildlife/cattle Interface Areas of Kafue Flats were examined for the presence of virulence genes capable of causing diseases in humans. Of the 55 *E. coli* isolates tested, 33 (60%) were found to harbour one of the virulence genes. Out of 33 isolates, 28 (84.8%) were positive to Enteroaggregative *Escherichia coli* (EAEC) heat-stable toxin 1 (EAST1) gene, with *E. coli* isolates from cattle being the most predominant in this group (18/28) (64.3%). Out of 53 *Salmonella* isolates, 39 (73.6%) were positive to *invA* gene of which 19 (48.7%) isolates were serologically positive to *Salmonella* polyvalent O antisera, while the rest were non-typeable. All the *Salmonella* isolates belonging to serogroup 8 were positive to *invA* gene.

These results demonstrate the potential of Kafue lechwe and pastoral cattle faeces as a possible vehicle for transmission of pathogenic and multi-drug resistance diarrheagenic *E. coli* and *Salmonella* in the Interface Areas of Kafue Flats, Zambia.

DEDICATION

This study is dedicated to my late father Mr. Chamberlain Mubita and my late mother Mrs Mwangala Mutakate Mubita for out of their love, care, tolerance, patience, sacrifice, guidance and counsel they brought me up to experience the challenges of life. Above all I acknowledge the grace and the eternal love of the Almighty God for wonderfully ordaining my life.

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LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
cAMP	cyclic Adenosine Monophosphate
cGMP	cyclic Guanosine Monophosphate
CFs	Colonization factors
CFU	Colony forming unit
DAEC	Diffusely adherent *E. coli*
DEC	Diarrheagenic *Escherichia coli*
DNA	Deoxyribonucleic acid
EHEC	Enterohemorrhagic *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
EIEC	Enteroinvasive *E. coli*
EAggEC	Enteroaggregative *E. coli*
EAST1	Enteroaggregative *Escherichia coli* (EAEC) heat-stable toxin 1
GMA	Game Management Area
HUS	Hemolytic uraemic syndrome
HIV	Human immunodefiency virus
invE	Invasion E gene
ipaB	Invasion plasmid antigen B
ipaH	Invasion plasmid antigen H
invA	Invasion A gene
LGMA	Lochinvar Game Management Area
LT	Heat –labile
Log cfu/g	Logarithm of colony forming units per gram of sample
NP	National Park
NTS	Non-typhoidal *Salmonella*
NSF	Non-sorbitol fermenting
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis

Stx	Shiga toxin
STEC	Shiga toxin-producing *E. coli*
ST	Heat –stable
UTH	University Teaching Hospital
UTI	Urinary tract infection
VTEC	Vero-cytotoxin producing *E. coli*
WHO	World Health Organization
ZAWA	Zambia Wildlife Authority

CHAPTER ONE

INTRODUCTION

Kafue lechwe (*Kobus leche kafuensis*) is a medium-sized, semi-aquatic animal living in large groups and is indigenous to Zambia. Kafue lechwe are found in Kafue Flats (Stafford 1991) in the Southern Province of Zambia. The Flats is supplied by the Kafue River where the Kafue lechwe are in contact with livestock (Munyeme *et al.*, 2008). Seasonal movement of livestock from upper to lower lands (flood plains) for the purpose of grazing during dry seasons is predominantly practiced in the Kafue Flats (Sitima 1997). It is through this practice of stock movements that pastoral cattle occasionally come in close contact with wildlife which raises the risk for disease transmission from cattle to wildlife or *vice versa* through contamination of grazing grounds and water hole sources (Munyeme *et al.*, 2008 and Susuki *et al.*, 1995).

Kafue lechwe is a highly sought after delicacy and utilized game animal in Zambia as a source of dietary protein at local community and at national levels (Siamudala *et al.*, 2003 and Stafford 1991). Meat and meat products from Kafue lechwe are also often served to international tourists in most of the tourist lodges and hotels. Government effort to encourage private game farming has also contributed to the increased contact between wildlife animals, livestock and humans.

Because of the above observations there is need to ascertain the food safety aspect in terms of its wholesomeness, safety and soundness by determining the possible presence of common food spoilage bacteria in these wildlife derived products. It is worth mentioning that consumption of meat and other meat products from the wildlife food-producing animals are likely to continue rising as the national economy improves and the proliferation of game ranches.

Currently there are growing concerns over the increasing number of human settlements in the Kafue Flats of what was once a wildlife habitat. These communities are engaged in a

number of activities to sustain their livelihoods ranging from fishing to poaching of wildlife (Siamudala *et al.*, 2003). Because of these illegal activities, people may be at risk of being exposed to zoonotic infections of wildlife animals as meat is consumed without veterinary ante-mortem and post-mortem inspections. In the recent years, Nswilile, Likenga, Bunda and Namalyo fishing human settlements in Lochinvar Game Management Area (LGMA) have experienced severe outbreaks of diarrheal diseases (B. Wishkoti 2008., Personal communication). The incidence of diarrheal diseases tends to rise sharply during the warm and rain season around December to March. There is no available information that has incriminated either food or water borne pathogens as the source of gastroenteritis. It is a generally considered opinion by both medical personnel and the affected individuals that these diarrheal outbreaks are as a result of failure by human population in the interface areas of wildlife and domestic animals to observe hygiene during food handling and cooking practices. Besides lack of personal hygiene, perhaps there is need to examine the quality of the drinking water as this is consumed directly from the water lagoons without prior treatment. Kafue lechwe being semi aquatic animals have more access to the lagoons and therefore the water body is more likely to be contaminated with pathogenic bacteria from faeces. Despite the serious magnitude of the problem, no studies have been done in the interface areas of wildlife and domestic animals of the Kafue Flats to find the causative microorganisms of these diarrheal outbreaks.

Pathogens such as *Salmonella* and DEC have emerged and spread world wide from reservoirs in healthy food animals (Gyles 2007; Cookson *et al.*, 2006a and Tauxe 1997). The consumption of the pathogenic strains of *Salmonella* and DEC contaminated food is capable of causing human illness by several different mechanisms. Infections with this type of bacteria pose a serious threat to public health with outbreaks arising from food and water contaminated with human or animal feces or sewage. It is clear that the potential of *E. coli* and *Salmonella* from either wildlife or pastoral cattle origin may find itself in the human population. Further, tuberculosis and brucellosis have been found in the Kafue lechwe (Munyeme *et al.,* 2008 and Muma *et al.,* 2006). These diseases are also quite common in pastoral cattle grazing in these areas (Sitima 1997), even though no

detailed study has ever been undertaken to determine the relationship of strains from wild animals and domestic animals. The demands for meat from Kafue lechwe and pastoral cattle will increase as a source of dietary protein for humans despite being exposed to the biological hazards in the absence of meat inspection. Therefore it is important that the occurrence of *Salmonella* and diarrheagenic *Escherichia coli* (DEC) as well as antimicrobial resistance from wildlife food-producing animals is well elucidated to create awareness about the risk factors associated with occurrence of these pathogens in wildlife. Furthermore, finger printing may be the prelude to understanding these potential zoonotic pathogens in detail.

The interaction of the animals in Kafue flats is usually promoted by climatic factors such as drought and flooding (Siamudala *et al.*, 2003). In times of flooding, Kafue lechwe move to higher grounds inhabited by humans and their domestic livestock. In drought years, the domestic animals move to the plains for fresh vegetation and water.

Molecular characterization of *E. coli* and *Salmonella* isolates from the Kafue lechwe and pastoral cattle faecal samples were investigated by serotyping, biovar differentiation, antibiotic susceptibility and finger printing technique to facilitate differentiation from similar micro-organisms which have been isolated from pastoral cattle with identical serogroups to compare possible epidemiological relationship. This present study was to determine the occurrence and relatedness of *E. coli* and *Salmonella* serovars isolated from Kafue lechwe and those from the pastoral cattle in the interface areas of wildlife and livestock of the Kafue Flats of Zambia. This was then to establish some information regarding their properties. Considering the human risks associated with consumption of meat products contaminated with pathogens, there is need to determine the safety of meat and meat products from wildlife and pastoral cattle.

Therefore the general purpose of the present study was to attempt to elucidate the presence of enteropathogenic bacteria from the faecal droppings of wildlife and determine the antibiotic resistance pattern and then compare with similar bacteria from domestic animals which are in close contact with human population in the interface areas

of the Kafue Flats of Zambia. There is evidence that some properties of these organisms in some parts of the world are usually indicative of some interactions between man and animals (Sanchez *et al.*, 2002). In order to fulfill the above postulate, the study was designed with four specific objectives.

1. Assess the occurrence of *Salmonella* and pathogenic *E .coli* serovars in Kafue lechwe and pastoral cattle with emphasis on their biochemical profiles.
2. Determine antimicrobial susceptibility levels in *Salmonella* serovars and pathogenic *E. coli* isolates from the Kafue lechwe in the interface area of Kafue flats.
3. Detect and identify the virulence genes in pathogenic *E. coli* and *Salmonella* isolates from Kafue lechwe and pastoral cattle.
4. Compare the antimicrobial resistance pattern of *E. coli* and *Salmonella* serovars obtained from pastoral cattle with those from wildlife which are in close contact.
5. Compare plasmid deoxyribonucleic acid (DNA) profiles of *E. coli* and *Salmonella* isolates from Kafue lechwe and similar micro-organism isolated from pastoral cattle with identical antimicrobial resistance pattern to determine strain relatedness.

Justification of the study

Kafue lechwe is the highly sought after and utilized game animal in Zambia as a source of dietary protein at local community and national levels (Siamudala *et al.*, 2003 and Stafford 1991). In the light of increasing demands for Kafue lechwe meat coupled with high prevailing levels of zoonotic diseases such as tuberculosis and brucellosis (Munyeme *et al.*, 2008 and Muma *et al.*, 2006), it is important that other food borne pathogens including DEC and *Salmonella* are investigated to determine their safety in wildlife food producing animals. Such food pathogens can be a source of public health concern particularly for the HIV/AIDS patients, the elderly and the children within the rapidly growing human settlements of the Game Management Areas (GMA). Humans in the GMA are likely to be exposed to the biological hazards through drinking of water contaminated with animal faecal matter and also consumption of meat contaminated with bacterial pathogens. It is hoped that the results might also assist policy makers to regulate the influx of human habitation in designated areas of wildlife.

CHAPTER TWO

LITERATURE REVIEW

2.1.0 General overview of *Escherichia coli* and *Salmonella*

Escherichia coli and *Salmonella* are members of the *Enterobacteriaceae* family which are widely distributed in the environment, as saprophytes or pathogens in the intestine of humans and animals (Bisping *et al.*, 1988). Some strains of *E. coli* and *Salmonella* serogroups are capable of causing disease in humans and animals under certain conditions when the immune system is compromised or disease may result from an environmental exposure (Cookson *et al.*, 2006a). *Escherichia coli* and *Salmonella* are today among the major causative agents of food-borne diseases in humans (Olsvik *et al.*, 1991; Schmidt 1998 and Palmer 1986). They are characterized by being Gram negative rods, oxidase-negative and facultative anaerobes which ferment glucose and a wide range of other sugars (Quinn *et al.*, 2006; Bettelheim 1994 and Edwards *et al.*, 1972).

2.2.0 Pathogenic *Escherichia coli*

Escherichia coli were first reported by Theodor Escherich (1885) as *Bacterium coli commune* which was isolated from the faeces of newborns and this bacterium was later renamed *Escherichia coli.* In the decades that followed, progressive research works have been carried out on the bacterium which has led to a deeper understanding of its involvement as one of the most frequent causes of some of the common bacterial infections including urinary tract infection (UTI), enteric diseases and septicemia. There are six recognized categories of *E. coli* which cause diarrhea in human: Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Verotoxigenic *E. coli* (VTEC), Enteropathogenic *E. coli* (EPEC), diffusely adherent *E. coli* (DAEC) and Enteroaggregative *E. coli* (EAggEC) (Nataro *et al.*, 1998). Each category has distinct virulence factors, serogroups, and epidemiological features and produces characteristic clinical symptoms (Levine 1987). Currently there are four major categories of DEC these being enterohaemorrhagic *E. coli* (EHEC), ETEC, EPEC and EIEC (Levine 1987) and are briefly reviewed below based on distinct properties, different interaction with the

mucosa, distinct clinical syndromes, distinct somatic (O): flagella (H) serogroups and also where possible, differences in epidemiology. However, the four main DEC do have certain underlying commonalities with respect to pathogenesis such as critical virulence properties that are encoded in plasmids. They also have an interaction with the intestinal mucosa and they either produce enterotoxins or cytotoxins. In each category, the strains tend to fall within certain O, H and capsular (K) antigens.

Verotoxigenic *E. coli* are characterized by the production of verotoxin or shiga toxins (*stx*) which has a cytotoxigenic effect on vero cells (Konowalchuk *et al.*, 1977). Verotoxin are absorbed through the intestines and damage specific cells that line the walls of the blood vessels particularly those of the kidneys. There are many shiga toxin-producing *E. coli* (STEC) serogroups which usually do not cause disease in animals. Only the serotype O157:H7 has been clinically associated with haemorrhagic colitis (HC) syndrome in humans and as such has been designated EHEC (Nataro and Kaper 1998). Other serogroups in this group includes; O4:H10, O5:HNM, O26:H11/NM, O55:H7/10, O91:HNM, O103:H 2, O104:H 2, O105:H 18, O111:HNM, O115:H 10, O118:H 12, O128:H 2/8/25/NM, O145:H 25/NM, O153:H 25, O157:H 7/NM, O163:H 19, and O165:H 19/25.

Enterotoxigenic *E. coli* strains have a distinctive feature in that they express heat–labile (LT) or heat–stable (ST) or both enterotoxins and the presence of fimbriae used for attachment to the host intestinal cells (Levine 1987). LT is similar physiologically, structurally and antigenically to cholera toxin and the 2 toxins have a similar mode of action; it increases the level of cyclic adenosine monophosphate (cAMP) in intestinal cells and this causes an increase in electrolyte and water excretion (diarrhea) (Guerrrant *et al.*, 1974). On the other hand, ST stimulates the production of cyclic guanosine monophosphate (cGMP), further leading to increase in fluid excretion and diarrhea. The bacteria first colonize and multiply in the small intestines where they elaborate LT or ST enterotoxins (Nataro *et al.*, 1998). ETEC strains are non-invasive, and they do not leave the intestinal lumen. Colonization of ETEC is usually associated with the presence of surface adhesions known as colonization factors (CFs) that mediate attachment to the

small intestine. The disease varies from minor discomfort to a severe cholera-like syndrome (Merson *et al.*, 1980a). There are specific serogroups that have been associated with ETEC diarrhea that includes; O6, O8, O11, O15, O20, O25, O27, O63, O73, O78, O85, O114, O115, O128, O139, O148, O149, O153, O159, O166, O167, O168, and O169.

Enteropathogenic *E. coli* are among the principal agents of epidemic and endemic infantile diarrhea in developing countries (Toledo *et al.*, 1983 and Gurwith *et al.*, 1978). They were first recognized to cause infantile enteritis in the 1940s (Bray and Beavan 1948). In 1955, the term enteropathogenic *E. coli* was first coined by Neter *et al.*, (1955) to indicate the strains of *E. coli* that had been epidemiologically associated with childhood diarrhea. EPEC was the first group of the DEC. According to the World Health Organization (WHO), a "Classical" EPEC O groups includes the following serogroups; O26, O55, O86, O111, O114, O119, O125, O127, O128, O142, and O158 (WHO, 1987). EPEC serogroups include both typical and atypical strains, as well as other DEC categories. Typical EPEC strains are usually of human origin and they possess a virulence plasmid called the EPEC adherence factor plasmid (EPEC-EAF) while atypical EPEC do not possess this plasmid. The atypical EPEC strains are considered less virulent than the typical ones (Levine *et al.*, 1985).

Enteroinvasive *E. coli* do not produce enterotoxin but are capable of invading intestinal epithelial cells and proliferate within to cause eventual death of the cells (DuPont *et al.*, 1971). Like shigella, EIEC produces an invasive, dysentery form of diarrheal illness in humans Doyle (1989). Manifestation of the illness includes fever, abdominal cramps malaise, toxemia and watery diarrhea with blood, mucus and many faecal leukocytes. According to the Infectious Disease Control Manual of the Louisiana Office of Public Health website (Anonymous 2004) there are eleven specific somatic serogroups principally associated with EIEC. These include 028, O112, O115, O124, O136, O143, O144, O147, O152, O164, and O167.

2.3.0 Pathogenic *Salmonella*

Worldwide, there are over 1500 *Salmonella* serogroups but only about 14 cause common infections (Sleigh *et al.*, 1990). The principal clinical syndromes associated with *Salmonella* infection are enteric (typhoid) fever and gastroenteritis. The typhoid bacillus was first observed by Eberth in the 1880s (Coats 1882) in spleen sections and mesenteric lymph nodes from a patient who died from typhoid. In humans, typhoid fever is caused by *Salmonella typhi* and *Salmonella paratyphi* (Ohl *et al.*, 2001 and Sleigh *et al.*, 1990). *Salmonella* serotype *typhi* is found only in humans and infection implies direct contact with infected person or with an item contaminated by a carrier. Clinical manifestations include fever, abdominal pain, transient diarrhea, or constipation and occasionally a rash. On the other hand gastroenteritis is caused by non-typhoidal *Salmonella* strains such as *Salmonella enteritidis,* and *Salmonella typhimurium* (Ohl *et al.*, 2001). *Salmonella enteritidis* is also considered as one of the most common serotype causing human morbidity and mortality in many countries of the world (Cox 1995). They infect a wide range of animal hosts, including poultry, cattle, pigs and cause a self-limiting enteritis in humans. The site of infection is either the small or large intestine where some strains produce enterotoxins similar to those of toxigenic strains of *E. coli* (Rahman *et al.*, 1992 and Sleigh *et al., 1990). Beef and poultry products, such as meat and eggs, are an* important source of *Salmonella* infection for man. Contamination of poultry and animal carcasses during slaughtering procedures is undesirable but in most cases unavoidable (Dickson *et al.*, 1991 and Oosterom *et al.*, 1983). It is during this process that the numbers of contaminated carcasses are often increased due to cross-contamination that occurs during the evisceration and chilling process (Hang'ombe *et al.*, 1999a and WHO, 1983).

Salmonellosis is one of the most widespread food-borne zoonoses in the world and the problem is increasing both in industrialized and developing countries (Molla *et al.*, 1999). *Salmonella* are capable of infecting both man and animals alike and are the major cause of diarrheal diseases all over the world (Ohl *et al.*, 2001). The pathogens causes a wide range of human enteric diseases, from self-limiting gastroenteritis with mild symptoms of short duration, to severe gastroenteritis with or without bacteriemia and typhoid fever,

which is a severe, debilitating and potentially life threatening illness (Coburn *et al.*, 2007 and Kudaka *et al.*, 2006). In the USA alone, more than 40,000 cases of human Salmonellosis were reported (Anonymous 1994) with almost a third of these cases involving children younger than 5 years. *Salmonella* bacteriemia is one manifestation of immunosuppression in patients with human immunodeficiency virus (HIV) infections (Glaser *et al.*, 1985 and Bottone *et al.*, 1984).

2.4.0 Public health importance of *Escherichia coli* and *Salmonella*

Acute gastrointestinal illness is an important public health concern worldwide and may be caused by viruses, bacteria as well as parasites. (Huilan *et al.*, 1991). It is the second most cause of death in children living in developing countries accounting for approximately 20% of all children's death (Kosek *et al.*, 2003). As bacterial pathogens, some serogroups of *E. coli* and *Salmonella* which induce diarrhea were initially recognized based on O, H and K antigens found on the bacterial surface and have specific virulence characteristics that usually are plasmid-mediated. Diarrhea due to these organisms is generally, referred as food-poisoning. Diarrheagenic *E. coli* and *Salmonella* like any other intestinal bacterial pathogens are found in the intestinal tracts of bovines and other ruminants as well as foods originating from these animals. These bacterial pathogens are among the major sources for human infection.

2.5.0 Isolation of pathogenic *Escherichia coli* and *Salmonella* serovars in Zambia

Generally, there are scanty reports on the isolation of DEC and *Salmonella* serovars from wildlife food-producing animals in Africa and in particular Zambia and yet it is a sanctuary of so many sought after wild animals for meat consumption. A few of such reports include the isolation of *Salmonella enteritidis* and *Salmonella typhimurium* from dead young elephant and young black rhinoceros, respectively, which were at the time quarantined in Nairobi pending to be released in the National park (Windsor *et al.*, 1972). However, attempts have been made in the recent past to isolate EPEC and ETEC from the pastoral cattle in the interface areas of wildlife and livestock of the Kafue Flats of Zambia (Mubita *et al.*, 2008). Similarly, *Salmonella* strains have been isolated from commercial, private livestock farms and poultry hatcheries in Zambia (Hang'ombe *et al.*, 1999a;

Ngoma *et al.*, 1993 and Falade *et al.*, 1989). Human acute gastroenteritis due to EPEC, *Shigella, and Salmonella* were the commonest cause of admission (25.3%) to the pediatric block of the University Teaching Hospital (UTH), Lusaka, Zambia (Ghanem 2008; Dube *et al.*, 1984 and Patel *et al.*, 1982).

2.6.0 Significance of *Escherichia coli* in food poisoning

2.6.1. Enterohaemorrhagic *Escherichia coli* (EHEC)

Within the group of *E. coli* strains, has emerged an EHEC which is represented by a single strain (serotype O157:H7) and are responsible for HC or bloody diarrhea (Cleary 1988; Scotland *et al.*, 1988; Carter *et al.*, 1987; Hobbs *et al.*, 1987; Karmali *et al.*, 1985 and Karmali *et al.*, 1983) which can progress to the potentially fatal hemolytic uraemic syndrome (HUS). HUS is a rare disorder that primarily affects young children between the ages of one and 10 years, particularly those under the age of four years. The onset of HUS is preceded by gastroenteritis illness which is characterized by vomiting, abdominal pain, fever, and diarrhea. EHEC was first reported as a cause of illness during an outbreak of diarrhea in Michigan and Oregon in 1982 (Riley *et al.*, 1983). This illness was associated with the ingestion of undercooked hamburgers at a fast food restaurant chain. Thereafter several outbreaks of EHEC have occurred in many parts of the world especially in the homes of the aged and have resulted in many deaths.

Reports of human infection due to *E. coli* O157:H7 have been recorded in Australia and Nepal (Maharjan *et al.*, 2007 and Elliot *et al.*, 2004). The largest known outbreak due to *E. coli* O157:H7 occurred in the western state of Washington in 1993 (Anonymous 1993). Seven hundred and thirty-two cases were identified, mostly children of whom 195 were hospitalized, 55 developed HUS and four died. EHEC O157:H7 is responsible for approximately 62,000 cases of food-borne infections annually in the United States of America (USA) (Mead *et al.*, 1999). A number of EHEC O157:H7 infections have been isolated from several cases of diarrheal disease outbreaks in Africa following intake of food contaminated with pathogens (Raji *et al.*, 2003).

2.6.2. Enterotoxigenic *Escherichia coli* (ETEC)

Enterotoxigenic *E. coli* (ETEC) was first recognized as an important cause of human diarrhea in 1968 in India and Bangladeshi where the infection is endemic (Sack *et al.*, 1971). Human illness caused by ETEC is characterized by watery diarrhea usually accompanied by low-grade fever, abdominal cramps, malaise, and nausea (Levine 1987). In its acute form, an ETEC diarrheal illness resembles cholera with rice water-like stool that lead to dehydration (Doyle 1989). ETEC are among the common known etiological agents of diarrhea that account for the estimated 1.5 million deaths per year in the world (Kosek *et al.*, 2003). It is also an important cause of diarrhea in all age groups and travelers (Firdausi *et al.*, 2005; Germani *et al.*, 1985a and Rowe *et al.*, 1978) especially among infants and children (WHO 1999; Nataro *et al.*, 1998; Cohen *et al.*, 1995; Doyle 1989 and Germani *et al.*, 1985b).

Outbreaks of enteritis in adults in the USA and Japan caused by enterotoxigenic strains of *E. coli* 06.H16 have been reported (Rowe *et al.*, 1978). In another development, *E. coli* O169:H41 was the most commonly identified serotype in the diarrheal outbreaks in the USA (Beatty *et al.*, 2004). In Sao Paulo Brazil, ETEC strains were found to be important pathogens in cases of infantile diarrheal (Gomes *et al.*, 1991). Studies carried out on infantile diarrhea in a special-care baby unit revealed that *E. coli* 06.H16 was the most common diarrheagenic serotype recovered from the patients (Rowe *et al.*, 1978). Similarly, ETEC was found to be the most common cause of diarrhea in the study of infants in Egypt (Rao *et al.*, 2003).

In Bangladesh, ETEC was the most common pathogen isolated from patients with acute diarrhea attending treatment at various health centers (Stoll *et al.*, 1982). In Lagos, Nigeria, ETEC were among the pathogens that accounted for acute gastroenteritis diseases in patients from the period 1979 to 1981 (Akinyemi *et al.*, 1998 and Agbonlahor *et al.*, 1982). ETEC were also among the most common causes of diarrhea in travelers visiting Kenya in the early 1970s and 1980s, (Sack *et al.*, 1978).

2.6.3. Enteropathogenic *E. coli* (EPEC)

EPEC are known to cause a profuse watery diarrheal disease and are leading cause of infantile diarrhea in developing countries (Regua *et al.,* 1990; Robins-Browne 1987 and Levine 1987). They induce a non-bloody watery diarrhea and mucous similar to ETEC, but differ in the possession of colonization factors and do not produce ST or LT toxins. With the advent of HIV/AIDS, EPEC has emerged as an important cause of diarrhea in patients in Central African Republic (Mossoro *et al.*, 1998).

Studies done in other parts of the world have incriminated the strains of EPEC as the causative microorganisms in diarrheal diseases of adults and infants (Akinyemi *et al.,* 1998; Levine *et al.*, 1984 and Agbonlahor *et al.,* 1982). Furthermore, studies have shown that EPEC serogroups (055, 0111) appear to be the most predominant among the serogroups of the "classical" enteropathogenic strains reported to be associated with acute diarrheal diseases in Africa (Akinyemi *et al.*, 1998; Germani *et al*, 1985b and Agbonlahor *et al.*, 1982). Symptoms in adults with food associated EPEC gastroenteritis include severe diarrhea, nausea, vomiting, abdominal cramps, headache, fever, and chills (Schroeder, *et al.*, 1968 and Costin *et al.*, 1964).

2.6.4. Enteroinvasive *E. coli* (EIEC)

Enteroinvasive *E. coli* (EIEC) resemble shigella in many ways so much that the illness caused by the former has often been mistaken for the latter. EIEC strains, in particular serogroups O124, are reported to have caused hospital outbreaks in adults in Britain (Rowe *et al.*, 1974) and a large food-associated outbreak in the USA in 1971 (Marier *et al.,* 1973). Other strains of the EIEC have also been reported to cause gastroenteritis in infants by Akinyemi *et al.,* (1998) while Agbonlahor *et al.*, (1982) have in their studies shown the predominance of EIEC in diarrheal patients in Lagos, Nigeria. There is scanty information on the ability of the EIEC to cause disease in other animals other the humans.

2.7.0 Significance of *Salmonella* in food poisoning

Salmonella is one of the major food-borne pathogens causing gastroenteritis in humans and the infection has been associated with many different food types including beef and

beef products (Smerdon *et al.,* 2001). In the USA, ground beef was implicated in the transmission of a multi-drug resistant *Salmonella newport* strain in which during the first 4 months of 2002, 47 people in five states were diagnosed with Salmonellosis, of which 37% were hospitalized and one person later died (Glynn *et al.*, 1998). According to Mead *et al.*, (1999) there are estimated 1.3 million human cases of food-borne *Salmonella* infections and 553 deaths annually in the USA.

In North Africa, outbreaks caused by *Salmonella enterica* serovars are common and mainly result from consumption of untreated or sewage-contaminated water (Khalifa *et al.*, 2009). Among the *Salmonella* serotype, non-typhoidal *Salmonella* (NTS) represents a very important public health problem in many parts of the world (Gomez *et al.*, 1997) and other *Salmonella* serogroups also continue to be a major health problem (Lampel *et al* 2000 and Peplow *et al.*, 1999). A study in North America reported a bacteria incidence of 6% among infants with NTS diarrhea (Torrey *et al.,* 1986) while the work done in sub-Saharan Africa reported that NTS was among the three most important causative pathogens of human bacterial infections in adults and children (Shaw *et al.*, 2008) where it is a leading cause of blood stream infections (Morpeth *et al.,* 2009).

In Zambia, Dube *et al.*, (1982) reported isolation of 45 strains of various *Salmonella* species from patients at the University Teaching Hospital (UTH) presenting various clinical manifestation and about 93% of the strains were isolated from infants less than two years old. Most of the diarrheal diseases are caused by a large number of NTS serovars (Gordon 2008) and food-borne transmission from animals to humans predominates in high-income countries, while anthroponotic transmission is postulated to occur both within and outside healthcare facilities in sub-Saharan Africa (Morpeth *et al.,* 2009). In sub-Saharan African community, acquired NTS is reported to be a major cause of high morbidity and death among children less than five years of age especially those from the poor income group (Kariuki *et al.*, 2006a) and in the adults (Kankwatira *et al.,* 2004).

Cases of typhoid fever in the USA are usually acquired during foreign travel or by consumption of food contaminated by a chronic carrier (Shutze *et al.,* 1998). In Mangalore, India, an outbreak of food poisoning due to *Salmonella enterica* serotype *weltevreden* was reported involving 34 students from a tertiary care hospital (Antony *et al.,* 2009). Typhoid is perceived as a common cause of febrile illness in several African countries (Mweu *et al.,* 2008). Recent reports indicate that there were approximately 21.5 million infections and 200,000 deaths from typhoid fever globally each year (Mweu *et al.*, 2008). As a result of a continued increase in the incidence of human Salmonellosis, it has imposed considerable burdens on public health resources (Gomez *et al.*, 1997).

2.8.0 Virulence genes in *Escherichia coli* and *Salmonella* isolates

The pathogenicity of *Salmonella spp.* is dependent upon the ability of these organisms to gain access to the cells that are normally non-phagocytic (Ginocchio, *et al.*, 1995). It is for this reason that most, if not all virulent *Salmonella* strains, have a chromosally encoded *invA* gene which is functional for entry into epithelial cells (Galan, *et al.*, 1991). Pathogenicity of EHEC is usually linked to Shiga toxin (Nataro *et al.*, 1998) and virulence factors including, invasions, adhesions, protein secretion systems, and iron intake (Hacker *et al.*, 2000). These organisms can be detected by immunologic methods or Polymerase Chain Reaction (PCR) evaluation of virulence factors (Moussa *et al.,* 2010; Hamza 2010; Gerrish *et al.*, 2007; Scotland *et al.*, 1989 and Yolken *et al.,* 1977).

2.9.0 Molecular Characterization of diarrheagenic *Escherichia coli* and *Salmonella*

There is equally little documented information regarding relatedness of bacterial pathogens isolated from Kafue lechwe with similar pathogens isolated from pastoralist cattle which come in close contact with wildlife in the interface area of the Kafue Flats. However, Muma *et al.*, (2008) attempted to characterize DNA of atypical *Brucella* species from cattle and Kafue lechwe in the livestock-interface area of the Kafue Flats using PCR. Their observations were that although the atypical *Brucella* strains showed some phenotypic and genetic differences, they suggested that these differences may have arisen from the pathogens adaptation in the wildlife host *Kobus leche kafuensis.*

Elsewhere, several other methods have been described for the molecular characterization of EHEC and *Salmonella* isolates from farm animals (Kudaka *et al.*, 2006 and Lee *et al.*, 1996), poultry (Mirmomeni *et al.*, 2007), food (Kudaka *et al.*, 2006) and human infections (Van Duynhoven *et al.*, 2008). Among these methods, pulsed field gel electrophoresis (PFGE) has been used to establish genetic relationships of bacteria (Liebana *et al.*, 2001). PFGE allows the generation of simplified chromosomal restriction fragment pattern without having to resort to probe hybridization methods. In this method restriction enzymes that infrequently cut DNA at specific sites are used for generating large fragments of chromosomal DNA, which are then separated by special electrophoresis (Swaminathan and Matar 1993). It has been applied to sub-typing of several gram-positive and gram-negative bacteria. PFGE is also widely used in epidemiological surveillance.

2.10.0 Antimicrobial resistance to *Escherichia coli* and *Salmonella*

The antibiotic resistance is a growing concern everywhere in the world (Mateu *et al.*, 2001; Falkow *et al.*, 2001 and De Oliveira *et al.*, 2000). The use of antimicrobials in human and veterinary medicine has been very useful for treating bacterial infections, but the appearance of drug resistant bacteria has brought about serious problems (Aarestrup 1999). In 1988, England and Wales reported the emergence of a multi-drug-resistant *S. typhimurium* DT 104 in cattle which subsequently was isolated from poultry, sheep, pigs, and horses (Meslin 1997). It is usually the uncontrolled use of antibiotics as growth promoters in food of animals that has contributed greatly to the development of antibiotic resistance status (Johnson *et al.*, 2006).

The antibiotics selects for drug resistant bacteria such as *E. coli*, which may be transmitted to humans through the food supply (Collignon *et al.*, 2006). *Escherichia coli* and *Salmonella* are frequent members of the intestinal flora, where many species of bacteria exist in close proximity to each other. This arrangement facilitates *E. coli* strains that are piliated to accept and transfer plasmids from and to other bacteria (Salyers *et al.*, 2004). This conforms to existing knowledge that *E. coli* and other enterobacteria are

important reservoirs of transferable antibiotic resistance (Rice 2007 and Salyers *et al.*, 2004). Unsanitary, warm and moist environments are also conducive for the spread of antibiotic resistant pathogens. The evolution of a *S. typhimurium* resistant strain to the commonly used antibiotics has made it difficult to control the bacteria in food animals and has remained a zoonotic problem (Meslin 1997).

2.10.1. Wildlife animals and birds

In Zambia, reports on antibiotic resistance on bacterial isolates from wildlife food producing animals are scanty. Theoretically, free-ranging wildlife species are never exposed to treatment with antimicrobials therefore the isolation of any drug resistant microbes from wildlife animals would presumably be associated with being picked from either the environment in which they come into contact with domestic cattle or pasture contamination from cattle through excreta. For instance, in Portugal, seagulls have been reported as possible reservoirs for multi-drug resistant bacteria (Poeta *et al.*, 2008) and therefore present a risk to public health because of the potential pathogens-spreading capacity of migratory birds.

2.10.2. Domestic cattle

In the case of pastoral cattle in Zambia, owners frequently inject their animals with drugs. Tetracycline is one such drug which has been abused as it is administered through the intra-muscular route in the treatment of tick-borne and bacterial diseases (Mubita *et al.*, 2008). They reported a high frequency of *E. coli* resistance to penicillin G, erythromycin, cotrimoxazole, and nitrofurantoin. However, antibiotic resistance towards bacteria from livestock and poultry in the commercial sector where antimicrobial usage is wide spread has been adequately reported (Hang'ombe *et al.*, 1999b and Ngoma *et al.*, 1993).

2.10.3. Human

Antibiotic resistance in humans could be as a result of using sub-therapeutic doses or poor quality drugs which provides sub-inhibitory selective pressure. Increased antibiotic resistance may compromise the outcome of many common infections that would have been easily treatable. The infections caused by the drug resistant gram-negative bacteria

and other bacteria are a growing threat to the general public health (Lautenback *et al.*, 2007) and have reached crisis point in many hospitals around the world (Gould 2008).

In Africa, multi-drug resistant NTS are one of the leading causes of morbidity and high mortality in children less than 5 years of age, second in importance only to pneumococcal diseases (Kariuki *et al.*, 2006b). Surveys of healthy people carried out in Nigeria, have shown that they often carry large proportions of resistant *E. coli* in their gastro-intestinal tracts (Iwalokun *et al.*, 2001 and Okeke *et al.*, 2000). For instance, in Zambia reports of microbial drug resistance cases of human pathogenic enteric bacteria are on the increase (Mwansa *et al.*, 2007) and this poses a serious concern in particular with the advent of acquired immune deficiency syndrome (AIDS) epidemic and HIV related illnesses. Many drug-resistant human faecal *E. coli* isolates originate from poultry, whereas drug-resistant poultry source *E. coli* isolates are likely to originate from susceptible poultry-source precursors (Jonson *et al.*, 2007). For instance, high levels of *E. coli* resistance to trimethoprim-sulphamethoxazole and tetracycline were recovered from faeces of broilers, pigs, and foods in Spain (Saenz *et al.*, 2001). Gram-negative enteric bacteria are among the microorganisms known to cause a significant proportion of diarrheal infections in humans and animals.

2.11.0 Vehicles of transmission for diarrheagenic *Escherichia coli* and *Salmonella* serovars

2.11.1. Food of animal origin

Foods containing products from farm animals are recognized as a major vehicle of transmission of bacterial pathogens (Besser *et al.*, 2005; McEvoy *et al.*, 2003; Sanchez *et al.*, 2002; Zhao *et al.*, 2001; Blanco *et al.*, 1995; Olsvik *et al.*, 1991 and Hughes *et al.*, 1971). In particular, incompletely prepared or incompletely heated food has been reported as the source of different diarrheal pathogens. The main route of *Salmonella* and DEC into the food chain is through contamination of meat by intestinal contents and faeces in the abattoir (Butler 1996). These pathogens gain entry into human by the

faecal-oral route, with the common source being water contaminated with animal excreta and food contaminated with faecal pathogens.

Most human food-borne outbreaks due to *E. coli* O157:H7 have been linked to consumption of undercooked meat and drinking unpasteurized milk, salami, ice cream, and cheese (Sanchez *et al.*, 2002 and Upton *et al.*, 1994). For instance, in the USA, a home made venison jerky and uncooked meat were both implicated as a source of transmission of *E. coli* O157:H7 and were from the same deer (Keene *et al.*,1997). In Pretoria, South Africa, *Salmonella* was most frequently isolated from chopping blocks, mince meat and offals in butcheries and shops (Rislakki 1969). In Nairobi, Kenya, *E. coli* O157:H7 was isolated from informally marketed raw milk (Arimi *et al.*, 2000). However, there is no information on the occurrence of DEC and *Salmonella* serovars in Kafue lechwe as a source of food poisoning.

2.11.2. Animals and the environment

The spread of DEC and *Salmonella* serovars on the farm environment and among animals may be facilitated by communal grazing grounds and water holes contaminated by animal waste, and also animal-to-animal contact by domestic or wildlife. *Escherichia coli* O157:H7 is a temporary inhabitant of the gastrointestinal tract of ruminants and other mammals and is usually shed in the faeces of healthy cattle (Sanchez *et al.*, 2002 and Blanco *et al.*, 1995). Cattle and sheep serve as sources for contamination of food products and water sources.

Human infection due to vero-cytotoxin producing *E. coli* (VETEC) O157 was confirmed in the United Kingdom, in patients that were linked to have been in contact with farm animals prior to the illness (Trevena *et al.*, 1999). The study by Cookson *et al.*, (2006), revealed that being in contact with calves or their faeces, or exposure to the environment cross contaminated with ruminant faeces represented an increased risk for human diseases.

Farm grounds are environments that tend to be contaminated by animal droppings and more often infants or children contract diseases when allowed to crawl or play in grounds where infected animal faeces are present. Zoonotic STEC, are transmitted to humans through the ingestion of foods and/or water contaminated with animal faeces or through direct contact with the infected animals or their environment (Fairbrother *et al.,* 2006). Insects can also spread diseases. EHEC O157:H7 are reported to have been transmitted by houseflies (Alam *et al.,* 2004) while wild rodents and mice have been recognized as carriers of *Salmonella* infections in poultry (Davies *et al.,* 1995).

2.11.3. Farm manure and sewage contaminated water

Ingestion of farm produce, such as vegetables that were fertilized by animal manure can also lead to gastroenteritis. Direct consumption of sewage-contaminated water or generally untreated water may also lead to gastroenteritis. For instance, surface waters in the developing countries have been found to harbor pathogenic bacteria (Ohno *et al.,* 1997 and Begum *et al.*, 2005).

2.11.4. Human-to-human

Transmission of DEC and *Salmonella* often occurs through faecal-oral route. In homes or restaurants, foods are contaminated by infected food handlers or food workers during food preparation. Therefore, the faeces of virtually any animal may be a potential source of infections, especially the zoonotic enteric human disease outbreaks.

2.12.0 Reservoirs of diarrheagenic *Escherichia coli and Salmonella* serovars

2.12.1. Wildlife reservoir

Throughout history, wildlife has been an important source of infectious diseases transmissible to humans (Kruse *et al.*, 2004). Therefore, it is widely acknowledged that many new human pathogens that have emerged or re-emerged worldwide originated from animals or from products of animal origin (Meslin 1997). In the USA, *E. coli* O157:H7 was reported to have been recovered from tools that were used to dismember the deer

carcasses (Keene *et al.,* 1997). This implicated wildlife animals in transmitting human diseases.

Therefore, it needs not to be stressed that the bi-directional transmission of infectious diseases between wildlife and domestic animals at the wildlife/livestock interface has zoonotic implications (Bengis *et al.,* 2002). Some of the zoonotic food- borne diseases that may be involved are anthrax, tuberculosis, brucellosis, yersiniosis, salmonellosis, campylobacteriosis and colibacillosis. In the USA, *E. coli* O157:H7 has been reported to have been identified from fresh feacal samples collected on multiple pastures from free-ranging white-tailed deer which had access to cattle pastures (Sargeant *et al.,* 1999).

2.12.2. Domestic animal reservoir

Cattle and other ruminants are reported to be the most important reservoir of zoonotic STEC (Fairbrother *et al.,* 2006) and other food-borne pathogens (Beutin *et al.*, 1993). Animal exhibits have been reported to be on the increase in the past decade and have also facilitated in the spread of human infections (Keen *et al.*, 2006). Dairy and beef cattle are primarily known reservoirs of EHEC O157 (Hussein *et al.,* 2005 and Bach *et al.,* 2002) and outbreaks of this disease in humans have been associated with cattle and consumption of bovine and poultry products (Gyles 2007). Available literature seem to indicate that typical EPEC serogroups have not been found in animals (Nataro 1998), suggesting that humans may be the only living reservoir for these organisms.

In the USA, outbreaks due to *Salmonella enterica* were associated with being in contact with animals from at least four animal exhibits since 1990 as reported by Bender *et al.*, (2004). These animals carry pathogens asymptomatically and shed in their faeces. In some cases these pathogens are known to cause disease in young animals. For instance ETEC is known to be a major cause of severe diarrheal diseases in suckling and weanling animals (Gaastra *et al.*, 1996).

2.13.0 Socioeconomic risk factors associated with *Escherichia coli* and *Salmonella*

2.13.1. Nourishment

Generally, diet habits are a major factor in the acquisition of diarrheal diseases. Eating meals that have been prepared and exposed to flies at social or traditional ceremonies and drinking water in a utensil which has been handled by many people whose hands may not have been washed after the use of the toilet can result into diarrheal infection.

2.13.2. Immunity and other factors

Both the young and the elderly are more susceptible to DEC and *Salmonella.* People with AIDS are much more susceptible, in general, to zoonotic diseases. Pre-existing malnutrition in children, especially those living in the poor economic areas can lead to more severe enteric infections due to their being immunocompromised (Brown 2003).

2.13.3. Direct contact with wildlife and domestic animals

The desire of humans to touch wild animals or have contact with farm animals may expose children or other visitors to organisms such as *E. coli* O157 and *Salmonella.* An increase of human settlements living near areas where there is a high concentration of wildlife and domestic animals may also pose health hazards.

2.13.4. Consumption of raw milk and uncertified game meat

Drinking of raw milk left at room temperature for longer time or fresh blood from infected pastoral cattle (a ritual practice at traditional ceremonies) can lead to diarrheal diseases. Consumption of under-cooked wildlife meat from both legal and illegal sources is likely to be one of the most important route of bacterial pathogens transmission to people.

2.13.5. Ecological and occupational risks factors

Diarrheal diseases due to bacteria tend to occur frequently during the rain seasons. The drinking water holes become flooded with debris and faecal matter from the grazing grounds or pit latrines, respectively. More often human settlers in the GMA tend to drink

water directly sourced from the lagoons. Such water may not be safe as it may be constantly polluted with the discharge from the sewage and mining waste as is the case with the Copperbelt towns (Norrgren *et al.,* 2000 and Mwase 1994). Hunters and Wildlife officials are high risk groups by virtue of the nature of their works.

2.13.6. Translocation of the Kafue lechwe

Transfer of Kafue lechwe to other areas such as game ranches without screening them for infectious pathogens increases opportunities of transferring the pathogens to uninfected areas. Consequently, this may put other game animals or livestock in the adjacent areas are at risk.

CHAPTER THREE

MATERIALS AND METHODS

3.1.0. Study Area

During June through December 2008 the study was carried out at Lochinvar (410 km^2) and Blue-Lagoon (420 km^2) National Parks (NPs) in the interface areas of wildlife and livestock of the Kafue Flats. The Kafue Flats is the only known natural habitat of the Kafue lechwe which in 2001 was estimated to have a population of approximately 40,000. The study was conducted on Kafue lechwe of different ages and sex from different zones within the study area (Figure 1).

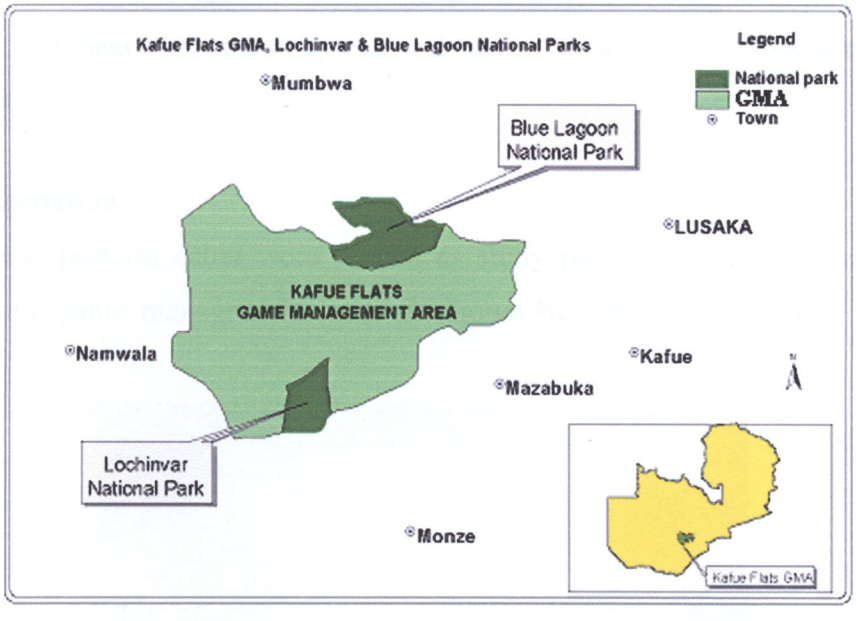


Figure 1: The study areas of Lochinvar and Blue-lagoon National Parks (Adapted from Milner 2003).

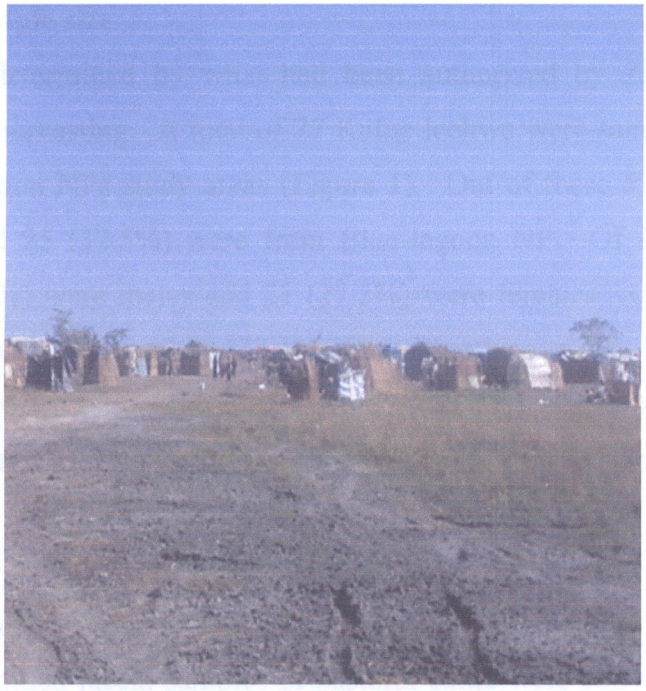


Figure 2: Human settlement within the study area, with human beings living in close association with wild animals.

3.2.0. Study population

Kafue lechwe and pastoral cattle were chosen as study populations due to their close interactions in the game management areas as shown by figure 2 above and figure 3 below.

Figure 3: The study subjects composing Kafue lechwe and pastoral cattle grazing side by side.

3.2.1. Kafue lechwe sample size

The sample size was determined by what had been authorized by Zambia Wildlife Authority (ZAWA) for harvesting. A total of 77 Kafue lechwe were sampled from both Lochinvar and Blue-lagoon NPs study areas (Figure 1). Out of these 52 (67.5%) were from Lochinvar NP and 25 (32.5%) were from Blue-lagoon NP. Of all the sampled Kafue lechwe, 56 (72.3%) were males and 21 (27.3%) were females. Of the males 38 (67.9%) were from Lochinvar NP and 18 (32.1%) from Blue-lagoon NP. Majority of the females 14 (66.7%) were from Lochinvar NP, while 7 (33.3%) were from Blue-lagoon NP. There was no statistical difference between the two strata's in terms of sex (X^2=0.9), P<0.01.

3.2.2. Cattle sample size

A total of 361 freshly voided faecal samples of pastoral cattle were conveniently picked from the grazing pastures using a sterile spatula in both Lochinvar and Blue-lagoon NPs irrespective of the sex and age. Out of 361 faecal samples, 261 (72.3%) were from Lochinvar NP and 100 (27.7%) were from Blue-lagoon NP.

3.3.0 Age distribution for Kafue lechwe

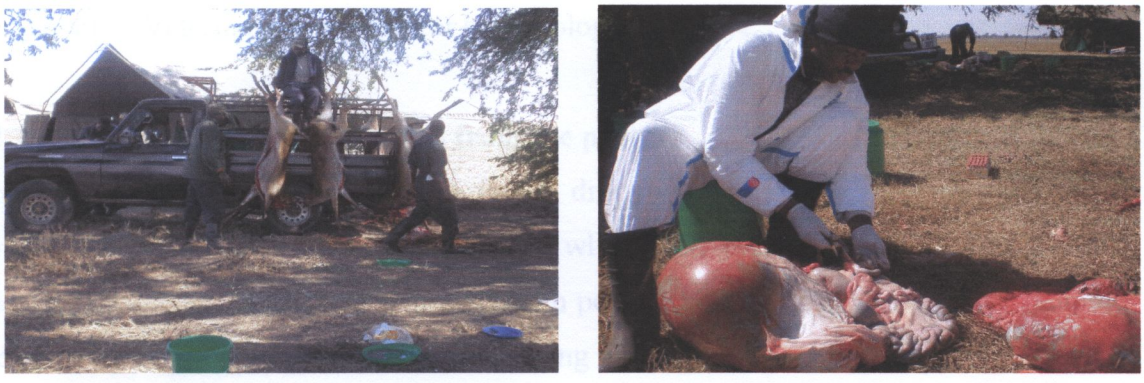
Age was determined using the horns in males and sometimes the teeth wear while in females only teeth wear was used for age determination (Waid *et al.*, 1985). The minimum age recorded from the 77 animals sampled was 2.5 years old Kafue lechwe, while the maximum (oldest) age recorded was 20 years old. The mean age of the Kafue lechwe sampled was 9.9 (10 years) (S.E ± 4.7).

3.4.0. Collection of faecal samples

3.4.1. Hunter harvested faecal samples from Kafue lechwe

There were a total of 77 faecal compound samples (rectum, ileo-caecal junction, caecum, and small intestine) collected from the hunted Kafue lechwe (Figures 4a, 4b and 5). About 2 g faecal contents was aseptically collected from the rectum of each Kafue lechwe and was placed in a 150 mm x 100 mm polythene self adhesive bag. Sterile

swabs were used to pick contents of the lower small intestine, ileum-caecum junction and the caecum from each carcass. These were stored in Carry and Blair transport medium (BBL - Becton Dickinson Co. Cockeysville, USA) which were also kept in ice-cooled cooler box before delivery to the laboratory for processing.



a. b.

Figure 4: (a) Evisceration of study subjects (Kafue lechwe carcasses) (b) Collection of faecal contents from the compound organs of a study subject (Gut of Kafue lechwe carcass)

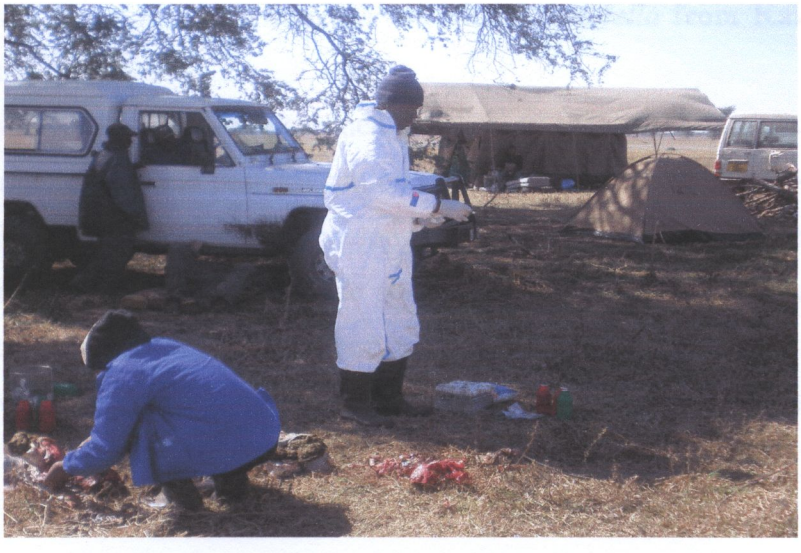


Figure 5: Camp site where all of the samples were processed for storage in transit to the laboratory.

3.4.2. Kafue lechwe faecal samples from the pasture

A total of 155 faecal samples of about 5 grams each, freshly and naturally evacuated Kafue lechwe droppings were conveniently and randomly picked using a sterile spatula

from different grazing blocks where the Kafue lechwe were found in the study area and were placed in a 150 mm x 100 mm polythene self adhesive bag. Identification of faecal samples was based on the experience of field guides, mostly the Wildlife Ecologists who were part of the survey team. Care was taken during sampling to avoid collection of pasture sediments. The samples were kept in ice-cooled cooler boxes before delivery to the School of Veterinary Medicine for bacteriological culture.

3.4.3. Pastoral cattle faecal samples from the pasture

Three hundred and sixty one (361) faecal droppings of about 5 grams each were conveniently picked from the grazing pasture where the pastoral cattle were found in the study area and were placed in a 150 x 100 mm polythene self adhesive bag. The samples were freshly collected from the animals grazing in the respective game parks (Figure 3). Out of these, 100 samples were from Blue-lagoon NP, while 261 samples were from Lochinvar NP.

3.5.0. Culture and isolation of *Escherichia coli* and *Salmonella* from Kafue lechwe and pastoral cattle

A total of 593 faecal samples were examined for the presence of DEC and *Salmonella* serovars from Kafue lechwe and pastoral cattle. In the laboratory, samples from the field were refrigerated at 4°C and processed no longer than 168 hours after collection. All samples were directly plated on appropriate selective medium. Thereafter, all samples were stored at -20°C except those in transport media which remained at 4°C. All faecal samples were processed using standard techniques, according to the methods described by Barrow *et al.,* (1993) and Edwards *et al.*, (1972).

3.5.1. Isolation and identification of *Escherichia coli*

Four suspicious *E. coli* colonies isolated on Desoxycholate Hydrogen Sulfide Lactose agar (DHL) (Nissui Pharmaceutical Co., Tokyo, Japan) from each sample were transferred to Eosine Methylene Blue agar (EMB) (Eiken Chemical Co., Tokyo, Japan) and were incubated at 37°C and inspected after 24 hr as described by Balows *et al.*, (1991) and Holt-Harris *et al.*, (1916) and Colonies on EMB which appeared dark

centered and flat, with or without metallic sheen were tentatively considered to be *E. coli.* Suspected *E. coli* strains were biochemically confirmed by testing into established standard medium; Triple Sugar Iron (TSI) (Merk-Darmstadt, Germany), Sulfide Indole Motility (SIM) (Eiken), Lysine Iron Agar (LIA) (Himedia-Laboratories Pvt. Mumbai. India), arabinose (Nacalai Tesque Inc, Kyoto, Japan), urea agar (Merk), mannitol (Wako Pure Chemical Industries, Osaka, Japan) and simmons citrate agar (Eiken). Their differentiation from other members of the family *Enterobactericeae* was according to the method described by Barrow *et al.*, (1993) and Edwards *et al.*, (1972).

3.5.2. Screening for the presence of Shiga toxin (*Stx*) producing *E. coli* serogroups using Sorbitol MacConkey agar (SMAC) and CHROMagar O157

A total of 575 *E. coli* isolates which had shown a pattern of biochemical activity similar to *E. coli* were screened for the presence of the non-sorbitol fermenting (NSF) isolates. Sorbitol MacConkey Agar (SMAC) (Himedia) was used as described by Boyce *et al.*, (1995) and March, *et al.*, (1986), with slight modification. The inability of most *E. coli* O157 to ferment sorbitol is exploited in SMAC (O' Sullivan *et al.*, 2006). Non-Sorbitol fermenting (NSF) colonies, indicative of STEC O157:H7 isolates are colourless on this medium. Furthermore, the isolates were tested as described by Bettelheim (1998) to determine their ability to utilize one of the chromogenic substrates in CHROMagar O157 medium (CHROMagar O157, Paris, France). The plates were incubated at 37°C and examined after 24 h.

Out of 575 *E. coli* strains, 104 (18.5%) were either non-sorbitol fermenting isolates (white) on SMAC or appeared mauve coloured colonies on CHROMagar O157. Sorbitol MacConkey agar and CHROMagar O157 are selective medium commonly used to screen for STEC serotype O157. STEC O157 serotype does not ferment sorbitol on SMAC but most of the non-O157 STEC serogroups do ferment sorbitol and can not be distinguished on SMAC media. CHROMagar O157 has been found to be highly sensitive for identifying strains of *E. coli* serotype O157 and to lesser degree *E. coli* serotype by their ability to utilize one of the chromogenic substrates in the media to produce mauve coloured colonies (Bettelheim 1998).

3.5.3. Isolation and identification of *Salmonella* from the contents of the rectum, small intestine, ileo-caecal junction and caecum

About 1.0 g of the contents of the rectum and each swab with faecal contents of the small intestine, ileo-caecal junction and caecum, was initially cultured in 10 ml Buffered Rappaport and Vassiliadis *Salmonella* enrichment broth (BRVSB) (Mast Diagnostic® Group, Merseyside, United Kingdom) at a dilution 1/10 weight/volume and incubated at 43°C overnight, followed by subculture onto Xylose-Lysine- Desoxycholate agar (XLD) (Himedia) as described by Rollender *et al.*, (1969). The plates were incubated at 37°C for 18 to 24 hr.

The colonies which appeared the same colour as the culture medium, or translucent or slightly opaque orange were considered to be *Salmonella.* Some colonies were either with or without black centers on XLD selective media. Four suspected *Salmonella* isolates from each sample were isolated and biochemically identified by conventional methods as described by Barrow *et al.,* (1993) and Edwards *et al.*, (1972) using standard media with slight modifications. Lightly the centre of the test colony was picked with sterile inoculating needle and inoculated into TSI, and LIA (Himedia), by streaking slant and stubbing butt and were incubated at 37°C for 18 hr. Caps of the tubes were made loose to maintain aerobic condition.

Isolates which produced alkaline (red) slant and acid (yellow) butt, with or without production of H_2S (Blackening of agar) in TSI and also produced an alkaline (purple) reaction in LIA butt were retained as potential *Salmonella* isolates and were further subjected to biochemical tests. They were inoculated into the following media; simmons citrate agar, urea agar, phenol red dulcitol broth, phenol red mannitol broth, phenol red lactose broth, phenol red sucrose broth and SIM (Nissui) and then incubated at 37°C for 18 hr. All isolates that produced a negative reaction for urease, indole test, lactose and sucrose and a positive reaction for dulcitol and mannitol; and/or variable reaction on citrate were tentatively diagnosed as *Salmonella* isolates.

3.6.0. Typing of *Escherichia coli* and *Salmonella* isolates

The following phenotypic methods were used to demonstrate whether individual isolates from Kafue lechwe and pastoral cattle may or may not be linked.

3.6.1. Serotyping of *Escherichia coli* and *Salmonella*

Identification of the somatic (O) antigens of the isolates was done by the slide agglutination method of Kauffmann (1947) with slight modification (Lior *et al.*, 1996 and Edwards *et al.*, 1972) using polyvalent and monovalent antisera. The serogroups serve as markers that correlate with specific virulent strains of bacteria (Whittam *et al.*, 1993).

3.6.1.1. *Escherichia coli* isolates

The *E. coli* isolates which were either non- sorbitol fermenting colonies on SMAC or appeared mauve coloured on CHROMagar O157 were serogrouped with commercially available *E. coli* antisera test kit (Denka Seiken Co. Tokyo, Japan) for specific O antigens. Isolates were sub-cultured on nutrient agar and serological reactions were performed using a slide agglutination method as described by Lior *et al.*, 1996; Merson *et al.*, 1980b; Edwards *et al.*, 1972 and Kauffmann 1947. First the strains were serotyped against somatic polyvalent antisera (Orskov *et al.*, 1984), followed by the corresponding monovalent antisera from the agglutinating group to differentiate into serogroups. The investigated serogroups are shown in Table 1. Only live bacterial suspension was used for the test. The strains belonging to the same serogroups from the same samples were reported.

Table 1: Composition of *Escherichia coli* antisera used in the test set

Polyvalent antisera	Monovalent O antisera
1	01, 026, 086a, 0111, 0119, 0127, 0128
2	044, 055, 0125, 0126, 0146, 0166
3	018, 0114, 0142, 0151, O157, 0158
4	06, 027, 078, 0148, 0159, 0168
5	020, 025, 063, 0153, 0167
6	08, 015, 0115, 0169
7	028ac, 0112ac, 0124, 0136, 0144
8	029, 0143, 0152, 0164

3.6.1.2. *Salmonella* isolates

Isolates that showed a biochemical pattern activity similar to members of the genus *Salmonella* were serotyped with *Salmonella* somatic polyvalent O grouping antisera (Denka Seiken) which bind to the surface antigen of the microorganism to form a visible antigen-antibody precipitate. First the strains were tested against polyvalent somatic antisera, followed by a range of specific O antisera groups to differentiate them into serogroups (Table 2).

Table 2: *Salmonella* antisera set

Polyvalent antisera	Specific *Salmonella* O antisera groups
O antisera	O 2; O 4; O 7; O 8; O 9; O 9, 46; O 3, 10; O 1; O 11; O 13; O 6,14; O 16; O 18; O 21

The slide agglutination method was done according to Lior 1996; Edwards *et al.*, 1972 and Kauffmann 1947 with slight modification. Briefly, two 10 µl volumes of sterile 0.85% saline solution were dispensed separately onto a thoroughly cleaned microscopic slide. With a platinum loop a single colony from a 24 hr *Salmonella* test culture was picked from nutrient agar (Nissui) and emulsified into each drop of saline to produce a

distinct and uniform turbidity. To one volume of the suspension 40 µl of *Salmonella* antiserum was added and to the other suspension a drop of 40 µl of saline was added as a negative control. The reagents were mixed by tilting the glass slide back and forth for 60 seconds and distinct agglutination was viewed under indirect light against a dark background. Any clumping observed in the saline control was regarded auto-agglutination. Non reactive strains and weak agglutination were recorded as negative. A confirmed *Salmonella* isolate from Hang'ombe *et al.*, (1999a) was used as a positive control while *E. coli* isolate confirmed by WHO was a negative control.

3.6.2. Biochemical profiles of *Escherichia coli* and *Salmonella* isolates

3.6.2.1. *Escherichia coli*

Biochemical profiling involved the reaction of a single colony from each *E. coli* strains that reacted similarly with O monovalent antiserum and was inoculated into 18 fermentative carbohydrates to establish the relatedness and these were; cellobiose, dextran, dulcitol, galactose, inositol, maltose, melibiose, raffinose, resorcinol, ribitol, rhamnose, trehalose, xylose, sorbitol, mannitol, mannose, starch and barbital to determine the biotypes. Similarities of biochemical reactions were recorded. The result signify a metabolic fingerprint of the isolate (O'Sullivan *et al.*, 2006).

3.6.2.2. *Salmonella*

Isolates that were positive to O polyvalent antiserum were inoculated into phenol red broth base (PRBB) (Difco Laboratories, Detroit, Michigan, USA) which had 20 different fermentative sugars and these were; arabinose, cellobiose, dextran, dulcitol, galactose, inositol, maltose, fructose, raffinose, resorcinol, ribitol, rhamnose, trehalose, xylose, salicin, sorbitol, mannitol, mannose, starch and barbital to determine the similarities of the isolates.

3.6.3. Characterization of DNA profile for *Escherichia coli* and *Salmonella* serovars

Pulsed-field gel electrophoresis (PFGE) was used on selected isolates to investigate the DNA plasmid relatedness of DEC and *Salmonella* strains from Kafue lechwe and those

isolated from pastoral cattle source with identical serogroups and antibiotic resistance pattern as described by Liebana *et al.*, (2001). A subsets of 12 strains of DEC and 10 *Salmonella* isolates from Kafue lechwe and those from pastoral cattle were used for the multiple–enzyme analysis. Each isolate was digested by four different restriction enzymes (*EcoRI, HindIII, PstI,* and *Xbal*) (Takara Bio Inc. Otsu, Shiga, Japan). The distribution of DNA fragment sizes from the four restriction enzyme profile of each tested strain was compared with that of the standard marker O Range Ruler 50bp DNA Ladder. Isolates exhibiting identical PFGE patterns were considered genetically indistinguishable, those exhibiting 1-3 band difference were considered closely related and those exhibiting 4-6 band difference were considered possibly related.

3.6.3.1. Harvesting of Bacterial cells

A standard protocol for small-scale preparations of plasmid DNA was used, with some modification (Sambrook *et al.*, 1989). A single bacterial colony was transferred into 5 ml Brain heart infusion broth ("Nissui", Japan) and incubated at 37^0C for 24 h. 1.5 ml of the broth culture was centrifuged into a microcentrifuge (Hitachi Himac, Model cf 15d, Japan) at 10,000 rpm for 5 minutes. The supernatant was discarded leaving the bacterial pellet as dry as possible.

3.6.3.2. Preparation of Plasmid DNA

The bacterial pellet was suspended in 100 µl of ice-cold solution I (50 mm glucose, 25 mm Tris. Cl (pH 8.0) (Gibcobril – Life Technologies, Grand Island, USA), 10 mm EDTA (pH 8.0) (Dojin-Dotite, Tokyo, Japan) followed by vigorous vortex. To the suspension 200 µl of freshly prepared Solution II (1% Sodium Dodecyl Sulfate) (Wako pure chemical) was added and the contents were tightly closed before mixing by inverting the tube rapidly 5 times. The tube was immediately placed on ice. 150 µl of ice-cold Solution III (5 M potassium acetate 60 ml, glacial acetic acid 11.5 ml and distilled water 28.5 ml) was added to the suspension which was followed by a gentle vortex in an inverted position for 10 seconds to disperse the viscous bacterial lysate. The tube was allowed to stand on ice for 5 minutes followed by centrifuging at 14,000 rpm for 5 minutes at 4^oC in a micro centrifuge tube. Out of the supernatant 300 µl was transferred

to a fresh tube to which an equal volume of phenol-chloroform was added and contents were mixed by vortex prior to centrifugation at 14,000 rpm for 2 minutes at 4°C. Then 200 µl of the supernatant was transferred to another fresh tube to which 2 volumes of 100% ethanol was added to precipitate the double-stranded DNA. The contents were mixed by vortex and allowed to stand for 2 minutes at room temperature followed by another centrifugation at 14,000 rpm for 5 minutes at 4°C. The supernatant was removed by gently aspiration and the tube was left to stand in an inverted position on a paper towel to allow all the fluid to drain away. The pellet of double stranded DNA was rinsed with 1 ml of 70% ethanol followed by gentle aspiration of the supernatant before allowing the pellet of DNA to dry in the air for 10 minutes. The DNA pellet was re-dissolved in 25 µl of TE Buffer pH 8.0, briefly vortexed before free spin and then stored at -20°C.

3.6.3.3. Gel electrophoresis

To perform gel electrophoresis, 1.0% agarose was prepared. Sufficient working electrophoresis TBE buffer was prepared by diluting 50 ml of x10 TBE buffer pH 8.0 with 450 ml of distilled water. About 1.0 g (1%) of Agarose powder was weighed and added to 100 ml working electrophoresis TBE buffer. The slurry was heated in a microwave oven until the agarose dissolved. The agarose was cooled and kept molten at 60°C prior to adding 3 µl ethidium bromide. The molten agarose was poured into casting trays which had eight (8) and seventeen (17) well comb respectively and allowed to solidify completely for 30 minutes at room temperature. The comb was carefully removed from the solidified gel to create the wells and then the gel was mounted in the electrophoresis chamber that contained enough TBE buffer pH 8.0.

3.6.3.4. Preparation of restriction enzymes reaction mixtures and digestion of the genomic DNA substrates

Four restriction enzymes digest *Pst1*, *Xbal*, *HindIII*, and *EcoR1* were prepared and mixed according to the manufacturers instructions with a slight modification. To 2 µl volume of restriction enzyme was added 4 µl Buffer (10x) and 7 µl sterilized distilled water to give a total volume of 13 µl mixture. The reagents were mixed by vortex and then pooled to

the bottom of the micro-centrifuge tube by free flushing in a micro-centrifuge. The mixture was kept on ice.

To 7 µl volume of DNA substrate was added 13 µl restriction enzyme mixture to give a final volume of 20 µl. The mixture was vortexed to mix and pooled to the bottom of the tube by a free flush in a microcentrifuge. The digestion of genomic DNAs' with the restriction enzymes *Pst1*, *Xbal, HindIII,* and *EcoR1* were incubated in a Dry–block bath (Scinics Co. Japan) at 37°C for 1 hr and then kept on ice. DNA substrate without the restriction enzyme but with only sterile distilled water was also included in the test as a control after which gel electrophoresis was done.

Briefly, to 10 µl of genomic DNA digested substrate was mixed with 5 µl of gel loading buffer on a strip of parafilm. After mixing, slowly 15 µl were loaded into the slots of the submerged agarose gel wells using a disposable micropipette. In parallel 10 µl of DNA molecular ladder (20 fragments) of increasing size from 50 to 1000 bp was included as a DNA size standard and was loaded into the first slot of the wells. The gel was stained with ethidium bromide. The electrophoresis of the DNA substrate was performed on a Mupid electrophoresis chamber (Advance Co. Japan) on a horizontal gel for 40 minutes at 110 V. The DNA fragments were visualized and photographed on an ultraviolet wavelength (UV) Chromato Dual intensity-transilluminator (San Gabriel, USA). The restriction endonuclease DNA digest patterns were compared, and their similarities were scored as described by Davis *et al.*, (2003). Isolates that differed in their PFGE fragments by one or two bands were regarded as closely related, as minor mutational changes would result in such patterns (Kiriuki, *et al.,* 2006a).

3.6.4. Detection of virulence genes in *Escherichia coli* and *Salmonella* serovars from Kafue lechwe and pastoral cattle

Identification of DEC and *Salmonella* isolates requires that these organisms be differentiated from non-pathogenic members of the normal flora (Nataro *et al.,* 1998). A number of nucleic acid based methods have been reported for the detection and characterization of DEC and *Salmonella* (Moussa *et al.*, 2010; Kwon *et al.,* 2008; Eyigor

et al., 2007; Veilleux *et al.*, 2006; Ruttler *et al.*, 2006 and Gibotti *et al.*, 2004). Some selected and provisionally identified *E. coli* (ETEC, EPEC, EIEC) and *Salmonella* serogroups were further examined for the presence of genes encoding for virulence factors using PCR according to the method described by Hamza *et al.*, (2010) and Oliveira *et al.*, (2003) with annealing temperature of 55°C for *E. coli* and 60°C for *Salmonella.* The DNA from selected *E. coli* and *Salmonella* isolates was extracted and used to target the presence of invasion E gene *(invE)*, LT, Heat stable toxin 1 (ST1), Shiga toxin 1 *(sxt-1),* Shiga toxin 2 *(stx-2),* invasion plasmid antigen B *(ipaB)*, invasion plasmid antigen H *(ipaH),* EAEC heat-stable toxin 1 (EAST1) and *invA* genes as shown in Table 3 (Gerrish *et al.*, 2007; Veilleux *et al.*, 2005; Fode-Vaughan *et al.*, 2003 Hudson *et al.*, 2000 and Barzu *et al.*, 1993).

The detection of genes involved bacterial culture and preparation of the bacterial template DNA. Test cultures were sub-cultured on Brain Heart Infusion agar (BHI) and incubated at 37°C for 18 hr. Three colonies previously sub-cultured were picked and emulsified into 1 ml of sterile distilled water and was heated at 95°C in a dry block bath (Scinics Co. Japan) for 10 minutes. To 9 µl PCR mixture (5 µl Phusion flsh mixture, 1.5 µl sterile distilled water, 2.5 µl primers (Forward and Reverse) was added 1 µl of bacterial DNA template. The PCR for 96 Wells (Finnzymes Piko) was performed using 10 µl total reaction volumes. The thermal cycling protocol was performed using the rapid cycle DNA amplification method, which consisted of initial denaturation step at 98°C for 30 seconds, followed by 30 cycles of template denaturation at 98°C, primer annealing at 60°C (*E. coli*) and 55°C (*Salmonella*), and extension at 72°C for 10 seconds. The PCR products were viewed with ethidium bromide after electrophoresis through 3% agarose gels in TAE buffer.

Table 3: MORA-Primer Diarrheal pathogens

Serial No.	Name of pathogen	Target gene	Amplicon size (bp)
1	*Enteroinvasive E. coli*	*invE*	127
2	*Enterotoxigenic E. coli* *Enterohemorrhagic E. coli*	LT Shiga 1 (*Stx*-1)	297 376
3	*Enteroinvasive E. coli* *Enterohemorrhagic E. coli*	*IpaB* Shiga 2 (*Stx*-2)	137 272
4	*Escherichia coli*	ST1	171
5	*Enteroinvasive E. coli*	*ipaH*	117
6	*Salmonella* *Enteroaggregative E. coli*	*invA* EAST1	422 173

The amplicons were checked by gel electrophoresis and their size determined by using O'Range Ruler 50bp DNA Ladder (Molecular marker). The electrophoresis of the DNA substrate was performed on a Mupid electrophoresis chamber on a horizontal gel for 20 minutes at 110 V. The DNA fragments were visualized and photographed on an ultraviolet wavelength (UV) Chromato Dual intensity-transilluminator. The presence of the amplification product was interpreted as a positive test.

3.6.5. Antimicrobial susceptibility test for bacterial isolates

Escherichia coli and *Salmonella* isolates from Kafue lechwe and pastoral cattle faecal samples were tested for antibiotic susceptibility by the single disk diffusion method as previously described by Bauer-Kirby *et al.*, (1966) to eight antimicrobial agents (Himedia) (Table 4) which included some gram positive, protozoan and anaerobic antibiotics.

Table 4: Antibiotics discs used, their symbol and strength per disk

Type of Antibiotic	Symbol	Strength/disc
Amoxicillin	AML	30 μg
Cephalexin	CP	30 μg
Co-trimoxazole	CO	25 μg
Gentamycin	G	10 μg
Metronidazole	MT	5 μg
Oxacillin	OX	5 μg
Penicillin G	PG	10 units
Tetracycline	TE	30 μg

The procedure was carried out according to the manufacturer of the antibiotic discs, 3 to 4 colonies of pure cultures of the test organisms were transferred into 5 ml of Nutrient broth (Nissui). The broth was incubated overnight, after which the turbidity was adjusted to yield McFarland turbidity standards number 0.5. A non toxic sterile cotton swab on a wooden applicator was dipped into the standardized inoculum. The soaked swab was firmly rotated against the upper inside wall of the tube to express excess fluid. Mueller Hinton agar (Difco – Becton, Dickinson and Co., Sparks, USA) plates were prepared prior to use in the test. The entire surface of the agar was streaked with the swab three times, turning the plate at 60°C angles between each streaking to obtain a confluent growth on the agar plate. The plates were allowed to dry 15 minutes with a lid in place. In this method, paper discs impregnated with standard amounts of antibiotic were applied

on the inoculated surface of the agar plate at equal distance apart. The agar plates were incubated at 37°C for 18 hr. The zones showing complete inhibition were recorded as susceptible according to the method of Bauer-Kirby *et al.,* (1966). Furthermore, resistance of the isolates was noted as detailed by the manufacturers of the antibiotic discs used in this study (Himedia).

3.7.0. Enumeration of viable *Escherichia coli* strains from rectal contents and faecal samples from the environment

Faecal samples from the rectum of Kafue lechwe and pasture were examined for total *E. coli* viable count according to the method described by Hayes (1985). To 0.1 g of the faecal sample was added 0.9 ml of sterile normal saline (NS) then vortexed and the tube was marked number 10^1. The suspension was diluted tenfold from tubes marked number 10^1 to 10^5 by transferring 0.1 ml from the first tube of the suspension into the next tube. Similarly the rest of the tubes were treated the same. Inoculated 0.1 ml volume from a suitable dilution of the faecal sample onto the duplicate surface of dried DHL ("Nissui", Japan).

The inoculum was evenly spread over the surface using sterile bent glass rods of the hockey stick type. The plates were incubated at 35°C for 24 hr and counted the number of colonies with a preferred dilution that contained not more than 300 colonies per whole plate. Viable counts were expressed as the number of colony forming units per 1 g of faeces obtained by multiplying the mean of colonies on the duplicate plates by the dilution factor. All counts were reported as logarithm count per gram of the faecal sample. Bacteria which are stressed or injured may not be detected by this approach (O'Sullivan *et al.*, 2006).

CHAPTER FOUR

RESULTS

4.1.0. Faecal sample collection from Kafue lechwe and pastoral cattle for isolation and identification of *Escherichia coli* and *Salmonella*

A total of 593 faecal samples from Kafue lechwe and pastoral cattle were collected in this study between June through December 2008. Of the 593, 77 (13.0%) were from hunter harvested Kafue lechwe while 516 (87.0%) were faecal samples of Kafue lechwe and pastoral cattle conveniently picked from the grazing pastures. Out of 516, 155 (30%) were from Kafue lechwe while 361 (70%) were from pastoral cattle.

4.1.1. *Escherichia coli* isolates from the Kafue lechwe

Seventy-seven (77) rectal faecal samples and 155 faecal samples from pasture were collected from Lochinvar and Blue-lagoon NPs. Samples were examined for the presence of DEC and from these specimens, 575 presumptive *E. coli* isolates were recovered and identifified.

Out of 575 presumptive *E. coli* isolates, 104 (18.1%) were NSF isolates on SMAC and appeared mauve coloured on CHROMagar O157 a characteristic which distinguishes STEC O157 from other coliforms (Figure 6a and 6b). Eighty (13.9%) isolates out of 575 presumptive *E. coli* isolates from Kafue lechwe were NSF, while 35 (6.1%) were mauve coloured. All (104) isolates exhibited typical green metallic sheen on EMB agar as shown in Figure 6c and were subjected for further biochemical identification. Out of 104 *E. coli* isolates presumptively identified as O157 strains, 79 (76%) were from Lochinvar NP, while 25 (24%) were from Blue-lagoon NP. Further, 80 of 104 (76.9%) were NSF isolates, while 35 (33.7%) were mauve coloured on CHROMagar O157. There was a significant difference ($P = 0.001$) in the isolation frequency of NSF *E. coli* isolates between Lochinvar ($n = 55$) and Blue-lagoon ($n = 25$) NPs.

(a) (b) (c)

Figure 6: (a) Sorbitol negative *E coli* isolate on sorbitol MacConkey agar (Right plate) (b): Mauve coloured *E coli* isolate on CHROMagar O157 (Right plate (c) *E. coli* colonies on EMB Agar.

Sixty-nine out of 104 (66.3%) isolates were from the faecal samples picked from the pasture while 35 (33.7%) were from the rectum. Thus there was no statistically significant difference (P = 0.217) in the isolation frequency of NSF *E. coli* isolates between faecal samples from the rectum and pasture. All the isolates from Blue-lagoon NP were NSF while the proportion of NSF *E. coli* isolates from Lochinvar NP was 69.6% (55/79). Similarly, there was also a significant difference (P < 0.001) in the isolation frequency of mauve coloured isolates on CHROMagar O157 between Lochinvar and Blue-lagoon NPs. Thirty-four of 35 (97.1%) mauve coloured isolates were from Lochinvar NP while only one mauve coloured isolate was from Blue-lagoon NP. There was a significant difference (P = 0.029) in the isolation frequency of mauve coloured isolates between isolates from the rectum and the pasture. Of 35 mauve coloured isolates, 18 (51.4%) were from the faecal samples picked from the pastures while 17 (48.6%) were from the rectum. The proportion of mauve coloured isolates from Lochinvar NP was 43% (34/79) higher than those from Blue-lagoon NP 4% (1/25). Thirteen of 77 (16.9%) compound samples (animals), and 27 (17.4%) samples out of the 155 faecal samples from the pasture were found positive to one or more DEC strains.

Majority of the NSF and mauve coloured isolates were confirmed as *E. coli* by conventional tests. Biochemically, these organisms produced indole, fermented arabinose, were motile and were positive to lysine decarboxylation. On TSI medium, these organisms showed acid butt, acid slant and abundance of gas suggestive of *E. coli*

strains (Table 5 and Figure 7). Twenty nine of 35 (82.9%) mauve coloured isolates and 60 of 80 (75%) NSF isolates were arabinose fermentative.

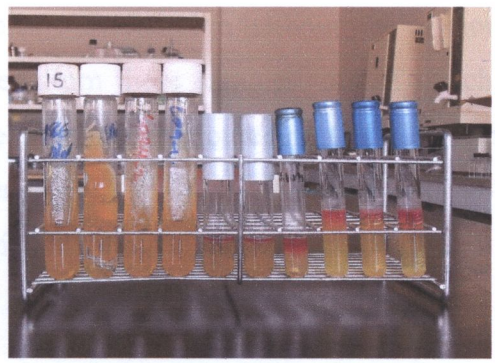


Figure 7: *E. coli* isolates reaction on TSI and SIM media.

Table 5: Biochemical reaction of non-sorbitol fermenting isolates and mauve coloured *E. coli* isolates from Kafue lechwe *(n* = 104)

Tests	Common reaction	Characteristics of isolates	
		Non-sorbitol fermenting isolates (*n* = 80)	Mauve coloured isolates (*n* = 35)
Glucose/Sucrose/Lactose (TSI)	+	80	35
H_2S production(TSI)	-	0	0
Gas production (TSI)	+/-	52	30
Motility	+/-	78	35
Urea	-	80	35
Lysine carboxylase	+	80	35
Simmons citrate	-	0	0
Arabinose	+	60	29
Indole	+	80	35

Key (+ = positive reaction, - = negative reaction, +/- = variable reaction)

4.1.2. *Escherichia coli* isolates from the pastoral cattle

A total of 361 pastoral cattle faecal samples, 261 from Lochinvar NP and 100 from Blue-lagoon NP, were examined for the presence of DEC. Seventy-one (19.7%) faecal samples out of 361 samples were found to harbour one or more DEC. Altogether 708 *E. coli* isolates were isolated from these samples, out of which, 144 (20.3%) were either NSF on SMAC and appeared mauve coloured on CHROMagar O157. Of the 144 *E. coli* isolates, 110 (76.4%) were from Lochinvar NP, while 34 (23.6%) were from Blue-lagoon NP. The area distribution of the 71 faecal samples found to harbour DEC were 52 (73.2%) from Lochinvar NP and 19 (26.8%) from Blue-lagoon NP.

Of 144 isolates, 68 (47.2%) were NSF while 94 (65.3%) were mauve coloured on CHROMagar 0157. Out of 68 NSF isolates, 52 (76.5%) were from Lochinvar NP, while 16 (23.5%) were from Blue-lagoon NP ($P = 0.570$). Seventy-five out of 94 (79.8%) mauve coloured isolates were from Lochinvar NP while 19 (20.2%) were from Blue-lagoon NP. There was a marginal significant difference ($P = 0.13$) in the isolation frequency of mauve coloured isolates on CHROMagar O157 between Lochinvar and Blue-lagoon NPs.

4.1.3. *Salmonella* isolates from the Kafue lechwe

A total of 232 Kafue lechwe faecal samples, 77 from the rectum and 155 from pasture in Lochinvar and Blue-lagoon NPs, were examined for the presence of *Salmonella spps.* These samples yielded one or more *Salmonella* isolates and altogether 59 isolates were identified to belong to the genus *Salmonella* based on phenotypic characterization (Table 6). *Salmonella* isolates were isolated from 6 (7.8%) of the 77 compounded faecal samples and 12 (7.7%) of 155 faecal samples from pasture. Out of the 59 isolates, there were significantly more isolates ($P < 0.001$) from Lochinvar NP (66.1%) than were from Blue-lagoon NP(33.9%).

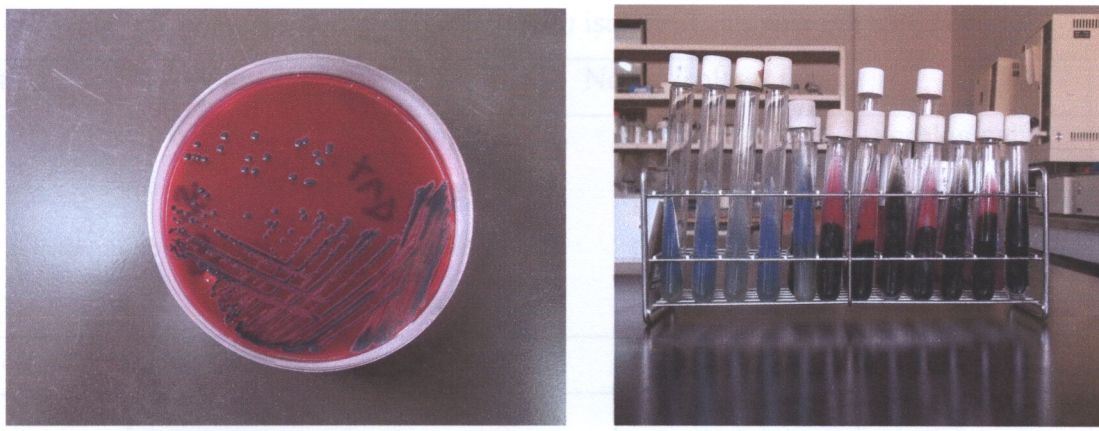
Results of biochemical test are presented in Table 6. All isolates were negative to urease, indole and did not ferment lactose and sucrose. On TSI medium, the organism showed acid butt and alkaline slant with abundant H_2S production. Eighteen (94.7%) of 19

Salmonella isolates positive to *Salmonella* polyvalent antisera were able to utilise citrate, produce hydrogen sulphide (causing a black precipitate of ferrous sulphide in the XLD, TSI and SIM medium) (Figure 8a and 8b) and were motile except for one isolate which could not ferment dulcitol, mannitol and utilise citrate.

Table 6: Phenotypic characterization of *Salmonella* isolates from Kafue lechwe and pasture ($n = 59$)

Test	*Salmonella* species reaction	*Salmonella* polyvalent antisera positive isolates ($n = 19$)	Serological negative isolates ($n = 40$)
Urease	-	19	40
TSI (Glucose)	+	19	40
H_2S	+	18	0
Gas production	+/-	10	16
Lysine decarboxylase	+	19	40
Indole	-	19	40
Phenol red dulcitol fermentation	+	18	5
Phenol red mannitol fermentation	+	18	38
Phenol red lactose fermentation	-	19	40
Phenol red sucrose fermentation	-	19	40
Simmons citrate	+/-	18	7
Motility	+/-	18	32

Key (- = negative reaction, + = positive reaction, +/- = variable reaction)



(a) (b)

Figure 8: (a) *Salmonella* colonies on Xylose-Lysine-Deoxycholate Agar (b) *Salmonella* isolates on simmons citrate and Triple sugar iron slopes.

4.1.4. *Salmonella* isolates from the pastoral cattle

A total of 361 cattle faecal samples were examined for the presence of *Salmonella* serovars. Out of these, 261 (72.3%) were from Lochinvar NP and 100 (27.7%) from Blue-lagoon NP. Four (1.5%) out of 261 samples from Lochinvar NP, and 5 of the 100 (5%) samples from Blue-lagoon NP were positive for one or more isolates of *Salmonella.* Altogether 30 *Salmonella* isolates were biochemically identified from the faecal samples. All the isolates were urease and indole negative and did not ferment lactose and sucrose, but showed a positive reaction of the lysine decarboxylase and fermentation of glucose only in TSI agar. The majority of isolates positive to *Salmonella* polyvalent antisera fermented mannitol, dulcitol and produced hydrogen sulphide from TSI and SIM (Table 7).

4.2.0. Serotyping

Serotyping of *E. coli* was based on the use of 43 specific panels of antisera mentioned in Table 1 for the detection of somatic O antigens expressed by these bacteria.

Table 7: Phenotypic characterization of *Salmonella* isolates from pastoral cattle ($n = 30$)

Test	*Salmonella* species reaction	No. of isolates tested		
		Serologically positive *Salmonella* isolate ($n = 13$)	*Salmonella* serogroup 8 positive isolates ($n = 1$)	Serologically negative isolates ($n = 17$)
Urease	-	13	1	17
TSI (Glucose)	+	13	1	17
H_2S	+	11	0	0
Gas production	+/-	3	0	0
Lysine decarboxylase	+	13	1	17
Indole	-	13	1	17
Dulcitol fermentation	+	11	1	12
Mannitol fermentation	+	11	1	4
Lactose fermentation	-	13	1	17
Sucrose fermentation	-	13	1	17
Simmons citrate	+/-	11	1	14
Motility	+/-	11	1	14

Key (- = negative reaction, + = positive reaction, +/- = variable reaction)

4.2.1. Serotyping of *Escherichia coli* isolates from the Kafue lechwe and prevalence estimates

One hundred and four *E. coli* isolates (18.1% of 575) from Kafue lechwe with similar growth characteristics as *E. coli* O157 on SMAC and CHROMAGAR O157 were

serogrouped into 8 serogroups using *E. coli* monovalent antisera test kit. Out of 104, 18 (17.3%) isolates were typeable (O15, O27, O78, O8/O125, O29/O143, O55/O125, and O125) while the rest were non-typeable. The most frequent serogroup was the non-typeable *E. coli* serogroup which accounted for 82.7% of the total isolates. The details of the distribution of the serogroups are presented in Table 8. None of the isolates were agglutinated by O157 antiserum.

Table 8: *E. coli* serogroups isolated from Kafue lechwe (n = 104)

Serotype	Category	No. of *E. coli* strains reacting to antiserum	Frequency (%)
015	ETEC	2	1.9
027	ETEC	5	4.8
078	ETEC	1	0.96
08/0125	ETEC/EPEC	1	0.96
029/0143	EIEC	1	0.96
055/0125	EPEC	2	1.9
0125	EPEC	6	5.8
Non-typeable	-	86	82.7
Total		104	

There was a significant association (P = 0.004) between serovars and the choice of the selective media used. All isolates belonging to serogroups O15 and O125 were NSF isolates on SMAC, whereas the isolates belonging to serotype 078 and the majority of the strains belonging to serotype 027 (P=0.004) were mauve coloured on CHROMagar O157. The most frequently isolated DEC serogroups were EPEC O125 (5.8%) followed by ETEC serotype 027 (4.8%) (Table 9).

Table 9: Distribution of *E. coli* serogroups from Kafue lechwe by selective media (n = 104)

Serotype	No. of isolates reacting to antiserum	No. of non-sorbitol fermenting isolates on SMAC (%)	No. of positive isolates on CHROMagar O157 (%)
015	2	2 (100)	0
027	5	2 (40)	4 (80)
078	1	0	1 (100)
08/0125	1	1 (100)	0
029/0143	1	0	1 (100)
055/0125	2	0	2 (100)
0125	6	6 (100)	0
None-typeable	86	69 (80.2)	27 (31.4)
Total	104	80	35
P value		0.004	0.004

The prevalence of the typeable isolates was 18 out of 104 (17.3%) and the rest respectively were non-typeable (Table 10). There was no significance difference in the isolation frequency of serogroups between pasture and rectum (P = 0.395) and between Lochinvar and Blue-lagoon NPs (P = 0.237). The most frequently isolated *E. coli* serogroups were O27 and O125 and were all from the pasture. Serogroups O15 and O125 were evenly isolated between Lochinvar and Blue-lagoon NPs (Table 10).

4.2.2. Serotyping of *E. coli* isolates from the pastoral cattle and prevalence estimates

One hundred and forty four *E. coli* isolates from pastoral cattle were sero-grouped into 16 serogroups. Most of the serogroups typed were from Lochinvar NP. There was a significant difference (P < 0.001) in the isolation frequency of serogroups between Lochinvar and Blue-lagoon NPs. The most frequent serotype was the non-typeable *E. coli* which accounted for 77.8% of the isolates. Isolates belonging to O125 serogroup were all from Lochinvar NP. Details of the distribution of the serogroups are presented in Table 11. None of the isolates were agglutinated by O157 antiserum.

Table 10: Distribution of *E. coli* serogroups from Kafue lechwe by sample source and area of sampling (n =104)

Serotype	No. of isolates reacting to antiserum	No. of positive isolates per sample source (%)		No. of positive isolates by area of sampling (%)	
		Pasture	Rectum	Lochinvar	Blue-lagoon
015	2	2 (100)	0	1	1
027	5	4 (80)	1 (20)	5	0
078	1	1 (100)	0	1	0
08/0125	1	1 (100)	0	0	1
029/0143	1	1 (100)	0	1	0
055/0125	2	2 (100)	0	2	0
0125	6	6 (100)	0	3	3
Non-typeable	86	52 (60.5)	34 (39.5)	66	20
Total	104	69	35	79	25

There was a significant difference ($P < 0.001$) in the isolation frequency of *E. coli* serogroups between faecal samples from Lochinvar and Blue-lagoon NPs. Majority of the serogroups were predominantly isolated from faecal samples collected from Lochinvar NP. All the serogroup O125 isolates from pastoral cattle were from the Lochinvar NP, while all the serogroup O29 isolates were from Blue-lagoon NP, indicating an ecological distribution of the isolates. Majority of serogroup O27 were from Blue-lagoon NP. Only one serogroup O55 was isolated and it was from Blue-lagoon NP and the only serogroup O78 was from Lochinvar NP. Serogroup O8 was evenly isolated between Lochinvar and Blue-lagoon NPs (Table 12).

Table 11: *E. coli* serogroups isolated from pastoral cattle (n = 144)

Serotype	Category	Diarrheagenic positive *E. coli* isolates (%)
0125	EPEC	4 (2.8)
0125/0169	EPEC/ETEC	2 (1.4)
0159	ETEC	1 (0.7)
027	ETEC	4 (2.8)
027/0115	ETEC	1 (0.7)
027/055/0125	ETEC/EPEC	1 (0.7)
08/027	ETEC	1 (0.7)
029	EIEC	6 (4.2)
055	EPEC	1 (0.7)
055/0125/0169	EPEC/ETEC	2 (1.4)
078	ETEC	1 (0.7)
08	ETEC	2 (1.4)
08/027/0125	ETEC/EPEC	1 (0.7)
08/027/055/0125	ETEC/EPEC	2 (1.4)
08/027/055/0125/0169	ETEC/EPEC	3 (2.1)
Non-typeable	-	112 (77.8)
Total		144

Table 12: Distribution of *E. coli* serogroups isolated from pastoral cattle between Lochinvar and Blue-lagoon NPs (n = 144)

Serotype	Category	No. of positive *E. coli* serogroups (%)	Lochinvar	Blue-lagoon
0125	EPEC	4 (2.8)	4	0
0125/0169	EPEC/ETEC	2 (1.4)	1	1
0159	ETEC	1 (0.7)	0	1
027	ETEC	4 (2.8)	1	3
027/0115	ETEC	1 (0.7)	0	1
027/055/0125	ETEC/EPEC	1 (0.7)	1	0
08/027	ETEC	1 (0.7)	0	1
029	EIEC	6 (4.2)	0	6
055	EPEC	1 (0.7)	0	1
055/0125/0169	EPEC/ETEC	2 (1.4)	0	2
078	ETEC	1 (0.7)	1	0
08	ETEC	2 (1.4)	1	1
08/027/0125	ETEC/EPEC	1 (0.7)	1	0
08/027/055/0125	ETEC/EPEC	2 (1.4)	2	0
08/027/055/0125/0169	ETEC/EPEC	3 (2.1)	3	0
Non-typeable	-	112 (77.8)	95	17
Total		144	110	34

4.2.3. Serotyping of *Salmonella* isolates from Kafue lechwe and prevalence estimates

Out of 59 *Salmonella* isolates identified on biochemical and physiological characterization, 19 (32.2%) were confirmed on serological typing with *Salmonella* somatic polyvalent antisera, while 40 (67.8%) were non-typeable. There was a positive association between reaction to anti-polyvalent antisera and source of isolate sample ($P < 0.001$). All 19 *Salmonella*-confirmed isolates were from Lochinvar NP, while 50% of each of the non-typeable isolates were from both Lochinvar and Blue-lagoon NPs, respectively. Of the 19 *Salmonella* isolates, 5 (26.3%) were identified to belong to

serogroup 8 serovars, while the rest 14 (73.7%) isolates did not react to the available *Salmonella* antisera groups.

Fifty-nine *Salmonella* isolates were identified based on phenotypic characterization, of which 39 were from Lochinvar NP, while 20 were from Blue-lagoon NP (Table 13). All the 59 *Salmonella* isolates were collectively isolated from the pasture and lechwe rectum, ileo-caecal junction, caecum and small intestine. There was marginal significant difference ($P = 0.011$) in the isolation frequency of *Salmonella* isolates between location and sample source. Of the 27 isolates from pasture, 16 (59.3%) were from Lochinvar NP, while 9 out of 16 (43.8%) isolates from the rectum were from Blue-lagoon NP. There was significant difference ($P < 0.001$) in the isolation frequency between isolates positive to polyvalent antisera and sample source. Majority (36.8%) of the isolates positive to serotyping with somatic antisera were from the rectum followed by ileo-caecal junction and the small intestine 21.1% (4/19) respectively. Of the 5 isolates belonging to serogroup 8, 4(80%) were from the rectum, while 1 (20%) was from the pasture ($P = 0.172$).

4.2.4. Serotyping of *Salmonella* isolates from the pastoral cattle and prevalence estimates

Thirty suspected *Salmonella* isolates were isolated and biochemically identified from faecal samples of the pastoral cattle, out of which 13 (43.3%) tested positive on agglutination test with *Salmonella* polyvalent antisera, whereas the rest of the isolates, 17 (56.7%) were non-typeable. Only one (3.3%) isolate was found to belong to *Salmonella* group 8.

Fifteen (50%) isolates out of 30 *Salmonella* isolates were from Lochinvar NP and another 15 (50%) were from Blue-lagoon NP (Table 13). There was a significance difference ($P < 0.001$) in the isolation frequency of *Salmonella* isolates positive to polyvalent antisera between faecal samples collected from Lochinvar and Blue-lagoon NPs. Of the 13 serologically positive isolates, 12 (92.3%) were from Lochinvar NP.

Table 13: Distribution of *Salmonella* isolates from faecal samples of Kafue lechwe by source and location (n = 59)

Source of isolates	No. of isolates	Lochinvar National Park		Blue-lagoon National Park	
		Polyvalent O antisera positive isolates	Non-typeable isolates (%)	Polyvalent O antisera positive isolates	Non-typeable isolates (%)
Pasture	27	1	15	0	11
Rectum	16	7	0	0	9
Ileo-caecal -junction	5	4	1	0	0
Caecum	3	3	0	0	0
Small intestine	8	4	4	0	0
Total	59	19	20	0	20

4.3.0. Biochemical profiles of *Escherichia coli* and *Salmonella* serogroups

The metabolic fingerprint of *E. coli* and *Salmonella* isolates belonging to the similar serogroups was determined by fermentation of carbon source to a range of carbohydrates.

4.3.1. *Escherichia coli* biovars

Table 14 and Figure 10 show fermentative reactions of *E. coli* serogroups from Kafue lechwe and pastoral cattle to 18 carbohydrates. Isolates numbers 1, 2, 3, 6, 8 and 9 were from Kafue lechwe, while isolates number 4, 5, 7, 10, 11, and 12 were from pastoral cattle. All DEC serogroups metabolised one or more carbohydrates. *Escherichia coli* serogroups O27 were in two distinctive fermentative groups. One group comprised isolates number 1 (Lp 13-2) and 4 (Sc 1-2) from Kafue lechwe and pastoral cattle, while the second group were number 3 (Lp Lr 28-4) and 5 (Lmc 167-2). Isolates in each group showed a similar pattern of fermentative characteristics suggesting that the strains are indistinguishable (figure 9). None of the isolates metabolised carbon from cellobiose, dextran, inositol resorcinol, ribitol and barbital. Isolates belonging to serovars O78 and O125 from Kafue lechwe and pastoral cattle were not related (Table 14).

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Table 14: Comparison of fermentative reaction of *E. coli* biovars from Kafue lechwe and pastoral cattle (n = 12)

Test	*E. coli* serotype tested											
	O27					O78		0125				
	Lechwe			Cattle		Lechwe	Cattle	Lechwe			Cattle	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**		**9**	**10**	**11**	**12**
Cellobiose	-	-	-	-	-	-	-	-		-	-	-	-
Dextran	-	-	-	-	-	-	-	-		-	-	-	-
Dulcitol	-	+	+	-	+	+	+	+		+	+	+	+
Galactose	+	+	+	+	+	+	+	+		+	+	+	+
Inositol	-	-	-	-	-	-	+	-		-	-	-	-
Maltose	+	+	+	+	+	+	+	+		+	+	+	+
Meliobiose	+	+	+	+	+	+	+	+		+	+	+	+
Raffinose	+	+	+	+	+	+	+	+		+	+	+	+
Resorcinol	-	-	-	-	-	-	-	-		-	-	-	-
Ribitol	-	-	-	-	-	-	-	-		-	-	-	-
Rhamnose	+	+	+	+	+	+	+	-		-	+	-	+
Trehalose	+	+	+	+	+	+	+	+		+	+	+	+
Xylose	+	+	+	+	+	+	+	+		+	+	+	+
Sorbitol	+	-	+	+	+	+	+	-		-	+	+	+
Mannitol	+	+	+	+	+	+	+	+		+	+	+	+
Mannose	+	+	+	+	+	+	+	+		+	+	+	+
Starch	-	-	-	-	-	-	+	+		+	+	+	+
Barbitol	-	-	-	-	-	-	-	-		-	-	-	-

Key (+ = positive reaction, - = negative reaction)

1 = Lp 13-2, **2** = Lp 208-4, **3** = Lr 28-4, **4** = Sc 1-2, **5** = Lmc 167-2, **6** = Lp 9-4, 7 = Lmc 248-2, **8** = Lp 24-2, **9** = Lp 50-1, **10** = Lmc 26-2, **11** = Lmc 38-1 and **12** = Lmc 59-1

Figure 9: Fermentation of carbohydrates by *E. coli* serogroups.

4.3.2. *Salmonella* biovars

Table 15 and Figure 10 show that isolates number 1, 3, 5 and 7 were from pastoral cattle while isolates 9, 9-1, 9-2, 9-3, 11, 12, 16 and 18 were from Kafue lechwe.

Salmonella biovars were positive to more than one carbohydrate (Table 15). Isolates number 1 (Lmc 226-1), 3 (Lmc 227-1), and 5 (Lmc 231-1) from pastoral cattle and isolates number 12 (Lr 79-4), 14 (Icj 59-1), 16 (C 67-2) and 18 (Si 69-1) from Kafue lechwe showed a similar pattern of fermentative reaction to 20 carbohydrates suggesting they were from a common source. None of the *Salmonella* biovars were positive to cellobiose, dextran, raffinose, resorcinol and barbital. The isolates belonging to serogroup 8 from the rectum of Kafue lechwe and pastoral cattle were not related. Only the five biovars (9, 9-1, 9-2, 9-3 and 11) belonging to serogroup 8 from faecal contents of Kafue lechwe were able to metabolise carbon from inositol. One *Salmonella* biovar number 11 (Lp 260-2) belonging to serogroup 8 from faecal samples of Kafue lechwe picked from the pasture was negative to dulcitol, rhamnose, trehalose and xylose suggesting that it was a different strain. Similarly the only isolate in serogroup 8 from cattle was positive to arabinose and salicin suggesting it is not from a common source with the other isolates.

Table 15: Comparison of fermentative reaction of *Salmonella* biotypes from Kafue lechwe and pastoral cattle (n = 13)

Test	Reaction of *Salmonella* isolates												
	Isolates from pastoral cattle				Isolates from Kafue lechwe								
	1	**3**	**5**	**7***	**9***	**9-1***	**9-2***	**9-3***	**11***	**12**	**14**	**16**	**18**
Arabinose	-	-	-	+	-	-	-	-	-	-	-	-	-
Cellobiose	-	-	-	-	-	-	-	-	-	-	-	-	-
Dextran	-	-	-	-	-	-	-	-	-	-	-	-	-
Dulcitol	+	+	+	+	+	+	+	+	-	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	-	-	-	-	+	+	+	+	+	-	-	-	-
Maltose	+	+	+	+	+	+	+	+	-	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-
Resorcinol	-	-	-	-	-	-	-	-	-	-	-	-	-
Ribitol	-	-	-	-	-	-	-	-	+	-	-	-	-
Rhamnose	+	+	+	+	+	+	+	+	-	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	-	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	-	+	+	+	+
Salicin	-	-	-	+	-	-	-	-	+	-	-	-	-
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch	+	+	+	+	+	+	+	+	+	+	+	+	+
Barbitol	-	-	-	-	-	-	-	-	-	-	-	-	-

Key (* = isolates belonging to serogroup 8, - = negative reaction, + = positive reaction)

Isolate numbers: **1** = Lmc 226-1, **3** = Lmc 227-1, **5** = Lmc 231-1, **7** = Sc 88-2, **9** = Lr = 65-1, **9-1** = Lr 65-2, **9-2** = Lr 65-3, **9-3** = Lr 65-4, **11** = Lp 260-2, **12** = Lr 79-4, **14** = Icj =59-1, **16** = C 67-2, and **18** = Si 69-1.

Figure 10: Fermentation of carbohydrates by *Salmonella* isolates.

4.4.0. Antibiotic resistance patterns

4.4.1. *Escherichia coli* resistance patterns

Kafue lechwe: The disc diffusion method was used to test the sensitivity of 93 *E. coli* from Kafue lechwe and 56 *E. coli* isolates from pastoral cattle to 8 types of antibiotics. The antibiotics tested were amoxicillin, cephalexin, co-trimoxazole, gentamicin, metronidazole, oxacillin, penicillin G, and tetracycline. The zones showing complete inhibition were recorded as susceptible, while those not showing inhibitions were recorded as defined by the manufacturers (Himedia) were noted as resistant (Figure 11). The antimicrobial susceptibility and resistance profiles of *E. coli* isolate from Kafue lechwe and pastoral cattle are presented in Tables 16 and 19).

The Kafue lechwe *E. coli* isolates were resistant to metronidazole (98.8%) followed by penicillin G (97.6%), amoxicillin (29%) and co-trimoxazole (15.1%). For cephalexin and oxacillin, the values were below 7%. None of the isolates were resistant to gentamycin and tetracycline. The distribution pattern of antibiotic resistance is presented in Table 16. Ninety-two (98.9%) *E. coli* isolates were resistant to at least one or more antimicrobial agents. Only amoxicillin ($P = 0.024$) and cephalexin ($P = 0.007$) showed significant differences in resistance patterns between Lochinvar and Blue-lagoon NPs. Majority of the isolates resistant to cephalexin (75%) and amoxicillin (70%) were from Lochinvar

NP. Generally, the isolates from Lochinvar NP were more resistant to antibiotics than those from Blue-lagoon NP except to co-trimoxazole, gentamycin and tetracycline.

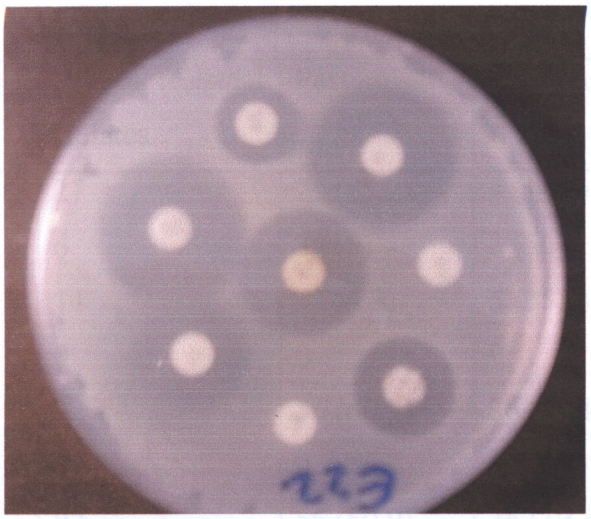

Figure 11: Antimicrobial test showing drug susceptibility and resistance of the isolates.

Table 16: Distribution of antibiotic resistance in *E. coli* isolates from Kafue lechwe (n = 93) by location of sampling

Antibiotic	No. of resistant strains (%)	Lochinvar (%)	Blue-lagoon (%)	P – value*
Amoxycillin	27 (29)	19 (70.4)	8 (29.6)	0.024
Cephalexin	6 (6.5)	4 (75)	2 (25)	0.007
Co-trimoxazole	14 (15.1)	7 (50)	7 (50)	0.122
Gentamycin	0 (0)	0	0	-
Metronidazole	92 (98.9)	63 (68.5)	29 (31.5)	1.000
Oxacillin	3 (3.2)	2 (66.7)	1 (33.3)	1.000
Penicillin G	92 (98.9)	63 (68.5)	29 (31.5)	1.000
Tetracycline	0 (0)	0	0	0.529

*P= Value test of association between antibiotic resistance and area of *E. coli* isolation

There was a significant difference (P = 0.001) in resistance pattern to amoxycillin between *E. coli* isolates from the pasture and the rectum of the Kafue lechwe. Generally

isolates from the pasture were more resistant to antibiotics than those from the rectum of Kafue lechwe (Table 17).

Table 17: Association between antibiotic resistance in *E. coli* isolates from Kafue lechwe ($n = 93$)

Type of antibiotic	No. of resistant isolates	Pasture	Rectum	P–value*
Amoxycillin	27	23 (85.2)	4 (14.8)	0.001
Cephalexin	6	3 (50)	3 (50)	0.065
Co-trimoxazole	14	9 (64.3)	5 (35.7)	1.000
Gentamycin	0	0	0	-
Metronidazole	92	56 (60.9)	36 (39.1)	1.000
Oxacillin	3	0	0	0.557
Penicillin G	92	56 (60.9)	36 (39.1)	1.000
Tetracycline	0	0	0	1.000

*P = Value test of association between antibiotic resistance and source of *E. coli* isolates.

There was a significant difference (P = 0.009) in resistance pattern to cephalexin among the *E. coli* serogroups. All the non-typeable isolates were resistant to cephalexin. Overall, non- typeable isolates were resistant to most antibiotics than the serotyped *E. coli* isolates (Table 18).

In the pastoral cattle, 56 *E. coli* isolates were tested for antimicrobial resistance patterns, 42 (75%) were from Lochinvar NP, whereas 14 (25%) were from Blue-lagoon NP. All the *E. coli* isolates were resistant to metronidazole. Of the 56 *E. coli* isolates resistant to metronidazole, 42 (75%) were from Lochinvar NP (Table 19). Other antimicrobials tested (co-trimoxazole, oxacillin, tetracycline, amoxicillin, cephalexin and penicillin G) showed resistance values below 7.1%. None of the isolates were resistant to amoxycillin, cephalexin, gentamycin, and penicillin G (Table 19).

Table 18: Association between antibiotic resistance in *E. coli* isolates from Kafue lechwe and serovars ($n = 93$)

Antibiotic	No. of isolates examined	No. of resistant strains per serotype								P-value*
		015	027	078	8/0125	029/ 0143	055/ 0125	0125	Non-typeable isolates	
AML	27	0	0	1	0	0	0	0	26	0.737
CP	6	0	0	0	0	0	0	0	6	0.009
CO	14	0	0	0	0	0	0	0	14	1.000
G	0	0	0	0	0	0	0	0	0	-
MT	92	2	4	1	1	1	2	3	78	1.000
OX	3	0	0	0	0	0	0	0	3	1.000
PG	92	2	4	1	1	1	2	3	78	1.000
TE	0	0	0	0	0	0	0	0	0	-

*P= Value test of association between type of antibiotic resistance and *E. coli* serotype

There was no significant difference between intermediate resistance and source of the specimen (P = 0.149), as more of the intermediate resistant isolates were from Blue-lagoon NP (P = 0.149). All resistant E. coli isolates to co-trimoxazole and tetracycline antibiotics were from Lochinvar NP.

Escherichia coli isolates marked A to L were typeable, while isolates marked M were non-typeable (Table 20). None of the isolates were resistant to amoxicillin, cephalexin or gentamycin. Furthermore, none of the typeable isolates were resistant to oxacillin and tetracycline. A high resistance rate was observed among the non-typeable *E. coli* isolates in particular against metronidazole (100%) and penicillin G (97.2%).

Table 19: Distribution of antibiotic resistance pattern in *E. coli* isolates from pastoral cattle by location of sampling ($n = 56$)

Antibiotic	No. of resistant isolates (%)	No. of resistant isolates per location		
		Lochinvar (%)	Blue-lagoon (%)	P-value*
Amoxycillin	0	0	0	-
Cephalexin	0	0	0	0.149**
Co-trimoxazole	4 (7.1)	4 (100)	0	0.562
Gentamycin	0	0	0	0.565
Metronidazole	56 (100)	42 (75)	14 (25)	-
Oxacillin	1 (1.8)	1	0	0.672
Penicillin G	0	0	0	1.000
Tetracycline	1 (1.8)	1 (100)	0	0.672

*P= Value test of association between antibiotic resistance and area of *E. coli* isolation.

** = There were 13 intermediate resistant isolates not shown in the table.

Table 20: Distribution of antibiotic resistance pattern in *E. coli* isolates from pastoral cattle by serovars ($n = 93$)

Serotype	No. of resistant strains examined	No. of resistant strains per antibiotic							
		AML	CP	CO	G	MT	OX	PG	TE
A	1	0	0	0	0	1	0	1	0
B	2	0	0	0	0	2	0	2	0
C	1	0	0	0	0	1	0	1	0
D	1	0	0	0	0	1	0	1	0
E	5	0	0	0	0	5	0	5	0
F	1	0	0	0	0	1	0	1	0
G	2	0	0	0	0	2	0	2	0
H	1	0	0	0	0	1	0	1	0
I	2	0	0	0	0	2	0	2	0
J	2	0	0	0	0	2	0	2	0
K	1	0	0	1	0	1	0	1	0
L	1	0	0	0	0	1	0	1	0
M	36	0	0	3	0	36	1	35	1
*P-value		-	0.491	0.587	0.629	-	0.037	1.00	1.00

*P = value test of association between antibiotic resistance and *E. coli* serotype.

Key: Serogroups: A = 08, B = 027, C = 029, D = 055, E = 0125, F = 027/0115, G = 055/0125/0169, H = 027/055/0125, I = 08/027/055/0125, J = 08/027/055/0125/0169, K = 0125/0169, L = 08/027/055, and M = None-typeable

4.4.2. *Salmonella* resistance patterns

Out of 59 *Salmonella* isolates from Kafue lechwe, 37 isolates were randomly selected for antibiotic resistance. Of these 18 (48.6%) were from Blue-lagoon NP and 19 (51.4%) were from Lochinvar NP. Most of the *Salmonella* isolates were from the pasture (56.8%), followed by the rectum (18.9%), small intestine (10.8%), and the ileo-caecal junction (8.1%) (Table 21). Out of 30 *Salmonella* isolates from pastoral cattle, random samples of 11 isolates were selected for antibiotic resistance. Of 11 isolates, 8 (72.7%) were from Lochinvar NP and 3 (27.3%) were from Blue-lagoon NP. Out of 11, 8 (72.7%) were positive to *Salmonella* polyvalent, while 3 (27.3%) isolates were non-typeable to *Salmonella* polyvalent antisera.

Table 21: Distribution of *Salmonella* isolates from Kafue lechwe tested for antimicrobial resistance pattern by source and location ($n = 37$)

	Variable	No. of *Salmonella* strains	Frequency (%)
Source	Pasture	21	56.8
	Rectum	7	18.9
	Ileo-caecal junction	3	8.1
	Caecum	2	5.4
	Small intestine	4	10.8
Location	Lochinvar	19	51.4
	Blue lagoon	18	48.6

The antibiotics to which *Salmonella* isolates from Kafue lechwe were most resistant to were metronidazole (100%), penicillin G (89.2 %), co-trimoxazole (67.7%), cephalexin (59.5%) and gentamycin (18.9%). None of the isolates were resistant to oxacillin and tetracycline. The distribution pattern of antibiotic resistance is presented in Table 22.

Antibiotic resistance pattern between isolates from Lochinvar and Blue-lagoon NPs was not the same and hence there were some variations. Two (100%) of the resistant *Salmonella* strains to amoxicillin ($P = 0.012$), 15 (68.2%) strains resistant to cephalexin

(P = 0.006) and 18 (72%) strains resistant to co-trimoxazole (P = 0.000) were from Blue-lagoon NP (Table 22).

All isolates from Lochinvar and Blue-lagoon NPs were resistant to metronidazole, in contrast none were resistant to oxacillin. There was significant difference (P = 0.046*) between intermediate resistant isolates and location, with more intermediate resistant isolates to tetracycline coming from Lochinvar NP than those from Blue-lagoon NP.

Table 22: Antibiotic resistance pattern in *Salmonella* isolates from Kafue lechwe by location of sampling (n = 37)

Type of antibiotic	Total No. of resistant isolates (%)	No. of resistant isolates (%)		P – value**
		Lochinvar	Blue-lagoon	
Amoxycillin	2 (5.4)	0	2 (100)	0.012
Cephalexin	22 (59.5)	7 (31.8)	15 (68.2)	0.006
Co-trimoxazole	25 (67.7)	7 (28)	18 (72)	0.000
Gentamycin	7 (18.9)	7 (100)	0	0.001
Metronidazole	37 (100)	19 (51.4)	18 (94.7)	-
Oxacillin	0	0	0	-
Penicillin G	33 (89.2)	17 (51.5)	16 (48.5)	1.000
Tetracycline	0	0	0	0.046*

* = There were 5 intermediate resistant isolates from Lochinvar National Park not shown in the table

**P = Test of association between antibiotic and source of specimen

All the *Salmonella* isolates were resistant to metronidazole. Majority of the isolates from the pasture were resistant to co-trimoxazole (95.2%), penicillin G (85.7%) and cephalexin (71.4%). All the isolates from rectum, caecum and small intestine were resistant to penicillin G. There was a significant difference (P < 0.001) between the antibiotic resistance pattern of *Salmonella* isolates and sources of the specimen. Isolates from

pasture and small intestine were more resistant to co-trimoxazole than those from other sources. Similarly, isolates from the small intestine, caecum and ileo-caecal junction were more resistant to gentamycin ($P < 0.001$) than those from other sources (Table 23).

Table 23: Distribution of antibiotic resistance pattern in *Salmonella* isolates by source of the specimen ($n = 37$)

Type of antibiotic	No. of resistant *Salmonella* isolates					P-value*
	Pasture (21)	Rectum (7)	Ileo-caecal junction (3)	Caecum (2)	Small intestine (4)	
Amoxycillin	2	0	0	0	0	0.844
Cephalexin	15	3	1	1	2	0.128
Co-trimoxazole	20	3	0	0	2	0.001
Gentamycin	0	0	2	1	4	0.001
Metronidazole	21	7	3	2	4	-
Oxacillin	0	0	0	0	0	-
Penicillin G	18	7	2	2	4	0.465
Tetracycline	0	0	0	0	0	0.505

*P = Test of association between antibiotic and source of specimen.

Of the 37 *Salmonella* isolates tested for antibiotic resistance, 11 were serologically typeable and 26 were non-typeable (Table 24). All the isolates were resistant to metronidazole, followed by penicillin G (89.2%), co-trimoxazole (67.6%), cephalexin (59.5%) and gentamycin (18.9%). The two isolates that were resistant to amoxycillin were non-typeable ($P = 0.004$). None of the isolates were resistant to oxacillin and tetracycline. There was a significant difference between resistant pattern and serogroups. Resistance values were high in non-typeable *Salmonella* isolates to cephalexin ($P =. 0.001$) and co-trimoxazole ($P = 0.001$).

Table 24: Distribution of antibiotic resistance pattern in *Salmonella* isolates from Kafue lechwe based by *Salmonella* polyvalent O serotype ($n = 37$)

		No. of resistant *Salmonella* strains		
Antibiotic	Total No. of resistant isolates	Non-typeable *Salmonella* isolates ($n = 26$)	Serologically positive *Salmonella* isolates ($n = 11$)	P-value*
Amoxycillin	2	2	0	0.004
Cephalexin	22	20	2	0.001
Co-trimoxazole	25	24	1	0.001
Gentamycin	7	2	5	0.012
Metronidazole	37	26	11	-
Oxacillin	0	0	0	-
Penicillin G	33	22	11	0.296
Tetracycline	0	0	0	0.623

*P = Value test of association between resistance to a particular antibiotic and *Salmonella* serotype

Of the 37 *Salmonella* isolates tested for antimicrobial resistance, three were classified as belonging to serogroup 8 (Table 25). None of the isolates belonging to serogroup 8 were resistant to amoxicillin, cephalexin, gentamycin, oxacillin or tetracycline, although they were resistant to metronidazole and penicillin G. The isolates tested showed no significant difference between the pattern of antibiotic resistance and *Salmonella* serogroups.

The antibiotics to which *Salmonella* isolates from pastoral cattle were most resistant to were metronidazole (90.9%), penicillin G (72.7 %) and gentamycin (54.5%). None of the isolates were resistant to amoxicillin, co-trimoxazole, oxacillin and tetracycline (Table 26).

Table 25: Distribution of antibiotic resistance pattern in *Salmonella* isolates from Kafue lechwe by serogroup ($n = 37$)

Antibiotic	Total No. of resistant isolates	No. of resistant *Salmonella* serogroup 8 ($n = 3$)	No. of other *Salmonella* ($n = 34$)	P- value*
Amoxycillin	2	0	2	0.609
Cephalexin	22	0	22	0.087
Co-trimoxazole	25	1	24	0.241
Gentamycin	7	0	7	1.000
Metronidazole	37	3	34	-
Oxacillin	0	0	0	-
Penicillin G	33	3	30	1.000
Tetracycline	0	0	0	-

*P = Value test of association between resistance to a particular antibiotic and *Salmonella* serogroup.

There was significance difference (P = 0.006) in antimicrobial resistance patterns between Lochinvar and Blue-lagoon NPs. All the isolates that were resistant to gentamycin were from Lochinvar NP. Similarly, 70% (7/10) of the *Salmonella* isolates resistant to metronidazole and 87.5% (7/8) of the isolates resistant to penicillin G were also from Lochinvar NP.

Table 26: Distribution of antibiotic resistance pattern in *Salmonella* strains from pastoral cattle by location of sampling ($n = 11$)

Type of antibiotic	Total No. of resistant isolates	Frequency (%)	No. of resistant isolates		P- value*
			Lochinvar	Blue-lagoon	
Amoxycillin	0	0	0	0	-
Cephalexin	1	9.1	1	0	0.055
Co-trimoxazole	0	0	0	0	-
Gentamycin	6	54.5	6	0	0.006
Metronidazole	10	90.9	7	3	1.000
Oxacillin	0	0	0	0	-
Penicillin G	8	72.7	7	1	0.055
Tetracycline	0	0	0	0	-

*P = Value test of association between antibiotic resistance and area of *Salmonella* isolation.

4.4.3. Comparison of antibiotic resistance patterns of similar serogroup of *Escherichia coli* and *Salmonella*

4.4.3.1. *Escherichia coli* serogroups

The isolates numbers 1 (Lp 13-2) and 3 (Lr 28-4) from Kafue lechwe and 4 (Sc 1-2) from pastoral cattle belonging to serotype O27 showed an identical pattern of resistance. Similarly, antimicrobial resistant pattern of isolates number 2 (Lp 208-4) and 5 (Lmc 167-2) from Kafue lechwe and pastoral cattle respectively, were identical suggesting they were from a common source, while isolates belonging to serotype O78 could be different strains (Table 27). Furthermore, a unique identical antimicrobial resistance pattern of isolates from Kafue lechwe and pastoral cattle belonging to serotype O125 was observed again suggesting the strains may have the same source.

Table 27: Comparison of antibiotic resistance pattern of *E. coli* serotype from Kafue lechwe and pastoral cattle (n = 12)

Antibiotic	*E. coli* serotype tested											
	O27					O78		O125				
	Lechwe			Cattle		Lechwe	Cattle	Lechwe		Cattle		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Amoxycillin	S	S	S	S	S	R	S	S	S	S	S	S
Cephalexin	I	S	I	I	S	I	I	I	I	I	I	I
Co-trimoxazole	S	S	S	S	S	I	S	S	S	S	S	S
Gentamycin	S	S	S	S	S	S	S	S	S	S	S	S
Metronidazole	R	R	R	R	R	R	R	R	R	R	R	R
Oxacillin	S	S	S	S	S	S	S	S	S	S	S	S
Penicillin G	R	R	R	R	R	R	R	R	R	R	R	R
Tetracycline	S	S	S	S	S	I	S	S	S	S	S	S

Key (S = susceptible, R = resistant, I = intermediate)

1 = Lp 13-2, **2** = Lp 208-4, **3** = Lr 28-4, **4** = Sc 1-2, **5** = Lmc 167-2, **6** = Lp 9-4, **7** = Lmc 248-2, **8** = Lp 24-2, **9** = Lp 50-1, **10** = Lmc 26-2, **11** = Lmc 38-1 and **12** = Lmc 59-1.

4.4.3.2. *Salmonella* serovars

Salmonella serovars number 1, 3, 5 and 7 were from pastoral cattle while 9, 9-1, 9-2, 9-3, 11, 12, 14, 16 and 18 were from Kafue lechwe. The study showed that serovars number 5 (Lmc 231-1) and 18 (Lp Si 69-1) had identical pattern of antimicrobial resistance, suggesting they were related. The rest of *Salmonella* serovars from Kafue lechwe and pastoral cattle appeared to be different strains (Table 28).

Table 28: Comparison of antibiotic resistance pattern of *Salmonella* serovars from Kafue lechwe and pastoral cattle (n = 13)

Antibiotic	*Salmonella* serovars tested												
	Pastoral cattle				Kafue lechwe								
	1	**3**	**5**	**7***	**9***	**9-1***	**9-2***	**9-3***	**11***	**12**	**14**	**16**	**18**
Amoxycillin	S	S	S	S	S	S	S	S	S	S	S	S	S
Cephalexin	I	S	I	I	S	I	I	I	S	I	R	R	I
Co-trimoxazole	S	S	S	S	I	I	I	I	R	S	S	S	S
Gentamycin	I	I	R	S	S	S	S	S	S	I	R	R	R
Metronidazole	R	R	R	R	R	R	R	R	R	R	R	R	R
Oxacillin	S	S	S	S	S	S	S	S	S	S	S	S	S
Penicillin G	R	R	R	R	R	R	R	R	R	R	R	R	R
Tetracycline	S	S	S	S	S	I	S	S	I	I	S	S	S

Key (* = isolates belonging to serogroup 8, R = Resistance, S = Susceptible, I = Intermediate)

Isolate numbers: **1** = Lmc 226-1, **3** = Lmc 227-1, **5** = Lmc 231-1, **7** = Sc 88-2, **9** = Lr = 65-1, **9-1** = Lr 65-2, **9-2** = Lr 65-3, **9-3** = Lr 65-4, **11** = Lp 260-2, **12** = Lr 79-4, **14** = Icj =59-1, **16** = C 67-2, and **18** = Si 69-1.

4.5.0. Statistical analysis of viable *Escherichia coli* counts from the Kafue lechwe faecal samples

The data base was established in Excel Spread sheets and for statistical analysis it was transferred to STATA SE/11 for windows statistical package (Stata Corp. College Station, Texas. USA). A p-value of <0.005 was considered indicative of a statistically significant difference. All *E. coli* counts were converted to the base -10 logarithm of the

number of colony forming units per gram of faecal samples (log cfu/g), and from these means and standard deviations were calculated. Data were analyzed using a **Two-sample t test** with equal variances. All counts were expressed in logarithm per gram (log/gm).

4.5.1. *Escherichia coli* count of faecal samples from the rectum of Kafue lechwe

Seventy-seven faecal samples from the rectum of Kafue lechwe were examined for total viable *E. coli* count of which 52 samples were from Lochinvar NP and 25 were from Blue-lagoon NP. Figures 12 and 13 show *E. coli* median log colony forming unit (cfu) from two locations. Only marginal differences were observed with respect to mean cfu of *E. coli* per gram faecal sample between the two locations (P = 0.099), with Blue-lagoon NP recording a relatively higher count [5.8 log cfu/g, (95% CI: 4.7 - 6.9)] compared to Lochinvar NP [4.9 log cfu/g (95% CI: 4.2 – 5.5)]. The comparisons of the median counts from the two areas are displayed in the box plot showing the medium values and the corresponding inter-quartile ranges.

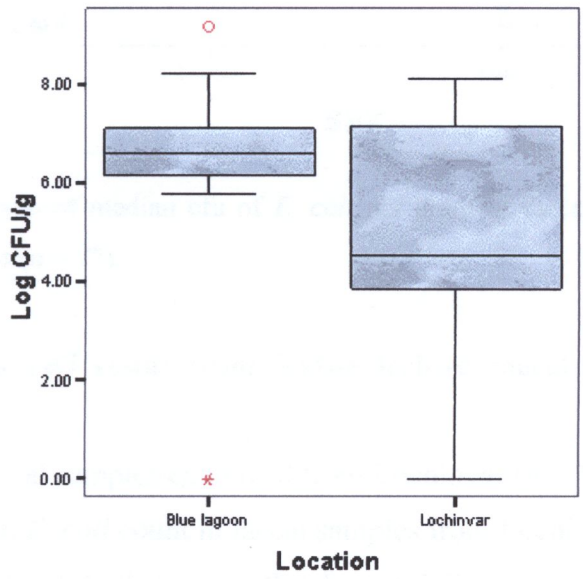

Figure 12: Comparison of median cfu of *E. coli* per gram of faecal samples in Kafue lechwe from Blue-lagoon and Lochinvar NPs (n = 77).

There was no statistically significant difference in *E. coli* cfu/g between faecal samples from female and male animals (P = 0.206), although in relative terms faecal samples from

females were observed to contain more *E. coli* cfu/g than males. A mean count of [5.7 log cfu/g (95% CI: 5.0 – 7.4)] and [4.9 log cfu/g (95% CI: 3.2 – 5.8)] were observed in females and males respectively. Figure 13 shows the graphical presentation of medium log cfu/g counts in the two sex category.

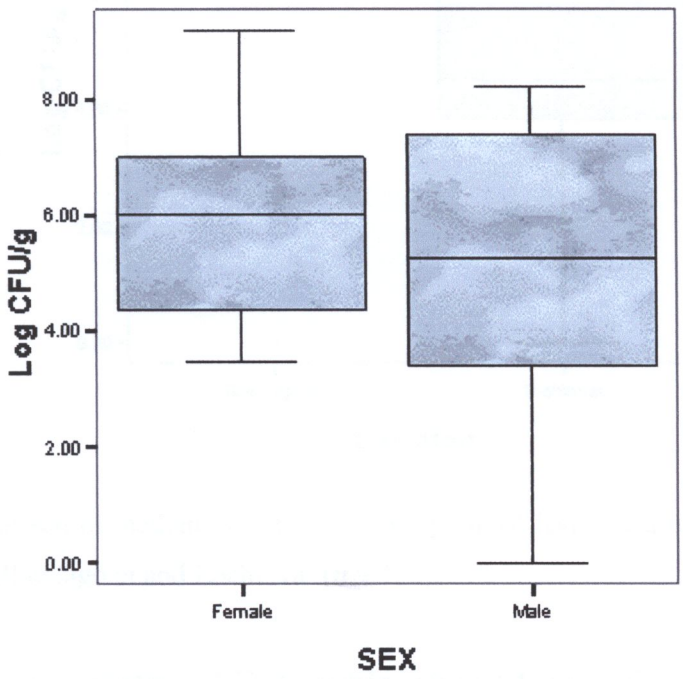

Figure 13: Comparison of median cfu of *E. coli* per gram of faecal sample between male and female Kafue lechwe ($n = 77$).

4.5.2. *Escherichia coli* count from Kafue lechwe faecal samples collected from pasture

There were 155 faecal samples examined from Lochinvar ($n = 110$) and Blue-lagoon ($n = 45$) NPs. The mean *E. coli* count in faecal samples from Lochinvar NP was 6.6 log cfu/g, (95% CI: 6.1 – 7.0) while that from Blue-lagoon NP was 6.2 log cfu/g (95% CI: 5.4 – 7.1). There was no significant difference between mean counts of *E. coli* from the two areas (P = 0.448) although the median value was slightly lower in samples from Blue-lagoon (Figure 14).

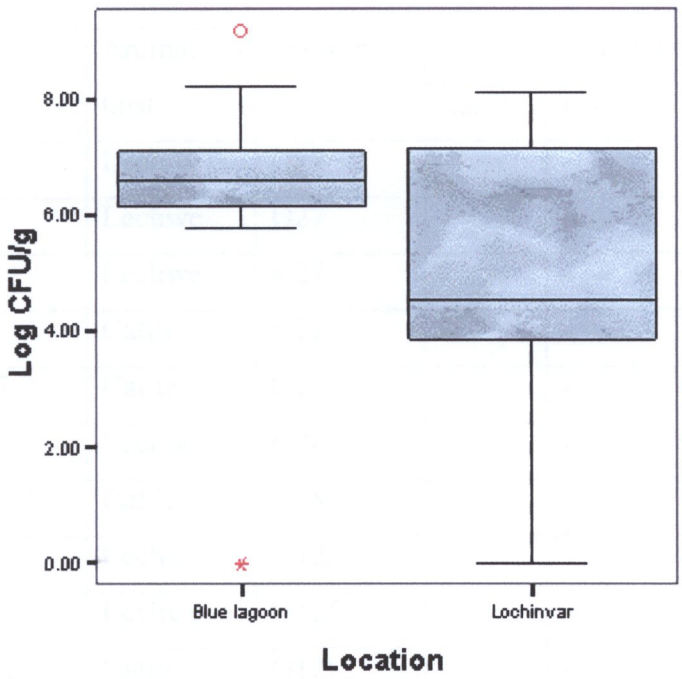

Figure 14: Comparison of median cfu of *E. coli* per gram of Kafue lechwe faecal samples from pasture between Blue-lagoon and Lochinvar (n = 155).

4.6.0. Plasmid fingerprinting of *Escherichia coli* and *Salmonella* isolates

The genomic fingerprints of 12 *E. coli* and 10 *Salmonella* isolates with similar serogroups from Kafue lechwe and pastoral cattle were analysed. Molecular characterization using PFGE showed some digestion of DNA with restriction enzyme *Pst1, Xbal, EcoR1* and *HindIII-(*Table 29 and 30). On agarose gel electrophoresis, all the isolates were observed to have multiple identical migration profiles.

4.6.1. *Escherchia coli* serogroups

Twelve isolates belonging to serogroups O27, O78 and O125 from Kafue lechwe and pastoral cattle were analyzed (Table 29). The isolates had 4 distinct bands. Two bands were greater than 1kb in size, while the other two bands were 950 bp and 150 bp respectively (figure 15). Lechwe and cattle isolates belonging to serotype O78 exhibited a similar pattern as indicated in figure 15.

Table 29: Comparison of DNA profile for *E. coli* serogroups from Kafue lechwe and pastoral cattle ($n = 12$)

Serial No.	Isolate No.	Animal host	Serotype	Restriction enzymes			
				EcoRI	*Hind III*	*PstI*	*XBal*
1	LP 13-2	Lechwe	O27	-	-	+	-
2	LP 208-4	Lechwe	O27	-	-	-	-
3	LR 28-4	Lechwe	O27	-	-	-	-
4	SC 1-2	Cattle	O27	-	-	+	-
5	LMC 167-2	Cattle	O27	-	-	+	+
6	LP 9-4	Lechwe	O78	-	-	-	-
7	LMC 248-4	Cattle	O78	-	-	-	-
8	LP 24-2	Lechwe	O125	+	+	-	-
9	LP 50-1	Lechwe	O125	+	-	-	-
10	LMC 26-2	Cattle	O125	-	-	-	-
11	LMC 38-1	Cattle	O125	-	-	+	-
12	LMC 59-1	Cattle	O125	+	-	+	+

Key: + = DNA digested reaction, - = none DNA digested reaction

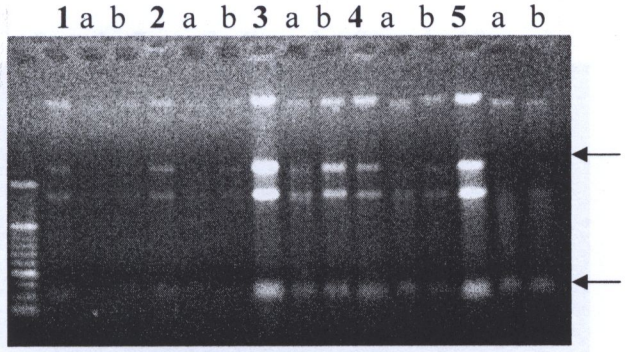

Figure 15: Isolates indicating a similar pattern with lane "a" showing *PstI* enzyme treatment while lane "b" shows *Xbal* treatment. The numerical numbers indicate untretated reactions.

Isolate number 12 (Lmc 59-1) belonging to O125 serotype from pastoral cattle had 6 bands, where 4 bands were completely digested by *PstI* enzyme and 3 bands were acted

upon by *Xbal* enzyme (figure 16) while isolate number 11 had the usual bands observed in figure 16. The *PstI* enzyme digested DNA from isolate 11 as observed in figure 16.

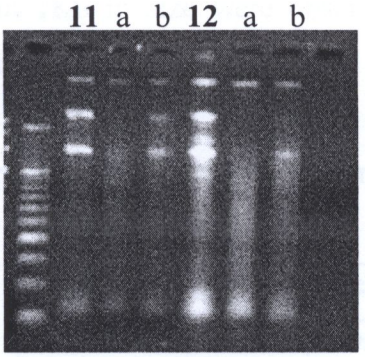

Figure 16: Enzyme reactions to isolates 11 and 12. Lane a indicates *PstI* enzyme treatment while lane b shows *Xbal* treatment.

As for *EcoRI* only isolate 8 and 9 were digested on two bands and *HindIII* acted on one band in isolate 9 (figure 17). From the restriction enzyme analysis results, it is clear, that the isolates cannot be assumed to be the same strains on this basis despite having similar bands for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11. The isolate number 12 was clearly different from the other strains.

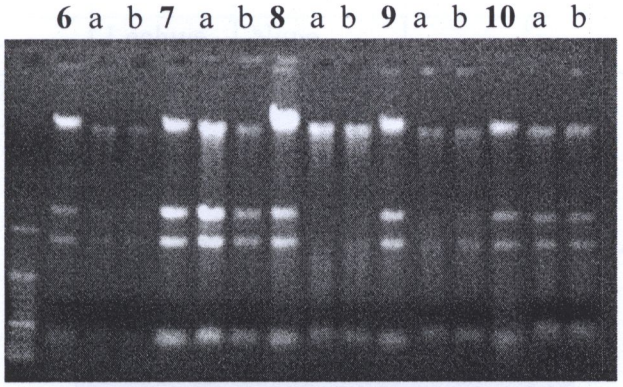

Figure 17: *EcoRI* (a) and *HindIII* (b) restriction enzyme treatment on selected isolates with isolates 8 and 9 showing some digestion.

4.6.2. *Salmonella* serogroups

Ten isolates from Kafue lechwe and pastoral cattle were analysed, out of which three belonged to *Salmonella* serogroup 8 (Table 30). In the case of *Salmonella* serovars, two distinct clear bands greater than 1Kb in size were observed. The heaviest band was digested by *EcoRI* and *HindIII* in all the isolates (figure 18).

Table 30: Comparison of DNA profile for *Salmonella* serogroups from Kafue lechwe and pastoral cattle ($n = 10$)

Serial No.	Isolate No.	Animal host	Serogroup	Restriction enzymes			
				EcoRI	*Hind III*	*PstI*	*XBal*
1	LMC 226-1	Cattle	None	-	-	-	-
3	LMC 227-1	Cattle	None	-	-	-	-
5	LMC 231-1	Cattle	None	-	-	-	-
7	SC 88-2	Cattle	O8	-	-	+	-
9	LR 65-1	Lechwe	O8	-	-	-	+
11	LP 260-2	Lechwe	O8	-	-	-	-
12	LR 79-1	Lechwe	None	-	-	-	-
14	ICJ 59-1	Lechwe	None	-	-	-	-
16	C67-2	Lechwe	None	-	-	-	
18	SI 69-1	Lechwe	None	-	-	-	-

Key: + = DNA digested reaction, - = none DNA digested reaction

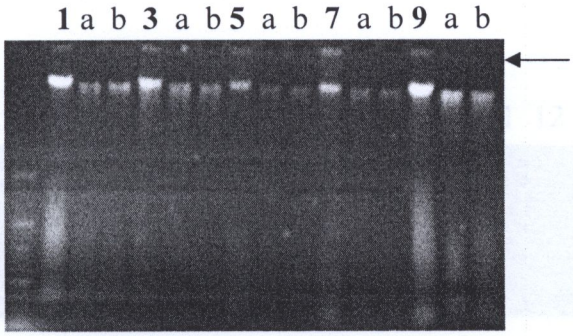

Figure 18: Selected *Salmonella* isolates 1, 3, 5, 7 and 9 showing the digestion of the heavy band (shown by arrow) by *EcoRI* "a" and *HindIII* "b" restriction enzymes. Numerical numbers 1, 3, 5, 7 and 9 indicate samples not subjected to enzyme treatment.

4.6.2. *Salmonella* serogroups

Ten isolates from Kafue lechwe and pastoral cattle were analysed, out of which three belonged to *Salmonella* serogroup 8 (Table 30). In the case of *Salmonella* serovars, two distinct clear bands greater than 1Kb in size were observed. The heaviest band was digested by *EcoRI* and *HindIII* in all the isolates (figure 18).

Table 30: Comparison of DNA profile for *Salmonella* serogroups from Kafue lechwe and pastoral cattle ($n = 10$)

Serial No.	Isolate No.	Animal host	Serogroup	Restriction enzymes			
				EcoRI	*Hind III*	*PstI*	*XBal*
1	LMC 226-1	Cattle	None	-	-	-	-
3	LMC 227-1	Cattle	None	-	-	-	-
5	LMC 231-1	Cattle	None	-	-	-	-
7	SC 88-2	Cattle	O8	-	-	+	-
9	LR 65-1	Lechwe	O8	-	-	-	+
11	LP 260-2	Lechwe	O8	-	-	-	-
12	LR 79-1	Lechwe	None	-	-	-	-
14	ICJ 59-1	Lechwe	None	-	-	-	-
16	C67-2	Lechwe	None	-	-	-	
18	SI 69-1	Lechwe	None	-	-	-	-

Key: + = DNA digested reaction, - = none DNA digested reaction

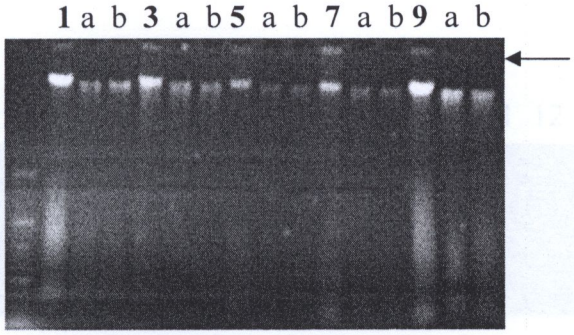

Figure 18: Selected *Salmonella* isolates 1, 3, 5, 7 and 9 showing the digestion of the heavy band (shown by arrow) by *EcoRI* "a" and *HindIII* "b" restriction enzymes. Numerical numbers 1, 3, 5, 7 and 9 indicate samples not subjected to enzyme treatment.

For *PstI* and *Xbal*, only isolates 7 and 9 were digested respectively (data not shown). Our PFGE results, however, indicate that these *Salmonella* serovars may have a single source within the population of study animals.

4.7.0. Detection of virulence genes in *Escherichia coli* and *Salmonella* isolates from Kafue lechwe and pastoral cattle

Escherichia coli and *Salmonella* isolates from Kafue lechwe and pastoral cattle in the wildlife/cattle Interface Areas of Kafue Flats were examined to detect virulence genes capable of causing diseases in humans.

4.7.1. *Escherichia coli* isolates

A total of 55 *E. coli* serogroups from 12 Kafue lechwe and 14 pastoral cattle faecal samples were randomly selected and tested for the presence of the virulence genes (*stx-1, stx-2, ipaB, ipaH, invE*, ST1, LT and EAST1). Of the 55 isolates, 33 (60%) were found to harbour one of the virulence genes. Further, out of 33 isolates, 15 (45.5%) were from Kafue lechwe while 18 (54.5%) isolates were from pastoral cattle. Of the 33 isolates, 28 (84.8%) were positive to Enteroaggregative *Escherichia coli* (EAEC) heat-stable toxin 1 (EAST1) gene as shown in figure 19, with *E. coli* isolates from pastoral cattle being the most predominant in this group (18/28). This was followed by 4 (12.1%) of *ipaB* gene from Kafue lechwe isolates, while the only one (3.0%) positive isolate to *stx*-2 gene was from the pastoral cattle. The information on the genes detected is summarized in Table 31.

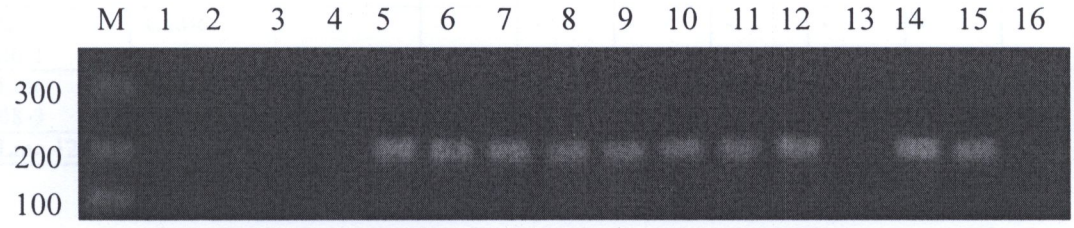

Figure 19: Agarose gel showing results of PCR products of DNA from *E. coli* serovars carrying a gene coding for EASTI (Lane 5-12 and 14-15), Lane M, 50 bp DNA marker and Lane 1-4, 13 and 16 serovars negative for EAST1 gene.

Table 31: Summary of PCR reaction products on the diarrheal *E. coli* pathogens ($n = 55$)

No.	Isolate No.	Host	Serotype	*E. coli* diarrheal pathogens							
				EHEC		EIEC			ETEC		EAEC
				Stx-1	*Stx-2*	*ipaB*	*ipaH*	*invE*	ST1	LT	EAST1
1	Lp 50-3	Lechwe	O125	-	-	+	-	-	-	-	-
2	Lp 50-4	Lechwe	O125	-	-	+	-	-	-	-	-
3	Lp212-3	Lechwe	-	-	-	+	-	-	-	-	-
4	Lp 265-4	Lechwe	-	-	-	+	-	-	-	-	-
5	Lp 50-1	Lechwe	O125	-	-	-	-	-	-	-	+
6	Lr 58-3	Lechwe	-	-	-	-	-	-	-	-	+
7	Lp24-2	Lechwe	O125	-	-	-	-	-	-	-	+
8	Lp 288-2	Lechwe	O55/O125	-	-	-	-	-	-	-	+
9	Lp 17-1	Lechwe	-	-	-	-	-	-	-	-	+
10	Lr 40-3	Lechwe	-	-	-	-	-	-	-	-	+
11	Lr 40-4	Lechwe	-	-	-	-	-	-	-	-	+
12	Lp 207-1	Lechwe	-	-	-	-	-	-	-	-	+
13	Lr 67-3	Lechwe	-	-	-	-	-	-	-	-	+
14	Lr 64-4	Lechwe	-	-	-	-	-	-	-	-	+
15	Lr 64-2	Lechwe	-	-	-	-	-	-	-	-	+
16	Lmc185	Cattle	-	-	+	-	-	-	-	-	-
17	Lmc124-2	Cattle	O8/O27/O 55/O125	-	-	-	-	-	-	-	+
18	Lmc 38-1	Cattle	O125	-	-	-	-	-	-	-	+
19	Lmc 167-2	Cattle	O125	-	-	-	-	-	-	-	+
20	Lmc 187-4	Cattle	-	-	-	-	-	-	-	-	+
21	Sc 1-2	Cattle	O27	-	-	-	-	-	-	-	+
22	Lmc 248-4	Cattle	O78	-	-	-	-	-	-	-	+
23	Lmc 59- 1	Cattle	O125	-	-	-	-	-	-	-	+
24	Lmc 59-2	Cattle	O27/O55/ O125	-	-	-	-	-	-	-	+
25	Sc 60-1	Cattle	O29	-	-	-	-	-	-	-	+
26	Sc 60-4	Cattle	O55	-	-	-	-	-	-	-	+
27	Lmc 26-2	Cattle	O125	-	-	-	-	-	-	-	+
28	Lmc 38-4	Cattle	O125	-	-	-	-	-	-	-	+
29	Lmc 228-2	Cattle	O8/027/05 5	-	-	-	-	-	-	-	+
30	Lmc 187-2	Cattle	O125/016 9	-	-	-	-	-	-	-	+
31	Lmc 26-1	Cattle	-	-	-	-	-	-	-	-	+
32	Lmc 48-3	Cattle	-	-	-	-	-	-	-	-	+
33	Lmc 124-3	Cattle	-	-	-	-	-	-	-	-	+

4.7.2. *Salmonella* isolates

Fifty-three *Salmonella* serovars from 22 Kafue lechwe and 6 pastoral cattle faecal samples were selected and tested for the presence of virulence *invA* gene. Of these, 43

(81.1%) isolates were from Kafue lechwe and 10 (18.9%) were from pastoral cattle. Furthermore, 39 (73.6%) of these isolates were positive to *invA* gene as shown in figure 20 where a 284 bp amplicon was detected. Of the 39 isolates, 19 (48.7%) were serologically positive to *Salmonella* polyvalent O antisera, while the rest were non-typeable (Table 32). All the *Salmonella* isolates belonging to serogroup 8 were positive to *invA* gene. Thirty-one of 43 (72.1%) isolates from Kafue lechwe and 8 (80.0%) of 10 isolates from pastoral cattle were positive to *invA* gene.

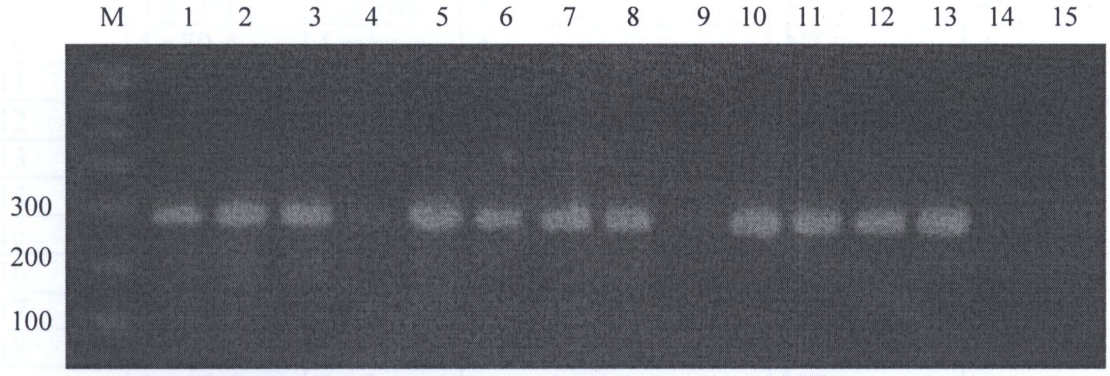

Figure 20: PCR products of *invA* from *Salmonella* isolates. Lane M is the molecular marker while lane 1 to lane 15, are *Salmonella* isolates from pastoral cattle and Kafue lechwe

Table 32: Summary of PCR products of *inv A* from *Salmonella* isolates ($n = 53$)

Serial No.	Isolate No.	Host	Reaction to somatic serotyping	*Salmonella* serogroup 8	Detection of *invA* gene
1	Lmc 226-1	Cattle	+	NT	+
2	Lmc 227-1	Cattle	+	NT	+
3	Lmc 231-1	Cattle	+	NT	+
4	Sc 88-2	Cattle	+	8	+
5	Lr 65-1	Lechwe	+	8	+
6	Lr 65-2	Lechwe	+	8	+
7	Lr 65 -3	Lechwe	+	8	+
8	Lr 65-4	Lechwe	+	8	+
9	Lp 260-2	Lechwe	+	8	+
10	Lr 79-4	Lechwe	+	NT	+
11	ICJ 59-1	Lechwe	+	NT	+
12	ICJ 59-2	Lechwe	+	NT	+
13	ICJ 59-3	Lechwe	+	NT	+
14	ICJ 59-4	Lechwe	+	NT	+
15	C 67-1	Lechwe	+	NT	+
16	C 67-2	Lechwe	+	NT	+
17	C 67-3	Lechwe	+	NT	+
18	SI 69-1	Lechwe	+	NT	+
19	Lp 59-2	Lechwe	-	NT	+
20	Lp 59-3	Lechwe	-	NT	+
21	Lp 59-4	Lechwe	-	NT	+
22	Lr 38-2	Lechwe	-	NT	+
23	Lr 48-1	Lechwe	-	NT	+
24	Lr 48-3	Lechwe	-	NT	+
25	Lr 76-4	Lechwe	+	NT	+
26	Lr 49-2	Lechwe	-	NT	+
27	SI 58-1	Lechwe	-	NT	+
28	SI 58-2	Lechwe	-	NT	+
29	SI 58-3	Lechwe	-	NT	+
30	SI 59-2	Lechwe	-	NT	+
31	SI 69-1	Lechwe	+	NT	+
32	SI 69-2	Lechwe	+	NT	+
33	SI 69-3	Lechwe	+	NT	+
34	SI 69-4	Lechwe	+	NT	+
35	Sc 88-1	Cattle	+	NT	+
36	Sc 9-1	Cattle	+	NT	+
37	Lmc 141-1	Cattle	-	NT	+
38	Lmc 141-4	Cattle	-	NT	+
39	Lr 50-4	Lechwe	-	NT	+
	Total		19	6	39

CHAPTER FIVE

DISCUSSION

5.0.0. Isolation of bacteria

In this study we attempted to isolate *E. coli* and *Salmonella* from free-range Kafue lechwe and pastoral cattle in the interface areas of the Kafue Flats. Following isolation of these bacteria, we performed comparative studies. 575 presumptive *E. coli* from Kafue lechwe and 708 presumptive *E. coli* from pastoral cattle were isolated on DHL agar and EMB agar according to the protocols described by Sakazaki *et al.*, (1971 and 1960) and Holt-Harris *et al.*, (1916), respectively. Furthermore, all the *E. coli* isolates were screened on SMAC agar as described by Boyce *et al.*, (1995) and March *et al.*, (1986) and on CHROMagar O157 according to the method of Bettelheim (1998). A total of 104 (18.1%) out of 575 *E. coli* isolates from Kafue lechwe, and 144 (20.3%) of 708 isolates from pastoral cattle were either NSF isolates on SMAC and appeared mauve coloured on CHROMagar O157 a characteristic which distinguishes STEC O157 from other coliforms. Fifty-nine *Salmonella* isolates from Kafue lechwe and 37 isolates from pastoral cattle were isolated on Xylose Lysine Deoxycholate Agar using the method described by Dunn *et al.*, (1971).

The study revealed that 80 (13.9%) isolates out of 575 presumptive *E. coli* isolates from Kafue lechwe were NSF isolates while 35 (6.1%) were mauve coloured. Sixty-eight (9.6%) out of 708 presumptive *E. coli* isolates from pastoral cattle were NSF isolates. Non-sorbitol fermenting isolates (Boyce *et al.*, 1995 and March *et al.*, 1986) were relatively higher in Kafue lechwe than in pastoral cattle, suggesting that the former are more susceptible to being colonized by STEC serotypes. Conversely, the recovery rate of mauve coloured isolates on CHROMagar O157) was 10.7% in pastoral cattle and slightly higher than in Kafue lechwe (6.1%) (Bettelheim 1998). However, the prevalence of NSF *E. coli* isolates in Kafue lechwe in this study was significantly higher than 1% (8 out of 1,112) isolates from faecal samples of wild animals which contained NSF *E. coli* in Trinidad and Tobago (Adesiyun 1999). In the present study, there was a significant

difference (P = 0.001) in the isolation frequency of non-sorbitol fermenting isolates on sorbitol macConkey agar and mauve coloured isolates on CHROMagar O157 from Kafue lechwe faecal samples between Lochinvar and Blue-lagoon NPs.

The prevalence of non-sorbitol fermenting *E. coli* isolates was 68.8% (55/80) in faeces of Kafue lechwe from Lochinvar NP higher than Blue-lagoon NP. This could have been due to high density of pastoral cattle which come into close contact with Kafue lechwe around Lochinvar NP. All non-sorbitol fermenting isolates and mauve coloured coloured isolates from Kafue lechwe and pastoral cattle were biochemically confirmed using standard methods (Edwards *et al.*, 1972 and Barrow *et al.*, 1993). Furthermore, our results have showed that 85.7% (30/35) of mauve coloured isolates from Kafue lechwe were aerogenic on TSI medium (production of stormy gas in the medium) (Carter *et al.*, 1979) compared to 65% of the NSF *E. coli* isolates

5.1.0. *Escherichia coli* and *Salmonella* isolates from Kafue lechwe

This study attempted to elucidate whether the Kafue lechwe could be a potential carrier of food-borne pathogens. *E. coli* was isolated from 13 of 77 (16.9%) Kafue lechwe compound samples and 27 (17.4%) samples out of the 155 Kafue lechwe faecal samples from the pasture were found positive to one or more diarrheagenic *E. coli* strains. These results have revealed that Kafue lechwe are carriers of at least seven diarrheagenic *E. coli* serogroups which include enterotoxigenic *E. coli* (O15, O27, and O78) and enteropathogenic *E. coli* (O55 and O125). Our finding is in agreement with other workers (Dunn *et al.*, 2004; Nagano *et al.*, 2004; Fischer *et al.*, 2001; Renter *et al.*, 2001; and Rice *et al.*, 1995) who have isolated pathogenic *E. coli* (STEC O157:H7) from deer antelope. Our results are similar to those described by Miko *et al.*, (2009) who in Germany reported the characterization of STEC into 8 serogroups from wildlife meat (O21, O146, O128, O113, O22, 088, O6 and O91). Furthermore, the prevalence of DEC in our study was generally higher than the work reported by Sergeant *et al.*, (1999) who in the USA isolated *E. coli* O157:H7 with an incidence rate of 2.4% from faecal samples of white-tailed deer sharing rangeland with cattle.

The isolation of pathogenic *E. coli* serogroup in the present study is in agreement with Kruse *et al* 2004) who reported that wildlife animals seem to be involved in the epidemiology of most zoonoses and serve as major reservoirs for transmission of zoonotic agents to domestic animals and humans. Since we did not have all the monovalent antisera for serotyping of the isolated diarrheagenic *E. coli,* it is possible that we could have underestimated the prevalence rate of *E. coli* serogroups carried by Kafue lechwe. An extended-spectrum ß-lactamases-producing *E. coli* has been isolated from wild seagulls in Porto, Portugal (Simoes *et al.,* 2010).

We have shown that among the DEC serogroups from Kafue lechwe, EPEC serogroup O125 were the most frequently isolated accounting for 5.8% followed by ETEC serogroup O27 (4.8%) and ETEC serogroup O15 (1.9%). According to our observations, the proportion of recovery of *E. coli* serogroups O15 and O125 using SMAC agar is significantly higher than CHROMagar O157 while the opposite is true for *E. coli* serogroup O27 ($P = 0.004$). Our results are suggestive that SMAC has high specificity for detecting *E. coli* isolates belonging to serogroup 015 and 0125 strains while CHROMagar 0157 has been observed to be sensitive for ETEC serogroup 027.

It is worthy noting that the ETEC serogroup O27 and O78 were most predominant in Lochinvar NP, while EPEC serogroup O125 and ETEC serogroup O15 were evenly distributed between Lochinvar and Blue-lagoon NPs, suggesting Kafue lechwe to be a reservoir animal. Most of the diarrheagenic strains were from the pasture possibly because there were more samples collected from the pasture than from the rectum. The use of SMAC agar and CHROMagar O157 to screen for presumptive STEC O157 strains was cost effective in the sense that they helped to reduce on the material costs and the number of *E. coli* isolates which needed to be confirmed by serogrouping. Though it is most likely that some unusual sorbitol positive *E. coli* O157 strains could have been missed which could not be differentiated from normal intestinal flora (Ammon *et al.,* 1999). Isolates that were non-typeable by the available antisera were in this study recorded negative though they could belong to other serogroups.

In the case of *Salmonella* isolation, there is scanty information on the prevalence of *Salmonella* serogroups in food-producing free range wildlife in Zambia, except for the report of Falade (personal communication) from captive wildlife (Bushpigs, duicker, warthog, zebra and bushbuck) and therefore there was no other report to compare our findings. The frequency of isolation of *Salmonella* from Kafue lechwe was moderately higher 7.8% (18/232) than pastoral cattle 2.5% (9/361). This finding is similar to the report by Gopee *et al.,* (2000) who indicated a prevalence of 7% in captive mammals in Trinidad. The prevalence of *Salmonella* isolates was highest in faecal samples picked from pasture (45.8%), followed by rectum (27.1%), small intestine (13.6%), ileo-caecal (8.5%) and caecum (5.1%). Other workers in various part of the world have isolated *Salmonella spp.* from captive wildlife mammals (Gopee *et al.,* 2000 and Mohan *et al.,* 1973), captured rock pigeons (Pedersen *et al.*, 2006), psittacine birds (Allgayer *et al.,* 2008) and from turtles and rabbits (Salehi *et al.*, 2009). Our results on the frequency isolation of *Salmonella* from wildlife are similar to the work by Gopee *et al.,* (2000) who reported frequency isolation of *Salmonella* serovars of 7% (29/404) from mammals, 3% (12/435) birds and 14% (25/173) reptiles.

Our results indicate that Kafue lechwe are potential carriers of *Salmonella* and may amplify transmission of the bacteria to pastoral cattle through faecal contamination of grazing pasture. Kafue lechwe become in contact with pastoral cattle when they graze together on the fresh grass which grows as the floods recedes (Pandey *et al.*, 2007 and Stafford 1991). The high prevalence of *Salmonella* in Kafue lechwe than pastoral cattle could imply that Kafue lechwe are more susceptible to *Salmonella.* This theory may be supported by the fact that Kafue lechwe are semi-aquatic animals (Gallagher *et al.,* 1972) and may on occasions shed *Salmonella* through faeces in the shallow waters of the lagoons where such pathogens remain viable for long periods (Angulo *et al.,* 2000). In addition, because Kafue lechwe live in large populations and are confined to a relatively small area, particularly in and around the Lochinvar and Blue-lagoon NPs (Siamudala *et al.*, 2003), this may result in high levels of cross contamination of the environment through faecal matter. In the food chain, faecal droppings of Kafue lechwe are a source of manure food for fish in the lagoons (Siamudala *et al.,* 2003) and subsequently these

fishes and semi-aquatic wildlife animals may consume and shed the pathogens in the water lagoons through the faeces. Furthermore, wild birds around the lagoons may shed the *Salmonella* since they have been documented to be carriers of the pathogenic bacteria (Foster *et al.*, 2006; Nielsen *et al.*, 2004 and Wallace *et al.*, 1997).

The study has further revealed that *Salmonella* isolates are more prevalent in Lochinvar (66.1%) than Blue-lagoon NP (33.9%). This may be a result of the high density of Kafue lechwe in Lochinvar than Blue-lagoon NP. Furthermore, faecal samples from Lochinvar NP were initially stored at -20°C prior to transporting to the School of Veterinary Medicine for microbiological analysis whereas faecal samples from Blue-lagoon NP were kept solely in cooler boxes for a longer period due to difficulties in hunting the Kafue lechwe because of the poor terrain. There was no significant difference in the frequency of isolation of *Salmonella* from faecal contents of the rectum, ileum-junction, caecum, small intestine and the faecal droppings of Kafue lechwe from the pasture. However, a marginal significance difference ($P = 0.011$) was observed in the frequency of isolation of *Salmonella* from faecal contents of the rectum, ileum-junction, caecum, small intestine and the faecal droppings of Kafue lechwe from the pasture between Lochinvar and Blue-lagoon NPs. For instance, the proportion of *Salmonella* isolates from the faecal samples picked from the pasture and rectal contents of Kafue lechwe were high in Lochinvar and Blue-lagoon NPs accounting for 59.3% (16/27) and 56.3% (9/16) of the isolates, respectively. Our results suggest that the prevalence of *Salmonella* in Kafue lechwe faecal samples from the pasture was relatively higher in Lochinvar NP than Blue-lagoon NP, while the prevalence of *Salmonella* in faeces from the Kafue lechwe rectum was higher in Blue-lagoon NP. .

Furthermore, there was a significance difference ($P < 0.001$) between frequencies of detection of *Salmonella* isolates positive to polyvalent O antisera and source. In the present study, the proportion of *Salmonella* isolates from compound samples was high in the rectum of Kafue lechwe which accounted for 36.8% (7/19) of the isolates positive to polyvalent O antisera. Our results suggest that, the recovery of serological positive *Salmonella* is somewhat high from Kafue lechwe rectal faeces than other sources.

Similarly, the study showed a significant difference ($P < 0.001$) between *Salmonella* isolates positive to polyvalent somatic antisera and location. All 19 (100%) isolates, positive to polyvalent somatic antisera were from Lochinvar NP, suggesting that the predilection site of the bacteria is both dependant on the animal host (rectal findings) and area of sampling. On another hand, 50% (20/40) of non-typeable *Salmonella* isolates were from Lochinvar and Blue-lagoon NPs respectively. These results indicate that serological positive *Salmonella* isolates from Kafue lechwe were most prevalent in Lochinvar NP than Blue-lagoon NP.

To our knowledge this is the first report of the isolation of DEC and *Salmonella* from the faeces of Kafue lechwe. Therefore the isolation of *Salmonella* in faecal contents of the rectum, caecum-ileum junction, small intestine and caecum of Kafue lechwe is of significance in food safety as this can result in contamination of carcasses during meat processing.

5.2.0. *Escherichia coli* and *Salmonella* isolates from pastoral cattle

We isolated 144 presumptive DEC and identified into 18 serogroups which included O125, O159, O27, O29, O55, O78, and O8. These serogroups have been associated with diarrheal diseases in humans (Nataro *et al.*, 1998; Doyle, 1989 and WHO, 1987). There were more *E. coli* serogroups from faeces of pastoral cattle than Kafue lechwe, suggesting that pastoral cattle were reservoir animals. Our finding is in agreement with the existing knowledge which has always implicated cattle as a major reservoir of DEC (Cookson *et al.*, 2006b; Bach *et al.*, 2002 and Trevena *et al.*, 1999).

Among the typeable *E. coli* isolates, serogroup O29 was the most frequently isolated and accounted for 4.2% of isolates, suggesting that serogroup O29 in pastoral cattle faeces was the most prevalent DEC serogroup. Furthermore, a significant difference ($P < 0.001$) in the frequency of isolation of serogroups between Lochinvar and Bue-lagoon NPs was observed. The prevalence of typeable DEC serogroups from pastoral cattle was higher in Blue-lagoon than Lochinvar NPs (Table 13). Our finding is probably due to the fact that

Blue-lagoon NP is more transhumance in nature than Lochinvar NP, because pastolist villages are much close to the wildlife/pastoral cattle interface area. Furthermore, our study has shown that all the EIEC serogroup O29 isolates were from Blue-lagoon, while all the EPEC serogroup O125 were from Lochinvar NP and ETEC serogroup O27 was mostly dominant in Blue-lagoon NP. These findings suggest that *E. coli* serogroups O29 and O125 are ecologically adapted to the respective areas. However, we recommend that more faeces from pastoral cattle and Kafue lechwe in respect to the locations be analyzed to ascertain the findings.

Isolation and identification of *Salmonella* from pastoral cattle in this study confirms with the existing information that cattle are reservoirs of *Salmonella* (McEvoy *et al.,* 2003; Hughes *et al.,* 1971 and Field 1948). The frequency of isolation of *Salmonella* from pastoral cattle was 2.5%, similar to that from faecal samples of cattle at a commercial Irish abattoir (McEvoy *et al.,* 2003). However, the low frequency of isolation of *Salmonella* from pastoral cattle in this study could be explained by the poor cooling facilities of the faecal samples in the field and also unforeseen delays in the delivery of the samples to microbiology laboratories for analysis. However, more comprehensive surveillance data are necessary to evaluate the actual burden of *Salmonella* prevalence in pastoral cattle.

5.3.0. Pathogenicity of the isolates

Selected diarrheagenic *E. coli* and *Salmonella* isolates from Kafue lechwe and pastoral cattle were investigated for the presence of virulent genes using PCR (Hamza 2010; Ahmed *et al.*, 2009; Ruttler *et al.*, 2006 and Fode-Vaughn *et al.*, 2003). Kafue lechwe is of economic importance to Zambia and is one of the most sought after game animal for wildlife meat (Siamudala *et al.*, 2003 and Stafford 1991). Human *Salmonella* infections occur from the ingestion of contaminated food, and many of these foods are of animal origin (Angulo *et al.,* 2000). It is for this reason that it becomes imperative to know the microbiological safety of the meat and meat products derived from Kafue lechwe. Other investigators have implicated wildlife meats as a source of human infections (Keene *et al.*, 1997 and Rabatsky-Her *et al.*, 1987).

An overview of our findings is indicative that the grazing pasture in Lochinvar and Blue-lagoon NPs would likely be contaminated with faecal diarrheagenic *E. coli* and *Salmonella* pathogens from Kafue lechwe and pastoral cattle. The corresponding diarrheagenic *E. coli* serogroups O8, O15, O27, O29, O55, O78, O125 and O159 from Kafue lechwe and pastoral cattle have been frequently implicated in human diarrheal diseases worldwide (Nataro *et al.*, 1998). For instance, ETEC serotype 078 is known to cause severe diarrheal outbreaks (Germani *et al.*, 1985 and Ryder *et al.*, 1976) while EPEC serogroup O125 is also known to cause a profuse watery diarrheal disease and is a leading cause of infantile diarrhea in developing countries (Regua *et al.*, 1990; Robins-Browne, 1987 and Levine, 1987). Furthermore, EPEC is known to induce a none-bloody watery diarrhea often accompanied with mucous and could have a devastating effect on HIV patients (Mossoro *et al.*, 1998). Other workers (Akinyemi *et al.*, 1998; Levine *et al.*, 1984 and Agbonlahor *et al.*, 1982) have incriminated the strains of EPEC as the causative microorganisms in diarrheal diseases of adults. EPEC-like organisms have also been isolated from other animals such as rabbits, pigs (Zhu *et al.*, 1994) and dogs (Nataro *et al.*, 1998).

Collectively, these results suggest that the eventual predominance of ETEC O27 and EPEC O125 on the pasture may have important implications such as safety of drinking water in the study area. Interestingly, we note that ETEC serogroup O15 and EIEC serogroup O29 are only confined to faecal samples from Kafue lechwe and pastoral cattle respectively; despite the latter being the most frequently isolated DEC serotype from the pasture. These findings further elucidate our earlier findings that elaborate on transmission dynamics as a function of environment, host and agent interaction in the aforesaid study area. We are tempted to assume that serogroup O29 was perhaps host specific though we would like to suggest that more research work could be done to substantiate the findings.

Heightened interactions between wild animals and cattle will likely lead to bacteria interspecies transfer (Gilbreath *et al.*, 2009). Lochinvar GMA, has the largest population

of pastoral cattle from the neighbouring districts and this is suggestive of frequent opportunities for faeco-oral transmission between wild animals and pastoral cattle. Human contacts with faecal matter of Kafue lechwe are diverse in much respect. For instance, professional hunters vary in hygiene and sanitation practices during evisceration of carcasses (Adesiyun *et al.,* 1995), and it could be possible that poachers and human settlers in the study area will have little knowledge about the occurrence of diarrheal pathogens in animal and human faeces thus creating many opportunities for human exposure to infections.

Whether these pathogens could be linked with the frequently reported sporadic human diarrheal diseases in Lochinvar GMA during rainy season (unpublished report) will certainly depend on the actual isolation of the pathogens from human presenting diarrheal manifestations and comparing them with those from wildlife and pastoral cattle. According to existing information, ETEC infections in areas of endemicity tend to be clustered in warm, wet months, when multiplication of ETEC in food and water is most efficient (Levine 1987). Identification of the DEC serogroup alone in this study was considered not a sufficient tool to determine if these *E. coli* strains are capable of causing disease in human and hence proceeded to do further investigations. According to Fratamico *et al.*, (2009), out of a particular *E. coli* serogroup, some strains are toxin-producing while others are none toxin-producing. However, Merson *et al.*, (1980) had a different view point and proposed the use of polyvalent antisera as a way to detect ETEC. Therefore in addition to serotyping of DEC and *Salmonella* isolates from Kafue lechwe and pastoral cattle, this study also aimed at detecting the potential of the selected isolates to cause disease in human by determining the presence of the virulence gene(s) using PCR-based methods.

Although the isolates were from apparently healthy animals, PCR revealed the presence of specific band amplicons of DEC isolates O27, O29, O55, O78 O125 and non-typeable *E. coli* isolates. Accordingly, 60% of 55 DEC were found to harbour one of the virulence gene(s) as referenced to molecular weights of the genes *ipaB*-137 bp (Fig. 20), EAEC heat-stable toxin 1-bp173 (EAST1) (Fig. 21) and Stx-2-bp 272 (Fig. 22). In addition,

73.6% of *Salmonella* serogroups were positive for the amplicon *invA* of 422 bp toxins gene (Fig.23). This study has showed that out of the 33 *E. coli* isolates containing virulence genes, 54.5% (18/33) were from pastoral cattle, and out of 39 *Salmonella* isolates which harboured virulent genes, 31 (79.5%) were from the Kafue lechwe. This implies that pathogenic bacteria with the potential to cause infection in humans are mostly *E. coli* from pastoral cattle and *Salmonella* from Kafue lechwe. Therefore, the study has revealed that Kafue lechwe is a potential carrier of pathogenic *Salmonella* to humans.

This study has further showed that EAST1 was the most frequent identified gene accounting for 50.1% (28/55) of the DEC isolates examined. 60.7% of the DEC isolates were from pastoral cattle while the rest were from Kafue lechwe. This is in agreement with the available knowledge that cattle are usually colonized by DEC isolates capable of presenting EAST1 toxins (Giraedeau *et al.*, 2003 and Bertin *et al.*, 2001). According to Bertin *et al.*, (2001), EAST1 gene was poorly present among the *Stx2* positive isolates (6.5%) but was 50% present in the *Stx1*- positive strains. In this study EAST1 gene was identified from different categories of DEC isolates (Table 16). However, our results are consistent with the work of Veilleux *et al.*, (2005), Yamamoto *et al.*, 1996 and Savarino *et al.*, (1996) who reported that EAST1 is not solely associated with EAEC but also with many other DEC categories.

The serotypic markers recorded in this study seem to correlate with specific categories of DEC (Nataro *et al.*, 1998) and *Salmonella* (Moussa *et al.*, 2010). For instance, we have shown that serotype 027, O29 O55, O78 and O125 isolates from Kafue lechwe and pastoral cattle possesses EAST1 gene. Detection of virulent EAST1 and other genes in this study in isolates from Kafue lechwe and pastoral cattle signaled a source of human infections. This finding is in agreement with Savarino *et al.*, (1996) who according to their investigations during a survey of *E. coli* strains, 14 (22%) of 65 strains tested hybridized with the *astA* gene encoding EAST1. The largest reported outbreak of diarrhea due to DEC in human beings occurred in Japan (Itoh *et al.*, 1997). This episode involved 16 schools with 2697 children suffering from diarrhea after consuming

contaminated lunch shown to contain an EAST1-producing *E. coli* strain. Furthermore, other workers have also shown an association between EAST1 positive strains and diarrhea in children (Vila *et al.,* 1998).

In the present study DEC isolates containing invasion plasmid antigens B (*ipaB)* were the second frequently identified accounting for 7.3% (4/55). DEC isolates positive for *ipaB* gene was from faeces collected from Kafue lechwe. Invasion plasmid antigen B is associated with the ability of *Shigella* and EIEC serogroups to invade epithelial cells and is the bacterial antigen most strongly and consistently recognized by the host during infection (Barzu *et al.*, 1993).

In our present study only 3% (1/33) of the DEC isolates from pastoral cattle contained *Stx*2 gene while all the isolates from Kafue lechwe were negative. Shiga toxin is a major virulence factor, and a defining characteristic of EHEC. These potent cytotoxins are the factor that leads to death and many other symptoms in patients infected with EHEC (Nataro *et al.,* 1998). Detection of *Stx*2 toxin producing strain from pastoral cattle in our study is consistent with the existing knowledge that cattle and other animals are known reservoirs of *Stx*-producing *E. coli* worldwide (Gilbreath *et al.,* 2009; Cookson, *et al.*, 2006a; Hussein *et al.,* 2005; Bertin *et al.*, 2001 and Beutin *et al.*, 1993). Cookson *et al.,* (2006b) in North Island, New Zealand, reported a 8.7% prevalence of *Stx*-2 in *E. coli* isolates from faecal samples of cattle and sheep. Our finding is important considering that that *Stx*- producing *E. coli* has been shown to survive in faecal material in the environment for more than 20 months (Beutin 2006).

EHEC was first reported by Riley *et al.*, (1983), who investigated two outbreaks of distinctive gastrointestinal illness characterized by severe crampy abdominal pain, watery diarrhea followed by grossly bloody diarrhea, and little or no fever. This illness was associated with the ingestion of undercooked hamburgers at a fast food restaurant chain.

The largest known outbreak due to *E. coli* O157:H7 occurred in the western state of Washington in 1993 (Anonymous 1993). Seven hundred and thirty-two cases were

identified, mostly children of whom 195 were hospitalized, 55 developed hemolytic uraemic syndrome (HUS) and four died. EHEC O157:H7 is responsible for approximately 62,000 cases of food borne infections annually in the United States (Mead *et al.*, 1999). EHEC is also an important pathogen in some countries of the southern hemisphere such as Argentina, Australia, Chile and South Africa (Nataro *et al.*, 1998).

In our present study 73.6% (39/53) *Salmonella* isolates were positive to *invA* protein and out of these, 25 (64.1%) isolates were positive to polyvalent "O" antisera, while 14 isolates were non-typeable, suggesting are capable of causing infection in humans. The isolates that were *invA* negative were considered non pathogenic. The non-serotyped *Salmonella* isolates may be attributed to the presence of rough mutant strains which lack the specific side chains responsible for "O" specificity (Topley and wilson's 1990). This study shows that PCR still is remains a relatively rapid and highly sensitive method in the diagnosis of non-typeable *Salmonella* isolates (Singer *et al.*, 2006). All the *Salmonella* isolates belonging to serogroup 8 were positive to *invA* gene. Hang'ombe *et al.*, (2008) reported that all the 59 *Salmonella* isolates examined from beef and poultry in Zambia were found to possess *spiC* and *invA* genes by Dot Blot Hybridization method. Furthermore, Isogai *et al.*, (2005) detected *Salmonella invA* from 12 of 14 (85.7%) poultry carcasses rinses in Zambia.

The prevalence level of *invA* gene in *Salmonella* isolates in our study was slightly lower compared to that obtained by Isogai *et al*, (2005) but similar in many respect with those reported by other workers elsewhere (Rexach *et al.*, 1994 and Threlfall, *et al.*, 1994). In this study, the occurrence of non-typeable *Salmonella* isolates could have contributed to the observed slightly low prevalence levels of *invA* gene. Furthermore, our results have shown that all the *Salmonella* isolates positive to polyvalent O antisera were positive to *invA* gene by PCR reaction (Salehi *et al.*, 2009). In Teheran, Iran, four *Salmonella* serovars were isolated from turtle livers, turtle's water source and rabbit faecal samples.

Detection of *invA* genes in *Salmonella* isolates is of paramount importance because the pathogenicity of *Salmonella* spp is dependent upon the ability of these organisms to gain

access to cells that are normally non-phagocytic (Rychlik *et al.*, 1998; Ginocchio *et al.*, 1995 and Barrow, 1995). *InvA* is one such protein which is present and functional in most, if not all, virulent *Salmonella* strains to enter intestinal epithelial cells (Galan *et al.*, 1991). Therefore, the detection of *invA* from the non-typeable *Salmonella* isolates in our study could be indicative of a *Salmonella* strain colonizing the Kafue lechwe which is yet to be identified serologically. In the same context our study had showed that 26.4% (14 out of 53) of *Salmonella* isolates were negative to *invA* gene and therefore are non-pathogenic.

5.4.0. Antibiotic resistance of the isolates

Nearly 149 *E. coli* and 48 *Salmonella* isolates from apparently normal, healthy Kafue lechwe and pastoral cattle were examined for resistance to antimicrobials of human and veterinary importance. Of these, 93 (100%) *E. coli* isolates from Kafue lechwe exhibited resistance to one or more of the eight antimicrobial agents tested.

Escherichia coli isolates from Kafue lechwe were mainly resistant to metronidazole (98.9%) and penicillin G (98.9%), moderate to amoxicillin (29%) and co-trimoxazole (15.1%), but significantly low to cephalexin (6.5%) and oxacillin (3.2%) (Table 18). Metronidazole is usually effective on treatment of protozoa and anaerobic bacteria, while penicillin G is for gram positive bacteria. Therefore the observed resistance is an expected natural phenomenon. Our findings disagree with White *et al.*, (1979) who carried out studies on antimicrobial resistance in bacterial isolates from wildlife and reported that it was rare. Interaction of livestock and wildlife populations can significantly shift the resistance. According to Rolland *et al.*, 1985 and Levy, 1984 have documented that wildlife do acquire resistant aerobic bacteria from food or the environment. However, none of the *E. coli* isolates from Kafue lechwe were resistant to gentamycin and tetracycline. But in Trinidad and Tobago, the prevalence of resistance among EPEC serogroups from free-ranging and captive wildlife was 6.8% to gentamycin (Adesiyun *et al.*, 1999). In this study, a significant difference ($P = 0.001$) was observed in the antimicrobial resistance to amoxicillin between *E. coli* isolates from the faecal samples of Kafue lechwe collected from pasture and rectum, with the isolates from the

pasture being the most resistant (Table 19). These findings suggest there could be an environmental determinant which needs further analysis, although this is beyond the scope of the current work. .

Out of 27 *E. coli* isolates resistant to amoxycillin, 26 (96.2%) were non-typeable and similarly 84.8% (78/92) of the isolates resistant to penicillin G, were non-typeable (Table 20). Our finding suggests that high resistance levels are pronounced in non-typeable isolates. Furthermore, results from this study seem to suggest that resistance levels of *E. coli* isolates from Kafue lechwe were relatively higher than in pastoral cattle. The pasture grounds of the Kafue Flats, have been shown over the years to cumulatively accumulate persistently organic pollutants (POPs). Apart from these substances, a lot of chemicals residues from surrounding farms drain towards this area. Studies by Kwenga *et al.,* (2007) indicated that Kafue lechwe have been cumulating these chemical residues over years. The Kafue Flats is the drainage point of the copper mines activities and agricultural activities within the study area.

Bacterial resistance to antimicrobial agents in cattle has been reported worldwide (WHO 1997; Spika *et al.*, 1987 and Tacket *et al.,* 1985) icluding Zambia (Ngoma *et al.,* 1993 and Falade *et al.*, 1989). This study has revealed significantly low levels of resistance in *E. coli* isolates from pastoral cattle against co-trimoxazole (7.1%), oxacillin (1.8%) and tetracycline (1.8%). Furthermore, the study has revealed high level of *E. coli* resistance to metronidazole (100%) in pastoral cattle. Surprisingly none of the isolates from pastoral cattle were resistant to amoxycillin; cephalexin, gentamycin and penicillin G, given that they are more exposed to antimicrobial treatment than Kafue lechwe. This finding is similar to the observation of Falade *et al* (1989) who found no *Salmonella* resistance against gentamycin. This study has showed evidence of relationship between antibiotic resistance and location. *Escherichia coli* isolates, from Kafue lechwe and pastoral cattle were more resistant to antimicrobial agents in Lochinvar NP than Blue-lagoon NP (Table 18 and 21).

There was significant difference in antimicrobial resistance to cephalexin, co-trimoxazolee and gentamycin between isolates from Lochinvar and Blue-lagoon NPs (Table 25). Isolates from Blue-lagoon NP were more resistant to cephalexin and co-trimoxazole, while *Salmonella* isolates from Lochinvar NP were 100% resistant to gentamycin. The observed absolute resistance of *Salmonella* isolates to gentamycin in Lochinvar NP is a source of concern given that gentamycin is an aminoglycoside antibiotic that is used to treat many types of bacterial infections, particularly those caused by Gram-negative bacteria (Moulds *et al.*, 2010).

Salmonella isolates from the faecal samples collected from the pasture were 95.2% (20/21) resistant to co-trimoxazole, while all the isolates from the faecal contents of the small intestine were resistant to gentamycin (Table 26). When compared to the 11 *Salmonella* isolates from pastoral cattle, 37 *Salmonella* isolates from Kafue lechwe were most resistant to metronidazole (90.9%), penicillin G (72.7%) and gentamycin (54.5%).

Low levels of antimicrobial resistance were observed against cephalexin (9.1%), and none to amoxicillin, co-trimoxazole, oxacillin and tetracycline. Bacterial resistance to metronidazole, amoxicillin and penicillin G was somewhat expected because in the case of the metronidazole, it is a frequently used antibiotic in human diarrheal diseases in particular due to anaerobic bacteria and certain intestinal parasites such as *Giardia lamblia* and *amoeba*, while amoxicillin and penicillin G are most effective against gram positive bacteria. This study has significantly showed that all *Salmonella* isolates resistant to gentamycin from Kafue lechwe and pastoral cattle were from Lochinvar NP (Table 29). This could suggest there is an abuse of the usage of these antibiotics in the area.

However, it is interesting to see drug resistance to co-trimoxazole and cephalexin by *E. coli* and *Salmonella* isolates from wildlife, as none of free-ranging wildlife animals had been treated with any of these antibiotics. Cephalexin is in a group of cephalosporin antibiotics which is used to treat human infections caused by bacteria, including upper respiratory, ear, skin and urinary infections. On the other hand co-trimoxazole is a

combination of trimethoprim and sulfamethoxazole (a sulfa drug) which eliminates bacteria that cause various infections including travelers' diarrhea. Our previous work (Mubita *et al.*, 2008) reported a widespread use of chemotherapeutic agents by pastoralists to treat bacterial infections and blood parasites in pastoral cattle in the study area which come into close contact with wildlife animals. Therefore it would suggest that free-range wildlife may have been exposed to bacteria strains of pastoral cattle but surprisingly the prevalence of antimicrobial resistance is more elevated in isolates from Kafue lechwe than pastoral cattle. Our findings suggest that the high antimicrobial resistance observed in wildlife may have been acquired from the environment or faecal contaminated water lagoons (Rolland *et al.*, 1985 and Levy, 1984).

Foods of animal origin are the source of most human *Salmonella* and *E. coli* infections and antimicrobial resistance is related to the consumption of contaminated meat with antimicrobial resistant pathogens or antimicrobial agents which could result into potential human health consequences (Anonymous 1989). Therefore, it would be proper to suggest that the bacterial resistances observed in wildlife animals and pastoral cattle to common antibiotics have consequential effect to humans after consumption of meat products contaminated with antibiotic resistant strains. Consequences include reduced choice of drugs for treatment of human cases and reduced efficacy of affordable empirical treatment. Our findings have healthy implication for hunters, consumers of game meat, game viewers and humans who share common source of drinking water with wildlife.

Often, hunters who handle wildlife animals in the National Parks, have little knowledge on the possible risks associated with contamination of meat with food-borne pathogens while dressing, handling and transporting wildlife meat. The presence of the antimicrobial resistant ETEC, EPEC and EIEC strains on pasture in Lochinvar and Blue-lagoon GMA may result in increased likelihood of other ruminants (buffalos and zebras) being colonized. Usually there is high density of pastoral cattle in Lochinvar NP for about 4-5 months (Pandey *et al.*, 2007) from Namwala, Mazabuka and Monze districts due to "seasonal movements" of livestock by the local tribes (Tonga and Illa). As such,

there is also a high interaction between pastoral cattle and Kafue lechwe. Pastoral cattle from these districts have from time in memory been exposed to wide range of tick-borne and bacterial diseases.

The antibiotics that are commonly stocked by pastoralists include co-trimoxazole, penicillin-streptomycin, oxytetracycline and enrofloxacin. In theory prolonged use of these antibiotics in pastoral cattle could have led to the rapid spread of antibiotic resistance strains through transfer of resistance genes by selective pressure. Abuse of drugs by pastoralists to prevent cattle diseases is compatible and consistent with knowledge of the acquisition of antimicrobial resistance within food-producing animals such as cattle (Levy, 1994). The situation has further been compounded by brief case business men who easily buy drugs from the counter and offer it to pastoralists in exchange with maize bags.

Blue-lagoon NP, on the other hand has less pastoral cattle population which is mainly drawn from Mumbwa district. Drug abuse by pastoralists in Blue-lagoon GMA is somewhat controlled due to its closeness and accessibility to professional veterinary services from Lusaka. Cattle owners in Blue-lagoon GMA are generally knowledgeable in terms of drug usage because of the established veterinary services within their villages. For instance, the School of Veterinary Medicine, University of Zambia has a research station at Shibuyunji (Blue-lagoon GMA) where they do routine clinical treatment of pastoral cattle. Therefore it is less unlikely that these animals will be treated with sub-standard drugs/dosage and hence the disease situation is usually under control. Also due to the ecological aspect of Blue-lagoon NP, human settlements in the GMA are few and far apart from the National Park boundary which is not the case with Lochinvar NP. It is also possible that the perceived high antimicrobial resistance to pathogens from Kafue lechwe could be due to a subpopulation of bacteria which has a predisposition towards resistance. The investigation by The National Centre for Zoonosis Resaerch (Anonymous 2010) recently demonstrated high prevalence of antibiotic resistance in the normal bacteria flora from woodland population of wild rodents that have never been treated with antibiotics. Therefore there is a possibility that the population of

antimicrobial resistant bacteria could have originated from wildlife animals or perhaps could have come about through the effluent discharge including industrial waste and domestic sewage from the Copperbelt towns into the Kafue River down the stream to Kafue Flats.

In Zambia, there is little published information on antimicrobial resistance in wildlife animals to compare with our results, mainly due to lack of sufficient epidemiological surveillance activities and limited diagnostic facilities. This is the first study of antibiotic resistance in *Salmonella* and *E. coli* isolates from Kafue lechwe and pastoral cattle. The results provide us with an opportunity to gain insight into the origin of antibiotic resistance and the flow of genetic elements between Lochinvar and Blue-lagoon NPs.

5.5.0. Relatedness of isolates

To ascertain the relatedness of the isolates, a number of activities were done including serotyping, biochemical profiles, DNA fingerprinting, detection of virulent genes and antibiotic susceptibility tests (Davis *et al.*, 2003; Sanchez *et al.*, 2002; Liebana *et al.*, 2001 and Johnson *et al.*, 1995).

a) Serotyping: Our attempt to isolate and identify DEC and *Salmonella* serogroups from Kafue lechwe and pastoral cattle has provided significant epidemiological information as a first measure to determine the relatedness between the similar strains. This study has shown that ETEC (O27 and O78) and EPEC (O55 and O125) are common serogroups haboured by Kafue lechwe and pastoral cattle. This is in agreement with the report by Rice *et al.*, (1995) that wild animals and cattle sharing common areas would likely experience interspecies transfer.

b) Biochemical profiling: All DEC serogroups were positive to one or more carbohydrates (Table 31). The study revealed that within *E. coli* serogroups O27, there were two distinct fermentative groups. One group comprised isolates number 1 (Lp 13-2) and 4 (Sc 1-2), while the other group comprised isolates numbers 3 (Lp Lr 28-4) and 5 (Lmc 167-2). Isolates in each group showed a similar pattern of fermentative

characteristics to 18 carbohydrates suggesting that the strains are indistinguishable (Table 31). The difference between the two groups was that one group was dulcitol positive while the other was negative. None of the isolates showed positive to cellobiose, dextran, inositol resorcinol, ribitol and barbital. Isolates belonging to serogroups O78 and O125 were not related because they showed unidentical pattern of metabolic fingerprint of the carbon source from various carbohydrates.

Salmonella serovars number 1 (Lmc 226-1), 3 (Lmc 227-1), and 5 (Lmc 231-1) from pastoral cattle and isolates number 12 (Lr 79-4), 14 (Icj 59-1), 16 (C 67-2) and 18 (Si 69-1) from Kafue lechwe showed a similar pattern of fermentative reaction to 20 carbohydrates suggesting they could be from a common source. None of the *Salmonella* serovars were positive to cellobiose, dextran, raffinose, resorcinol and barbital. The serovars belonging to serogroup 8 from the rectum of Kafue lechwe and cattle were not related because their biochemical profiles were not identical.

c) Antibiotic resistance: Antimicrobial resistance tests with amoxicillin, cephalexin, co-trimoxazole, gentamycin, metronidazole, oxacillin, penicillin G and tetracycline revealed that *E. coli* serogroups numbers 1 (Lp 13-2) and 3 (Lr 28-4) from Kafue lechwe and 4 (Sc 1-2) from pastoral cattle belonging to serogroup O27 had an identical pattern of resistance (Table 23). Interestingly the *E. coli* isolate number Sc 1-2 strain was from faecal samples of cattle collected from Blue-lagoon NP while the rest were from Lochinvar NP, suggesting they were from a common source. Similarly antimicrobial resistant pattern of isolates number 2 (Lp 208-4) and 5 (Lmc 167-2) from Kafue lechwe and cattle respectively, were identical suggesting they were from a common source, while isolates belonging to serogroup O78 could be different strains. A unique pattern of antimicrobial resistance of the isolates from Kafue lechwe and pastoral cattle belonging to serogroup O125 was observed suggesting that the strains may have the same source.

d) DNA fingerprinting: Molecular characterization using PFGE technique showed digestion with restriction enzyme that one or more strains of *E. coli* and *Salmonella* from Kafue lechwe were indistinguishable from the similar serogroups isolated from pastoral

cattle. For instance, we observed an association between isolate number 1 (Lp 13-2) from Kafue lechwe and isolate number 4 (Sc 1-2) from pastoral cattle both belonging to serogroup O27. These isolates were digested only by *PstI* and yielded an identical PFGE pattern, suggesting they were from a common source.

As for *Salmonella* serogroups, serovars number 5 (Lmc 231-1) and 18 (Lp Si 69-1) from pastoral cattle and Kafue lechwe respectively had identical patterns of antimicrobial resistance, suggesting they were related (Table 30).

The rest of *E. coli* and *Salmonella* serogroups from Kafue lechwe and pastoral cattle were not related to each other suggesting that the presence of *E. coli* serogroup O78 and *Salmonella* serogroup 8 is likely due to the appearance of multiple strains in the wildlife and cattle population rather than to a single source. If we define a strain as each serotype that has a unique antimicrobial resistance pattern, then we identified two different strains belonging to O27 serogroups and another two different strains belonging to serogroup O78 (Table 23). In the case of *Salmonella*, 10 different *Salmonella* strains were identified from 13 serovars tested (Table 30).

e) Pathogenecity: Some selected *E. coli* and *Salmonella* serogroups from Kafue lechwe and pastoral cattle with a similar serogroup were examined for genes encoding for toxins. The study had earlier showed evidence of relatedness in terms of phenotypic reaction of the serogrouping, biotyping and resistance pattern. However, the study showed genetic differences in the case of ETEC serogroups O27 (Lp 13-2, Lp 28-4 and Lp 208-4) belonging to Kafue lechwe and (Sc 1-2 and Lmc167-2) from pastoral cattle. The isolates from Kafue lechwe were negative for genes encoding toxins while the corresponding ETEC serogroup from pastoral cattle were positive, suggesting that they are capable of causing disease in humans. But *Salmonella* isolates from Kafue lechwe and pastoral cattle belonging to serogroup 8 were positive to the gene encoding for *invA* toxin. The results in our study seem to suggest that these genetic differences in DEC could have arisen from the pathogen adaptation in Kafue lechwe. Therefore current findings in our study confirms with the research hypothesis which states that bacterial pathogens from

Kafue lechwe antelope may somewhat be related in many respects to the some bacterial strains from pastoral cattle.

Salmonella, EHEC, EAEC and EIEC serovars from Kafue lechwe and pastoral cattle were identified based upon the O-somatic antigens and PCR reaction. Identification of ETEC and EPEC isolates of similar serogroup from Kafue lechwe and pastoral cattle in our study has provided significant epidemiological information as a first measure to determine the relatedness between strains.

5.6.0. Public health concerns due to the occurrence of pathogenic bacteria in domestic animals (cattle)

Cattle are a natural reservoir of STEC and *Salmonella* (Fairbrother *et al.,* 2006; Hornitzky *et al.,* 2005 and Singh *et al.,* 1983). However, no data is available on the prevalence of these bacteria in pastoral cattle which are in close contact with wild animals in the domestic/wildlife interface areas. In this study, STEC and *Salmonella* were identified from pastoral cattle living in close contact with wildlife, which may be difficult to prevent and control these organisms. The human populations living in these areas are seriously at risk of getting these infections from the environment. This is compounded by the fact that ETEC serogroups (O8, O27, O78, O115, O159 and O169), EPEC serogroups (055 and 0125) and EIEC (O29) were isolated from pastoral cattle and have shown to contain *Stx-2*, *ipaB* and EASTI gene(s), while *Salmonella* harbours *invA* gene. Hemolytic uremic syndrome and HC are the most severe and specific clinical manifestations of infection caused with *Stx* (Karmali 1989). *Salmonella* can survive in the environment for a period of up to one month (Jensen *et al.*, 2006), while STEC takes more than 20 months to completely eradicate (Beutin 2006). The information from this study highlights the importance of wildlife in maintaining infections, which then disseminate into human beings. Fairbrother *et al.,* (2006), Hornitzky *et al.,* (2005) Evans *et al.*, (1996) and Karmali, (1989) clearly demonstrated the public health concerns regarding domestic animals as the main vehicle in STEC and *Salmonella* infections.

5.6.1. Public health concerns due to the occurrence of pathogenic bacteria in wildlife

Wildlife animals can serve as a natural host for certain diseases that affect humans (zoonoses). The study has revealed that Kafue lechwe were potential carriers of pathogenic *Salmonella* and *E. coli* serovars. This population of carriers may play a significant role in the epidemiology of acquired diarrheal infections within and outside the Kafue Flats. The fact that *Salmonella,* ETEC serogroups (06, 015, 027, 078) and EPEC serogroups (055 and 0125) isolates from Kafue lechwe have shown to contain *invA*, *ipaB* and EASTI gene(s) poses a source of public health concern. EAST1 was first discovered in EAEC strain 17-2 isolated from the stool of a Chilean child suffering from diarrhea (Savarino *et al.*, 1991). Other studies have associated EAST1 with *E. coli* diarrheagenic classes other than EAEC, such as EHEC, ETEC, DAEC and EPEC and also with other bacterial genera like *Salmonella.* EAST1 positive strains have been reported in farm animals such as swine and cattle (Veilleux, *et al.,* 2006). Furthermore, meat from the food animals has been identified as an occasional vehicle of enteric infections (Vernone 1969 and Koretskaia and Kovalevskaia 1958). Investigators in the USA reported an outbreak due to *E. coli* O157:H7 infection of which homemade venison jerky from deer meat was implicated as the source of transmission (Keene *et al.*, 1997). In another study, Rabatsky-Her *et al.*, (1987) reported a case of *E. coli* O157:H7 infection which was acquired by eating meat of a wild tailed-deer (*Odocoileus virginianas*). During hunting seasons of wildlife in Zambia, some animals are accidentally shot in the abdominal cavity and as a result contents of the intestines and caecum are discharged on the whole flesh meat making it contaminated with faecal matter (unfit for human consumption). The situation is further worsened by the unhygienic environments during evisceration where enteric micro-organisms tend to contaminate carcasses (Adesiyun *et al.*, 1995) and this could lead to food spoilage. Worse still, carcasses are transported in non refrigerated vans for long distances to the market areas and improper temperature control favours proliferation and contamination of game meat (Rabatsky-Her *et al.*, 1987) and consequently increases the risks of human infections after consumption of such meat.

Occurrence of ETEC, EPEC, EIEC and *Salmonella* strains in the Interface Areas of wildlife/pastoral cattle of the Kafue Flats could be associated with sporadic diarrheal

outbreaks that have been reported over the years in the human settlements of Lochinvar GMA (unpublished data). However, in the absence of laboratory investigation on humans who present clinical manifestation of the illness, we are restrained from making any firm conclusions about the risks associated with these pathogens. Clinical manifestation of these human cases has largely been cholera-like diarrheal syndromes. ETEC is also known to cause a watery diarrhea, and in the most severe cases the illness is indistinguishable from that due to *Vibrio cholerae* (Sack 1975 and De *et al.*, 1956).

In African countries, ETEC is among the major etiologic agent of infantile diarrhea (Geyd *et al.*, 1998 and Ako-Nai *et al.*, 1990). In Lochinvar GMA, human diarrheal cases seem to be rampant during the rainy season when the drinking water holes are virtually flooded with water and faecal matter from the pasture (unpublished data). However, future studies would need to be carried out to consider the significance of Kafue lechwe and pastoral cattle as possible vehicles in the spread of human enteric pathogens within the study areas.

Human population expansion and encroachment in what was wildlife habitat can influence the epidemiology of zoonoses with wildlife. Currently there is a growing concern of the increasing human population in the interface areas of the Kafue Flats which could subsequently favour an increase in the level of contact between humans and infected wildlife animals. According to our observation on the site, the general living standards of humans in the Kafue Flats were relatively poor in terms of sanitation and quality of drinking water. Due to lack of proper toilet facilities, humans are compelled to excrete in the open and water logged areas of the Kafue Flats resulting into further faecal contamination of drinking water. Currently there are no published reports on the human infections due to *E. coli* serogroups 06, 015, 027, 078 and 0125 in the Republic of Zambia though human diarrheal cases have been reported (Patel *et al.*, 1982; Dube *et al.*, 1982 and Mwansa *et al.*, 2007). This makes it difficult to postulate on the epidemiology of DEC and *Salmonella* in human infections.

CHAPTER SIX

RECOMMENDATIONS

Detection of potentially enteropathogenic bacteria in Kafue lechwe and pastoral cattle in the study area is alarming. Therefore in view of our findings we recommend that;

1 Game meat inspection should be re-introduced as a matter of public health concern because of the health risks associated with consuming and handling of fresh game meat and carcasses. Currently game carcasses whether obtained legally or illegally are processed in most cases in unsanitary environments and without veterinary certification, for this reason such meat should be adequately cooked.

2 Zambia Wildlife Authority (ZAWA) should embark on public health campaign to create awareness among the hunting fraternity and consumers of game meat of the possible dangers associated with the consumption of meat and meat products.

3 Transporters of Kafue lechwe carcasses from Game Management Areas (GMA) could be advised to use facilities whose temperature can be controlled to about 4^0C to avoid rapid proliferation of pathogens which can lead to contamination.

4 Drinking water should be boiled or treated at household level by chlorination because sources in Africa tend to have highest faecal organisms contamination at the onset of the wet season (Wright *et al.,* 1986) and this could explain the episodes of human diarrheal infections in Lochinvar GMA during the rainy season among both adults and children.

5 Handling of animal faeces (cattle manure) is a well established risk factor for acquisition of diarrheal infection due to *E. coli* O157:H7 (Cookson *et al.*, 2006; Osek 2002 and Wang *et al.*, 1996) and therefore washing of hands before eating should be

encouraged by all persons living within the study area as not doing so would be a source of infection.

6 In view of the new knowledge in this study on the occurrence of DEC and *Salmonella* in Kafue lechwe, it is necessary that the Government or Stake holders such as ZAWA to consider control measures to regulate the current uncontrolled movements of fish traders from Kafue Flats to urban areas and vise versa, as well as monitor the increase of seasonal movements of domestic animals in what was previously wildlife habitat which is likely to introduce pathogens from infected areas to areas where they had not been previously reported (Siamudaala *et al.,* 2003)..

7 Bacterial resistance to penicillin G, co-trimoxazole and cephalexin is a source of worry from a public health point of view, especially in the Lochinvar GMA where human, wildlife and pastoral cattle population are on the increase. The antimicrobial agents to which many serovars were resistant are commonly used for treatment of various human infections such as upper and lower respiratory, skin, wound, diarrheal and septicemia infections (Anosike *et al.*, 2009; Laufer *et al.*, 2006; Badri *et al.*, 1999; Kim 1987; Gower *et al.,* 1976 and Anderson 1945). A special consideration should be made to look at the plight especially with individuals at higher risk such as the elderly, the children, the HIV-infected persons, and AIDS patients living within the study area. The majority of human antimicrobial-resistant infections are acquired from ingestion of foods contaminated with antimicrobial-resistant bacteria (Angulo *et al.*, 2000).

Further investigations proposed

In Zambia, wildlife farming has the potential of growing given that this sector receives a lot of attention and support from the government and therefore there is a likelihood of the increase of contact between wildlife and owners. The major impact of health risk factors associated with free-range and captive wildlife has been unknown. Therefore it is important that more information should be collected on Kafue lechwe to be used as baseline for policy makers in order to protect the risk groups such as the collectors of hides and trophies and consumers of game meat and other game products from acquiring

diarrheal infections through exposure to faecal contamination and consumption of faecal contaminated meat. We propose to investigate further on the following aspects;

a) To serotype all the *Salmonella* isolates which were positive to polyvalent antisera from Kafue lechwe and pastoral cattle.
b) To determine the serogroups of the non-typeable *E. coli* and *Salmonella* isolates from Kafue lechwe and pastoral cattle which were found to contain virulent genes.
c) To investigate the genetic elements which encode high levels of resistance to cephalexin and co-trimoxazole in *Salmonella* isolates from Kafue lechwe.
d) To investigate the role of wildlife animals (Kafue lechwe) as a source of antimicrobial resistance for pastoral cattle.

CHAPTER SEVEN

CONCLUSION

1. This is the first report on the isolation of DEC and *Salmonella* in Kafue lechwe which may be colonized by ETEC serovars (015, 027, and 078) and EPEC serogroups (055 and 0125).

2. Kafue lechwe and pastoral cattle are asymptomatic carriers of diarrheagenic *E. coli* and *Salmonella spp.*

3. Enterotoxigenic *E. coli* serogroups O27 and O78 from Kafue lechwe are most predominant in Lochinvar NP, while enteropathogenic *E. coli* serogroups O125 and enterotoxigenic *E. coli* serogroup O15 from Kafue lechwe are evenly distributed between Lochinvar and Blue-lagoon NPs.

4. Enterotoxigenic *E. coli* serogroups O15 and enteroinvasive *E. coli* serogroups O29 were only prevalent in faecal samples of Kafue lechwe and pastoral cattle respectively.

5. Antimicrobial resistance of *Salmonella* isolates from Kafue lechwe against metronidazole (100%), penicillin G (89.2%), co-trimoxazole (67.7%) and cephalexin (59.5%) were significantly higher than in pastoral cattle which they come into close contact. On the other hand *E coli* isolates from Kafue lechwe was significantly higher to penicillin G (98.9%) than in pastoral cattle.

6. That *Salmonella* isolates are more prevalent in faecal samples of Kafue lechwe picked from Lochinvar than Blue-lagoon NP. *Escherichia coli* serogroup O29 was the most frequently isolated from pastoral cattle which accounted for 4.2% of *E. coli* isolates and was predominant in Blue-lagoon NP.

7. Enterotoxigenic *E. coli* serogroup O27 and enteropathogenic *E. coli* serogroup O125 from Kafue lechwe and pastoral cattle seem to have an eventual predominance in the faecal samples picked from the pasture and this may have important implications such as safety of drinking water in the study area.

8. Some diarrheagenic *E. coli* and *Salmonella spp* from Kafue lechwe and pastoral cattle do harbour one of the virulence genes that encode for toxins (*ipaB,* EAST1, *invA* and *stx*-2).

9. Enteroaggregative *Escherichia coli* (EAEC) heat-stable toxin (EAST1) was the most frequently identified gene accounting for 50.1% (28/55) of the diarrheagenic *E coli* isolates examined.

10. Some strains of diarrheagenic *E. coli* serogroups showed a similar pattern of phenotypic and genotypic characteristics suggesting that the strains are indistinguishable.

11. All the *E. coli* (100%) isolates in Kafue lechwe from Blue-lagoon NP were non-sorbitol fermenting while the proportion of non-sorbitol fermenting *E. coli* isolates from Lochinvar NP was 69.6% (55/79).

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