

**OCCURRENCE, MORPHOMETRIC IDENTIFICATION AND
HISTOPATHOLOGICAL LESIONS OF *EIMERIA* SPECIES IN JAPANESE QUAILS
(*Coturnix coturnix japonica*) IN ZARIA, NIGERIA**

BY

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OCTOBER, 2014

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BY

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M.Sc./Vet. Med/15458/2011-2012**

**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A
MASTER OF SCIENCE DEGREE IN VETERINARY PARASITOLOGY AND
ENTOMOLOGY**

**DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA**

OCTOBER, 2014.

DECLARATION

I declare that the work in this thesis entitled “**Occurrence, morphometric identification and histopathological lesions of *Eimeria* species in Japanese quails (*Coturnix coturnix japonica*) in Zaria, Nigeria**” has been performed by me in the Department of Veterinary Parasitology and Entomology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Halilu Adinoyi, UMAR

Name of Student

Signature

Date

DEDICATION

This thesis is dedicated to Almighty God and my parents, Engr. I. J. Umar and Barr. (Mrs.) H. A. Umar.

ACKNOWLEDGEMENTS

I am very grateful to God Almighty for the gift of life and the ability to have undertaken this research work successfully.

My profound gratitude goes to my supervisors, Professor I. A Lawal, Dr. O. O. Okubanjo and Dr. A. M. Wakawa for guiding me through this research work.

I appreciate all technical staff of the Department of Veterinary Parasitology and Entomology especially Mr. U. K. Amadi, Mr. Y. Magaji and Mr. S. Ummar of the Helminthology Laboratory.

I sincerely appreciate my family for their care, love, and support. Arc. and Mrs. Mohammed Suleiman, thanks for your support also.

My gratitude also goes to my colleagues and friends, Drs. Bashir Mohammed, Adanu Williams, Daniel Avazi, Aplakah Ikhangliah, Imodagbe Fadillah and Francis Abidemi. You all are appreciated for your moral support.

Finally, my gratitude goes to everyone who contributed to the success of this work and whose names do not appear on this page. God bless you abundantly.

ABSTRACT

Coccidiosis is one of the most important diseases of poultry worldwide. The aim of this study was to determine the occurrence and identify the species of *Eimeria* causing coccidiosis in quails in Zaria. The samples for the study were collected in Sabon Gari and Zaria Local Government Areas of Kaduna State. Four hundred faecal samples were collected from 10 farms, 40 pooled from each farm. They were processed using simple floatation technique and examined at x10 and x40 objective of microscope. Oocysts shape indices (length/width) of sporulated oocysts were determined by measuring their length and width using a calibrated ocular micrometer at x40 magnification and calculating the ratios of the length and width. Fifty birds were selected at random from the sampled farms and sacrificed. The intestines were harvested and observed for gross lesions. Segments of the intestine were taken to the laboratory and opened up, and the intestinal contents were removed, examined and analyzed using Geimsa, Haematoxylin & Eosin stain and then observed microscopically for the developmental stages of the parasite. A total prevalence of 45.75% was recorded for *Eimeria* infection, most of which were nonclinical. Information gotten from observations and questions asked showed that 100% percent of the farms had an incidence of water spillage, while 50% had a high stocking density. Frequency of litter disposal was poor in 60% of the farms and 80% of the farmers did not practice the prophylactic use of coccidiostats. Four species of *Eimeria* were detected, with only *Eimeria bateri* identified and conforming to the guidelines provided. The main gross lesion seen was non-haemorrhagic ballooning of the caeca. Intestinal scrapping smear revealed a developmental stage of the parasite (merozoites) in the jejunum. Histopathology also revealed a developmental stage (schizont) of the parasite in the caecum and desquamation of the epithelial lining with areas of necrosis. In

conclusion, the study showed that there was a relatively high prevalence of *Eimeria* infection in quails in Sabon Gari and Zaria Local Government Areas of Zaria. Damp environment and poor biosecurity practices were the predisposing factors for the disease transmission. However the infections were inapparent and *Eimeria bateri* was the only *Eimeria* spp confirmed in this study.

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LIST OF ABBREVIATIONS

%: Percent

µm: Micrometer

⁰C: Degree Celsius

DFRRI: Directorate for Food, Road and Rural infrastructure

g: Gram

H & E: Haematoxylin and Eosin

LGAs: Local Government Areas

NaCl: Sodium Chloride

NVRI: National Veterinary Research Institute

P.I: Post Infection

PABA: Paraminobenzoic acid

RBC: Red Blood Cell

rpm: Revolutions Per Minute

spp: Species

w/v: Weight per volume

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Research Study

Coccidiosis is one of the most important diseases of poultry worldwide. The disease is caused by protozoa of the Phylum: Apicomplexa which undergoes a direct life cycle with transmission between hosts by way of resistant oocysts. In the host, the parasite grows and multiplies intracellularly in epithelial and sub epithelial cells, usually in the gut inducing enteritis (Gordon and Jordan, 1982). Flocks infected as a result of mild to severe exposure usually show a marked decrease in food and water consumption and birds become depressed and tend to huddle. Decreased weight gains occur as a result of the disruption of the intestinal mucosa where minimal absorption is taking place. Diarrhoea may result as the host is trying to flush the organism from the body, which may induce dehydration. Lesions of the intestinal mucosa and loss of pigmentation may also become apparent during the latter stages of infection (Conway and McKenzie, 1991; Edgar, 1992).

The disease in some cases only occurs if a bird is subjected to heavy infection or its resistance to infection is lowered. It is important at the outset to differentiate between infection and disease. The presence of infection does not invariably lead to the development of clinical signs of disease. Low level of challenge can actually be beneficial by stimulating protective immune responses in the host (Catchpole *et al.*, 1993). The disease is cosmopolitan, occurring in practically all kinds of vertebrates including fowls, cattle, pigs, sheep, goats, cats and dogs (Ajayi and Todd, 1973). The problem of identification of species is simplified by the fact that parasites are host specific, each species occurs in a single or in a limited group of related hosts. In most of the described genera of Coccidia the genus *Eimeria* contains the species of major economic importance in

poultry. Internal arrangement of the sporocysts and sporozoites in a sporulated oocysts is the characteristic used to distinguish this genus from several others that may occasionally be encountered. The oocysts of all genera, when freshly passed, consist of a thickened outer wall and rounded mass nucleated protoplasm. Distinguishing characteristics of coccidia oocyst become apparent after sporulation. In the genus *Eimeria*, four sporocysts develop, each containing two banana-shaped sporozoites and this is distinguishable from the genus *Isospora* which contains two sporocysts each containing four sporozoites (Bruno *et al.*, 2014).

Various species of *Eimeria* have been isolated from the different species of quails such as *E. tsunodai*, *E. uzura*, *E. bateri* from Japanese quails (Teixeira *et al.*, 2004) and *E. lophortygis*, *E. okanaganensis* described from California quails while *E. crusti*, *E. oreortygis* are described from mountain quail (Duszynski and Gutierrez, 1981) and *E. conturnicis*, *E. bateri* are described from grey quail (Tsutsumi, 1972) and *E. colini*, *E. lettyae* from bob white quail (Ruff, 1985) also *E. tahamensis* from Arabian quail (Amoudi, 1987).

The Japanese quail, also known as Coturnix quail, *Coturnix japonica*, is a species of Old World quail found in East Asia. They are a migratory species, breeding in Manchuria, Southeastern Siberia, Northern Japan, and the Korean Peninsula, and wintering in the South of Japan and Southern China. They also dwell in grasslands and cultivated fields (Anon, 2014).

The Japanese quail was first introduced to Nigeria in 1992 (Musa *et al.*, 2008). The purpose was to diversify the poultry sub-sector and help supplement domestic chicken production through massive quail farming by Nigerian farmers. Since then, quail farming have been growing in popularity in Nigeria.

1.2 Statement of Research Problem

Coccidiosis is a major parasitic disease of poultry, in spite of advances made in prevention, control through chemotherapy, management and nutrition and it has a substantial economic impact on the poultry industries in Nigeria and other parts of the world (Trees, 1999; David, 2000; Etuk *et al.*, 2004).

According to Calnek *et al.* (1997) poultry production is constrained by many extrinsic factors among which malnutrition, poor management practices and the absence of biosecurity are outstanding. Parasitism like coccidiosis ranks high among the factors that threaten poultry production (Adene and Dipeolu, 1997). Birds that survive severe coccidiosis may never be productive, while survivors of one strain of *Eimeria* may become infected with another different strain thereby requiring further treatment (Chookyinox *et al.*, 2009). Although coccidiosis is controllable under most circumstances, the cost of control makes the disease one of the most expensive parasitic diseases encountered in the poultry industry (Etuk *et al.*, 2004).

The overall national increase in poultry production has probably triggered off vigorous research into alternative and cheaper feed resources urgently needed to sustain the growth of the industry, there is the need to continually focus attention on the health of the animals in order to realize the full potentials of the industry (Barksh, 2009). In spite of the exceptional attributes and advantages of keeping Japanese quail, its production in Nigeria is still comparatively rudimentary. Among the major challenges of quail production in Nigeria are diseases, high cost of feed, non-readily available market when the farmers are ready to sell their stock and

inadequate knowledge and information about the advantages of the consumption of quail meat and egg (Owen and Dike, 2013).

1.3 Justification of the Study

Quail production has become important in Nigeria, but there is lack of information on the occurrence of coccidiosis in Japanese quail in the study area. This is due to the fact that the Japanese quail which is now being bred for meat and eggs has the potential to serve as an excellent and cheap source of animal protein for Nigerians (Babangida and Ubosi, 2006). Quails are so precocious that they can lay eggs when hardly more than five weeks old and the meat is renowned for its low caloric value in addition to having high quality protein of high biological value (Haruna *et al.*, 1997).

The eggs are rich in vitamin D, antioxidants which according to Sahin *et al.* (2008) improve animal origin food quality in terms of colour, oxidative stability, tenderness, storage properties, and has positive effects on people with stress problems, hypertension, digestive disturbance, gastric ulcer, liver problems, blood pressure and lipid control, migraine, asthma, anaemia, various types of allergies, eczema, heart problems, bronchitis illness, depression, panic and anxiety illness. The nutritional value of quail eggs is 3 - 4 times greater than chicken eggs (Tunsaringkarn *et al.*, 2013). Quails are now also commonly used as an experimental animal for biological research and for producing vaccines against many diseases which they themselves are resistant to (particularly certain strains of Newcastle disease) (Shanaway, 1994). It promotes body and brain development in children (Onyewuchi *et al.*, 2013). Chicken meat and eggs though the major source of animal protein is still now unable to meet up the protein demand of the world (Igado and Aina, 2010) thus the need for alternative sources. Commercialization of

quail bird production is a recent development in Nigeria (Akpan and Nsa, 2009), and while quail farming is an uncommon farming business in parts of Nigeria and with lots of potentials, the people that have embraced are enjoying both the nutritional and health benefits derived from consuming it.

The high rate of returns and low cost of investment in the production of quail meat and egg are some of the reasons many farmers especially in the north are fast resorting to quail farming. Emphasis has been on domestic fowl production, whereas nutritive and economic benefits can be derived from quail production since the quail is fast growing and resistant to many diseases than domestic fowls (Oluyemi and Roberts, 2000).

Due to researches that have shown that the Japanese quail has the potential to serve as an excellent and affordable source of animal protein in Nigeria and with an increasing interest in quail production evidenced by the proliferation of quail farms, it is pertinent to continually evaluate the prevalence and management issues associated with common quail diseases such as coccidiosis in any given zone.

This study is therefore designed to report the occurrence of *Eimeria* species and their histopathological lesions in Japanese quails in Zaria, Nigeria.

1.4 Aim of the Study

To determine the occurrence of *Eimeria* oocysts, identify the species and their histopathological lesions in Japanese quails, in selected farms in Zaria, Nigeria.

1.5 Objectives of the Study

The objectives of the study were to:

- i. confirm the presence of *Eimeria* oocysts in Japanese quails in Zaria.
- ii. identify the *Eimeria* species found in the faeces and gastrointestinal tract of Japanese quails.
- iii. identify pathological lesions associated with *Eimeria* infection in Japanese quails.
- iv. evaluate the factors that predispose Japanese quails to coccidiosis in Zaria.

1.6 Research Questions

- i. Are quails in Zaria infected with *Eimeria* species?
- ii. What are the species of *Eimeria* found in Japanese quails in Zaria?
- iii. What are the gross and histopathological lesions caused by *Eimeria* species in Japanese quails?
- iv. Are there predisposing factors for the occurrence of coccidiosis in Japanese quails in Zaria?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Japanese Quail

Quail belong, along with chickens, pheasants and partridges to the Family Phasianidae, Order Galliformes and Class Aves of the Animal Kingdom (Howes, 1964). Species and subspecies of the genus *Coturnix* are native to all continents except the Americas. One of them *Coturnix coturnix* or common quail are migratory birds of Asia, Africa and Europe (Howes, 1964). Several interbreeding subspecies are recognized, the more important being the European quail, *Coturnix coturnix coturnix*, and the Asiatic or Japanese quail, *Coturnix coturnix japonica*. One subspecies that commonly migrates between Europe and Asia was eventually domesticated in China. These birds were raised as pets and singing birds. The domesticated coturnix were brought at about the eleventh century to Japan from China across the Korean bridge (Howes, 1964).

The Japanese quail was first introduced into Nigeria at the National Veterinary Research Institute (NVRI) Jos, Plateau state on the 18th of December, 1992, with the support of the Directorate for Food, Road and Rural infrastructure (DFRRI), (Musa *et al.*, 2008). Four hundred and fifty fertile quail eggs were imported from the republic of Benin out of which 377 were found suitable for incubation. After 17 days of incubation, 141 chicks hatched with an average weight of 4.5 g per chick. These chicks were brooded using a wooden brooder to which four 100 watts electric bulb were fixed. Water and commercial starter mash were fed for up to 5 weeks of age and replaced with layer's mash. The first egg was dropped at 8 weeks of age with average weight of eggs measuring 10 g (Musa *et al.*, 2007).

Thereafter eggs were set on 3-day basis. Subsequent setting from parent and filial generations led to a stock number of 15,000 quails and 12,000 eggs in the incubator after five months. The age of lay was found to improve from 8 weeks to 5-6 weeks with the first 2-3 filial generation, fertility and hatchability also improved from 58% and 70.5% respectively. This was probably due to acclimatization to the environment (Musa *et al.*, 2007).

More than ten years now, over 500 hundred thousand quail birds have been produced by National Veterinary Research Institute, Vom and distributed to farmers all over Nigeria, with vigorous encouragement and support of the National Animal Production Research Institute (NAPRI), various research studies were undertaken especially in areas of disease, nutrition, and adaptation and biomedical research. At present quails have been acclimatized and adapted in places like Kano, Benue, Lagos, Oyo, Yobe, Enugu, Akwa Ibom, Kaduna, Kebbi, Borno, Abuja, Niger, Kwara, Jigawa and Plateau states (Musa *et al.*, 2007).

Varieties of Japanese quails include: British Range, English White, Manchurian Gold Pharaoh, Tuxedo (Pappas, 2002; Sibley and Monroe, 1990). Shim (2005) stated that the domesticated subspecies, *Coturnix coturnix japonica*, is called Japanese quail but is also known by other names: Common quail, Eastern quail, Asiatic quail, Stubble quail, Pharaoh's quail, Red-throat quail, Japanese grey quail, Japanese migratory quail, King quail, and Japanese King quail. Shim (2005) now concluded that the correct popular nomenclature for *Coturnix coturnix japonica* should be Japanese quail or Coturnix, but not Coturnix quail since in Latin "*coturnix*" may be translated as quail.

2.1.1 Geographic Distribution of Japanese Quails

In general, Japanese quails inhabit parts of Russia (Johnsgard, 1988) and eastern Asia, including Japan, Korea and China (Hoffmann, 1988) as well as India (Finn, 1911). During winter, they migrate to China, South-Eastern Asia, the extreme North-Western coast of Africa, and a Sub-Saharan band North of Congo and including the Nile River valley from Egypt to Kenya. A small population has been found in Angola. This quails are also found in Nigeria, Kenya, Tanzania, Malawi, South Africa, Mozambique, and Namibia as well as parts of Madagascar. This quail may breed in parts of Europe, Turkey, and central Asia to parts of China (Alderton, 1992).

2.2 Coccidiosis in Japanese Quails

Coccidiosis is a parasitic disease of the intestinal tract of animals caused by coccidian protozoa. The disease spreads from one animal to another by the ingestion of infected faeces containing the sporulated oocyst. Diarrhoea, which may become bloody in severe cases, is the primary symptom. Most animals infected with coccidia are asymptomatic, but young or immunocompromised animals may suffer severe symptoms and death. Coccidia can infect a wide variety of animals, such as birds and livestock, and they are usually species-specific (Ettinger and Feldman, 1995).

The majority of the coccidia of importance in domestic animals belong to the genus *Eimeria*. The genus *Eimeria* is one of the 25 genera recognized under the Family: Eimeriidae of the Phylum: Apicomplexa. Various species of *Eimeria* has been isolated from the different species of quails such as *E. tsunodai*, *E. uzura*, *E. bateri* from Japanese quails (Teixeira *et al.*, 2004) and *E. lophortygis*, *E. okanaganensis* described from California quails, while *E. crusti*, *E. oreortygis* are described from mountain quail (Duszynski *et al.*, 1981) and *E. conturnicis*, *E. bateri* are

described from grey quail (Tsutsumi, 1972) and *E. colini*, *E. lettyae* from bob white quail (Ruff, 1985) also *E. tahamensis* from Arabian quail (Amoudi, 1987).

2.3 Distribution of *Eimeria* species in Japanese Quails

Eimeria species has been isolated from Japanese quails in South America, specifically in Brazil (Teixeira *et al.*, 2004). It has also been isolated in United States of America in North America and also in Iraq in the Middle East (Duszynski *et al.*, 1981; Mohammad, 2012).

2.4 Life Cycle of Coccidiosis in Japanese Quails

The coccidia exhibit a complex life cycle, comprised of both intracellular and extracellular stages. The intracellular reproductive process has asexual and sexual reproduction taking place inside the bird and the extracellular stage is maturation of the oocysts outside the bird by sporulation (Reid, 1978).

The typical life cycle of coccidia consists of three phases, sporogony, merogony (schizogony) and gametogony (Figure 1) (Lillehoj and Trout, 1993). During sporogony, which occurs outside the host, four sporocysts each containing two sporozoites are formed (Gordon and Jordan, 1982). Sporulated oocysts, when ingested by susceptible hosts, initiate the infective cycle. After ingestion sporozoites invade the intestinal epithelium and round up to form a trophozoite followed by nuclear division to form an immature meront (schizont) by which the merogony stage commences. A varying numbers of merozoites are being produced asexually by multiple fission from each meront. The first generation schizont measures up to 54 μm in diameter and may contain up to 900 first generation merozoites. The mature schizont ruptures into the lumen of the crypts of the caecal glands 3 days post infection (P.I) and the merozoites penetrate other epithelial cells to form young second generation schizonts. Colonies of the second generation

schizonts mature by day 4 post infection and release about 300 second generation merozoites into the lumen of the cecum. When large numbers of second generation schizonts are involved, a massive hemorrhage into the caecal lumen may be evident at about day 4 post infection (Soulsby, 1982). Second generation merozoites penetrate new epithelial cells and initiate either third generation of schizonts or the gametogonous cycle; the majority undertaking gametogony cycle.

Gametogony starts when merozoites invade cells and develop into either macrogamonts or microgamonts. The former gives rise to a single macrogamete whereas the latter undergoes multiple divisions resulting in the formation of numerous flagellated microgametes.

Fertilization occurs when the microgamete invades cells containing macrogamete; a wall forms as the oocysts mature (Lillehoj and Trout, 1993). Although the general live cycle is the same for all *Eimeria*, host specificity, site of development, patent and prepatent periods and pathogenicity vary between species. Only a small proportion of the millions of oocysts produced by a bird survives and become infective. Essential conditions for the survival are sufficient moisture, oxygen and suitable temperatures. Oocyst survival time is greatly extended in the presence of high humidity. The optimum level of temperature and relative humidity required for sporulation of oocysts are 29⁰C and 50-75% respectively (Urquhart *et al.*, 1987).

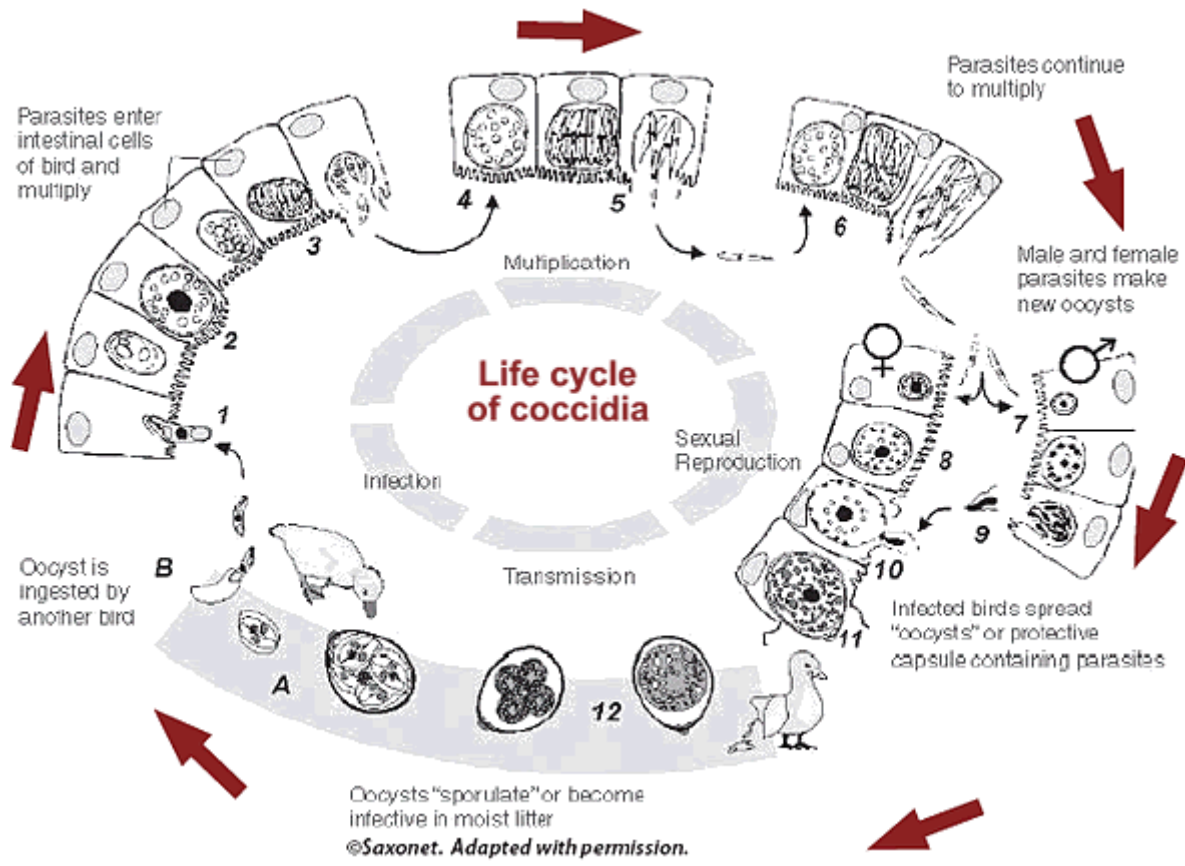


Figure 1: Life cycle of *Eimeria* species in birds

Source: (Anon, 2013)

2.5 Transmission and Occurrence of *Eimeria* Infection

Coccidiosis could be regarded as ubiquitous in poultry management (Soulsby, 1982). Ingestion of viable sporulated oocyst is the only natural method of transmission. Both diseased and recovered birds may continue to shed oocysts. There was a steady escape of oocysts demonstrated by the occasional finding of oocysts in the caecal contents up to 7.5 months, but birds stricken with severe coccidiosis may die before oocysts have completed their development (Conway and McKenzie, 1991).

Human beings are the main mechanical transmitters in disseminating oocysts, which could be spread by manure clinging to shoes or by utensils carried from one pen to another. Flies, beetles, cockroaches, rodents, pets and wild birds have also been incriminated as mechanical vectors (Reid, 1978). Oocysts may survive as long as 86 weeks in shaded soil. But sunlight assists in destruction of oocysts. Incubator temperature held for several days will kill oocysts, so there is no danger of hatchery transmission to the baby chicks. Oocysts are so resistant to disinfectants that they survive stringent attempts to kill them.

In birds reared outdoors under range conditions, coccidiosis outbreaks are common in the spring and summer than cooler fall and winter conditions. If confinement rearing is practiced, outbreaks may occur at any season of the year. However, there is some evidence of more rapid cycling in rainy season than in dry season (Soulsby, 1982).

The increased use of cages for laying birds has greatly reduced the number of outbreaks that occur in these birds (Reid, 1990). It was presumed that the possible causes of infection in cage birds are human beings and may be from feed processing manufacturers too. However, in deep

litter poultry houses, which offer optimal condition of temperature and humidity for oocyst sporulation, the risk of infection is further increased (Urquhart *et al.*, 1987).

Infection with single species of coccidia is rare in natural conditions, and mixed infections are common. Nevertheless, in many outbreaks the clinical entity can be ascribed principally to one species or occasionally a combination of two or three (Soulsby, 1982). The clinical disease is dependent on the number of oocysts ingested by individual birds. If the environmental hygiene is poor, this number may be very large which is particularly true for *Eimeria tenella*. But in very light doses no clinically recognizable symptoms may occur and thus, the morbidity and mortality increase in proportion to the size of the dose ingested (Soulsby, 1982).

Outward signs of coccidiosis in birds include droopiness and listlessness, loss of appetite, loss of yellow color in shanks, pale combs and wattles, ruffled, unthrifty feathers, huddling or acting chilled, blood or mucus in the feces, diarrhea, dehydration, and even death. Other signs include poor feed digestion, poor weight gain, and poor feed efficiency. Some signs can be confused with other diseases. For example, necrotic enteritis which is a gut disease that also causes bloody diarrhea (Ettinger *et al.*, 1995).

2.6 Diagnosis of Coccidiosis

A presumptive diagnosis is usually possible after a necropsy examination of recently sacrificed birds and microscopic examination of smear preparations. Confirmation may require cross-immunization experiments (Conway and McKenzie, 1991). Diagnosis of coccidiosis in birds is best accomplished by postmortem examination of representative number of birds. These postmortem lesions can be ballooning of the intestines, haemorrhages in the intestine, thickening of the intestinal wall, necrosis and sloughing off of the intestinal mucosa.

Diagnosis by fecal examination may lead to quite erroneous results (Soulsby, 1982). In some instances the major pathology is produced before oocysts are shed in the feces (e.g. *E. tenella*) and conversely, the presence of large number of oocysts may not necessarily indicate a serious pathogenic condition which can be due to host genetics, nutritional factors, concurrent diseases, and species of the *Eimeria*. Oocyst per gram (OPG) counts in faeces or litter have a poor relation with the impact of the parasite on the performance of a flock. Identification of different species based on morphology of oocysts is very challenging and requires expertise. Lesion scoring is an interpretation based on macroscopic visible lesions caused by *Eimeria*, usually following a scoring system from zero to four (Johnson and Reid, 1970). An important debate is still ongoing on what levels are to be considered clinical (and requiring treatment) and what levels are subclinical. Some consider lesions higher than 1.5 per species as indicative for clinical disease, and levels below as subclinical, not requiring treatment. *E. praecox* and *E. mitis* are not scored for and are completely disregarded using the lesion scoring method, although both species are shown to be able to cause losses through an increased feed conversion rate and in the latter case even morbidity (Gore and Long, 1982; Fitz-Coy and Edgar, 1992; Williams, 1998). Moreover, it has been demonstrated that there can be a poor relationship between macroscopic and microscopic lesions, therefore emphasizing the use of macroscopic lesion scoring alone is not suitable to detect all economical relevant coccidiosis infections (Idris *et al.*, 1997).

Necropsy sessions are performed in cooperation with the pharmaceutical industry in a number of countries. Basically, such systems consist of a planned, organized and benchmarked assessment of the lesion scores and gut health on poultry complex (group of farms on the same anticoccidial program) basis. A number of times per year and always at the same laboratory, preferably the same, well-trained specialists assess a significant number of poultry houses, thus improving the

reproducibility compared to a field lesion scoring session. This methodology is suitable for assessing the overall efficacy of the anticoccidial program, including reduced sensitivity and resistance of drugs in use. In order to make firmer conclusions, session data are compared with historical data.

A very innovative technique in diagnosis has been introduced which is called coccimorph (Gruber *et al.*, 2007). This is a computational approach for parasite diagnosis, in this case *Eimeria* spp. from chicken and rabbit. Images from sporulated oocysts from a confirmed species were assessed on different features: curvature characterization, size and symmetry and internal structure characterization. Users can upload their digital images from unidentified oocysts and have the program identify the species concerned. This is very accessible and the low cost is a major advantage. A disadvantage is only sporulated oocysts can be identified, which limits the use of this technique to litter sample identification only.

2.7 Clinical Signs and Gross Lesions of Coccidiosis

During the first week of life, few young quails presented diarrhoea, weakness and small blood spots in the upper small intestine (jejunum and ileum). Later, softening of feces at the 14th day and an increased cecum were seen in quails necropsied at the 21st days. From the 35th to the 42nd days, a significant number of quails had diarrhea, which disappeared soon. (Teixeira *et al.*, 2004)

There are few reports about the pathogenicity of *Eimeria* species in quails. Mazurkiewicz *et al.* (1967) reported clinical signs such as lack of appetite, ruffled feathers, uncoordinated movements, inhibition of egg laying and loss of weight in naturally infected young and mature quails reared at the laboratory. Norton and Pierce (1971) infected young Japanese quails experimentally with *E. bateri* and observed mild loss of weight and, although anorexia and softening of feces were observed at the third day of infection, the disease was considered mild and easy to overcome. Tsunoda and Muraki (1971) also reported low pathogenicity in Japanese quails experimentally infected with 1×10^5 oocysts of *E. uzura*, observing diarrhea and anemia from the 5th to 8th day of infection. Mortality was not reported in these researches, and the disease was considered similar to coccidiosis caused by *E. acervulina* in chickens. Later, Ruff and Fagan (1984) used pure and mixed cultures of *E. uzura* to infect quails. They reported a low mortality, lower weight gain and poor reproductive performance. Concerning weight gain, *E. tsunodai* was considered more pathogenic than *E. bateri*.

2.8 Microscopic Examinations of Coccidiosis

Parasitic stages (sporulated oocysts, merozoites and schizonts) demonstrated in fresh smear preparations are usually adequate in confirming a diagnosis. A standard parasitological technique for demonstration of the parasite from intestinal mucosa scraping can be used efficiently (Conway and McKenzie, 1991). Gross pathological lesions represented by softening of feces in duodenum, small intestine and more clearance in caecum, with thickening of mucosa and light hemorrhage in caecum. Histopathological lesions were characterized by severe hyperplasia of epithelial cells with construction of intestinal gland cavities in small intestine and caecum with presence of developmental stages of parasite in epithelial layer lining of intestinal glands. There was infiltration with inflammatory cells represented by eosinophils and presence of edema between the muscle fibers in small intestine and caecum (Conway and McKenzie, 1991).

Endogenous stages (merozoites, schizonts and gametocytes) of the parasites were found in the small intestine (jejunum). These were usually located in the villi, mainly above the nucleus of apical epithelial cells, or in the medium portion close to the glands. (Teixeira *et al.*, 2004).

These observations resemble those described by Tsutsumi (1972) not only because of the site of infection, but also morphology of the *Eimeria* species were similar. Thus, endogenous stages observed in the small intestine were assumed to be developmental stages of *E. bateri* and *E. uzura*, while the species found in the caecum might be *E. tsunodai* (Teixeira *et al.*, 2004).

Histopathological changes were also observed in the mucosa of the small intestine. Villous erosion, frequently concomitant with hyperplasia of the Crypts of Lieberkühn, was often

observed, as well as inflammatory infiltrate characterized by the presence of granulocytes and mononuclear cells, usually associated with edema (Teixeira *et al.*, 2004).

The colonisation of the gastrointestinal tract causes structural and functional changes, since the presence of parasites induces both general and local disturbances. A common feature of the infection is a severe depression of the digestive and absorptive capabilities of the mucosa. Furthermore, the magnitude of all disturbances and functional changes are usually related to the intensity of the parasitic infection (Teixeira *et al.*, 2004).

Oocyst size and shape are less useful as diagnostic characteristics in birds than in many other animals, because they are similar except with *E. maxima*. Measurement of a number of oocysts (10 suggested) using an ocular micrometer is required (Conway and McKenzie, 1991). Length and width must be determined by selecting a side and not an end view of the oocyst. Thick fecal smears frequently prevent securing the necessary side view of the oocyst. Average length, average width, range in length, and range in width should be determined (Reid, 1978). In critical studies in species identification additional techniques other than microscope examinations are needed, such as:

- Time required for sporulation and prepatent period
- Cross-immunity tests
- Location of the parasite in relation to the host epithelial cells nuclei and
- Tissue culture or egg embryo developmental differences may prove useful in identification of some species.

2.9 Economic Importance of Coccidiosis

Coccidiosis is one of diseases of poultry that play inhibitory role in the growth of this industry. It inflicts the birds in both clinical and subclinical forms. The clinical form of the disease manifests through prominent signs of mortality, morbidity, diarrhoea or bloody faeces, and subclinical coccidiosis manifests mainly by poor weight gain and reduced efficiency of feed conversion and gives rise to highest proportion of the total economic losses (Williams, 1999). Although coccidiosis is probably the most frequently reported disease of chickens worldwide (Biggs, 1982), there are considerable difficulties in arriving at a reliable figure for the specific financial losses. There are very few reports on economic losses due coccidiosis (Oyekole, 1984; Braunius, 1987; Graat *et al.*, 1996). The increased crowding of birds under mass production methods creates a favorable condition for the occurrence of coccidiosis. It is one of the top five poultry disease most frequently diagnosed in the field and laboratory representing 5-15% of all mortalities, with subclinical coccidiosis more common than clinical coccidiosis. Losses due to this form of the disease are heavy and cannot be estimated (Gordon and Jordan, 1982).

A mild coccidiosis infection kept under control is not very harmful, and is actually necessary for creating immunity in replacement flocks and free ranging birds. However, a severe attack of the disease can cause weight loss, reduced egg production; morbidity and mortality (Lillehoj and Trout, 1993).

2.10 Treatment of Coccidiosis

In general, there are about 25 or more approved anticoccidial drugs (Soulsby, 1982). Selection of the best is based on the ability of the drug to improve weight and feed conversion and to suppress the development of lesions. Anticoccidial agents (coccidiostats) show activity early in the life cycle of the organism that few benefits are derived from treatment in later stages after symptoms appear. Early recognition of the disease may permit initiation of treatment before all birds have peaked up massive doses of sporulated oocysts. Delay of a few hours may be costly and delay of a full day may render treatment useless. For this reason, the preventive approaches have largely superseded treatment in the use of the drugs (Chapman, 2005).

The emergence of drug resistance strains of coccidia in birds presents a major problem. Continuous use of anticoccidial drugs leads to increased incidence of drug resistant strain development, which results in reduced activity of the drug against the agent (Graat *et al.*, 1996). Methods used to avoid the development of drug resistance include switching around the different classes of drugs: that is drug rotation and ‘shuttle program’, which is a planned switch of drugs in the middle of the growing period of birds (Soulsby, 1982).

2.11 Prevention and Control of Coccidiosis

Modern intensive poultry production is largely dependent upon chemoprophylaxis for the control of coccidiosis (Chapman 1999; Allen and Fetterer, 2002), although there is a rising problem of drug resistant strains of *Eimeria*. In addition the use of live vaccines for control of coccidiosis is also well established (Williams, 2002). The life cycle of *Eimeria* comprises intracellular, extracellular, asexual and sexual stages, so it is not surprising that host immunity is also complex and involves many facets of non-specific and specific immunity (cellular and humoral immune mechanism) (Lillehoj, 1998), and their pathogenicity varies in birds of different genetic

background. Therefore, in the natural host, the immunity is species specific (e.g. chickens immune to one species of *Eimeria* are susceptible to others). Additionally, *Eimeria* species exhibit different tissue and organ specificity in the infected host, so, understanding the interplay between the host and the parasites in the intestine is crucial for the design of novel control approaches against coccidiosis (Dalloul and Lillehoj, 2005). The introduction of alternative prevention measures such as non-chemical feed supplements that effectively enhance productivity and non-specific immunity may help to limit the use of anticoccidials in control of chicken coccidiosis.

2.11.1 Control using Anticoccidial Drugs

The effective use of anticoccidial drugs over the past 50 years has played a major role in the growth of poultry industry and has allowed the increased availability of high quality, affordable poultry products to the consumer. Numerous products were introduced, many of which are available and used today. However, there is increasing concern about rising levels of drug resistance (Chapman, 1997). The anticoccidial drugs can be classified as Synthetic drugs (chemicals) and Polyether ionophores. Synthetic drugs have specific modes of action against parasite metabolism, sulphamides and related drugs compete for the incorporation of paraminobenzoic acid (PABA) and metabolic of folic acid, amprolium compete for absorption of thiamine by the parasite. Quinoxaline and clopidol inhibit energy metabolism in the cytochrome system of coccidia. The quinolones and ionophores arrest or kill the sporozoite or early trophozoite, nicarbazin, robenidine and zoalene destroy the first or second generation schizonts and the sulphonamides act on the developing schizonts and also on the sexual stages. The ionophores kill coccidia by interfering with the balance of important ions such as sodium and potassium. The host cells are able to manage these ions in the presence of ionophores, but the

parasites cannot. Synthetic drugs were introduced first, then the ionophores followed and are now an important component of coccidiosis control. The recent anticoccidial drugs in control of coccidiosis are diclazuril and toltrazuril (Chapman, 1999). The use of toltrazuril as the sole anticoccidial for two consecutive days in the drinking water between days 10 and 14 would be the best time for good coccidiosis control (Mathis *et al.*, 2004). Mehlhorn *et al.* (1988) reported that toltrazuril is effective against all species of *Eimeria* infecting chickens. Mehlhorn *et al.* (1984) also reported that toltrazuril is active against all intracellular developmental stages including those of schizogony and gametogony. It has been reported that despite high efficacy toltrazuril does not interfere with the development of natural immunity but can even enhance it (Greif, 2000). Mathis *et al.* (2003) demonstrated that toltrazuril was an effective aid to certain anticoccidial program as well as effective as a standalone anticoccidial. Vanparijs *et al.* (1989ab, 1990), McDougald *et al.* (1990ab) reported that diclazuril has a potent, broad-spectrum anticoccidial activity against *Eimeria* species. Chapman *et al.* (2004) reported that roxarsone has important anticoccidial activity particularly against *E. tenella* and works very well in combination with ionophores. Also combinations of anticoccidials such as salinomycin and roxarsone with a digestive enhancer such as bacitracin are widely used in the starter and grower feeds of broilers for control of coccidiosis and improvement of growth in broilers (Chapman and Johnson, 2002). It is quite clear that some degree of resistance to all anticoccidial drugs, including ionophores, has developed (Chapman, 1997). To minimize the effects of resistance, poultry producers rotate the use of various anticoccidials with successive flocks, where drugs from different classes are used sequentially on a single crop of birds, one class might be used in starter feed, another in growers, returning to the first for the finisher diet, followed by a withdrawal diet (Sangster, 2001).

Polyether ionophores: Since 1971 the preferred drugs for coccidiosis prevention have been ionophore antibiotics. These drugs still achieve sufficient control despite resistance being common; for example, salinomycin, narasin, monensin, lasalocid, maduramicin and semduramicin remain useful agents except in situation of heavy parasite challenge (Chapman, 1997). In addition it has been demonstrated that some ionophores can be used in combination with live virulent vaccines, therefore the use of ionophores-tolerant resistant strains would probably have a wider application of the development of anticoccidial vaccines for suitable control of coccidiosis (Danforth, 2000; Vermeulen *et al.*, 2000a). The advantage of such ionophores is that they prevent infection during the first 3-4 weeks of age when immunity is not developed, such use limits the increase of infection pressure due to the expanding field strains during the development of immunity, which further reduces the overall risk of contracting coccidiosis (Vermeulen *et al.*, 2000a; 2001). It is known that coccidiosis is aggravated by microflora, for example *Clostridium perfringens* interacting with intestinal mucosa damage as well as the developmental stages of *Eimeria* parasites. This problem can be reduced by the use of antibacterial properties of the ionophores. The use of ionophores such as monensin, narasin and salinomycin in combination with live virulent vaccines (Nobilis COX ATM) can protect birds against coccidiosis as well as necrotic enteritis. Advantages claimed for this method are protection by the ionophore against coccidiosis (due to wide-type strains) in the period before effective immunity has developed, protection against species not included in the vaccine and effectiveness against bacterial diseases such as necrotic enteritis due to the additional properties of the ionophores (Chapman *et al.*, 2002; Vermeulen *et al.*, 2000ab).

2.11.2 Vaccines against *Eimeria* Parasites

It is known that when chickens are infected with low number of *Eimeria* parasites, protective immunity is induced after two or three consecutive infections (Joyner and Norton, 1973; Long *et al.*, 1986). Therefore, it would seem obvious that vaccines could offer excellent alternatives to drugs as a means of controlling coccidiosis. Thereby live vaccines have been used mostly in breeder stocks and, to a lesser extent, in commercial broilers and replacement hens. This strategy is based on the well-documented protective immunity that develops in chickens after a primary coccidial infection (Williams, 2002). Three groups of live vaccines are used in control of disease:

- i. Live, virulent strains
- ii. Live, attenuated strains
- iii. Live, tolerant to ionophores

2.11.3 Alternative Controls including Nutritional and Probiotics (Immunomodulators) or Natural-Feed Additives

Natural medicinal products as feed supplements have been widely used as growth and health promoters in farm animals in China (Li, 1998). A current estimation of the number of immunoactive natural medicinal products ranges between 200 and 300 and most products originate from plants and fungi (Li, 2000). The immunoactive components of these plants and fungi include polysaccharides, glycosides, alkaloids, volatile oils, and organic acids, of which polysaccharides are considered to be the most important (Xue and Meng, 1996; Li, 2000). Polysaccharides may act as immune enhancers or immunomodulators, and these components may display antibacterial activity (Xia and Cheng, 1988) and could affect both innate and adaptive immunity including cellular and humoral responses (Lien and Gao, 1990). Also some mushrooms and herb polysaccharides which were used as feed supplements or vaccines

adjuvants showed antibacterial (Yuan *et al.*, 1993) antiviral (Yu and Zhu, 2000), or antiparasitic (Pang *et al.*, 2000) effects.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Zaria, located in Kaduna State, within latitudes $11^{\circ}7'$ to $11^{\circ}12'$ N and longitude $07^{\circ}41'$ E. It is a medium sized city with an estimated population of 408,198 (NPC, 2006). It is divided administratively into Zaria and Sabon Gari Local Government Areas (Kaduna State Ministry of Economic Development, 1996). It has an estimated land area of about 300 square kilometers and it is approximated that about 40-75% of its working population derive their principal means of livelihood from agriculture (Anon, 2000). Zaria which is located in the North Guinea Savannah zone of Nigeria has an annual ambient temperature, ranging between $18.0 \pm 3.7^{\circ}\text{C}$ and $31.8 \pm 3.2^{\circ}\text{C}$, The harmattan season (the cold-dry period of the year) lasts from November to February, while the hot-dry season lasts from March to May and the rainy season from June to October (Anon, 2010).

3.2 Study Design

3.2.1 Questionnaire

Permission was sort from and granted by the quail farmers for questionnaires to be administered to them. A semi structured questionnaire was designed, distributed to them and it covered information on bio data, health indicators and predisposing factors responsible for the occurrence of coccidiosis in Japanese quails in the study area.

3.2.2 Sampling

Sampling was carried out in Sabon Gari and Zaria Local Government Areas of Kaduna State. Quail farms in these areas were limited in number, therefore a total of ten quail farms, five from each LGA were identified and sampled from. Faecal sampling was done by collecting fresh faecal droppings from each of these farms. Sampling was carried out from the months of April to June 2013. The samples were brought to the laboratory for examination. Samples that could not

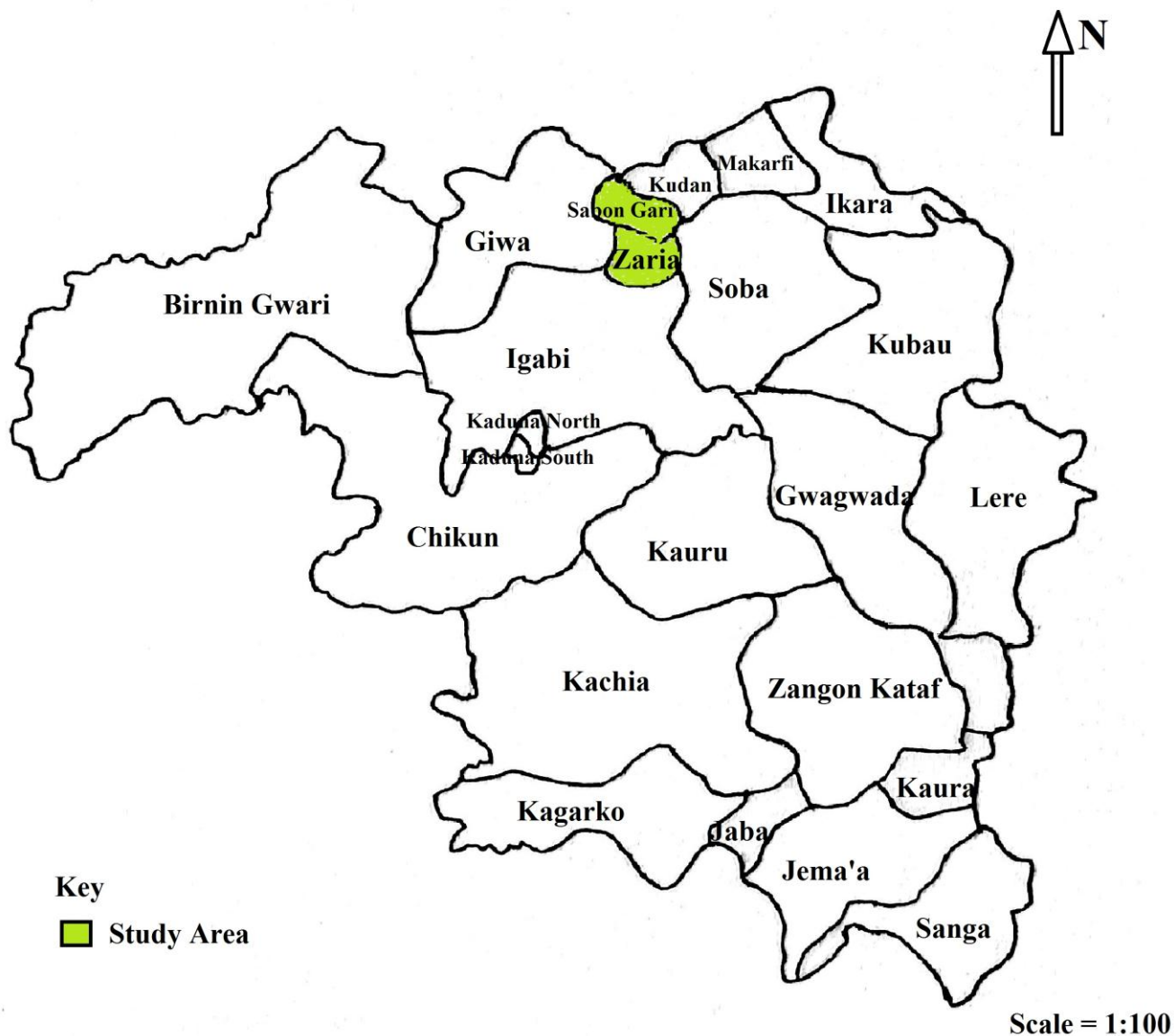


Figure 2: Map of Kaduna State showing Sabon Gari and Zaria Local Government Areas
 Source: (Anon, 2012)

be processed were refrigerated and analysed not later than two days after collection. Live birds were purchased and sacrificed for gross and histopathological studies.

3.3 Sample Size

Sample size was determined using the formula of Thrusfield (1997).

$$N = \frac{Z^2 pq}{d^2}$$

Where N = sample size

Z = appropriate value for the standard normal deviate for the desired confidence = 1.96

p = prevalence

q = 1 - p

d = level of significance (0.05)

The occurrence of coccidial infection in quails is not known in the area, so the sample size will be determined using 50% prevalence.

$$\begin{aligned} \text{Therefore } N &= \frac{1.96^2 \times (0.50 \times 0.50)}{(0.05)^2} \\ &= 384.16 \end{aligned}$$

A total number of 400 samples was finally collected.

3.4 Sample collection, Handling and Processing

A total of 400 faecal samples (40 pooled from each quail farm) was collected using polythene bags, while 50 birds were purchased from one of the quail farms. The birds were sacrificed and their gastrointestinal tracts harvested. The samples were labeled and transported to the helminthology laboratory, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Samples not examined immediately were refrigerated at 4°C to maintain the integrity of the oocyst (Duszynski and Wilber, 1997).

3.4.1 Laboratory Examination of Faecal Samples

The faecal samples were examined for the presence of coccidia oocysts using the simple floatation technique (Urquhart *et al.*, 1987).

Furthermore, the intensities of the infection of each sample positive for coccidia oocyst, were categorized as described by Lawal *et al.* (2008) as follows: 1-10 oocysts per field = +1 (inapparent infection), 11-20 oocysts per field = +2 (low grade infection), above 20 oocysts per field = +3(severe infection).

3.4.2 Specie Identification

For each of the positive samples, the oocysts shape index (length/width) of each were determined by measuring its length and width using a calibrated ocular micrometer at x40 magnification and calculating the ratio of the length and width. A total oocyst shape index of 20 randomly selected oocyst determined.

The calculated oocysts shape index value of each oocyst was compared with the standard diagnostic guide provided by Teixeira and Lopes (2002) to determine the species encountered in the study.

The procedure used in the determination of oocyst shape index is as follows:

Each of the positive samples were thoroughly mixed with aqueous potassium dichromate (2.5% w/v) solution, and then placed in thin layers in Petri dishes to allow aeration of the oocysts and allowed to sporulate for 7-10 days at room temperature (24⁰C to 30⁰C) as described by (Adefolabi and Chiejina, 1987; Harper and Penzorn, 1999).

To retrieve and concentrate the oocysts, dichromate solution was then centrifuged at 300 rpm for 10 min and the supernatant discarded (Harper and Penzorn, 1999). The sediment contained in the tube was then filled with distilled water until a convex meniscus was formed. A cover slip was then placed on the tube, allowing 10 min for oocysts to float unto the cover slip. The cover slip was then removed carefully in a vertical manner, placed on a microscope (Harper and Penzorn, 1999). The slide was then scanned in parallel sweeps and the oocyst seen were identified. Measurements was carried out with an ocular eyepiece, calibrated with a micrometer, under a 40x objective (magnification factor x 3.64).

3.4.3 Postmortem Examination

The gastrointestinal tracts of the sacrificed birds were examined macroscopically for pathological lesions. The various segments of the small intestine (duodenum, jejunum and ileum) and large intestine (caecum and colorectum) were separated and each halved. The first halves were placed in polythene bags, labeled and immediately taken to the Veterinary Protozoology Laboratory for Intestinal Scrapping Smear, while the other halves of the intestinal segments were placed in bottles containing 10% formalin and then taken to Histopathology laboratory of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for processing. Histological examination was then carried out to confirm the presence of the developing stages of the parasites within the various segments according to the method described by Mitchell *et al.* (2003).

3.5 Data Analysis

The occurrence of *Eimeria* species in quails was determined by the formula

$$\text{Occurrence} = \frac{\text{Positive samples}}{\text{Total samples analyzed}} \times 100$$

Results were presented using tables and charts. Data was analyzed using descriptive statistics and Chi-square was used to determine the strength of association between the factor and the disease. Values of $P < 0.05$ were considered significant. These was carried out using the Statistical Package for Social Sciences (SPSS) version 17.

CHAPTER FOUR

4.0 RESULTS

4.1 General Observations during Sampling

The clinical sign that was observed during sampling was diarrhoea. The nature of the faeces was dark brown in colour and watery. The birds were actively feeding, drinking and apparently healthy.

4.2 Questionnaire Analysis of the Information gotten from Japanese quail farmers in Zaria, Kaduna State, Nigeria.

Extracts from the questionnaire revealed that the farmers aged between 20 to 40 years old, maintained an average population of 200 quails per farm using the deep litter system. The birds were acquired from different sources where available and were fed on commercially compounded feed (Feed millers). The quails were said to begin laying between 6 to 7 weeks of age. According to the farmers mortality was rare in the farms but when noticed they treated the birds themselves. However 60% of the farmers claimed to have experienced non-specific clinical signs such as diarrhoea, weight loss and weakness in their flock. 100% percent of the farms had an incidence of water spillage and 80% of the farmers did not practice the prophylactic use of coccidiostats (Figure 3). Ventilation was poor in 10% of the farms (Figure 4), while 50% had a high stocking density (Figure 5). Frequency of litter disposal was poor in 60% of the farms (Figure 6)

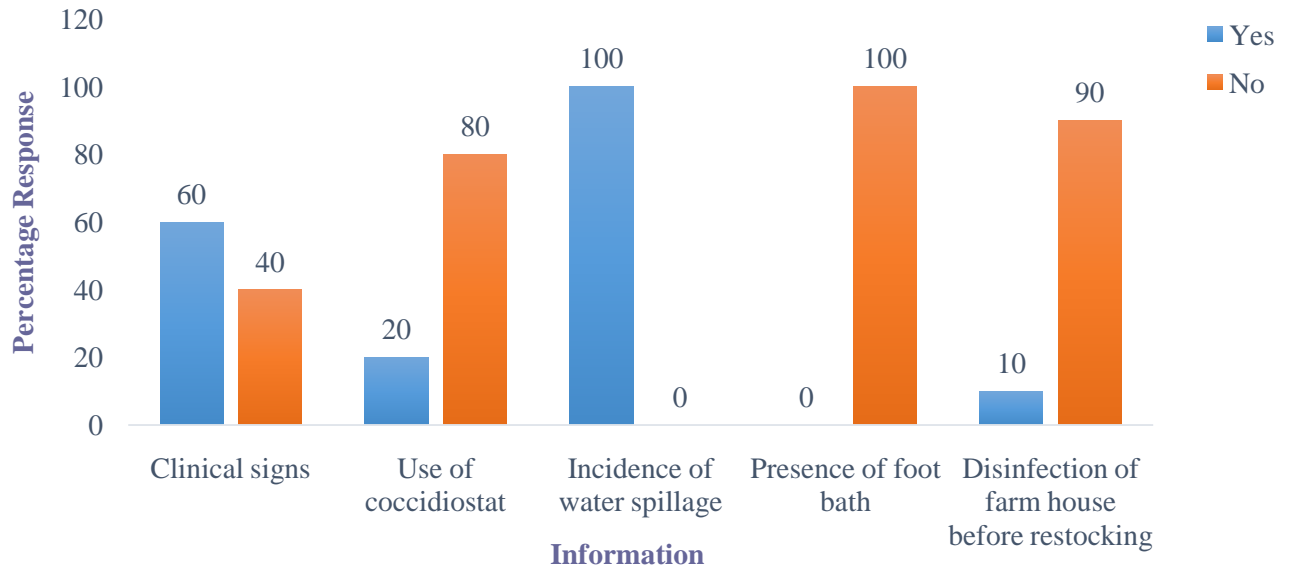


Fig 3: Bar Chart showing percentage response by quail farmers to questions on management practices in Zaria, Kaduna State, Nigeria.

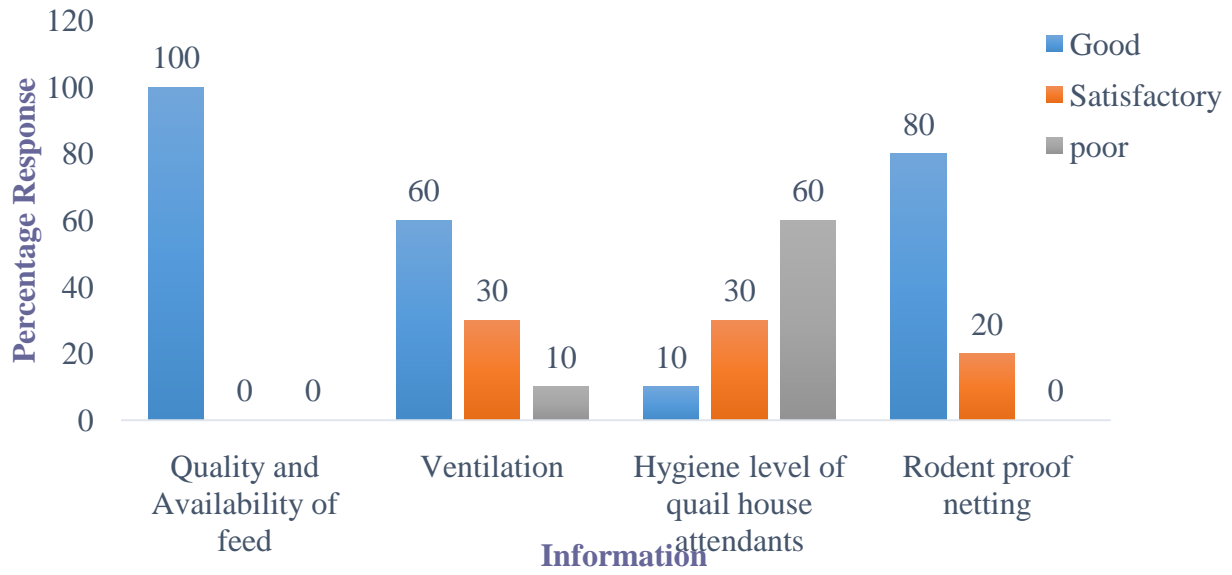


Figure 4: Bar Chart showing percentage response by quail farmers to questions on management practices in Zaria, Kaduna State, Nigeria.

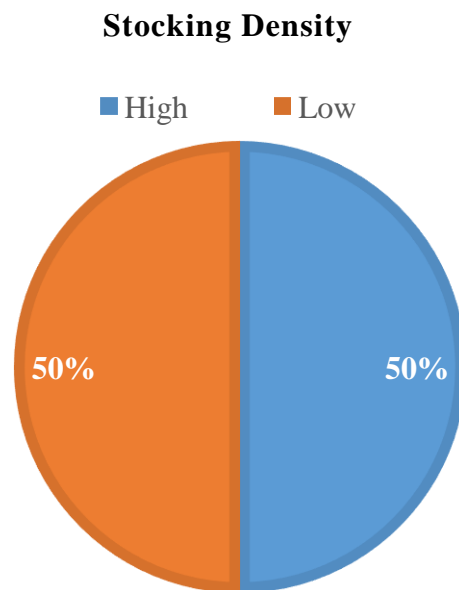


Figure 5: Pie Chart showing percentage stocking density in Japanese quail farms in Zaria, Kaduna State, Nigeria.

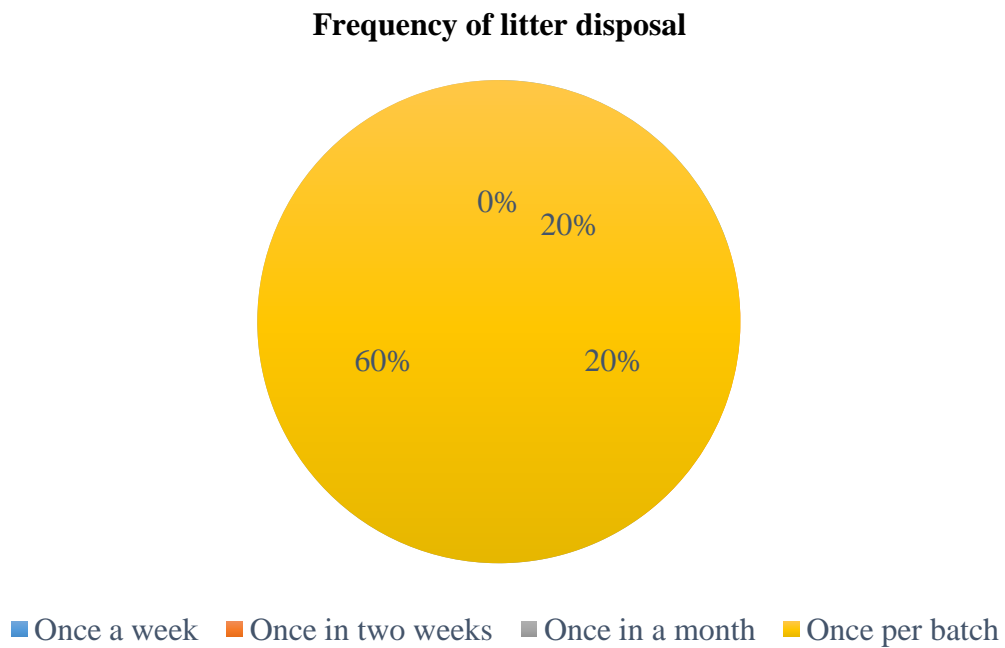


Figure 6: Pie Chart showing percentage frequency of litter disposal practiced by Japanese quail farmers in Zaria, Kaduna State, Nigeria.

4.3 Results of Flootation Technique

Varying occurrence rates of *Eimeria* infection were recorded in the farms sampled (Table 4.1). A high occurrence of 87.5% was recorded in Farm D, while none of the samples from Farm C was positive (0% occurrence).

In Table 2, an overall occurrence of 45.75% was recorded in all the farms. The occurrence rate for Sabon Gari (55%) was significantly higher than that of Zaria Local Government Area (35.5%). Intensities of *Eimeria* infections in Japanese quails Sabon Gari and Zaria LGA's, varied from inapparent, low grade to severe infection (Table 4.3 and 4.4). Although inapparent infections predominated, low grade characterized the infections recorded in Japanese quails in Farms (A, B, D and E). Severe infections were low in occurrence and were 4.40%, 2.90% and 3.50% in Farms B, D and E respectively (Table 4.3).

Varied intensities of infections also characterized the quails sampled in Zaria Local Government Area were inapparent infections predominate (Table 4.4). Consequently three Farms F, I and J had 100% inapparent type of infections. Low grade infection was scanty and was only seen in Farm J. Severe infections were observed in two farms (G and J).

Table 4.1: Occurrence of *Eimeria* oocysts in Japanese quails from farms in Sabon Gari and Zaria Local Government Areas, Kaduna State, Nigeria.

Farms	No. sampled	Positive samples	Occurrence (%)
A	40	23	57.5
B	40	23	57.5
C	40	0	0
D	40	35	87.5
E	40	29	72.5
F	40	1	2.5
G	40	20	50
H	40	4	10
I	40	15	37.5
J	40	33	82.5
Total	400	183	45.75

Chi-square (χ^2) = 151.7, df =9, *p*-value = 0.001

Table 4.2: Occurrence of *Eimeria* oocysts in Japanese quails in Sabon Gari and Zaria Local Government Areas, Kaduna State, Nigeria.

Local Government Areas	No. Sampled	Positive Samples	Occurrence (%)
Sabon Gari	200	110	55
Zaria	200	73	36.5
Total	400	183	45.75

Chi-square (χ^2) = 13.79, df = 1, *p*-value = 0.0002

Table 4.3: Intensity of *Eimeria* oocysts in Japanese quails isolated from farms in Sabon Gari local Government Area, Kaduna State, Nigeria.

	Sabon Gari Local Government Area										
	A		B		C		D		E		
	No.	%	No.	%	No.	%	No.	%	No.	%	
Intensities of infection											
Inapparent infection	19	82.61	20	86.96	0	0	32	91.43	24	82.76	
Low grade infection	4	17.39	2	8.7	0	0	2	5.71	4	13.79	
Severe infection	0	0	1	4.35	0	0	1	2.86	1	3.45	
Total	23		23		0		35		29		

Table 4.4: Intensity of *Eimeria* oocysts in Japanese quails isolated from farms in Zaria local Government Area, Kaduna State, Nigeria

	Zaria Local Government Area									
	F		G		H		I		J	
Intensities of infection	No.	%	No.	%	No.	%	No.	%	No.	%
Inapparent infection	1	100	19	95	4	100	15	100	28	84.85
Low grade infection	0	0	0	0	0	0	0	0	4	12.12
Severe infection	0	0	1	5	0	0	0	0	1	3.03
Total	1		20		4		15		33	

Table 4.5: Dimensions of the sporulated oocysts, morphological characteristics and speciation of *Eimeria* isolated from the faeces of Japanese quail from farms in Zaria, Kaduna State, Nigeria.

Species	Oocyst size (μ)		Range	Shape Index	Morphology	Polar granule	Oocyst wall	Likely species
	Length (Mean \pm StE.)	Width (Mean \pm StE.)						
<i>a. Eimeria</i> spp	22.20 \pm 0.58	16.38 \pm 0.42	(18.20-25.48) (14.56-18.20)	1.36	Subspherical	Present	Double	<i>Eimeria bateri</i>
<i>b. Eimeria</i> spp	22.36 \pm 1.67	15.08 \pm 0.95	(14.56-25.48) (10.92-18.20)	1.48	Ovoid	Present	Double	Unknown
<i>c. Eimeria</i> spp	16.64 \pm 1.08	16.12 \pm 0.74	(14.56-21.84) (14.56-18.20)	1.03	Ellipsoidal	Present	Double	Unknown
<i>d. Eimeria</i> spp	20.57 \pm 0.40	14.74 \pm 0.56	(18.20-21.84) (10.92-21.84)	1.40	Subspherical	Absent	Double	Unknown

4.4 Micrometry (Dimensions, Morphology and Speciation)

a. *Eimeria bateri*: Sporulated oocysts were subspherical measuring 22.20 ± 0.58 by 16.38 ± 0.42 μm and shape index 1.36. Oocyst wall was smooth, double layered. A single and refractive polar granule was present, but micropyle and the residual body of the oocyst were absent (Plate III).

b. *Eimeria* species: Sporulated oocysts were ovoid, measuring 22.36 ± 1.67 by 15.08 ± 0.95 μm and 1.48 shape index. Oocyst wall was smooth, double layered. A Polar granule was present. Micropyle and residual body of the oocyst were absent (Plate V).

c. *Eimeria* species: Sporulated oocysts were ellipsoidal, measuring 16.64 ± 1.08 by 16.12 ± 0.74 μm , and 1.03 shape index. Oocyst wall was smooth, double layered. A polar granule was present. The micropyle and residual body of the oocysts were absent (Plate VI, VII).

d. *Eimeria* species: Sporulated oocysts were subspherical, measuring 20.57 ± 1.08 by 14.74 ± 0.56 μm , and 1.40 shape index. Oocyst wall was smooth, double layered, and colorless outer layer. A Polar granule was absent and the micropyle and residual body of the oocysts were absent (Plate IV).

4.5 Gross and Histopathological lesions

The main gross pathological lesion is the ballooning of the caeca with no bloody exudate in the lumen (Plate VIII). Histopathologically, there was desquamation of intestinal epithelium (Plate XI) and caecal necrosis (Plate X). Developmental stages of the parasite, specifically the merozoites (Plate IX) and schizonts were seen in the intestinal epithelium (Plates X and XI).

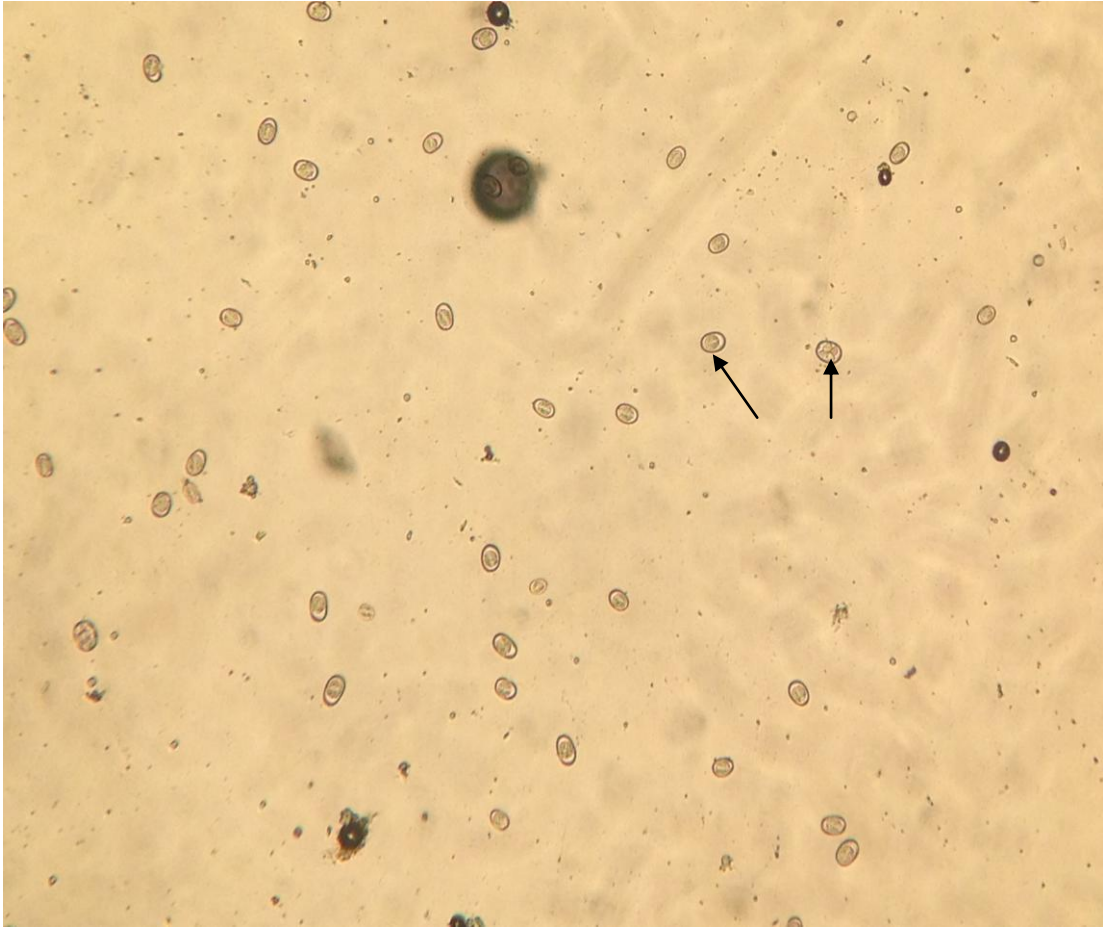


Plate I: Numerous unsporulated *Eimeria* oocysts (arrows) isolated from Japanese quails in farm F using simple floatation technique (x10).



Plate II: Sporulated *Eimeria* oocysts (arrow) isolated from Japanese quails in farm H using simple floatation technique (x40).

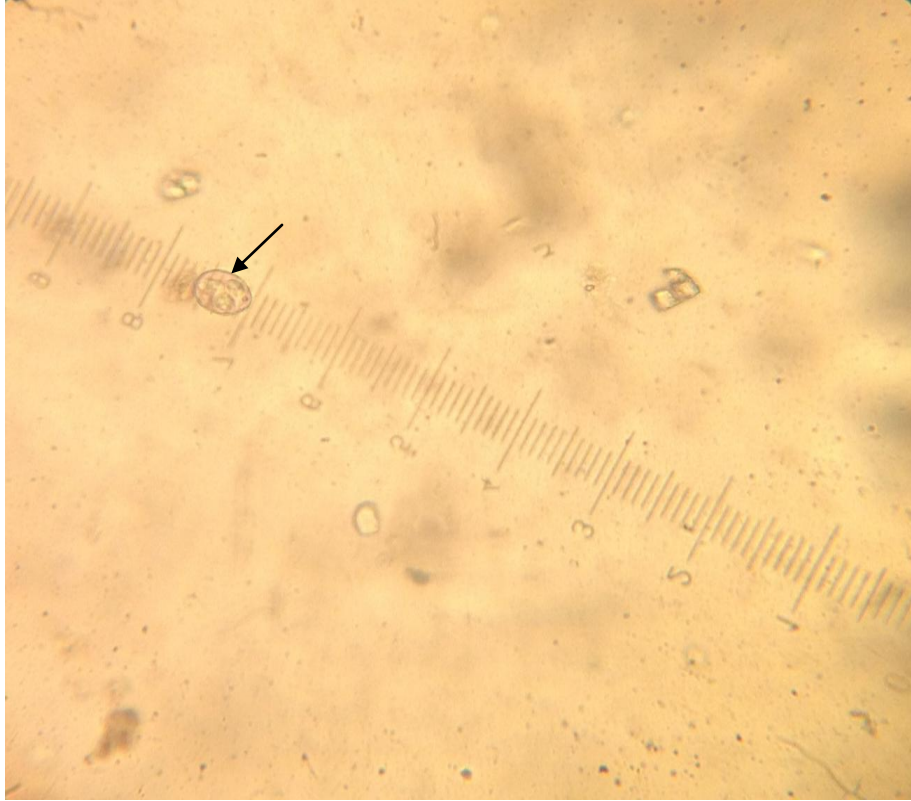


Plate III: In vitro sporulated subspherical *Eimeria bateri* oocysts (arrow) with a polar granule isolated from Japanese quails in farm B using an ocular micrometer (x40).

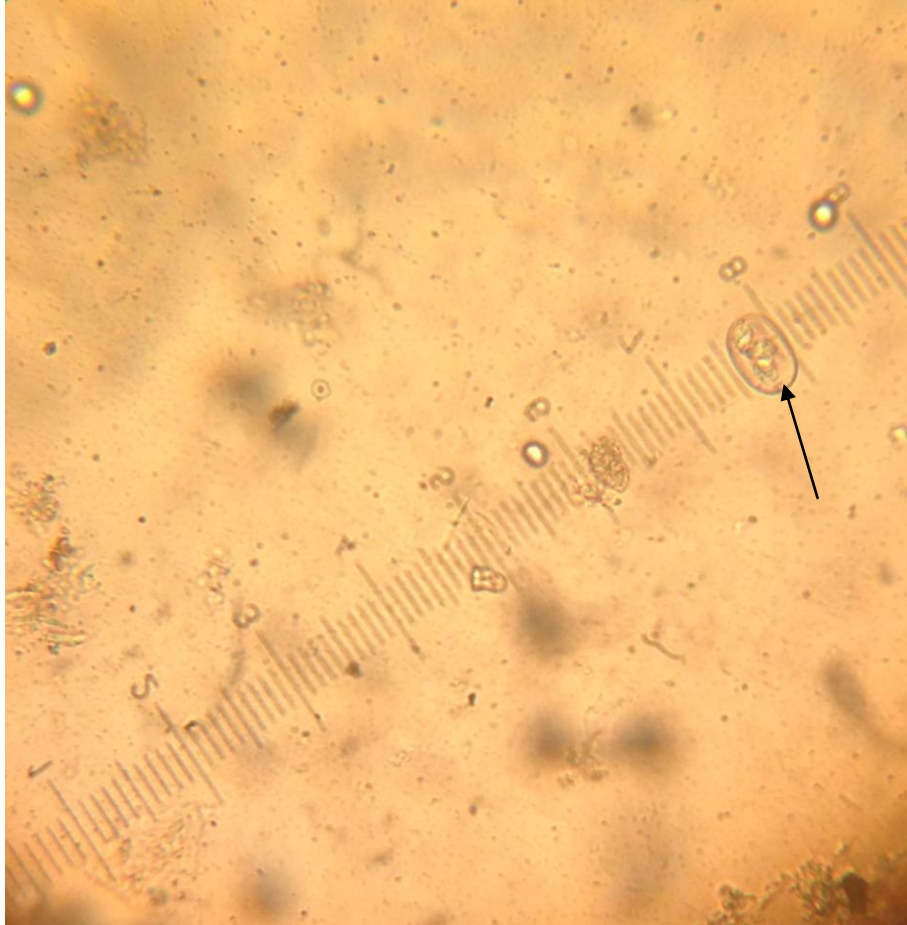


Plate IV: In vitro subspherical *Eimeria* oocysts (arrow) with no polar granule isolated from Japanese quails in farm F using an ocular micrometer (x40).

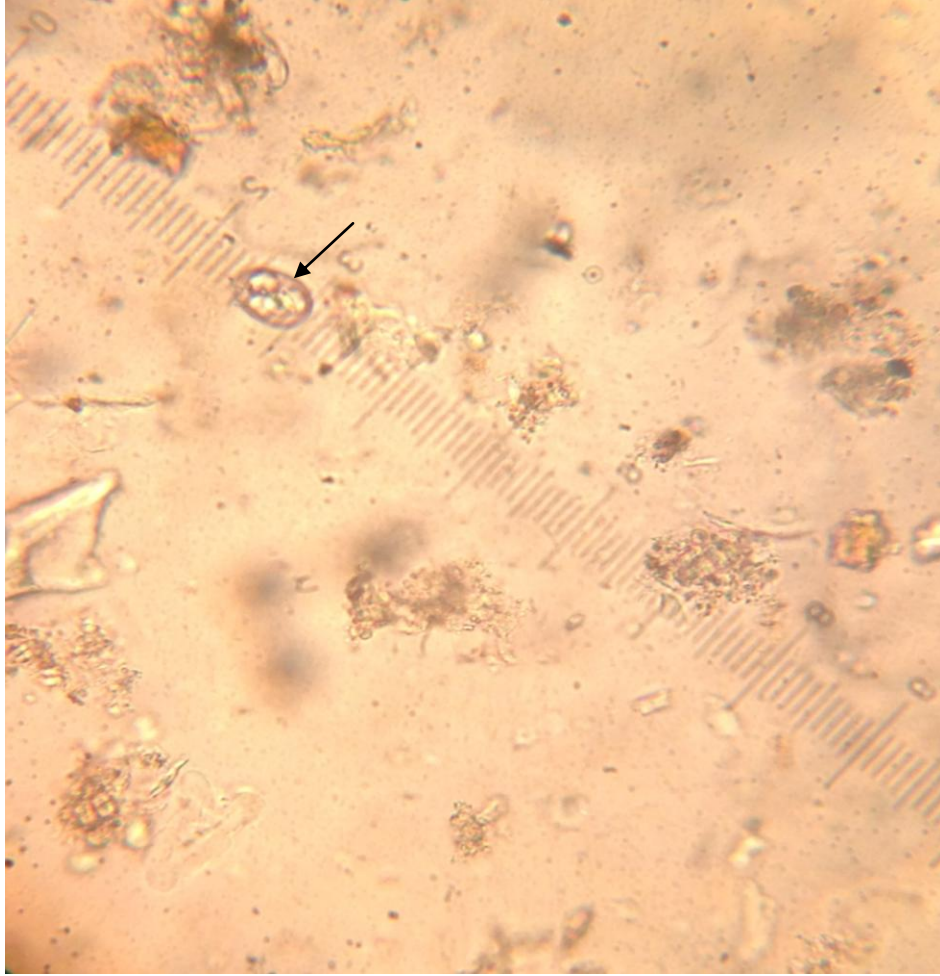


Plate V: In vitro sporulated ovoid *Eimeria* oocysts (arrow) isolated from Japanese quails in farm G using an ocular micrometer (x40).



Plate VI: In vitro sporulated spherical *Eimeria* oocyst (arrow) isolated from Japanese quails in farm J using an ocular micrometer (x40)

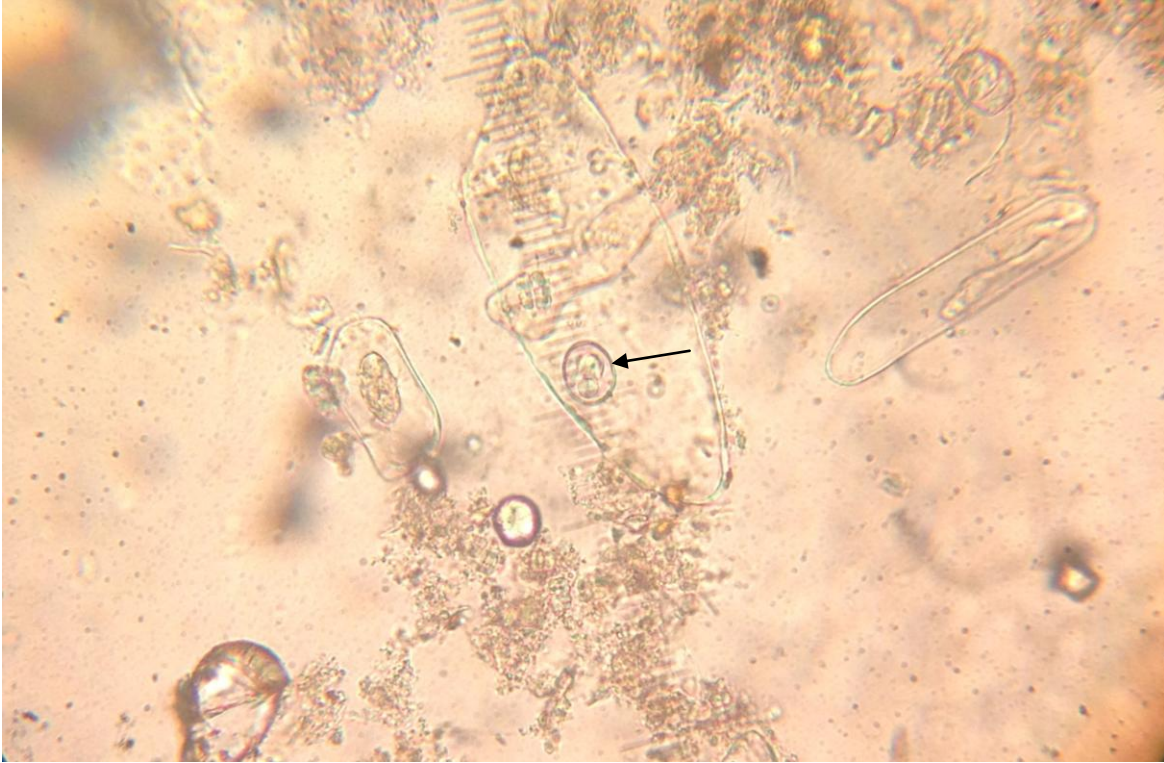


Plate VII: In vitro spherical *Eimeria* oocyst (arrow) using isolated from Japanese quails in farm I using an ocular micrometer (x40).



Plate VIII: Ballooning of the caeca (arrows) of a Japanese quail naturally infected with *Eimeria bateri* from farm D.

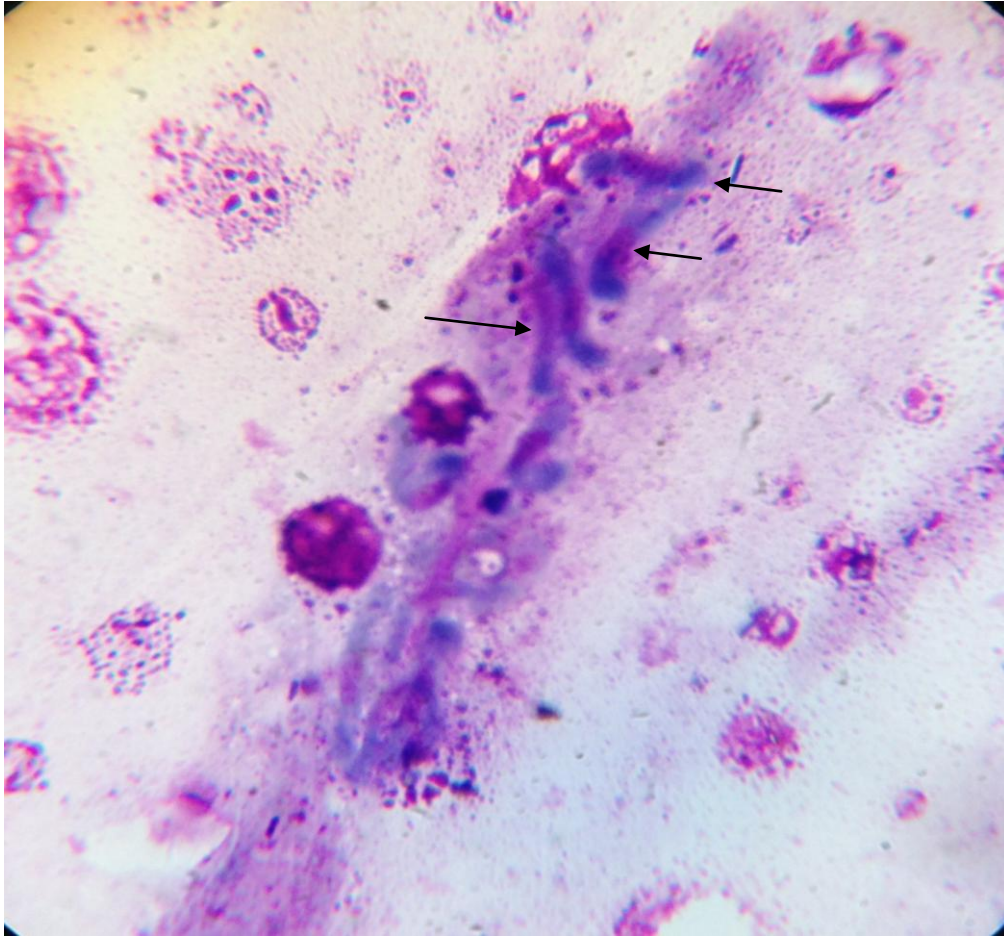


Plate XI: Jejunum of a Japanese quail showing the developmental stage (merozoites) (arrows) of *Eimeria* spp. in the epithelium using Geimsa stain (x100) oil immersion isolated from farm D.

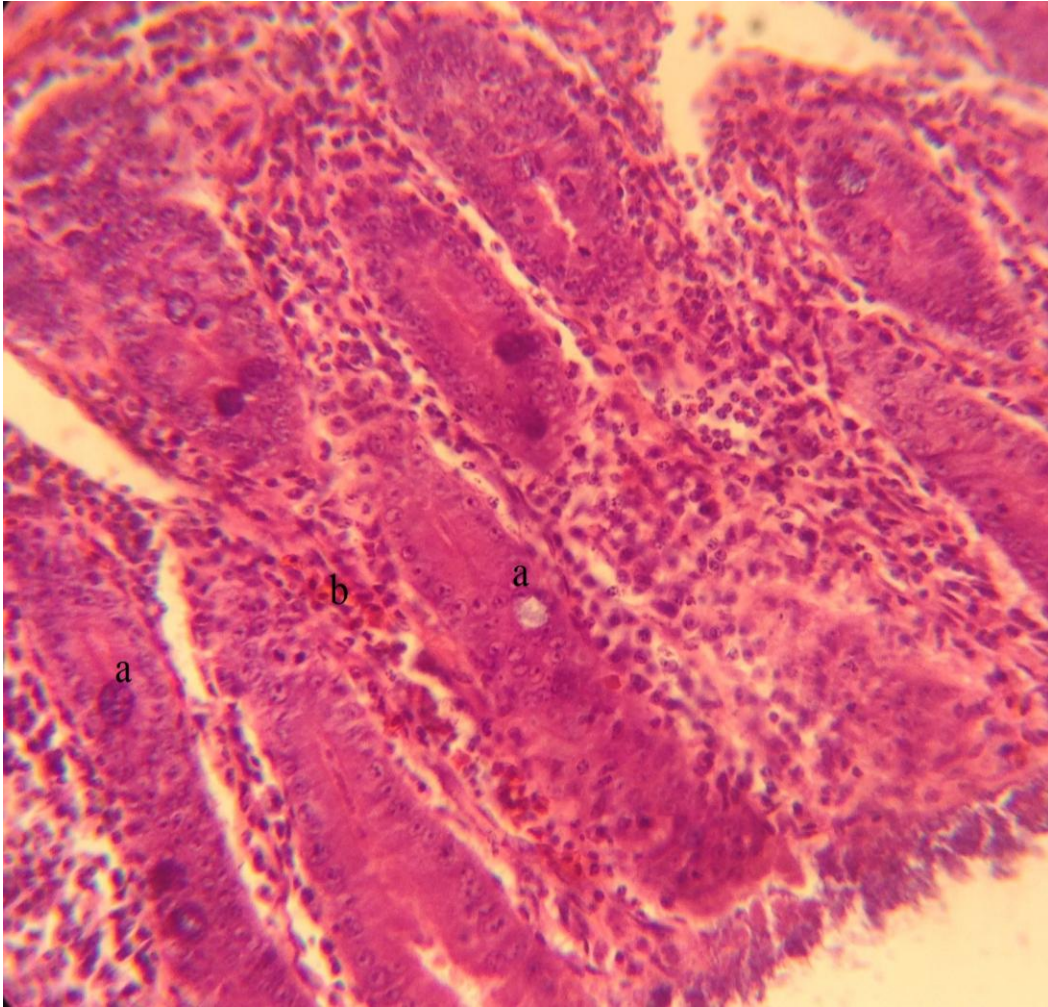


Plate X: Histological section of a caecum showing the (a) developmental stage (schizont) of *Eimeria* spp. (b) areas of necrosis H&E (x40) isolated from farm D

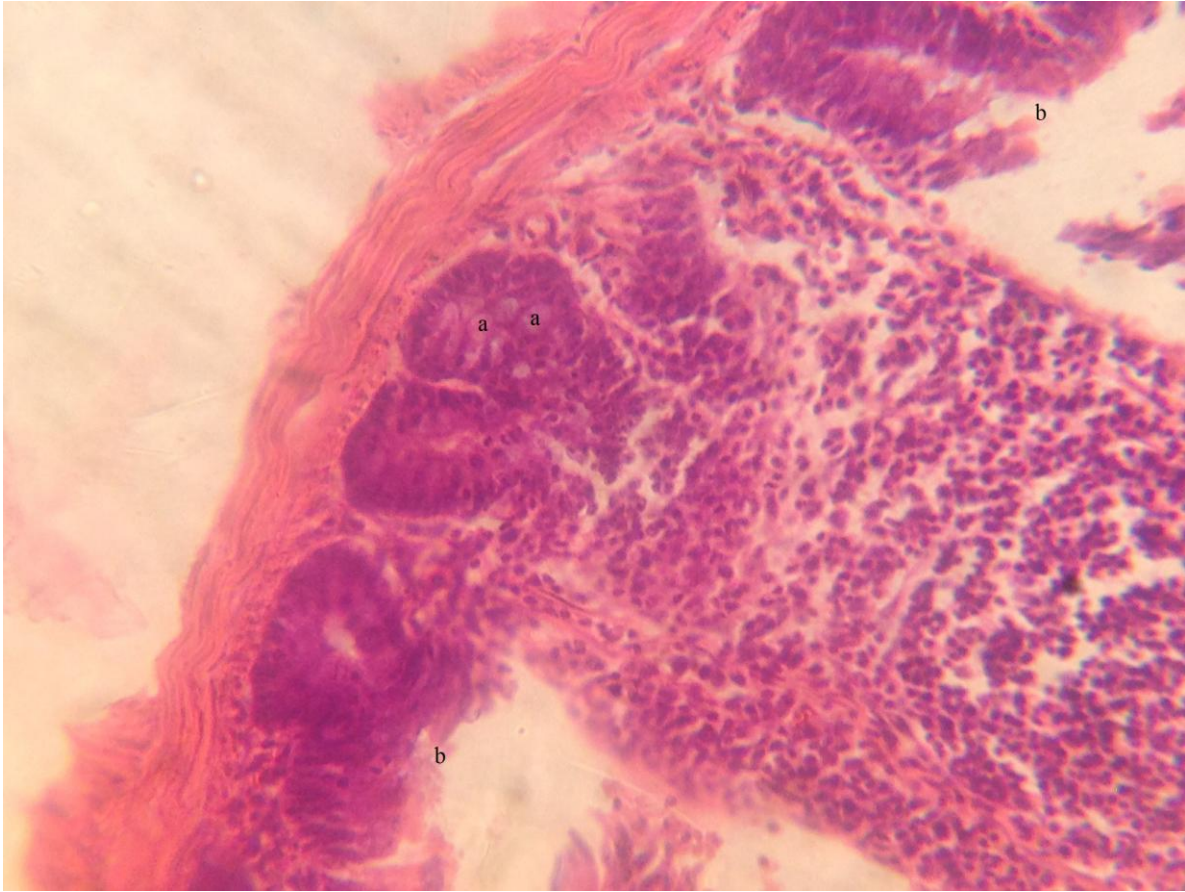


Plate XI: Histological section of a duodenum showing the (a) developmental stage (schizonts) of *Eimeria* species. (b.) Desquamation of the epithelium H&E (x40) isolated from Farm D

CHAPTER FIVE

5.0 DISCUSSION

This study showed the occurrence, morphological and pathological characteristics of *Eimeria* species in Japanese quails in Sabon Gari and Zaria Local Government Areas of Kaduna State. An overall occurrence of 45.75% of *Eimeria* infection was recorded in this study. This shows that *Eimeria* infection can be a problem for quail farmers in the study areas. Although, there is limited data for comparison, the finding is apparently similar to that of Mohammad (2012), who detected an *Eimeria* infection of 49.9% in Japanese quails in Mosul, Iraq and Musaev *et al.* (1998) who also detected an *Eimeria* infection of 52% in Japanese quails in Azerbaijan using the floatation technique.

The difference in the percentages of infection in the various studies conducted may be related to different factors such as environmental conditions, seasonal fluctuations in biotic factors and type of anticoccidial drugs that might be used (Nematollahi *et al.*, 2008).

The differences in the prevalence rate for *Eimeria* infection in Sabon Gari and Zaria LGAs may not be readily explained. However variations in awareness as regards management of poultry and quails might have contributed to the differences in prevalence.

The impact on the production of the varying intensities of infection should be recognized. Although most birds had inapparent and low grade infection, the insidious effects may have more negative production impact than envisaged.

It is known that the economic importance of coccidiosis is largely associated with the sub-clinical form of the disease as it has been reported to have negative effect on the performance of infected birds (Haug *et al.*, 2008). Impaired feed conversion is among the major effect of subclinical coccidiosis and since feed costs comprise some 70% cost of producing commercial birds, the economic impact of inapparent/subclinical infection is therefore considerable.

Out of the four *Eimeria* oocysts isolated from Japanese quails in this study, only *Eimeria bateri* oocyst with shape index of 1.36 was identified on the guidelines of Teixeira and Lopes (2002). The other three *Eimeria* oocysts with shape indices of 1.48, 1.03 and 1.40 could not be identified as they did not conform to any of the given species by the above authors.

In spite of the shortcomings identified above, differences in morphology of the oocyst which were ovoid, subspherical and spherical, presence and absence of polar granule can be observed. However difficulties in differentiating the species based on oocysts overlap in sizes and shapes of some species, especially the dislodgement of micropolar cap and distortion in the course of the experiment were also observed as previously reported by Duszynski and Wilber (1997).

Gross lesions associated with *Eimeria* infection in quails in this study, was limited to mainly ballooning of the caecal tonsils. The only clinical sign observed was diarrhea. The reports of previous works seem to be at variance with the present observation as regards the pathogenicity of *Eimeria* in quails. Mazurkiewiewicz *et al.* (1967) reported a wide range of clinical signs such as lack of appetite, ruffled feathers, uncoordinated movements, inhibition of laying and loss of weight in naturally infected young and mature quails reared at the laboratory. In young Japanese quails infected with *E. bateri*, mild loss of weight, anorexia and softening of faeces were observed and was considered mild and easy to overcome (Norton and Pierce, 1971). Tsunoda

and Muraki (1971) also reported low pathogenicity in Japanese quails experimentally infected with 1×10^5 oocysts of *E. uzura*, with signs limited to diarrhea and anemia and no mortality was reported.

However, Ruff and Fagan (1984) used pure and mixed cultures of *E. uzura* to infect quails and reported mortality, lower weight gain and poor reproductive performance. Several factors such as environment, differences in the parasite strain and management system may be responsible for the discrepancies in the observation of the various works.

The findings from this study shows that merozoites are the main endogenous stages of the parasites found in the small intestine and schizonts in the large intestine. The absence of other pathogenic stage such as the gametocytes means that the pathology of the infected quails will be mild. Histopathological lesions were located in the villi, mainly above the nucleus of apical epithelial cells, or in the medium portion close to the glands. These observations resemble those of Tsunoda and Muraki (1971), Norton and Pierce (1971) and Tsutsumi (1972) as regards the site of infection. Desquamation of epithelial lining and caecal necrosis were also observed in the study.

The analysis of the questionnaires and also observation of the farms showed that the predisposing factors for the prevalence of coccidiosis in the farms were high moisture level in the poultry house and poor level of biosecurity. It was observed that the intensive management system practiced by farmers did not provide adequate space for the birds thus the possibility of spread of the disease due to crowding. Lack of disinfectant also ensures persistence of oocysts in the quail houses and dissemination of sporulated oocysts through the attachment of the infective

stages to the clothes and shoes Poultry attendants or personnel also often serve as disseminators of sporulated oocysts through the attachment of the infective stages to their clothes and shoes.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusions

The study showed that there is a high prevalence of *Eimeria* oocysts in Japanese quails in Zaria. Although different *Eimeria* species were identified in the study, only *Eimeria bateri* was speciated as it conformed to the guideline used. Despite the inapparent and nonspecific clinical signs observed in the birds the parasites were considered important because the endogenous stages of the parasites were associated with intestinal lesions. Incidence of water spillage on litter, poor hygienic standards, non-prophylactic use of coccidiostats, high stocking density and non-evacuation of litter material as at when due favour the sporulation and multiplication of *Eimeria* species and therefore predisposes Japanese quails to the disease in Zaria.

6.2 Recommendations

From the study, the following recommendations are prescribed:

1. Enlightenment of clinicians and farmers on the presence of coccidial agents, and the benefits of introducing and sustaining the use of prophylactic measures against coccidiosis in Japanese quails in Zaria
2. Further studies should be conducted using molecular techniques to properly identify the unknown species of *Eimeria* which were detected in the study

3. Proper control measures must be taken with emphasis on avoiding water spillage, proper stocking density, frequent disposal of litter material and improved hygienic practices should be instituted in quail farms.

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Appendix 1: Test for Statistical significance for the presence of *Eimeria* oocysts in quails between Sabon Gari and Zaria Local Government Areas

L.G.A	Negative	Positive	Total
Sabon Gari	90	110	200
Zaria	127	73	200
Total	217	183	400

Chi-square = 13.79, df = 1, P value = 0.0002, Odds ratio = 0.4703, 95% CI =0.3150 to 0.7021

Appendix 2: Test for Statistical significance for the presence of *Eimeria* oocysts in quails between the farms in Sabon Gari and Zaria Local Government Areas

Farms	Negative	Positive	Total
A	17	23	40
B	17	23	40
C	40	0	40
D	5	35	40
E	11	29	40
F	39	1	40
G	20	20	40
H	36	4	40
I	25	15	40
J	7	33	40
Total	217	183	400

Chi-square = 151.7, df =9, P value = $P < 0.001$

Appendix 3: Questionnaire to assess the predisposing factors responsible for the occurrence of coccidiosis in Japanese quails in Zaria.

Instruction: Please answer the following set of questions by filling in the empty spaces or by ticking the appropriate option. Your participation will be highly appreciated and all information will be treated as confidential.

A. General Information

1. Farm Location: _____
2. Phone number: _____
3. Sex: male female
4. Age: less than 20 years between 20 & 40 years above 40 years
5. Level of education: Primary Secondary Tertiary Informal
6. Age of the farm: 0-6 months 7-12 months 1-2 years more than 2 years
7. Purpose of keeping quails : Family consumption Cash earning Other (specify)

8. Production system: Battery Cage Deep Litter

B Flock Data

9. Age of birds: _____
10. No of Birds: _____
11. Source of the birds: _____
12. Age and weight at which birds are sold: Age: _____ Weight: _____
13. Age at which birds start laying/capacity: _____

C Health Indicators for Flock

14. Disease problem in the farm: Yes No. If yes:

15. Major diseases in your farm (in order of importance):

A) _____ B) _____

C) _____ D) _____

16. What do you do when diseases occur?

Call a veterinary doctor

Take the sick animals to a vet clinic

Call someone with knowledge about animal diseases

Treat them yourself

17. Has any of these signs occurred in your flock?

Bloody diarrhoea Yes No

Weight loss Yes No

Weakness Yes No

None

D. Information on feed and feeding.

18. Source of feed:

Manufactured in the farm.

Brought from local suppliers.

Other (specify) _____

19. Feed management

Ad libitum

Controlled

Other (specify) _____.

20. Quality and Availability of feed: Good Satisfactory Poor

21. Quality of feeding and watering equipment in relation to feeding and watering spillage:

Good Satisfactory Poor

22. Type of feeding system: Automated Manual

23. Use of Coccidiostats: Yes No

24. Method of application: In feed In water In feed and water

25. Storage facility for feed? Yes No

26. Is storage room adequately ventilated? Yes No

E. Information on Environment/ Management Factors.

27. Litter moisture: Average High

28. Stocking density (Number of birds per meter square): _____

29. Ventilation system: Good satisfactory Poor

30. Type of litter material Wood shavings Saw dust Rice chaff

Specify _____

31. Incidence of water spillage around drinking points Yes No

F. Information on disease control measures.

32. Disease control measures in your farm:

Drugs Cleaning and disinfection Vaccination Other

(Specify) _____

33. How long have you been using the above measures?

1 year 2 years more than 2 years

34. Frequency of litter disposal: Once/ week Once/ month Once/ batch

Other (specify) _____

35. Drugs used in the farm starting with the most frequently used.

A) _____ B) _____

C) _____ D) _____

G. Level of biosecurity measures in the farm:

36. Presence of foot bath disinfectant at each quail house: Yes No.

37. Its regular functioning. Good Satisfactory Poor

38. The hygiene control level of quail house attendants and supervisors to keep the

Sanitation. Good Satisfactory Poor

39. Any cracks, holes or opening to the poultry houses through which wild birds,

rodents or predators enter into the houses. Maximum Minimum No

40. Rodent Control program Yes No

H. Information on farm Structure (Nature of Building)

41. Ventilation [] Good [] Satisfactory [] Poor

42. House Orientation [] East [] West

43. Bird Proof Netting [] Good [] Satisfactory [] Poor

44. Rodent Proof Netting [] Good [] Satisfactory [] Poor

Appendix 4: Guide for the identification of *Eimeria* oocyst in Japanese Quails (Teixeira and Lopes, 2002)

a. *Eimeria tsunodai* Tsutsumi, 1972: Sporulated oocysts were ovoid, measuring 20.2 ± 1.5 by 14.88 ± 0.79 μm and 1.36 shape index. Oocyst wall was smooth, double layered, with brownish inner layer and colorless outer layer, and measured 0.99 ± 0.1 μm . Despite usually only one polar granule was present, it could appear in pairs and refractive. Micropyle and residual body of the oocyst were absent. The sporocysts varied from ovoid to ellipsoid and measured 10.41 ± 0.6 by 5.39 ± 0.3 μm . They had a finer end where a small and faint Stieda body projected. The residual body of the sporocyst was present and dispersed among the sporozoites, which were in pairs with globules visible at the enlarged extremity.

b. *Eimeria uzura* Tsunoda & Muraki, 1971: Sporulated oocysts were ovoid, measuring 22.2 ± 6.76 by 16.16 ± 1.13 μm , and 1.32 shape index. Oocyst wall was smooth, double layered, with brownish inner layer and colorless outer layer, measuring 1.08 ± 0.12 μm . Two to five polar granules were observed in the oocysts, sometimes with a massive aspect, but not refractive). The micropyle and residual body of the oocysts were absent. The sporocysts were ovoid measuring 11.76 ± 0.74 by 5.94 ± 0.45 μm , with a finer end, where a piriform Stieda body projected. The residual body of the sporocysts was present and had concentric granules between the sporozoites, which had refractive globules at the enlarged extremity.

c. *Eimeria bateri* Bathia, Pandey & Pande, 1965: Sporulated oocysts were subspherical, ovoid or ellipsoid, measuring 21.50 ± 1.84 by 16.36 ± 1.32 μm and shape index 1.32. Oocyst wall was smooth, double layered, with brownish inner layer and colorless outer layer, measuring 0.99 ± 0.1 μm . A single and refractive polar granule was present, but micropyle and the residual body of the oocyst were absent. Sporocysts were ovoid and measured 10.35 ± 0.73 by 6.66 ± 0.58 μm , with a prominent knob-like Stieda body. The residual body of the sporocyst was dispersed among the sporozoites, which had refractive globules at the enlarged extremity.