OCCURRENCE OF *SALMONELLAE*, ECTO- AND GASTRO-INTESTINAL PARASITES AND ASSESSMENT OF BIOSECURITY IN KANO ZOOLOGICAL GARDEN, NIGERIA

BY

AMINU HANGA BELLO (DVM)

DEPARTMENT OF VETERINARY MEDICINE,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

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BY

Aminu Hanga BELLO (DVM)
(P13VTVM8004)

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DEPARTMENT OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

JULY, 2017

DECLARATION

I declare that the work in this Dissertation titled "OCCURRENCE OF *SALMONELLAE*, ECTO- AND GASTRO-INTESTINAL PARASITES AND ASSESSSMENT OF BIOSECURITY IN KANO ZOOLOGIAL GARDEN, NIGERIA" has been carried out by me in the Department of Veterinary Medicine. The information derived from literature has been duly acknowledged in text and a list of references provided. No part of this Dissertation was previously presented for another degree or diploma at this or any other Institution.

Aminu Hanga BELLO _____ _____

Signature **Date**

CERTIFICATION

This Dissertation titled "OCCURRENCE OF *SALMONELLAE,* ECTO- AND GASTRO-INTESTINAL PARASITES AND ASSESSSMENT OF BIOSECURITY IN KANO ZOOLOGIAL GARDEN, NIGERIA" by Aminu Hanga BELLO meets the regulations governing the award of Master of Science degree in Wildlife Medicine of Ahmadu Bello University Zaria, and is approved for its contribution to scientific knowledge and literary presentations.

Prof. P.A. Abdu	_____	_____
Chairman, Supervisory Committee	Signature	Date
Prof. C.A. Kudi	_____	_____
Member, Supervisory Committee	Signature	Date
Prof. L.B. Tekdek	_____	_____
Head, Department of Veterinary Medicine	Signature	Date
Prof. Kabir Bala	_____	_____
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This work is dedicated to Almighty Allah with whose blessings and bounties all good deeds are accomplished.

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ABSTRACT

Infectious and parasitic diseases in zoo animals affect their welfare, reproduction and longevity and pose health threat to researchers, visitors and staff of zoological garden. The aim of this study was to assess biosecurity and determine occurrence of *Salmonellae,* ecto- and gastro-intestinal parasites (GIP) in Kano zoological garden, Nigeria (KZG). A total of 388 samples were collected from 161 wild animals by non-random sampling method. The distribution of the samples was: one hundred and eleven cloacal swabs from wild birds; one hundred and seventy faecal samples from carnivores (28), herbivores (62), non-human primates (35) and wild birds (45); one hundred and seven ectoparasite (EP) samples from herbivores (2), carnivores (53), non-human primates (27) and wild birds (25). Conventional biochemical tests were used to identify *Salmonella* species after which suspected/confirmed isolates were subjected to antimicrobial sensitivity test using a panel of 12 antimicrobial agents. Simple flotation technique and microscopic examination methods were used to identify gastro-intestinal parasite eggs/oocysts and ecto-parasites respectively. Of the total faecal and rectal swab samples (196) examined for *Salmonella,* seven isolates (3.57%) were confirmed; two were from lions (*Panthera leo*), one each from bush buck (*Tragalophus scriptus*), Cape eland (*Tragalophus oryx*), Egyptian geese (*Alopochen aegyptiacus*), parrot (*Psittacus erithacus*) and crested porcupine (*Hystrix cristata*) respectively. The occurrence of *Salmonella* was 7.14% in carnivores, 5.76% in herbivores and 2.7% in wild birds while the overall occurrence in KZG was 4.8%. All the *Salmonella* isolates (100%) showed multidrug-resistance (MDR) pattern with resistance profile of 3-4. However, none of the isolates showed mild, extensive or pan drug resistance. Eggs and oocysts were identified from 85 faecal samples: *Ascaris* from tortoise; *Enterobius* from chimpanzee; *Strongyle* from tantalus, red patas and tortoise; *Taenia* from lion; *Toxocara* from lion, Nubian vulture and mangoose;

Trichurid from baboon, buffalo, porcupine, red patas and tantalus; *Coccidia* from peacock and red patas; and *Isospora* from lion and mangoose. The occurrence of GIP egg was highest among non-human primates (37%) and lowest among wild birds (13.5%). Among herbivores, the occurrence was 26.9% while in carnivores was 14.28%. The gastro-intestinal parasite richness count (GIPRC) among carnivores, herbivores, non-human primates and wild birds was 5/3, 5/3, 7/4 and 4/2 respectively. The overall occurrence of GIP eggs and oocysts in KZG was 63% and GIPRC was 21/8. Bug (*Cimex lectularius*) was identified from baboon and red patas; and *Rhipicephalus sanguineus* tick from buffalo and common jackal. The occurrence of EP and ecto-parasite richness count (EPRC) among buffalos, common jackal, baboon, red patas and spotted eagle owl were 100% and 2/3; 33% and 2/3; 25% and 1/3; 16% and 2/3; 20% and 1/3 respectively. Of the nine components of zoo biosecurity assessed in KZG, quarantine practices had highest biosecurity risk (100%) and risk level (2.6) while work and hygiene practices for staff and visitors poses lowest biosecurity risk of 58.3% and risk level of 2.0. Audit and validation of biosecurity practices in property management and wildlife sections revealed breaches in traffic control, isolation and sanitation in many sections (70%) of KZG.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABU	Ahmadu Bello University
^{o} C	Degree Celsius
EIDs	Emerging Infectious Diseases
EP	Ectoparasite
EPRC	Ectoparasite richness count
FAO	Food and Agriculture Organization
FGN	Federal Government of Nigeria
GIP	Gastrointestinal parasite
GIPRC	Gastrointestinal parasite richness count
>	Greater than
<	Less than
KZG	Kano zoological garden
KAZOWMA	Kano zoological and wildlife management agency
LGAs	Local Government Areas
µl	Microlitres
MDR	Multidrug resistance
ml	Millilitres

nm	Nanometre
OIE	Office International des' Epizootics
%	Percentage
Sp	Species
SPSS	Statistical Package for Social Science
WB	Wild Bird
WHO	World Health Organization
X^2	Chi Square value

1.0 INTRODUCTION

1.1 Background to the Study

Infectious disease within zoo collection impacts on individual health and welfare, and can have long term impacts on reproduction, longevity, behaviours, population and species viability (Reiss and Woods, 2011). Subclinical and chronic diseases can exert their effects for years and even decades. Sickness, death and reproductive failure in collection animals leads to greater costs (husbandry, veterinary care, acquisition), and reduces the financial viability of the zoo as a business. Infectious diseases like salmonellosis that may spread to humans or domestic animals can have serious social, economic and ethical costs (Reiss and Woods, 2011).

Salmonellosis is caused by *Salmonella* species and characterized clinically by one or more of three major syndromes: septicaemia, acute and chronic enteritis (Kahn and Line, 2005). The portal of infection in Salmonellosis is almost always the mouth, so that the severity of the disease in an individual or of an outbreak in a group depends on the degree of contamination and environmental conditions; temperature and dryness, which determine the survival time of *Salmonellae* (Radostits, 1997). The response to infection with *Salmonella* varies depending on the size of the challenging dose and the immunological status of the animal (Radostits, 1997).

Parasitism is an important environmental component of the life cycle of most organisms including birds and wildlife (Loye and Zuke, 1991). While ecto-parasites live on the surface of the host's body, endo-parasites are found within the body of the host (Narula, 2013). Helminth parasites like *Trichuris* and *Strongyle* are significant pathogens of wildlife and responsible for unthriftness, decrease in fecundity rates and sometimes death (Davies and Anderson, 2004). Overcrowding, dampness and unsanitary conditions are the predisposing factors for the proliferation of helminthosis and infectious diseases (Radostits, 1997). Such adverse conditions occur frequently under captivity

than under free-living conditions (Devos and Lambrechts, 2003). Helminthes are the predominant macro parasites found in birds and with heavy infestations they cause morbidity and mortality in wild birds (Norton and Ruff, 1997). Single and mixed infestation of mites and nematodes has also been reported in a variety of wild animals kept in captivity for long periods (Mbaya, 2006). Young animals and those stressed by translocation, disease or injury are the most likely to be affected by parasites (Mbaya, 2006; Mbaya *et al*, 2007).

Biosecurity is the set of precautions taken to minimize the risk of introducing and establishing infectious and parasitic diseases into animal population (Reiss and Woods, 2011). Good zoo biosecurity help keep zoo animals, zoo staff and visitors safe and healthy, minimize costs of treatment, keep zoos open and running during infectious disease outbreak, promote the good reputation of individual zoo and assist zoos in acquiring and managing exotic species (Reiss and Woods, 2011). A zoo's ability to protect itself from a disease outbreak will be greatly improved if it has appropriate biosecurity arrangements (Reiss and Woods, 2011).

1.2 Statement of Research Problem

Wildlife are reservoirs of diseases that affect other animals and humans and in many cases animals in captivity are not screened for such diseases. Most veterinary schools in Nigeria do not offer wildlife medicine as a specialty area; this impacts negatively on wildlife health in zoological gardens, wildlife parks, game reserves and other animal sanctuaries. Due to shortage of funding some zoos lack adequate treatment and facilities, do not employ veterinarians as staff while some do not offer professional training on wildlife disease management.

Captive wild birds carry pathogens that may not result in disease in the wild, but once taken into captivity and exposed to a number of stress factors, resistance is lowered and disease can break-out

(Schmid, 2005). The removal of wild birds from their natural habitat has had a hugely detrimental impact on the population of many species (Beissinger, 2001). Many species of captive – reared birds, aquatic species and other captive collections commonly become infected with *Salmonellae* and die from salmonellosis in zoological gardens (Friend, 2001). Game birds such as grouse and pheasants reared in captivity for sporting purposes and cranes for species conservation are often victims of salmonellosis (Friend, 2001). Free-flying birds rarely manifest clinical diseases and may frequently serve as reservoirs of many parasite species (Fallis and Benneth, 1960; Levine, 1963; Carlton and Herman, 1970; Herman and Brischoff, 1994; Mbaya, 2006; Oladele *et al*, 2012).

1.3 Justification of the Study

Paucity of information regarding wildlife diseases in KZG and Nigeria justifies this research. Zoo visitors interact with captive and free–range wild birds while eating, drinking and sitting thus creating a favourable condition for the spread of infectious agents. Wildlife plays a key role by providing a 'zoonotic pool' from which new diseases may emerge (Deszak, 2000). Emergence of key zoonotic diseases such as Ebola from wildlife populations has also increased awareness worldwide of the importance of the study of captive wildlife diseases in protecting both livestock and public health (Embrey *et al.,* 2012).

Presence of free–range wild birds scavenging on animal feed in KZG and unruly behaviour of some visitors who offer toys and feed to animals poses a serious biosecurity breach. Lack of proper understanding of biosecurity and negligence of some zoo keepers who violate hygiene procedures could also facilitate disease transmission in zoological gardens. Treatment and control measures against parasitic diseases are carried out on regular basis in KZG but baseline data to evaluate the success or failure of the program is not available. Also, the impact of biosecurity practices on the

health status of wildlife in KZG is not assessed. A comprehensive study of parasitic and infectious diseases in captive wildlife would aid in the development of possible control measures which may help in enhancing their conservation, survival and performance in captivity. For more than forty years KZG has served as recreational, educational and tourist destination with average of 250,000 – 300,000 visitors annually (KAZOWMA, 1972). Additionally, description of parasites and diseases in free-living and captive animals may help to evaluate the importance of host-parasite relationship in each environment (Carlton and Hermen, 1970). Each zoo's unique characteristics will influence its biosecurity requirements and individual zoos are encouraged to develop their own biosecurity plan (Reiss and Woods, 2011). Biosecurity is the best approach for preventing the spread of diseases in zoos; within wildlife, between wildlife and humans or vice-versa. The last outbreak of Ebola triggered donation of several wildlife to KZG raising concern over the health status of wildlife in KZG.

1.4 Aim of the Study

The aim of the study was to assess biosecurity and to determine the occurrence of *Salmonella* species, ecto- and gastro-intestinal parasites in wildlife in KZG.

1.5 Objectives of the Study

The objectives of the study were to:

1. Conduct an assessment of biosecurity practices observed in KZG.

2. Isolate and characterize by biochemical means, *Salmonella* species from wildlife in KZG.
3. Determine antimicrobial resistance of *Salmonella* species from wildlife in KZG.
4. Collect and identify ecto- and gastro-intestinal parasites from wildlife in KZG.

1.6 Research Questions

1. What is the level of biosecurity measures observed in KZG?
2. Are there *Salmonella* species infecting wildlife in KZG?
3. What is the antimicrobial resistance profile of *Salmonella* species from wildlife in KZG?
4. What is the occurrence of ecto- and gastro-intestinal parasites in wildlife in KZG?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Zoo and Zoological Garden

Zoo, also known as zoological garden or zoological park is an institution devoted to the exhibition, preservation and study of wild animals (Burnie, 2009). A Zoo is also defined as a place where a great number of valuable wildlife species are kept together, after being taken out of their natural habitats (Penayatoba–Pencheva, 2013).

At least 600 million people visit more than 1,000 zoos around the world per year. Although most people visit zoos for entertainment, zoos also educate the public about animal behavior, natural habitats and the plight of animals in danger of extinction. Zoos play a role in the conservation of threatened or endangered animals and conduct scientific research on animal diseases, nutrition, reproduction and behavior (Burnie, 2009).

Zoos employ several kinds of workers to care for animals. Zoologists arrange captive breeding programs and make decisions about type of species put on public display and the housing they need while veterinarians are concerned with health of the animals. Zoo keepers tend to the day-to-day welfare of the animals in their charge, including feeding, grooming and maintaining enclosures (Burnie, 2009).

The earliest known collections of captive animals date back more than 4,000 years. In ancient Egypt, the pharaohs acquired giraffes, monkeys and gazelles from the southern edges of the Sahara. From India they imported chickens, at the time considered exotic birds which proved to have a very practical value. In the Middle East, the kings of Assyria collected animals in the course of their conquests, keeping them in landscaped zoological gardens. Among them were Asian elephants and

Bactrian camels, as well as crocodiles and hippopotamuses, which were kept in specially constructed pools (Burnie, 2009).

In 1490 BC, Queen Hatshepsut of Egypt launched what is thought to be the first animal collecting expedition. Five ships ventured as far as Somalia and returned with leopards, exotic birds, monkeys, and a giraffe. The animals were all housed in the queen's palace menagerie. Two centuries later another Egyptian ruler, Ramses II, took pride in his collection of animals, particularly a lion that was said to have followed his master everywhere, even into battle. In 1000 BC, Chinese emperor Wen Wang established a 600-hectare animal preserve. Called the Garden of Intelligence, this animal collection was established to study and learn from wildlife (Burnie, 2009).

The first zoo that closely resembles modern zoo was the imperial Menagerie, established in 1752 in Vienna, Austria (Burnie, 2009). Modern zoo design dates back to the early 1900s, when animal trainer Carl Hagenbeck opened a zoo in Stellingen, Germany. Hagenbeck housed animals in outdoor enclosures that used ditches and moats instead of bars. Many of these enclosures were built to imitate natural habitats, such as rocky mountainsides or open plains (Burnie, 2009). The first modern zoos founded in the United States were the Philadelphia Zoological Gardens in Pennsylvania, established in 1859; Central Park Zoo in New York, which opened in 1861; and Lincoln Park Zoo in Chicago, Illinois, which opened in 1868 (Burnie, 2009).

The major goals of modern zoo include animal welfare, education, conservation, research and entertainment. However, these goals may be in conflict. For example visitors enjoy learning and observing natural behavior in captive animals but visitors often want to observe and interact with animals in close proximity (Fernandez, 2009). Unfortunately, close proximity and social interaction with human induce stress for many species, particularly non-human primates. Modern zoos are also concerned with the effect of exhibit design and behavior of animals on zoo visitors as well as the effect of visitors on behavior of exhibited animals (Fernandez, 2009).

2.2 Wildlife and Captive Wildlife

The Encarta dictionary define wildlife as wild animals, birds and other living things including vegetation, living in a natural, undomesticated, untamed and uncultivated state (Encarta, 2009).

When wild animals are kept in confinement or captivity they are referred to as "captive wildlife". Captivity has become a way of life for many species of animals because for hundreds of years man has made a practice of capturing animals from the wild for use in captivity especially in zoos. This gives zoo visitors an exciting glimpse of life from the wild (Anon, 2012).

2.3 Wildlife Diseases

Historically, wildlife diseases have been considered important only when agriculture or human health have been threatened. However, because of outbreaks of diseases in endangered species, increasing veterinary involvement, and advances in host-parasite population biology, the threat of wildlife diseases is now taken more seriously (Deszak, 2000).

Many wildlife species are reservoirs of pathogens that threaten domestic animal and human health, as well as emerging wildlife infectious diseases (EWIDs), which pose a substantial threat to the conservation of global biodiversity. Disease emergence most frequently results from a change in ecology of host, pathogen or both (Deszak, 2000). Human population expansion has promoted the emergence of EIDs via increasing population density, especially in urban areas (dengue, cholera), and encroachment into wildlife habitat (Ross River virus disease). This encroachment may have been a key factor in Africa for the global emergence of Marburg and Ebola viruses and human immunodeficiency virus (HIV) (Deszak, 2000).

EIDs of wild animals can be classified into three major groups on the basis of key epizootiological criteria:

i. EIDs associated with "spill-over" from domestic animals to wildlife populations living in proximity

ii. EIDs related directly to human intervention, via host or parasite translocations and

iii. EIDs with no overt human or domestic animal involvement.

Wildlife diseases can have negative consequences for biodiversity, human and livestock health, animal welfare and the economy. Wildlife plays a key role by providing a 'zoonotic pool' from which new diseases may emerge. Wildlife play a major role in disease transmission and so is important when addressing certain diseases in domestic animals or humans (Deszak, 2000).

The majorities (60%) of emerging human infectious diseases are caused by zoonotic pathogens and 75% of these have a wildlife origin or link (Warwick, 2012). Human encroachment on shrinking wildlife habitats can cause increased wildlife population densities which can boost disease transmission risks. Also, increased human population density is linked to a rise in the number of zoonotic infections in humans. Wildlife diseases are also important in their own right, with impacts on biodiversity and animal welfare (Deszak, 2000). Climate change is expected to lead to substantial changes in wildlife disease patterns and frequency. As conservation programs expand and contact between humans, domestic animals and wildlife increases, conflicts between biodiversity, conservation, public health and domestic animal health may intensify (Anon, 2008).

2.4 Biosecurity in Zoological Gardens

Biosecurity is the set of precautions taken to minimise the risk of introducing an infectious disease into an animal population (Reiss and Woods, 2011).

Good biosecurity is integral to the successful management of all zoos. Biosecurity is important for all zoos, regardless of size. With today's growing focus on biosecurity management, it is important that zoo biosecurity focuses on all risks, not just those arising from exotic species. All zoos (including smaller zoos and fauna parks holding few or no exotic species) need to address the biosecurity risks relevant to their circumstances. All zoo staff needs to be aware of the principles of biosecurity and how this applies to their work at the zoo.

Biosecurity is an insurance policy against disease outbreak and its consequences. Biosecurity is concerned with recognizing and managing risk. Individual zoos can achieve best practice by conducting an institution-specific biosecurity risk assessment to establish the level of risk that exists in each area of its operations. Zoos are encouraged to develop their own institution-specific biosecurity Plans (Reiss and Woods, 2011).

Good zoo biosecurity aims to prevent the following:

I. Introduction of infectious disease and contaminants to zoo animals.
II. Spread of disease from an infected area to an uninfected area within the zoo.
III. Spread of infectious disease from zoo animals to animals outside the zoo.
IV. Spread of infectious disease from animals to humans or humans to animals.

It is important to consider all factors that may impact on zoo biosecurity, including: species, origin and number of collection animals, location and layout of the zoo, source of water supply, source of food supply, method of waste management, disease status of collection animals, disease status and proximity to animals in the surrounding area, type of wildlife and pest species zoonotic disease

potential, animal movements and transactions and movement of staff, visitors, contractors and deliveries (Reiss and Woods, 2011).

2.5 Levels of Biosecurity in Zoological Gardens

2.5.1. Routine biosecurity procedures

The majority of biosecurity measures outlined below should be applied on a routine or daily basis by most zoos in most circumstances. Maintaining these levels of routine biosecurity will give a high assurance that disease agents are not carried into animal enclosures and will reduce the risk of disease transmission between enclosures. They include:

I. Record keeping, animal identification, staff training and documentation.
II. Property management: this includes input and output, perimeter management, enclosure and ground maintenance, drainage and waste disposal.
III. Work and hygiene procedures for staff and visitors.
IV. Zoonotic disease risk management.
V. Animal health and preventative medicine.
VI. Quarantine: general quarantine practices, veterinary care and investigation during quarantine and management of sick animals.
VII. Animal deaths, post-mortem examination and carcass disposal.
VIII. Management of animals, vehicles and equipment during animal transport.
IX. Zoo-specific biosecurity plan and emergency biosecurity response plan.

2.5.2 Higher level biosecurity procedures

Some biosecurity measures may not be a necessary part of routine practice in zoos, but may be implemented in situations or circumstances outside the normal. Higher level biosecurity procedures may be adopted by individual zoos, according to zoo-specific circumstance and risk. In the event of an increased disease risk (e.g. infectious disease event in one enclosure, changed health status of individuals), an increased level of biosecurity should be implemented as determined by the circumstances (Reiss and Woods, 2011).

2.5.3 Emergency biosecurity response plans

These are emergency response plans which zoos are encouraged to develop for use in the event of a suspected outbreak of an emergency disease, serious endemic disease or an unusual increase in mortality or illness.

The Emergency Biosecurity Response Plan should include protocols for work practices, restriction on animal, staff and visitor movement and should detail the agencies and authorities which need to be informed (Reiss and Woods, 2011).

2.6 Salmonellosis in Zoological Gardens

Animals kept at the zoo are usually bred in captivity, acquired from other facilities or captured in the wild and have been reported to be associated with bacterial infections, which are major health hazard, as their excretion result in contamination of the environment leading to morbidity and mortality of other animals as well as significant economic losses for the zoo (Adesiyun *et al*., 1984: Gopee *et al*., 2000).

Bacterial pathogens such as *Salmonella* spp, *Escherichia. coli* and *Clostridium perfringens* are zoonotic and can therefore be interchanged between zoo keepers and captive wildlife (Gopee *et al*., 2000). These pathogens are commonly associated with outbreaks of diarrhoea, septicaemia, enteritis, fever, dysentery, abortion and numerous other infections individually or in association. The genus *Salmonella* has become increasingly significant due to their ubiquitous distribution, wide host range, complex pathogenesis and their complicated epizootiology involving humans, animals and the environment (Oludairo *et al.*, 2013).

Probable sources of infection for zoo animals are poor biosecurity practices such as poor hygiene, feeding animals with unwholesome fruits and foods by zoo keepers and visitors, native rodents and wild birds which gain access to the enclosures (Goope *et al*., 2000).

2.7 *Salmonella*

A German named Gaffky in 1884 cultivated the typhoid bacillus–*S. enterica* serovar Typhi that was first observed by Eberth in 1880 from the spleen and mesenteric lymph nodes of infected patients (Le Minor, 1994). Later in 1885, two American veterinarians, Salmon and Smith, isolated the bacterium causing hog cholera (*Salmonella choleraesius*) from infected pigs (Salmon and Smith, 1886). The name *Salmonella* was subsequently adopted in honor of Dr. Salmon (Mestrovic, 2015).

Salmonella species have assumed increased significance due to their ubiquitous distribution, the growing number of serotypes, wide host range (including wildlife), complex pathogenesis, and complicated epizootiology involving humans, domesticated and wild animals and the environment. The carrier state is the major source of infection for animals and humans. Excretion of the organism results in the contamination of water, food and the environment with wildlife animals playing important roles (Oludairo *et al*., 2013).

The genus Salmonella is composed of motile bacteria which conform to the definition of the family Enterobacteriaceae *and* tribe Salmonellae. During their biochemical reactions hydrogen sulphide is produced, methyl red reaction is positive, lysine and ornithine are decarboxylated, arginine is dehydrolysed, indole is not formed, urea is not hydrolysed, Voges-Proskauer test is negative and neither phenylalanine nor tryptophan is deaminated. However, acid is not produced from sucrose, adonitol, raffinose or alpha - methyl glucoside. Lactose is fermented by most strains belonging to subspecies ❜❜❜a and ❜❜❜b but not by those of ❜, ❜❜, ❜V or V. Dulcitol is fermented by members of subspecies ❜, ❜❜ and ⊗ but not by those of ❜❜❜a and ❜❜❜b or ❜V. Inositol is not fermented by strains of subspecies ❜❜❜a and ❜❜❜b, ❜V and ⊗ (Ewing, 1986).

Subspecies ⊗❜ was later described by Le Minor and others in 1986 consisting of strains that are inositol and sorbitol negative, with 22% fermenting lactose and 67% fermenting dulcitol (Le Minor *et al.,* 1986).

Currently, the nomenclature system used at the Centers for Disease Control (CDC) for the Genus *Salmonella* is based on recommendations from the WHO Collaborating Centre. According to the CDC system, the genus *Salmonella* contains two species, *S. enterica*, the type species and *S. bongori.* All antigenic formulae of recognized *Salmonella* serotypes are listed in a document called Kauffman–White scheme also known as *Salmonella* antigenic formula (Popoff, 2001). There have been two supplements to this scheme since then (Guibourdenche *et al.,* 2010; Issenhuth–Jeanjean *et al.,* 2014), which now brings the total number of serovars to 2,659.

The number of serovars in each species and subspecies are as follows:

S. enterica	2,637
S. enterica subspp *enterica*	1,586

S. enterica subspp *salamae*	522
S. enterica subspp *diarizonae*	338
S. enterica subspp *arizonae*	102
S. enterica subspp *houtenae*	76
S. enerica subspp *indica*	13
S. bongori	22
Total	2659

(Adapted from Issenhuth–Jeanjean *et al.,* 2014).

2.8 Salmonellosis

Salmonellosis in both human and animal host is generally associated with *Salmonella enterica* (also termed subspecies I) and acute infections can present in one or four ways : enteric fever, gastroenteritis, bacteaemia and extraintestinal focal infection. As with other infectious diseases, the course and outcome of the infection are dependent upon a variety of factors including: inoculating dose, immune status of the host and genetic background of both host and infecting organism (Cammie and Miller, 2000). Broadly speaking, the *Salmonella enterica* infections can be subdivided into two groups: the enteric fever (typhoidal) group and non–typhoidal *Salmonella* (NTS), which typically cause gastroenteritis but can also cause invasive disease under certain conditions. There are five serotypes of *Salmonella* associated with enteric fever: *Salmonella enterica* subspecies *enterica* serovar Typhi (Typhi), *S.* Paratyphi A, *S.* Paratyphi B, *S.* Paratyphi C and *S.* Sendai (Selander *et al.*, 1990). Most *Salmonella* strains cause gastroenteritis while some strains, particularly *S. enterica*

serotypes *typhi* and *paratyphi,* are more invasive and typically cause enteric fever which is a serious infection that poses problems for treatment due to antibiotic resistance.

2.8.1 Typhoidal salmonellosis

Typhoidal *Salmonella* are host-specific *Salmonella* affecting only humans but occasionally may be seen in other animals. The World Health Organization (WHO) estimated that globally there are more than 22 million cases of typhoid fever each year with more than 200,000 deaths (WHO, 2003).

2.8.2 Host-adapted salmonellosis (*S. Gallinarum* and *S. Pullorum*)

Fowl typhoid and pullorum disease (bacillary white diarrhoea), caused by *Salmonella enterica* subspecies *enterica* serovars Gallinarum and Pullorum, respectively, are widely distributed throughout the world, especially in countries with less developed poultry industries (Shivaprasad, 2000; OIE, 2012). *Salmonella gallinarum* and *S. pullorum* are adapted to avian species and considered to pose minimal zoonotic risk (Eswarappa *et al.,* 2009), although the genome is continually evolving, which could theoretically widen the host range in future (Liu *et al.*, 2002).

2.8.3 Non- typhoidal salmonellosis (Paratyphoid *Salmonella*)

Non-typhoidal *Salmonellae* (NTS) are important food-borne pathogens with acute gastroenteritis being the most common clinical manifestation (Kariuki *et al.,* 2006). However, invasion beyond the gastro-intestinal tract occur in approximately 5% of the patients with NTS gastroenteritis resulting in

bacteraemia (Hoemann, 2001). Non-typhoidal Salmonellosis is acquired from multiple animal reservoirs. The main mode of transmission is from food products contaminated with animal products or wastes (Miller *et al.,* 2000). In developing countries NTS is an important cause of invasive disease, particularly in tropical regions of Africa, where *Salmonella enterica* serovar *Typhimurium* (*S. Typhimurium*) and *Salmonella enterica* serovar *Enteritidis* (*S. enteritidis*) are consistently the most common causes of childhood bacteraemia as well as important causes of meningitis, arthritis and pneumonia (Kariuki *et al.,* 2006).

2.8.4 Treatment of salmonellosis

Fluoroquinolones are widely regarded as optimal for the treatment of typhoid fever. They are well tolerated and more rapidly and reliably effective than the first-line drugs, viz. chloramphenicol, amoxicillin and trimethoprim-sulfamethoxazole (WHO, 2003). The third generation cephalosporins (ceftriaxone, cefixime, cefotaxime and cefoperazone) and erythromycin are also effective drugs for typhoid (Miller *et al.,* 2000). In general, in areas with high prevalence of multi-drug resistant *Salmonella* infections, all patients suspected of having typhoid fever should be treated with a quinolone or third generation cephalosporin until the result of culture sensitivity studies become available (Miller *et al.,* 2000).

Gastroenteritis caused by *Salmonella* is usually a self-limiting disease and therapy should be directed to the replacement of fluid and electrolyte losses. Therefore, antimicrobials should not be used routinely to treat uncomplicated non-typhoidal *Salmonella* gastroenteritis or to reduce convalescent stool excretion (Richards *et al.,* 1993). However, antimicrobial therapy should be considered for any systemic infection (Parry *et al.,* 2002). For susceptible organisms, treatment with an oral quinolone,

trimethoprim-sulfamethoxazole or amoxicillin is adequate (Parry *et al.,* 2002). Amoxicillin and trimethoprim-sulfamethoxazole are effective in eradication of long-term carriage. The high concentrarion of amoxicillin and quinolone in bile and the intracellular penetration of quinolone are theoretical advantages over trimethoprim-sulfamethoxazole (WHO, 2003).

2.8.5 Pathogenesis and immunology of salmonellosis

All *Salmonella* infections begin with the ingestion of contaminated food or water. After leaving the stomach, *Salmonella* must traverse the mucosal layer overlaying the epithelium of the small intestine. After crossing the mucosal layer overlaying the small intestine, *Salmonella* interacts with both enterocytes and macrophage cells (Francis *et al.,* 1992). The organisms are rapidly internalized and transported into sub-mucosal lymphoid tissue where they may enter into systemic circulation. *Salmonella* also have the ability to induce non–phagocytic epithelial cells by a process known as bacterial-mediated endocytosis. This process involves the formation of large membrane ruffles around the organism and cytoskeleton rearrangement (Francis *et al.,* 1992). Salmonella is then internalized and transported into sub-mucosal lymphoid tissue where they may enter into systemic circulation (Rathman *et al.,* 1997).

Salmonella Pathogenicity Island (SPI1) function is required for the initial stages of salmonellosis, i.e the entry of *Salmonella* into non-phagocytic cells by triggering invasion and the penetration of the gastro-intestinal epithelium. Furthermore, SPI1 is required for the onset of diarrheal symptoms during localized gastro-intestinal infections. The function of SPI1 is required for later stages of the infection, i.e. systemic spread and the colonization of host organs. The role of SPI2 for survival and replication in the host phagocytes appears to be essential for this phase of pathogenesis (Hansen–Westar and Hansel, 2001).

2.9 Isolation of *Salmonella*

2.9.1 *Salmonella* transport/pre–enrichment media

Transport/ pre–enrichment media are used to support the life of *Salmonella* especially if the samples are to be transported for a long duration. These media allow abundant growth with uniform morbidity. These include:

i. Peptone water

ii. Tryptone soya broth

iii. Nutrient broth (Cruickshank *et al.,* 1975) and

iv. Rappaport–Vassiliadis (RV) (Waltman, 2000).

2.9.2 *Salmonella* enrichment media

These liquid media are used to assist in the isolation of *Salmonella* from faeces, sewage and other materials with mixed bacterial flora by aiding the growth of *Salmonella* while limiting that of *E. coli* and other organisms before plating on solid media. They include:

I. Tetrathionate broth with or without brilliant green: this increases selectivity for *Salmonella* but is too inhibitory for *S. typhi.*

II. Selenite - F broth: this is the most used enrichment medium for *Salmonella* isolation.

III. Strontium chloride broth was found to be superior to selenite – F broth especially for the isolation of *S. typhi.*

IV. Selenite-M.

V. Ruy's medium.

VI. Rappaport Vassiliadis.

VII. ¼ Ringer's solution.

2.10 Antimicrobial Resistance of *Salmonella*

Antimicrobial resistance is the ability of microbes such as bacteria, viruses, parasites or fungi to grow in the presence of a chemical (drug) that would normally kill it or limit its growth (Palmgreen *et al.,* 2006).

Resistance to various classes of antimicrobial agents has been encountered in many bacteria of medical and veterinary relevance. Over the years various studies have reported the presence of genes and mutations conferring resistance to antimicrobial agents in zoonotic bacteria such as *Salmonella* (Palmgreen *et al.,* 2006). There are three major mechanisms reported by which bacteria may become resistant to antimicrobial agents: enzymatic inactivation; reduced intracellular accumulation of the antimicrobials; protection, alteration or replacement of the cellular target sites (Schwartz and Claus – Danela, 2001).

Multi–drug resistance which is defined as antibiotic resistance against three or more antibiotics is becoming prominent with *Salmonella* (Palmgreen *et al.,* 2006) and is limiting the choice of drug therapy for *Salmonella* infections in both humans and animals and raises more public health

questions (Tacket *et al.,* 1985). Furthermore, some variants of *Salmonella* have developed multidrug-resistance as an integral part of their genetic material. Consequently, these variants are likely to retain their drug–resistant genes even when antimicrobial drugs are no longer used (Anon, 2012).

When fluoroquinolones were first licensed for therapy in humans, no immediate rise in *Salmonella* resistance was observed. But following the licensing of fluoroquinolones for use in food animals, the rates of fluoroquinolone–resistant *Salmonella* in animals and feed and subsequently in human infections rapidly increased in several countries. While resistance to fluoroquinolones often emerges as a result of mutations in the bacterial genome, resistance to other antimicrobials often spread by transfer of DNA between bacterial strains (Anon, 2012).

Most *Salmonella* strains are sensitive to chloramphenicol, ampicillin, streptomycin, tetracycline, cotrimoxazole and some other antibiotics. Chloramphenicol was considered to be the most effective drug in the treatment of typhoid fever. However, some strains are highly resistant to some of these antibiotics as a result of mutation or acquisition of transmissible resistance plasmid (Anon., 2012). This, therefore makes it necessary to test the antibiotic sensitivities of any *Salmonella* isolated (Cruickshank *et al.,* 1975).

Following the isolation of six *Salmonellae* from wildlife at Agodi Garden and University of Ibadan Zoo, Falade and Durojaiye (1976) carried out disc antibiotic sensitivity tests for eight antibiotics on blood agar culture. The result indicated that all the bacteria were resistant to sulphaforazole and penicillin; four were resistant to terramycin and erythromycin while the other two were sensitive. Three were also resistant to streptomycin. However, all the isolates were sensitive to chloramphenicol and nitrofurazone (Falade and Durojaiye, 1976). A Danish study found out that

although persons with susceptible *Salmonella* infections had a higher mortality than the general population, persons with resistant *Salmonella* had an even higher mortality (Anon, 2012).

2.11 Prevention and Control of Salmonellosis in Zoological Gardens

In developing countries, reducing the number of non typhoidal salmonellosis cases in the general population requires provision of safe drinking water, effective sewage disposal and hygienic food preparation (Mastroeni, 2006). Non-typhoidal salmonellosis is a major public health problem world-wide and reduction of these diseases presents a serious and challenging problem because they have several animal reservoirs (Strugnell and Wijburg, 2006). Some of the measures to prevent and control the spread of *Salmonella* infection in zoological gardens include the following:

I. After visiting zoos people should wash their hands with sanitizer, detergent or soap and water before eating or handling food.
II. Zoo management should provide adequate hand washing and public convenience facilities for staff and visitors respectively.
III. Routine surveillance, quarantine and screening of newly acquired animals to detect the presence of *Salmonella*.
IV. Cages and enclosures housing animals should be routinely cleaned and disinfected with phenolic compounds or household bleach diluted at 1:32 (CDC, 2009).
V. Visitors should be discouraged from offering food to the animals as this could be a source of infection to the animals (CDC, 2009).

VI. Good biosecurity practices such as provision of hygienic food, reducing stress, adequate housing and control of rodent species are likely to reduce rate of infection (Hoelzer *et al.*, 2011).

VII. Screening and treating zoo staff to prevent infection of the animals since human carriers of non-typhoid *Salmonella* may transmit the infection as reverse zoonoses (CDC, 2009).

2.12 *Salmonella* in Wildlife of Nigerian Zoological Gardens

Salmonella are found in many species of wildlife including birds, reptiles and aquatic animals where they often cause diseases, acute and chronic diarrhea and deaths. Despite this, reported cases of *Salmonella* infection in wild animals in Nigeria and Africa are few (Oludairo *et al,* 2013).

Falade and Durojaiye (1976) reported no published report on *Salmonella* in wildlife in Nigeria, but noted few unpublished isolation of *S. aba* and *S. takoradi* from a lizard and dead baby elephants and *S. typhimurium* from a healthy monkey and gorilla from the University of Ibadan Zoo.

Moreover, unexplained diarrhea among captive wildlife at the Agodi Gardens prompted a bacteriological survey of wildlife at both the Agodi Gardens and the University of Ibadan Zoo. The result showed eight *Salmonella* isolates of six different serotypes; six were isolated from the wildlife sampled at the Agodi Gardens, while two were isolated from the wildlife sampled at the University of Ibadan Zoo (Oludairo *et al.*, 2013).

The wildlife and the respective *Salmonella* isolated from them included: Putty nosed monkey, *S. Weybridge*; Green monkey, *S. Weybridge*; Patas monkey, *S. Offa*; Baboon, *S. saint-paul*; Marsh mongoose, *S. Saint-paul*; Civet cat, *S. glostrup*; Hyena, *S. Wimborne* and *S. dublin* and Aviary, *S. wimborne* (Oludairo *et al.*, 2013).

Okoh and Onazi, (1980) reported the isolation of 14 strains of *Salmonella* from a variety of carcasses, faecal samples and morbid wildlife from KZG. The animals and the respective samples that yielded *Salmonella* isolates were pigeon liver; *S. typhimurium*, parrot small intestine; *S. give*, peacock liver; *S. gallinarum*, flamingo faeces; *S. apeyeme*, pelican small intestine; *S. tilene*, vulture liver; *S. gallinarum*, gazelle small intestine; *S. epicrates*, giraffe liver; *S. dublin*, galago bush baby small intestine; *S. durban*, kangaroo liver; *S. vejle*, hyena faeces; *S. oranienburg*, cheetah faeces; *S. chandans*, cheetah faeces; *S. rissen*, lion faeces; *S. vejle*, chimpanzee faeces; *S. liverpool*, chimpanzee faeces; *S. elizabethville*. Moreover, Ocholi *et al.* (1987) also reported the isolation of *Salmonella pullorum* from the lung, liver, kidney and intestine of a captive chimpanzee (*Pantroglodytes*) at the Jos Zoo. The one-year old female chimpanzee that was reported ill with constant diarrhea and anorexia died after five days (Oludairo *et al.*, 2013).

2.13 Ecto-parasites in Wildlife

Parasites can impact host survival and reproduction directly through pathologic effects and indirectly by reducing host condition. Severe parasitosis can lead to blood loss, tissue damage, spontaneous abortion, congenital malformations, and death. However, less severe infections are more common and may impair nutrition, travel, feeding, predator escape, and competition for resources or mates, or increase energy expenditure (Gillepsie, 2006).

Like domestic animals, zoo animals are vulnerable to a wide variety of ecto/endo-parasites and similar drugs are used for treatment. Care must be exercised in the choice of medications due to species-specific sensitivities to some drugs. In as much as mixed parasitic infestations have been reported in several domestic animal species (Soulsby, 1963; Soulsby, 1982), single infection of mites and also mixed infection of nematodes have also been reported in a variety of wild animals

kept in captivity for long periods. Young animals and those stressed by transportation, disease or injury are the most likely to be affected by parasites (Mbaya, 2006; Mbaya *et al*, 2007).

In addition to being vectors that transmit diseases, ecto-parasites can be direct causes of illness and death. Heavy infestation of lice, mites, fleas, flies and other biting insects have also been responsible for causing illness and even death of wild birds, especially among nestlings (Ballweber, 2001). Conditions caused by these insects range from feather loss and skin damage from acariasis or mange, to myiasis or infestation with fly maggots and anemia. Mites of the genus *Knemidocoptes* are the primary cause of mange in birds, and the mites belong to the same family (*Sarcoptidae*) of mites that cause mange in mammals and humans. The *Knemidocoptes* species of mites is specific to birds and they are not a human health hazard. Mites of the genus *Knemidocoptes* are the primary cause of mange in wild birds (Cole and Friend, 2001).

2.14 Tick Infestation in Wildlife

Ticks are among the most important vectors of human and animal diseases caused by protozoa, rickettsiae, bacteria, viruses and helminthes. They rank second only to mosquitoes as vectors of life-threatening or debilitating human and animal diseases. Moreover, ticks transmit a greater variety of infectious agents than any other arthropod group (Madder *et al*, 2010).

The vast majority of indigenous ticks in the sub - Saharan region are parasites of wildlife, and indeed a large number of species would be unable to complete their life cycles if there were no wild hosts available. Many of the tick species deemed to be parasites of domestic cattle, sheep, goats, horses and pigs are frequently more abundant or prevalent on smaller wild animals (Madder *et al*, 2010).

Ticks may live in many types of environment. For instance, most bat - associated soft tick species are typically found in caves, whereas ticks of wild terrestrial mammals are commonly found in forested areas. Domestic animals like cattle and dogs may carry ticks infested by pathogens which may represent a risk to humans (Dantas–Torres *et al.,* 2012).

Several vector-borne pathogens have received considerable attention for the toll they exact on human health for which a growing body of evidence indicates may be exacerbated by anthropogenic environmental change. A rigorous understanding of the transmission dynamics of pathogens from infected wildlife hosts to vector organisms is critical to explorations of the ecology of vector - borne diseases (Allan *et al.,* 2010).

Tick infestations are of great importance in animals. In addition to their role as vectors of infectious diseases, heavy infestations can cause direct losses (Radostits, 1997). Many are active blood feeders and may cause death from anemia. Some species cause tick paralysis and it is possible that other ticks may elaborate toxins other than those causing paralysis (Radostits, 1997). Heavy tick burdens cause sufficient irritation and stress such that affected animals become anorexic which may lead to reduced productivity. Although many ticks favor a particular host they are usually not completely host-specific and many parasitize a wide variety of animals (Radostits, 1997).

Several wild ruminant species are susceptible to *Ehrlichia ruminantium,* the causal organism of heartwater or cowdriosis or can act as carriers of the organism. Some are also carriers to certain *Theileria* species, while zebra are carriers of *Babesia caballi* and *Theileria equi,* the cause of equine piroplasmosis. Wild suids are carriers of *Babesia trautmanni*,apart from being the cause of porcine babesiosis, are also a reservoir for the virus of African swine fever. Ticks are also important as pests, affecting humans, livestock and wildlife (Madder *et al.,* 2010).

Tick paralysis in song birds has been associated with the bite of hard-bodied tick, *Ixodes brunneus.* Fatal paralysis from bites by this tick has been reported where the engorged ticks are generally found on the birds' head and they may be attached to its eyelids. Death results from a powerful neurotoxin that is secreted by the tick while it feeds on the bird. Just a few ticks feeding on a small bird can cause anemia, reduced growth, weight loss and contribute to a depressed state of health (Cole and Friend, 2001).

2.15 Tick–Borne Zoonoses

The risk of tick–borne diseases is increasing worldwide and this situation seems to be driven by several interacting factors. Wildlife populations can naturally migrate, transporting ticks and tick–borne pathogens from one area to another. Human travelers may also play a role in the transportation of wildlife species and introduction of exotic tick species into previously free areas, which may eventually carry relevant pathogens. Tick–borne diseases are increasingly diagnosed in travelers returning from endemic areas (Dantas–Torres *et al.*, 2012).

Ticks and wildlife are the main reservoirs of tick–borne pathogens of medical and veterinary concern. Wildlife may also serve as amplifying hosts of several human pathogens as the vast majority of tick–borne diseases are from wildlife reservoirs. However, tick–borne pathogens traditionally associated with diseases in domestic animals may also eventually emerge as human pathogens. For example, human babesiosis caused by *Babesia divergens* has been known in Europe for a long time as a zoonosis from cattle. *Ehrlichia canis* has been implicated in a few human cases from Venezuela and a new strain of *E. canis* has been detected in dogs from Peru (Dantas–Torres *et al.*, 2012). Nonetheless, the expanding spectrum of tick–borne pathogens affecting wildlife, domestic animals and humans will require new studies on the epidemiology, diagnosis and ecology of these newly recognized diseases. For example, *Ricketsia. massiliae,* a bacterium first isolated

from brown dog ticks, *Rhipcephalus sanguineus* in France has been implicated in human cases of spotted fever in Europe and South America. A laboratory study also suggested the potential of the tick *R. turanicus* in the transmission of *R. massiliae* (Dantas–Torres *et al.*, 2012).

A new soft tick species was recently discovered in a bat cave in Brazil and found to be highly anthropophilic. One of the researchers responsible for the species description was accidentally bitten by a female tick, and an intense inflammatory response was recorded 24–48 hours after the tick bite, with intense swelling, redness, heat and pain. Probably the inflammatory response was induced by the tick saliva (Dantas–Torres *et al*, 2012). In South America most cases of human infestation are related to *Amblyomma* ticks, which are frequently associated with wildlife. Some of these *Amblyomma* species like *Amblyomma cajannense and Amblyomma triste* have been implicated as vectors of rickettsiae such as *R. rickettsii* and *R. perkeri* (Dantas–Torres *et al.*, 2012).

2.16 Tick Control and Tick–Borne Diseases Prevention

The control of ticks is largely based on the use of chemicals on animals and the environment. Several active ingredients with killing and or repellant effects might be prescribed in different formulations such as sprays, soaps, shampoos, powders, impregnated collars, dip solutions, pour–on and spot–on applications (Dantas–Torres *et al.*, 2012).

Moreover, results of new strategies for the control of ticks in wildlife like white-tailed deer and the studies indicate that the methods might be useful in reducing the level of environmental infestation by some tick species, thus contributing to the control of certain tick–borne diseases such as lyme borreliosis (Dantas–Torres *et al.*, 2012).

Alternative tick control strategies have been proposed, including use of anti–tick vaccines of biological control agents such as entamopathogenic fungi. A vaccine is commercially available for the prevention of tick–borne encephalitis in Europe and Eastern Russia. Because the transmission of certain tick–borne pathogens like *Borrelia burgdorferi* is not immediate, the prompt removal of all attached ticks might greatly reduce the risk of infection and thus should be strongly emphasized by physicians and veterinarians (Dantas–Torres *et al.*, 2012).

2.17 Lice Infestation in Wildlife

Chewing lice are small, wingless, dorsoventrally compressed insects that parasitize birds and mammals. Avian chewing lice belong to one of two sub–orders; *Amblycera* which occur on feather and skin or *Ischnocera* which are more restricted to feathers. Chewing lice are obligate, permanent parasites that complete their life cycle on the body of the host. Their cycle consists of the egg, three nymphal instars and the adult stage (Clayton *et al.*, 2008).

As a group chewing lice are among the most host-specific of all parasites. Some species of chewing lice are less specific however, occurring on multiple host genera, families or even orders. Most bird lice feed on feathers, dead skin and skin products while some also feed exclusively on blood and a few species of *Amblycera* feed exclusively on blood. Chewing lice are normally found in small subclinical infestations that are kept in check by regular host grooming including preening with the bill and scratching with the feet. When present in large numbers, however, they can cause severe irritation and reduced host survival and reproductive success. They can also affect the host indirectly by serving as vectors of other parasites including some species of filarial worms. The time and energy that birds must devote in preening to keep lice in check may also be costly (Clayton *et al.*, 2008).

Transmissions of chewing lice among hosts often require physical contact between birds, such as between mates and parents and their offspring in the nest. When present in large number *Amblycera* lice can cause extensive feather and skin damage, leading to dermatitis, pruritus, insomnia and excessive preening and scratching. Lice also have negative effect on wild birds. Severe haemorrhagic ulcerative stomatitis and death have been documented in juvenile American White Pelicans infested with the *Menoponid* louse, *Piagetella peralis*, specie that lives within the pouch of these hosts. Although it is not clear weather lice were the principal cause of death, they clearly contributed to poor condition in heavily infested young pelicans (Clayton *et al.*, 2008).

In a case study on the impact of lice on wild bird, the population of the *Ischnoceran* lice *Columbicola columbae* and *Companulotes camper* increase dramatically on Rock Pigeons *(Columba livia)* with naturally or experimentally impaired preening ability. These two feed on abdominal contour feathers and reduce the density of the plumage leading to an increase thermal conductance and a corresponding increase in the metabolic rates of their avian hosts to maintain normal core body temperatures. The end result, not surprisingly, is a significant drop in the rate of survival during the winter months. The impact of feather lice on energetic may also be responsible for a significant drop in the rate of male courtship display and thus the ability of heavily infested males to attract mates. Feather damage from *Menoponid* louse *Hirindoecus malleus* can result in holes in the tail feathers of Barn Swallows. These holes may increase feather breakage as well as permeability of the feathers to air, thus altering aerodynamic efficiency (Clayton *et al.*, 2008).

Chewing lice can also have indirect effects on the host by acting as vectors or intermediate hosts of other parasites. For example, the *Amblyceran* lice, *Trinoton anserium* transmits the common heartworm, *Sarconema eurycera* to swans when the louse takes a blood meal, while the *Ischnoceran* lice that serves as intermediate hosts for other filarid nematodes transmits these worms when they

are ingested during preening. Viruses and bacteria have also been isolated from chewing lice, but it is not clear weather lice play a role in their transmission (Clayton *et al.*, 2008).

Ecologically speaking, bird lice can be divided into five categories based on morphology and how they escape preening: (a). Agile *Amblycera* that runs quickly across the skin or feathers (b). Large *Amblycera* that slip sideways between the feathers (c). Sluggish triangular–headed *Ischnocera* that avoid preening by dwelling on head and neck (d). Elongate *Ischnocera* that hide between the barbs of wings and tail feathers (e). Sluggish *Ischnocera* that burrow into downy regions of neck and abdominal feathers (Clayton, 2008).

2.17.1 Diagnosis of lousiness

In principle, lice are easy to detect because their life cycle is restricted to the body of the host. The five most commonly used methods for collecting lice include body washing, postmortem ruffling for dead birds, dust ruffling, visual examination and the use of fumigation chambers for live birds (Clayton *et al.*, 2008).

2.17.2 Treatment and control of lice infestation

The safest choice is pyrethrum dust or spray, a "fast knock–down, slow killing" insecticide with no side effects on birds and mammals. Its kill rate is not 100%, so most commercial products use a combination of pyrethrin, a derivative of pyrethrum and the synergist piperonyl butoxide. A 1% concentration of this mixture kills effectively, with no side effects on host nestlings or adults. Overcrowding of birds should be avoided because it facilitates transmission of lice with a

subsequent increase in average louse load. For this reason, highly social birds are probably more at risk than solitary birds (Clayton *et al*, 2008).

2.18 Host Defense and Immunity to Lice Infestation

The simplest defense against lousiness is to avoid infection and the most important defense of infested birds against lice is preening. Wild birds with bill deformities can have enormous louse populations because they are not able to preen efficiently.

Similarly, natural experiments confirm that scratching with the feet is critical for controlling louse populations on regions that cannot be preened. Birds that cannot scratch because of leg injuries sometimes have large number of lice and nits on the head and neck, but not on regions that the bird can still preen. Allopreening in which one bird preens another, may also play a role in louse control, although this possibility has not been tested carefully. Other behaviour that may help control lice includes dusting, sunning, anting and fumigation with aromatic green vegetation (Clayton *et al*, 2008).

2.19 Helminth Infection in Wildlife

Nematoda, trematoda and cestoda are the three major Classes of parasitic helminths of economic and zoonotic importance in the sub–Saharan region (Anene *et al.,* 1994). Helminth parasites are significant pathogens of wildlife, and are responsible for unthriftness, decrease in fecundity rates and sometimes death (Ibrahim, 2012). Overcrowding, dampness and unsanitary conditions are the predisposing factors for the proliferation of helminthic infections (Radostits, 1997). Such adverse conditions occur frequently under captivity than under free-living conditions (Ibrahim *et al*, 2012).

Intestinal parasites are a major continuous problem in many species kept in natural exhibit on dirt substrate especially in young and stressed individuals (Ibrahim *et al*, 2012). Parasites with indirect life cycle pose a problem less frequently if the exhibit area is clean and free of intermediate hosts (Anon, 2000).

Many cases of resistance to helminth infections in wildlife often breakdown when they are translocated from their natural habitat to the unnatural conditions obtained in captivity (Ibrahim *et al*, 2012). Thus, the free ranging reindeer, in Northern England hardly harboured *Strongyle* infections because of the sparseness of the infective larval stages in those areas, yet when they were relocated to zoos they showed high levels of strongylosis (as cited by Ibrahim, 2012).

The most common species of nematode associated with parasitic gastro–enteritis in most sub–Saharan countries are *Haemonchus contortus, Oesophagostomum columbianum and Trichostrongylus colubriformis.* Others are *Trichostongylus axei, Bunostomum trigonocephalum, Cooperia curticei, Trichuris ovis, Trichuris globulosis, Strongyloides papillosus* and *Gaigeria pachycelis* (Soulsby, 1982).

Strongylate nematodes are among the most characteristic parasites of the gastro-intestinal system of ruminants throughout the world. Although there is recognition of the potential influence of gastro-intestinal nematodes on morbidity and mortality in sylvatic bovids and cervids, typically there have been only superficial assessments of these parasites within the context of wildlife management (Hoberg, 2001).

Many helminths recovered from antelopes are those usually encountered in domestic ruminants, especially cattle and sheep, while other helminths of cattle, sheep and antelopes are more host-specific and rarely encountered in other species (Boomker, 2010).

The nematodes *Dictyocaulus africana, Dictyocaulus filaria, Dictyocaulus viviparus,* and *Bronchonema magna* occur in the bronchi and trachea of a variety of antelopes. Initially, the worms cause alveolitis, followed by bronchiolitis and finally bronchitis as they become mature and move to the bronchi. The patent phase is associated with two main lesions, namely a parasitic bronchitis, characterized by the presence of many adult worms embedded in white frothy mucus. Secondly, a parasitic pneumonia occurs, characterized by collapsed areas around infected bronchi. Recovery starts taking place once the adult lungworms have been expelled. The lung tissue organizes and clinical signs abate. *Pneumostrongylus calcaratus* in impalas is so common that it is considered normal, and apart from localized discolouration and slight fibrosis the lesions cause no discomfort to both the host species (Boomker, 2010).

The *Gongylonema* species, of which there are several, occur in the submucosa of the tongue, oesophagus or the rumen. The typical zig-zag pattern in the mucosa is the only indication of *Gongylonema* presence of the worms but they are non-pathogenic. Like the *Gongylonema* species, adult *Calicophoron,* which live in the rumen and reticulum, are non-pathogenic. Several species occur in wildlife, all of which use a fresh water snail, usually of the genus *Bulinus*, as intermediate host (Boomker, 2010).

A number of *Haemonchus* species occur in the abomasum of antelopes, but their pathogenicity has not been studied. It became apparent that certain *Haemonchus* species are associated with certain host groups. For example, in the Kruger National Park, *Haemonchus vegliai* is associated with the browsing antelope (kudu, nyalas and bushbuck) while impalas in the park harboured *Haemonchus krugeri* (Boomker, 2010).

The family *Trichostrongylidae* is well represented in all antelope and the commonly encountered genera are *Cooperia, Cooperiodes, Nematodirus, Impalaia, Paracoperia* and *Trichostrongylus*.

Large numbers of worms of any or all the genera mentioned above can occur in antelope, but clinical signs are rarely seen *(*Boomker, 2010).

Oesophagostomum is a large genus of which two species are commonly encountered in antelopes. These are *Oesophagostomum columbianum* and *Oesophagostomum walkerae*. The former nematode species has been recorded from at least 18 antelopes, but no mention is made on the pathogenicity of the parasites in their respective hosts. Several species of the genus *Trichuris* parasitize wildlife. *Trichuris globulosa*, one of the more commonly encountered species, occurs in eight antelope species and the infection is invariably very mild. Because of its monoxenous life cycle, and the infective larva that occurs in a thick-walled egg, large numbers can build up in enclosures and under intensive conditions. In private collections or zoos, *Trichuris* species is one of the most troublesome (Boomker, 2010).

Different species of *Parabronema* parasitize buffaloes, elephants, giraffes and rhinoceroses in South Africa and buffaloes in North Africa. All make use of a stomoxid fly, *Haematobium* or *Lyperosia*, as intermediate host. Large numbers of worms are often present in the abomasum or stomach, and may or may not cause gastric ulcers (Boomker, 2010).

Basson *et al.*, (1971) however, saw fatal cases of ostertagiosis caused by *Ostertagia ostertagi* in the abomasums of eland that were kept in small camps. Basson *et al.* (1970) found 5% prevalence in the buffaloes they processed. Hydatidosis, or cystic echinococcosis does not seem to be of importance in the larger nature reserves but could theoretically become problematic on game farms.

Oesophagostomum radiatum is fairly common in buffaloes but the infection is mild. Approximately 16% of the buffaloes in the Kruger National Park have lesions of one or more of the three species of *Onchocerca* which occur in buffaloes. The infection manifests as small nodules in the subcutis of

mainly the thoracic, sternal and abdominal regions, but are also present in the eyelids, the prepuce and testis (Boomker, 2010).

Monodontus giraffae is an extremely common parasite of the bile ducts of giraffe and causes mild to severe cholangitis, depending on the number of worms present. *Schistosoma* species are common in those animals that are dependent on water, and have been recorded from baboons, buffaloes, giraffes, hippopotami, zebras and at least 13 species of antelopes in Southern Africa. Severe phlebitis and thrombosis of the mesenteric veins was described in one of these buffaloes *(*Boomker, 2010).

As is the case with the antelope and pigs, the carnivores are also not affected by worms, given that they are well-fed and that the worm-infections are not overwhelming. It is quite conceivable that the high mortality of young lions is due to malnutrition combined with parasite infections, especially the hookworms *Ancylostoma*. But little is published on helminths diseases of free-living lions and leopards. *Ancylostoma* are virulent blood suckers and can cause severe anaemia in a very short time. *Toxocara* and *Toxascaris* presumably behave in the same way in lions as they do in cats and dogs, and therefore have a more severe influence on the young animals than older ones. These ascarids compete with the host for available nutrients (Boomker, 2010).

Several *Taenia* species occur in the small intestine of lions and leopards, and as is the case with similar species in dogs, the tapeworms do not seem to do significant damage. Species include *Taenia regis*, *Taenia crocutae*, *Taenia hyaenae* and *Taenia gonyamai* (Boomker, 2010).

Echinococcus is one of the worst helminth zoonoses and man may be an accidental host. Whenever dealing with carnivores, including cats and lions, one should always wear gloves, and definitely not eat, drink or smoke. The strain of *Echinococcus* that is found in lions is known as *Echinococcus*

granulosus felidis, as it is the strain that infects felids, while *E. granulosus granulosus* infects canids (Boomker, 2010).

The tapeworm genera *Mesocestoides* and *Dipylidium* have been recorded from lions and leopards, but are of little importance. *Spirocerca lupi* has been recorded from a growth on the oesophagus of a lion that was kept at a zoo, while *Cylicocyclus* species occurs in nodules in the stomach of lions and leopards, and non-pathogenic *Physaloptera* species in that of cheetahs.

In Kruger National Park, a surprisingly large number of lions suffer from cutaneous dirofilariosis, caused by the filarid nematode *Dirofilaria sudanensis*. Clinically it manifests as a large soft lump under the skin, but does not seem to cause much discomfort. The nematode is an extremely long one that lies curled up in the subcutis (Boomker, 2010).

In wildlife *Trichinella spiralis* has the sylvatic cycle which involves lion, spotted hyaena, jackal, multi-mammate mouse, warthog and Africa civet. South of the Sahara and especially in East Africa, *Trichinella nelsoni* appears to be the more important one in wildlife. Trichinellosis is largely asymptomatic in wildlife and man is the main sufferer. Experimental infections of domestic pigs with *T. nelsoni* and *T. spiralis* from meat of wild animals have indicated that the nematode can adapt, and may thus become an important zoonosis in future. In large game reserves, the incidence and prevalence of muscle cysticercosis is low. Cysticerci of *Taenia solium, Taenia hydatigena, Taenia crocutae, Taenia hyaenae* and *Taenia regis* have been recorded. As is the case with cysticerci in domestic animals, little pathology is caused (Boomker, 2010).

Physocephalus sexalatus is a spirurid nematode that utilizes an intermediate host, usually a dung beetle, in its life cycle. It occurs in the stomach of warthogs and bush pigs and only when present in massive numbers do they cause gastritis. Six species of *Oesophagostomum,* of which *Oesophagostomum mocambiquei* and *Oesophagostomum mwanzae* were the most common, and two

of *Murshidia* have been described from the large intestine of warthogs and bush pigs and were present in vast numbers. The anoplocephalid tapeworms *Moniezia mettami* and *Paramoniezia phacochoeri* are regularly encountered in young warthogs, in which they do not cause disease (Boomker, 2010).

A large variety of nematodes occur in the gastro-intestinal tract of zebras. These include the *ascarid Parascaris, Spirurids, Draschia* and *Habronema;* strongylids, *Strongylus* and *Triodontophorus,* and a whole host of cyathostomins, such as *Cylicodontophorus, Cylicostephanus, Cyathostomum, Cylicocyclus, Poteriostomum,* and *Oesophagodontus*. The Habronematidae are represented by *Habronema* and *Draschia,* while *Oxyuris equi* (Oxyuridae) and *Trichostrongylus thomasi* (Trichostrongylidae) are usually present in small numbers. The family *Atractidae* are tiny worms and occur in tens of thousands rather than tens or hundreds (Boomker, 2010).

2.20 Gastro-intestinal Parasites of Non–Human Primates

Non-human primates are one of the most common groups of animals in zoological gardens for their role in public entertainment. They are however, known to harbor different gastro-intestinal parasite species which affect their survival and reproductive activity by causing gastroenteritis, hemorrhage, extra-intestinal infection, spontaneous abortion and death. The close phylogenetic relationship between humans, the encroachment of humans into natural habitats and the closeness of humans to them even in the zoological gardens have caused frequent pathogen exchange with humans. This phenomenon has also resulted into emerging zoonoses which currently threatens global health and has resulted in a decline in non-human primate population in the wild and in captivity (Adetunji, 2014).

Non–human primates are particularly vulnerable to parasitic infections because many species live in cohesive groups characterized by frequent social interactions that facilitate parasite transmission between individuals (Adetunji, 2014). Infections by gastro-intestinal parasites have been reported in a range of non–human primate hosts which include gorilla *(Gorilla gorilla*), chimpanzee (*Pan troglodytes*), green monkey (*Chlorocebus sabaeus*), red patas (*Erythrocebus patas*), mandril monkeys (*Papio leucophaeus*), white collared mangabey (*Cercocebus torquatus*), mona monkey (*Cercopithecus mona*) and anubis baboon (*Papio anubis*) (Adetunji, 2014).

Annelids, helminths, and protozoa have parasite representatives in man, apes and monkeys. Protozoa parasite such as *Entamoeba histolytica*, *Giardia* species, *Cryptosporidium* species and *Balantidium coli* are frequently reported in non-human primates.

Gastro-intestinal parasites in non-human primates are regarded as major causes of gastroenteritis, watery diarrhea, hemorrhage, dysentery and extra-intestinal infection such as liver abscess and even death. *Entamoeba histolytica* causes intestinal and extra-intestinal amebiasis. Balantidium is an intestinal parasitic protozoa in man, while giardiasis caused by *Giardia* species and cryptosporidiosis caused by *Cryptosporidium* species are known as causes of failure of young animals to thrive. Considering the health significance of *Entamoeba histolytica*, *Giardia* species and *Cryptosporidium* species in man, their zoonotic involvement in non-human primate and man should be highly considered (Akpan *et al.*, 2010).

Akinboye *et al*, (2010) reported the presence of helminths (*Ascaris lumbricoides* and *Trichuris trichiura)* and protozoa *(Entamoeba histolytica* and *Giardia lamblia)* among zoological garden workers while only helminths *(Strongyloides* specie, *Trichuris* specie and *Ascaris* specie*)* were found in wildlife in the University of Ibadan Zoological Garden. In a study carried on primate bush

meat and pets in Cameroon, seven *Nematodirus* species, three protozoan species, one cestode specie and one trematode specie were reported (Dawet *et al*, 2013).

As human population density continues to increase exponentially, speeding the reduction and fragmentation of primate habitat, greater human - primate contact is inevitable and higher rates of pathogen transmission are likely. Baseline data on patterns of parasitic infections in wild primate populations are critical to provide an index of population health and to begin to assess and manage disease risks. In addition, considering the evolutionary and ecological linkages between primates and their parasites, one can view parasites as indicator species, potentially alerting us to imminent threats to primate conservation (Gillepsie, 2006). However, regular health services such as hygienic and deworming measures lower the prevalence of helminths infection in non-human primates.

2.21 Protozoa Infection in Wildlife

Protozoa is a collective name for animal-like, single-celled organisms, some of which may form colonies. Several phylla are commonly recognized. They include: flagellated zoomastigina, amoeboid sarcodina, ciliated ciliophora, cnidospora and sporozoa. More than 20,000 species are known including such familiar forms as Paramecium and Amoeba (Anon, 2009).

Acute diarrhoea can result from massive infections of *Trichomonas, Giardia* or *Balantidium* species. Amebiasis which is fairly common in primates and reptiles can be fatal in compromised animal (Anon, 2000).

2.22 Coccidiosis in Wildlife

Coccidiosis, a protozoal disease of many mammalian and all domestic livestock species, is caused by infection with species of the genera *Eimeria* or *Isospora*. Clinically, it is characterized by

enteritis although subclinical infections are frequent. Coccidiosis occurs universally, most commonly in animals housed or confined in small areas contaminated with oocysts (Radostits, 1997). Coccidia are generally host-specific parasites, and very specific to a particular region in the intestines. *Eimeria* infections are more specific compared to *Isospora* infections (Vorster, 2012). The Coccidia are host-specific and there is no cross immunity between species of Coccidia (Radostits, 1997).

Coccidiosis is mostly a disease of young animals raised and kept under intensive management systems although older animals may occasionally be clinically affected. Disease usually occurs when the resistance of the host is lowered following stress, overcrowding, weaning, transportation, housing under conditions of poor hygiene, food changes, nutritional deficiencies, concomitant infections with other parasitic and infectious agents and adverse weather conditions (Vorster and Mapham, 2012).

Clinical illness caused by infection with these parasites is referred to as coccidiosis, but their presence without disease is called coccidiasis. In most cases, a bird that is infected by coccidia will develop immunity from disease and it will recover unless it is reinfected. The occurrence of disease depends, in part, upon the number of host cells that are destroyed by the juvenile form of the parasite, and this is moderated by many factors. Severely infected birds may die very quickly. Damage to the bird's intestine often results in interrupted feeding, disruption of digestive processes or nutrient absorption, dehydration, anemia and increased susceptibility to other disease agents. In cranes, coccidia that normally inhabit the intestine sometimes become widely distributed throughout the body. The resulting disease, disseminated visceral coccidiosis (DVC) of cranes, is characterized by nodules, or granulomas, on the surface of organs and tissues that contain developmental stages of the parasite (Cole and Friend, 2001).

The incubation period of coccidiosis is usually about three weeks, although it may vary from one week to more than one month in some cases. Diarrhea may start off as watery, becoming increasingly hemorrhagic. Fresh blood and blood clots may be present in the faeces and a mucoid or fibrinous exudate may be seen as the clinical expression of the disease progresses. Faeces may become blackish-red to blackish, or greenish-black and it may become foul-smelling with the presence of shreds of mucosa. Tenesmus, pronounced borborygmi, constant grinding of teeth, abdominal pain, prolapse of the rectum, fever, increased respiratory rate and loss of appetite may be seen. Severely affected animals may become extremely emaciated and anemic (Vorster and Mapham, 2012).

Birds may be infected with coccidia at any time. Although little is known about the conditions that may lead to the development of clinical disease in wild birds, birds may become diseased more frequently during periods of stress. Most epizootics of intestinal coccidiosis in waterfowl in the Upper Midwest, United States have broken out in early spring, during a stressful staging period of spring migration (Vorster and Mapham, 2012).

Because each coccidia species has a preference for parasitizing a particular bird species and because of the self-limiting nature of most infections, coccidiosis in free- ranging birds has not been of great concern. However, habitat losses that concentrate bird populations and the increasing number of captive-reared birds that are released into the wild enhance the potential for problems with coccidiosis (Cole and Friend, 2001).

Field signs of coccidiosis for free-ranging wild birds have not been reported. Non-specific clinical signs reported for captive birds include inactivity, anemia, weight loss, general unthrifty appearance, and watery diarrhea that may be greenish or bloody. Tremors, convulsions, and lameness are also

occasionally seen. Rapid weight loss may lead to emaciation and dehydration followed by death. Young birds that survive severe infections may suffer retardation of growth (Cole and Friend, 2001).

2.22.1 Diagnosis of coccidiosis

History, clinical signs, necropsy findings and demonstration of the parasite in fresh faecal samples forms the foundation of the diagnosis. Small quantities of faeces are required for analysis but accurate species identification of the Coccidia may require the expertise of experienced laboratory personnel. An estimate of the number of oocysts in faeces is possible but care should be taken as it may be difficult to interpret the results. Scrapings from the intestinal lesions or tissue sections of the intestine may be examined for the presence of meronts, gamonts or oocysts. It is not uncommon for mixed infections to be seen (Vorster and Mapham, 2012).

Serologic analytical methods by ELISA and Western Blot have been developed, but they are not as definitive as visual examination of faeces. The use of PCR assays has been more extensively pursued in the poultry industry (Vorster and Mapham, 2012).

2.22.2 Treatment of coccidiosis

Anticoccidial compounds may be used either prophylactically or therapeutically although *Eimeria* has developed drug resistance against most anticoccidials currently used. Some of anticoccidia for control of avian coccidiosis are too toxic for use in wild ruminants.

Amprolium, decoquinate, lasalocid, lincomycin, monensin, and salinomycin have all been used to treat calves, lambs, and kids. Sulphonamides commonly used for treatment of coccidiosis in ruminants are only partially effective. Gut-active sulphonamides (e.g. succinylsulphathiazole and phthalysulphathiazole) should not be used. Orally administered nitrofurazone at a dose of 10 mg/ kg

per day for five days is effective but is not advocated for use in many countries due to persistent residues.

Toltrazuril and diclazuril are symmetric triazinetriones advocated for the treatment of coccidiosis. Toltrazuril has an effect on all intracellular forms of the parasite, primarily by interfering with cellular respiration and pyrimidine synthesis. The possible effect of toltrazuril on immune function has been investigated in poultry and mice. It was found not to interfere with the development of normal immunity, although it enhanced antibody production following treatment (Vorster and Mapham, 2012).

2.22.3 Control of coccidiosis

Control of coccidiosis is mainly aimed at preventing the accumulation of large number of oocyst in the environment by creating an adverse environment for their development:

I. Animals should be fed clean and dry food, and feed spillage to the ground from feed troughs should not take place.
II. Leakages from water troughs should be avoided and faecal contamination of feed and water troughs should be minimized.
III. Proper drainage of cages and enclosures is essential.
IV. Special attention should be paid to all the young and susceptible animals; and any potential form of stress such as may be experienced at weaning, sudden changes of diet and transportation should be minimized.
V. In heavily infected environments, sterilization may be attempted, but this is usually not a practical control measure. Exposure to sunlight for at least eight hours per day, and desiccation with humidity of less than 25%, may be more cost-effective methods.

VI. For a group of animals housed in same cage or enclosure, those showing clinical signs should be removed. They are not to be returned to the pens until at least two weeks after the clinical signs are no longer present, as oocysts shedding may persists for some time (Vorster and Mapham, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Kano State is located between Latitude 110^{o} 59´-120^{o} 02´ N of the equator and between Longitudes 80^{o} 31´-80^{o}33´ E, 840 km away from the edge of the Sahara desert (Okunola *et al*., 2012). Kano metropolis is the second largest city in Nigeria after Lagos. Kano State has a mean height of about 472.5 m above sea level. The climate is semi-arid and the vegetation is Sudan savannah with mean annual rainfall of 903 mm and temperature range of 18.85°C-33°C. Kano city has expanded over the years and has become the third largest conurbation in Nigeria (Ministry of Information, Kano State, 2005). The Kano Urban area covers 137 sq. km and comprises eight Local Government Areas (LGAs): Municipal, Gwale, Dala, Tarauni, Nassarawa, Fagge, Ungogo, and Kumbotso LGAs (Figure 3.1).

The study area was Kano Zoological Garden (KZG) which is located along Zoo Road, a commercial hub in Kano Municipal Local Government Area and covers an area of 100 hectares. It consists of 47 wildlife species, with 237 individual animals. These are divided into four sections: carnivores (Appendix 1), herbivores ((Appendix 2), non-human primates (Appendix 3) and wild birds/reptiles (Appendix 4). KZG was established on 14th November, 1972, with 60 wildlife species and 200 individual wildlife collection from different parts of the world and since then it has served as a popular tourist and educational destination for locals and foreigners.

The organogram of KZG comprises Managing Director as the overall head with four Departments headed by Directors; zoo services, planning research and statistics, administration/general services and wildlife departments. The departments comprise various sections and units such as: game reserve, open areas, education/conservation, recreation and veterinary sections (Figure 3.2).

3.2 Study Design

The design of the study was cross sectional, where all animals in the KZG were sampled.

3.3 Identification of Animals

Animals were identified using identification plates placed in front of animal enclosures. The information was crosschecked with "National Audubon Society field guide to African Wildlife" texts (1995).

3.4 Restrain and Immobilization

Physical restrain techniques and equipments like crush, gloves, hobbles, ropes, snares, squeeze cage, normal cage, net (drop net and hoop net) were employed depending on the wildlife species.

3.5 Sample Size and Sampling Method

A total of three hundred and eighty eight samples were collected by non-random sampling method. Samples were collected in the early morning hours from February to August, 2016. One hundred and eleven (111) cloacal swabs were collected from wild birds. One hundred and seventy faecal samples were collected: carnivores (28), herbivores (62), non-human primates (35) and reptiles/wild birds (45). One hundred and seven (107) ecto-parasites were collected: carnivores (53), herbivores (2), non-human primates (27) and wild birds (25).

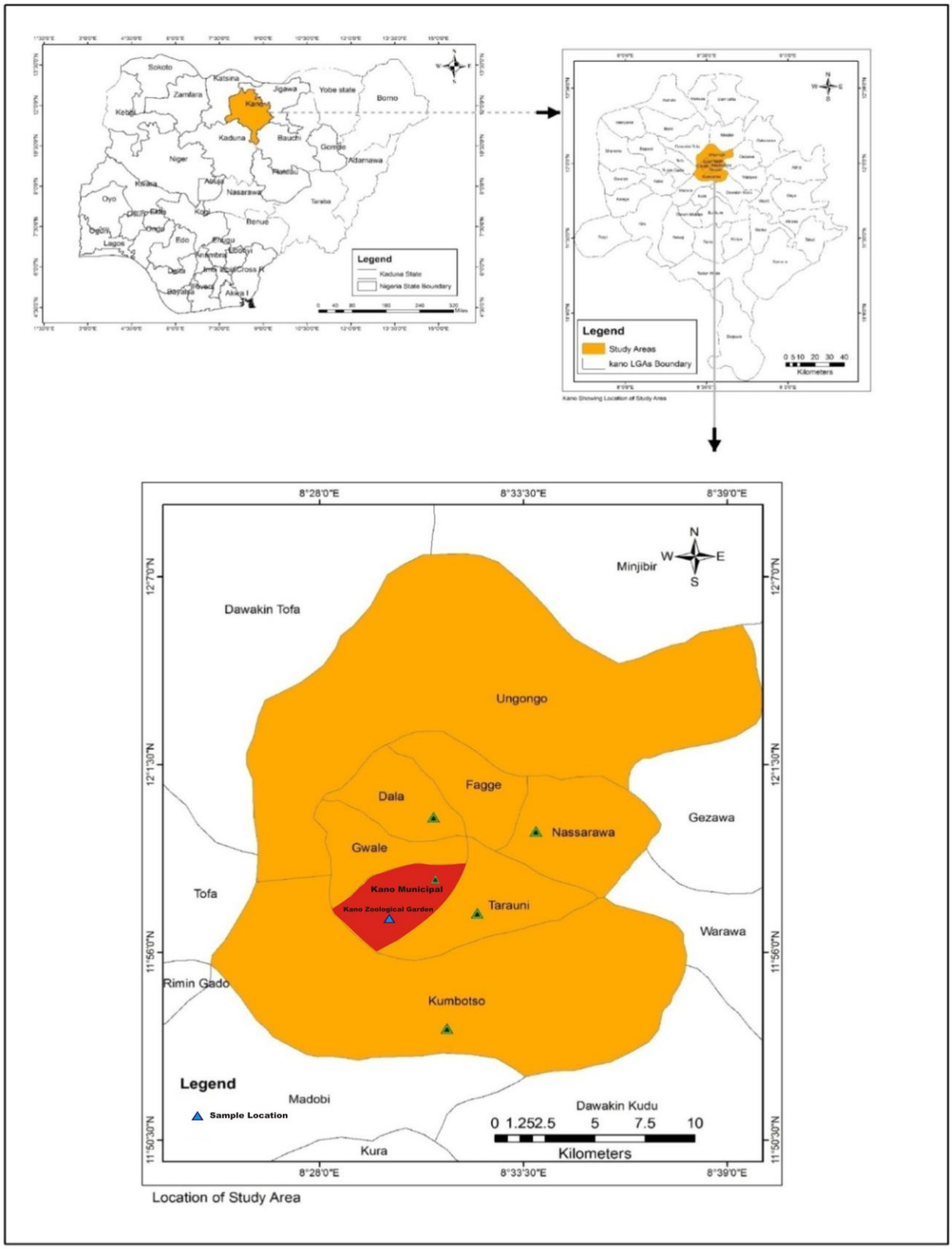


Figure 3.1: Map of Kano Metropolis showing Kano Zoological Garden (Mallam *et al*., 2016)

3.6 Assessment of Biosecurity in Kano Zoological Garden

A biosecurity checklist was designed, pretested and adjusted to correct limitations identified during pretesting. The checklist sought to estimate risk levels using scales (Table 3.1) and assessed how the following components of biosecurity increased risk parasitic and infectious diseases in KZG: Animal identification, record keeping and staff training (Appendix 6); Work and hygiene procedures (Appendix 7); Animal health and Preventive medicine (Appendix 8); Animal death, post-mortem examination and carcass disposal; Zoonotic disease management; Management of animal during translocation; Property management; Quarantine practices (Appendix 9) and Zoo-specific and emergency biosecurity protocol. However, emphasis was focused on the major sections of KZG namely: carnivores (Appendix 11), herbivores (Appendix 12), non–human primates (Appendix 13) and wild birds / reptiles (Appendix 14) sections.

3.7 Audit and Validation of Biosecurity Practices

Audit and validation of biosecurity practices in KZG was carried out according to property management with emphasis on sanitation, isolation and traffic control. All the four sections of KZG was audited and validated by examining and reviewing the shortcomings of their property and solutions were proffered. Other facilities such as abattoir, incinerator, post-mortem unit, quarantine zone and water supply were audited and validated.

Table 3.1: Biosecurity risk assessment scale used for Kano Zoological Garden, Nigeria.

Biosecurity risk (%)	Remark	Risk level	Remark
0	No risk	0	No risk
1-25	Very low risk	1.0	Risky
26-50	Low risk	1.1-2.0	Moderately risky
51-75	Moderate risk	2.1-3.0	Highly risky
76-100	High risk		

3.8 Culture and Isolation of *Salmonella* Species

3.8.1 *Salmonella* differential and selective solid media

Differential and selective media were used for the isolation of salmonella from faecal and cloacal swab samples that may be contaminated with other bacteria. Pre-enrichment and enrichment were carried out before primary isolation. The different culture media used for the isolation of salmonella included the following:

I. MacConkey's bile salt lactose agar medium in which *Salmonella* colonies were pale yellow or nearly colorless after 18-24 hours at 37°C. The colonies were 1-3 millimetres in diameter and easily distinguished from the pink-red colonies of the lactose fermenting *coliform bacilli* which also grow well on this differential medium.

II. On Leifson's Deoxycholate – Citrate agar (DCA), *Salmonella* colonies appeared pale to nearly colorless, smooth, shiny, translucent with or without black centers and were slightly smaller in size compared to those on MacConkey agar.

III. *Salmonella* colonies appeared colorless with or without black centers on *Salmonella – Shigella* agar (SSA) (Waltman, 2000).

Salmonellae were isolated from cloacal swab of birds, rectal swab and faecal samples of carnivores and herbivores using ISO Standard Protocol (2003). A tube containing swab and 1 millilitre of diluent was inoculated into 9 millilitre of selenite broth, and then incubated at 37°C for 24 hours. Ten grams of faecal sample was weighed and suspended in 90 millilitres of selenite broth incorporated with sodium bile selenite and incubated at 37°C for 24 hours. Presence of colorless colonies were marked as *Salmonella* suspects which were inoculated into nutrient agar slant and then incubated at 37°C and stored for further identification (Cheesebrough, 2006).

3.8.2 Biochemical reactions

Carbohydrates were fermented by *Salmonella* with the production of acid and/or gas. Typically, *Salmonella* fermented glucose, mannitol, arabinose, maltose, dulcitol and sorbitol while lactose, sucrose, salicin and adonitol were not fermented. Galactosidase (ONPG) test was negative (Cruickshank *et al.,* 1975). Pure, single colonies suspected to be *Salmonella* were picked from the agar plates for fermentation tests. Usually triple sugar iron (TSI) agar containing glucose, lactose, sucrose, ferrous sulphate and phenol red indicator were used. When any of the three sugars were fermented the colorless medium turned yellow but if it was only glucose that was fermented, red (alkaline) coloration was observed on the slants especially under aerobic conditions and because of protein breakdown. The medium remained yellow underneath the tube in an anaerobic condition (acid). Production of H_2S was indicated by the blackening of the medium (Jones *et al.,* 2000).

Colonies which produced characteristic *Salmonella* results in TSI were inoculated into urea agar. Urease was not produced by *Salmonella* i.e. *Salmonella* was urease-negative. *Salmonella* decarboxylated amino acid, lysine, ornithine and arginine but not glutaric acid. In other biochemical tests, indole was not produced, methyl red was positive, Voges–Proskauer was negative, H_2S may or may not be produced in ferrous chloride gelatin medium (Cruickshank *et al.,* 1975).

3.9 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility patterns of the *Salmonella* isolates were determined in Bacterial zoonoses laboratory of the Department of Veterinary Public Health and Preventive Medicine. All isolates were subjected to antimicrobial susceptibility testing using disk diffusion method. A bacterial lawn was prepared by transferring bacterial colonies to glass tube containing 5 millilitres sterile peptone water with a sterile inoculating loop. The suspension was vortexed and visually matched with 0.5 MacFarland standards for turbidity (CSLI, 2005). Sterile cotton-tipped swab was

immersed in the suspension, excess fluid removed by rolling the swab on the upper part of the tube, and spread onto Mueller Hinton agar (Oxoid, UK) to obtain a semi-confluent growth. Disks impregnated with predetermined amounts of antibiotics were dispensed onto the bacterial lawn and the plates were incubated for 18-24 hours at 35˚C. After the incubation, the diameter of the inhibition zones were measured and interpreted as sensitive, intermediate or resistant using the criteria described by the Clinical and Laboratory Standards Institute (CSLI, 2005), European Committee on Antimicrobial Sensitivity Testing (EUCAST, 2015), Centres for Disease Control and Prevention (CDC) and European Centres for Disease Control (ECDC, 2013).

The isolates were tested with a panel of 12 different antimicrobials commonly used in veterinary and human medicine to treat bacterial infections namely chloramphenicol 30 µg, gentamicin 10 µg, neomycin 30 µg, streptomycin 10 µg, ciprofloxacin 5 µg, enrofloxacin 5 µg, erythromycin 10 µg, doxycycline 30 µg, oxytetracycline 30 µg, amoxicillin 10 µg, ampicillin 10 µg and trimethoprim 25 µg (CSLI, 2005).

3.10 Collection of Faecal Samples

Fresh faecal samples for helminth, coccidia and salmonella identification were collected either directly using spatula or in some cases freshly voided in the animal cages into a labelled polythene bag (Levine, 1963; Soulsby, 1982; Murray, 1986; Ibrahim *et al*, 2006; FAO, 2007). The samples were immediately transported on ice pack to Helminthology and Bacterial zoonoses Laboratories of the Department of Veterinary Parasitology and Entomology and Veterinary Public Health and Preventive Medicine, ABU Zaria respectively.

3.11 Processing of Faecal Samples

3.11.1 Simple flotation technique

Faecal samples were analysed by the sedimentation and flotation methods for detection of helminth eggs with saturated sodium chloride as the flotation medium (Soulsby, 1982). Flotation and sedimentation were carried out at the Helminthology Laboratory, Department of Veterinary Parasitology, Ahmadu Bello University, Zaria. Four grams of faeces was placed in a universal bottle and then about five millilitres of flotation medium added. Glass rod was used to mix the faeces with the medium and sieved into a centrifuge tube or straight-walled test tube. More of the medium was added until a convex meniscus was formed, and a cover slip gently placed on the preparation and left for 3-5 minutes. Finally, the cover slip was carefully removed and placed on a glass slide and examined for egg or oocyst**.**

3.11.2 Sedimentation technique

This technique was used to identify eggs that did not float well due to high specific gravity or presence of operculum especially the eggs of flukes or tapeworms. The procedure allowed debris and eggs present in faecal samples to settle at the bottom of the apparatus. After settling, the preparation was then examined for eggs or oocyst (Soulsby, 1982).

3.12 Collection and Identification of Ecto-parasites

A thorough and systematic physical examination was conducted on each animal. Ecto-parasites attached to animal's body were removed by dislodging them gently and placed in a sample bottle containing 70% ethanol as preservative (Soulsby, 1982). The samples were transported to Entomology laboratory, Department of Veterinary Parasitology and Entomology, ABU, Zaria. Using a dissecting microscope, ecto-parasites were examined and identified using keys described by Soulsby (1982). Parasites seen were photographed using digital camera.

3.13 Data Analyses

Descriptive statistics were used for statistical analysis. Data obtained in figures, tables and plates. Percentage occurrence was calculated by dividing number of positive samples by total number of samples collected and multiplied by hundred. Parasite richness count was calculated by comparing the parasite species identified from individual animal to the total number of parasite species identified from wildlife section.

CHAPTER FOUR

4.0 RESULTS

4.1 *Salmonella* and Incidental Organisms Isolated from Wildlife in Kano Zoological Garden

Isolates of bacteria from five faecal samples and two cloacal swabs showed characteristic biochemical reactions similar to *Salmonella* species after conventional biochemical tests (Table 4.1).

Five of the *Salmonella* isolates showed typical *Salmonella* reactions to tests during the conventional biochemical test, while two isolates showed little deviation from the standard. For example in the case of hydrogen sulphide two samples (PRT40M from parrot and BB71M from bush buck) recorded negative reaction. However, all the seven samples (BB71M from bush buck, EGG41F from Egyptian geese, ELA72F from eland, LN22M from lion, LN23F from lioness, PCP42M from porcupine and PRT40M from parrot) that showed typical *Salmonella* reaction after conventional biochemical tests were positive to motility and citrate, and negative to indole and urea. The rate of recovery of *Salmonella* from the carnivores section was 7.14% (1/28) while the class-specific occurrence for herbivores, non–human primates and wild birds sections were 5.76% (3/52), 0 and 2.7% (2/37) respectively. The overall occurrence of *Salmonella* in all the four classes of wildlife in KZG was 4.8% (7/144) (Table 4.2).

Upon conventional biochemical tests five incidental bacterial organisms were identified and recorded. These were: one *Edwardsiella* (BB71M), one *Providencia* species isolate (CHMF6), two *Enterobacter* species isolates (WTM52M and GNT36), two *Shigella* species isolates (GHB43M and MON137) and fourteen *Proteus* species isolates (from ABS 65, BB71M, CRC41, CRO39, DUK19F, ELA72M, FIE37, GRFM, HDV29a, JAC27M, PCK21F, WHG and ZEB6M), (Appendix 1).

Table 4.1: Number of *Salmonella* isolates from wildlife species in Kano Zoological Garden, Nigeria.

Wildlife Species	Specimen code	Sex	Number of animals	Number of samples tested	Number of positive isolates
Bush buck	BB 71M	Male	6	6	1

(*Tragelaphus scriptus*)					
Cape eland (*Tragelaphus oryx*)	ELA 72F	Female	4	4	1
Egyptian geese (*Alopochen aegyptiacus*)	EGG 41F	Female	19	19	1
Lioness (*Panthera leo*)	LN 23 F	Female	1	1	1
Lion (*Panthera leo*)	LN 22M	Male	2	2	1
Parrot (*Psittacus erithacus*)	PRT 74M	Male	1	2	1
Porcupine (*Hystric cristata*)	PCP 42M	Male	2	2	1
Total			**35**	**35**	**7**

Table 4.2: Distribution of *Salmonella* isolated from Kano Zoological Garden by class of wildlife.

Wildlife section	Number of samples tested	Samples positive for *Salmonella* from biochemical test	Class specific occurrence (%)
Carnivores	28	2	7.14
Herbivores	52	3	5.76

Non-human primates	27	0	0.00
Wild birds	37	2	2.70
Total	**144**	**7**	**4.8**

4.2 *Salmonella* Antimicrobial Sensitivity Testing and Profiling

The antimicrobial susceptibility patterns of the *Salmonella* isolates were determined using disk diffusion method. The isolates have antimicrobial resistance profiles ranging from 2-4 and were resistant to at least one antimicrobial agent from two antimicrobial categories. However, none of them showed mild, extensive or pan drug resistance. These resistant *Salmonella* isolates were obtained from lion (LN22M), lioness (LN23F), bush buck (BB71M), Egyptian goose (EGG41F), cape eland (ELA72F), crested porcupine (PCP42M) and parrot (PRT40M) respectively (Table 4.4).

Of the seven isolates five (LN23F, LN22M, EGG41F, ELA72F and PRT40M) showed same resistance pattern to chloramphenicol, ciprofloxacin, doxycycline and trimethoprim. One of the seven isolates (BB71M) showed a different pattern of resistance to gentamicin, ciprofloxacin and doxycycline, while another isolate (PCP42M), showed different pattern of resistance to chloramphenicol, doxycycline and trimethoprim (Table 4.4).

On the other hand all the isolates were sensitive to amoxicillin 10 µg, ampicillin 10 µg, Enrofloxacin 5 µg, Erythromycin 10 µg, Neomycin 30 µg, Oxytetracycline 30 µg and Streptomycin 10 µg.

Table 4.3: Antimicrobial resistance pattern of *Salmonella* isolated from wildlife in Kano Zoological Garden, Nigeria

Code	Wildlife species	Antimicrobial resistance pattern
ELA 72F	Eland (*Tragelaphus oryx*)	CHL, CIP, DOX, SXT
LN 22 M	Lion (*Panthera leo*)	CHL, CIP, DOX, SXT
LN23F	Lioness (*Panthera leo*)	CHL, CIP, DOX, SXT
EGG 41F	Egyptian goose (*Alopochen egyptiacus*)	CHL, CIP, DOX, SXT
PRT 40M	Parrot (*Psittacus erithacus*)	CHL, CIP, DOX, SXT
BB 71M	Bush buck (*Tragelaphus scriptus*)	GEN, CIP, DOX
PCP 42 M	Porcupine (*Hystrix cristata*)	CHL, DOX, SXT

CHL = Chloramphenicol; CIP = Ciprofloxacin; DOX = Doxycycline

GEN = Gentamicin; SXT = Sulphamethozole/Trimethoprim

4.3 Gastro-intestinal Parasites Eggs/Oocysts from Wildlife in Kano Zoological Garden.

The overall occurrence of GIP in all the four sections of KZG was 63% and the overall GIPRC was 21/8.

4.3.1 Carnivores

All the three lions in this section had at least one egg type with prevalence of 100% and gastro-intestinal parasite richness (GIPRC) count of 3/3. One lion (LN22M) had *Isospora* oocysts (Plate) and *Toxocara* eggs (Plate), while the other two (LN23F and LN23M) had *Isospora* oocysts (Plate) and *Taenia* eggs (Plate) respectively.

One white-tailed mangoose had *Isospora* oocysts (Plate) and *Toxocara* eggs (Plate) with 33% prevalence and 2/3 gastro-intestinal parasite richness count (GIPRC).

The carnivores section has 14.28% overall occurrence of gastro-intestinal parasites (GIP) and 5/3 overall GIPRC (Table 4.5).

4.3.2 Herbivores

In the herbivores section, three egg types of were identified. Tortoises had *Ascaris* and *Strongyle* eggs with prevalence of 50% (10/20) (Plate) with 2/3 GIPRC. The lone buffalo (BUF-M) had *Trichuris* egg type with prevalence of 100% (1/1) (Plate) and 1/3 GIPRC. Cape eland had *Strongyle* egg type with a prevalence of 50% (1/2) and 1/3 GIPRC.

The herbivores section had an overall occurrence of 26.9% (14/52), and overall GIPRC of 5/3 (Table 4.6).

Table 4.4: Gastro-intestinal parasite richness count and occurrence among carnivores in Kano Zoological Garden, Nigeria

Carnivore species	Number examined	Number infested	Egg/oocyst identified	Occurence (%)	Parasite richness count

Common jackal	6	-	-	-	
Lion	3	3	*Isospora,Taenia,Toxocara*	100	3/3
Nile crocodile	4	-	-	-	-
Ratel	4	-	-	-	-
Spotted hyena	3	-	-	-	-
Striped hyena	2	-	-	-	-
Sand fox	1	-	-	-	-
Mangoose	3	1	*Isospora, Toxocara*	33	2/3
Genet cat	2	-	-	-	-
Overall occurrence	**28**	**4**		**14.28**	**5/3**

Table 4.5: Gastro-intestinal parasite richness count and occurrence among herbivores in Kano Zoological Garden, Nigeria

Herbivores species	**Number examined**	**Number infested**	**Egg/oocyst identified**	**Occurrence (%)**	**Parasite richness count**
Buffalo	**1**	**1**	*Trichuris*	100	1/3
Bush buck	-	-	-	-	-

Cape eland	4	2	*Strongyle*	50	1/3
Dorcas gazelle	3	-	-	-	-
Duiker	1	-	-	-	-
Elephant	1	-	-	-	-
Giraffe	3	-	-	-	-
Porcupine	2	1	*Trichuris*	50	1/3
Red gazelle	7	-	-	-	-
Tortoise	20	10	*Ascaris, strongyle*	50	2/3
Warthog	2	-	-	-	-
Zebra	2	-	-	-	-
Overall occurrence	**52**	**14**		**26.9**	**5/3**

4.3.3 Gastro-intestinal parasites eggs/oocysts of non–human primates

The overall occurrence of GIP in this section is 37% (10/27) while GIPRC was 7/4 (Table 4.7). Among the non–human primates, baboon had only *Trichuris* egg with 75% (3/4) occurrence of GIP and GIPRC of 1/4. The red patas had *Coccidia* oocyst (plate), *Strongyle* and *Trichuris* egg types with occurrence of 25% (3/12) and GIPRC of 3/4. The chimpanzee had 50% occurrence (1/2) and

GIPRC of 1/4, while tantalus had 50% (3/6) occurrence of GIP and GIPRC of 2/4. The chimpanzee had *Enterobius* egg type (Plate [illegible]), while tantalus had *Trichuris* and *Strongyle* egg types.

4.3.4 Gastro-intestinal parasites eggs/oocysts of wild birds

The wild bird section had the lowest GIP occurrence of 13.5% (5/37) and lowest GIPRC of 4/2. Two Nubian vultures had *Toxocara* egg with GIP occurrence of 100% (2/2) and GIPRC of 2/2, while peacock had only *Coccidia* sp (plate [illegible]b) with occurrence of 22% (2/9) and GIPRC of 1/2. (Table 4.8).

Table 4.6: Gastro-intestinal parasite richness count and occurrence among non-human primates in Kano Zoological Garden, Nigeria

Non-human primate	Number examined	Number infested	Ova/oocyst identified	occurrence (%)	Parasite richness count
Chimpanzee	2	1	*Enterobius*	50	1/4
Baboon	4	3	*Trichuris*	75	1/4
Mona	2	-	-	-	-
Red patas	12	3	*Coccidia, Strongyle, Trichuris*	25	3/4

Sooty mangabey	1	-	-	-	-
Tantalus	6	3	*Trichuris, Strongyle*	50	2/4
Overall occurrence	**27**	**10**		**37**	**7/4**

Table 4.7: Gastro-intestinal parasite richness count and occurrence among wild birds in Kano Zoological Garden, Nigeria

Wild bird Species richness count	**Number examined**	**Number infected**	**Ova/oocyst**	**Occurrence identified**	**Parasite (%)**
Bustard (*Chlamydotis undulate*)	2	-	-	-	-
Crown crane (*Balerica pavonina)*	4	-	-	-	-
Egyptian vulture (*Aegypiosus tracheliotus*)	4	-	-	-	-
Fish eagle (*Haeliacetus vocifer*)	1	-	-	-	-

Hooded vulture (*Neophron monarchus*)	2	-	-	-	-
Kite (*Milvus migrans*)	6	-	-	-	-
Marabou stork (*Leptoptilus crumineferus*)	1	-	-	-	-
Nubian vulture (*Torgos tracheliotus*)	2	2	*Toxocara* sp	100	2/2
Parrot (*Psittacus erithacus*)	1	1	-	-	-
Peacock (*Pavus cristatus*)	9	2	*Coccidia* sp	22	2/2
Spotted eagle owl (*Bubo Africana*)	1	-	-	-	-
Overall occurrence	**37**	**5**		**10.81**	**4/2**

4.4 Ecto-parasites Identified from Wildlife in Kano Zoological Garden

The overall occurrence of ecto-parasites in all the four sections of KZG was 38% and the overall EPRC was 5/2 (Table 4.9). At least one animal from each section of KZG was infested with one ecto-parasites (EP) specie. Common jackal was infested with *Rhipicephalus sanguineus* tick with occurrence of 33% (2/6) and EPRC of 1/2 (Plate ▮▮▮b). Buffalo was infested with *Rhipicephalus sanguineus* tick (Plate ▮▮▮a) with occurrence of 100% (1/1) and EPRC of 1/2. Red patas monkey was infested with *Cimex lectularius* bug (Plate ▮▮▮c) and had 16% (2/12) occurrence and 1/2 EPRC. Baboon was infested with *C. lectularius* bug (Plate ▮▮▮) with 25% (1/4) occurrence and EPRC of 1/2. Spotted eagle owl was infested with *C. lectularius* bug (Plate ▮▮▮) with 20% (1/5) occurrence and EPRC of 1/2.

Table 4.8: Distribution of ecto-parasites identified from wildlife in Kano Zoological Garden, Nigeria

Wildlife	Ecto-parasite identified	Occurrence (%)	Ecto-parasite richness count
Baboon (*Papio Anubis*)	*Cimex lectularius*	25	1/2
Buffalo *(Syncerus cafer)*	*Rhipicephalus sanguineus*	100	1/2
Common jackal (*Cannis aureus)*	*Rhipicephalus sanguineus*	33	1/2
Eagle owl *(Bubo africanus)*	*Cimex lectularius*	20	1/2
Red patas *(Erythrocebus patas)*	*Cimex lectularius*	16	1/2
Total		**38**	**5/2**

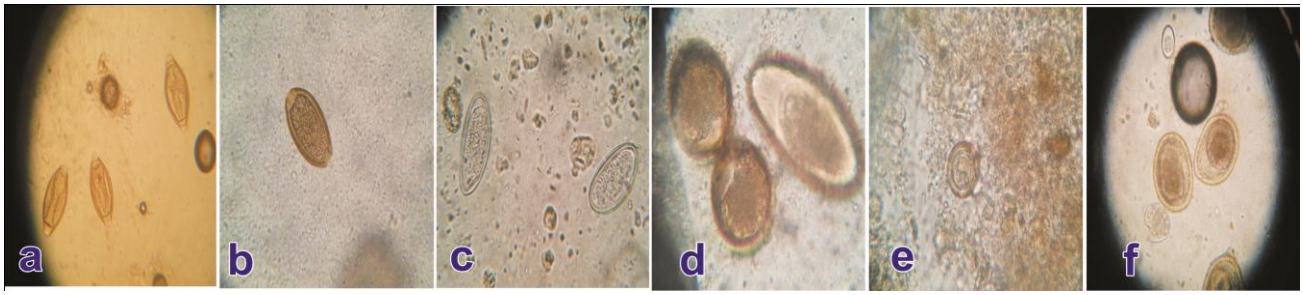


Plate I: Helminth parasites eggs identified from wildlife in KZG. (a) *Trichuris* egg from a buffalo (b) *Capillaria* egg from a bush buck (c) *Enterobius* egg from a chimpanzee (d) *Strongyle* and *Ascaris* eggs from a tortoise (e) *Taenia* egg from a lion (f) *Toxocara* egg from a mongoose.

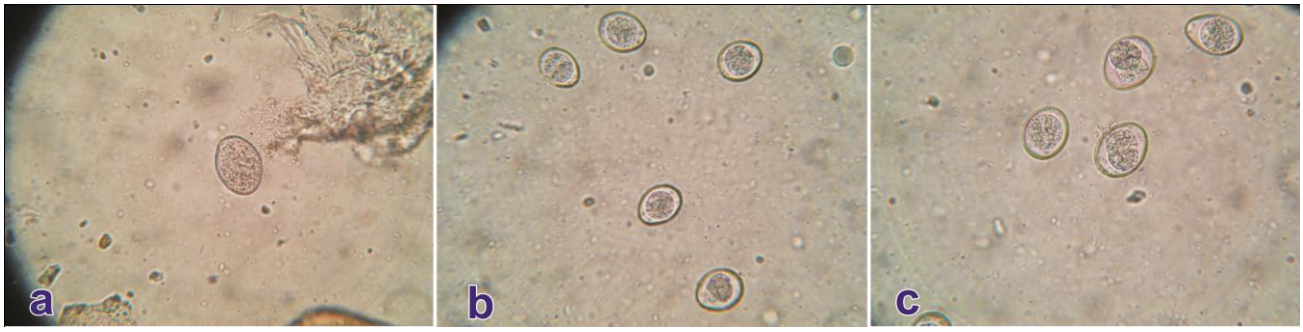


Plate ⅈⅈ: Protozoan parasites oocysts identified from wildlife in KZG. (a) *Isospora* oocyst from a lion. (b) *Coccidia* oocyst from a peacock. (c). *Coccidia* oocyst from a red patas.



Plate *: Ecto-parasites identified from wildlife in KZG.(a) *Rhipicephalus* tick from buffalo and jackal. (b) Beetle from buffalo, jackal, red patas and spotted eagle owl. (c). Bug from baboon and red patas.**

4.5 Biosecurity of Kano Zoological Garden

4.5.1 Biosecurity practices in Kano Zoological Garden

The overall biosecurity risk on record keeping, animal identification, staff training and property management was 77.7% with risk level of 2.3 (Appendix 5), on work and hygiene procedure was 58.3% with risk level of 2.0 (Appendix 6). The biosecurity risk on animal health, preventive medicine and zoonotic disease risk management was 72% with risk level of 2.0 (Appendix 7) while on quarantine was 100% with risk level of 2.6 (Appendix 8). Animal death, postmortem examination and carcass disposal had biosecurity risk of 65.0 with risk level of 2.5 while management of animals, vehicles and equipments during translocation had 52% with risk level of 2.3 (Table 4.9). Zoo-specific and emergency biosecurity response plan had biosecurity risk of 81% with risk level of 2.6 (Table 4.9).

4.5.2 Biosecurity practices in property management and wildlife sections of Kano Zoological Garden

4.5.2.1 Property management

The KZG has two gates for vehicles, the front gate used by both staff and visitors and the back gate used by staff only. For foot traffic, ten small gates were present. However, entry and exit gates were not separated. Vehicles were not disinfected before entry and no hand washing facilities at the gate. Apart from the official parking space for staff and visitors, different parts of the zoo grounds serve as parking spaces for the neighbourhood. There was a secure perimeter fence and enclosure security but due to poor patrol the fence was compromised by hoodlums posing a serious biosecurity threat to the animals. Each enclosure was secured and identified with a number but some enclosures like giraffe's was not properly constructed to prevent animal escape (Plate ⅈ⊗). Only few cages and few

enclosures were set aside for quarantine. There were no facilities dedicated for postmortem examination and no hand washing facilities in any section of KZG. Stray animals like camels and donkeys were allowed to roam freely on zoo grounds.

The abattoir was dilapidated due to lack of maintenance and the refrigerators were abandoned due to shortage of power (Plate ⊗ and ⊗) and sometimes carcass was processed on the ground outside the abattoir (Plate ⊗). KZG was not divided into distinct biosecurity zones and there was no documented biosecurity response plan. The two small incinerators were inadequate, carcasses and rubbish burn on the ground and waste products were not disinfected (Plate ⊘). Some enclosures lacked drainage while some were poorly drained, leading to accumulation and stagnation of water especially during rainy season, and attracting other wild birds from outside (Plate ⊘). There was no documented pest control program and no staff was assigned the duty of pest control officer. Some enclosures in dire need of rehabilitation were more than forty years old (Plate ⊘) while in some weeds and grasses had overgrown with no modern system for monitoring water quality for aquatic animals (Plate ⊘). A soccer viewing center located behind the Veterinary Clinic poses a serious nuisance which breaches the principles of captive animal biosecurity and welfare (Plate ⊘).

Table 4.9: Distribution of biosecurity risk and risk levels according to biosecurity components in Kano Zoological Garden, Nigeria

Biosecurity components	Biosecurity risk (%)	Risk level

Record keeping, animal identification and staff training	77.7	2.3
Property management	77.7	2.3
Work and hygiene procedures for staff and visitors	58.3	2.0
Animal health and preventive medicine	72.2	2.0
Zoonotic disease management	72.2	2.0
Quarantine Practices	100	2.6
Animal deaths, postmortern examination and carcass disposal	65.0	2.5
Management of animal and vehicles during animal translocation	52.0	2.3
Zoo-Specific and emergency biosecurity response plan	81.0	2.6
Overall	**72.9**	**2.3**

Risk level: No risk = 0; Risky = 0.1; Moderately risky = 1.1 – 2.0; Highly risky = 2.1 – 3.0; Biosecurity risk (%); 0 = No risk; 1-25 = Very low risk; 26-50 = Low risk; 51-75 = Moderate risk; 76 – 100 = High risk

4.5.2.2 Carnivores section

The roof of the inner compartment of spotted hyena enclosure had collapsed, exposing the animals to extreme rain and sunlight (Plate ⦸). A dead tree which had a deep cave was left in the center of the enclosure (Plate ⦸⊗) and a hole dug by the animals were left unfilled (Plate ⦸). Spotted

eagle owl, a nocturnal carnivore was placed in a cage exposed to sunlight in three directions (Plate ⦸⊗).

4.5.2.3 Herbivores section

Different species were mixed in same enclosure sharing same feeding and drinking troughs. Zebra was mixed with dorcas gazelle (Plate ⦸⊗), emu was mixed with tortoise (Plate ⦸⊗) while crown crane was mixed with gazelle and tortoise (Plate ⦸⦸). The cape eland enclosure lacked functional crush for proper handling and restraint.

4.5.2.4 Non – Human Primates section

Most of the enclosures in this section lacked adequate and environmental enrichment items. The space between enclosures was not enough causing frequent congestion of visitors. The barriers between visitors and enclosures were small, as a result of which some visitors get too close to wildlife in this section to offer food and drinks to the animals. (Plate ⦸⦸). The chimpanzee cage was too small for the animal subjecting it to stress.

4.5.2.5 Wild birds section

In the wild bird section multiple species were mixed in the same enclosure; in the main aviary abdim stork, crown crane, Egyptian geese, mallard duck and spur winged geese shared the same waters (Plate ⦸⦸). In other cages, parrot was housed with vulture and cock (Plate ⦸) and ram with Egyptian geese and crown crane (Plate ⦸⦸⊗). The ground beside marabou stork cage was used for dumping and burning refuse (Plate ⦸⦸⊗).

In all the four sections of the zoo, there were no hand washing and sanitary facilities for staff and visitors and public convenience for visitors was inadequate. The main drainage that cut across the zoo was at some portions congested with refuse and sand (Plate ⦸⦸⊗).



Plate ⊗: Giraffe barrier; crossed by the animal to stray on zoo grounds





Plate ⊗: Stray donkeys roaming freely on zoo ground

P **Plate ⊗❣: Dilapidated abattoir building.**

P **Plate ⊗: Dilapidated abattoir roof. Note drying of hide on a pipe.**





Plate ⊗; Carcass processing on ground; wooden cart used for meat distribution.



Plate ⦸: Incinerator out of use; carcass burnt on bare ground.

Plate ⦸: Stagnation of water due to lack of drainage.



Plate ⦸ⓘ: Dilapidated enclosure housing spotted hyena.



Plate ⦸ⓘⓘ: Crocodile pond; poor water quality, grasses, weeds and dilapidated shed.



Plate ⃠: Soccer viewing center near KZG veterinary clinic; nuisance for animals.



Plate ⃠⊗ Dead tree with cave inside spotted hyena enclosure.



Plate ⦸⊗: A big hole frequently dug by hyena inside enclosure.



Plate ⦸⊗❣: Spotted eagle owl cage accessed by sunlight from three directions.



Plate ⦸⊗𓃰𓃰: Mixed species: zebra and gazelle.



Plate ⦸⊗𓃰𓃰𓃰: Mixed species: emu and tortoise.



Plate ⦸⦸: Mixed species: crown crane, dorcas gazelle and tortoise.



Plate ⦸⦸: Sooty mangabey sipping ice cream offered by unscrupulous visitor.



Plate ⦸⦸❶: Mixed species: abdim stork, crown crane and spur wing geese.



Plate ⦸⦸❶❶: Mixed species: Egyptian geese and mallard duck in the aviary.



Plate ⦸⦸❦❦: Mixed species: parrot, Egyptian vulture and cock.



Plate ⦸⦸❦⊗: Mixed species: ram, crown crane and Egyptian geese.



Plate ⦸⦸⊗: Refuse dumped beside marabou stork enclosure.



Plate ⦸⦸⊗❢: Main drainage of KZG filled with sand and refuse.

CHAPTER FIVE

DISCUSSION

This study with 4.8% occurrence has established increased prevalence of *Salmonella* by three fold compared to the study of Okoh and Onazi (1980), who reported a prevalence of 1.76 % from KZG. However, Okoh and Onazi (1980), sampled only few carcasses and selected morbid animals, while in this study the whole wildlife collection of KZG was sampled. However, the variety of species and wildlife population of KZG has decreased compared to that of the 1980s. The implication of these findings is that zoo staff, visitors and animals could be infected with *Salmonella* as well as serve as sources of infection when they come in contact with zoo animals and materials contaminated by them. In a related study carried out at Colorado zoo in 1996, a *Salmonella*-contaminated wooden handrail surrounding a Komodo dragon exhibit caused an outbreak of salmonellosis involving 65 confirmed human cases and possibly several hundred unconfirmed cases (*Friedman et al.*, 1998).

Gitter and Brand (1969) reported 7.8 % prevalence of *Salmonella* from sixty four wildlife examined at Nairobi National Park and Orphanage. Falade and Durojaiye (1976) reported a prevalence of 9.5% from the University of Ibadan Zoo, while 7% prevalence was reported by Gopee *et al.*, (2000) at the Emperor Valley Zoo, Trinidad and Tobago. Windsor and Ashford, (1972) reported death of three wild animals at Nairobi Game Park, and concluded that game animals rarely suffer from clinical salmonellosis in the wild but the disease is more of capture, captivity and contact with man.

Accordingly, zoonotic infections and infestations from wildlife are not new, but today they form part of the phenomenon of emerging human diseases because they constitute a novel health threat (Chomel *et al.*, 2007; Wolfe *et al.*, 2005; Zinsstag *et al.*, 2007). This pathological renaissance is largely attributable to two factors: one factor is that the efficiency and economics of modern transportation offers convenient access to remote areas of the world introducing people to novel

environments within hours or days. Another factor is that wildlife species are conveniently transported from distant regions of the world to the domestic market, circuses and zoos, also within a very short period of time ((Weber and Rutala, 2001)).

The sources of infection with *Salmonella* and other organisms in KZG could be contaminated food offered to the animals by the keepers as well as visitors who offer assorted food items and drinks to the animals. Other sources of infection with *Salmonella* in KZG might be migratory wild birds which perch on trees to defecate over the cages and rodents which gain access to animal cages thereby contaminating water and food. Poor biosecurity practices and staff of the facility especially zoo keepers could also be sources of the *Salmonella* (Goppee *et al.,* 2000). Falade and Durojaiye (1972) also suggested that *Salmonella* found in man and isolated from wildlife could have been acquired from contact with humans and other animals or feeding carnivores with market meat. Goppee *et al*, (2000) suggested that sources of *Salmonella* in captive animals at the Emperor Valley zoo, Trinidad and Tobago may have been food offered to them, particularly raw meat or carrier animals brought to the zoo by the public or native rodents and wild birds that gain access to animal enclosures.

The highest class-specific occurrence of *Salmonella* observed in KZG lions (7.1 %), may be attributed to the dead carcasses of livestock from abattoirs, farms and veterinary clinics which are fed to them. The low frequency of isolation of *Salmonella* sp from wild birds (2.7%) was reported by other investigators. For example in a study of captive wild birds in Trinidad none of the birds was a carrier of *Salmonella* sp (Goppee *et al.*, 2000). In another study, water birds among others had the highest (6%) detection rate of *Salmonella* sp isolated from the digestive tract of flamingos and water birds, and mortality due to the organism was reported (Wobeser, 1997).

Multidrug-resistant *Salmonellae* were isolated from three sections of KZG. All the *Salmonella* isolates were resistant to at least one antimicrobial agent from three antimicrobial classes, and showed antimicrobial resistance profile of 3-4. According to Cole *et al.,* (2011), water contact and acquisition via food seem to be major aspects of transmission of resistant bacteria of human or veterinary origin to wildlife. Migratory birds also act as transponders or as reservoirs of resistant bacteria and consequently play a significant role in the dissemination of resistance (Rhadhouani *et al.,* 2010). On the other hand wild rodents pick up human waste and interacts with human faeces in the sewage system and can therefore easily acquire and disseminate multi-resistant bacteria to wildlife (Mallon *et al.,* 2002).

It is also worthy to mention that four of the five miscellaneous organisms isolated in this study namely: *Proteus, Shigella, Enterobacter, Edwardsiella* and *Providencia* sp were never reported from any zoo in Nigeria. However, before this study *Shigella* sp was reported from a Nigerian zoo. It was suggested that chimpanzees acquire shigellosis from contaminated food from human source or carriers in their population. Man is the main reservoir of the infection and non-human primates acquire it by contact through food or water. On the other hand, zoo visitors especially children, pregnant women and elderly people could be infected with *Shigella* sp by contact with zoo animals especially primates and materials contaminated by them. Surprisingly, the overall occurrence of *Proteus* sp was higher than the overall occurrence of *Salmonella* sp in KZG. *Proteus* is a bacterial opportunistic pathogen which inhabits the intestines of humans and animals. But under favorable conditions it can cause a number of infections including urinary tract infections, wound infections, meningitis in neonates or infants and rheumatoid arthritis (Rozalski, 2012). These miscellaneous organisms could pose potential threat to the survival and welfare of the wildlife in KZG.

Result of parasites egg/oocyst identification from this study showed that wildlife at KZG harbor parasites which are not novel to science but are important to human and wildlife health especially

Toxocara and *Trichuris* egg types. Ajayi (1984) reported that *Trichuris* and *Oesophagostomum* and *Trichuris* and *Entamoeba* were the most frequently encountered parasites in Agodi and University of Ibadan Zoo respectively. However, *Balantidium, Enterobius* and *Giardia* infections in the gorillas and chimpanzees respectively at the Agodi Gardens and University of Ibadan zoo were thought to be of human origin because the sampled animals had never escaped from captivity. In a study of primates and carnivores at two Italian zoos, Fagiolini *et al.*, (2010) found one or more intestinal parasites including *Cryptosporidium, Toxocara,* and *Strongyloides* sp in 61.5% of sample and concluded that gastro-intestinal helminths and zoonotic protozoans are common in zoo mammals. Beck *et al*., (2011) also studied 131 faecal samples of 57 mammalian species at Zagreb Zoo for the presence of *Giardia* sp and reported prevalence of 29%, while all animals were asymptomatic. The higher occurrence of helminthes compared to protozoa obtained in this study is similar to the findings of Munene *et al.,* (1998) who reported higher prevalence of helminthes than protozoa at a Zoological garden in Kenya. This may be due to favorable climatic factors which provide optimal conditions for viability of parasite egg and ova (Soulsby, 1982).

The low occurrence of helminth eggs in the aviary could be attributed to the housing and deworming practice. The aviary has enough floor space as well as spacious pond and some of the birds have created a spot to defecate which is far from their feeding spot. This may have contributed to the low occurrence of gastro-intestinal parasites eggs in these birds. In the herbivores section, the high occurrence of gastro-intestinal parasites eggs may be related to grasses and weeds which over grow in the enclosures and marshy nature of the soil which favor development of intermediate hosts and larvae of helminthes.

Identification of ecto-parasites showed *Cimex lectularius* bugs in red patas, baboon and eagle-owl; and *Rhipicephalus sanguineus* ticks in buffalo and common jackal. However, the unexpected presence of beetle (*Coleoptera* sp) on buffalo, jackal, eagle owl and red patas monkey requires

further investigation because the insect can play a role in mechanical transmission of diseases such as helminthosis and mycobacteriosis (Fischer, 2004).

The nine components of biosecurity essential for management of wildlife in zoo setting were assessed, their shortcomings identified and appraisal of associated biosecurity risk and risk level was made. This had revealed a serious biosecurity threat to wildlife collection, staff and visitors of KZG.

Audit and validation of biosecurity practices according to property management and wildlife sections revealed lapses in maintenance of structures as well as management of visitors and wildlife. For instance in the non-human primate section, narrow passage and poor barrier allowed visitors opportunity to offer food and drinks to the animals, which might have led to occurrence of respiratory and other infectious diseases in this section especially during festive seasons. The chimpanzee was housed in a small enclosure subjecting it to stress and limiting its ability to express natural behavior. In the carnivores section, spotted eagle owls which are nocturnal animals were housed in an enclosure exposed to sunlight in three directions. This is a source of stress to the birds because it does not mimic their natural environment. Summarily, the poor biosecurity practices may have contributed to the introduction, establishment and spread of infectious and parasitic diseases in KZG.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From this research work:

1. The overall occurrence of *Salmonella* in KZG was 4.86%. The occurrence of *Salmonella* among carnivores was 7.14%, herbivores 5.7%, wild birds 2.7% and non–human primates 0.
2. All the *Salmonella* isolates (100%) from this study were multi-drug resistant.
3. The overall occurrence of gastro-intestinal parasite in KZG was 63% and gastro-intestinal parasite richness count was 21/8. The occurrence and gastro-intestinal parasite richness count among carnivores was 14.28% and 5/3, herbivores 26.9% and 5/3, non–human primates 37% and 7/4 and wild birds 13.5% and 4/2.
4. The occurrence and ecto-parasite richness count in common jackal, buffalo, baboon, red patas and spotted eagle owl was 33% and 1/2, 100% and 1/2, 25% and 1/2, 16% and 1/2 and 20% and 1/2 respectively.
5. Quarantine practices in KZG poses the highest biosecurity risk of 100% with risk level of 2.6; work and hygiene practices for staff and visitors poses the lowest biosecurity risk of 58.3% and risk level of 2.0.

6.2 Recommendations

From the findings of this work:

1. All wildlife harboring *Salmonella* sp in KZG should be treated and newly introduced ones screened against *Salmonella* sp and examined for ecto and GIT parasites.

2. Newly acquired animals should be quarantined by the zoo veterinarian for a minimum of 90 days for mammals, 60–90 days for non–human primates and 90 days for snakes.

3. Antimicrobial sensitivity test should be carried out by KZG veterinary services on organisms isolated from sick animals prior to treatment especially when managing gastroenteritis and enteric fever.

4. Zoo staff, students and visitors in KZG should be educated on the risk of contracting zoonotic infections especially due to *Salmonella*, *Shigella* and GIT parasites and the risk of contact with wildlife and materials contaminated by them.

5. Regular faecal examination should be conducted on all animals in KZG to detect and treat parasitic infections before clinical signs appear.

6. Zoo–specific biosecurity protocol and emergency biosecurity response plan should be established and made accessible to staff and researchers in KZG.

7. Preventive health program for staff especially zoo keepers should be improved to minimize disease transmission between people and zoo animals or vice–versa.

8. Dedicated Facilities for quarantine and postmortem examination should be constructed, dilapidated structures should be reconstructed and sanitary facilities should be provided in every section of KZG.

9. Further studies to determine the origin and characterization of the *Salmonella* and other organisms isolated from KZG should be carried out.

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APPENDICES

Appendix 1: Miscellaneous bacteria isolated from wildlife species in Kano Zoological Garden, Nigeria.

Wildlife species	Specimen code	Sex	Number of samples tested	Positive samples	Miscellaneous bacteria
Abdim stork	ABS65M	Male	2	1	*Proteus* sp

(*Ciconia abdimi*)					
Bush buck (*Tragalaphus scriptus*)	BB71M	Male	6	1	*Proteus* sp
Crocodile (*Crocodilus niloticus*)	CRO 39F	Female	4	1	*Proteus* sp
Crown crane (*Balaerica pavonina*)	CRC41F	Female	4	1	*Proteus* sp
Duiker (*Cephalophus monticola*)	DUK19F	Female	1	1	*Proteus* sp
Giraffe (*Giraffe camelopardilis*)	GRFM	Male	3	1	*Proteus* sp
Jackal (*Cannis aureus*)	JAC27M	Male	6	1	*Proteus* sp
Eland (*Tragelaphos oryx*)	ELA73M	Male	4	1	*Proteus* sp
Fish eagle (*Heliacetus oocifer*)	FIE37 M	Male	1	1	*Proteus* sp
Hooded vulture (*Neophron monarchus*)	HDV29A	Male	2	1	*Proteus* sp
Peacock (*Pavo cristatus*)	PCK21 F	Female	6	1	*Proteus* sp
Warthog (*Phacocerous aetiophicus*)	WHGM	Male	2	1	*Proteus* sp
Zebra (*Equus quagga*)	ZEB 6M	Male	2	1	*Proteus* sp
Ground hornbill (*Bucovus cafer*)	GHB 43M	Male	2	1	*Shigella* sp
Mona (*Cercopithecus mona*)	MON 137	Female	2	1	*Shigella* sp
Mangoose (*Ichneumia albicauda*)	WTM 52F	Female	3	1	*Enterobacter* sp
Genet cat (*Genetta genetta*)	GNT 36	Female	2	1	*Enterobacter* sp
Bush buck (*Tragelophus scriptus*)	BB 71F	Female	1	1	*Edwardsiella* sp
Chimpanzee (*Pan troglodyte*)	CHM 6F	Female	2	1	*Providencia* sp
Total			**55**	**19**	

Appendix 2: Carnivores collection of Kano zoological garden, *Hausa* names, source and estimated cost.

Animal	***Hausa* name**	**Sex (M:F)**	**Source**	**Estimated cost (N)**
Cobra Snake (*Naja africanus*)	*Jan Nasuru*	1:3	Kano	1,000-1,500

Common Jackal (*Cannis aureus*)	*Dila*	3:3	Kano	5,000-10,000
Lion (*Panthera leo*)	*Zaki*	2:1	Kano, Niger Republic	1,000,000-2,000,000
Nile Crocodile (*Crocodilus niloticus*)	*Kada*	2:2	Kano	20,000-35,000
		1:0	Ibadan	7,000-10,000
Nile Monitor Lizard (*Veranus niloticus*)	*Guza*	0:2	Yobe	5000-10,000
Ratel/Honey Badger (*Mellivora capensis*)	*Dage*			
Spotted Hyena (*Crocuta crocuta*)	Bakar *Kura*	1:2	Borno	500,000-600,000
White tailed Mangoose (*Ichneumia albicauda*)	*Tunku*	1:2	Yobe	5,000-10,000
Striped Hyena (*Hyena hyena*)	*Sayaki*	1:1	Katsina	250,000-300,000
Sand fox (*Vulpes ferrilata*)	*Yanyawa*	0:1	Yobe	5,000-10,000
Total		**12:17**		**2,986,500.00**

Key: M = Male; F = Female; N = Naira

Appendix 3: Herbivores collection of Kano Zoological Garden, *Hausa* names, source and estimate cost.

Animal	***Hausa* name**	**Sex (M:F)**	**Source**	**Estimated cost**
Buffalo (*Syncerus cafer*)	*Bauna*	1:0	Niger Republic	1,700,000
Bush buck (*Cannis aureus*)	*Mazo*	3:3	Borno	250,000

Cape eland (*Tragelaphus oryx*)	*Gwanki*	1:3	Tanzania	200,000
Cane rat/Grass cutter (*Thronomys swindrianus*)	*Jauji*	2:3	Ibadan	10,000
Common hippopotamus (*Hippopotamus amphibious*)	*Dorina*	0:1	Niger	2,000,000
Crested porcupine (*Hystrix cristata*)	*Beguwa*	1:1	Yobe	30,000
Dorcas gazelle (*Gazelle dorcas*)	*Farar barewa*	2:1	Niger	350,000
Duiker (*Cephalophus monticola*)	*Gada*	0:1	Kaduna	40,000
Elephant (*Loxodonta africanus*)	*Giwa*	1:0	Borno	2,000,000
Giant tortoise (*Testudo* sulcato)	*Kunkuru*	10:10	Borno	200,000
Giraffe (*Giraffe camelopardilis*)	*Rakumin dawa*	2:1	Borno, Niger Republic	800,000
Red fronted gazelle (*Gazelle rufifrons*)	*Jar barewa*	4:3	Borno	450,000
Warthog (*Phacocherus aethiopicus*)	*Mugun dawa*	1:1	Borno, Jos	400, 000
Zebra (*Equus quagga*)	*Jakin dawa*	1:1	Bauchi	300,000
Total		**29:29**		**8,730,000.00**

Appendix 4: Non-human primate collection of Kano Zoological Garden, *Hausa* names, source and estimated cost.

Animal	***Hausa* name**	**Sex (M:F)**	**Source**	**Estimated cost (N)**
Chimpanzee (*Pan troglodytes*)	*Biri mai ganga*	1:1	Ibadan, Jos	250,000
Dog faced baboon	*Bika*	3:1	Kano	30,000

(*Papio Anubis*)				
Mona monkey (*Cercopithecus mona*)	*Mona*	1:1	Ibadan, Kano	15,000
Red patas *(Eryhthrocebus patas*)	*Gata*	2:10	Kano	20,000
Sooty mangabey (*Hippopotamus amphibious*)	-	1:0	Kano	40,000
Tantalus (*Cercocebus torquatus*)	*Kirka*	1:5	Kano	15,000
Total		**9:18**		**370,000**

Key: M = Male; F = Female; N = Naira

Appendix 5: Wild bird collection of Kano Zoological Garden, *Hausa* names, source and estimated cost.

Animal	***Hausa* name**	**Sex (M:F)**	**Source**	**Estimate cost**
Abdims stork (*Ciconia abdimii*)	*Shamuwa*	**1:1**	Jigawa	3,000
Buzzzard (*Buteo auguralis*)	*Ci kadangaru*	1:0	Bauchi	1,500

Egyptian vulture (*Aegypious tracheliotus*)	*Ungulun masar*	1:1	Egypt	12,000
Crown crane (*Balaerica pavononina*)	*Gauraka*	2:2	Borno	200,000
Egytian goose (*Alopochen aegyptiacus*)	*Dinyar masar*	11:8	Egypt	12,000
Emu (*Dromaius novohollandiae*)	*Jiminar Australia*	1:1	Katsina	500,000
Ground hornbill (*Bucovus cafer*)	*Bultu*	1:1	Bauchi	10,000
Hooded vulture (*Neophron monarchus*)	*Ungulu*	1:1	Yobe	6,000
Ostrich (*Struthio camelus*)	*Jimina*	1:0	Borno	350,000
Nubian vulture (*Torgus tracheliotus)*	*Farar ungulu*	1:1	Borno	75,000
Fish eagle (*Heliaecetus oocifer*)	*Mikiya*	1:0	Portharcourt	20,000
Bustard (*Chlamydotis undulata*)	*Kadabkara/jauji*	1:0	Niger Rep.	15,000
Kite (*Milvus milvus*)	*Shirwa*	1:2	Kano	1,500
Parrot (*Erythacus psittacus*)	*Aku*	1:0	Portharcourt	15,000
Spotted eagle owl (*Bubo africanus*)	*Mujiya*	2:3	Kano	1,500
Marabou stork (*Leptoptilus crumenifer*)	*Babba da jaka*	1:0	Borno	50,000
White stork (*Ciconia ciconia*)	*Tsagagi*	1:0	Jigawa	3,500
Total		**29:21**		**1,276,000.00**

Key: M = Male; F = Female; N = Naira

Appendix 6: **Animal identification, staff training, property management and biosecurity level in Kano Zoological Garden, Nigeria**

Animal identification staff training and property management	Biosecurity risk (%)	Risk Level
Individual animal not permanently identified with microchip/tatto	100	1
Zoo lack biosecurity manual accessible to all staff	100	1

Not all zoo staff have awareness of biosecurity	100	3
Staff not trained on general and site-specific biosecurity	100	3
Staff do not have understanding of major routes for disease transmission	100	3
Zoo not divided into biosecurity zones	100	2
Different species mixed in a single enclosure	50	3
Enclosures not appropriately cleaned	50	3
Equipment, furnishing, enrichment items not dedicated to single enclosure	50	3
Waste product not disinfected	100	3
Waste and waste material from enclosure not assessed for biosecurity risk	100	2
Food not stored under conditions that minimize contamination	0	0
Water source not inspected for contamination	100	3
No regular monitoring of water quality for aquatic animals	25	2
No effective program for pest control	100	3
Adequate facilities for quarantine and postmortem not available	100	3
Some facilities have no provision for safe capture and restraint	25	1
No hand washing facility for staff and visitors	100	3
Overall	**77.7**	**2.3**

Risk level; No risk = 0; Risky = 0.1; Moderately risky = 1.1 – 2.0; Highly risky = 2.1 – 3.0; Biosecurity risk (%); 0 = No risk; 1-25 = Very low risk; 26-50 = Low risk; 51-75 = Moderate risk; 76 – 100 = High risk

Appendix 7: Work and hygiene procedure and biosecurity risk level in Kano Zoological Garden, Nigeria

Work and hygiene procedures	Biosecurity risk (%)	Risk level
Not all staff working with animals wear dedicated foot wear	50	3
Staff not aware of biosecurity risk of visiting multiple enclosure in a day	100	3
Work and hygiene not altered during time of increase risk	25	2
Staff and visitors participating in interactive program not discouraged from eating, drinking or smoking	25	2
Hand washing/disinfection facilities not available to participants of interactive program	100	3
Number of vehicles entering and leaving zoo not minimized	100	3
Overall	**67.0**	**2.6**

Risk level; No risk = 0; Risky = 0.1; Moderately risky = 1.1 – 2.0; Highly risky = 2.1 – 3.0; Biosecurity risk (%); 0 = No risk; 1-25 = Very low risk; 26-50 = Low risk; 51-75 = Moderate risk; 76 – 100 = High risk

Appendix 8: Animal health, preventive medicine, zoonotic disease management and biosecurity risk level in Kano Zoological Garden, Nigeria

Animal health, preventive medicine, and zoonotic disease management	Biosecurity risk (%)	Risk level

Preventive medicine and health program not well established	50	3
Zoo veterinary service lack equipment suitable for zoo animals	100	3
Zoo not a member of network that enable access to biosecurity Information	100	2
Staff not fully aware of responsibilities for disease notification	25	3
No staff health program	30	2
Staff not provided with document on the risk of zoonotic disease in zoo	100	3
Staff not aware of species with increase zoonotic risk potential	75	2
Staff not aware that change in their health status can alter their risk of zoonotic disease	100	3
Staff not trained in zoonotic disease risk management	100	3
Overall	**72.2**	**2.0**

Risk level; No risk = 0; Risky = 0.1; Moderately risky = 1.1 – 2.0; Highly risky = 2.1 – 3.0
Biosecurity risk (%); 0 = No risk; 1-25 = Very low risk; 26-50 = Low risk; 51-75 = Moderate risk; 76 – 100 = High risk

Appendix 9: Quarantine practices and biosecurity risk level in Kano Zoological Garden, Nigeria

Quarantine practices	Biosecurity risk (%)	Risk level
Biological products arriving at zoo such as feather, semen not assessed for biosecurity risk	100	2
No signage to indicate areas of quarantine status	100	2

No signage to indicate areas of restricted access	100	2
Staff of quarantine area not trained in principle of barrier keeping	100	3
No separate, dedicated facility for quarantine of new animals	100	3
No dedicated equipment/tools for use only within quarantine areas	100	3
Facilities for hand sanitation not available in quarantine area	100	3
Newly arrived animals not accompanied by complete medical record	100	2
No adequate test for animals under quarantine	100	3
Zoo staff not trained on signs of illness in zoo animals	100	3
Overall	**100**	**2.6**

Risk level; No risk = 0; Risky = 0.1; Moderately risky = 1.1 – 2.0; Highly risky = 2.1 – 3.0; Biosecurity risk (%); 0 = No risk; 1-25 = Very low risk; 26-50 = Low risk; 51-75 = Moderate risk; 76 – 100 = High risk

Appendix 10: Classes and concentrations of antimicrobial agents used for antimicrobial sensitivity test on *Salmonella* isolates.

Antimicrobial category	Antimicrobial agent	Concentration
Phenicols	Chloramphenicol	30 µg
Aminoglycosides	Gentamicin	10 µg
	Streptomycin	10 µg
	Neomycin	30 µg
Quinolones	Ciprofloxacin	5 µg
	Enrofloxacin	5 µg
Macrolides	Erythromycin	10 µg

Tetracyclines	Doxycycline	30 µg
	Oxytetracycline	30 µg
B – Lactam	Amoxycillin	10 µg
	Ampicillin	10 µg
Folate Pathway Inhibitor	Trimethoprim	25 µg

Appendix 11: Carnivores album of Kano Zoological Garden, Nigeria.

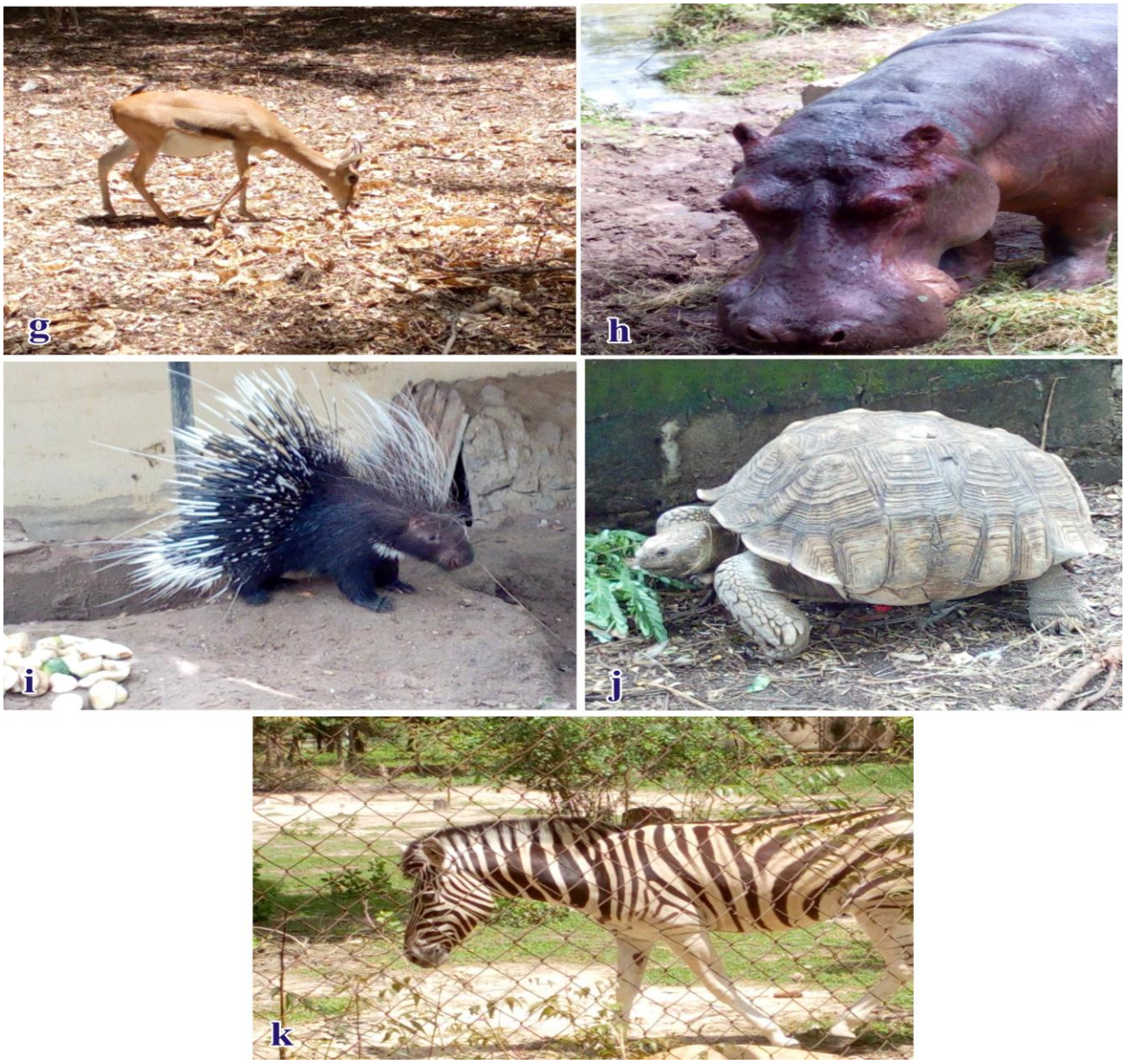


a. Lion b. jackal c. spotted hyena d. striped hyena e. genet cat f. mongoose g. Nile crocodile.

Appendix 12: Herbivores album of Kano Zoological Garden, Nigeria.



a. Cape buffalo b. bush buck c. duiker d. cape eland e. elephant f. giraffe.



g. Dorcas gazelle h. hippopotamus i. porcupine j. tortoise k. zebra.

Appendix 13: Non-human primate album of Kano zoological Garden, Nigeria.



a.Dog face baboon b. chimpanzee c. mona d. red patas e. sooty mangabey f. tantalus.

Appendix 14: Wild bird album of Kano Zoological Garden, Nigeria.



a. Abdim's stork b. buzzard c. crown crane d. Egyptian geese e. Egyptian vulture f. Nubian vulture.



g. Fish eagle h. kite i. emu j. ostrich k. mallard duck l. spur winged geese.



m. hooded vulture n.grey parrot o. marabou stork p. white stork q. peacock r. bustard