PREVALENCE AND MOLECULAR CHARACTERISATION OF *EIMERIA* SPECIES IN COMMERCIAL CHICKENS IN OYO AND OGUN STATES, NIGERIA

ADEYEMI, SAMUEL OLUFEMI

FEBRUARY, 2014

PREVALENCE AND MOLECULAR CHARACTERISATION OF *EIMERIA* SPECIES IN COMMERCIAL CHICKENS IN OYO AND OGUN STATES, NIGERIA

BY

ADEYEMI SAMUEL OLUFEMI

DVM, MPVM, MBA, B PHARM, LL.B

MATRIC NO: 22528

A THESIS IN THE DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) OF THE UNIVERSITY OF IBADAN

FEBRUARY, 2014

ABSTRACT

Eimeria species are important coccidian parasites worldwide causing significant economic losses from clinical and subclinical coccidiosis in poultry. Vaccines for its control in Nigeria are imported without consideration of the local species often resulting in vaccine failure. This study determined the prevalence of and characterised the *Eimeria* species present in Oyo and Ogun States towards an effective control and prevention of coccidiosis.

A semi-structured questionnaire was administered randomly to 166 poultry farmers (82, Oyo; 84, Ogun) based on the registered members of the Poultry Association of Nigeria, to obtain data on experience of coccidiosis and the prophylactic measures adopted. Poultry flocks were grouped into six epizootiological categories comprising: vaccinated and medicated 1-8 week-old (1, 2), vaccinated and medicated 9-18 week-old (3, 4) and above 19 week-old caged and deep-litter (5, 6). Pooled faecal samples were collected from 9, 9, 9, 20, 54, and 19 chicken flocks in Oyo and 9, 16, 18, 27, 39, and 12 in Ogun belonging to categories 1 to 6 respectively. Oocysts were isolated by modified flotation method, sporulated in 2.5% potassium dichromate and quantified by the McMaster method. *Eimeria* species were identified by morphometry, infection prevalence and relative abundance was determined. *Eimeria* DNA was extracted, amplified and quantified using Real-Time Quantitative Polymerase Chain Reaction (RTQPCR). Data were subjected to descriptive statistics, Chi-square, Student's *t* and Kappa tests. Significance was set at $p<0.05$.

Most of the respondents, 77 in Oyo; 69 in Ogun had experienced coccidiosis outbreaks, of which 74.2% and 71.6% were in below-8-week chicks. In Oyo and Ogun, 67.7% and 73.3% adopted vaccination; 32.3% and 26.7% adopted chemoprophylaxis while 37.1% and 21.9% reported outbreaks despite preventive measures. Oocyst burden was 49,494 and 2,830 oocysts-per-gram

of faeces for cages and 71,585 and 8,255 for deep-litter in Oyo and Ogun respectively. Overall prevalence in Oyo was 65.0% (100.0%, 100.0%, 100.0%, 75.0%, 40.7% and 73.7%) and in Ogun, 61.2% (100.0%, 75.0%, 83.3%, 33.3%, 51.3% and 75.0%) in categories 1-6 respectively. Prevalence of 93.7% in chicks (100.0% in Oyo; 85.7% in Ogun) was significantly higher than the 55.7% (54.3% in Oyo; 56.9% in Ogun) for above 18 week-old. It was also significantly higher on deep-litter (73.7%, Oyo; 75.0%, Ogun) than in cages (40.7%, Oyo; 51.3%, Ogun). Species prevalences were; *E. acervulina* (100.0%, 83.3%), *E. maxima* (16.7%, 16.7%), *E. necatrix* (50.0%, 50%), *E. tenella* (66.7%, 66.7%) and *E. mitis* (50.0%, 66.7%) in Oyo and Ogun respectively. Relative abundance from morphometry were; Brunetti-Maxima (3.0%, 4.3%), Necatrix-Tenella-Praecox (45.0%, 23.6%) and Acervulina-Mitis (52.0%, 72.1%); from molecular characterization were; *E. acervulina* (48.0%, 20.0%), *E. maxima* (0.2%, 0.3%), *E. necatrix* (5.4%, 22.7%), *E. tenella* (20.2%, 13.5%) and *E. mitis* (25.5%, 43.5%) in Oyo and Ogun respectively. Comparison of RTQPCR results with tentative morphometric identification showed complete agreement in 77.8% (28 of 36) samples.

Morphometry remains acceptable for routine tentative diagnosis; however, molecular characterisation is more precise for *Eimeria* species identification. The most prevalent species: *E. acervulina, E. maxima, E. necatrix, E.tenella* and *E. mitis* are therefore recommended for inclusion in coccidiosis vaccines.

Key words: Coccidiosis, Oocyst morphometry, Molecular characterisation.

Word Count: 498.

DEDICATION

This work is dedicated to God Almighty, maker of heaven and earth for his mercies and for his wonderful works in my life and that of my family. He has given human being a limitless sense of adventure and thirst for knowledge and each person and circumstances determines how far each person shall go. To Him alone be all the glory, honour and adoration forever and ever.

CERTIFICATION

This is to certify that this work was carried out by

ADEYEMI SAMUEL OLUFEMI

AT

THE DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, UNIVERSITY OF IBADAN

UNDER THE SUPERVISION OF

JOPAdej. 06/05/2014

Professor J.O. Adejinmi

DVM, MSc (Ib), MSc (Edin), Ph.D (Ib) MCVSN

06/05/2014

Professor G. A. T. Ogundipe

DVM, MPVM, Ph.D (Ib); FCVSN

ACKNOWLEDGEMENT

My sincere appreciation first and foremost goes to my supervisors, Prof. G.A.T. Ogundipe and Dr. J.O. Adejinmi for their painstaking supervision and commitment to the success of this research work. This research was carried out at the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan. The support of this work by the Department of Veterinary Medicine, University of Ibadan and Biopharm Institute of Biopharmacy, Jilove near Prague, Czech Republic is greatly appreciated. I gratefully acknowledge the immense technical and professional contribution of Dr. Vladimir Virba, Dr Pavel Trefil, Dr. Michal Pankandl, Ms Katerina Hodacova, Ms. Gabina, Mr. Etienne Effenberger, Ms Jana Ostadalova and Engr. Jan Byrtus and the entire staff of Biopharm Institute who made my stay during the period of training and laboratory work on this project very interesting and enjoyable.

My immense appreciation also goes to Dr. Olatoye Isaac Olufemi and Dr. E. A. Awosanya for their unflinching support and commitment to the successful completion of this work. I wish to thank the past and present Heads of Department of Veterinary Public Health and Preventive Medicine. Iam also indebted to the current Dean of the Faculty of Veterinary Medicine Prof. Victor Olusegun Taiwo for his encouragement and Prof. S. A. Agbede, Drs I.G. Adeyemi, Olayinka O. Ishola, Olanike K. Adeyemo and O.B. Adedeji for their moral and administrative contributions to the successful completion of this work. I also acknowledge the immense contribution of Mr Wole Ogun, John Akinsanya, Johnson Oyewole, Mrs Bolanle Adekanmbi, Dr. Odunsi, Dr. Orenuga, Dr. Dotun, Dr Gade, Mrs Temitope Ben-Dominic, Mr Layiwola Jeminusi and Samson Ilo during the period of sample collection.

My appreciation also goes to Mr. Bankole Ojomo, Dr. J.O.Abiola, Mr. Yemi Okunlade, Mr. J. A. Olanrele and Mr. John Odeyemi for their assistance during the laboratory stage of the

work.Finally I like to acknowledge the immense contribution of my wife Deaconess Julianah Oyebola Adeyemi and our baby of the house, Solomon Olubayo Adeyemi for providing a peaceful home front and tremendous support during the entire duration of this work.

TABLE OF CONTENTS

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

LIST OF APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AST	Anticoccidial Sensitivity Testing
CMI	Cell Mediated Immunity
Ct	Threshold Cycle
DNA	Deoxyribo Nucleic Acid
FDL	Federal Department of Livestock
GALT	Gut Associated Lymphoid Tissue
LAMP	Loop Mediated Isothermal Amplification
Mt DNA	Mitochondrial Deoxyribo Nucleic Acid
OPG	Oocyst per gram of faeces
PCR	Polymerase Chain Reaction
QPCR	Quantitative Polymerase Chain Reaction
rDNA	Ribosomal Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
RTQPCR	Real-Time Quantitative Polymerase Chain Reaction
TMLS	Total Mean Lesion Score
NK-cells	Natural Killer Cells
IEL	Intra Epithelial Lymphocytes
AGP	Antibiotic Growth Promoter
NE	Necrotic Enteritis
UK	United Kingdom

PREVALENCE AND MOLECULAR CHARACTERISATION OF *EIMERIA* SPECIES IN COMMERCIAL CHICKENS IN OYO AND OGUN STATES, NIGERIA

ADEYEMI, SAMUEL OLUFEMI

FEBRUARY, 2014

PREVALENCE AND MOLECULAR CHARACTERISATION OF *EIMERIA* SPECIES IN COMMERCIAL CHICKENS IN OYO AND OGUN STATES, NIGERIA

BY

ADEYEMI SAMUEL OLUFEMI

DVM, MPVM, MBA, B PHARM, LL.B

MATRIC NO: 22528

A THESIS IN THE DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) OF THE UNIVERSITY OF IBADAN

FEBRUARY, 2014

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background Information

Nigeria is the largest livestock producer in West Africa sub-region and third largest producer in the sub-Sahara Africa following Ethiopia and Sudan. Compared with countries like Italy, Spain and Germany, the livestock population of Nigeria is about 58%, 56% and 14% higher suggesting that Nigeria has more livestock potentials than even some well-known livestock producers of the world. However, the livestock population is managed by small holders and a considerable proportion is managed on free range traditional system (Lamorde, 1998).

The livestock sector is vital to the socio-economic development of Nigeria. It contributes about 9 - 10% of the agricultural Gross Domestic Product (GDP). Poultry production is an important component of the Nigerian Livestock subsector, providing employment, income, animal protein for the urban and rural dwellers as well as manure for crops (Eduvie, 2002). Nigeria's chicken population is about 150.682 million of which 25% are commercially farmed, 15% semi-commercially, and 60% in backyards (UNDP, 2006).

The poultry sub-sector is the most commercialised of all of Nigeria's agricultural sub-sector and represents an important source of high quality animal protein, providing about 36.5% of the total protein intake of Nigerians. It is one of the highest investments in agriculture with net worth of N250 billion. Poultry production in Nigeria can be classified into extensive and intensive systems based on scale, stock and the extensive production system presently accounts for about 85% (Avian Influenza final report, 2007).

In Nigeria, the population growth rate and demand for food is over 3% while growth rate in food production is between 1.0 and 1.5% leaving a shortfall of 1.5 and 2% in annual food supply. Plant sources contribute about 75% while livestock accounts for the remaining 25% of the food supply shortfall. This is mainly due to improper development of the livestock industry (Lamorde, 1998).

Majority (65%) of the commercial poultry flock in Nigeria are located in South western region with Ibadan being the main entry point of exotic chicken and the location of major poultry breeders and retailers throughout the country and beyond (Owoade *et al.,* 2004).

Poultry diseases remain one of the major threats to boosting poultry production in Nigeria. Parasitic diseases are of particular importance because of their high incidence in poultry occasioned by the tropical environmental conditions under which the farmers operate (Seifert, 2006). Coccidiosis is a disease common in intensively managed farms especially where management or hygiene standards are compromised (Adene and Oluleye, 2004). A damp litter that has high moisture content and warmth of between 25 and 30^{o}C favours oocyst sporulation (David 2000). Birds of any age are susceptible to coccidiosis but most birds get infected in the few weeks of life (Chookyinox *et al*., 2009).

A coccidiosis free environment is not likely achieved and chicks that become infected may develop acquired immunity or succumb to disease if the balance is in favour of the parasite (Chookyinox *et al.,* 2009). Epizootiological studies have established the economic importance of coccidiosis as a major parasitic disease of poultry in Nigeria (Majaro, 1980, 1983, 2001). The importance of coccidiosis is based on the economic implications of its outbreak in poultry farms (Majaro, 1980; Barksh, 1989). Although coccidiosis is controllable under most circumstances, the cost of control makes the disease one of the most

expensive parasitic diseases encountered in poultry industry (Majaro, 1980, 1981). Adene and Oluleye in 2004 reported a prevalence of 12% for coccidiosis in chicken Nigeria. Etuk *et al.*, 2004 reported in Akwa Ibom state, South Eastern Nigeria a coccidiosis overall prevalence of 12.7% in the rainy season. The report also included a prevalence of 26.69% among birds managed on deep litter; 18.75% in 1-5 week-old birds and 22.29% in layers. In Zaria, Nothern Nigeria, studies indicated that *Eimeria tenella* and *E. necatrix* are the most pathogenic *Eimeria* species causing bloody caecal and intestinal coccidiosis respectively (Bishu, 1982). Coccidial infections may be classified in one of three ways (Williams, 1999a): (1) as clinical coccidiosis, characterized by mortality, morbidity, diarrhoea or bloody faeces, as well as by adverse effects on economic performance; (2) as subclinical coccidiosis, not immediately obvious, but causing reductions in weight gain and feed conversion efficiency of the host, without frank signs of disease; or (3) as coccidiasis (Levine, 1961), a mild infection causing no adverse effects on the host.

Coccidiosis is a disease caused by infection with one or more of the many species of coccidia which is a subdivision of the phylum Protozoa, intracellular protozoal parasites of the sub-phylum Apicomplexa and the genus *Eimeria*. The genus *Eimeria* contains the species of major economic importance in domestic birds, such as chickens, ducks, geese, guinea fowl, peafowl, pheasants, pigeons and turkeys. While coccidiosis occurs in practically all kinds of birds, the parasites are host-specific and species occur in a single or in a limited group of related hosts. Avian hosts are known to harbour more than one specie of coccidia. Species of *Eimeria* that cause coccidiosis in chickens include *Eimeria acervulina, E. brunetti, E. hagani, E. maxima, E. mitis, E. mivati, E. necatrix, E. praecox* and *E. tenella*. Among domesticated birds, chickens are the most susceptible to significant economic losses from coccidiosis, although losses can also occur within turkeys, geese,

ducks, and guinea fowl. Coccidiosis has also produced serious losses in pheasants and quail raised in captivity. The effects of a coccidiosis infection can take the highly visible form of devastating flock mortality, but another undesirable effect is morbidity and/or weight loss which results from infection.

1.2 Life Cycle of *Eimeria* Parasite

During the life cycle, the *Eimeria* parasite passes through a number of stages. The life cycle begins when the chicken ingests the infectious stage, known as the sporulated oocyst, during ground feeding. The wall of the sporulated oocyst is ruptured by a combination of mechanical grinding action and chemical action in the gizzard and intestinal tract, resulting in the release of four sporocysts. The sporocysts pass into the duodenum where they are exposed to bile and digestive enzymes resulting in the release of two sporozoites per sporocyst.

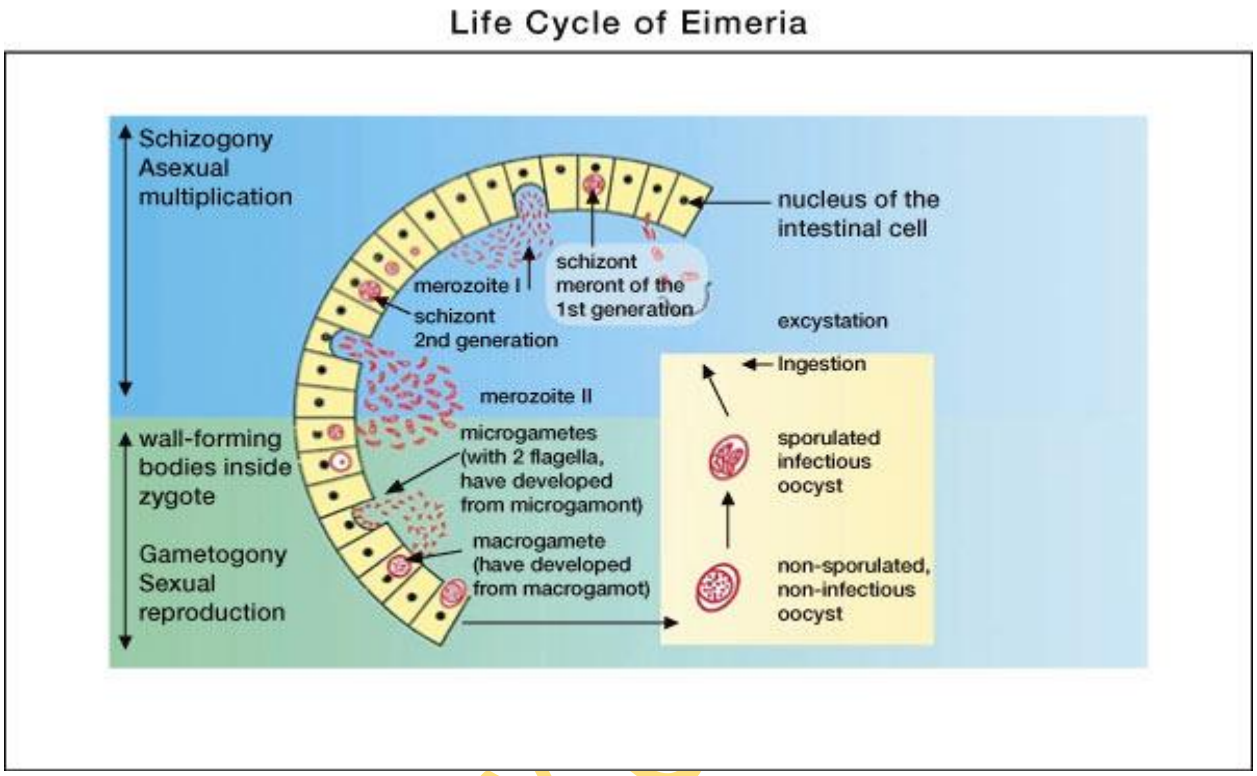
The sporozoites are mobile and search for suitable host epithelium cells in order to penetrate and reproduce in them. Following infection of an epithelium cell, the parasite enters the schizont phase of its life cycle, producing from 8 to 16 to more than 200 merozoites per schizont. Once released from the schizont, the merozoites are free to infect further epithelium cells. After two to five of these asexual reproduction cycles, the intracellular merozoites grow into sexual forms known as the female or macrogametocyte and the male or microgametocyte. Following fertilisation of the macrogametocyte by the microgametes released from the microgametocyte, a zygote is formed which creates a cyst wall about itself. The newly formed oocyst is passed out of the infected chicken with the fecal droppings. With the correct environmental conditions of temperature and humidity and sufficient oxygen in the air, the oocyst will sporulate into the infectious stage, ready to

infect a new host and thereby spreading the disease. Thus, no intermediate host is required for transfer of the parasite from bird to bird.

1.3 Effects of *Eimeria* Parasite Multiplication in the Chicken

Eimeria parasite multiply in the intestine and causes tissue damage lowered feed intake, poor absorption of nutrients from the feed, dehydration and blood loss (Seifert, 2006). The result of the *Eimeria* parasite infecting the digestive tract of a chicken may be a reduction in weight gain, increased feed conversion, cessation of egg production and, in some cases, death. The increase in intensive production of poultry has been accompanied by severe losses due to this parasite; indeed, coccidiosis has become an economically important parasitic disease. In the past, several methods have been used in attempts to control coccidiosis. Prior to the advent of chemotherapeutic agents, improved sanitation using disinfectants, together with the mechanical removal of litter, was the main method employed; sufficient oocysts, however, usually remained to transmit the disease. The introduction of coccidiostatic agents in the feed or drinking water, in addition to good management, resulted in some success at disease control. Such agents have been found to suffer from a drop in effectiveness over the years, due partly to the development of drug resistant strains of coccidia. Furthermore, several chemotherapeutic agents have been found to leave residues in the meat, making it unsuitable for consumption.

There are nine species of *Eimeria* said to infect the chicken host, seven of which are well-known and have been studied thoroughly, namely: *Eimeria acervulina, Eimeria brunetti, Eimeria maxima, Eimeria mitis, Eimeria necatrix, Eimeria praecox* and *Eimeria tenella.*These species are universally accepted and there are no doubts about their validity. However, there are two species that are also described in the literature but their existence is



Courtesy of Bayer healthcare AG, Germany

Figure 1.1 Life Cycle of *Eimeria* species of Chicken

questioned by many researchers (Shirley *et al*., 1983; McDonald and Ballingall, 1983), namely: *Eimeria mivati* and *Eimeria hagani*. These two species are also overlooked in papers which deal with species discrimination techniques (Fernandez *et al*., 2003; Morgan *et al.*, 2009; Vrba *et al.*, 2010).

E. mivati was first described by Edgar and Seibold (1964) as a pathogenic coccidium having oocyst morphology similar to *E. mitis*. The controversy surrounding the status of *Eimeria mivati* has recently been resolved when it was discovered to be a variant of *Eimeria mitis* (Vrba *et al.*, 2011). Despite the advances in poultry husbandry, nutrition and chemotherapy that have made clinical outbreaks of coccidiosis rather infrequent, subclinical coccidiosis continues to be one of the poultry industry's most common and expensive diseases worldwide (McDougald, 2003). The broiler industry in particular relies on continuous in-feed prophylaxis with application of anticoccidial drugs. As a result of the increasing concern about drug resistance and drug residues, the EU Commission proposed a phasing out for use of anticoccidials by 31 December 2012 (EU Commission, 2003).

The application of specific diagnostics, as well as study of the epizootiology of the infections, is important for carrying out rational and effective control measures (McDougald, 2003). Species differentiation within the coccidia has traditionally been based on comparing several parasite characteristics and host responses (Long *et al*., 1976; Long and Reid, 1982). This diagnostic procedure is not only expensive and time-consuming, but can also be unreliable since the different species have overlapping properties and substantial intra-species variation (Joyner & Long, 1974; Pelle´rdy, 1974; Long & Joyner, 1984; Thebo *et al*., 1998). Knowledge of *Eimeria* species at the genomic level is continuously emerging, and objective molecular methods for *Eimeria* species differentiation have been developed (Stucki *et al*., 1993; Tsuji *et al*., 1997; Schnitzler *et al*., 1998, 1999; Gasser *et al*., 2001,

2005; Su *et al*., 2003; Lien *et al*., 2007; Haug *et al*.2007). The practical implementation of these techniques in routine diagnostics and epizootiology of chicken coccidiosis have so far been limited (Lew *et al*., 2003; Gasser *et al*., 2005; Blake *et al*., 2006; Morris *et al*., 2007). Substantial work on coccidiosis based on experimental infections and drug and vaccine trials has been presented over many years. However, reports on infection prevalence, oocyst abundance and frequencies of the different *Eimeria* species in commercial poultry flocks are few. Often, the reports are not comparable due to the differences in management and production systems, sample materials, sampling periods, sampling methods and prophylactic measures applied.

More knowledge of the aetiology and population dynamics of mixed coccidial infections in commercial poultry is needed in view of the recent upsurge in the usage of coccidiosis vaccines for prophylactic purposes. Identification of *Eimeria* has traditionally relied on oocyst morphology, host range and life-cycle attributes. However, it is increasingly recognized that *Eimeria* species can vary in size and shape across their host range, an attribute known as 'polymorphism' that presents a unique challenge for identification. Advances in molecular tools hold promise for characterising *Eimeria* that may otherwise be misclassified based on morphology.

The previous predominantly used anticoccidial method of coccidiosis control in poultry was often effective irrespective of the *Eimeria* species present because of its broad mechanism of action but the precise identity of *Eimeria* present in poultry farms in Ogun and Oyo states is critical for a successful adoption of coccidiosis vaccine prophylaxis due to lack of cross immunity among the different species.

The present study involves Morphometric and Molecular identification of *Eimeria* species of commercial chickens in Oyo and Ogun States as well as the determination of the Prevalence, Relative abundance and Infection levels of coccidial infections.

1.4 Research Questions

(1) What are the factors associated with good knowledge of coccidiosis and the adoption of coccidiosis vaccines?

(2) What is the identity of *Eimeria* species in commercial chicken flocks in Oyo and Ogun states?

(3) What is the prevalence and relative abundance of *Eimeria* species in Oyo and Ogun states?

(4) What is the level of agreement between morphometric and molecular methods of *Eimeria* species identification?

1.5 General Objective

The overall objective of the study is to determine the prevalence of Eimeria species using morphometric and molecular methods in Oyo and Ogun states of Nigeria.

1.5.1 Specific Objectives

(a) To collect data from Chicken farmers in Oyo and Ogun states via questionnaire on the factors associated with a good knowledge of coccidiosis as well as the factors associated with their adoption of coccidiosis vaccines prophylaxis

(b) To identify and quantify *Eimeria* species of commercial chickens in Oyo and Ogun states using morphometric method.

(c) To determine the prevalence, oocyst burden and relative abundance of *Eimeria* infection of chickens in Oyo and Ogun States.

(d) To determine and compare the prevalence of coccidial infection in caged chickens with those kept on deep litter.

(e) To use Real-time quantitative PCR to characterise and determine the relative abundance of *Eimeria* species in Oyo and Ogun states

(f) To determine the level of agreement between morphometric and molecular method by comparing their sensitivity and specificity (Haug *et al*., 2008).

1.6 Justification

Coccidiosis still remains an enzootic disease of major economic importance to the poultry industry in Nigeria and worldwide. More research is needed to elucidate the various factors and events that have contributed to the sustenance of the disease in the commercial chicken population in Ogun and Oyo states

Hitherto, identification of the *Eimeria* species present in Nigeria has been done mostly by the traditional morphometric methods which may not be very precise whereas vaccine prophylaxis requires precise identification of local *Eimeria* species. There is therefore the need to identify *Eimeria* species with greater precision in view of the increasing adoption of vaccine prophylaxis in Nigeria and this can be accomplished by the application of molecular methods of identification.

1.7 Study Hypotheses

(a) H1: There is no significant difference in the prevalence of coccidial infection in Oyo and Ogun States.

(b) H2: There is no significant difference in the prevalence of coccidial infection in young birds (below 8weeks) and older birds (above 18 weeks).

(c) H3: The *Eimeria* species causing coccidial infections in Oyo and Ogun States are the same.

(d) H4: There is no significant difference between the prevalence of coccidial infection on deep litter and cage system.

(e) H5: There is a low level of agreement between molecular and morphometric methods of *Eimeria* species identification.

1.8 Description of Study Area

1.8.1 Oyo State

Oyo State is located South-Western Nigeria at coordinates 8^0 00 ‘00^0 N and $4^0$00’00 E. Its capital is Ibadan. It is bounded in the south by Ogun State and in the north by Kwara State, in the west by Ogun state and partly by the Republic of Benin while in the east it is bounded by Osun State. Oyo State has an equitorial climate with dry and wet seasons and relatively high humidity of between 71 and 88%. The dry season lasts from November to March while the wet season starts from April and ends in October. Average daily temperature ranges between 25^0C (77.0 ^{0}F) and 35 ^{0}C (95.0 ^{0}F) almost throughout the year. (http://en.wikipedia.org/wiki/Oyo_State).

1.8.2 Ogun State

Ogun state is located at coordinates 7^0 00’00 N and 3.35’ 00” E. It is a State in the South Western part of Nigeria. It borders Lagos State to the south, Oyo and Osun States to the North, Ondo State to the east and Republic of Benin to the west. The climate of Ogun State follows a tropical pattern with the raining season starting about March and ending by November, followed by dry season. The mean annual rainfall varies from 128cm in the southern parts of the state to 105cm in the northern

areas. The average monthly temperature ranges from 23^0C in July to 32^0C in February and a relatively high humidity of between 80 to 90%. http://logbaby.com/encyclopedia/ogun-state-nigeria-overview-history-and-summary-information_35.html#.U0JXGSaPK1s.

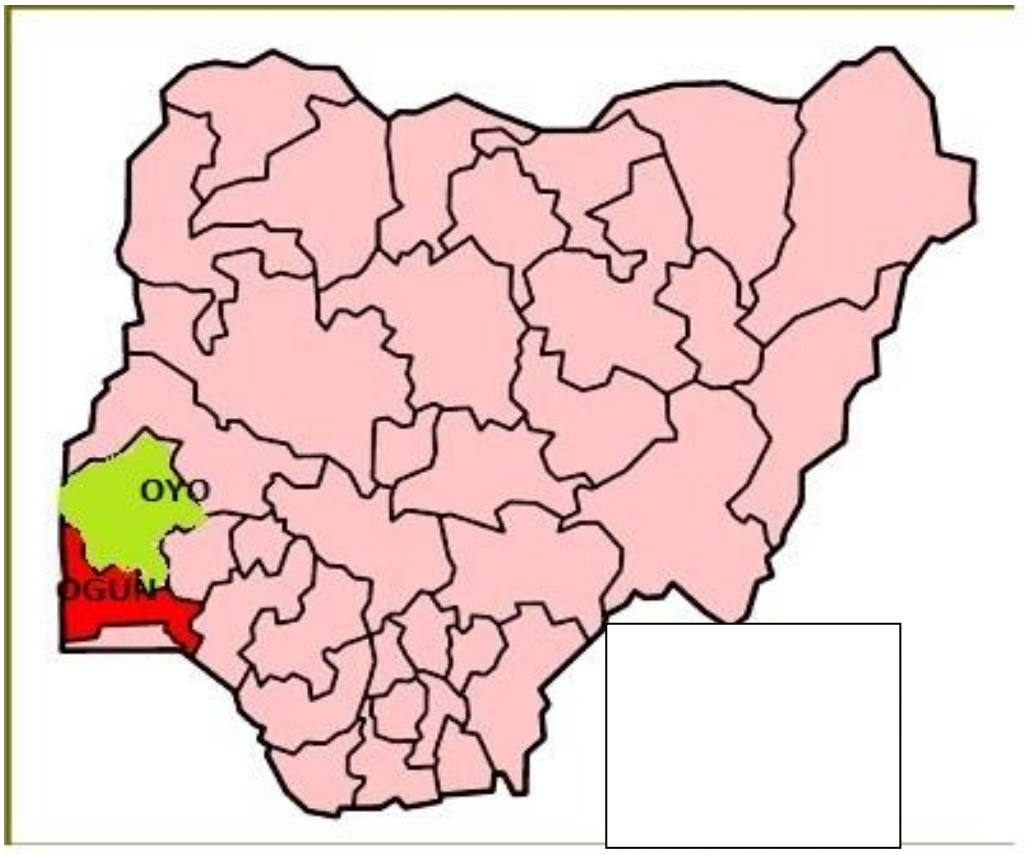


Figure 1.2 Map of Nigeria showing Oyo and Ogun States.

Source: http://en.wikipedia.org/wiki/Ogun_State and http://en.wikipedia.org/wiki/Oyo_State

CHAPTER TWO

LITERATURE REVIEW

2.1 Occurrence and Classification of Coccidial Infections

As the world's poultry production continues to grow, so do concerns about the control of coccidial infections which remain one of the most commonly reported diseases of chickens (Biggs, 1982; Xie *et al.*, 2001). Coccidial infection of chickens is an intestinal disease caused by parasitic protozoa of the genus *Eimeria* (Apicomplexa). It occurs worldwide and flocks free from coccidia are extremely rare. Coccidial infections may be classified in one of three ways (Williams, 1999a)

(1) As clinical coccidiosis, characterized by mortality, morbidity, diarrhoea or bloody faeces, as well as by adverse effects on economic performance.

(2) As subclinical coccidiosis, not immediately obvious, but causing reductions in weight gain and feed conversion efficiency of the host, without frank signs of disease.

(3) As coccidiasis, a mild infection causing no adverse effects on the host (Levine, 1961).

2.2 *Eimeria* Species of Poultry

There are nine species of *Eimeria* said to infect the chicken host, seven of which are well-known and have been studied thoroughly, namely: *Eimeria acervulina, Eimeria brunetti, Eimeria maxima, Eimeria mitis, Eimeria necatrix, Eimeria praecox* and *Eimeria tenella*. These species are universally accepted and there are no doubts about their validity. However, there are two species that are also described in literature but their existence is questioned by many researchers (Shirley *et al.*, 1983; McDonald and Ballingall, 1983), namely: *Eimeria mivati* and *Eimeria hagani*. These two species are also overlooked in papers which deal with species discrimination techniques (Fernandez *et al.*, 2003; Morgan *et*

al., 2009; Vrba *et al*., 2010). *E. mivati* was first described by Edgar and Seibold (1964) as a pathogenic coccidium having oocyst morphology similar to *E. mitis*. The controversy surrounding the status of *Eimeria mivati* has recently been laid to rest when it was reported to be a variant of *Eimeria mitis* (Vrba *et al.,* 2011). The authenticity of *E. hagani* is still uncertain. Chickens of all ages and breeds are susceptible to coccidiosis. But it is largely a disease of young birds because immunity quickly develops after exposure and gives protection against later outbreaks.

It usually affects chicks below 10 weeks of age with maximum incidence in 3-6 week-old chicks. It is rarely seen in birds less than 3 weeks of age unless they are brooded on contaminated litter but both intestinal and caecal coccidiosis in a flock of 5-day old broiler breeder has been reported in a farm in Zaria (Musa *et al., 2010*). Oocysts are not infective until they sporulate under optimum condition of warmth (21-32°C) with adequate moisture and oxygen, and this requires 1 to 2 days, which they gets on the ground, soil or litter.

Sporulated oocysts may survive for long periods, depending on the environmental factors and are protected by the thick oocyst wall, and are therefore, able to survive for months or even years. Due to the short prepatent period and high biotic potential, the number of oocysts in the litter rises rapidly on arrival of susceptible new flock of birds (Dhama *et al*., 2012). Coccidia are almost invariably present in poultry rearing operation but clinical disease occurs only after ingestion of relatively large numbers of sporulated oocysts by susceptible birds. Both clinically infected and recovered birds shed oocysts in their droppings, which contaminate feed, water, litter and soil. Threat of coccidiosis is greater in cooler wet weather (rainy and winter seasons) and is lesser during hot dry weather (dry season and summer).

The disease is transmitted through horizontal course. Transmission of oocysts takes place by shoes, feed, trucks, crates, pets, rodents, and moving equipments. Consumption of as few as 10,000 sporulated oocysts will produce coccidiosis. Birds pick up the infection through ingestion of oocyst contaminated feeds and drinking water. Oocysts remain viable in litter for many months but high temperature above 56°C and desiccation can kill them (Dhama *et al*., 2012).

Upon infection, sporozoites invade the cells of intestinal (or caecal) surface epithelium and development of some species occurs here *(E. brunetti* and *E. praecox).* Other species develop in the epithelium of the crypts *(E. acervulina, E. maxima, E. necatrix, E. tenella).* *Eimeria* primarily infect the intestine of the susceptible host and cause damage, resulting in dehydration, blood loss and increased susceptibility to other infectious agents (Dhama *et al*., 2012). Microscopic examinations of lesion scrapings will show numerous gametocytes and oocysts. Diarrhoea, severe weight loss, poor feed conversion and loss of skin pigmentation are the common signs. During severe infection of *E. maxima*, numerous petechiae appear on the serosal surface of the intestine and the walls get thicken; ballooning of the intestine may occur.

The disease appears in two forms; caecal and intestinal. Caecal form is generally observed in young chicks whereas adult birds usually suffer from intestinal form of coccidiosis. Clinical signs in young chicks include depression and droopiness, anorexia, huddling together, having ruffled feathers, watery diarrhoea and by the fourth day blood begins to appear in the droppings. The greatest amount of blood appears by day five or six and by the eighth or ninth day the bird is either dead or on the way to recovery. Severe haemorrhagic diarrhoea may produce anemia. Comb and wattles become pale. Mortality is highest between the fourth and sixth days (Dhama *et al*., 2012). Birds that recover may develop a

chronic illness as a result of a persistent caecal core. The core usually detaches itself by eight to ten days and is shed in the droppings. Clinical signs in adult birds include gradual loss of appetite, ruffled feathers, progressive emaciation and disinclination to move, combs become pale. Chocolate colored droppings are characteristic. *E. praecox* has been reported to reduce the clinical effects of *E. maxima* infection when chickens were infected at the same time with both species (Jenkins *et al*., 2008). It was observed that when there is co-infection of chickens with two *Eimeria* species that infect similar regions of the gut, one species non-pathogenic (*E. praecox*) and the other species pathogenic (*E. maxima*), elicits non-specific immunity against both *E. praecox* and *E. maxima*, thereby reducing clinical signs of coccidiosis.

2.3 Effects of Management on the Occurrence and Dynamics of Coccidial Infections

The widespread occurrence of coccidial infections in chickens even under modern conditions of production reflects both the adaptability of the parasite and the way birds are raised. Large numbers of day-old, fully susceptible chicks are placed together on litter in poultry growing houses. The flock grow-out time may be a brief, five to nine weeks for broiler or meat-type birds, which make up a majority of the world poultry population, or much longer periods for other types of chickens such as replacement layers and breeers. Regardless of the ultimate goal, the risk of coccidiosis is indeed great to the young bird under these conditions. Once a house becomes contaminated, it is virtually impossible to totally decontaminate the environment (Reid, 1989).

Studies with broiler birds have shown that exposure to sporulated oocysts usually begins shortly after chicks are placed on the litter (Long and Rowell 1975; Long, Tompkins, and Millard 1975; Long and Millard 1978; Braunius 1984). Litter oocyst counts are generally

low during the first two to three weeks, increase rapidly to a peak between four and six weeks, and decrease to low levels again by seven to eight weeks. Lapses in the anticoccidial drug program as a consequence of restricted feeding, skip-a-day feeding, or a withdrawal period extending beyond seven days increase the risk of coccidiosis outbreaks.The occurrence of other diseases and post-vaccination stress can have a significant adverse impact on feed consumption and the overall efficacy of an anticoccidial program.

2.3.1 Effects of Chick Behaviour on the Occurrence and Dynamics of Coccidial Infections

Chick behaviour may influence both vaccine uptake and spreading of oocysts in the litter. Tolman (1964, 1965) reported that the presence of companions in a new environment increases feeding activity by chicks up to 4 days old. This clearly would have a beneficial effect on uniformity of vaccine uptake when day-old chicks are vaccinated by feed spray in a broiler house. The subsequent distribution of vaccinal oocysts in the litter after initial vaccine uptake is influenced by chick density and movements within the broiler house.

2.3.2 Effects of Host Environment on the Occurrence and Dynamics of Coccidial Infections

The pioneering studies of Johnson and Tyzzer in the 1920s laid the groundwork for the understanding of coccidiosis and the important management and environmental factors that affect the incidence and epizootiology of this disease (Chapman, 2003). Of the many improvements in husbandry and housing that have occurred over the years, one of the most significant may have been the introduction of nipple type drinking systems. These systems have the potential to reduce litter wetness that is known to be a major factor in the occurrence of clinical coccidiosis. An example of the effect of management upon the effectiveness of coccidiosis control programs was given by Williams; higher parasite

numbers were produced by vaccinated birds where “clean-out” between flocks was demonstrably inadequate (Chapman *et al.,* 2002).

2.4 Pathogenicity of Different *Eimeria* Species of Chickens

Eimeria tenella infections are found only in the cecae and can be recognized by accumulation of blood in the cecae and by bloody droppings. Caecal cores, which are accumulations of clotted blood, tissue debris, and oocysts, may be found in birds surviving the acute stage. *Eimeria necatrix* produces major lesions in the anterior and middle portions of the small intestine. Small white spots, usually intermingled with rounded, bright or dull red spots of various sizes, can be seen on the serosal surface. In severe cases, the intestinal wall is thickened, and the infected area dilated to about twice the normal diameter (Dhama *et al*., 2012).

Eimeria acervulina has a great reproductive potential and is regarded as pathogenic because it produces a marked depression in gain of body weight, higher feed conversion. Infection is characterized by numerous, whitish, transverse patches in the upper half of the small intestine and may not be easily distinguished on gross examination. *Eimeria brunetti* occurs in the lower small intestine, rectum, ceca, and cloaca. In moderate infections, the mucosa is pale and disrupted but lacking in discrete foci, and may be thickened. In severe infections, extensive coagulative necrosis and sloughing of the mucosa occurs throughout most of the small intestine. *Eimeria maxima* develops in the small intestine, where it causes dilatation and thickening of the wall; petechial hemorrhage; a reddish, orange, or pink viscous mucous exudate and fluid. The oocysts and gametocytes (particularly microgametocytes), which are present in the lesions, are distinctly large (Dhama, 2012).

Eimeria mitis is recognized as pathogenic in the lower small intestine. Formerly, it was confused with *E. mivati*, but there is now doubt as to validity of the latter species name.

Lesions resemble moderate infections of *E. brunetti* but can be distinguished by finding small, round oocysts in the lower small intestine. *Eimeria praecox* is parasitic in the upper half of the intestine and may decrease rate of growth but is of less economic importance than the other species. *Eimeria hagani* is of dubious status but is thought to develop in the anterior part of the small intestine (Dhama *et al.*, 2012).

2.5 Control of *Eimeria* Infections

There are basically two means of prevention of coccidiosis; chemoprophylaxis and vaccine prophylaxis (vaccination). Prophylactic medication with anticoccidial drugs is relied upon extensively in broiler chickens, but the emergence of strains individually resistant to many of the drugs poses a continuing problem. Consequently, the poultry industry has become dependent upon strategies that make use of combinations of different anticoccidial drugs in order to overcome, or limit, infections with drug-resistant parasites. Breeding flocks can be given anticoccidial drugs only during the early rearing period and not when they are in lay, in order to avoid the accumulation of drug residues in their eggs. Consequently, rearing these birds poses special problems since one objective of the early rearing phase is to establish an effective balance between coccidial infection and immunity.

2.5.1 Anticoccidials in Feed Additives

The effective use of anticoccidial feed additives has played a major role in the growth of the poultry industry and has allowed the increased availability of high quality, affordable poultry products to the consumer. These anticoccidial can be classified as:

(a) Chemicals which has specific modes of action against parasite metabolism, such as amprolium, clopidol decoquinate, halofuginone.

(b) Polyether ionophore such as monensin, lasalocid, salinomycin, narasin, and maduramycin) which act through general mechanisms of altering ion transport and disrupting osmotoic balance. Chapman (1994, 1998) and Ruff and Danforth (1996) reported that some degree of resistance to all anticoccidial drugs, including ionophores, has developed. Williams (1998a) reported that to minimize the effects of resistance, poultry producers rotate the use of various anticoccidial with successive flocks, combine chemical and ionophore treatments, or employ shuttle programs during a flock growth. Application of these treatment programs depend on seasonal conditions and prevalence of various species of coccidia. In recent years, pharmaceutical companies have not brought new anticoccidial to market. However two potential drug targets, enzymes of the sporozoite mannitol cycle (Allocco *et al*., 1999; Schmatz, 1997) and trophozoite host one deacetylase, have been identified (Schmatz, 1997).

2.5.2 Anticoccidial Vaccines

In the early 1980s, it was generally believed that it is impossible to immunize chickens against coccidiosis with dead antigen (Rose, 1982). Subsequently, contrary evidence began to emerge stimulating great hopes for the discovery of a recombinant vaccine Murray *et al.*, 1986; Crane *et al.*, 1991; Karkhanis *et al.*, 1991; Wallach *et al.*, 1992). The early hopes were not quickly realized, and it eventually became clear that the development of anticoccidial vaccines was likely to be achieved soonest by using live oocysts.

Several brands of live, multivalent anticoccidial vaccines have been registered in various countries for use in chickens or turkeys. All of them include more than one product for different classes of bird or types of husbandry. There has been development of new specific products and a steady stream of adaptations of the methods for administration of the older vaccines (Shirley & Long, 1990; Bedrn´õ k *et al.*, 1995; Chapman, 2000a; Vermeulen *et al.*,

2001). The extreme complexities of the immune system of the chicken are still far from completely understood and the search for specific protective antigens is still ongoing. The development of new live vaccines and improvements in their effectiveness has been made possible by simple practical measures. Since live coccidia may be relied upon to stimulate the whole natural gamut of immunological reactions in a chicken, it has been possible to achieve rapid improvements in vaccine efficacy by concentrating on epizootiology and vaccine administration, rather than on fundamental immunology (Parry *et al.*, 1989, 1992; Shirley, 1989; Williams, 1996, 1998a, 1999b). This approach has been particularly successful for the development of vaccines specifically for broilers.

2.5.2.1 Embryo-Adapted Vaccinal Parasites

Following the discovery of Long (1965) that *E. tenella* is able to complete its life cycle in the chorio-allantoic membrane of a chicken embryo; it was shown that repeated passaging of parasites through embryos was possible (Long, 1971). After 42 embryo passages of *E. tenella* the oocyst yield increased dramatically, yet pathogenicity to embryos and chickens was considerably reduced, although this attenuation was lost after two passages through chickens (Long, 1972). Attenuation was shown to be due to a loss of the ability to produce the characteristically large second generation schizonts, both in embryos and chickens (Long, 1973). These schizonts developed normally after the reversal of attenuation following two passages through chickens (Long, 1973). However, after 62 embryo passages of the adapted line, the attenuation was not reversible after nine passages through chickens (Long, 1974). On the other hand, Bedrn´õ k *et al.* (1986) failed to achieve attenuation of an *E. tenella* line, even after 117 embryo passages.

The stability of an embryo-adapted line should be carefully examined before its use in an attenuated vaccine. In practice, it is difficult to obtain embryo-adapted parasites with a

satisfactory combination of immunogenicity and attenuation of virulence (Shirley & Long, 1990). Moreover, the major limitation of embryo-adaptation as a means of producing potential vaccinal parasites has been the total lack of success in obtaining complete development of *E. acervulina, E. maxima* and *E. praecox* in the embryonic chorio-allantoic membrane (Shirley and Long, 1990). Nevertheless, an embryo-adapted line of E. tenella, derived after more than 100 passages, is included with precocious lines of *E. acervulina, E. brunetti* and *E. maxima* in the Livacox® vaccines

2.5.2.2 Precocious Vaccinal Parasites

The most widely used method of attenuation is selection for precocity of parasites in chickens. It is based on the seminal work of Jeffers (1974, 1975). The essential characteristics of attenuated parasites of the precocious type are a reduced prepatent time; a reduced reproductive potential resulting in attenuation of virulence; retention of immunogenicity; and genetically controlled stability of traits. They behave differently from non-attenuated vaccines.

Precocious lines of all *Eimeria* species of the fowl have been produced, viz., *E. acervulina* (McDonald *et al*., 1982; McDonald and Ballingall, 1983a; Long and Johnson, 1988; Onaga *et al*., 1992a; Stewart and Jorgensen, 1997; Jorgensen and Anderson, 2001; Kawazoe and Manarini, 2001); *E. brunetti* (Johnson and Long, 1985; J.K. Johnson *et al*., 1986a; Shirley *et al*., 1986); *E. maxima* (Cheng and Edgar, 1979; McDonald *et al*., 1986a; Kucera and Bedrn´õ k, 1987; Long and Johnson, 1988; Shirley and Bellatti, 1988; Shirley, 1989; Onaga *et al*., 1992a; Traore *et al*., 1994; Jorgensen and Anderson, 2001); *E. mitis* (McDonald and Ballingall, 1983b; McDonald and Shirley, 1984; McDonald *et al*., 1985; Long and Johnson, 1988); *E. necatrix* (Shirley and Bellatti, 1984; Long and Johnson, 1988; Onaga *et al*., 1992b; Jorgensen and Anderson, 2001); *E. praecox* (Shirley *et al*., 1984; Long and Johnson,

1988); E. tenella (Jeffers, 1974, 1975; Cheng and Edgar, 1979; Johnson *et al*., 1979; Bedrn´õ k *et al*., 1986; McDonald *et al*., 1986b; Kawaguchi *et al*., 1988; Long & Johnson, 1988; Onaga *et al*., 1992b; Shimura and Isobe, 1994; Weng *et al*., 1994; Jorgensen and Anderson, 2001).

Although the characteristics of precocious lines tend to be rather more predictable than embryo adapted lines, they do not always provide a satisfactory combination of immunogenicity and attenuation of virulence. For instance, the virulence of the precocious line of *E. tenella* selected by Bedrn´õ k et al. (1986) was not significantly different from its parent strain. Furthermore, a precocious line of *E. maxima* selected by Kucera and Bedrn´õ k (1987), although reduced in pathogenicity compared with its parent strain, failed to provide a satisfactory degree of protective immunity against a heavy challenge.

2.6 Development of Vaccines for the Control of Coccidiosis

2.6.1 Live Non-Attenuated Vaccines

Observations of *Eimeria* infections and subsequent immunity in several early studies indicated that the development of an anticoccidial vaccine was feasible (Tyzzer E., 1929, Johnson W. 1932, Tyzzer 1932). It has been established that any infection with *Eimeria* causes a strong, species-specific protective immunity that has also been found to be strain specific, at least with regard *E. maxima* (Fitzcoy 1992, Martin *et al.,* 1997); therefore, any vaccine administered should include the common pathogenic species and strains that affect poultry. Immunity to *Eimeria* is stimulated by the initial developing parasite stages, particularly the schizonts, and subsequently boosted and maintained by multiple re-exposures to oocysts in the litter. Thus, the recycling of infection following administration of live oocysts is critical for the development of protective immunity (Chapman and Cherry 1997).

The first commercial anticoccidial vaccine, CocciVac (Alabama, USA), was introduced to the United States market in 1952 and was a live vaccine comprising several wild-type strains of *E. tenella* oocysts. Criticism of the early vaccine was based on the observation that inclusion of only one species of *Eimeria* would not protect flocks from other species (Hinshaw W., 1952). Therefore, the vaccine went through a number of reformulations over the past 61 years and variants of the original product Coccivac-B, Coccivac-D and Immucox (Ontario, Canada) are still in use today and are registered in over 40 countries. However, the use of live non-attenuated vaccines is limited somewhat by the pathogenicity of the parasites used. Thus, until the late 1990s, vaccination with live vaccines was accompanied by chemotherapy to control pathology often induced by the live parasites (Martins *et al*., 1997), though this is usually not required today as a result of improved means of administration of oocysts (Danforth *et al*., 1998, 1997, Williams R. B., 2002). Hence, although virulent strains are still widely used, especially in North America, attenuated strains are now, arguably, the preferred products.

2.6.2 Live Attenuated Vaccines

The effectiveness of attenuated vaccines relies on administration of low doses of oocysts that are re-cycled through the litter, with protective immunity induced after 2 to 3 consecutive infections (Joyner and Norton 1973, Long *et al.*, 1986). Re-cycling of oocysts with an attenuated vaccine in use results in a lower risk of disease occurring as there is a reduction in proliferation of the parasites and less damage to the intestinal lining after passage through the gut.

Early attempts to attenuate *Eimeria* parasites included heat treatment (Jakiencz & Schofield 1934) and X-irradiation (Albanese and Simetana, 1937), both of which were unsuccessful. The first successful attempt to develop attenuated parasites of *Eimeria* began, when Long,

1974 showed that *E. tenella* was able to complete its life cycle in the chorio- allantoic membrane of the chicken embryo, and that serial passage in eggs resulted in significant attenuation of the parasite (Long P. L,. 1974). The loss of pathogenicity of the parasites was attributed to a reduction in the size and invasiveness of the second generation schizonts (Long P. L., 1974). Based on this, an embryo-adapted line of *E. tenella*, derived after more than 100 passages, is included in the commercially available Livacox (Jilove near Prague, Czech Republic) vaccine along with precocious lines of *E. acervulina*, *E. necatrix* and *E. maxima* (Shirley M. W., 1980, Williams R. B, 2002). Although embryo-adapted, attenuated lines of *E. necatrix* have been described (Kogut *et al*, 1983, Shirley M.W., 1980), there has been a failure to produce the equivalent in *E. acervulina*, *E. maxima* and *E. praecox* (Shirley and Bedrnik 1997). This is thought to be mainly because of the failure of the sporozoites to develop in the embryo, or oocysts produced not sporulating properly (Shirley *et al.*, 1981). Therefore, a different means of attenuation was required for vaccine development. Today, the second of the two commonly used methods of attenuation of *Eimeria* species for inclusion in vaccination formulations, precociousness, is the most widely used method. Precociousness is a selectable trait of the parasite and refers to a population of parasites that complete their lifecycle from sporozoite to oocyst up to 30 h faster than parasites from the same parent strain (Shirley and Bedrnik, 1997). The selection of such parasites was first described by Jeffers (Jeffers T. K., 1975) who showed that serial passage of oocysts through a chicken and collection at earlier and earlier time points post-challenge resulted in parasites of attenuated virulence. Importantly, infection of chickens with these parasites induced a high level of immunity against a challenge with the parent line (Johnson *et al.,* 1979). Whilst initial attempts to derive further protective lines of precocious parasites failed (Johnson *et al.*, 1979, Chang and Edgar 1979), precocious lines were eventually described for all seven species of *Eimeria* (Williams R. B. 2002).

Characteristically, precocious parasites of *Eimeria* have a marked reduction in oocyst reproduction and pathogenicity, and yet are still highly immunogenic. Studies also demonstrated the genetic stability of precocious lines, where precociousness was retained through serial passage without selection for early maturation of oocysts (McDonald and Shirley, 2009); thus, lines do not revert back to virulence. With this inherent improvement in safety, and parasites being more predictable and reliable than embryo-adapted lines, precocious lines of *Eimeria* became the basis of the development of the first attenuated anticoccidial vaccine, Paracox (Intervet/ Schering Plough Animal Health, Milton Keynes, UK). Paracox was launched in 1989, to protect laying and breeding hens and it contained precocious lines of all seven species of *Eimeria*, including two lines of *E. maxima* due to antigenic variation seen in this species (Williams R.B. 1994, Shirley M.W. 1989, Shirley and Millard 1986). As its introduction, several other formulations and attenuated vaccines have become commercially available for use in different poultry flocks. Generally, *E. maxima*, *E. tenella* and *E. acervulina* are the only species included in vaccines for broiler birds as younger flocks rarely encounter the pathogenic species *E. brunetti* or *E. necatrix* (Williams R.B. 1998, Chapman H. 2000).

In 2003, Eimeriavax® was the first live coccidiosis vaccine registered for use in Australian poultry. It is comprised of drug-sensitive, precocious lines of *E. acervulina*, *E. maxima*, *E. tenella* and *E. necatrix*, each isolated from backyard flocks of Australian chickens (Jorgensen *et al* 2006a, Jorgensen *et al* 2006b). Field trials showed that the vaccine could protect broiler breeders, broilers, free-range and barn flocks of egg laying hens by eye-drop or coarse aerosol application (Richard & Woods, 2001). Efforts continue to be directed towards the derivation of further vaccines based on precociousness and the reliance on these types of vaccines will most probably increase in years to come (McDonald and Shirley, 2009).

2.6.3 Subunit Vaccine Development

An anticoccidial vaccine composed of protective antigens, either native or recombinant, has been pursued as an alternative to live vaccines and the problems and costs associated with them. The identification of protective antigens is vital in the development of any future vaccine and many of the *Eimeria* antigens investigated thus far are primarily associated with asexual stages of the parasite such as sporozoites and merozoites. In particular, proteins associated with invasion and the apical complex, characteristic of this phylum of parasites have been investigated as potential subunit vaccine components. These include antigens associated with micronemes (Tomley *et al.,* 1996, Tomley *et al.,* 1991), rhoptries (Tomley F. M. 1994) and refractile bodies (Vermeulen *et al.,* 1993).

Ultimately, these studies have revealed that use of these asexual stage antigens to immunise chickens only provide a moderate and, often, inconsistent protection against challenge with *Eimeria* infections (Jenkins M.C., 2001, Vermeulen A. N., 1998). Studies have shown that there is distinct antigenic variability between the endogenous developmental stages of the parasite and that antigenic modification during successive asexual generations may aid the parasite in evading immune responses (Tomley F.M., 1994, McDonald *et al.,* 1988). The various antigens and strategies used in attempts to develop subunit vaccines against the asexual stages of *Eimeria* have been reviewed thoroughly in recent years (McDonald and Shirley, 2009; Vermeulen 1998, Jenkins M. C., 1998, Shirley *et al.*, 2007, Shirley 2005) but the results have not been remarkable.

2.6.4 A Novel Sexual Stage Subunit Vaccine

The subunit vaccine, CoxAbic, is comprised of affinity purified gametocyte antigens (APGA) from *E. maxima* in proprietary oil in water adjuvant. The vaccine is cost effective

on a commercial scale through a novel strategy of maternal immunization, where vaccination of laying hens can lead to protection of broiler offspring.

More specifically, injection of gametocyte antigens into the breast muscle of breeder hens stimulates the production of large amounts of specific IgG (also referred to as IgY) maternal antibodies that are transferred to their offspring, via the egg yolk, to provide protective immunity (Smith *et al.,* 1994, Wallach *et al.,* 1992, Wallach *et al.,* 1995). Immunisation occurs prior to hatching, thus eliminating stress imposed by vaccination of the hatchlings, which are protected against coccidiosis from day 1 of age. The vaccine functions as a transmission blocker by inhibiting development of macrogametes into oocysts, thereby reducing levels of oocysts shed in the litter. Thus, broiler chicks, once exposed to the parasite in the field, are able to develop active immunity against reinfection without suffering the economically damaging effects of the disease.

2.7 Vaccination Methods

2.7.1 Vaccination through Drinking Water and Feed

The first formulations of live vaccines were either administered in the drinking water or feed to chicks of about one week old. Immucox® originally required two administrations (Lee, 1987, 1989). A major advance has been the development of various administration methods for the vaccination of chicks with a single dose at day-old. This is particularly important in initiating immunity as early as possible in broilers. Such early vaccination was once thought to be impracticable, since some evidence suggested that very young chicks were generally more resistant to coccidial infection than older ones (Krassner, 1963; Doran and Farr, 1965; Rose, 1967; Long and Millard, 1979a), possibly because the excystation of oocysts was inefficient in one-day or two-days-old chicks. Furthermore, the immune system of such

chicks was believed to be so immature that coccidia are not as well able to evoke a strong immunity as when the host is approaching one week of age (Rose, 1987).

Previous work had shown that, in fact, chicks infected at day-old are perfectly capable of mounting an effective immune response (e.g., Stuart *et al.*, 1963; Edgar, 1964) and the principle is now well established (e.g., Sanda, 1977; McDonald and Ballingall, 1983a; Long *et al.*, 1986; Lillehoj, 1988; Bafundo, 1989; Bafundo and Jeffers, 1990; Nakai *et al.*, 1992; Stiff and Bafundo, 1993; Watkins *et al*., 1995; Chapman and Cherry, 1997a, 1997b; Danforth *et al*., 1997b; Martin *et al*., 1997). It is now known that even late embryos have a functional immune system (Ruff *et al*., 1988; Fredericksen *et al*., 1989; Doelling *et al*., 2001). It has been shown that theproduction by hatchling chicks of pancreatic enzymes (which include trypsin, the key enzyme foroocyst excystation) are triggered by feed intake (Sklan, 2001). The sooner chicks are given access to feed after vaccination the better should be their uptake of vaccine.

2.7.2 Intra-Occular Method

Intra-ocular vaccine administration involves spraying vaccine into an eye of a chicken. The oocysts pass down the nasolachrymal duct to reach the intestine via the buccal cavity (Cherry, T. E and Chapman H.D in Chapman *et al*., 2002). This method, carried out in newly hatched chicks in the hatchery can achieve very uniform dosage (Chapman and Cherry, 1997a, 1997b). However, for logistical reasons, it has fallen out of use in the USA, although it is still recommended for Livacox® in broilers. The Biojector II®, with an Immunizer® attachment (Schering-Plough Animal Health, USA) is suitable for intra-ocular administration of live vaccines in hatcheries (Chapman and Cherry, 1997a). In the USA, this used to be done simultaneously with Marek's disease vaccination by injection into the neck of a chick, using the combined apparatus.

2.7.3 Hatchery Spray Administration

This method involves the spraying of the vaccine mixed with colourants over trays of chicks in the hatchery. Chicks probably ingest the oocysts partly by the direct oral and ocular routes but mainly by self-preening and pecking drops of diluted vaccine off their neighbours (Bafundo and Jeffers, 1990). This method is used for Coccivac®, Nobilis® COX ATM, Paracox®-5 and Viracox®, (Cherry T.E. and Chapman H.D. in Chapman *et al*., 2002). Hatchery spraying presumably has an incidental benefit in providing a first source of water, which might help to delay dehydration of chicks during their transport to the farm. Such is the apparent improvement in the uniformity of vaccination with Coccivac®-B, that it has been considered possible to discontinue the previous requirement for routine therapy with amprolium, because post-vaccinal reactions have been considerably reduced (Newman, 1999).

2.7.4 Edible Gel Administration

An edible gel can be placed in the chick trays at the hatchery or on feed trays in the poultry house immediately after placement. Immucox® is applied in this way by incorporation in a bright green gel (Danforth, 1998; Chapman, 2000a). Like the hatchery spray method, the gel presumably provides an early water source for chicks during transport to the farm or immediately on arrival. Danforth (1998) compared four methods of vaccinating day-old chicks with Immucox® in battery trials; they were crop gavage, gel delivery, spray cabinet and slurry delivery. All methods of immunization resulted in a significant protective immune response to challenge with a homologous infection, but it was concluded that the gel delivery system was the most effective of those tested. Chapman and Cherry (1997b), however, found that the intra-ocular route of vaccination gave a better vaccine uptake than the edible gel method in their studies.

2.7.5 Spray-on-Feed Administration

Another method of administration for day-olds is that of spraying vaccine on the first feed that the chicks receive after placement. This has been used for the Coccivac®, Paracox® and Viracox® vaccines. These vaccines contain live oocysts in aqueous suspensions and so the manufacturers' instructions with regard to timing of spraying onto feed should be followed precisely to prevent oocysts death from desiccation.

2.7.6 Intra-Yolk Sac Administration

This method delivers live vaccine by intra-yolk sac injection of hatchling chicks (Dibner *et al*., 1999). The vaccine may comprise oocysts of any species, which must first be fractured to release a proportion of the contained sporocysts. These injected sporocysts then escape via the yolk sac stalk into the intestine where the contained sporozoites are released. The advantages claimed for this vaccination method are uniformity of dosing and a low shedding rate of oocysts that reduces the risk of coccidiosis outbreaks due to litter contamination (Dibner *et al*., 1999); presumably the use of non-attenuated parasites was envisaged. The low shedding rate of oocysts following vaccination might be partly attributed to the fact that only about half of the vaccinal oocysts were broken to release sporocysts. However, a lower oocyst production of a non-attenuated vaccine could be achieved by simply giving chicks an appropriately low oral dose by any other efficient administration method. Watkins *et al*., (1995) had previously found that oocysts were produced by chicks 7 days after hatching, following the in ovo injection of oocysts or sporocysts into the yolk sac of 17-days-old embryos. This method of Dibner *et al*. (1999) produces early oocyst shedding that seeds the litter to initiate recycling infections, rather than immunizes chicks directly.

2.7.7 In-Ovo Administration

In ovo vaccination against coccidiosis is perhaps the ideal route of administration for broilers. It would deliver a precise vaccinal dose per chick, and achieve the earliest possible onset of immunity. Long (1965) demonstrated that sporozoites of *E. tenella* would complete their life cycle in the chorio-allantoic membrane after injection into the allantoic cavity of a 10-days-old developing chick embryo. However, intact oocysts do not establish infections by this route (Long, 1966; Shibalova, 1970). Furthermore, Doran (1973) concluded that the apparent success of Long (1966) in establishing an infection with sporocysts of *E. tenella* was actually due to contamination of the inoculum with free sporozoites.

Since the original work of Long (1965) with *E. tenella* sporozoites, it has been shown that not all of the seven species of *Eimeria* that parasitize the chicken are capable of establishing infections in the chorio-allantoic membrane. Among the *Eimerian* species that can develop in that tissue, infections are established only by sporozoites (Doran, 1973). *E. acervulina*, *E. maxima* and *E. praecox* have consistently been found not to be able to complete their life cycles in the chorio-allantoic membrane (Doran, 1973). *E. acervulina* and *E. maxima* are, however, two of the commonest species to be found in commercial chickens (Williams *et al.,* 1996), and must be included in any vaccine for broilers.

2.8 Vaccination Equipment for Broilers

Various kinds of equipment are used for the administration of live anticoccidial vaccines as oocysts. For the drinking water route, either the standard equipment in the house, or a temporary adaptation, may be used, particularly for pipeline systems. For other routes, specific types of machine have been developed. All of them may be used for attenuated or non-attenuated vaccines.

2.8.1 Bell-Type Drinkers

Early trials in broilers showed that vaccination via the drinking water using bell-type drinkers was effective (Govoni *et al*., 1987; Williams, 1994). This is a technique that has long been in use for broiler breeders. Anticoccidial vaccines administered in open water containers such as bell-type drinkers or troughs have a thickening agent in the formulation to keep the oocysts in suspension, because the average depth of immersion of a chick's beak when drinking is 1 cm (Ross and Hurnick, 1983).

2.8.2 Pipeline Nipple Drinkers

Pipeline nipple drinkers have been used in several different ways. Vaccine may be administered directly into the drip trays that are fixed under each nipple of some types of pipeline system (Williams *et al*., 1999). Alternatively, the vaccine may be delivered from a temporary reservoir into the pipeline itself, using a gravity fed delivery system (Williams and Gobbi, 2002). A more sophisticated method is to set up a reservoir with a pump and link up the pipelines in a house to establish a temporary circulating system, thus keeping the diluted vaccine in motion around a long pipeline circuit (Williams *et al*., 2001). An ultrasensitive oocyst counting technique has been designed to monitor vaccine delivery in pipeline and other water delivery systems (Williams *et al*., 2001).

2.8.3 Hand-Held Sprays

Hand-held sprays may be of the "gardener's spray" type with a small integral reservoir or a "back-pack" type canister with a capacity of up to 10 litres. These are suitable for spraying diluted vaccine on small quantities of feed in plastic trays or very large quantities spread on the floor of a broiler house, respectively. Care should be taken if feed is spread underneath gas-heated canopy brooders, lest the vaccine is desiccated before chicks can ingest it. Hand-

held sprays may also be used for directly spraying chicks in their trays before they are turned out onto the litter in a poultry house. The Spraycox ® Junior (Schering-Plough Animal Health, USA), designed primarily for spraying chicks in the on a small scale, may also be used for this purpose if a compressed air supply is available on the farm.

2.8.4 Egg Injection Machine

The Inovoject® (Embrex, Inc.) was originally developed as a result of the finding that *in ovo* administration of Marek's disease virus gives protective immunity against the disease (Sharma & Burmester, 1982). For coccidiosis, it was originally used to deliver an experimental recombinant antigen into the air-sac of an egg (Fredericksen *et al.*, 1989). This machine has also been used more recently to deliver oocysts into the amniotic cavity of embryos (Poston *et al.*, 2001). The oocysts are ingested by the developing embryo (Weber *et al.*, 2001), and the coccidial progeny are shed by the hatchling chick. Dibner *et al.*, (1999), developed a device that delivers oocysts by injection into the yolk sac of hatchling chicks.

2.9 The Basis of Anticoccidial Vaccine Efficacy

The efficacy of a vaccine is considerably influenced by the efficiency of the method of administration (Cherry T.E. and Chapman H.D. in Chapman *et al.*, 2002). The principle of anticoccidial vaccine efficacy consists of several factors.

2.9.1 Species Necessary in Coccidiosis Vaccine

Immunity to avian coccidia is strongly species specific when induced by parasites developing in the gut (Rose, 1973, 1978), so that all the species that chickens of any particular class should be included in any vaccine intended for them. Potentially, all seven of the ubiquitous chicken species of *Eimeria* should be considered (Shirley and Millard,

1986; Shirley, 1988a; Williams, 1998a), but fewer species are necessary for a broiler vaccine, because younger birds rarely encounter the extremely pathogenic species *E. brunetti* and *E. necatrix* (Williams, 1998a; Williams *et al.*, 1996; Chapman, 2000a). *E. acervulina*, *E. maxima* and *E. tenella* are included in all commercially available broiler vaccines.

Some other species, such as *E. mitis* and *E. praecox* that have been erroneously considered to be virtually innocuous have been under-estimated in their commercial importance (Bafundo and Donovan, 1988; Shirley, 1988a; Williams, 1998a). *E. mitis* is commonly world-wide (Chapman, 1982; McDougald *et al.*, 1987; Kucera, 1991; Shirley *et al.*, 1995; Williams *et al.*, 1996; Jorgensen *et al.*, 1997; McDougald *et al.*, 1997; Thebo *et al.*, 1998) and is rather poorly immunogenic (Shirley, 1988b). These factors, with its significant pathogenicity (Joyner,1958; Shirley *et al.*, 1983; Bafundo and Donovan, 1988; Watkins *et al.*, 1990; Fitz-Coy and Edgar,1992; Jorgensen *et al.*, 1997; Williams, 1997a, 1998a), which in fact approaches that of *E.acervulina* (Shirley *et al.*, 1983; McDonald *et al.*, 1985; Bafundo & Donovan, 1988; Jorgensen *et al.*, 1997; Williams, 1997a, 1998a), makes its inclusion in a broiler vaccine essential. *E. praecox*, however, although potentially pathogenic (Long, 1968a; Gore and Long, 1982; Williams, 1997a, 1998a), is rather less fecund (Williams, 2001) but more immunogenic than *E. mitis* (Shirley, 1988b), so it is not usually included in broiler vaccines. The results of some limited trials by Bedrn´õ k (1989) rather surprisingly indicated that *E. maxima*, *E. mitis* and *E. praecox* are not particularly pathogenic.

There is a risk that when a live vaccine is used, any *Eimeria* species resident in a poultry house but not present in the vaccine may be able to cause disease in certain circumstances. Hence, Danforth (H.D. Danforth in Chapman *et al.*, 2002) has suggested that the incidence

of so-called “lesser species” may increase as a result of immunological selection. However, Bedrn´õ k *et al.* (1993) found that during the use over several years of 2 million doses of vaccines containing only two or three species, those absent from the vaccine did not overwhelm the vaccinal species in the field environment. This was presumably because the *Eimeria* species in a vaccine do not eradicate the same species in the field, but instead seed the environment with vaccinal parasites, which inter-breed with the respective species in the resident populations.

2.9.2 Immunogenic Variations in Strains of *Eimeria* Species

Variation in immunogenicity and pathogenicity of strains of *Eimeria* species should be clearly distinguished, since there is no consistent relationship between them (e.g., Joyner, 1969; Joyner and Norton, 1969; Karim *et al.*, 1991; Fitz-Coy, 1992; Martin *et al.*, 1997). Whilst the pathogenicities of various strains may be demonstrably different when compared under the same conditions (Shumard and Callender, 1970), the pathogenicity of any one strain may be considerably influenced by changes in exogenous factors, such as the strain of chicken (e.g., Patterson *et al.*, 1961; Long, 1968b; Bumstead and Millard, 1992) and the diet of the host (e.g., Sharma *et al.*, 1973; Williams, 1992b). Immunogenicity, however, seems generally to be a less markedly variable characteristic of strains, but *E. maxima* is rather the exception to this rule (Schnitzler and Shirley, 1999). Various workers have come to different conclusions concerning the importance of antigenic variation.

Joyner, (1969), found that two pathogenically similar strains of *E. acervulina* were immunogenically different. Birds solidly immune to one strain supported a degree of infection with the other, as indicated by oocyst production. Jeffers (1978) and Karim and Trees (1989) obtained similar results with *E. acervulina,* but also measured the weight gains of birds, which were not obviously correlated with their oocyst production. Similar studies

of *E. tenella*, however, revealed no detectable immunogenic differences (Joyner and Norton, 1969; Jeffers, 1978). There were no obvious differences between the pathogenicities and the immunogenicities of two strains of *E. mitis*, judged by weight gains and FCRs of homologously and heterologously challenged birds (Fitz-Coy and Edgar, 1989). Long and Millard (1979b), though, demonstrated immunogenic variation in *E. maxima*, using oocyst production and lesion severity as criteria, as did McDonald *et al.* (1986a), using oocyst production only. Jeffers (1978), working with field strains of *E. maxima* and *E. acervulina*, concluded that the magnitude of the antigenic differences found was probably too small to be an important consideration in the efficacy of vaccines.

Jeffers's (1978) conclusion is supported by the fact that there is only a poor correlation between various parasitological criteria and growth performance of birds (Williams and Catchpole, 2000), and mild to moderate lesions may be observed, with no associated deleterious effect on growth, in healthy vaccinated birds (Williams, 1997b; Williams and Andrews, 2001). In the frequent cases where immunogenic variation is reflected only by differences in oocyst production in cross-protection tests, there would probably be little risk of clinical disease arising in the field. This is because the "trickle infection effect" (Joyner and Norton, 1973, 1976) of recycling infections is likely to stimulate a greater degree of immunity than the usual short term laboratory methods of immunising birds. This hypothesis is supported by the results of Karim *et al.* (1991), who compared British and Bangledeshi strains of *E. tenella*, which were pathogenically similar but immunologically different. However, when solid immunity judged by oocyst production was induced by trickle infections, birds were protected against disease after heterologous challenge.

Shirley and Hoyle (1981) found that populations of *E. maxima* recovered from a farm during eight months gave almost complete cross-protection, suggesting that antigenic drift

occurs slowly if at all, despite the marked immunogenic variation shown by this species (Rose, 1982).

Danforth (1998; H.D. Danforth in Chapman *et al.*, 2002) reported that vaccine efficacy may be compromised by the more dramatic differences in immunogenicity that sometimes exist between strains of *E. maxima* from widely separated geographical locations (Fitz-Coy, 1992; Martin *et al.*, 1997).

In practice, commercial birds are exposed to trickle infections with vaccinal lines that stimulate at least partial protective immunity to field strains, which simultaneously produce their own immune effects while inter-breeding with the vaccinal parasites.

It is therefore difficult to agree with the view expressed by Lee (1993) concerning how a field strain might not be controlled by a vaccine unless the reason for vaccine failure was that such large numbers of the resident strain oocysts remained from the previous flock that the vaccinal line was overcome before it could induce protective immunity, as once observed by Williams (R.B. Williams in Chapman *et al.*, 2002).

To achieve clinical protection by Immucox® vaccine against variant strains of *E. maxima*, refractory strains have been included in different reformulations (Lee, 1993; Danforth *et al.*, 1997a). The commercial Paracox® vaccines contain two lines of *E. maxima* (Shirley, 1989; Williams, 1992a), as does Nobilis® COX ATM (Schetters *et al.*, 1999), in order to control any possibly troublesome geographical variants.

In a series of field trials of an attenuated vaccine in the UK, some oocysts not attributable to administration of the vaccine were recovered from poultry house litter (Shirley and Long, 1990). It was concluded that the most likely explanation for the presence of wild-type parasites in vaccinated birds is that there is antigenic diversity between them and vaccinal

lines of the same species. Individual antigenic differences can generally be characterised as genetic traits of the Mendelian type (Jeffers, 1978). Since the vaccinal lines can interbreed with wild-types of the same species of *Eimeria*, it seems likely that extremes of phenotypic expression of immunogenicity in a mixed vaccinal and wild-type population of any species would eventually be reduced if a vaccine were used repeatedly on a farm, because of the production of recombinant populations of each species present. The aggregate characters of each population would, of course, depend on the relative dominance or recessiveness of the relevant genes. Very little research has been done on this topic, and it is to be hoped that PCR methods will soon be developed to analyse vaccinal and wild type strains in field populations of coccidia.

2.10 Development of Immunity

Following vaccination, immunity is stimulated initially by the developing parasitic stages of the vaccinal lines, and is subsequently boosted and maintained by multiple re-infections initiated by oocysts in the litter, originating from the vaccine and from adventitious infections by local wild-type strains. This recycling of infection is crucial to the development of fully protective immunity, since it has been shown that vaccinated birds reared in cages remain susceptible to challenge, whilst birds reared on the floor become immune (Chapman and Cherry, 1997a, 1997b). Furthermore, multiple mild re-infections are markedly more effective in stimulating protective immune responses than are single heavy infections (Joyner and Norton, 1973, 1976).

However, studies carried out in isolation from extraneous coccidia (Williams and Catchpole, 2000) have shown that a live vaccine did not necessarily require the presence of wild strains to boost its effect in birds on litter. Recycling of vaccinal infection was sufficient to induce protective immunity, even against a heterologous strain of *E. maxima*. The same work also

demonstrated that there was no interference between *Eimeria* species that might have compromised the simultaneous, independent development of immunity to all of them in the fowl, confirming the findings of Williams (1995a). The continuous low-level recycling of infections that occurs in clinically immune chickens, by maintaining a viable coccidial population in the local environment, prevents the complete eradication of parasites that would otherwise occur due to the lethal effects of the litter on oocysts (Williams, 1995b). It is frequently stated that stress (which may comprise a multitude of poorly understood factors) increases the susceptibility of chickens to coccidiosis, or alternatively may cause immunosuppression.

Although immunological responses are often depressed by stressors, there are circumstances wherein stress seems to enhance resistance to specific diseases (Siegel, 1995). For instance, Gross (1985) concluded that when chickens find them- selves in stressful increased population densities, the resultant increase in blood corticosterone levels stimulates higher primary cellular resistance and higher cell-mediated immunity sensitization to coccidiosis. Thus the chicken increases resources devoted to coccidiosis resistance as they are needed. Further pertinent work with Leghorn chickens showed that birds vaccinated with *E. tenella* were best able to resist lesion formation after homologous challenge when stress in the form of social disruption had been applied on the fourth day after vaccination (Pierson *et al.*, 1997).

2.10.1 Early Immunity for Broilers

The short life of broilers, currently often 40 to 45 days but sometimes as brief as 32 days or as long as 65 days depending on market preferences, makes early vaccination essential, in order to achieve clinical protection as soon as possible in the birds' lives. For vaccines used mostly for breeders and layer replacements, very early protection is not so important, as

these birds are reared to sexual maturity and beyond. The route of administration for the older vaccines was, therefore, usually *via* the drinking water at about one week of age and, in the case of non-attenuated vaccines, problems sometimes arose because birds were not equally exposed to infection, some remaining susceptible and others developing clinical coccidiosis (Shirley, 1988a; Ruff, 1991; Newman, 1999). The manufacturers of Coccivac® used to recommend a 2-day treatment with amprolium 10 days after vaccination to suppress any post-vaccinal reaction (Chapman and Cherry, 1997a; Chapman, 1999a, 2000a). The introduction of a hatchery-spray administration method using a Spraycox® machine has allegedly improved uniformity of vaccine application (Newman, 1999), making it possible to omit post vaccinal drug treatment (Mathis, 1999a, 1999b).

Much work has now been done to adapt vaccines to the needs of the broiler farmer by facilitating earlier and more uniform dosing. Although, in the case of the non-attenuated vaccine Coccivac-B® administered by hatchery spray, there is a transitory slowing of growth in young broilers (Mathis, 1999a; Newman, 1999), this is followed by a compensatory weight gain that reverses any performance losses by 35 to 42 days of age (Mathis, 1999a, 1999b; Mathis and Lang, 2001a, 2001b). However, the attenuated vaccine Paracox® administered in the drinking water does not produce any growth check in broilers (Williams and Gobbi, 2002). Furthermore, the precocious lines in the Paracox® and Livacox® vaccines speed up the onset and development of immunity.

2.11 The Fate of Vaccinal Oocysts

The infectivity and fecundity of oocysts are crucial to the efficacy of a live anticoccidial vaccine, since it is the oocyst stage of *Eimeria* that, following excystation, initiates the vaccinal infection, hence stimulating immunity, and during subsequent recycling of

infections, maintains it. The kinetics is fundamentally different in attenuated and non-attenuated vaccines because of their different life cycles.

2.11.1 The Fate of Vaccinal Oocysts Immediately after Ingestion of Vaccine

The results of a study of the fate of ingested oocysts during the prepatent period of infection (Williams, 1995c) have allayed concerns about the effects of the possible failure of a large proportion of oocysts to excyst in very young chicks (Doran & Farr, 1965 Rose, 1967). When one million sporulated *E. tenella* oocysts were administered to 2-days-old chicks, only 1.5% of them were recovered from faeces during 2 to 26 hours after infection. The range of recovery rates was 1.5 to 8.6% in chicks of 2 to 28 days of age, the recovery being 2.5% from the oldest birds. Since only intact oocysts and no free sporocysts were observed in the faeces, it is reasonable to conclude that between 91.4% and 98.5% of the sporulated oocysts excysted successfully, releasing their sporozoites potentially to invade epithelial cells of the caeca.

Similarly, Shiotani *et al.* (1992) were able to recover about 1.3% of a dose of 500,000 sporulated *E. tenella* or *E. maxima* oocysts from the faeces of 4-week-old chickens within 24 hours of inoculation. During the same period, a few sporozoites and sporocysts of *E. tenella* were also found in the faeces, comprising, with the oocysts recovered, a total of 17.8% of the original inoculum (measured in terms of sporozoites). Some sporocysts were found with the oocysts of *E. maxima*, together totalling only 2.8% of the inoculum recovered from the faeces. Thus, the proportions of inocula that probably established infections were 82.2% for *E. tenella* and 97.2% for *E. maxima.* The findings of Shiotani *et al.* (1992) are therefore in general agreement with those of Williams (1995c), despite the wide range of bird ages (2 days to 4 weeks) in these studies. Lotze and Leek (1969) provided further evidence for the excretion of viable parasites between 1 and 5 hours after

inoculation of 5 to 8-week-old birds, although the study was not quantitative. Goodrich (1944) examined 1 to 3 hours after infection, the gut contents of 4 to 17 days-old chicks, and discovered entire sporulated oocysts and free sporozoites as far down as the colon in one bird. The observations of Levine (1942) indicated that the faeces of an inoculated chicken may contain coccidia that can infect another chicken, but whether they were sporulated oocysts, sporocysts or sporozoites is not known. Since sporulated *E. tenella* oocysts administered to pheasants, which are innately resistant to this parasite, are still infective to chickens after being recovered undamaged from the pheasants' faeces (Williams, 1978), it is probable that vaccinal oocysts that by chance do not excyst during a first passage through a chicken also remain infective. Hence, any vaccinal oocysts failing to infect chickens immediately after vaccination will be passed out onto the litter and become available again to infect birds after only a few hours, certainly within one day. The same thing would probably occur to sporulated oocysts produced by coccidial life cycles completed later in the birds' life. Similarly to sporulated oocysts, viable sporocysts and sporozoites expelled in the faeces of one chicken would probably be able to establish infections if immediately ingested by another.

2.11.2 The Fate of Vaccinal Oocysts after Completion of the First Life Cycle

Results obtained by Williams (1995c) indicated that about 15–20% of viable unsporulated oocysts might pass undamaged through the intestine of a bird and subsequently be able to sporulate and become infective. Such a phenomenon helps to achieve a uniform vaccinal infection in the early stages of the development of protective immunity and assists recycling both of vaccinal and wild-type infections. Anything that accelerates these processes is of great value in rearing short-lived broilers.

The asynchrony of infections caused by the failure of a few sporulated oocysts to excyst, and the ingestion of some unsporulated oocysts that are again excreted to sporulate later, begins to develop immediately after vaccination. It involves both vaccinal and wild-type parasites, and establishes so-called trickle infections that have been shown to be extremely efficacious in stimulating protective immunity (Joyner and Norton, 1973, 1976).

2.11.3 Optimum Conditions for Oocysts Sporulation

It is well known that oocysts require oxygen, moisture and warmth to sporulate and thus become infective after excretion in the faeces of the host (Kheysin, 1972). With regard to oxygen requirement, Horton-Smith and Long, (1954), mentioned the possibly improved facilitation of oocyst sporulation resulting from litter disturbance. Williams *et al*,. (2000) studied flocks of broiler breeders reared at densities of 8 or 22 chicks per m2. The litter of the low density flock was turned daily by hand, but the litter of the high density flock was left untouched. Despite this, the high density flock still produced far more oocysts per gramme of litter than the other, so the effect of manual litter disturbance in the low density flock was not sufficient to outweigh the effect of a higher chick density.

It is frequently stated that sporulation is directly correlated with the moisture content of litter. Thus, it has sometimes been recommended that, when using vaccines in dry conditions, litter should be watered to stimulate sporulation (e.g., Christensen, 1999). However, as Chapman (2000a) pointed out, this practice may cause clinical coccidiosis or other health problems. On the other hand, it should also be remembered that if chicks are brooded under gas-heated canopies, some excreted oocysts might be killed by desiccation due to excessively high temperatures.

In a laboratory experiment (Williams, 1995b), *E. acervulina* oocysts achieved about 60% sporulation within one day and 100% sporulation within 3 days in litter with moisture content of about 31.0%.

2.12 Patterns of Oocyst Accumulation in the Litter

2.12.1 Accumulation of Oocysts in Vaccinated and Drug-Treated Chickens

The results of many floor pen and field trials have established a fairly predictable pattern of oocyst accumulation in the litter after vaccination of chicks with attenuated parasites. Hence, in broiler, broiler breeder and layer replacement birds, following vaccination with Paracox® *via* the drinking water at about one week of age (Williams, 1998a; Williams *et al.*, 1999), low numbers of attenuated vaccinal oocysts may be observed multiplying up with a small peak of production at 2–4 weeks, during which time protective immunity is developed as the infections recycle. At between 4 and 7 weeks, a slightly higher peak may occur, representing a challenge from the local virulent coccidial population, which while recycling further boosts the protective immunity built up between 1 and 4 weeks. Finally, after about 7 or 8 weeks, oocyst production is usually considerably reduced by flock immunity, and eventually, virtually no oocysts are detectable in the litter, although small numbers no doubt remain. Many of these are killed by adverse conditions in the litter (Williams, 1995b) and most of the remainder is removed when the litter is cleared from the house.

In comparism, flocks with an anticoccidial agent in their diet tend to produce a single major peak of oocysts between 4 and 8 weeks usually (but not always) higher than either of the two smaller peaks seen in vaccinated flocks (Williams, 1998a; Williams *et al.*, 1999). The height of this peak, and whether or not it is associated with clinical coccidiosis, depends on the species of coccidia present and their degrees of sensitivity to the drug in use. In un-

medicated, unvaccinated flocks a similar single peak occurs about 9 days earlier than in medicated unvaccinated flocks (Williams *et al.*, 1996). This latter pattern is apparently similar to that reported after vaccination with a non-attenuated vaccine (Huchzermeyer, 1976).

A study carried out in floor pens isolated from extraneous coccidial infection established that the first peak in the litter oocyst counts after vaccination with Paracox® comprises the progeny of vaccinal oocysts (Williams, 1994). The second peak, by inference, results mainly from a wild-type challenge, which is rapidly brought under control by the birds' immune responses (Williams *et al.,* 1999). This peak coincides temporally with the late single peak normally seen in birds reared with anticoccidial drugs in their feed (Long and Rowell, 1975; Long *et al.*, 1975; Shirley, 1988b; Shirley *et al.*, 1995; Williams *et al.*, 1996, 1999).

2.12.2 Differences in Oocysts Accumulation in Coccidiosis Vaccines

Studies involving vaccination of day-old broiler chicks with Paracox®-5 sprayed on the feed revealed a litter oocyst pattern different from that seen after vaccination with Paracox® via drinking water at one week of age (R.B. Williams in Chapman *et al*., 2002). In the field, where local wild-type parasites are present, the pattern for Paracox®-5 vaccinated chicks showed the onset of vaccinal oocyst production at about 7 days, rising to a peak at 28 days and then falling. The medicated controls showed a later initiated but faster rise, finally peaking higher than the vaccinated birds at 28 days, and also finishing higher. The shortened prepatent period of the precocious vaccinal coccidia and the vaccination of day-old chicks are clearly of benefit in the early initiation of immunity in short-lived broilers. Although vaccination at day-old produces vaccinal oocysts one week earlier than vaccination at 7 days old, the first wild type oocysts still do not start to appear until birds are about 14 days old.

The gradual post-vaccinal oocyst accumulation in day-old vaccinated chicks, with no obvious early peak, may result partly from their poorer capability than week-old vaccinated birds to produce oocysts (Krassner, 1963; Doran and Farr, 1965; Rose, 1967).

2.12.3 Competition between Vaccinal and Wild-Type Coccidia

Competition between the development of vaccinal and wild-type parasites is an important factor. The magnitude of wild-type populations might be expected to be correlated with the use of new litter, as with most European broilers, or re-used litter, as in the USA and Latin America. Built up litter are rarely used in Nigeria. However, when sentinel birds were used to monitor the potential coccidial challenge in commercial broiler houses, they revealed no consistent difference between oocyst concentrations in new and re-used litter, and no correlation between the numbers of oocysts in the litter and severity of infections in the sentinel birds (Long and Johnson, 1981). This may reflect the relative insensitivity of the oocyst counting technique or the fact that oocyst counts do not indicate parasite viability (Long and Johnson, 1981). The latter possibility is in accord with the results of Williams (1995b), who found that many oocysts left in litter die if not ingested by chickens. Nevertheless, when houses are cleaned out between crops, the speed and magnitude of accumulation of oocysts in new litter is influenced considerably by the degree of contamination with oocysts from the previous flock, as demonstrated by the consequences of failure to clean out commercial broiler houses adequately (R.B. Williams in Chapman *et al.*, 2002). It seems that even if the vaccinal parasites are precocious, excessive numbers of wild-type oocysts remaining after an inadequate clean-out may be capable of causing disease before birds can acquire fully protective immunity but this is a rare occurrence.

Perhaps oocysts washed out of litter can survive the period between occupancies of a house by chickens if they end up in cracks in wood or concrete where the microclimate facilitates

their survival. If large numbers of residual wild-type oocysts remaining after clean-out are known to be a persistent problem on a farm, special measures may be required before introducing an anticoccidial vaccine. The turn-around time between flocks must allow efficient physical cleaning, and the use of an oocysticide that generates ammonia may be worthwhile (Sainsbury, 1988).

2.12.4 Significance of Coccidial Lesions in Vaccinated Chickens

Whilst coccidial lesions resulting from a primary virulent infection in susceptible chickens may signal severe disease, lesions in immune birds are often of little consequence. Mild lesions, scored 1 or 2 on the scale of Johnson and Reid (1970), are sometimes seen in chickens vaccinated with Paracox ® under commercial conditions when the flock is showing no signs of ill health.

Until quite recently, it was not known whether such lesions seen in Paracox®-vaccinated chickens (e.g., Bushell *et al.*, 1990; Bushell, 1992) were due to a wild-type challenge or to the vaccine *per se*. However, a floor pen trial carried out in broilers isolated from extraneous coccidial infections (Williams, 1994) showed that mild lesions occurred between 26 and 40 days after vaccination with Paracox® in 4% of the 150 chickens sampled during that period. Furthermore, a few blood-tinged droppings were also seen, but no adverse effects onthe health and performance of the birds were demonstrated.

Williams and Andrews, 2001 showed that when a group of healthy broilers, isolated from extraneous coccidial infections, was monitored daily, Paracox® vaccine-induced lesions occurred randomly from 5 days to at least 23 days after vaccination; 24% of the 87 chickens sampled during 29 days had lesions, mostly (19 out of 21) scored 1 or 2 according to Johnson & Reid (1970). These results are at variance with the view of Chapman (2000a) that attenuated vaccines should not give rise to lesions. However, it was clear from smooth

growth curve of the sampled birds (Williams and Andrews, 2001) that such lesions had no deleterious effect. It has been shown that lesions may occur in fully protected Paracox® vaccinated birds in response to a subsequent virulent coccidial challenge without any associated adverse effects on performance (Williams, 1997b).

Broadly similar observations have been made in the case of non-attenuated vaccines. For instance, Newman, (1999) has published data pertaining to Coccivac®-B that suggest coccidial lesions may appear in up to 35% of chicks between 14 and 28 days after vaccination by hatchery spray, but it is not stated whether the lesions result from vaccinal or wild infections. Furthermore, Schetters *et al.,* (1999) and Vermeulen *et al.,* (2000b) have observed the occurrence of mild transient coccidial lesions in chicks up to 21 days after vaccination with Nobilis® COX ATM. When vaccinated birds were challenged with heterologous parasites, mild lesions occurred, although the birds remained healthy and were protected against clinical coccidiosis (Schetters *et al.*, 1999).

It was observed by Williams and Andrews (2001) that normal-looking coccidial lesions in healthy, vaccinated chicks frequently had very few or no endogenous parasites associated with them. This suggests that, unlike in fully susceptible birds in which lesions normally signal disease, lesions in immune birds indicate a successful repulsion of a parasite challenge by the host. This is in accord with the observation of Jeffers and Long (1985) that the presence of lesions *per se* may not indicate failure of an attempt at immunization.

2.12.5 Restoration of the Drug Sensitivity of *Eimeria* species

Jeffers, (1976a), suggested that the introduction of massive numbers of drug sensitive, attenuated coccidia onto farms where drug resistant strains dominate would be a useful adjunct to planned immunization. Ball, (1966), had already demonstrated that when a small number of drug-resistant oocysts were included in a larger inoculum of sensitive *E. tenella*

oocysts, the resistant individual apparently diminished in numbers during passages through unmedicated chicks. Long *et al.,* (1985), later suggested that in a population of approximately equal numbers of drug sensitive and drug resistant *E. tenella*, the sensitive organisms dominate in the absence of medication.

It has been reported that if the non-attenuated vaccine Coccivac®-B is used on broiler farms where drug resistance has been a problem, sensitivity of the local coccidial population may be substantially restored after vaccination of between one and five flocks (Chapman, 1994, 1996a). The drug sensitive parasites in the vaccine may replace the resistant parasites in the farm population (Chapman, 2000a). Based upon this work, rotation programmes of vaccines and drugs have been reported by Chapman (2000a, 2000b) and Chapman H.D. in Chapman *et al.,* (2002).

Although the attenuated parasites in Paracox® vaccine are precocious, and therefore have greatly reduced reproductive potentials (Williams, 1992a), it has been reported to ameliorate the drug resistance of wild-type coccidial populations after the field use of this vaccine (Vertommen, 1996). This effect has also been demonstrated during field use of the attenuated vaccine Livacox® (Oliveira, 2001). Following broiler trials on built-up litter in Brazil, it was concluded that the accumulation of vaccinal oocysts in the litter gave rise to improved results after at least two consecutive Anticoccidial vaccines for broilers flocks of birds had been vaccinated. However, when birds were placed on new litter, the best results were achieved earlier (Oliveira, 2001). Ern´õ k & Bedrn´õ k (2001) recommended the rotation of anticoccidial vaccines and drugs every three crops, based on work with Livacox® vaccine in the Czech Republic. Rather than simply replace resident populations of coccidia, it seems more likely that vaccinal parasites would interbreed with them. It would surely be impossible to maintain separate strains of a species in a field population when

oocysts of different genetic constitutions randomly initiate sexual life cycles in the same bird, with the inevitable interchange of genetically stable traits.

Williams (1992a, 1998a, 1999b) suggested that not only might drug-sensitive vaccines ameliorate drug resistance, but if they comprised attenuated precocious lines they might also reduce the virulence of the field population. This seems feasible because, just as drug resistance is genetically controlled (Shirley and Jeffers, 1990) and is persistent for long periods (Williams, 1969; Chapman, 1986), the traits of precocious lines are also genetically stable (McDonald *et al.*, 1986a; Shirley, 1988a) and demonstrably recombine with those of drug resistance (Jeffers, 1976b; Sutton *et al.*, 1986; Shimura and Isobe, 1994; Shirley and Harvey, 1996).

Shimura and Isobe (1994) showed that the attenuation of a precocious line and the drug resistance of a field strain of *E. tenella* were expressed in a recombinant line after interbreeding. Moreover, they also demonstrated that although the recombinant line still expressed sulphonamide-resistance, this would not adversely affect the performance of sulphonamide-treated chickens, because the recombinant parasites were innocuous. Richards and Woods (2001) have used PCR techniques to show the concomitant cycling of vaccinal and wild-type parasites in vaccinated chickens, but they did not state whether genetic recombinations were also recognized.

2.12.6 Concomitant Use of Anticoccidial Drugs and Vaccines

Despite the introduction of Coccivac® in 1952, the majority of coccidiosis control programmes were still based upon anticoccidial drugs incorporated in the feed (Reid, 1960). However, during the early use of Coccivac ®, it was discovered that the vaccine could immunize chickens while they were being given various drugs prophylactically in their feed

(Edgar, 1958a, 1958b; Stuart *et al.*, 1963). During the early 1960s, Coccivac® was marketed with the drug Trithiadol® as a planned immunization programme.

Delayed treatment has been reported to enhance the development of immunity by *Eimeria* species in the presence of anticoccidial drugs. Seeger (1947) and Dickinson *et al.* (1959) recommended the use of a sulphonamide in the feed, initiated 24–48 hours after coccidial infection, whilst Grumbles *et al.* (1948) delayed the introduction of treatment for two weeks. Eventually, the older drugs with rather low potency and a cidal mode of action were superseded by extremely potent ones such as clopidol and decoquinate, with quite different biochemical modes of action and anticoccidial effects (Wang, 1975, 1976; Fry and Williams, 1984; Williams, 1997c). There was then no possibility of Coccivac® being used along-side prophylactic drugs in broilers, because the coccidia in it were initially highly sensitive to those potent new drugs and did not allow birds to develop immunity (Brewer and Reid, 1967; Raines, 1968).

Several types of potent drugs launched during the 1960s tended to develop drug resistance fairly quickly (Ryley, 1980; McDougald, 1982; Williams, 1998b, 1998c), and by the early 1970s had themselves become superseded by the first ionophorous drugs (McDougald, 1993). Hence, oocyst accumulation in commercial poultry-house litter once more began to rise to the levels encountered in the presence of the older drugs.

The basis of efficacy of the ionophores was thus realised to be similar in practice to concomitant vaccination and chemotherapy demonstrated in the early days of Coccivac® use (Edgar, 1958a, 1958b, 1964; Stuart *et al.*, 1963). The capability of coccidia to multiply in the presence of anticoccidial drugs may be explained in one of three ways. First, some prophylactic drugs have inherently poor efficacy even when the parasites have not been previously exposed to them. Thus, they may not be able to suppress totally the completion

of coccidial life cycles and ultimate oocyst production, despite being protective against clinical disease. Second, the multiplication of the coccidia may have resulted from acquired drug resistance. Third, coccidia may develop during intermittent medication, when the relative timing of exposure of the host to drug-sensitive parasites and ingestion of drug allows the coccidia to avoid the drug's effect.

It has long been known that interrupted or delayed treatment with sulphonamides of birds suffering acute coccidiosis will provide therapeutic protection whilst allowing immunity to develop in the survivors (Davies and Kendall, 1954; Davies, 1958; White and Williams, 1983; Haberkorn, 1986; C.A. Johnson *et al.*, 1986; Mundt and Haberkorn, 1989; Duan and Liu, 1994). This kind of strategy depends upon the chosen drug having activity against intracellular parasites. However, it is known that ionophores are active against the coccidial stages that are found in the gut lumen, i.e., sporozoites (Itagaki *et al.*, 1974; Smith and Strout, 1979) and merozoites (Long and Jeffers, 1982), but probably not against intracellular parasites (Chapman, 1993). Therefore, if chicks are inoculated with coccidia a few hours before an ionophore is administered, the sporozoites are able to penetrate intestinal cells rapidly enough (Doran, 1966) to avoid the action of the drug. This phenomenon has been used to advantage by Bafundo (1989) and Bafundo and Jeffers (1988, 1990) to immunize chicks in the presence of an ionophore. Similarly, Edgar and Fitz-Coy (1985) successfully immunised broilers with CocciVac-D during in-feed medication with monensin commenced 11 days after vaccination. Such relative timing is not critical, of course, if parasites are ionophore resistant, in which case such coccidia may be used to immunize ionophore medicated birds (Danforth, 1998).

Ionophore resistant vaccine is used to immunize broilers whilst simultaneously allowing the use of an ionophore in the feed to control necrotic enteritis (Schetters *et al.*, 1999;

Vermeulen *et al.*, 2000a, 2000b). Such a vaccine would be expected to produce an effect similar in principle to the traditional prophylactic use of ionophores in broilers.

2.13 Anticoccidial Vaccines of Chickens and Clostridial Diseases

The relationship between clostridial diseases and coccidiosis is poorly understood but it is known that usage of antibiotic growth promoters (AGPs), which include ionophorous anticoccidial drugs, offer some control of *Clostridium* infections. The use of an anticoccidial vaccine often precludes the administration of anticoccidial drugs in broiler feed and some believe that using an anticoccidial vaccine would increase the risk of necrotic enteritis (NE) in broilers. Others believe that anticoccidial vaccination may predispose birds to NE.

According to K¨ohler (2000), clostridial diseases encountered in broiler chickens include NE, ulcerative enteritis, necrotic dermatitis and botulism; and to these may be added necrotic hepatitis and cholangiohepatitis. NE is caused by toxin produced by *Clostridium perfringens* type A (K¨ohler, 2000) and is a serious problem in broilers world-wide (Van der Sluis, 2000). The major source of infection has been stated to be *C. perfringens* spores in poultry feed (K¨ohler, 2000), although Shane *et al.* (1984) obtained results suggesting that vertical relationships between NE, chicken diets, coccidiosis and anticoccidial vaccines have been discussed by Williams (1999b), who concluded that no clear understanding was yet attainable. Williams (1999b) and Kaldhusdal (2000) both recommended further work on dietary ingredients to help elucidate the epizootiology and control of NE.

Traditional AGPs such as virginiamycin, zinc bacitracin, avoparcin, avilamycin, etc. apparently may give some help in controlling clostridial diseases (Elwinger *et al.*, 1998).

It has been reported that necrotic enteritis can still occur in commercial birds even when drug shuttles including an ionophore are used (Williams *et al.*, 1999; Ern´õ k and Bedrn´õ k,

2001). Williams *et al.*, (1999) found that, in large-scale commercial broiler trials in the UK, NE occurred in two out of nine trials in flocks medicated with either salinomycin or monensin in addition to the AGP virginiamycin; possibly drug resistant strains of *C. perfringens* were implicated. The precise nature of the possible association between coccidia and NE is uncertain (Williams, 1999b). It is not yet known whether coccidial infection *per se* or only severe clinical coccidiosis can damage the intestine sufficiently to allow the establishment of a clostridial infection capable of causing NE. If the latter is true, an attenuated anticoccidial vaccine or a mild non-attenuated coccidial infection causing minimal intestinal damage would not initiate NE. Indeed, this might suggest that an anticoccidial vaccine, far from causing NE, may actually protect chickens against it by preventing predisposing clinical coccidiosis.

Various cereals included in poultry diets are known to have different effects on the susceptibility of birds to diseases. For instance, wheat based diets exacerbate both NE (Branton *et al.*, 1987; Riddell and Kong, 1992) and coccidiosis (Williams, 1992b) when compared with maize based diets. Interestingly, in the commercial trials reported by Williams *et al.* (1999), birds fed a wheat-based diet plus whole wheat grains suffered outbreaks of coccidiosis and NE, despite the inclusion of an ionophore and virginiamycin in the feed. Conversely, during large scale broiler trials with Paracox® vaccine in Italy, when the cereal base of the diets was maize and all feeds contained the AGP avilamycin, no NE or coccidiosis was observed in vaccinated or anticoccidial drug-treated birds (Williams and Gobbi, 2002).

2.14 Probiotic

Probiotic are live microorganisms that, when administered through the digestive route, are favorable to the host's health (Guillot, 1998). The microorganisms used in animal feed are

mainly bacterial strains of Gram-positive bacteria belonging to the types *Lactobacillus*, *Enterococcus, Pediococcus* and Bacillus. Some other probiotics are microscopic fungi such as strains of yeasts belonging to the Saccharomyces cerevisiae species (Guillot, 1998, Thomke and Elwinger, 1998).

2.15 Cytokines

Cytokines are major modulators of immune responses to infection, and therefore they represent natural sources of immunostimulation that could be used as adjuvants in vaccines. IFN-γ has received the most attention as a possible adjuvant for an anticoccidial vaccine. Chicken IFN-γ has been cloned (Digby M.R. and Lowenthal J. W. 1995; Lowenthal *et al*., 1995; Song et al 1997). Treatment with recombinant IFN-γ alone has been shown to moderate reductions in weight gain (Lillehoj H.S. and Choi K.D. 1998; Lowenthal *et al.*, 1999) during *E. acervulina* and *E. tenella* infections and to moderate oocyst output (Lillehoj H.S. and Choi K.D. 1998; Lowenthal *et al.*, 1999) during *E. acervulina* infection. One problem with the in vivo use of cytokines is their rapid degradation and clearance (Lowenthal *et al*.,). Administering them in DNA form or in a viral or bacterial vector could overcome this problem and provide a more practical method for treating large flocks.

2.16 Natural Products Feed Additives

Many different types of substances have been investigated in the search for alternative controls of coccidiosis. A herbal mixture of *Vernonia amygdalina* and *Azadirachta indica* has been reported to be effective in reducing the faecal oocyst shed by 4 week old broilers that were naturally infected (Oyagbemi T.O. and Adejinmi J.O. 2012). Peptidyl membrane-interactive molecules are known to have antibacterial activities. Three of seven tested produced ultrastructural damage to sporozoite pellicles after a 5 to 10 minutes exposure to 5 μM concentrations in vitro. Oral doses (10, 50, or 75 μM) of two of them, which were

methylated to prevent proteolysis, reduced lesion scores from challenge infections with *E. acervulina*, E. *tenella*, and *E. maxima*, suggesting that they have potential for coccidiosis control. Artemisinin is a Chinese herb isolated from Artemisia annua; it is a naturally occurring endoperoxide with antimalarial properties. It has been found effective in reducing oocyst output from both *E. acervulina* and *E. tenella* infections when fed at levels of 8.5 and 17 ppm in starter diets. The mode of action is also thought to involve oxidative stress.

2.17 Comparism between Prophylaxis and Treatment of Avian *Eimeria* Infection

Acute infection of coccidia is generally treated with anticoccidials, but the economic losses are mainly because of subacute infection. Prophylactic use is preferred because most of the damage occurs before signs become apparent, and delayed treatment may not benefit the entire flock. Only a few of the prophylactic drugs are also effective therapeutically. Usually, coccidiosis is controlled by prophylactic in-feed medication of an anticoccidial drug on continuous basis in broilers and up to 16 to 18 weeks in layers. Water medication is generally preferred over feed medication for treatment. Antibiotics and increased levels of vitamins A and K are sometimes used in the ration to improve rate of recovery and prevent secondary infections.

2.18 Drug Resistance to Anticoccidials

Continuous use of anticoccidial drugs may result in selection for and survival of drug-resistant strains of coccidia. Drug resistance is a genetic phenomenon, and once established in a line of coccidian, remains for many years. To overcome this problem, shuttle programme and frequent rotation of drugs may be practised. This is because the new drug will be effective against the build up of coccidia that had reduced sensitivity against the previous product that had been used for a long time. The development of resistance to ionophores has been very slow and an explanation for this is the fact that they allow for

some leakage of sensitive oocysts. This leads to a less stringent resistance selection than with chemicals. The mode of action of the different ionophores is similar: they facilitate cation transport across the parasitic cell membrane. This causes ionic gradient and content modifications (Gumila *et al*., 1996) with parasite cell death as a final consequence. The ability for acquiring resistance to one drug by the use of another drug, the so-called cross resistance (Chapman, 2007) is an ongoing area of debate among coccidiologists. Cross resistance is said to be less obvious between products of different classes, for instance between maduramicin and monovalent ionophores.

2.19 Drug Sensitivity Testing

Anticoccidial sensitivity testing (AST) is a well-known technique to try to assess resistance of a certain coccidial isolate to different anticoccidial drugs (McDougald, 1987; Chapman, 1998; Naciri *et al*., 2003; Peek and Landman, 2003). Although a valid method for a certain isolate, this technique is not routinely used. The main reasons are the long duration and very high cost associated with the complicated, *in vivo* character of the test. The short period of testing without allowing the initially naive birds to recover from an artificially high infective dose makes interpretation of the results not easy.

2.20 Diagnosis of Coccidial Infection

2.20.1 Morphometric Methods

This is a very innovative technique of computational approach to parasite diagnosis (Gruber *et al.,* 2007). Images from sporulated oocysts from confirmed species were assessed on different features: curvature characterisation, size and symmetry and internal structure characterisation. Users can upload their digital images from unidentified oocysts and have

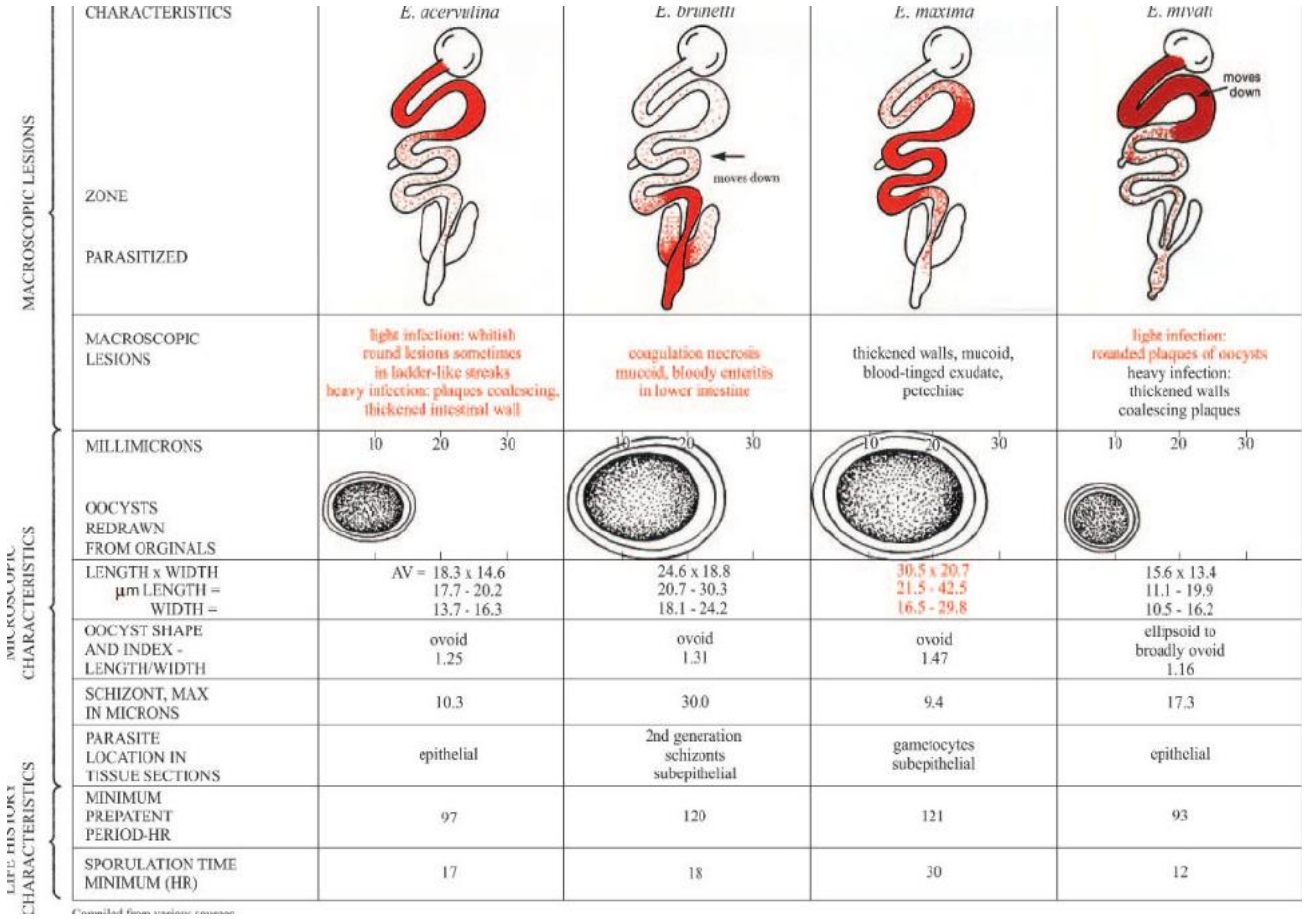
the program identify the species concerned. This is very accessible and the low cost is a major advantage.

2.20.2 Oocyst Faecal Count

Oocyst counting and morphometric identification by McMaster chamber under microscope fitted with graticule eye piece is one of the traditional age long parasitological methods of diagnosis. It is labour intensive, costly and can take several days. The oocyst per gram (OPG) of faeces derived from the McMaster count has a poor relation with the impact of the parasite on the performance of a flock (Haug *et al.,* 2008, Degussen *et al*., 2007, William *et al.,* 1996). Identification of different species based on morphology of oocysts is very challenging, lacks precision and requires expertise.

2.20.3 Lesion Scoring

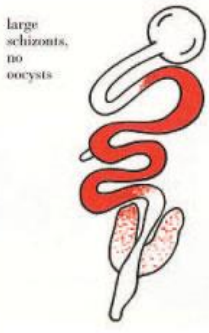
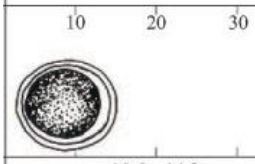
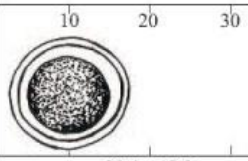
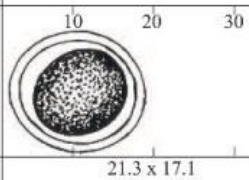
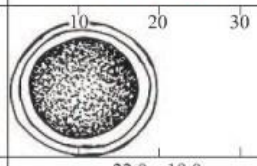
Lesion scoring is an interpretation based on macroscopic visible lesions caused by *Eimeria*, usually following a scoring system from zero to four (Johnson and Reid, 1970). The individual scores for all the species are usually compiled for a certain number of birds per flock resulting in a Total Mean Lesion Score (TMLS). The method is extremely labor intensive, sometimes subjective and only reliable when performed by skilled people. The correlation between lesion scores and performance is believed to be stronger than with OPG but still there is a difficult appreciation of the level of lesions towards impact on performance, especially at subclinical levels. A limitation is for instance the fact that *E. mitis*, although quite pathogenic, does not cause typical lesions and is mostly disregarded when using this method. Although lesion scoring remains the most frequently applied diagnostic method today it might not detect all the economically important coccidiosis infections if used alone (*Idris et al.,* 1997) .



Source: Poultry coccidiosis: Diagnostic and testing procedures. 3rd. edition. Conway D.P and McKenzie M. E (2007)

Figure 2.1 Morphometric characteristics of some *Eimeria* oocysts and macroscopic features of area of intestine parasitised.

DIAGNOSTIC CHARACTERISTICS IN RED

E. mitis	*E. necatrix*	*E. praecox*	*E. tenella*
			
no discrete lesions in intestine mucoid exudate	ballooning, white spots (schizonts), petechiae, mucoid blood - filled exudate	no lesions, mucoid exudate	onset: hemorrhage into lumen later: thickening, whitish mucosa, cores clotted blood
			
15.6 x 14.2 11.7 - 18.7 11.0 - 18.0	20.4 x 17.2 13.2 - 22.7 11.3 - 18.3	21.3 x 17.1 19.8 - 24.7 15.7 - 19.8	22.0 x 19.0 19.5 - 26.0 16.5 - 22.8
subspherical 1.09	oblong ovoid 1.19	ovoid 1.24	ovoid 1.16
15.1	65.9	20	54.0
epithelial	2nd generation schizonts subepithelial	epithelial	2nd generation schizonts subepithelial
93	138	83	115
15	18	12	18

Modified after Long and Reid (1982).

Source: Poultry coccidiosis: Diagnostic and testing procedures 3rd edition, Conway D.P and McKenzie M. E (2007)

Figure 2.2 Morphometric characteristics of some *Eimeria* oocysts and macroscopic features of area of intestine parasitised.

2.20.4 Molecular Methods

2.20.4.1 Polymerase Chain Reaction (PCR)

Central to the PCR-based identification of *Eimeria* species is the choice of the appropriate DNA target region (genetic marker or locus). For specific identification, the target DNA should differ enough in sequence to allow the differentiation of species, but display no or minor variation within a species. By contrast, for the purpose of identifying ‘‘strains’’ or population variants, a considerable degree of variation in the sequence should exist within a species. Various target regions, including nuclear ribosomal DNA (rDNA), mitochondrial DNA (mtDNA) and repetitive DNA elements, have been employed to achieve the identification of parasites to species or strains (Anderson *et al*., 1998; Gasser, 1999; Blouin, 2002; Gasser *et al*., 2002, 2004b; Hu et al., 2004). Studies have consistently shown that the first internal transcribed spacer (ITS-1) and second (ITS-2) internal transcribed spacer of nuclear ribosomal DNA yield reliable genetic markers for the identification of *Eimeria* species.

2.20.4.2 Real-time Quantitative Polymerase Chain Reaction Technique

This technique was developed in the early 1990s (Higuchi *et al*., 1992) and allows the amplification in the PCR to be monitored in real time. Real-time PCR systems detect the amplification using fluorescent chemistries. The main advantages of Real-time PCR over conventional PCR are;

(a) It does not require post amplification processing.

(b) It can be employed for quantitation over a wide range.

(c) It can be used to differentiate amplicons of varying sequence(s) by melting curve analysis.

The principle of the original method was to incorporate a specific, intercalating dye (such as ethidium bromide) into the PCR to measure the change in fluorescence after each cycle using a digital camera and a fluorometer coupled to the reaction tube (Higuchi *et al*., 1993). The technique has been modified to include other dyes, such as SYBR Green I (Becker *et al.*, 1996), into the reaction and analysis using a capillary thermal cycler/fluorometer, which eliminates the need for electrophoresis of amplicons and enables relative or absolute quantitation by allowing the identification of the cycle (Ct) at which the amplification commences. The more sensitive and reproducible method of realtime QPCR measures the fluorescence at each cycle as the amplification progresses. This allows quantification of the template to be based on the fluorescent signal during the exponential phase of amplification, before limiting reagents, accumulation of inhibitors, or inactivation of the polymerase have started to have an effect on the efficiency of amplification. The first cycle at which the instrument can distinguish the amplification generated fluorescence as being above the ambient background signal is called the "Ct" or threshold cycle. This Ct value can be directly correlated to the starting target concentration for the sample. The greater the amount of initial DNA template in the sample, the earlier the Ct value for that sample DNA standards (of a defined concentration) and test samples are subjected to cycling at the same time and their Ct values established and compared. A standard curve can be constructed from the data, and the relative amounts of template in test samples are calculated in relation to the standard curve.

2.20.4.3 Miscellaneous Molecular and Biochemical Methods

There are other biochemical and molecular methods of *Eimeria* species identification. These include Multilocus and Isoenzyme electrophoresis, Electrophoretic protein characterisation, Southern blot analysis, Pulsed-field gel electrophoresis and several RNA and DNA analyses. Although these techniques are a major addition for scientific research none has been adapted for practical application and still have to be regarded as experimental. The main application of these techniques for field diagnosticians today is the possibility of defining presence of species currently disregarded such as *E. praecox* and *E. mitis* (Morris and Gasser, 2006).

2.20.4.4 Loop-Mediated Isothermal Amplification (LAMP) Assays

Loop-mediated isothermal amplification (LAMP) is a relatively simple technique which facilitates rapid DNA amplification with a high level of sensitivity (He *et al*., 2009). Importantly, LAMP utilises the enzyme *Bst* DNA polymerase, which is active under isothermal conditions at a relatively high temperature and supports cost effective, rapid, target-specific amplification (Wastling *et al*., 2010). LAMP is based upon an autocycling strand-displacement reaction using a set of four oligonucleotides which recognise six DNA sequences within the target genomic region and form a loop-structured amplicon. Additional loop primers may be added to improve amplification (Nagamine *et al*., 2002). The efficiency and high yield of a LAMP reaction supports the use of intercalating dyes such as SYBR Green or hydroxynaphthol blue, enabling identification of a positive reaction with the naked eye (Goto *et al.*, 2009).

2.21 Immune Response to Coccidial Infection

The life cycle of *Eimeria* parasites is complex and comprised of intracellular, extracellular, asexual, and sexual stages. The host immune responses are also quite diverse and complex. After invasion of the host intestine, *Eimeria* elicit both nonspecific and specific immune responses which involve many facets of cellular and humoral immunity (Lillehoj, 1991; Lillehoj 1998; Lillehoj and Lillehoj, 2000; Dalloul and Lillehoj, 2005)

2.21.1 Non Specific Factors

These include physical barriers, phagocytes, leukocytes, chemokines and complement components. Antigen-specific immunity is mediated by antibodies, lymphocytes, and cytokines.

Due to the specific invasion and intracellular development of coccidia in the intestine, the gut-associated lymphoid tissue (GALT) plays an important role in the development of immunity. The GALT serve three main functions in host defense against enteric pathogens: processing and presentation of antigens, production of intestinal antibodies, and activation of cell-mediated immunity (CMI). In the naïve host, coccidia activate local dendritic cells and macrophages eliciting various chemokines and cytokines (Lillehoj, 1998). In immune hosts, parasites enter the gut early after infection, but are prevented from further development, indicating that acquired immunity to coccidiosis may involve mechanisms that inhibit the natural progression of parasite development (Rose *et al.,* 1984; Trout and Lillehoj, 1996; Lillehoj and Choi, 1998; Yun *et al.,* 2000a).

2.21.2 Antibody Responses

Following coccidiosis, both circulating and secretory antibodies specific for coccidian parasites are detected in serum, bile and intestine (Lillehoj and Ruff, 1987; Lillehoj, 1988; Yun *et al*., 2000c). However, antibody titers in serum and intestine do not correlate with the

level of protection after oral infection with coccidia (Dalloul *et al.*, 20003; Lillehoj and Ruff, 1987)

2.21.3 Cell Mediated Immunity

The evidence that the removal of the bursa by chemical or hormonal means (Rose and Long, 1970; Lillehoj, 1987) did not interfere with the development of protective immunity against *Eimeria* indicated the importance of cell-mediated immunity in coccidiosis. The role of T cells in the protection against coccidiosis has also been studied in immunosuppressed chickens using T cell-specific drugs that selectively abrogate or severely impair T cell function. The impairement has negative effect on the host protective immunity against coccidiosis (Trout and Lillehoj, 1996). Direct evidence for the presence of *Eimeria*-specific T cells was demonstrated by an *in vitro* antigen-driven lymphoproliferation assay (Rose and Hesketh, 1984; Lillehoj, 1986; Vervelde *et al*., 1996).

2.21.4 Natural Killer Cells

The role of Natural Killer-cells in parasitic diseases has been well documented (Lillehoj *et al.*, 2004). The NK cell activity was higher in the jejunum and ileum than in the duodenum and cecum. Following infection with *Eimeria* parasites, the NK cell activities of both splenic and intestinal IEL decreased to a subnormal level during the early phase of infection (Lillehoj, 1989). NK cell activity returned to normal or slightly higher than normal levels about one week after the primary inoculation.

CHAPTER THREE

SURVEY OF FARMERS' KNOWLEDGE OF *EIMERIA* INFECTIONS OF COMMERCIAL CHICKENS AND THEIR ADOPTION OF VACCINE PROPHYLACTIC MEASURES IN CHICKEN FARMS IN OYO AND OGUN STATES

3.1 Introduction

The poultry industry in Nigeria has recorded considerable expansion in recent times (FAO, 2000). Poultry diseases remain one of the major threats to boosting poultry production in Nigeria (Halle *et al* 1998, Laseinde, 2002). Parasitic diseases are of particular importance because of their high incidence in poultry occasioned by the tropical environmental conditions under which the farmers operate (Seifert, 1996). Epizootiological studies have established the importance of coccidial infection as a major parasitic disease in Nigeria based on the economic implications of its outbreak in poultry farms (Majaro, 1980); Barksh, 1989.

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 (Majaro, 1980, 1981). Advances in poultry husbandry, nutrition and chemotherapy have made clinical outbreaks of coccidiosis rather infrequent but subclinical coccidiosis continues to be one of the poultry industry's most common and expensive diseases worldwide (McDougald, 2003). The broiler industry in particular relies on continuous in-feed prophylaxis with application of anticoccidial drugs.

There is currently, a growing global concern about the twin issues of drug resistance and drug residues so much so that the EU has proposed a phasing out of drug use in coccidiosis

prevention by 31st December 2012 (EU Commission 2003). With the increasing interest in poultry production evidenced by the proliferation of poultry farms, it is pertinent to continually evaluate the prevalence and management issues associated with coccidiosis in commercial chicken farms.

3.2 Objectives

1. To collect data from Chicken farmers in Oyo and Ogun states via questionnaire on the factors associated with good knowledge of coccidiosis and their adoption of coccidiosis vaccines prophylactic method.

3.3 Justification

Coccidiosis still remains an enzootic disease of major economic importance to the poultry industry in Nigeria and worldwide. More research is needed to elucidate the various factors and events that have contributed to the sustenance of the disease in the poultry population in Oyo and Ogun States

3.4 Materials and Methods

3.4.1 Sampling Procedure

One hundred and sixty six poultry farmers were selected through random sampling of poultry farms in Oyo and Ogun States. Eighty two and eighty four farmers were sampled in Oyo and Ogun States respectively based on the list of poultry farmers registered with Poultry Association of Nigeria in the respective States. Willingness to participate in the survey and condition of confidentiality were among the criteria for the selection. The respondents included farm owners, farm managers and technical staff (including Veterinary doctors, animal scientists and farm supervisors).

3.4.2 Questionnaire Interview

The study was conducted using semi structured questionnaire (Appendix 1). The questionnaire was subjected to a validation test before data collection commenced. The questionnaire has both closed and open-ended questions. The questionnaire was designed to collect data in respect of the Poultry Owners' personal information, farm management experience and practice as well as knowledge of coccidiosis in commercial chickens. In the first section, the data collected were related to Age, Educational status, Religion and Marital status of the respondents.

The second section comprises of data in respect of the age of the farm, poultry breeds kept, different ages of flocks, poultry population, housing, feed, water and litter management as well as bio-security. The third and final section was to assess the awareness levels and knowledge of the farmers in respect of coccidial infections and the prophylactic methods adopted against coccidiosis.

3.4.3 Data Analysis

The data were entered into Microsoft Excel Program and analysed using Epi-Info version 3.3. Chi-square and Student T test were used to determine the level of significance of the result obtained. Determination of a significant difference was set at $P < 0.05$.

3.5 Results

3.5.1 Result of Poultry Farmers' Characteristics

The detailed result of data obtained on poultry farmers' characteristics is shown in table 3.1. Out of the 166 poultry farmers interviewed, 92 (56.4%) were 50 years and below while74 (45.6%) were above 50 years. The majority of the poultry farmers 140 (84.4%) had tertiary education while 26 (15.6%) have only secondary education. A total of 114 (68.7%) of the

respondents were Christians, 49 representing 29.5% were Muslims while only 3 representing 1.8% are Traditionalists. The table further shows that 155 (93.4%) of the respondents were married, 10, representing 6% are still single while only one is widowed.

3.5.2 General Data about the Respondents' Farms

Details of the data obtained in respect of the respondent farms are shown in detail in table 3.2. From the questionnaire survey, 70.5% of the poultry farms were over 5 years old, 28.9% were between one and 5 years old while a mere 0.6% was less than a year old. The majority of the respondent farmers (81.3%) reared only layer chickens; 1.8%, broiler chickens, 5.4%, breeders while 11.5% reared two or more breeds of chicken. Majority of the respondent farmers (66.3%) have chickens aged above 18 weeks on their farms. Only 2.4% and 4.8% of the farms have only birds aged 0 to 8 weeks and 9 to 18 weeks respectively. About 26.5% of the farmers kept chickens of multiple ages on their farms,

About 50.6% of the poultry population was based on farms with flock size of between 1000 and 5000 birds while 9% has a chicken population of between 50,001 and 330,000. Almost all the respondent farmers (99.4%) used conventional open sided housing system.

The study also showed that 44% of the poultry farmers reared their flock strictly on deep litter, 31.9% used cages exclusively while 24.1% used both systems.

The study further revealed that 53% got their feed supply from external sources while 47% produced their feed themselves. About 59% of the farmers used manual watering system, 36.2% used automatic watering system while 4.8% used both. Water spill occurs regularly on 70.5% of the farms while 92.8% of the respondents reacted to accidental water spills by packing out the wet litter immediately and replacing it with dry ones. About 61.4% of the respondents often have caked litters on their farm especially around the drinking areas while

54.2% of the respondent farmers turn around their litter monthly. Finally only 73% of the farms have foot dip at the entrance of their pens.

3.5.3 Respondents' Awareness and Knowledge of Coccidiosis

Details of the data obtained in respect of the Respondents'awareness and knowledge of coccidiosis is shown in table 3.3 below. A total of 159 (95.9%) respondents have the awareness and the knowledge of coccidiosis. A majority, 136 (82%) got the information either from Veterinary doctors, Veterinary clinic or from School. Majority of the respondents 146 (88%) confirmed that an outbreak of coccidiosis had occurred on their farms before, 121 (72.9%) of which was before the birds were 8 weeks old. Concerning the season of the year when the outbreak occurred, 61 (36.7%) was during the rainy season, 8 (4.8%) during the dry season and 82 (49.4%) of the outbreak occurred irrespective of season.

To about 75.9% of the respondents, coccidiosis is still a disease of economic importance but only a paltry 15.1% had a good knowledge of the disease while the rest 84.9% have only a fair knowledge, often regarding bloody faecal dropping as the only sign of coccidiosis. About 30.1% of the respondents had suffered coccidiosis outbreak despite preventive measures.

Table 3.1 Distribution of Personal Characteristics of Respondents (n = 166)

Variable	Operationalisation	Frequency	Percentage
Age	Below 10 years	0	0
	10-30 years	18	10.8
	31-50 years	74	44.6
	Above 50 years	74	44.6
Educational Attainment	Primary Education	0	0
	Secondary Education	26	15.6
	Tertiary Education	140	84.4
	Others	0	0
Religion	Christians	114	68.7
	Muslims	49	29.5
	Traditionalists	3	1.8
	Others	0	0
Marital Status	Single	10	6
	Married	155	93.4
	Widowed	1	0.6
	Divorced	0	0

Source: Field Survey 2010-2011

Table 3.2 General Data of the Respondents' Farms

Variables	Operationalisation	Frequency	Percentage
Age of Farms	Less than 1 year	1	0.6
	1-5 years	48	28.9
	Above 5 years	117	70.5
Type of chickens on farms	Broilers	3	1.8
	Layers	135	81.3
	Breeders	9	5.4
	2 or more breeds	19	11.5
Age range on Farms	0-8 weeks	4	2.4
	9-18 weeks	8	4.8
	19 weeks & above	110	66.3
	Multiple ages	44	26.5
Poultry population	200 – 999	10	6
	1000 – 5000	84	50.6
	5001 – 10000	34	20.5
	10001 – 50000	23	13.9
	50001 – 330000	15	9
Type of Rearing Pens	Open Sided	165	99.4
	Controlled Environment	1	0.6
Rearing Method	Deep Litter	73	44
	Cage system	53	31.9
	Both Deep litter and Cages	40	24.1
Method of Livestock feed for the birds	Produced on the farm	78	47
	From external sources	88	53
Watering system	Manual system	98	59
	Automatic system	60	36.2
	Both systems	8	4.8
Water spill in the pens	Occurs very often	117	70.5
	Occurs rarely	49	29.5
How do you handle accidental water spills on the litter?	Allow to dry	4	2.4
	Park out wet litter and replace with dry ones	154	92.8
	Mix the wet litter with other surrounding litter.	3	1.8
	Not deep litter system	5	3
Do you normally have caked litter?	Yes.	102	61.4
	No.	61	36.8
	Not deep litter system.	3	1.8
How often do you replace your litter?	Monthly	90	54.2
	Quarterly	35	21.1
	Yearly	5	3
	Not deep litter system	36	21.7
Disinfectant Foot dip at the entrance of pens	Present	121	73
	Absent	45	27

Table 3.3 Distribution of Respondents on Awareness and Knowledge of Coccidiosis

Awareness of Coccidiosis.	**Yes**	**NUMBER 159**	**PERCENTAGE 95.9**
	No	**7**	**4.1**
Source of awareness.	**Radio/TV**	**9**	**5.4**
	Vet/School/Vet Clinic	**136**	**82**
	Fellow farmers	**9**	**5.4**
	Personal experience	**4**	**2.4**
	No information at all	**8**	**4.8**
Previous occurrence on farm.	**Yes**	**146**	**88**
	No	**20**	**12**
Age of flock when Coccidiosis occurred.	**Below 8 weeks**	**121**	**72.9**
	Above 8 weeks	**25**	**15**
	No Outbreak	**20**	**12.1**
Season of the year when coccidiosis occurred.	**Dry Season**	**8**	**4.8**
	Rainy season	**61**	**36.7**
	Anytime	**82**	**49.4**
	Don't know	**15**	**9**
Is Coccidiosis of economic significance?	**Yes**	**126**	**75.9**
	No	**37**	**22.3**
	Don't know	**3**	**1.8**
Knowledge of Clinical signs of coccidiosis	**Good**	**25**	**15.1**
	Fair	**141**	**84.9**
Coccidiosis outbreak despite prevention	**Yes**	**50**	**30.1**
	No	**116**	**69.9**

3.5.4 Adoption of Preventive Measures and Post Adoption Experience

The details of data collected in respect of the preventive measures used and the post adoption experience is as shown in table 3.4 below.

For the prophylactic measures against coccidiosis, 30.1%, 54.8% and 15.1% of the respondents use coccidiostat in feed or water, Coccidiosis vaccine and alternation between the two methods respectively. About 25.3% of the respondents made the choice themselves while only 15.7% were influenced by fellow farmers' testimony. It is however interesting to note that the choice of about 59% of the respondents was influenced by their External consultants.

Concerning the duration of usage of the adopted prophylactic methods; 50.6% said it has been used for over 5 years while majority (91.6%) affirmed that the chosen method has been very effective. Surprisingly, about 29.5% of the respondents still suffered outbreaks of coccidiosis despite preventive measure. Majority, (88%) of the respondents appeared quite pleased with their adopted preventive measures and 72.3% did a cost-effectiveness analysis of the prophylactic method chosen.

Table 3.4 Adoption of Preventive Measures and Post Adoption Experience

Variable	Operationalisation	Frequency	Percentage
What preventive measures are you using against coccidiosis	Coccidiostat in feed or water	50	30.1
	Coccidiosis Vaccine	91	54.8
	Alternation between both	25	15.1
Who chose the method adopted?	Self or farm staff	42	25.3
	External Consultant	98	59
	Fellow farmers' testimony	26	15.7
Length of usage of adopted method.	Below 2 years	25	15.1
	Between 2-5 years	57	34.3
	Above 5 years	84	50.6
Effectiveness of adopted method.	Very effective	152	91.6
	Barely effective	14	8.4
Any outbreak in spite of preventive measures?	Yes	49	29.5
	No	117	70.5
Contemplation of change of preventive method?	Yes	20	12
	No	146	88
Was the cost-effectiveness of the preventive method evaluated?	Yes	120	72.3
	No	46	27.7

Table 3.5 Factors Associated with Good Knowledge of Coccidiosis

Variables	Good Knowledge n (%)	Fair Knowledge n (%)	Odd ratio	95% CI	P value
Age					
-Young Adults(<50yrs)	15 (61)	78 (55)	1.3	0.5-3.6	0.81
-Old Adults (>50yrs)	9 (39)	64 (45)			
Education					
-Pry & Secondary	3 (11)	23 (16)	0.64	0.1-3	0.44
-Tertiary	22 (89)	118 (84)			
Religion					
-Christians	18 (72)	96 (68)	1.2	0.4-3.7	0.95
- Muslims	7 (28)	45 (32)			
Age of farms					
< 5years	8 (33)	41 (29)	1.2	0.4-3.6	0.92
>5years	16 (67)	101 (71)			
Rearing Method					
-Deep Litter/Cage	**14 (56)**	**113 (80)**	**0.3**	**0.1-0.9**	**0.03[a]**
-Deep Litter & Cage	**11 (44)**	**28 (20)**			
Source of information					
-From Vet Clinic/Vets	**7 (28)**	**87 (62)**	**0.2**	**0.08-0.7**	**0.02[b]**
-Other Sources	**13 (72)**	**54 (38)**			
Outbreak after preventive measures.					
Yes	12 (50)	37 (26)	2.9	1-8	0.07
No	12 (50)	105 (74)			

a,b – p values with superscripts have statistical significant difference.

3.5.5 Factors Associated with Good Knowledge of Coccidiosis

The detailed data obtained in respect of the factors associated with good knowledge of coccidiosis is as shown in table 3.5.

Out of the several factors considered in respect of their association with having a good knowledge of coccidiosis, only two were highly significant ($P < 0.05$). The first one was that farmers that have both deep litter and cage system on their farms have significant higher knowledge of coccidiosis than those that are exclusively using one of the systems. The second one was that the farmers who got their information from the Veterinary doctors and Veterinary clinics have significantly higher knowledge of coccidiosis than those getting theirs from other sources.

3.5.6 Factors Associated with Adoption of Coccidiosis Vaccine Prophylaxis by Farmers

The details of the data obtained in respect of factors associated with the adoption of Coccidiosis vaccine propylaxis by farmers is as shown in table 3.6 below.

Several factors were considered for their association with the adoption of coccidiosis vaccine prophylaxis but only two were significant. Those farmers that took decision on the coccidiosis preventive method by consulting external consultants have a significantly higher adoption rate of coccidiosis vaccine than others. Interestingly, respondents that are Muslims have a significantly higher probability of adopting coccidiosis vaccine prophylaxis than chemoprophylaxis. This may be due to the fact that most muslim farmers in a particular area may be attending the same Jumat worship center on Fridays and such coming together regularly could afford them the opportunity to compare notes and share experiences.

Table 3.6 Factors Associated with the Adoption of Coccidiosis Vaccination

Variables	Coccidiosis Vaccine	No Coccidiosis Vaccine	Odd Ratio	95% Confidence Limit	P Value
Age					
< 50 yrs	63 (54)	30 (60)	0.8	0.4-1.8	0.73
> 50 yrs	53 (46)	20 (40)			
Educational Level					
Pry & Sec	22 (19)	4 (8)	0.3	0.1-0.9	0.2
Tertiary	94 (81)	46 (92)			
Religion					
Christains	**72 (62)**	**42 (84)**	**0.3**	**0.1-0.9**	**0.03[a]**
Muslims	**44 (38)**	**8 (16)**			
Rearing Method					
Deep litter	49 (54)	24 (69)	0.5	0.2-1.3	0.26
Cages	42 (46)	11 (31)			
Prevention method					
Coccidiostat	111 (97)	48 (95)	1	0.2-9.8	0.48
Vaccines	4 (3)	3 (5)			
Who influenced choice?					
External	**80 (64)**	**18 (35)**	**4.2**	**1.8-9.5**	**0.0008[b]**
Fellow farmers	**35 (36)**	**33 (65)**			
Cost Effectiveness					
Yes	90 (78)	30 (60)	2.4	1-5.4	0.066
No	26 (22)	20 (40)			

a,b –p values with superscripts have statistical significant difference.

3.6 Discussion

The preponderance of the below 50 years respondents (55.7%) may be indicative of the relationship between physical agility of youth and the physical demands of poultry farming operations. This observation corroborates the earlier result of Oladoja and Olusanya (2007), where 73.9% of poultry farmer respondents were within the age range of 31-50 years. Similarly, Adeyemi *et al.,* (2002) reported the age range of 24 to 62 years for most poultry farmers. The high numbers of poultry farmers with tertiary education (84.4%) probably reflects the technical nature of poultry farming and its need for high academic standard. This result is at variance with that of Oladoja *et al.,* (2007) who reported 1.14% without formal education, 50% with secondary education and 20.68% with tertiary education. A farmer with tertiary education is more likely to keep abreast of innovations and new preventive methods against certain infectious diseases like Coccidiosis and be willing to adopt it.

Majority of the poultry farmers (68.9%) are Christians. This corroborates the result of Oladoja *et al*., (2007) who reported 67% Christians. Religion plays an important socio-cultural role in one's way of life, relationship and occupation. It is unclear really why there was such a wide disparity between the practitioners of the two religion involved in poultry farming as poultry is devoid of the religious prejudices that occur in piggery.

It is not surprising that an overwhelming majority (93.5%) of the respondents were married. This is because poultry farming is dominated by middle aged people and majority of this age range are expected to be married. Marriage confers responsibility on individuals for their households. This observation corroborates the works of Oladoja *et al*., (2007) who reported 87.5% married respondents. Similarly, Oladoja (2000) affirmed the preponderance of married persons in the poultry business. Marital status may play a role in the management of coccidiosis especially in small poultry farms where the workers may not be effective

except closely supervised. It therefore means that if one of the spouse mangaing the chicken farm is not available, the other spouse could fill the void and thereby maintain some form of stability in management.

Only 0.8% of the poultry farms were started in the past one year while 70.5% were over 5 years old. It might be an indication that fewer people are presently going into poultry farming than in the past 5 years. This might be because a lot of young people now prefer 'Okada' riding and recharge card sales which bring in quicker returns with relatively less stress and starting capital. This is a danger signal to the authourities and they must strive to reverse this ugly trend to avert an acute shortage of poultry products in the very near future. This trend is also affecting the availability of young people for employment in the physically demanding poultry business sector and this might create managemental inadequacies on farms, culminating in the outbreaks of avian coccidiosis.

The rearing method adopted showed a preponderance of deep litter system. This is not unexpected since the capital requirements for a deep litter management system is far lower than that of cage system. However, farmers must note that coccidiosis is more prevalent under the deep litter management system and so, those using this method must take adequate proactive prophylactic measures against coccidiosis; such as vaccination, medicating coccidiostats in feed or water, avoiding spillage of water on the litter, prompt removal of caked litter among others to prevent devastating coccidiosis outbreaks. This is of particular importance to the conventional breeder farmers who have to use the deep litter system because of the need for mating within the flock. It is of note that some breeder farms have started using the cage system by adopting the artificial insemination method (Personal observation).

About 47% of the respondent farmers produced their feed while about 53% depended on external supplies. For those farms that adopted chemoprophylaxis of continual in-feed coccidiostatic medication, it is imperative that they produce their own feed so as to ensure thorough mixing of the coccidiostat in the feed. Similarly, those on vaccine prophylaxis should either produce their own feed independently or have a strict control on the supply source so that there will be no trace of coccidiostat in the feed resulting from residual carriage from some previous feed made for customers using feed grade coccidiostats.

About 59% of the respondents use manual drinkers while the rest either use automatic drinkers (Nipple or bell drinkers) or a combination of the two. Manual drinkers are disposed to frequent water spillage on the litter and this can cause dampness that could create a condusive environment for the proliferation of coccidian oocyst which may increase the risk of outbreak of coccidiosis. Over 70% of the respondents confirmed having regular water spills on the litter. The use of nipple drinkers may on the contrary cause excessive dryness in the litter which may make the darkling beetle, *Alphitobius diaperinus* to attack the birds in their search for water (Goodwin *et al*., 1996). Unfortunately, this beetle has been confirmed to transmit various disease agents including *Eimeria* species (Goodwin *et al*., 1996).

Good litter management is a critical factor in the control of coccidiosis outbreak. It is therefore important that conditions that encourage the sporulation of oocysts such as caked litter and litter dampness occasioned by water spillage and infrequent turning of litter should be avoided. Preventive measures against coccidiosis work best in conjunction with good hygienic measures; however, only 73% of the respondents actually had a foot dip with disinfectants at the entrance of their poultry pens. Majority of those that did not have foot dips were small and medium class poultry farms. Efforts should therefore be concentrated

by livestock extension officers on this class of poultry farmers to enhance their bio-security consciousness.

In this study, most of the farmers (95.9%) were aware of a disease called coccidiosis. This confirms the cosmopolitan and ubiquitous distribution of the *Eimeria* species and the enzootic presence in most poultry farms in the 2 states under study. The most important source of information for the farmers was through the Veterinary doctors, Veterinary clinic and Veterinary colleges and institutions. About 82% of the respondents got information on coccidiosis through these sources as against 5.4%, 5.4% and 2.4% that got it via Radio/TV, fellow farmers, and from outbreaks on their farms respectively. This is critical information for Veterinary extension workers and policy makers while planning for a high diffusion rate of new information, method and or control measures to the farmers. About 88% of the respondent farmers have suffered coccidiosis outbreak on their farms in the past. It may therefore means that outbreak of coccidiosis is currently been under-reported in the past. About 73.0% confirmed that the outbreak occurred in young flocks of below 8 weeks while only 15% suffered it after 8 week. This is a confirmation of the result of the experimental part of this study which confirmed that the prevalence of coccidial infections in birds between 1 and 8 weeks is significantly higher than in those above 19 weeks.

The survey showed a higher prevalence of coccidial infection during the rainy season than the dry season. This corroborates Shirley *et al*., (1992) who reported the effect of the environment (Temperature and moisture) on the course and severity of coccidial infections. There is no doubt that Coccidosis is a disease of economic importance but 22.3% said it was of no economic significance. It was apparent that those who have suffered an outbreak before can confirm the economic importance of the disease but majority of those yet to suffer outbreak could not confirm it. It is hoped that they will not learn the economic importance of coccidiosis from a devastating outbreak on their farms.

Only 15.1% of the respondents had a good knowledge of the clinical signs of coccidiosis, majority of the respondent (84.9%) recognize only bloody faecal dropping as sign of coccidiosis. Since there are several other clinical signs excluding bloody faeces, especially in subclinical cases, this disease could therefore be so easily wrongly diagnosed especially when Veterinarians are not involved.

The fact that 70% of the respondents have chosen Coccidiosis vaccine as a preventive method either wholly or in rotation with chemoprophylaxis is an interesting shift from the previous predominant usage of in-feed coccidiostatic drugs as a preventive method. The gradual development of resistance to nearly all the anticoccidials drugs in use and the non-development of any new anticoccidial since 1990 made the shift to vaccination more commendable. Coccidiosis vaccine usage has been confirmed to restore sensitivity to hitherto resistant species (Derek, D., 2005). Majority of the farmers confirmed that their choice of preventive methods was influenced by their Veterinary consultants. This is another testimony of the role of Veterinarian and other professionals in the dissemination of technical information to poultry farmers.

More than half of the farmer respondents had been using their adopted preventive method (either vaccine propylaxis or chemopropylaxis) for over 5 years and an overwhelming majority (91.6%) confirmed they have been very effective while only 8.4% were not quite satisfied. It was disheartening however that about (30.0%) still had outbreaks despite preventive measures. Before querying the efficacy of the chosen method, it must be borne in mind that poor hygiene, improper litter management and suboptimal vaccination against some important immunosuppressive diseases of poultry such as infectious bursal disease could compromise preventive measures against coccidiosis. About 12% of the respondents are contemplating changing the preventive measures they are currently using; it will be

important for them to consult veterinary experts who could help them choose a more efficient and cost effective coccidiosis preventive method.

CHAPTER FOUR

MORPHOMETRIC IDENTIFICATION OF *EIMERIA* SPECIES OF COMMERCIAL CHICKENS AND DETERMINATION OF PREVALENCE OF COCCIDIAL INFECTION IN OYO AND OGUN STATES

4.1 Introduction

Coccidiosis of chickens is an intestinal disease caused by parasitic protozoa of the genus *Eimeria* (Apicomplexa). The global cost of coccidiosis to the poultry industry is estimated at more than US$2.4 billion per annum (Shirley *et al*., 2005). Transmission is via the ingestion of infective oocysts that have sporulated over several days following their excretion in the faeces. The disease is extremely contagious and infective oocysts in the environment are resistant to most disinfectants making its eradication difficult (Williams *et al*., 1997). Traditionally, seven species of *Eimeria* have been recognized, based on parasite morphology, pre-patent period, site of infection and pathogenicity (Callow *et al.*, 1984, Jorgensen *et al.*, 1997) although new taxonomic units have been characterized recently (Cantacessi *et al*., 2008). In addition to the use of preventive chemotherapy and vaccination (Shirley *et al*., 2007), the specific diagnosis of infection has a key role in the prevention, surveillance and control of coccidiosis (Morris *et al.*, 2006). Traditionally, diagnosis has been achieved by detecting and enumerating *Eimeria* oocysts excreted in the faeces from infected chickens, measuring oocysts dimensions and shape, assessing the site and extent of pathological lesions caused by *Eimeria* in the intestines of chickens at necropsy, comparing pre-patent periods, minimum sporulation time and the extent of cross-protection (Eckert *et al*., 1995). These approaches are cumbersome, time-consuming, and can be unreliable particularly when multiple species of *Eimeria* simultaneously infect chickens because of the overlapping size ranges of oocysts, pre-patent periods and minimum sporulation times

among some *Eimeria* species, as well as sites of infection within the intestine (Long *et al*., 1984). Although this traditional method represents the gold standard, molecular tools are increasingly being developed and relied upon for the species level identification of *Eimeria* species. The infective form of *Eimeria* is the highly resistant oocyst, which is shed in the faeces of infected animals. The oocyst is excreted from the host as an undifferentiated stage, and in order to become infective it must sporulate. During sporulation four sporocysts, each containing two sporozoites, are formed within the oocyst. Sporulation of the oocyst depends mainly on three basic factors; temperature, humidity, and access to oxygen (Kheysin, 1972).

Under ideal conditions, sporulation occurs in 24 to 48 hours for most poultry *Eimeria* species (Edgar, 1955). The degree and rate of sporulation of excreted oocysts are important factors affecting the infection pressure in a flock of birds, thus influencing the epizootiology of the infections. It is generally believed that moist litter will favor the development of coccidiosis, because of the higher sporulation ability thus induced (Card and Nesheim, 1972; Matter and Oester, 1989).

4.2 Objectives

(a) To quantify and identify *Eimeria* species of commercial chickens in Oyo and Ogun states using morphometric method.

(b) To determine the prevalence, relative abundance (spectrum of *Eimeria* species) and infection levels (oocyst burden) of *Eimeria* species in Oyo and Ogun states.

(c) To determine and compare the prevalence of *Eimeria* species in caged and deep litter chickens.

(d) To determine and compare the prevalence of *Eimeria* species in chicks (below 8 weeks) and older chickens (above 19 weeks)

4.3 Material and Methods

4.3.1 Epizootiological Categories.

The following epizootiological categories were taken into consideration during samples collection.

1A	Chicks 1 to 8 week-old on vaccine prophylaxis
1B	Chicks 1 to 8 week-old on chemoprophylaxis.
2A	Chickens 9 to 18 week-old on vaccine prophylaxis.
2B	Chickens 9 to 18 week-old on chemoprophylaxis.
3A	Chickens aged above 19 weeks in cage system.
3B	Chickens aged above 19 weeks on deep litter system.

4.3.2 Sample Size and Sampling Method

Although the calculation of the sample size required for the study at the reported prevalence of 12% reported by Adene and Oluyemi 2004 was 160, two hundred and forty one pooled faecal samples of different epizootiological categories were actually collected from a total of 130 poultry farms in Oyo and Ogun states. One hundred and twenty pooled samples (9, 9, 9, 20, 54 and 19) belonging to epizootiological categories 1A to 3B respectively) were collected from 63 farms in Oyo state while 121 pooled samples (9, 16, 18, 27, 39 and 12) from categories 1A to 3B respectively) were collected from 67 poultry farms in Ogun state. One hundred grams (100 g) of fresh faeces was collected from 6 evenly distributed areas in the selected house on each poultry farm and packed in a nylon container and labeled. This was done in all the pens belonging to each of the epizootiological category on each poultry farm. Samples from each pen were homogenized together and 100 g representative sample

taken. The 100 g representative samples taken from different poultry pens of each category were pooled and similarly homogenized. It was from this that the representative 100 g pooled sample was taken for each of the categories. This final sample was well labeled and put in a plastic container inside a cool box packed with ice packs and taken to the laboratory for further processing (Haug *et al.*, 2008; Carvalho *et al.*, 2010)

4.4 Laboratory Methods

4.4.1 Enumeration of *Eimeria* Oocysts

The 100 g pooled faecal sample from each of the epizootiological categories was added 100 ml of distilled water and homogenised by the use of a mixer for 5 minutes. The homogenized suspension was passed through a sieve (50 microns) and nylon tissue. It was allowed to stand overnight to sediment. The supernatant was decanted and the sediment mixed together. From the sediment was taken 10 ml and put in a centrifuge tube and centrifuged at 4000 x g for 10 minutes. The supernatant was decanted and the sediment re-suspended in saturated sucrose solution (containing 685 g sucrose per liter of solution). It was then centrifuged at 1500 x g for 30 minutes. The supernatant which will contain the oocysts if present was collected by the use of a syringe without a needle piece. The collected supernatant was subsequently diluted up to 5 times with diluted water and vortexed, after which it was centrifuged at 4000 x g for 10 minutes. The supernatant was decanted and the *Eimeria* oocysts present was in the sediment (Shirley, 1995). Oocysts were kept in 2.5% Potassium dichromate for preservation (Carvalho *et al*., 2010). The McMaster chamber method was used for the oocyst enumeration according to Long *et al.*, (1976).

4.4.2 Sporulation of *Eimeria* Oocysts

The oocyst suspension in a solution of 2.5% potassium dichromate was poured into petri dishes and kept at room temperature for 5 days for sporulation. Sporulation took place in the presence of oxyen at room temperature.

4.4.3 *Eimeria* Oocysts Burden

McMaster method described by Hodgson, 1970 and Long *et al.*, 1976 was used. One ml of the oocyst sample was taken into a graduated beaker and 9 ml of saturated salt solution (containing 36 g of NaCl in 100 mL of distilled water) added to make a dilution factor of 1:10. One ml of the diluted sample was then pipetted and filled into the 2 chambers of the McMaster slide. All oocysts under the six grids in each of the two chambers were counted and the mean sum recorded. The number of oocysts recorded for each positive sample was used to calculate the oocysts per gram of faeces. The oocyst per gram of faeces was done using the formular below:

$$P \times K \times D$$

P = The mean of the number of oocyst counted in the 2 chambers of the McMaster slide.

K = It is a constant and for this method it is 6.66

D = It is the dilution factor and varies depending on the rate of dilution. It is actually 100 but may increase to 1000 if the sample is further diluted due to a higher concentration of oocysts.

Using this formular, if the 2 chambers of the MacMaster slide as 3 and 5 oocysts respectively, then the oocyst per gram of faeces will be:

(3+5)/2 x 6.66 x 100 = 2664.

The least possible oocyst per gram of faeces using this method is 334 and this can only occur if one oocyst is seen in a chamber and nothing is seen in the second chamber. In this case the mean oocyst count would be ½.

4.4.4 Identification of *Eimeria* Species

The oocysts enumerated above (both sporulated and unsporulated) were identified morphologically by randomly measuring the dimension of 50 oocysts using calibrated ocular micrometer at 400x magnification according to Long and Reid, (1982); Norton & Joyner (1981) and Edgar and Siebold, (1964). They were categorized in accordance with Anita Haug *et al.*, (2008) into three groups: *Acervulina-Mitis* (AM) group (small oocysts, <18.8µm; tentatively *Eimeria acervulina* and/or *Eimeria mitis*); *Necatrix, Tenella & Praecox* (NTP) group (medium-sized oocysts, 18.9 µm to 23.8 µm; tentatively *Eimeria necatrix, Eimeria tenella* and/or *Eimeria praecox*) or a *Brunetti-Maxima* (BM) group (large ovoid oocysts, >23.9µm; tentatively *Eimeria brunetti* and/or *Eimeria maxima*).

4.5 Result

4.5.1 *Eimeria* Oocyst Burden for Oyo State.

The oocyst per gram of faeces (OPG) for 1 to 8 week-old birds in Oyo State that used vaccine prophylaxis (OY 1A) was between 668 and 58363 with a mean of 9894.22. The value for 1 to 8 week-old birds in Oyo state that used chemoprophylaxis (OY 1B) was between 2668 and 8671 with a mean of 3409.33. The oocyst count per gram of faeces for 9 to 18 week-old chickens that used vaccine prophylaxis (OY 2A) was between 2001 and 26680 with a mean of 8307.11. The oocyst count per gram of faeces for chickens that were aged between 9 and 18 weeks in Oyo State that used chemoprophylaxis (OY 2B) has a value of between 5670 and 2174420 with a mean of 219843. The oocyst count per gram of faeces

of chickens aged above 19 weeks in Oyo State that were in cages (OY 3A) was between 334 and 1247290 with a mean of 49494. The oocyst count for chickens in Oyo state aged above 19 weeks that were on deep litter (OY 3B) was between 334 and 690862 with a mean of 160575.6.

4.5.2 *Eimeria* Oocyst Burden for Ogun State

The oocyst per gram of faeces (OPG) count in Ogun State for chicks aged between 1 and 8 weeks that used vaccine prophylaxis (OG 1A) was between 334 and 141404 with a mean of 19306.22. The count for chicks aged between 1 to 8 weeks that used chemoprophylaxis (OG 1B) was between 1001 and 2668 with a mean of 1167.5. A count of between 334 and 1257300 with a mean of 82690.63 was obtained for birds aged between 9 and 18 weeks that used vaccine prophylaxis (OG 2A) while birds aged between 9 and 18 weeks on chemoprophylaxis (OG 2B) has a count of between 668 and 2668 with a mean of 432.48. Birds aged above 19 weeks in Ogun State kept in cages (OG 3A) has a count of between 334 and 54694 with a mean of 2830.74 while those of the same age that are kept on deep litter (OG 3B) has a count of between 334 and 60033 with a mean of 8254.83.

4.5.3 *Eimeria* Infection Levels for Oyo and Ogun States

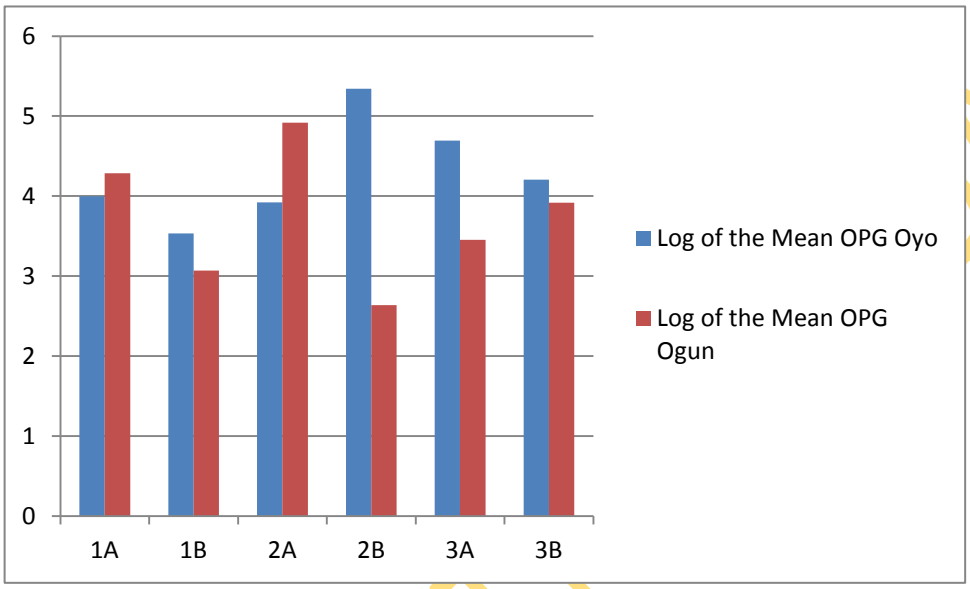
Eimeria infection level (mean opg) for chickens kept on deep litter was 75185 and 8255 for Oyo and Ogun State respectively. The infection level for birds of the same age kept in cages was 49494 and 2830 for Oyo and Ogun State respectively. The coccidial infection level for birds on deep litter was higher than for those kept in cages for the two States. The coccidial infection level for Oyo State samples was generally higher than those of Ogun State with mean oocyst per gram (OPG) value of 60106 and 4107 for Oyo and Ogun State respectively (appendix iv). When subjected to student T test (Appendix v), the differences in the coccidial infection levels among the various groups of birds were not statistically

significant. The difference in the oocyst per gram of faeces from chickens on vaccine prophylaxis and chemoprophylaxis was statistically significant ($P < 0.001$) both in Oyo and Ogun States (Appendix Xviii).

4.5.4 Prevalence of Coccidial Infection

4.5.4.1 Prevalence of Coccidial Infection for Oyo and Ogun States

Out of a total of 120 pooled faecal samples screened in Oyo State, 78 (65.0%) were positive for *Eimeria* oocysts. Out of 121 pooled samples screened in Ogun State, 74 (61.2%) were positive for coccidial infection. The prevalence of coccidial infection for birds aged between 1 and 8 weeks on vaccine prophylaxis was 9 (100%) for Oyo and Ogun State respectively. For birds aged between 1 and 8 weeks on chemoprophylaxis the prevalence was 9 (100%) and 12 (75%) for Oyo and Ogun State respectively. The prevalence rate for birds between 9 and 18 weeks on vaccine prophylaxis was 9 (100%) and 15 (83.3%) for Oyo and Ogun state respectively. The prevalence for 9 to 18 weeks old birds on chemoprophylaxis was 15 (75%) and 9 (33.3%) for Oyo and Ogun State respectively. The prevalence for birds above 19 weeks that were in cages was 22 (40.7%) and 20 (51.3%) for Oyo and Ogun State respectively. The prevalence for birds above 19 weeks old on deep litter was 14 (73.7%) and 9 (75%) for Oyo and Ogun State respectively. When subjected to statistical analysis, there was neither significant difference between the overall prevalence of coccidial infections in Oyo and Ogun States nor between those protected by vaccination and those on anticoccidials. The prevalence of coccidial infection in chicks between 1 to 8 weeks was significantly higher than that of cickenss above 19 weeks. The prevalence in birds kept on deep litter is significantly higher than those in cages.



1A = 1-8 weeks chicks on vaccine prophylaxis; 1B = 1-8 weeks chicks on chemoprophylaxis. 2A = 9-18 week chickens on vaccine prophylaxis; 2B = 9-18 weeks chickens on chemoprophylaxis; 3A = Above 19 weeks chickens in cages; 3B = Above 19 weeks chickens on deep litter

Fig. 4.1: The Log of the Mean OPG in Oyo and Ogun States

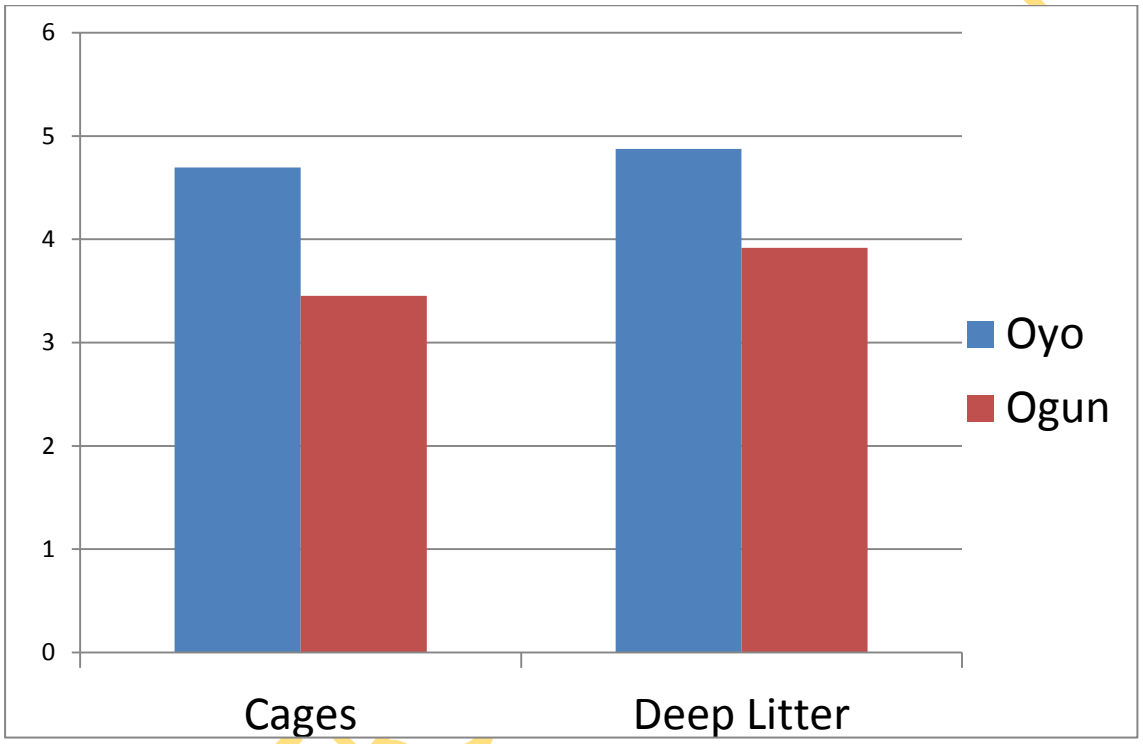


Figure 4.2 Comparism of Oocysts Burden in Cage and Deep Litter System in Oyo and Ogun States

Table: 4.1. Statistical analysis of OPG values for Oyo and Ogun states

	OYO STATE			OGUN STATE		
Epizootiological Categories	**Mean (OPG)**	**Standard deviation**	**Standard Error of Mean**	**Mean (OPG)**	**Standard deviation**	**Standard Error of Mean**
1A	**9894.2**	**17502.21**	**14.70**	**19306.22**	**43330.5**	**23.13**
1B	**3409.33**	**2918.97**	**6.00**	**1167.5**	**957.86**	**1.93**
2A	**8307.11**	**9952.57**	**11.08**	**82690.63**	**281287.1**	**29.46**
2B	**219843**	**538374.3**	**36.69**	**432.48**	**785.15**	**1.04**
3A	**49494**	**235865.2**	**8.99**	**2830.74**	**8864.15**	**2.41**
3B	**160575.6**	**412680**	**22.57**	**8254.83**	**17612.09**	**11.06**

1A = 1-8 weeks chicks on vaccine prophylaxis; 1B = 1-8 weeks chicks on chemoprophylaxis. 2A = 9-18 week chickens on vaccine prophylaxis; 2B = 9-18 weeks chickens on chemoprophylaxis; 3A = Above 19 weeks chickens in cages; 3B = Above 19 weeks chickens on deep litter

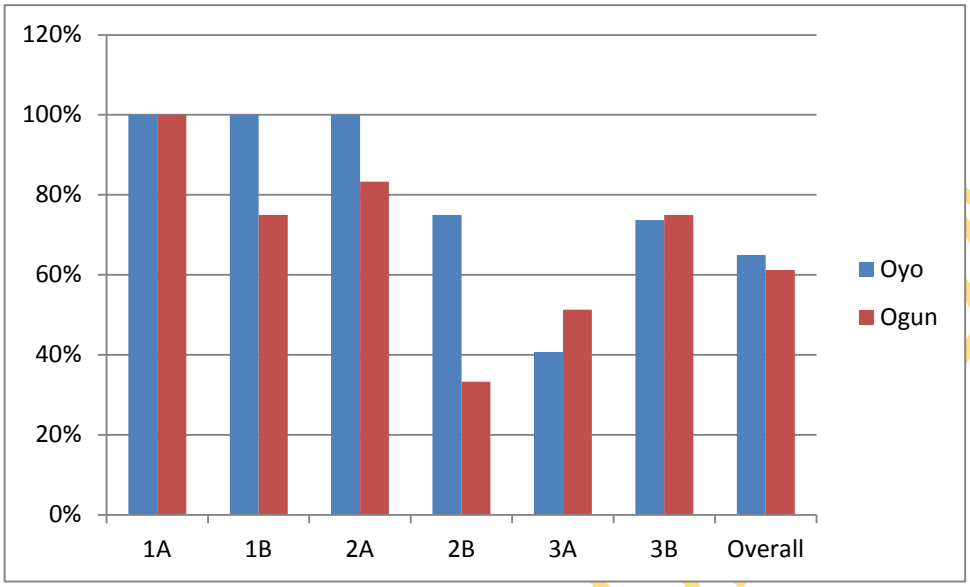
Table 4.2 Prevalence of Coccidial Infection for Oyo and Ogun States

EPIZOOTIOLOGICAL CATEGORIES	OYO STATE n (%)	OGUN STATE n (%)
1-8 weeks old birds on vaccine prophylaxis	9 (100)	9 (100)
1-8 weeks old birds on chemoprophylaxis	9 (100)	12 (75)
9-18 weeks old birds on vaccine prophylaxis	9 (100)	15 (83.3)
9-18 weeks old birds on chemoprophylaxis	15 (75)	9 (33.3)
Above 19 weeks birds kept in cages	22 (40.7)	20 (51.3)
Above 19 weeks birds kept on deep litter	14 (73.7)	9 (75)
Overall for the state	78 (65.0)	74 (61.2)

Table 4.3 Statistical Analysis of Coccidial Infection Prevalence in Oyo and Ogun States

Prevalences for comparison	Actual values	Z values	Interpretation
Prevalence in Oyo State versus Ogun State	78/120 ; 74/121	0.47	Not significant
Prevalence in 8 weeks and below versus 19 weeks and above	**39/43; 65/124**	**2.88**	**Significant**
Prevalence in deep litter versus cages	**23/31; 42/93**	**2.477**	**Significant**

Key: Figure > 1.6 is significant for Z test; figure < 1.6 are insignificant.



1A = 1-8 weeks chicks on vaccine prophylaxis; 1B = 1-8 weeks chicks on chemoprophylaxis. 2A = 9-18 week chickens on vaccine prophylaxis; 2B = 9-18 weeks chickens on chemoprophylaxis; 3A = Above 19 week chickens in cages; 3B = Above 19 weeks chickens on deep litter. Overall = Prevalence for the state

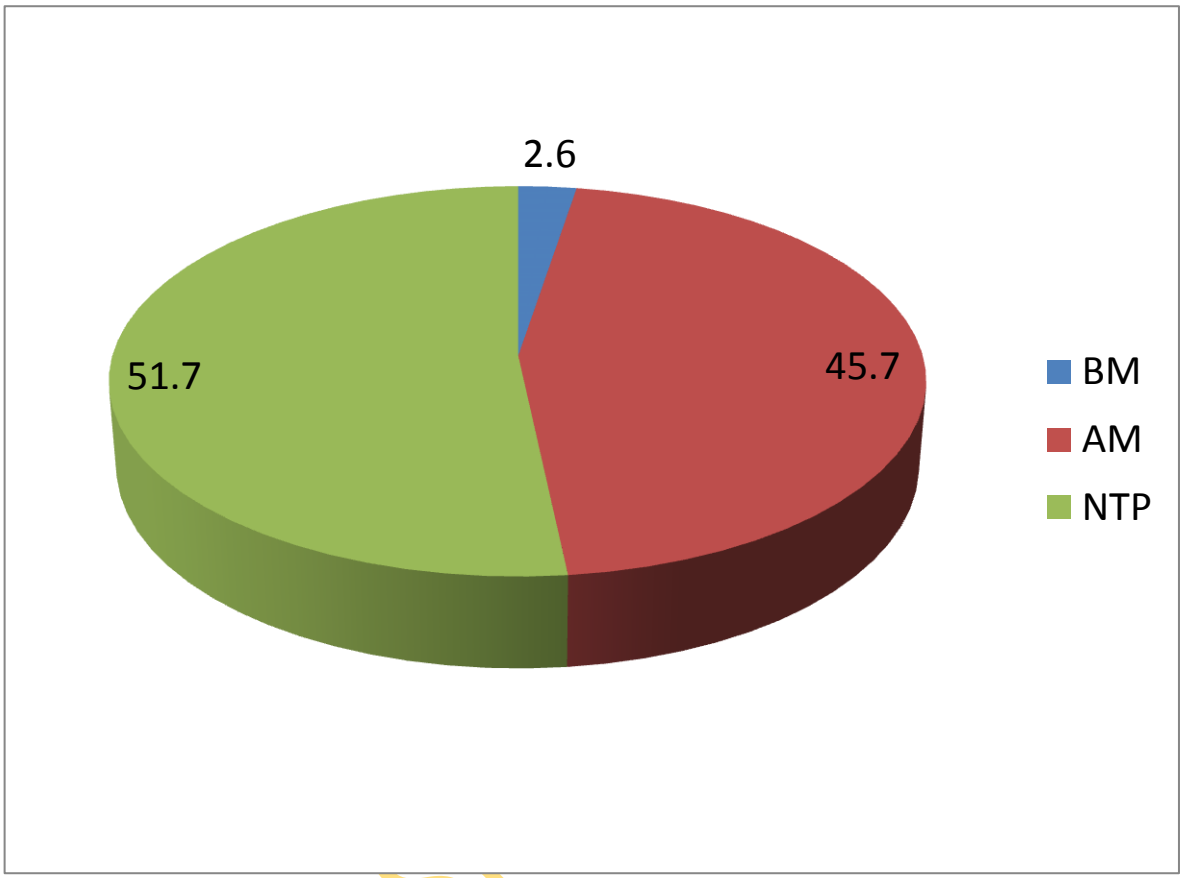
Figure 4.3 Prevalence of *Eimeria* Infection for Oyo and Ogun States

4.5.5 Relative Abundance of *Eimeria* Species in Oyo and Ogun States

The relative abundance of *Eimeria* oocysts in Oyo State is 3%, 52% and 45% for BM group (*E. brunetti* and *E. maxima*), AM group (*E. acervulina* and *E. mitis*) and NTP group (*E. necatrix, E. tenella and E. praecox*) respectively while Ogun State has 4.3%, 72.1% and 23.6% for BM, AM and NTP groups respectively. The relative abundance among the various epizootiological categories are as follows: In Chickens aged between 1 and 8 weeks in Oyo State on vaccine prophylaxis, *Eimeria* species abundance was 2.6%, 45.7% and 51.7% for BM, AM and NTP groups respectively while for Ogun State it was 21.1%, 40.8% and 38.1% for BM, AM and NTP groups respectively. In Chickens aged between 1 and 8 weeks in Oyo State on chemoprophylaxis, the relative *Eimeria* species abundance was 0%, 29.4% and 70.6% for BM, AM and NTP groups respectively. The same category of Chickens in Ogun State has an *Eimeria* species abundance of 35.7%, 0% and 64.3% for BM, AM and NTP groups respectively.

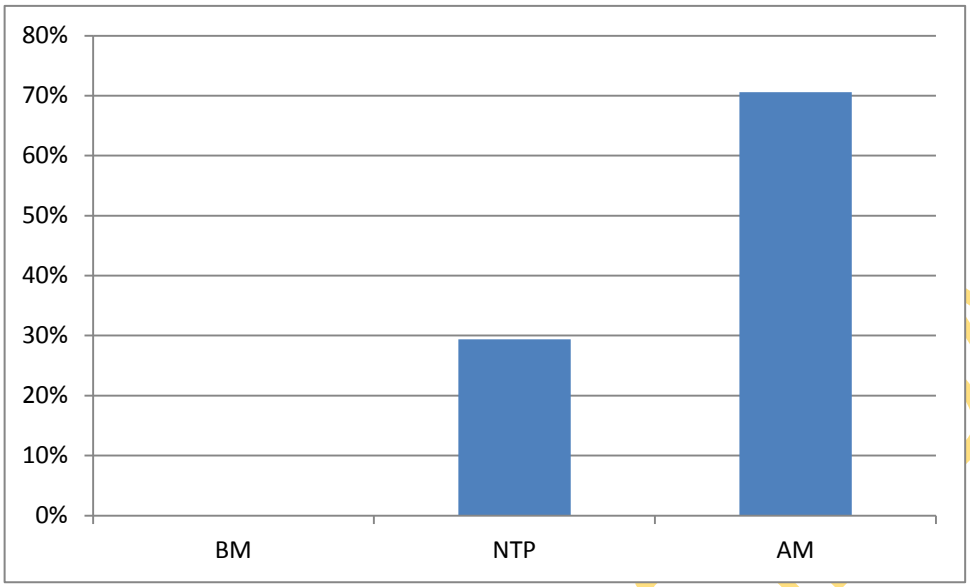
In Chickens aged between 9 and 18 weeks in Oyo State on vaccine prophylaxis, relative *Eimeria* species abundance was 5.3%, 62.5% and 32.2% for BM, AM and NTP group respectively. The same category of Chickens in Ogun State has a relative abundance of 2%, 79.6% and 18.4% for BM, AM and NTP group respectively. In Chickens of same age but on chemoprophylaxis in Oyo State, the relative *Eimeria* species abundance was 0%, 52.8% and 47.2% for BM, AM and NTP group respectively. This group of Chickens in Ogun State has an *Eimeria* species relative abundance of 0%, 50% and 50% for BM, AM and NTP group respectively.

The Chickens aged above 19 weeks in cages in Oyo State has a relative *Eimeria* species abundance was 5.2%, 57.6 and 37.2% for BM, AM and NTP group respectively. Similar Chickens in Ogun State have an *Eimeria* species relative abundance of 6.3%, 62.8% and 30.9% for BM, AM and NTP group respectively. In Chickens above 19 weeks in Oyo State kept on deep litter, the relative *Eimeria* species abundance was 5.7%, 45.9% and 48.4% for BM, AM and NTP group respectively. Similar birds in Ogun State have a relative *Eimeria* species abundance of 9.4%, 30.9% and 63.7% for BM, AM and NTP group respectively.



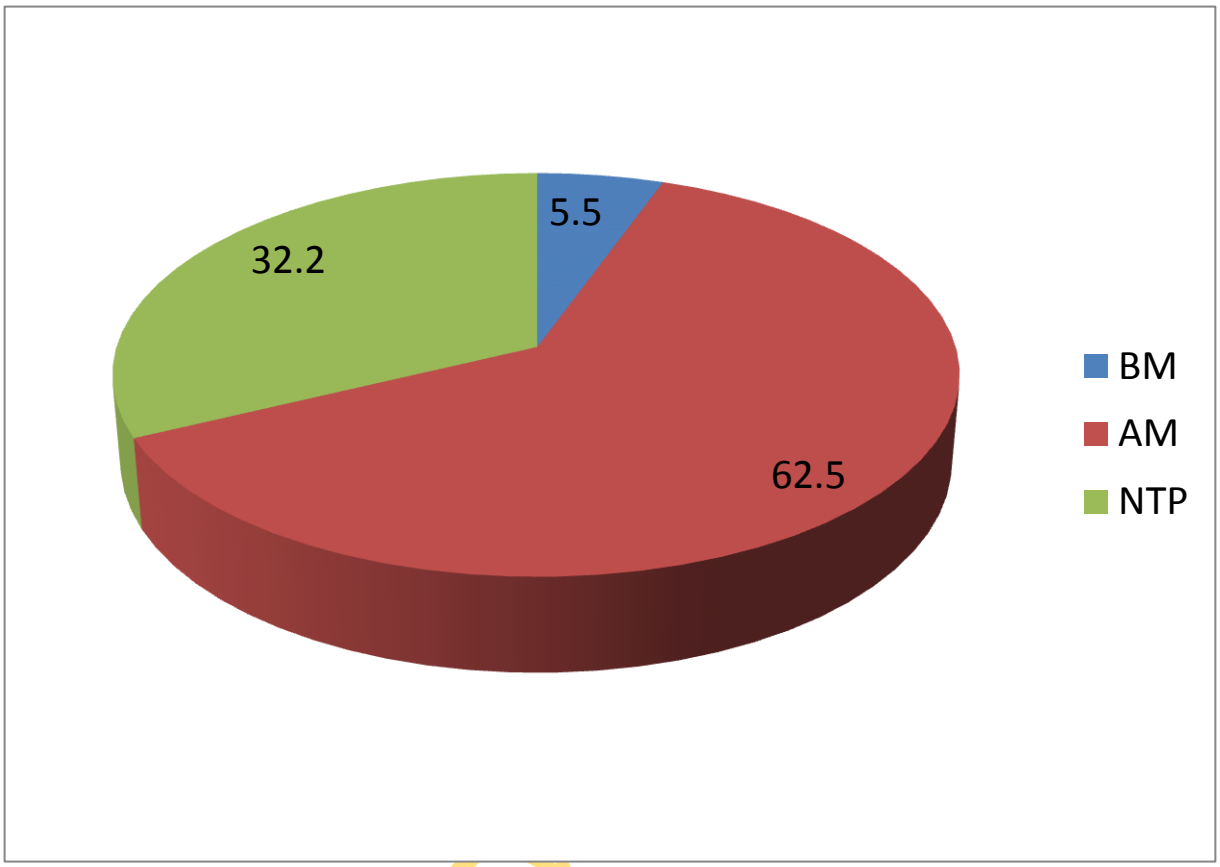
BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.4 Relative Abundance of *Eimeria* Species in Chickens 1-8 Week-old on Vaccine Prophylaxis in Oyo State



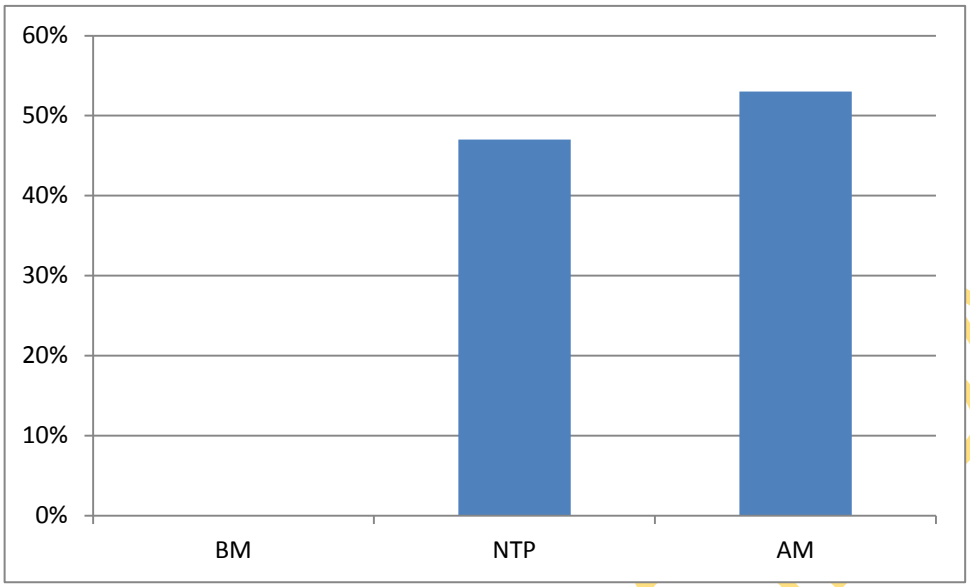
BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.5 Relative Abundance of *Eimeria* Species in Chickens 1-8 Week-old on Chemo Prophylaxis in Oyo State



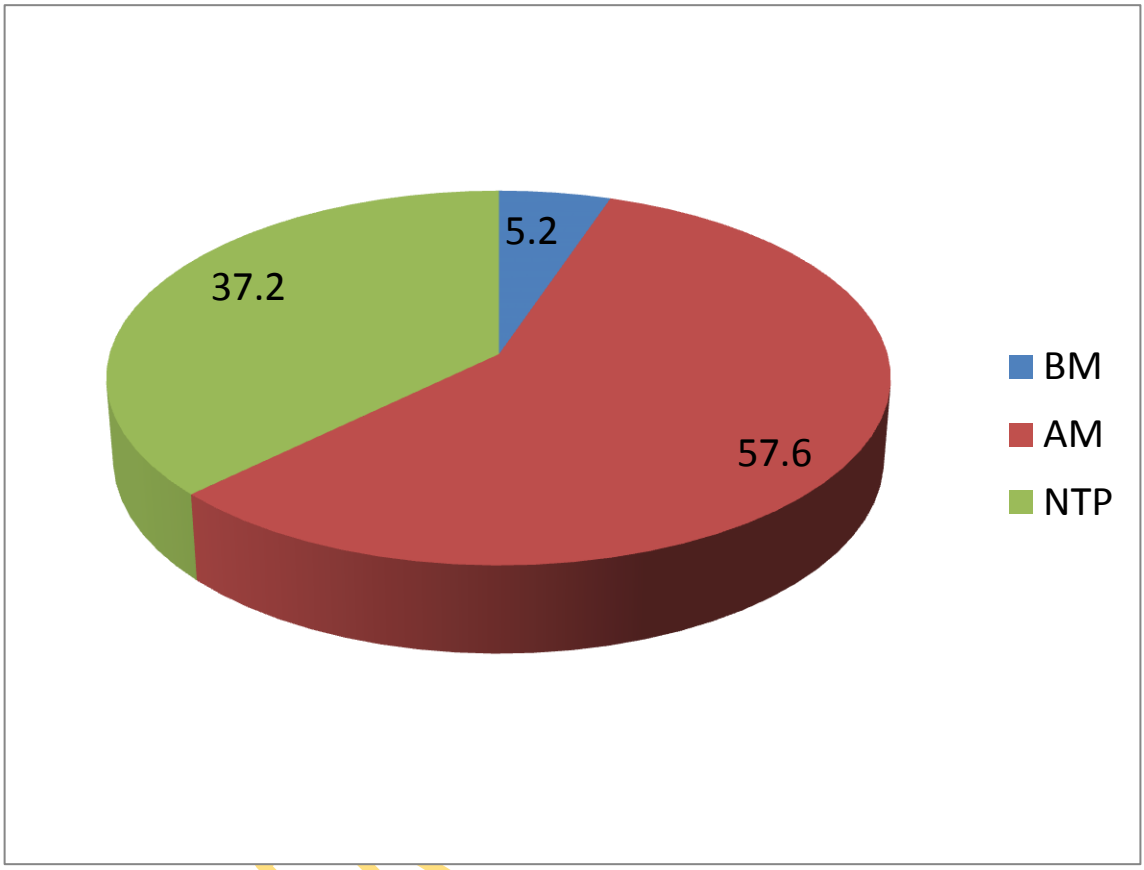
BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.6 Relative Abundance of *Eimeria* Species in 9-18 Week-old Chickens on Vaccine Prophylaxis in Oyo State.



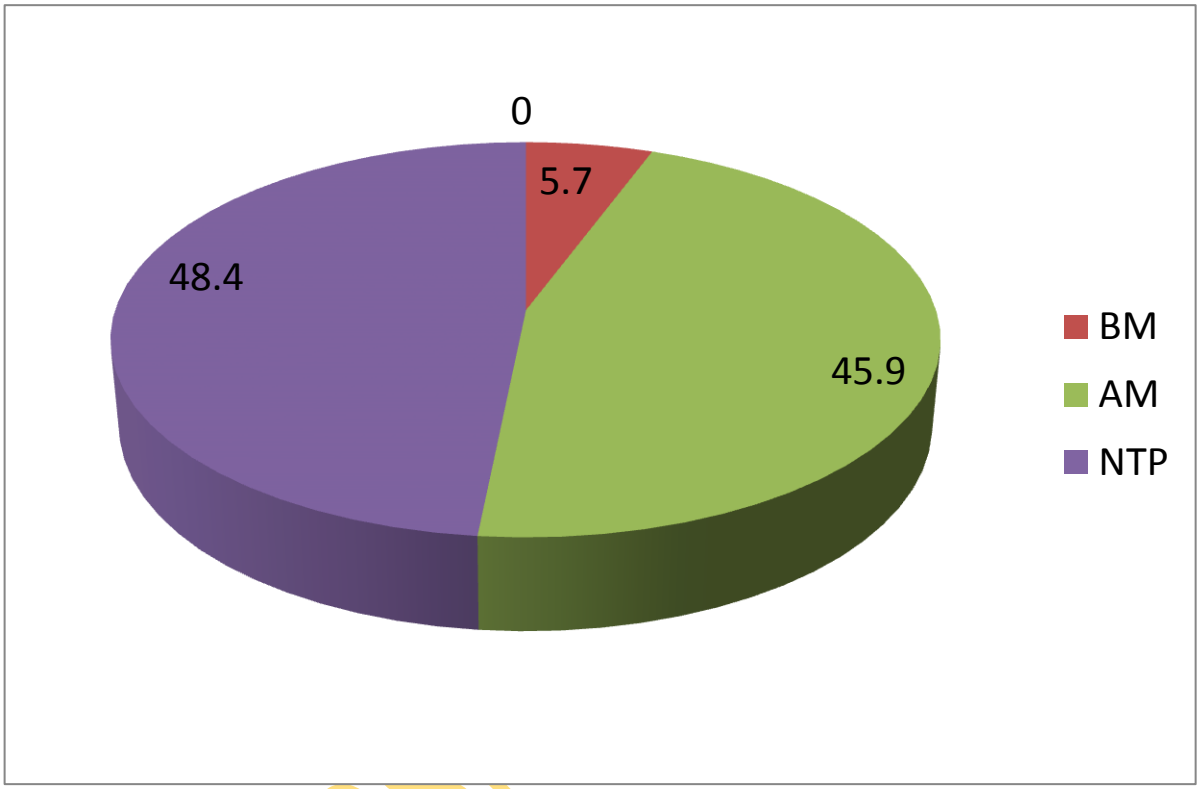
BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. E.tenella and E. praecox*

Figure 4.7 Relative Abundance of *Eimeria* Species in 9-18 Week-old Chickens on Chemo Prophylaxis in Oyo State



BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.8 Relative Abundance of *Eimeria* Species in Chickens aged above 19 Weeks in Cages in Oyo State.



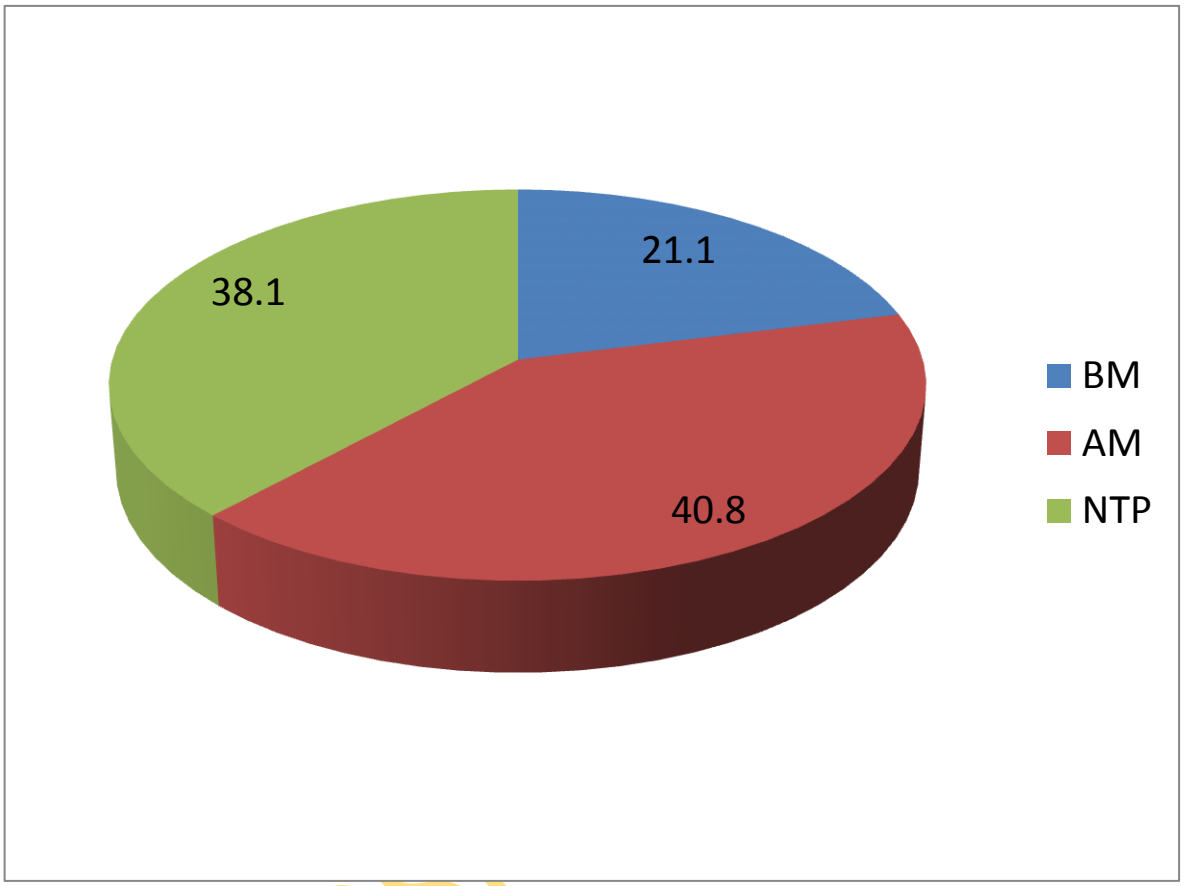
BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.9 Relative Abundance of *Eimeria* Species in Chickens aged above 19 Weeks on Deep Litter in Oyo State.

Table 4.4 Relative Abundance of *Eimeria* Species in the Six Epizootiological Categories of Oyo State

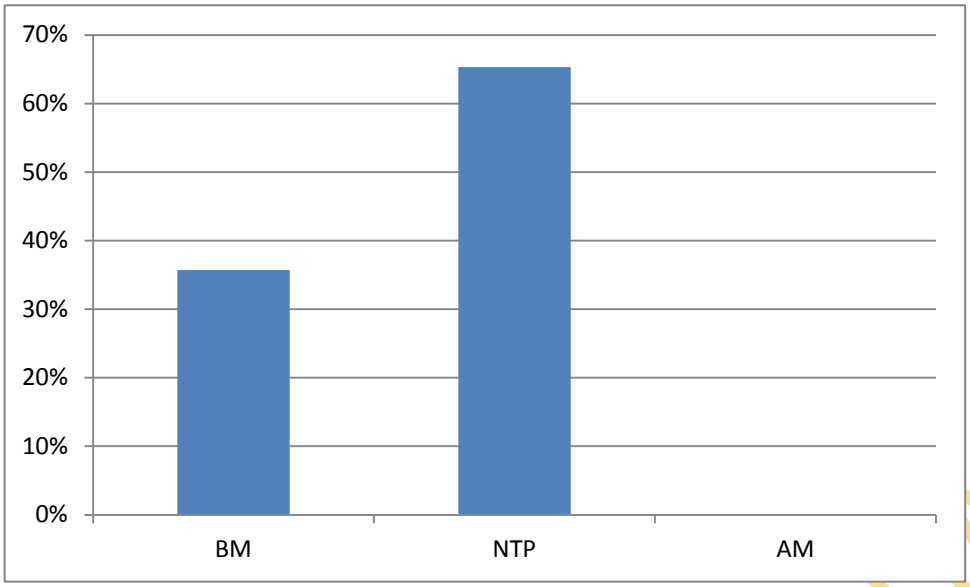
	OPG	BM n (%)	AM n (%)	NTP n (%)
OY 1A	90049	2336 (2.6)	41180 (45.7)	46533 (51.7)
OY 1B	11339	0	3335 (29.4)	8004 (70.6)
OY 2A	37352	2001 (5.3)	23342 (62.5)	12009 (32.2)
OY 2B	2186426	0	1153989 (52.8)	1032437 (47.2)
OY 3A	1336342	69018 (5.2)	769702 (57.6)	497622 (37.2)
OY 3B	1427186	81123 (5.7)	655226 (45.9)	690837 (48.4)
Cummulative	5088694	154478	2646724	2287442
Relative Abundance		3%	52%	45%

BM= ***E. brunetti*** **and** ***E. maxima;*** **AM=** ***E. acervulina and E. mitis;*** **NTP=** ***E. necatrix, E. tenella and E. praecox***



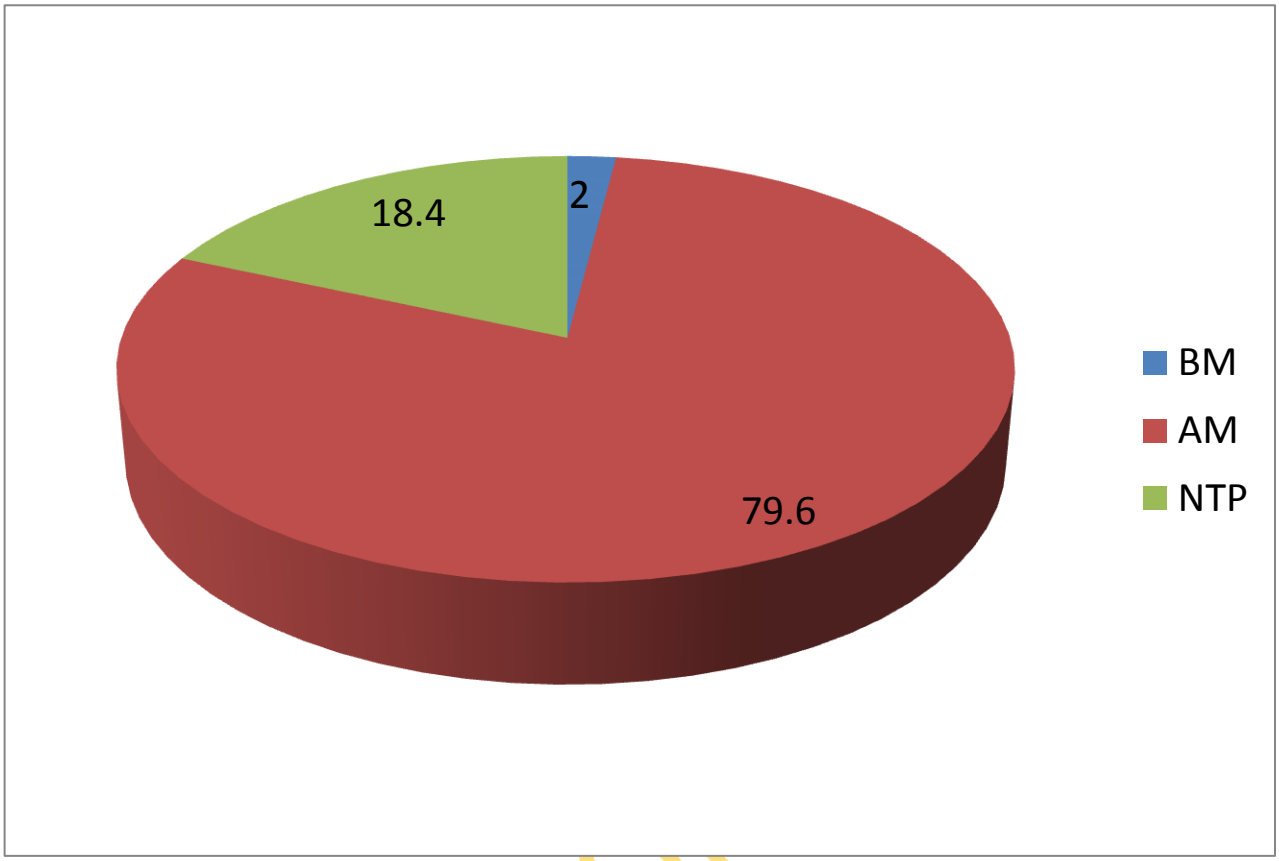
BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.10 Relative Abundance of *Eimeria* Species in 1-8 Week-old Chickens on Vaccine Prophylaxis in Ogun State



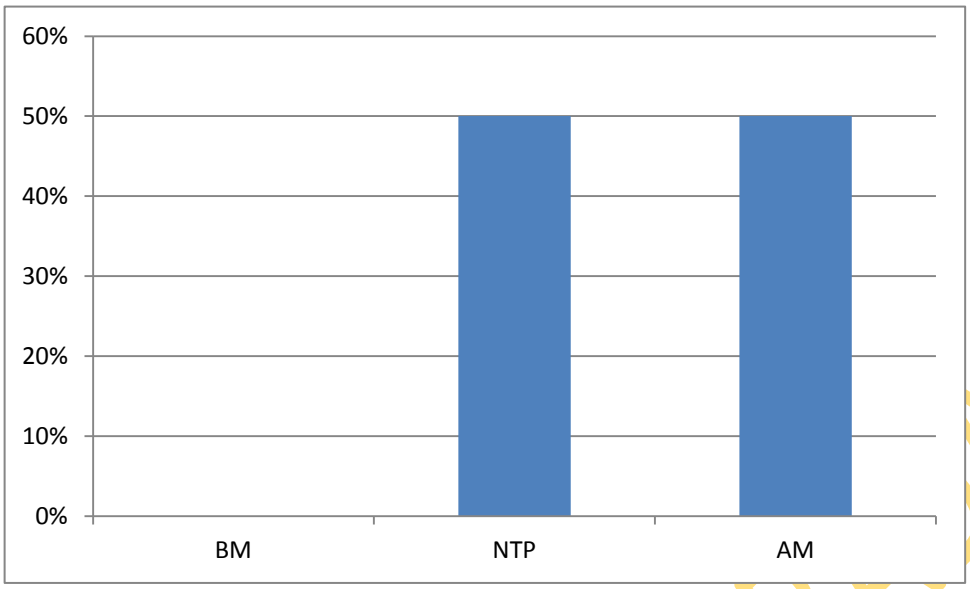
BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.11. Relative Abundance of *Eimeria* Species in 1-8 Week-old Chickens on Chemoprophylaxis in Ogun State



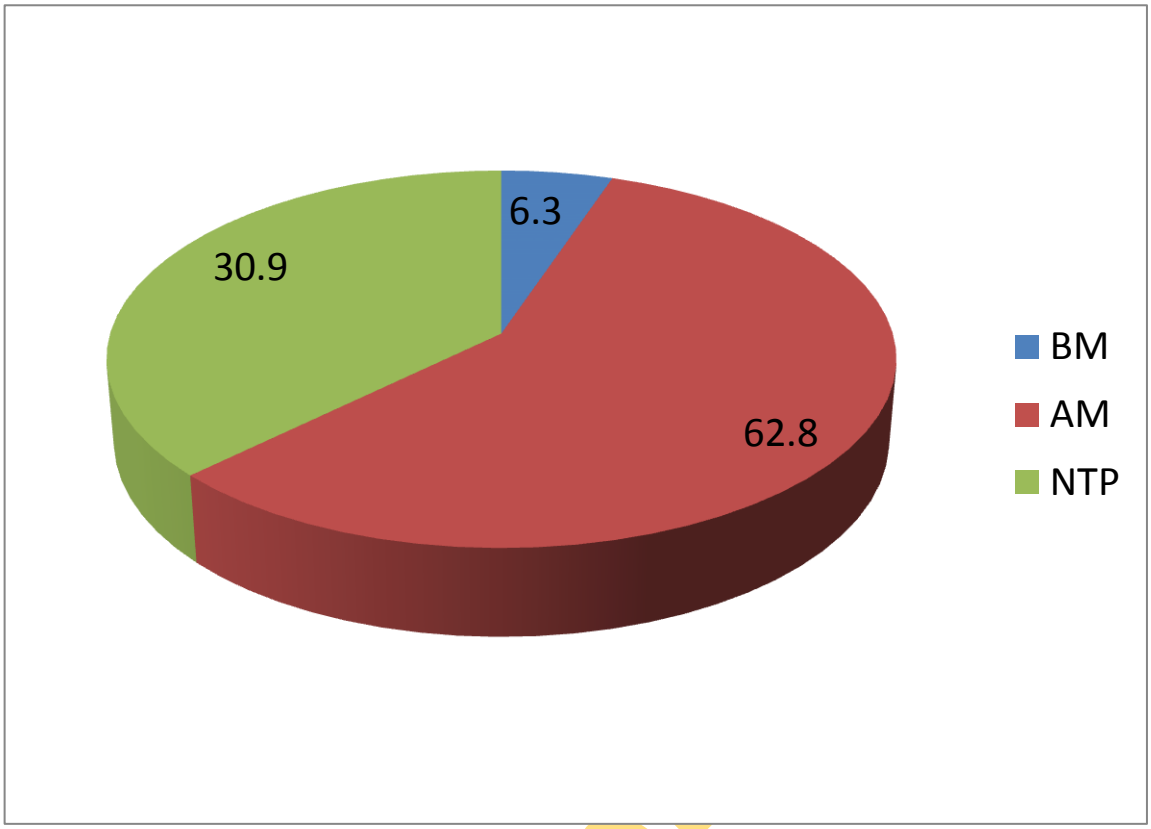
BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.12. Relative Abundance of *Eimeria* Species in 9-18 Week-old Chickens on Vaccine Prophylaxis in Ogun State.



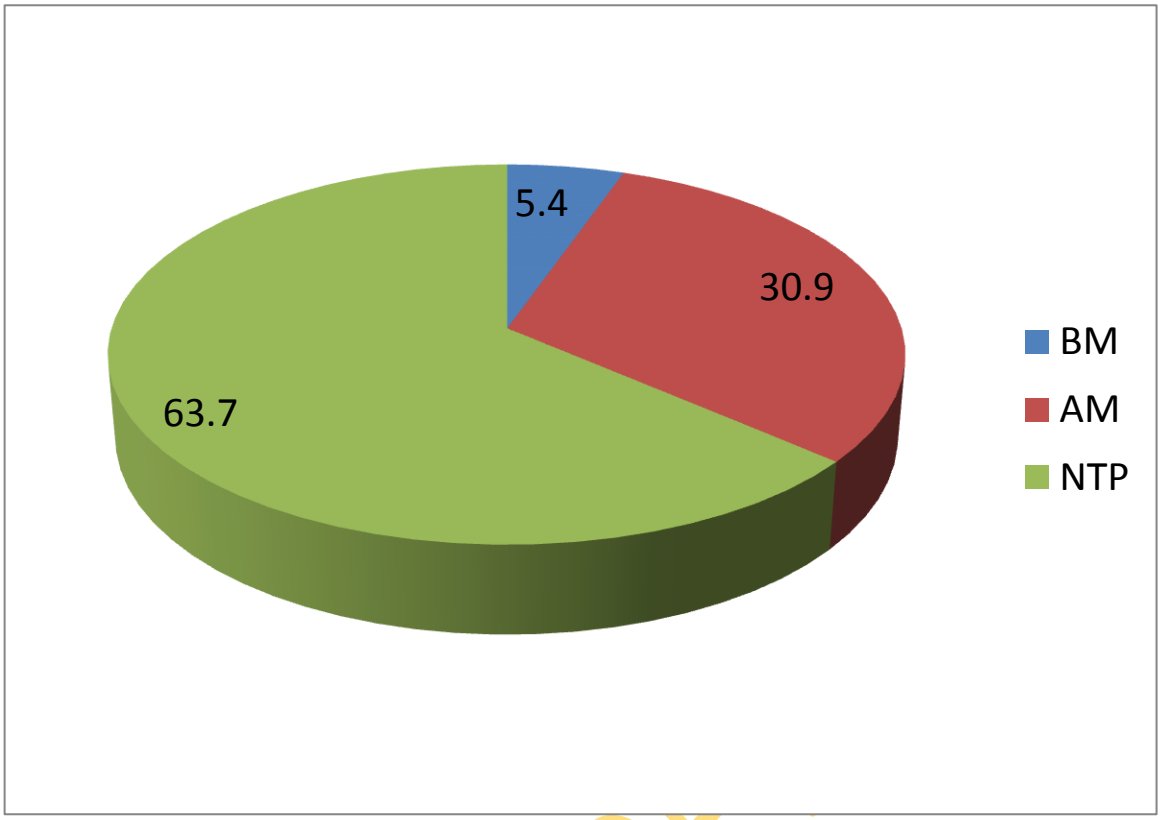
BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.13 Relative Abundance of *Eimeria* Species in 9-18 Week-old Chickens on Chemo Prophylaxis in Ogun State.



BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.14 Relative Abundance of *Eimeria* Species in Chickens above 19 Weeks in Cages in Ogun State



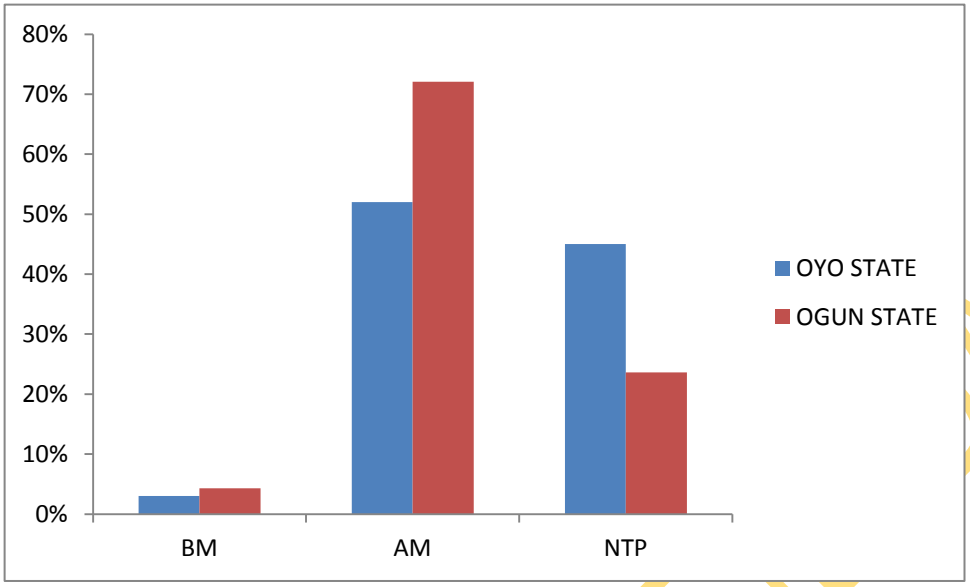
BM= *E. brunetti* and *E. maxima;* AM= *E. acervulina and E. mitis;* NTP= *E. necatrix, E. tenella and E. praecox*

Figure 4.15 Relative Abundance of *Eimeria* Species in Chickens above 19 Weeks on Deep Litter in Ogun State

Table 4.5 Relative Abundance of *Eimeria* Species in Ogun State

EPIZOOTIOLOGICAL CATEGORIES	OPG	BM N (%)	AM N (%)	NTP N (%)
OG 1A	154745	32612 (21.1)	63159 (40.8)	58974(38.1)
OG 1B	4670	1668 (35.7)	0	3002(64.3)
OG 2A	1383024	28096 (2)	1100316 (79.6)	254612(18.4)
OG 2B	11676	0	5838 (50)	5838 (50)
OG 3A	105730	6677 (6.3)	66365 (62.8)	32688(30.9)
OG 3B	99058	5337 (9.4)	30593 (30.9)	63128(63.7)
Cummulative	1747895	74390	1260767	412738
Relative Abundance		4.3%	72.1%	23.6%

BM = *E. brunetti* and *E. maxima;* AM = *E. acervulina and E. mitis;* NTP = *E. necatrix, E. tenella and E. praecox*



BM = *E. brunetti and E. maxima*, AM = *E. acervulina and E. mitis*, NTP = *E. necatrix, E. tenella and E. praecox.*

Figure 4.16 Relative Abundance of *Eimeria* Species in Ogun and Oyo States

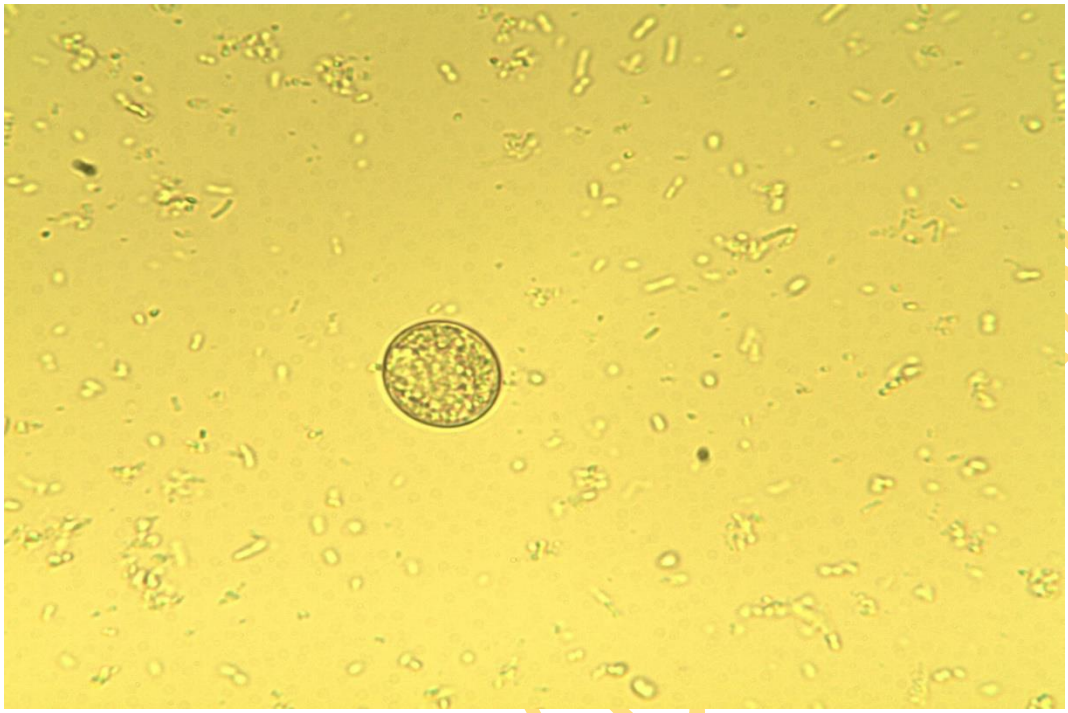


Figure 4.17 Unsporulated Oocyst of *E. necatrix, E. tenella and E. praecox* (NTP) Group x400

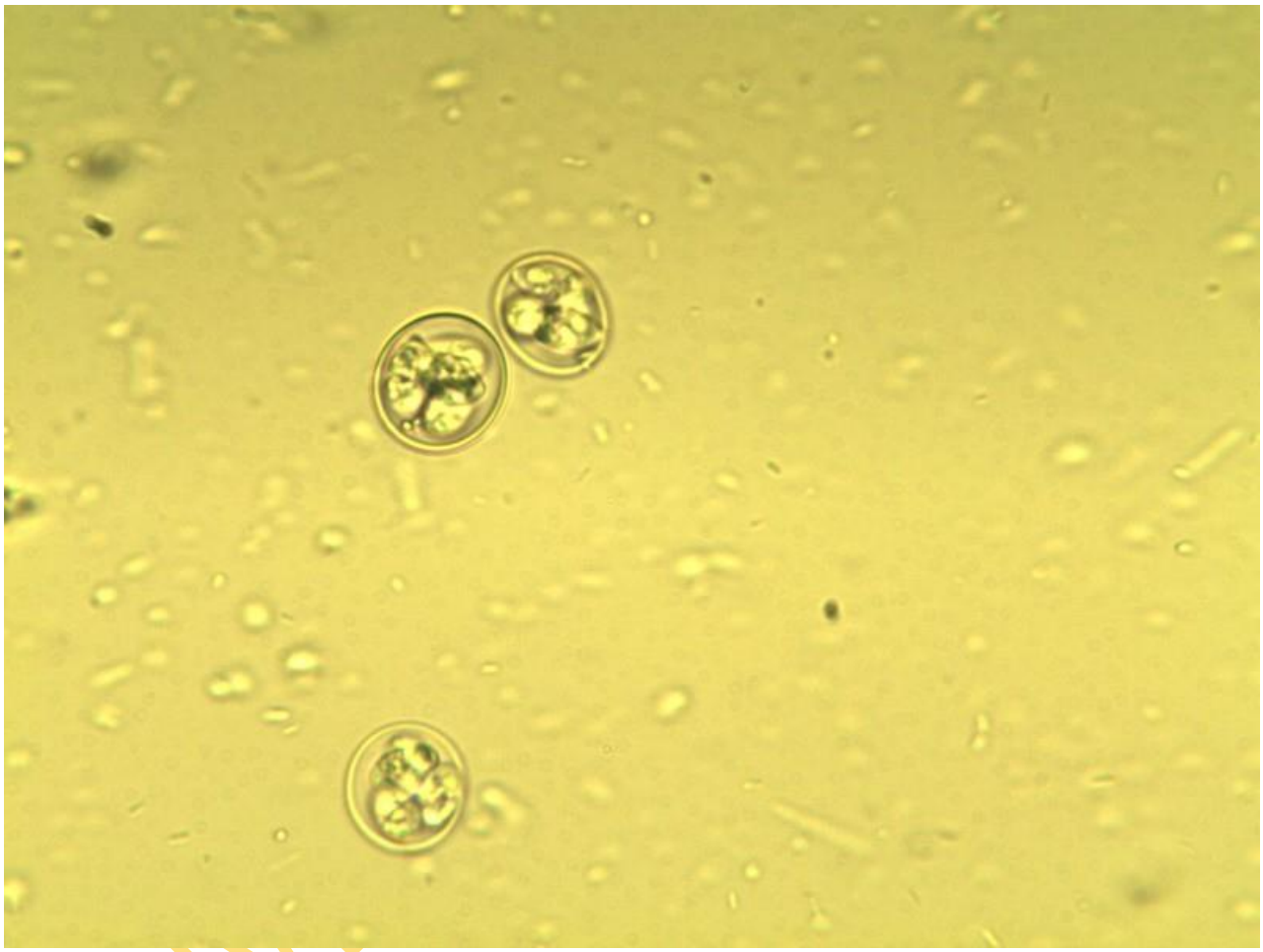


Fig. 4.18 Sporulated Oocysts of the *E. necatrix, E. tenella and E. praecox* (NTP) Group x400

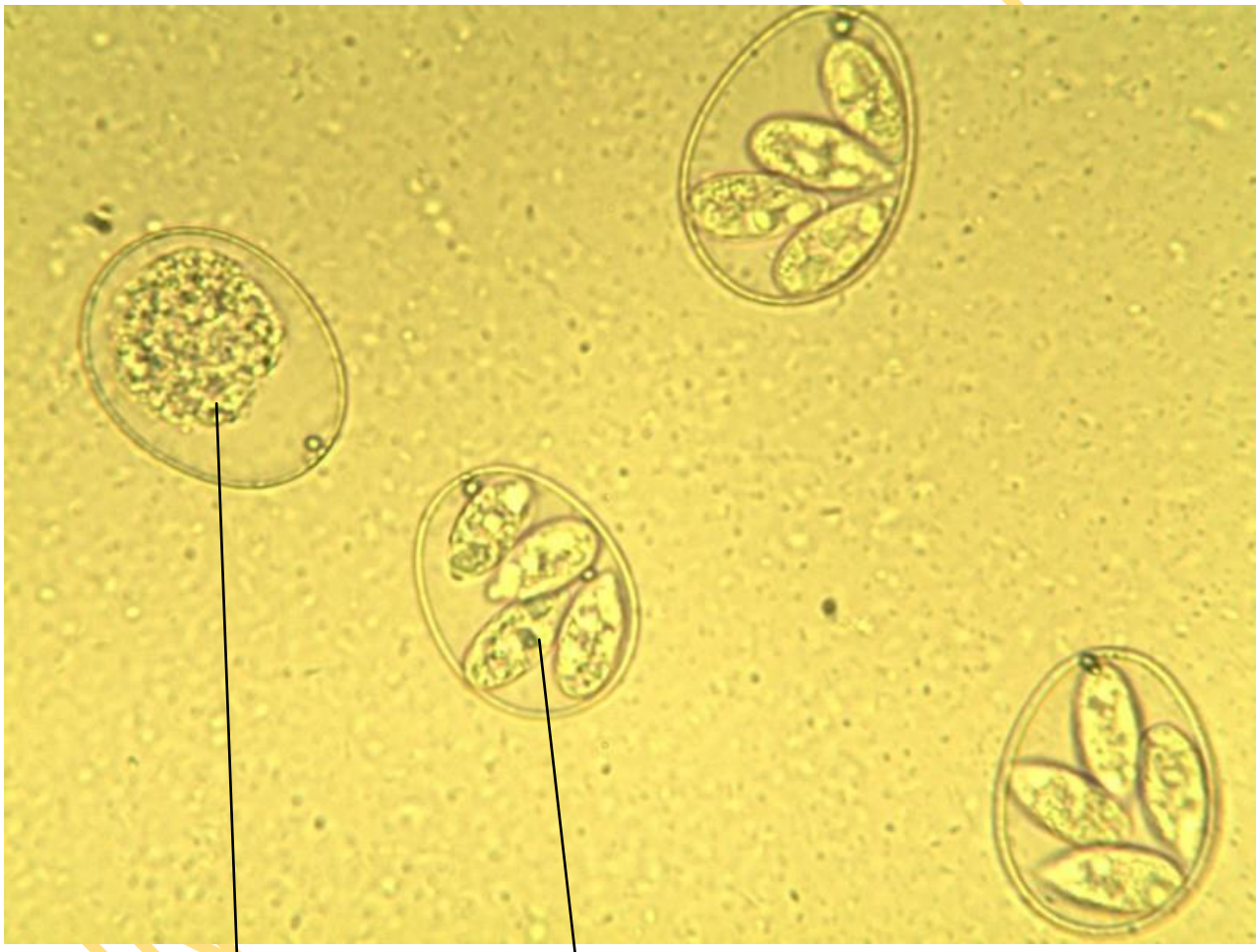


Fig 4.19 **Sporulated and Unsporulated Oocysts of *E. maxima* and *E. brunetti* (BM) Group x400**

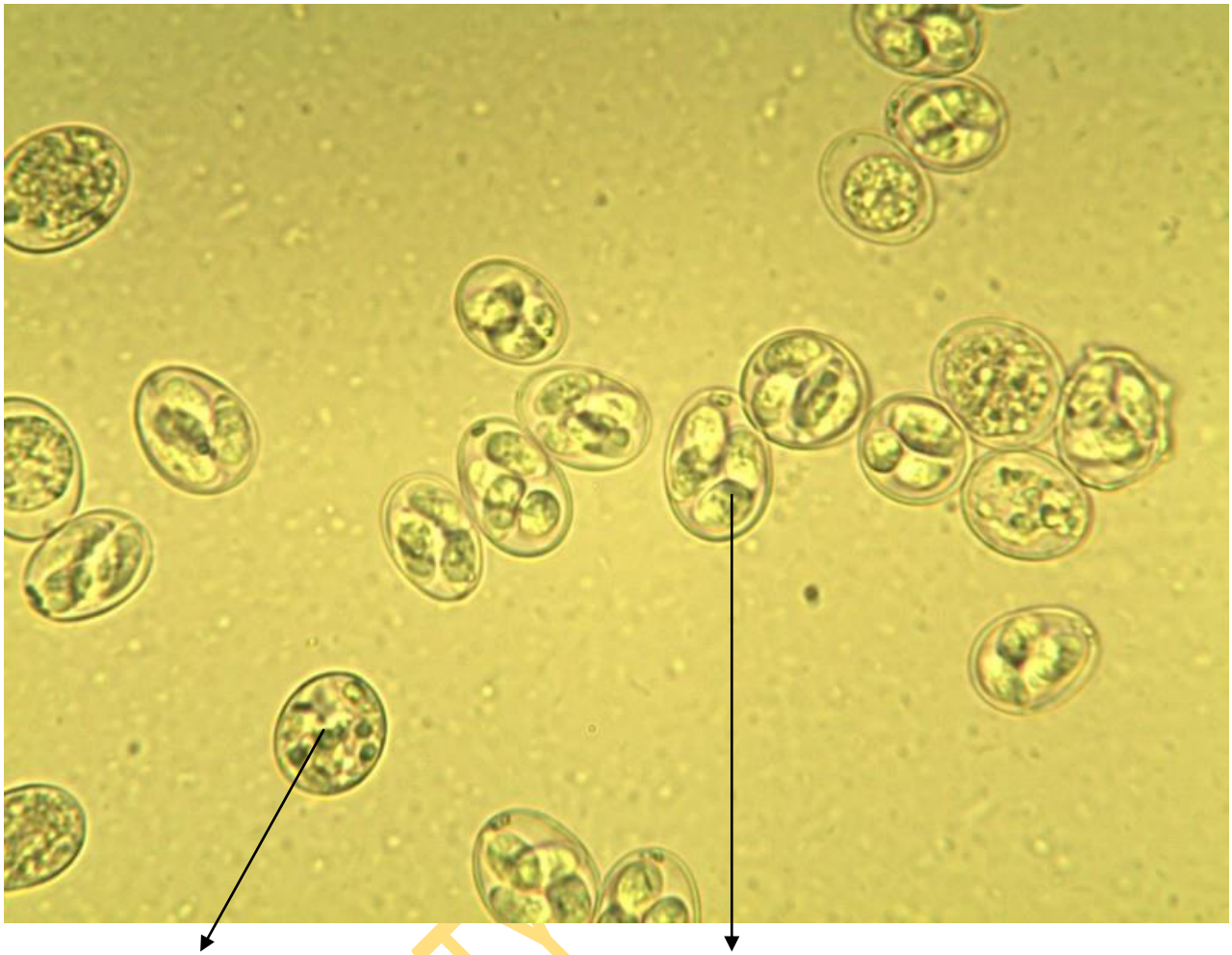


Fig 4.20 Sporulated and Unsporulated Oocysts of *E. acervulina and E. mitis* (AM) Group x400

4.6 Statistical Analysis and Hypotheses Testing

Statistical analysis of the overall prevalence of coccidial infection in Oyo and Ogun State as well as those of young birds (8 weeks and below) and older ones (Above 18 weeks) were obtained by ~~Z~~ test for two different proportions. There was no significant difference ($p>0.05$) in the prevalence of coccidial infections between Oyo and Ogun State.

The overall prevalence of coccidial infections in birds eight weeks and below in the two states (90.7%) is significantly higher ($p<0.05$) than older birds of 19 weeks and above (52.4%).

The prevalence of coccidial infection in birds above 19 weeks kept on deep litter (74.2%) is significantly higher than the same age group kept in cages (45.2%).

Therefore the null hypothesis (H1) which states that there is no significant difference between the prevalence of coccidial infection in Oyo and Ogun States is accepted.

However, the null hypothesis (H2) which states that there is no significant difference between the prevalence rate of coccidial infection in young birds (below 8 weeks) and older birds (above 18 weeks) is hereby rejected.

The null hypothesis (H4) which states that there is no significant difference between the prevalence of coccidial infection under cage and deep litter management system is hereby rejected.

4.7 Discussions

Understanding the occurrence and distribution of various *Eimeria* species in poultry farms is of tremendous epizootiological importance for effective control strategy including the development of coccidiosis vaccine. The result showed an overall prevalence of 65.0% and 61.2% for Oyo and Ogun State respectively. This prevalence is higher than that of Adene and Oluleye, 2004 and Etuk *et al.*, 2004 who reported a prevalence of 12% and 12.7% for Nigeria and Akwa Ibom State respectively. This may be due to differences in the type of samples collected. While the two studies used either faecal or intestinal scraping of individual chickens, the present study used pooled faecal samples of several chickens. Etuk *et al.*, also reported a prevalence of 26.69% among birds managed on deep litter; 18.75% in 1 to 5 week-old birds and 22.29% in layers in South Eastern Nigeria. It was also higher than that of Muazu *et al.*, (2008) who reported 52.9% and 36.6% for young and adult chicks respectively in Plateau State but similar to the findings of Sun *et al.*, (2008) who reported a prevalence of 65.8% and 62.03% for Shandong and Anhui province of China respectively.

The prevalence for the different epizootiological categories varied widely and this is to be expected because of the peculiar differences in the mechanism of action of the prophylactic methods adopted and the apparent differences in the ages of the flocks. The 100% prevalence obtained for epizootiological categories OY 1A and OG 1A is not unexpected because coccidiosis vaccines was used for prophylaxis and this will invariably result in proliferation of the vaccinal oocyst in addition to some field oocyst that were not incorporated in the vaccine. The above explanation is also applicable to the prevalence of 100% and 83.3% got for OY 2A and OG 2A respectively due to the fact that coccidiosis vaccine was similarly used.

On the contrary, the prevalence of 100%, 100%, and 75% for OG 1B, OY 1B and OY 2B respectively were higher than expected when compared to the 33.3% obtained in OG 2B considering the fact that feed grade anti-coccidials were used as a method of control. The high prevalence of coccidial infection obtained in this study is in agreement with reports from Ethiopia (Ashenefi *et al*., 2004); from Jordan (Al Natour *et al*., 2002); from France (Williams *et al*., 1996) and from Argentina (Mc Dougald *et al.*, 1997) suggesting that coccidial infections are widespread in most countries. The high prevalence of the infection under study may either be an indication of widespread proliferation of coccidian oocysts in the farm environment or the inadequacy of the cleaning and disinfection procedures on the farms. From the questionnaire analysis, it was noted that some farms controlled coccidiosis by routine medication as opposed to continuous in-feed coccidiostatic medication. Some of the faecal samples might have been taken during the period preceding the routine treatment. For those on continual in feed coccidiostatic medication, it has been shown that some ionophores like Coban and Avatec allow leakages of oocysts so that the birds can build up immunity.

It is also worthy of note that the fact that the anticoccidial medication is in the feed does not mean that the birds are getting it. Apart from inappropriate dosing and ineffective mixing, other situations such as empty feed pans, hot weather and concurrent disease problems that may prevent birds from feeding on medicated feed does not necessarily stop them from picking the litter and ingesting coccidian oocysts. Development of resistance to the anticoccidials being used may also be responsible for the high prevalence. Al Natour *et al*., (2002), observed a prevalence of 78% in Jordan while Anita *et al*., (2012) also recorded a prevalence of 75% for birds of similar age on anticoccidial preventive medication.

The prevalence of 40.7% and 51.3% in OY 3A and OG 3A (Birds above 19 weeks in cages in Oyo and Ogun states) respectively corroborates Soulsby, (1973) who reported that

immunity to coccidiosis increases with the age of the birds. This result is comparable to that of Bachaya H.A *et al.,* (2012) in Pakistan who observed a prevalence of 60.16% and 37% for young and old layers respectively. The prevalence of 73.5% and 75% obtained for OY 3B and OG 3B (Birds above 19 weeks on deep litter in Oyo and Ogun States) is in agreement with the result of Etuk *et al*., (2004) who recorded the highest prevalence of coccidiosis among birds raised on deep litter system. This might be due to the fact that birds on deep litter system by virtue of their direct access to the litter are at a higher risk of coccidial infection than their counterparts in the cages. The infection level in the 2 States was high as indicated by the number of oocyst per gram of faeces. No clinical signs of coccidiosis were seen in the flocks during sample collection. Such high OPG in farms that control coccidiosis by chemoprophylaxis may be an indication of failure of control measures adopted. This confirms the assertion of Maarten Degussen *et al.,* (2007) that OPG counts in faeces or litter has a poor relationship with the impact of the parasite on the performance of a flock. This observation corroborates the result of William *et al.,* (1996) in France and Anita Haug *et al.,* (2008) in Norway who both recorded millions of oocyst per gram of faeces in broilers without clinical coccidiosis. It is a fact of parasitology that only a sporulated oocyst is infective, so the percentage of the oocysts that are sporulated may be a critical factor as well as its accessibility to the birds.

The identification of *Eimeria* species based on traditional morphometric method has been the routine standard especially in Africa where the molecular diagnostic method is yet to be fully embraced. The result showed a preponderance of the small sized oocysts belonging to the Acervulina-Mitis group comprising of *E. acervulina* and *E. mitis*. It is closely followed by the medium oocysts belonging to the Necatrix-Tenella-Praecox group made up of *E. necatrix, E. tenella* and *E. praecox*. The big oocysts comprising the Brunetti-Maxima group made up of *E. brunetti* and *E. maxima* constituted less than 5% of the oocyst population.

This result is in agreement with that of Anita Haug *et al.*, (2008) who reported a predominance of the small oocyst AM groups in the coccidial infections of large broilers in Norway. Majaro, (1984) reported that oocyst sizes and shapes vary as the duration and the severity of infection increases. This assertion therefore put to question the reliability of identification based purely on microscopic morphometric methods. This diagnostic procedure is not only expensive and time-consuming, but also unreliable since the different species have overlapping properties and the intra-species variation is substantial (Joyner & Long, 1974; Pelle´rdy, 1974; Long & Joyner, 1984; Thebo *et al.*, 1998).

CHAPTER FIVE

MOLECULAR CHARACTERISATION OF *EIMERIA* SPECIES OF COMMERCIAL CHICKENS AND DETERMINATION OF RELATIVE ABUNDANCE

5.1 Introduction

Traditionally, seven *Eimeria* species are recognized to infect the chicken, exhibiting variable levels of pathogenicity. Species can be distinguished by oocyst morphology, pre-patent period, site of infection or minimum sporulation time, but all of these methods are labour intensive, time consuming and can be very difficult and unreliable with a mixed sample. Accurate determination of which species of *Eimeria* are present in poultry faeces or litter is difficult, because the size and morphology of most *Eimeria* species oocysts are nearly identical.

Although, pioneering work using conventional Polymerase Chain Reaction (PCR) with nucleotide sequence-derived markers has greatly improved specific diagnosis, it lacks the ability to quantify the number of *Eimeria* oocysts present in samples. Classical non-quantitative PCR methods for molecular diagnosis of the seven *Eimeria* species that infect chickens have been developed using targets including internal transcribed spacer or ITS-1 sequences (Schnitzler *et al*., 1999; Haug *et al.,* 2007) or sequence characterized amplified regions – SCARs (Fernandez *et al.,* 2003a). Diagnosis can be complicated in chickens with mixed-species infections and polymorphism within the target region can affect sensitivity (Morgan *et al*., 2009). Quantitative PCR is capable of identifying, differentiating among and quantifying the *Eimeria* species present in chickens even with mixed-species infections. It is more sensitive than conventional PCR because while Real-Time PCR can detect as little as two fold amplification, Agarose gel resolution required about 10 folds (Morgan *et al*., 2009).

Quantitative PCR provides a powerful tool for epizootiological investigations and has several applications, including investigations into coccidiosis outbreaks (causative species and disease thresholds), assessment of disease risk, the monitoring of the effectiveness of vaccination programmes and minimization of vaccine costs through the use of targeted treatment regimens.

There are four previously published real time quantitative PCR (qPCR) markers developed for *Eimeria* that infect the chicken which are based on either of the following assays.

(a) The one utilizing Sequenced Characterized Amplifying Regions (SCAR) targets which have been confirmed to be non-polymorphic (Blake *et al*., 2008).

(b) The one using the ribosomal ITS-2 DNA sequence as the target (Morgan *et al.,* 2009).

(c) The one using the SCAR target in supplementation with a gene specific assay targeting the ribosomal 5S repeat (Vladimir Vrba *et al.* 2010).

The ITS regions are present in multiple copies per genome and many share the same sequence, there is some degree of variability both within a genome as well as between strains and species (Cantacessi *et al*., 2008). Although there is the advantage of higher sensitivity when using a multi-copy ITS targets, the Sequence Characterized Amplified Region (SCAR) which is a non-polymorphic single-copy target is better suited for specific detection and precise quantification of multiple species in a mixture (Valdimir Vrba *et al* 2010). All seven qPCR markers used in this study (courtesy of Vladimir Vrba of Biopharm Research Institute of Biopharmacy and veterinary drugs) was sequenced from multiple strains and confirmed to be non-polymorphic and identical to the original SCAR sequence. Sequences conserved within each species were chosen with the aim of developing genuinely universal markers, providing global coverage. An exact match for the primers and TaqMan® probe during PCR cycling enables precise relative quantification of multiple species in a mixture regardless of the strains present. All markers utilized in these qPCR

assays are according to Biopharm institute, absolutely species-specific and support reproducible quantification across a wide linear range, unaffected by the presence of non-target species or other contaminating DNA. The sensitivity of these assays indicated that DNA equivalent to a single sporulated oocyst can be consistently detected. (Vladimir Vrba *et al*., 2010). The ability to prepare and isolate pure DNA from a variety of sources is an important step in many molecular biology protocols. For successful identification, it is necessary to have an efficient procedure for DNA extraction from oocysts. Protocols from commercial extraction kits are commonly used to break the rigid oocyst wall (Fernandez *et al*., 2003b; Meireles *et al.*, 2004; Haug *et al*., 2007).

5.2 Objectives

(a) To extract the genomic DNA from *Eimeria* oocysts isolated in study two above.

(b) To run a Real-time qPCR analysis of the DNA isolated.

(c) To characterize the *Eimeria* species from the qPCR plots obtained and determine the relative presence (abundance) of the species.

(d) To compare morphometric and molecular identification of *Eimeria* species and determine their level of agreement.

5.3 Material and Methods

The samples of the oocysts isolated in the morphometric section in chapter four were pooled together on the basis of their epizootiological categories, making a total of 12 samples in all, six for each state. Genomic DNA was extracted from these 12 samples and subjected to real time quantitative PCR analysis.

5.3.1 Extraction of DNA from *Eimeria* Oocysts

One µl of the pooled oocyst sample for each of the epizootiological categories was washed with an equal volume of Phosphate Buffer Saline (PBS) in a 2 ml Eppendorf tubes three times by centrifuging at 13,000 g for 1 minute in order to remove the potassium dichromate used in preserving the oocysts.

The resulting pellet was re-suspended in 100 µl of PBS. It was transferred into a screw cap tube with 0.5µg of glass bead added and vortexed for 3 minutes to break the resilient oocyst walls in order to extract the DNA from the sporozoites. A drop of the sample was examined under the microscope to confirm disruption of the oocyst wall under x40 objective. After confirmation of the disruption of the oocyst wall, 200µl of ATL from Qiagen DNeasy blood and tissue kit was added and vortexed briefly. After this, 20µl proteinase K was added to the mixture and vortexed. It was incubated at 56^0C for 30 minutes to enable efficient tissue lysis.

The lysate was put in a new tube and 200µl of AL buffer was added and vortexed. Also, 200µl of ethanol was added to the sample and vortexed. The resulting suspension was transferred into a DNeasy column and centrifuged at 13,000 g for 1 minute. The flow-through was discarded and the spin column put inside a new 2ml collection tube. It was then washed with 500µl AW1 and spinned at 13,000 g for 1 minute. The flow-through was similarly discarded and the spin column put inside another new 2 ml collection tube. This was also washed with 500µl AW2 and spinned at 13000 g for 3 minutes to dry the DNeasy membrane of residual ethanol so as not to interfere with subsequent reactions.

It was then eluted with 100µl AE buffer into clean 2 ml micro centrifuge tubes and centrifuged for 1 minute at 8000 g. The flow-through contained the genomic DNA samples of the oocysts and stored in a refrigerator for preservation.

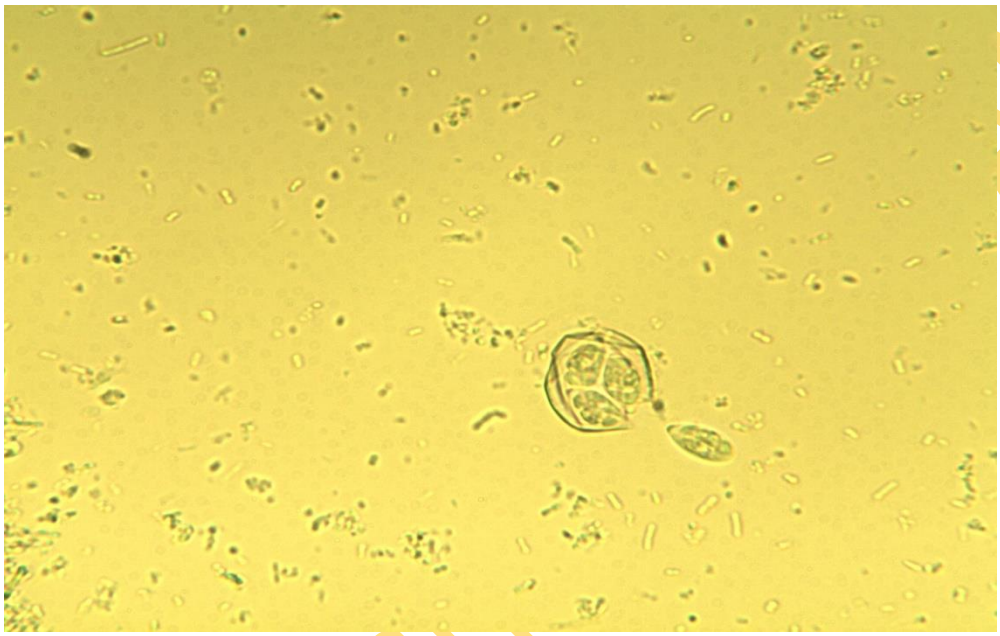


Figure 5.1 Sporulated *Eimeria* Oocyst disrupted and Sporocyst released after Vortexing

5.3.2 Quantitative Real Time Polymerase Chain Reaction Technique

The DNA sample was removed from the fridge and 1µl was taken by pipette into a well in the 96 plate qPCR plate in triplicates. 1 mL of the positive and negative controls was put in the 11^{th} and 12^{th} column respectively. The following were measured, vortexed and mixed together in a 2ml qPCR tube (**A**): 500µl master mix containing 150mM Tris-Hcl, 40mM $(NH4)_2$ SO_4, 0.02% Tween 20, 5mM $MgCl_2$, 400µM d ATP, 400µM d CTP, 400µM d GTP, 400µM d TTP and 50µ/mL Taq DNA polymerase; 297µl qPCR water and 3µl ROX dye. It was briefly vortexed after mixing. Into another 2ml qPCR tube **(B)** was measured the following after being vortexed initially: 10µl of Primer, 5µl of the probe and 175µl of qPCR water. It was vortexed again after the additions. Subsequently 8µL from the mixture in A and 1µL of B was added to all the rows and columns of the qPCR plate.

The qPCR plate was covered with the optical cover provided and pressed down without allowing finger print impression marks on it. The plate was centrifuged briefly for a few seconds to allow a proper settlement of the constituents in the wells of the qPCR plates. The thermo-cycler was already put on to warm it up to the required temperature of 95 ^{0} C. The qPCR plate was put inside the thermo-cycler. The thermal cycling program used was initial denaturation at 95 ^{0} C for 1 minute followed by 40 cycles of denaturation at 95 ^{0}C for 15 seconds and combined annealing and extension at 60^0 C for 30 seconds.

Fluorescence data were collected at the end of each cycle. Each sample was run in triplicate. The result was then analysed on the computer after the end of the cycling period. The quantitative real-time PCR reactions were performed in a Stratagene Mx3005P® real-time qPCR cycler. Both positive and negative template controls were included. The resulting data were processed using Mx ProTM software (Stratagene, USA). Threshold cycles (Ct) were calculated from baseline-corrected normalized fluorescence (dRn) data.

5.3.3 Determination of Relative Abundance of *Eimeria* Species

Relative abundance refers to the percentage contribution of each of the *Eimeria* species to the sample under study in which coccidial infection has been confirmed. These data are automatically generated by the computer software during the qPCR process.

5.3.4 Comparism of Morphometric and Molecular Methods of *Eimeria* Species Identification

The result of *Eimeria* species identification carried out by morphometric method was compared with the one carried out by molecular method to determine their level of agreement between the two. Agreement is defined when an *Eimeria* specie is identified by molecular method and at least one oocyst of the corresponding oocyst length category was also detected by morphometry and vice versa.

Table 5.1 Primer and Probe Sequences

Species	Sequence source (SCARdb ID)	Primer and probe sequences	Amplicon size [
E. acervulina	Ac-AD18-953	ACE-F: GCAGTCCGATGAAAGGTATTTG ACE-R: GAAGCGAAATGTTAGGCCATCT ACE-P: [6-FAM]ACAGTCCCCGCTGATGGTGTAACG[BHQ1]	103
E. brunetti	Br-J18-626	BRU-F: AGCGTGTAATCTGCTTTTGGAA BRU-R: TGGTCGCAGACGTATATTAGGG BRU-P: [6-FAM]CAACCGCAGCAAGCGAAGTTGA[BHQ1]	118
E. maxima	EmMIC1	MAX-F: TCGTTGCATTCGACAGATTC MAX-R: TAGCGACTGCTCAAGGGTTT MAX-P: [6-FAM]ATTGTCCAGCCAAGGTTCCCTTCG[BHQ1]	138
E. mitis	Mt-A09-716	MIT-F: CAAGGGGATGCATGGAATATAA MIT-R: CAAGACGAATGGAATCAATCTG MIT-P: [6-FAM]CCCGCGAGGGTTTCAGTTGATG[BHQ1]	115
E. necatrix	Nc-AD10-702	NEC-F: AACGCCGGTATGCCTCGTCG NEC-R: GTACTGGTGCCAACGGAGA NEC-P: [6-FAM]CCGTAGCATAGCTCAGGCAGCCAC[BHQ1]	134
E. praecox	Pr-A09-1108	PRA-F: CACATCCAATGCGATATAGGG PRA-R: ACAGAAAAACGCAAAGAGCAA PRA-P: [6-FAM]AGCAGCAGCTGCCTCTCATTGACC[BHQ1]	117
E. tenella	Tn-E03-1161	TEN-F: TCGTCTTTGGCTGGCTATTC TEN-R: CAGAGAGTCGCCGTCACAGT TEN-P: [6-FAM]CTGGAAAGCGTCTCCTTCAATGCG[BHQ1]	100

Courtesy of Vladmir Vrba, Damer P Blake and Martin Poplstein of BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, a.s., Pohori-Chotoun, Jilove u Prahy 25449, Czech Republic.

5.4 Result

5.4.1 Result of Quantitative PCR Analysis for Ogun State

The result of the qPCR analysis of the six samples of epizootiological categories OG 1A (1 to 8 weeks birds on vaccine prophylaxis), OG 1B (1 to 8 weeks birds on chemoprophylaxis), OG 2A (9 to 18 weeks birds on vaccine prophylaxis), OG 2B (9 to 18 weeks birds on chemoprophylaxis), OG 3A (Birds aged above 19 weeks in cages) and OG 3B (Birds aged above 19 weeks in cages) from Ogun State is shown in the qPCR plots of figures 5.1 to 5.6. It confirmed the presence of five *Eimeria* species. They are *Eimeria acervulina, Eimeria maxima, Eimeria necatrix, Eimeria tenella* and *Eimeria mitis.*

Each of the six epizootiological categories has two or more *Eimeria* species present. OG 1A has the presence of *E. acervulina, E. necatrix* and *E. mitis* while OG 1B has *E. acervulina, E .tenella* and *E. mitis.* OG 2A has *E. acervulina, E. tenella, E. maxima, E. necatrix* and *E. mitis* while *E. tenella* and *E. mitis* was found in OG 2B. OG 3A has *E. acervulina* and *E. necatrix* while OG 3B has *E. acervulina* and *E. necatrix. E. acervulina, E. maxima, E. necatrix, E. tenella* and *E. mitis* was present in 83.3%, 16.7%, 50%, 66.7%, and 66.7% of the coccidial infection in the epizootiological groups respectively. *Eimeria brunetti* and *E. praecox* was not detected in any of the six groups. All the 5 species was present in those birds that were vaccinated with coccidiosis vaccine whereas *E. maxima* were not found in the group that received chemoprophylaxis.

1. OG 1A

species	qPCR Ct cycle	abundance
Eimeria acervulina	29,76	25,677%
Eimeria maxima	No Ct	not detected
Eimeria necatrix	34,64	4,348%
Eimeria tenella	27,89	62,839%
Eimeria brunetti	No Ct	not detected
Eimeria mitis	34,01	7,137%
Eimeria praecox	No Ct	not detected

2. OG 1B

species	qPCR Ct cycle	abundance
Eimeria acervulina	36,92	3,844%
Eimeria maxima	No Ct	not detected
Eimeria necatrix	No Ct	not detected
Eimeria tenella	35,76	8,862%
Eimeria brunetti	No Ct	not detected
Eimeria mitis	34,60	87,294%
Eimeria praecox	No Ct	not detected

3. OG 2A

species	qPCR Ct cycle	abundance
Eimeria acervulina	31,89	22,433%
Eimeria maxima	37,89	1,799%
Eimeria necatrix	No Ct	not detected
Eimeria tenella	34,12	4,930%
Eimeria brunetti	No Ct	not detected
Eimeria mitis	32,44	70,838%
Eimeria praecox	No Ct	not detected

4. OG 2B

species	qPCR Ct cycle	abundance
Eimeria acervulina	No Ct	not detected
Eimeria maxima	No Ct	not detected
Eimeria necatrix	No Ct	not detected
Eimeria tenella	38.38	4.377%
Eimeria brunetti	No Ct	not detected
Eimeria mitis	35.87	95.623%
Eimeria praecox	No Ct	not detected

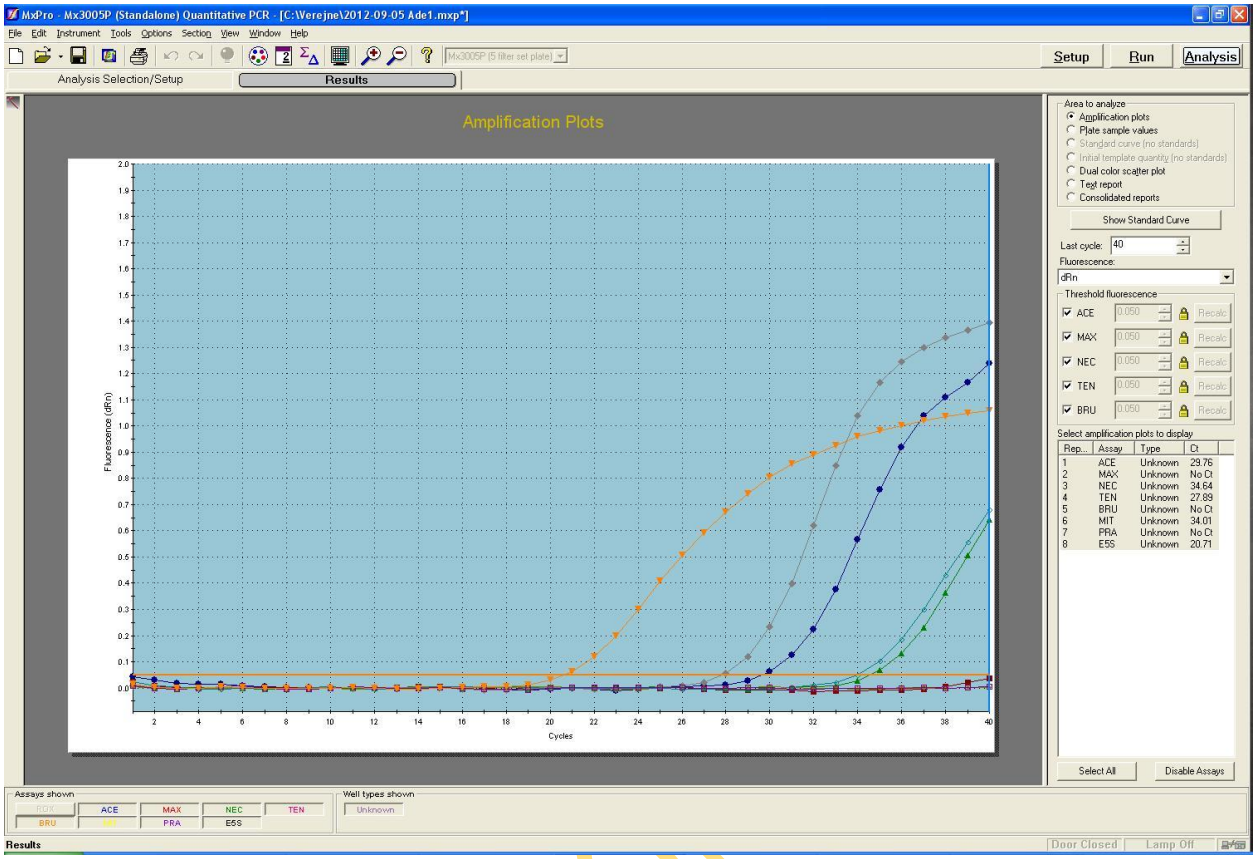
5. OG 3A

species	qPCR Ct cycle	abundance
Eimeria acervulina	36.37	13.829%
Eimeria maxima	No Ct	not detected
Eimeria necatrix	35.89	86.171%
Eimeria tenella	No Ct	not detected
Eimeria brunetti	No Ct	not detected
Eimeria mitis	No Ct	not detected
Eimeria praecox	No Ct	not detected

6. OG 3B

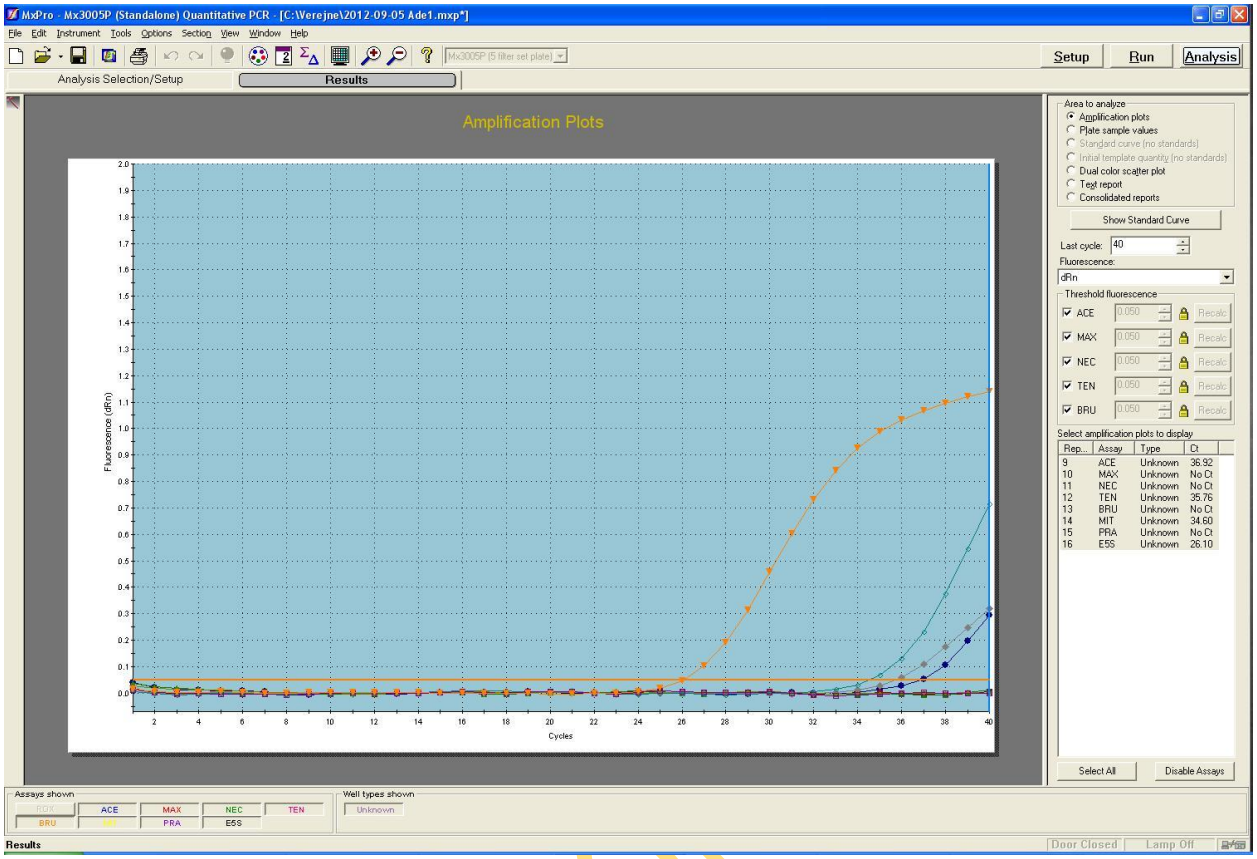
species	qPCR Ct cycle	abundance
Eimeria acervulina	33.68	54.498%
Eimeria maxima	No Ct	not detected
Eimeria necatrix	36.22	45.502%
Eimeria tenella	No Ct	not detected
Eimeria brunetti	No Ct	not detected
Eimeria mitis	No Ct	not detected
Eimeria praecox	No Ct	not detected

Figure 5.2 Result of Quantitative PCR Analysis for Ogun State



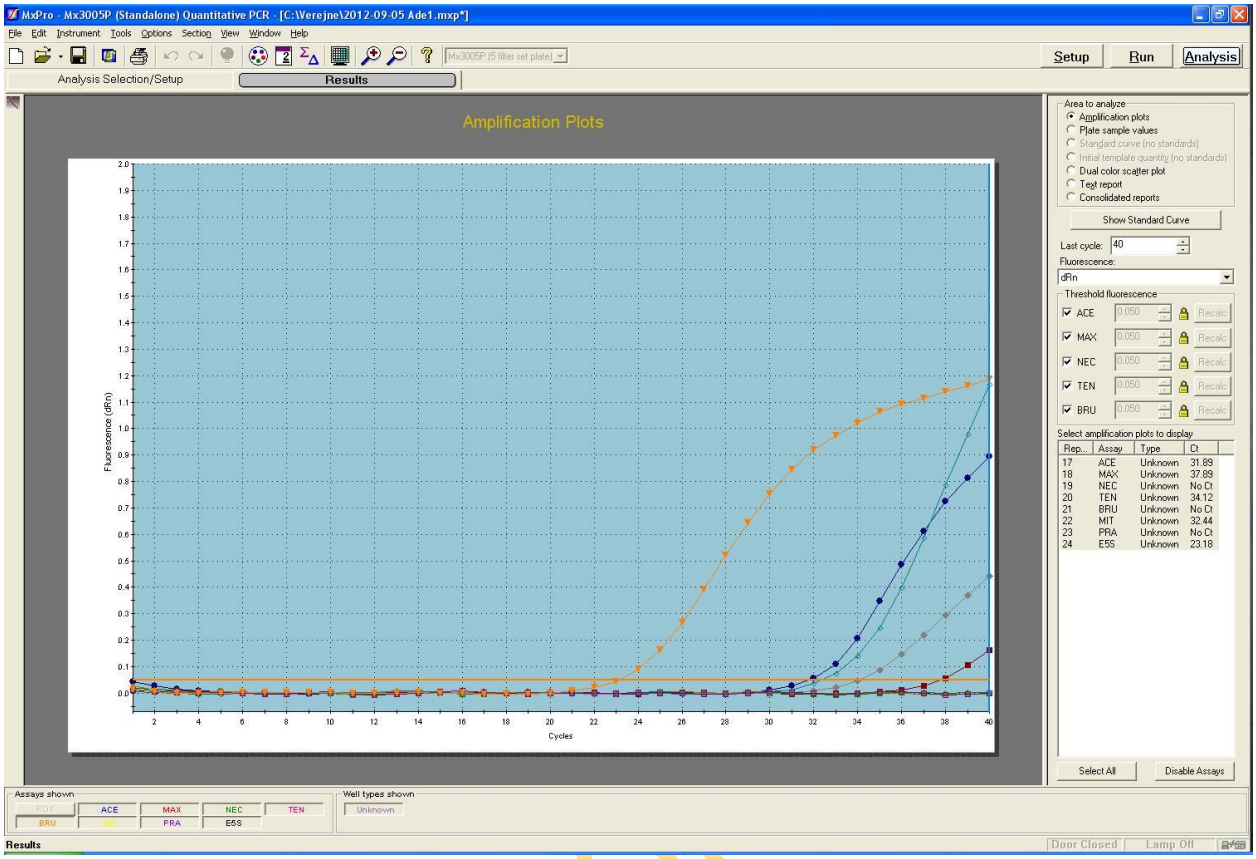
Ash colour = *E. tenella*; Blue = *E. acervulina*; Light green = *E. mitis;* Deep green = *E. necatrix*; Magenta = *E. maxima*

Figure 5.3 RTQPCR Plots for 1 to 8 Week-old Chickens on Vaccine Prophylaxis in Ogun State (OG 1A)



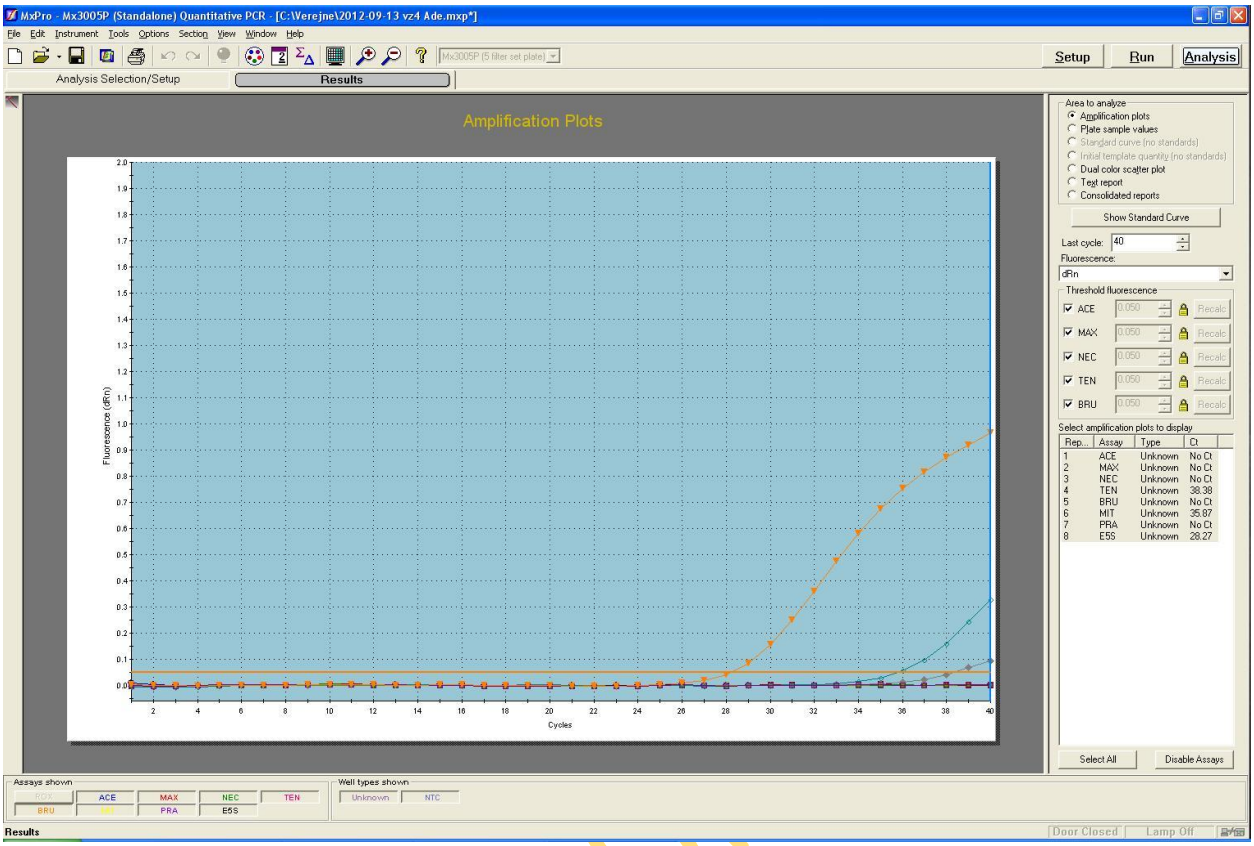
Ash colour = *E. tenella*; Blue = *E. acervulina*; Light green = *E. mitis;*

Figure 5.4 RTQPCR Plots for 1 to 8 Weeek-old Chickens in Ogun State on Chemoprophylaxis (OG 1B)



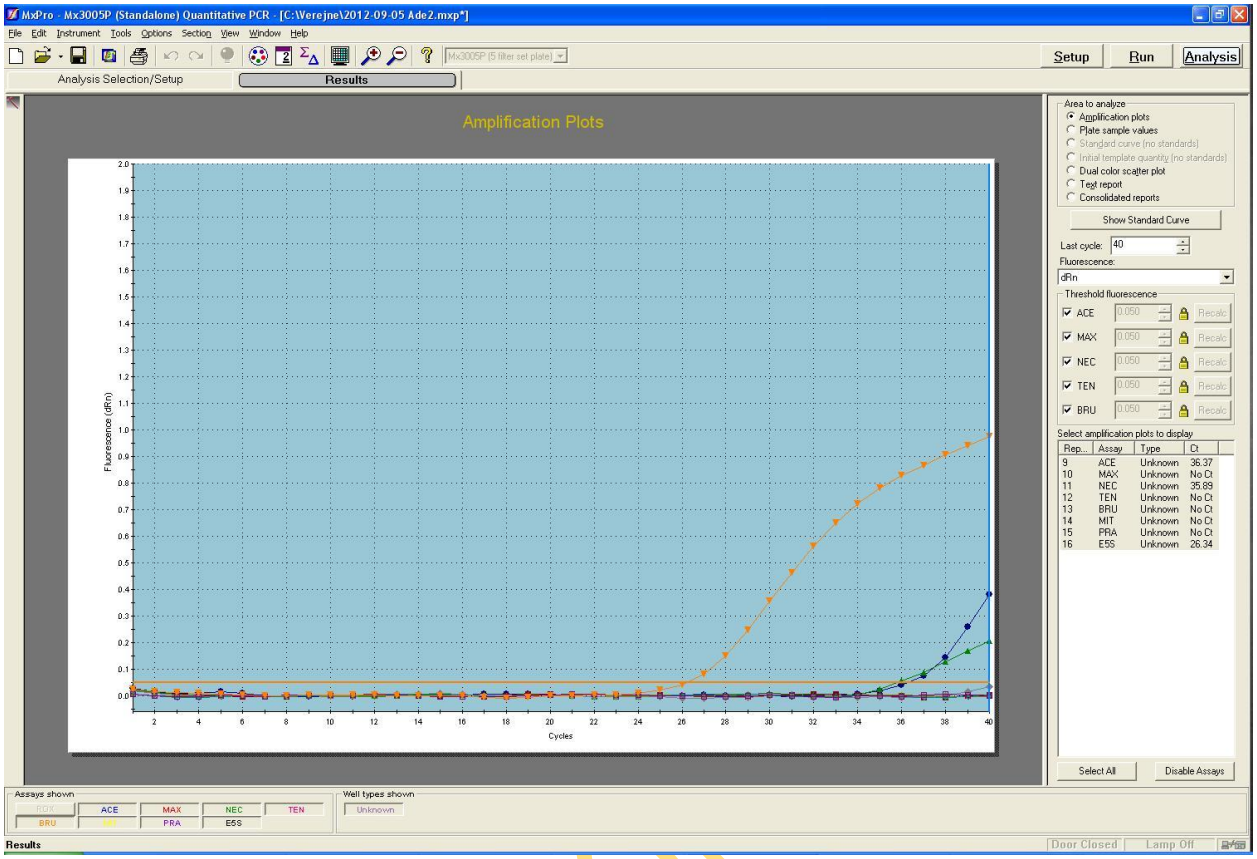
Ash colour = *E. tenella*; Blue = *E. acervulina*; Light green = *E. mitis*; Magenta = *E. maxima*

Figure 5.5: RTQPCR Plots for 9 to 18 Week-old Chickens in Ogun State on Vaccine Prophylaxis (OG 2A)



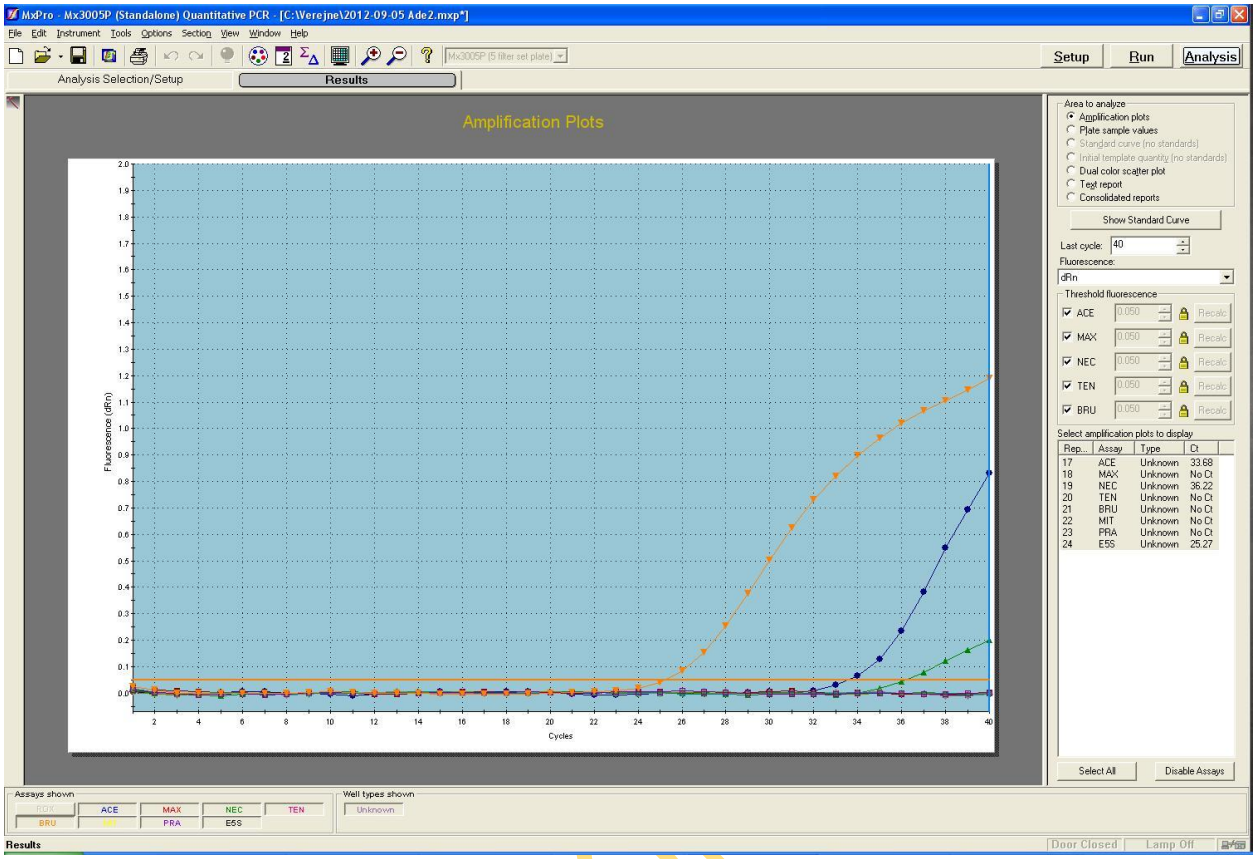
Ash colour = *E. tenella*; Light green = *E. mitis*;

Fig. 5.6: RTQPCR Plots for Chickens 9 to 18 Week-old in Ogun State on Chemoprophylaxis (OG 2B)



Blue = *E. acervulina*; Deep green = *E. necatrix*

Figure 5.7: RTQPCR plots for Chickens Aged above 19 Weeks in Cages in Ogun State (OG 3A)



Blue = *E. acervulina*; Deep green = *E. necatrix*

Figure 5.8: RTQPCR Plots for Chickens above 19 Weeks on Deep Litter in Ogun State (OG 3B)

5.4.2 Result of RTQPCR Plot for Oyo State

The result of the RTQPCR analysis of the samples of the six epizootiological categories in Oyo State namely OY 1A (Birds 1 to 8 weeks old in Oyo State on vaccine prophylaxis), OY 1B (Birds 1 to 8 weeks old in Oyo State on chemoprophylaxis), OY 2A (Birds 9 to 18 weeks old in Oyo State on Vaccine prophylaxis), OY 2B (Birds 9 to 18 weeks old in Oyo State on chemoprophylaxis), OG 3A (Birds above 19 weeks old in Oyo State in cages) and OG 3B (Birds above 19 weeks in Oyo State on deep litter) are shown in the RTQPCR plots in figures It confirmed the presence of 5 *Eimeria* species namely *E. acervulina, E. maxima, E. necatrix, E. tenella* and *E. mitis*. OY 1A has *E. acervulina, E. tenella and E. mitis* while OY 1B has *E. acervulina,* and *E. tenella.* OY 2A has *E. acervulina, E. maxima, E. necatrix, E. tenella* and *E. mitis* while OG 2B has *E. acervulina, E.necatrix* and *E mitis*. OY 3A has *E. acervulina* and *E. necatrix* while OY 3B has *E.acervulina* and *E.tenella.* The result further showed that *E. acervulina, E. maxima, E. necatrix, E. tenella* and *E .mitis* was present in 100%, 16.7%, 50%, 66.7% and 50% of the coccidial infections respectively. *E. praecox* and *E. brunetti* was not detected. All the 5 species was present in those birds that were vaccinated with coccidiosis vaccine whereas *E. maxima* was not found in the group that received chemoprophylaxis.

7. OY1A

species	qPCR Ct cycle	abundance
Eimeria acervulina	34,19	20,820%
Eimeria maxima	No Ct	not detected
Eimeria necatrix	No Ct	not detected
Eimeria tenella	34,15	20,870%
Eimeria brunetti	No Ct	not detected
Eimeria mitis	35,02	58,311%
Eimeria praecox	No Ct	not detected

8. OY 1B

species	qPCR Ct cycle	abundance
Eimeria acervulina	33,50	48,234%
Eimeria maxima	No Ct	not detected
Eimeria necatrix	No Ct	not detected
Eimeria tenella	33,29	51,766%
Eimeria brunetti	No Ct	not detected
Eimeria mitis	No Ct	not detected
Eimeria praecox	No Ct	not detected

9. OY 2A

species	qPCR Ct cycle	abundance
Eimeria acervulina	29,45	37,825%
Eimeria maxima	36,97	1,058%
Eimeria necatrix	34,16	7,104%
Eimeria tenella	33,07	3,109%
Eimeria brunetti	No Ct	not detected
Eimeria mitis	31,23	50,905%
Eimeria praecox	No Ct	not detected

10. OY 2B

species	qPCR Ct cycle	abundance
Eimeria acervulina	31.60	49.269%
Eimeria maxima	No Ct	not detected
Eimeria necatrix	36.91	6.562%
Eimeria tenella	No Ct	not detected
Eimeria brunetti	No Ct	not detected
Eimeria mitis	34.10	44.169%
Eimeria praecox	No Ct	not detected

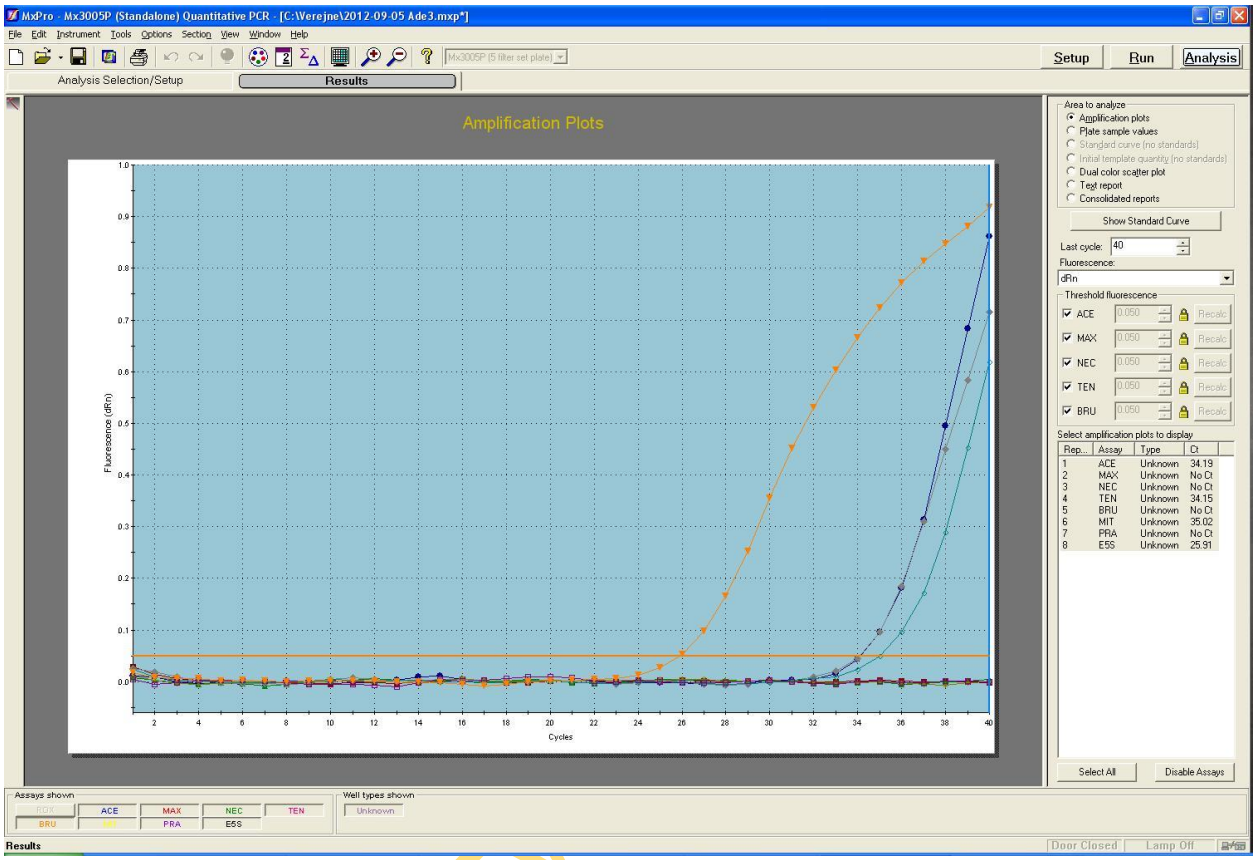
11. OY 3A

species	qPCR Ct cycle	abundance
Eimeria acervulina	32.06	81.394%
Eimeria maxima	No Ct	not detected
Eimeria necatrix	36.55	18.606%
Eimeria tenella	No Ct	not detected
Eimeria brunetti	No Ct	not detected
Eimeria mitis	No Ct	not detected
Eimeria praecox	No Ct	not detected

12. OY 3B

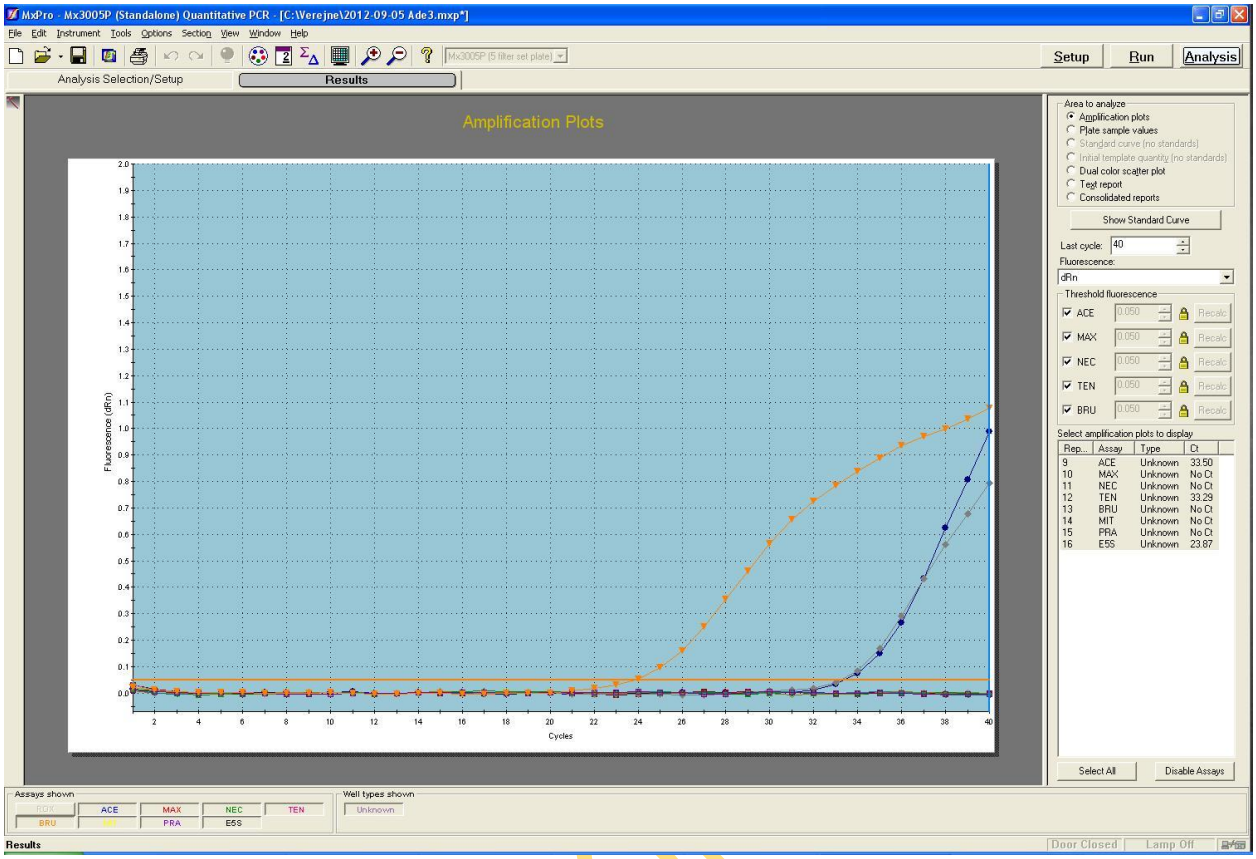
species	qPCR Ct cycle	abundance
Eimeria acervulina	33,85	54,832%
Eimeria maxima	No Ct	not detected
Eimeria necatrix	No Ct	not detected
Eimeria tenella	34,10	45,168%
Eimeria brunetti	No Ct	not detected
Eimeria mitis	No Ct	not detected
Eimeria praecox	No Ct	not detected

Figure 5.9 Result of RTQPCR Analysis for Samples in Oyo State



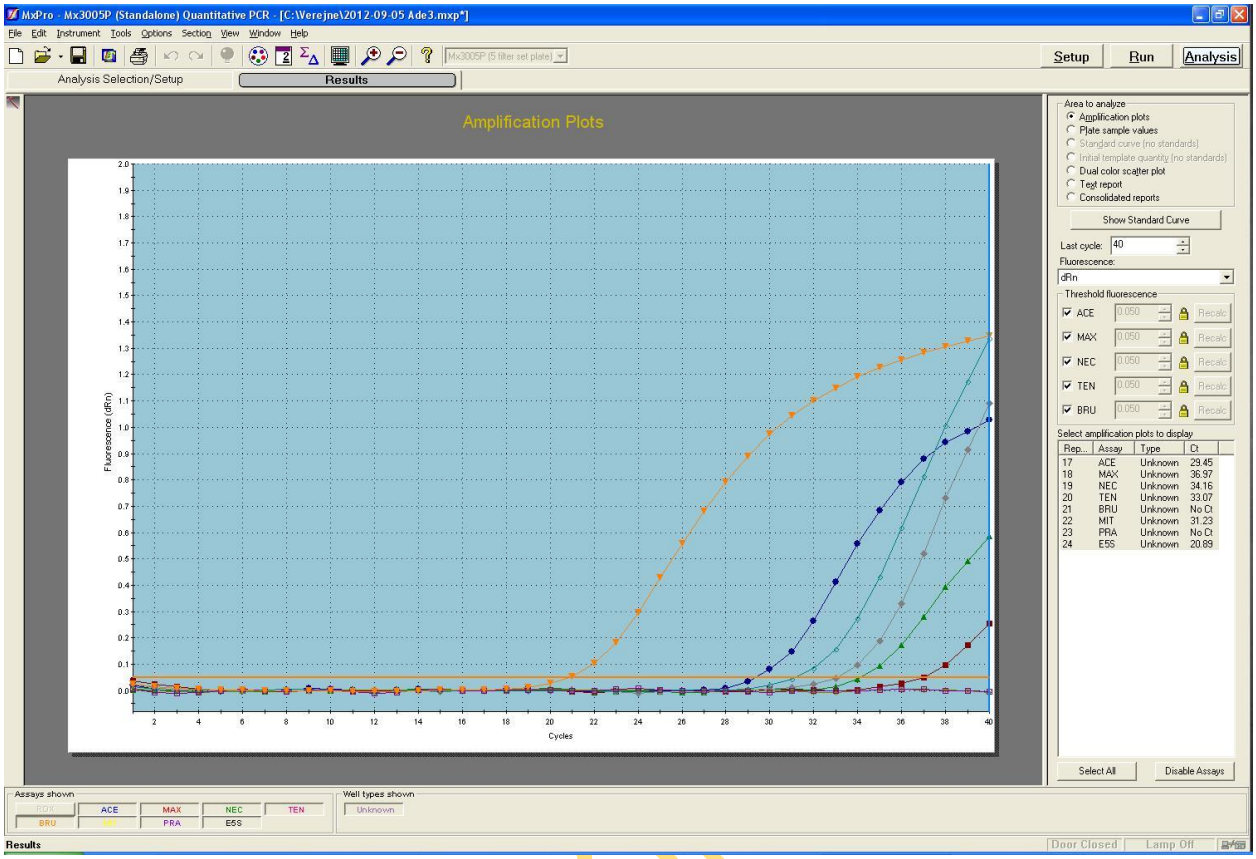
Ash colour = *E. tenella*; Blue = *E. acervulina*; Light green = *E. mitis*;

Figure 5.10 RTQPCR Plots for 1 to 8 Week-old Chickens in Oyo State on Vaccine Prophylaxis (OY 1A)



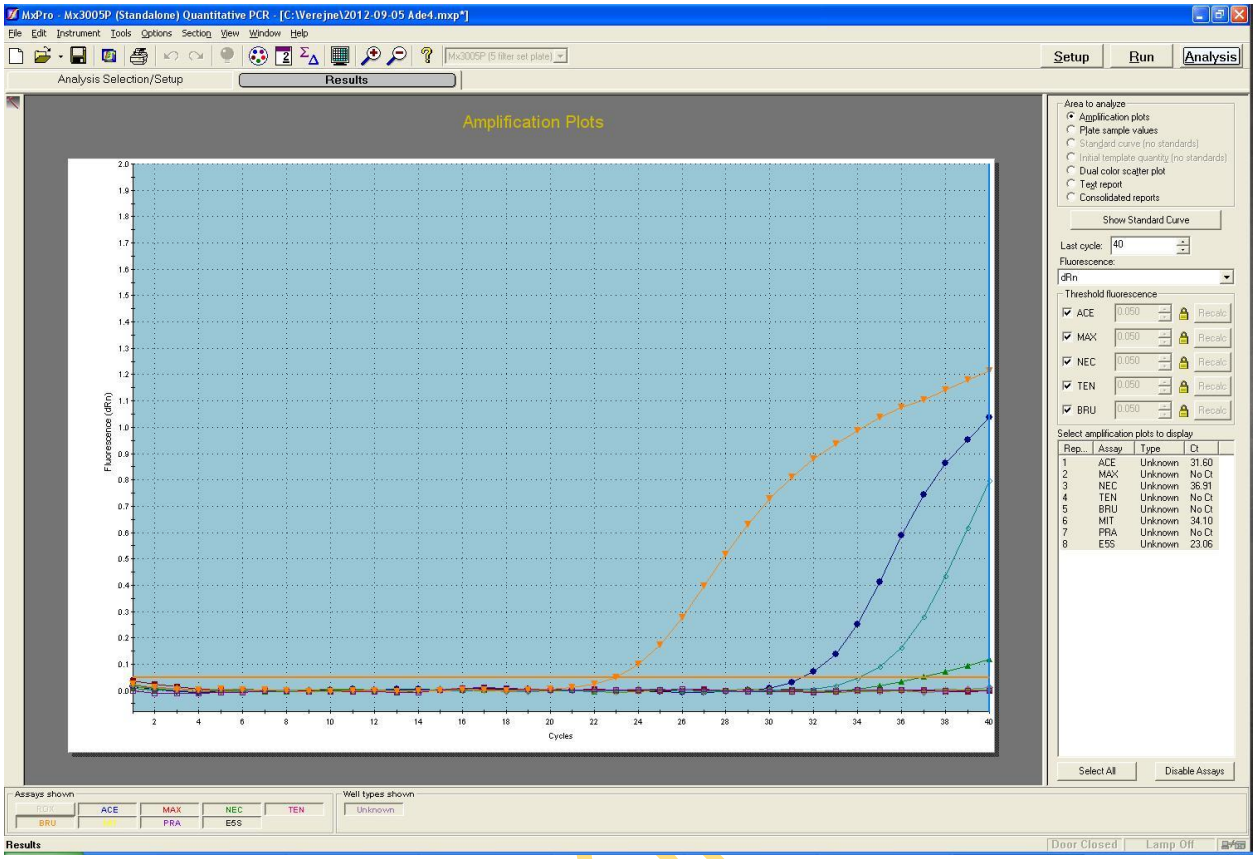
Ash colour = *E. tenella*; Blue = *E. acervulina*; Light green = *E. mitis*

Figure 5.11 RTQPCR Plots for 1 to 8 Week-old Chickens in Oyo State on Chemoprophylaxis (OY 1B)



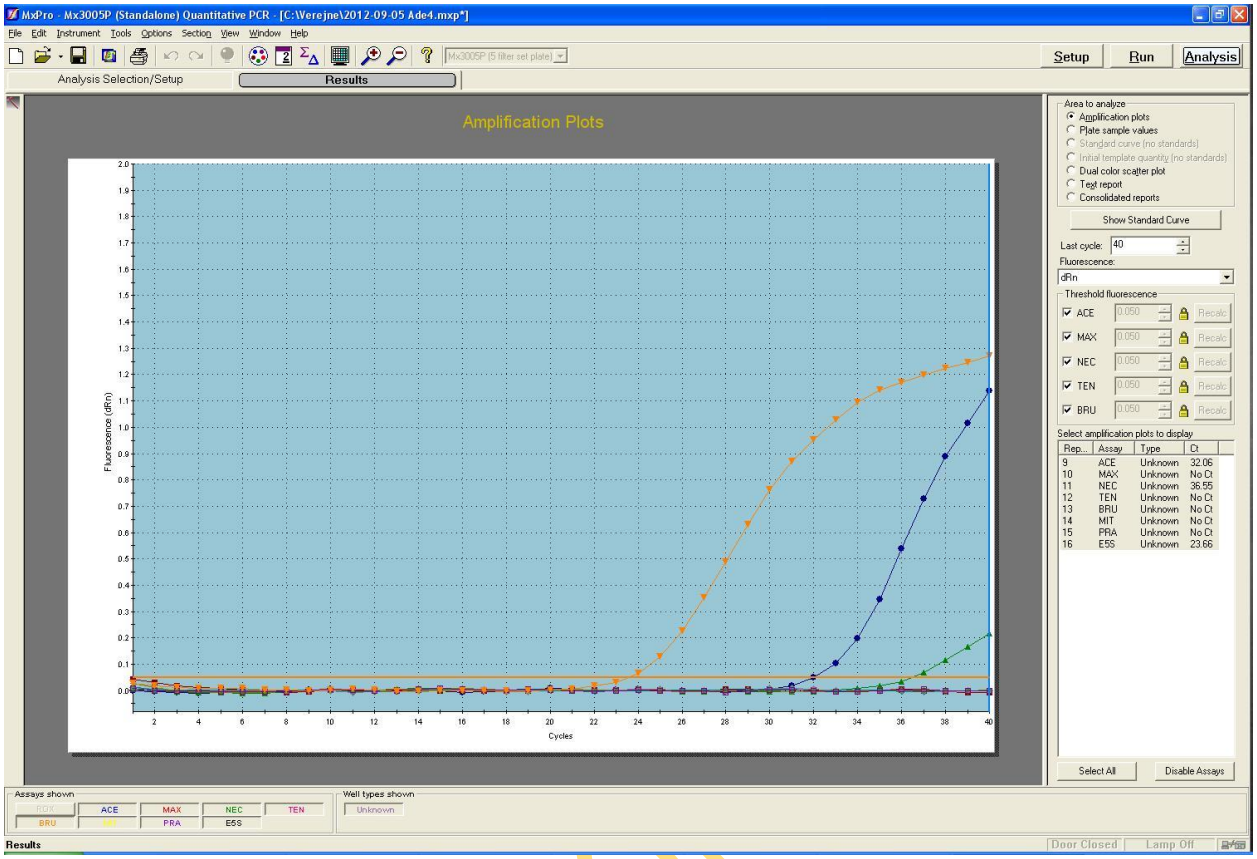
Ash colour = *E. tenella*; Blue = *E. acervulina*; Light green = *E. mitis*; Deep green = *E. necatrix;* Magenta = *E. maxima*

Figure 5.12 RTQPCR Plots for 9 to 18 Week-old Chickens in Oyo State on Vaccine Prophylaxis (OY 2A)



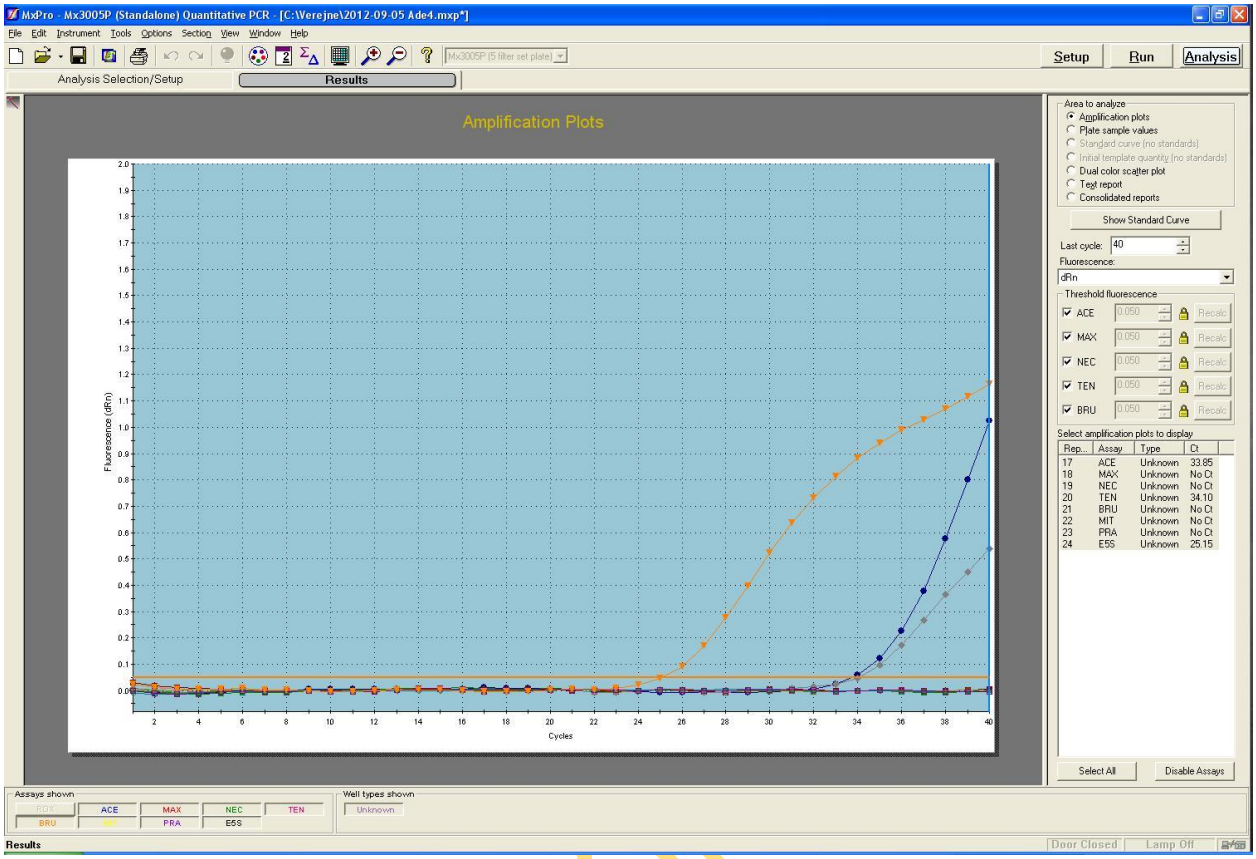
Blue = *E. acervulina*; Light green = *E. mitis*; Deep green = *E. necatrix*

Figure 5.13 RTQPCR Plots for 9 to 18 Week-old Chickens in Oyo State on Chemoprophylaxis (OY 2B)



Blue = *E. acervulina;* Deep green = *E. necatrix*

Figure 5.14 RTQPCR plots for above 19 week-old Chickens in Oyo State in Cages (OY 3A)



Ash colour = *E. tenella*; Blue = *E. acervulina;*

Figure 5.15 RTQPCR Plots for Chickens aged above 19 Weeks in Oyo State on Deep Litter (OY 3B)

Table 5.2 RTQPCR Analysis for Samples from Ogun and Oyo States

EC	*E. acervulina*	*E. maxima*	*E. necatrix*	*E. tenella*	*E. brunetti*	*E. mitis*	*E. praecox*
OG 1A	+	nd	+	+	nd	+	nd
OG 1B	+	nd	nd	+	nd	+	nd
OG 2A	+	+	nd	+	nd	+	nd
OG 2B	nd	nd	nd	+	nd	+	nd
OG 3A	+	nd	+	nd	nd	nd	nd
OG 3B	+	nd	+	nd	nd	nd	nd
OY 1A	+	nd	nd	+	nd	+	nd
OY 1B	+	nd	nd	+	nd	nd	nd
OY 2A	+	+	+	+	nd	+	nd
OY 2B	+	nd	+	nd	nd	+	nd
OY 3A	+	nd	+	nd	nd	nd	nd
OY 3B	+	nd	nd	+	nd	nd	nd

EC = Epizootiological category; Nd = Not detected; + = detected

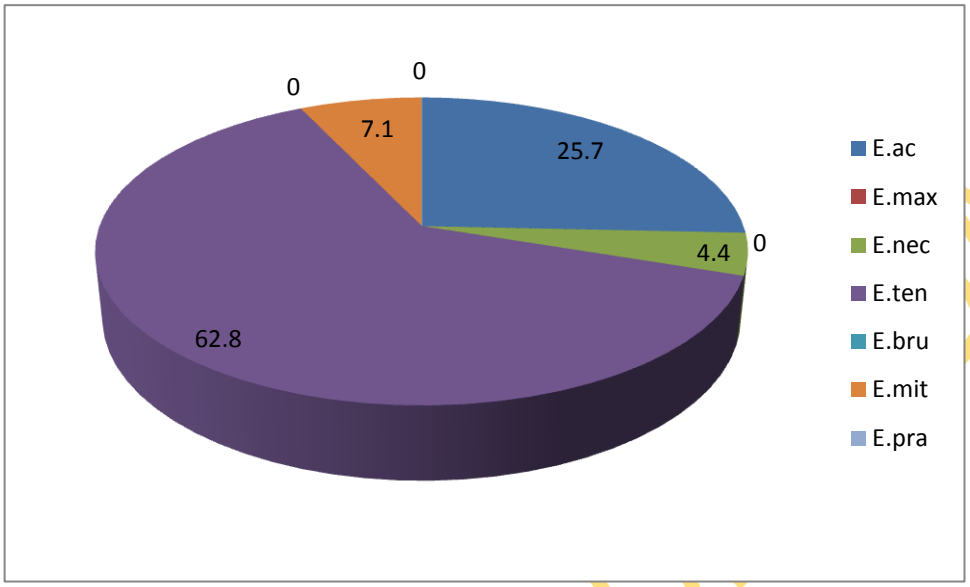
Table 5.3 Relative Abundance of *Eimeria* Species for Ogun and Oyo States

EC	*E. acervulina*	*E. maxima*	*E. necatrix*	*E. tenella*	*E. brunetti*	*E. mitis*	*E. praecox*
OG 1A	25.7%	0	4.4%	62.8%	0	7.1%	0
OG 1B	3.8%	0	0	8.9%	0	87.3%	0
OG 2A	22.4%	1.8%	0	4.9%	0	70.9	0
OG 2B	0	0	0	4.4%	0	95.6%	0
OG 3A	13.8%	0	86.2	0	0	0	0
OG 3B	54.5%	0	45.5	0	0	0	0
OY 1A	20.8%	0	0	20.9%	0	58.3%	0
OY 1B	48.2%	0	0	51.8%	0	0	0
OY 2A	37.8%	1.1%	7.1%	3.1%	0	50.9%	0
OY 2B	49.3%	0	6.5%	0	0	44.2%	0
OY 3A	81.4%	0	18.6%	0	0	0	0
OY 3B	54.8%	0	0	45.2%	0	0	0

5.4.3 Relative Abundance of *Eimeria* Species in Ogun State

Chickens aged between 1 to 8 weeks on vaccine prophylaxis were infected by four *Eimeria* species namely; *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria tenella* and *Eimeria mitis* with a relative abundance of 25.7%, 4.4%, 62.8% and 7.1% respectively. Chickens aged between 1 and 8 weeks on chemoprophylaxis were infected by 3 species namely; *Eimeria acervulina*, *Eimeria tenella* and *Eimeria mitis* with a relative abundance of 3.8%, 8.9% and 87.3% respectively. Chickens aged between 9 and 18 weeks on vaccine prophylaxis were infected by four *Eimeria* species namely; *Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella* and *Eimeria mitis* with a relative abundance of 22.4%, 1.8%, 4.9% and 70.9% respectively.

Chickens aged between 9 and 18 weeks on chemoprophylaxis were infected by two *Eimeria* species namely; *Eimeria tenella* and *Eimeria mitis* with a relative abundance of 4.4% and 95.6% respectively. Chickens aged above 19 weeks and kept in cages were infected by two *Eimeria* species namely; *Eimeria acervulina* and *Eimeria necatrix* with a relative abundance of 13.8% and 86.2% respectively. Chickens aged above 19 weeks but kept on deep litter were infected by two *Eimeria* species namely; *Eimeria acervulina* and *Eimeria necatrix* with a relative abundance of 54.5% and 45.5% respectively.



E.ac = Eimeria acervulina; E. max = Eimeria maxima; E.nec. = Eimeria necatrix;

E.ten = Eimeria tenella; E. bru = Eimeria brunette; E.mit = Eimeria mitis; E.pra = Eimeria praecox

Figure 5.16 Relative Abundance of *Eimeria* Species in 1 to 8 Week-old Chickens in Ogun State on Vaccine Prophylaxis (OG 1A)

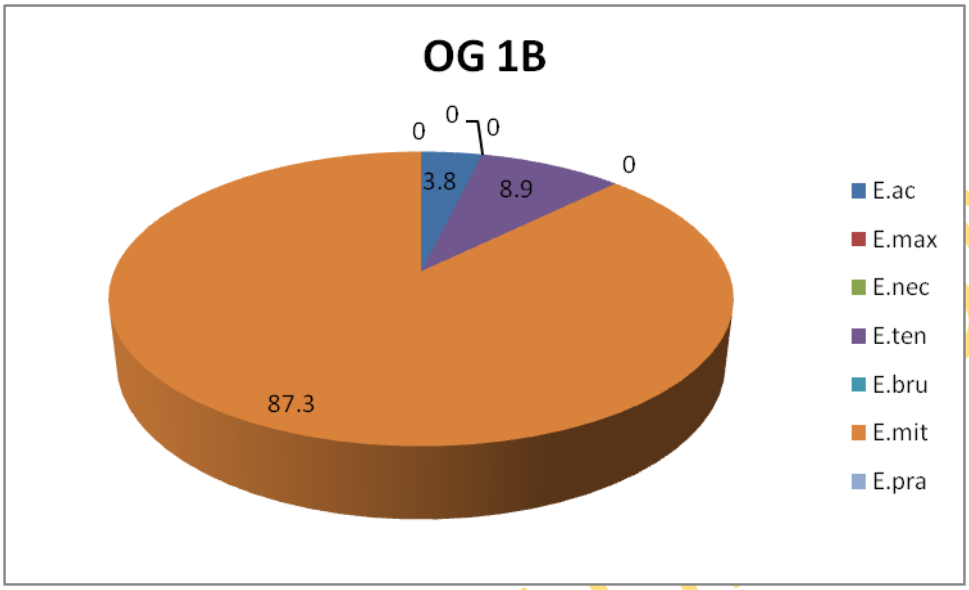


Figure 5.17 Relative Abundance of *Eimeria* Species in 1 to 8 Week-old Chickens in Ogun State on Chemoprophylaxis

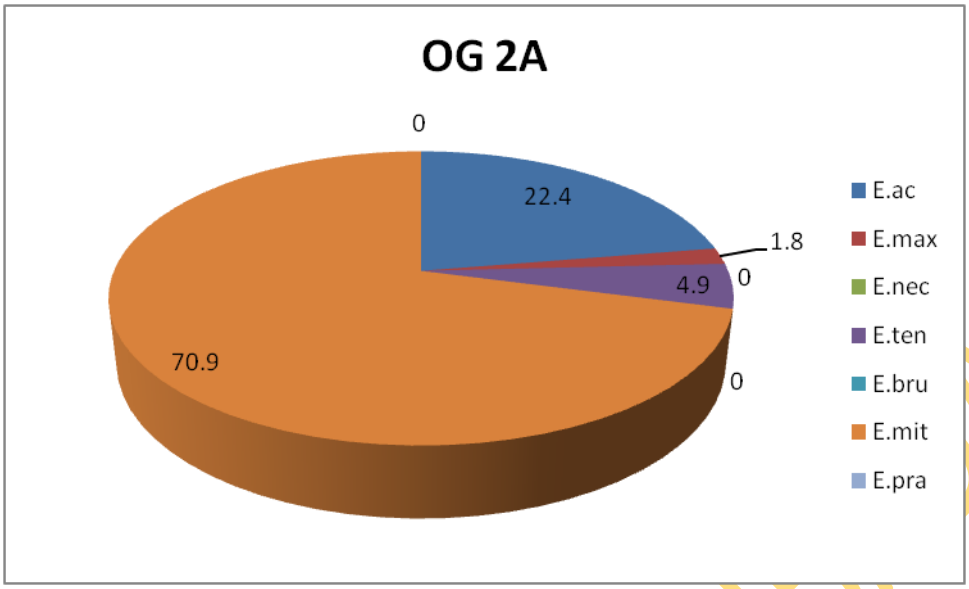


Figure 5.18 Relative Abundance of *Eimeria* Species in 9-18 Week-old Chickens in Ogun on Vaccine Prophylaxis

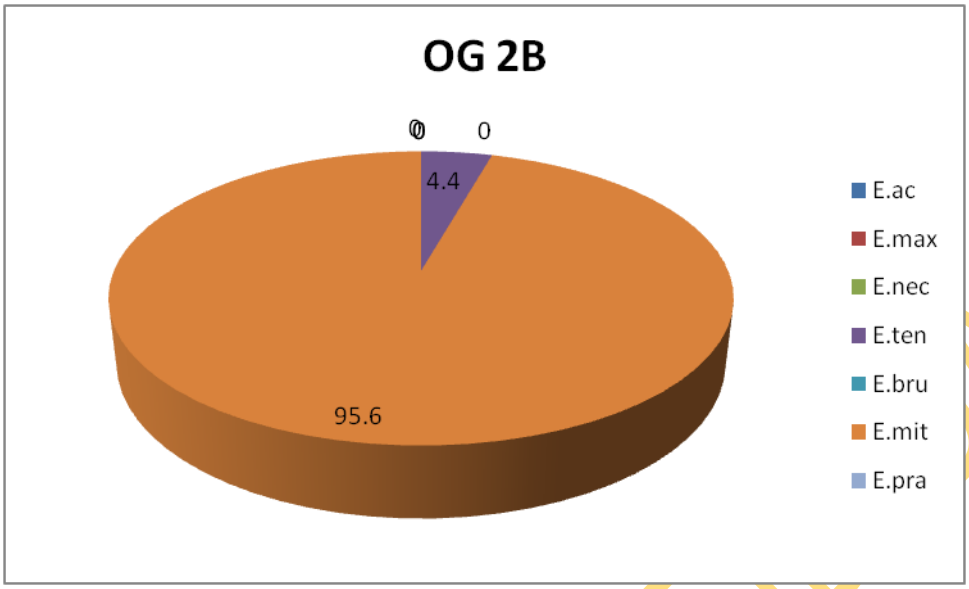


Figure 5.19 Relative Abundance of *Eimeria* Species 9-18 Week-old Chickens in Ogun on Chemo Prophylaxis

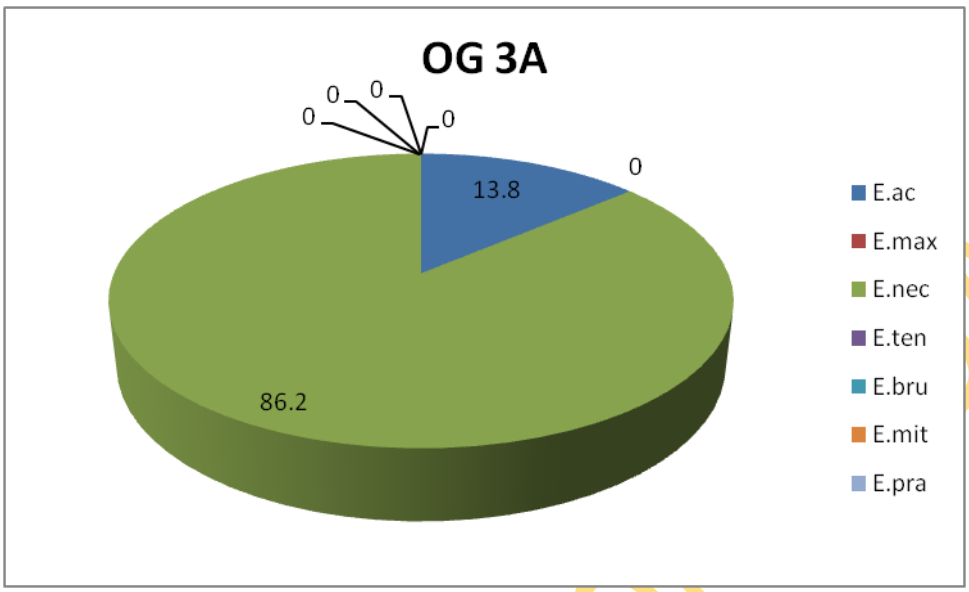


Figure 5.20 Relative Abundance of *Eimeria* Species in Chickens above 19 Weeks in Ogun State in Cages

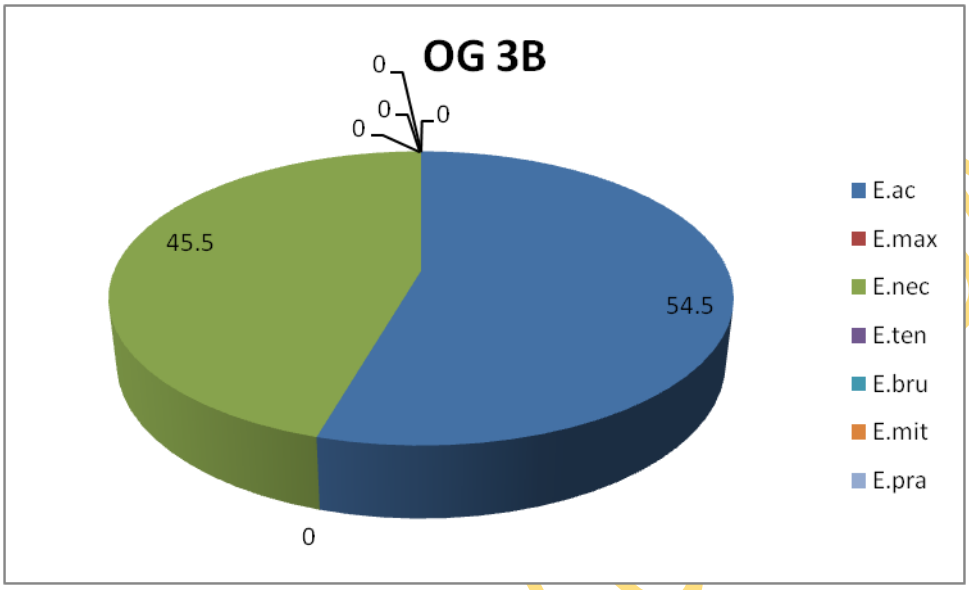


Figure 5.21 Relative Abundance of *Eimeria* Species in Chickens aged above 19 Weeks in Ogun State on Deep Litter

5.4.4 Relative Abundance of *Eimeria* Species in Oyo State

Birds aged between 1 to 8 weeks on vaccine prophylaxis were infected by three *Eimeria* species namely; *Eimeria acervulina*, *Eimeria tenella* and *Eimeria mitis* with a relative abundance of 20.8%, 4.4%, 20.9% and 58.3% respectively. Birds aged between 1 and 8 weeks on chemoprophylaxis were infected by two species namely; *Eimeria acervulina* and *Eimeria tenella* with a relative presence of 48.2% and 51.8% respectively. Birds aged between 9 and 18 weeks on vaccine prophylaxis were infected by five *Eimeria* species namely; *Eimeria acervulina*, *Eimeria maxima*, *Eimeria necatrix*, and *Eimeria mitis* with a relative abundance of 37.8%, 1.1%, 7.1%, 3.1% and 50.9% respectively. Birds aged between 9 and 18 weeks on chemoprophylaxis were infected by three *Eimeria* species namely; *Eimeria acrevulina*, *Eimeria necatrix* and *Eimeria mitis* with a relative abundance of 49.3%, 6.5% and 44.2% respectively. Birds aged above 19 weeks and kept in cages were infected by two *Eimeria* species namely; *Eimeria acervulina* and *Eimeria necatrix* with a relative abundance of 81.4% and 18.6% respectively. Birds aged above 19 weeks but kept on deep litter were infected by two *Eimeria* species namely; *Eimeria acervulina* and *Eimeria tenella* with a relative abundance of 54.8% and 45.2% respectively.

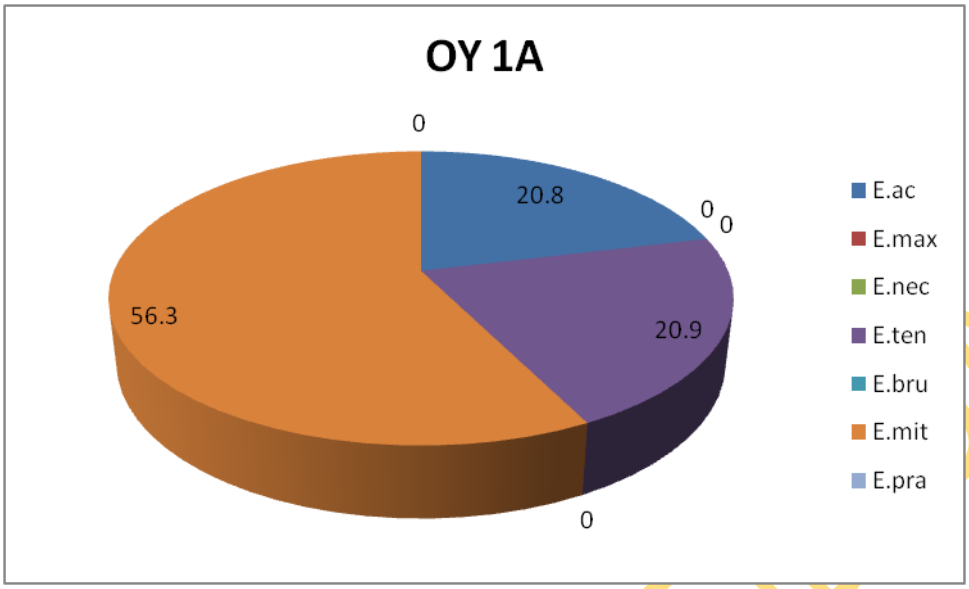


Figure 5.22 Relative Abundance of *Eimeria* Species 1-8 Week-old Chickens in Oyo State on Vaccine Prophylaxis

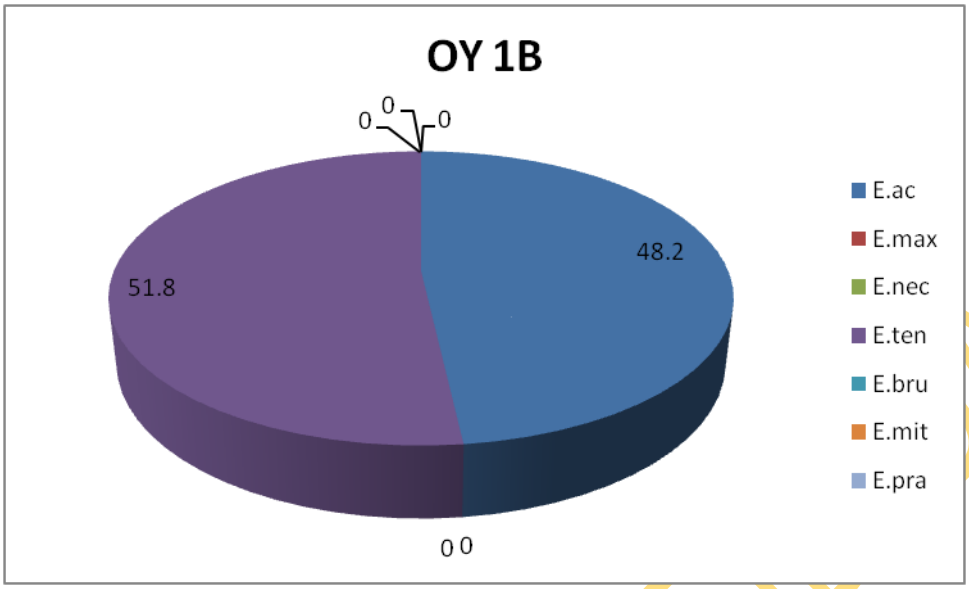


Figure 5.23 Relative Abundance of *Eimeria* Species in 1 to 8 Week-old Chickens in Oyo State on Chemoprophylaxis

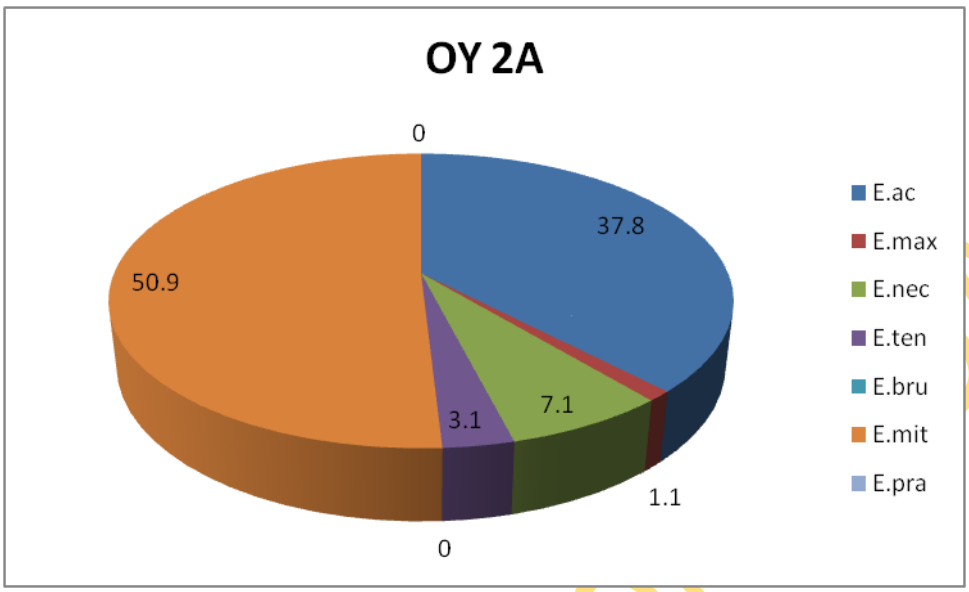


Figure 5.24 Relative Abundance of *Eimeria* Species in 9 to18 Week-old Chickens in Oyo State on Vaccine Prophylaxis

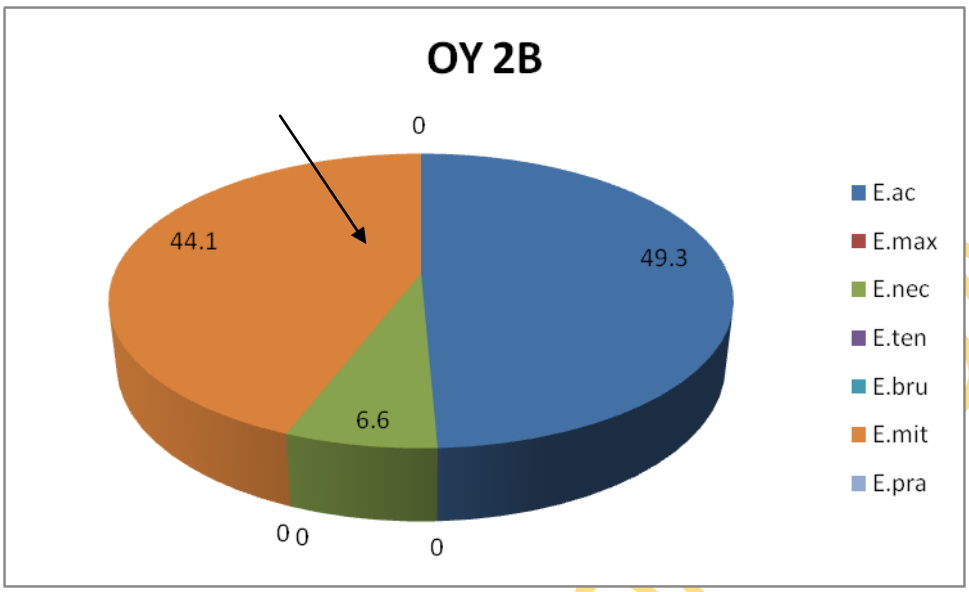


Figure 5.25 Relative Abundance of *Eimeria* Species in 9 to 18 Week-old Chickens in Oyo State on Chemoprophylaxis

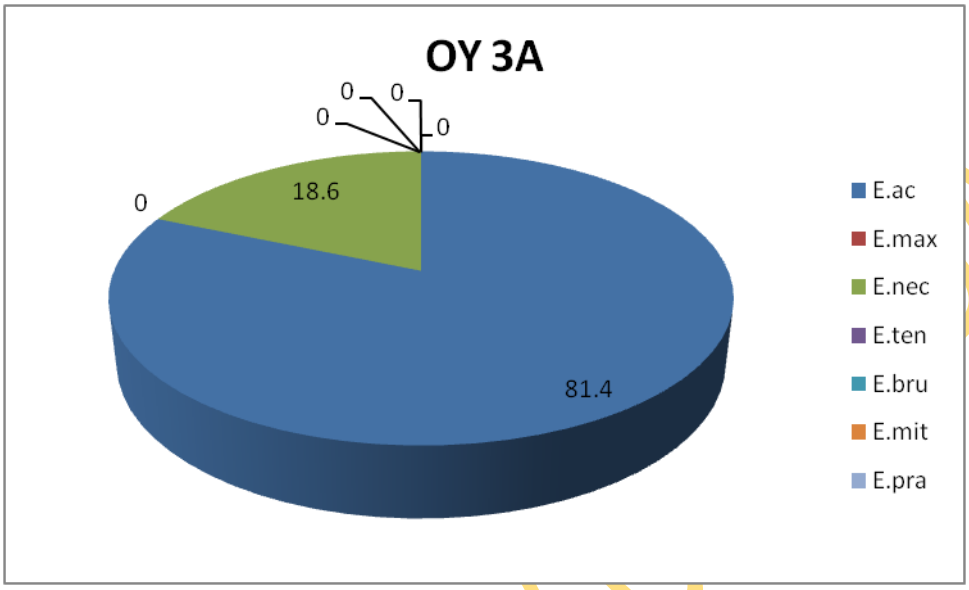


Figure 5.26 Relative Abundance of *Eimeria* Species in Chickens above 19 Weeks in Oyo State in Cages

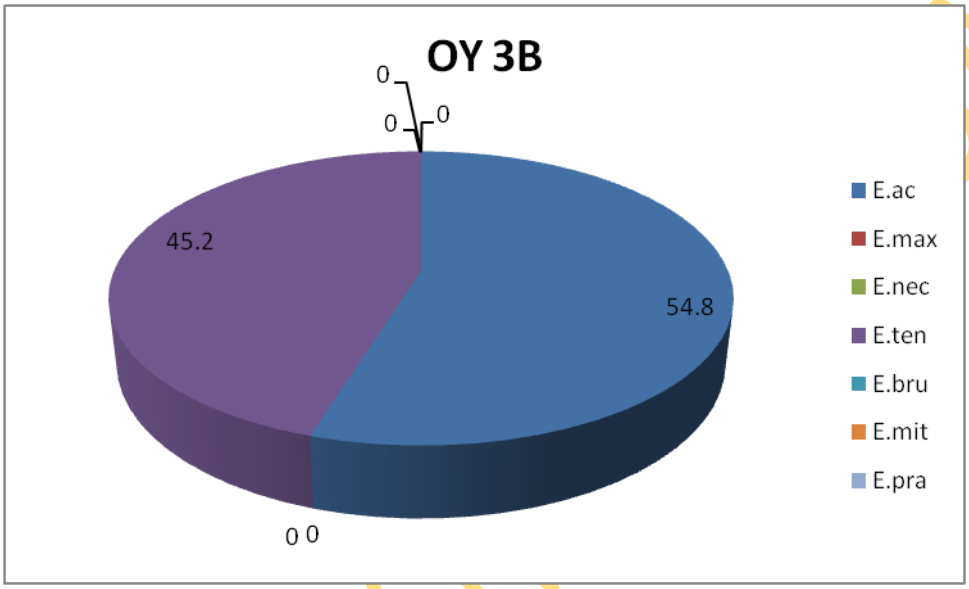


Figure 5.27 Relative Abundance of *Eimeria* Species in Chickens above 19 Weeks in Oyo State on Deep Litter

.

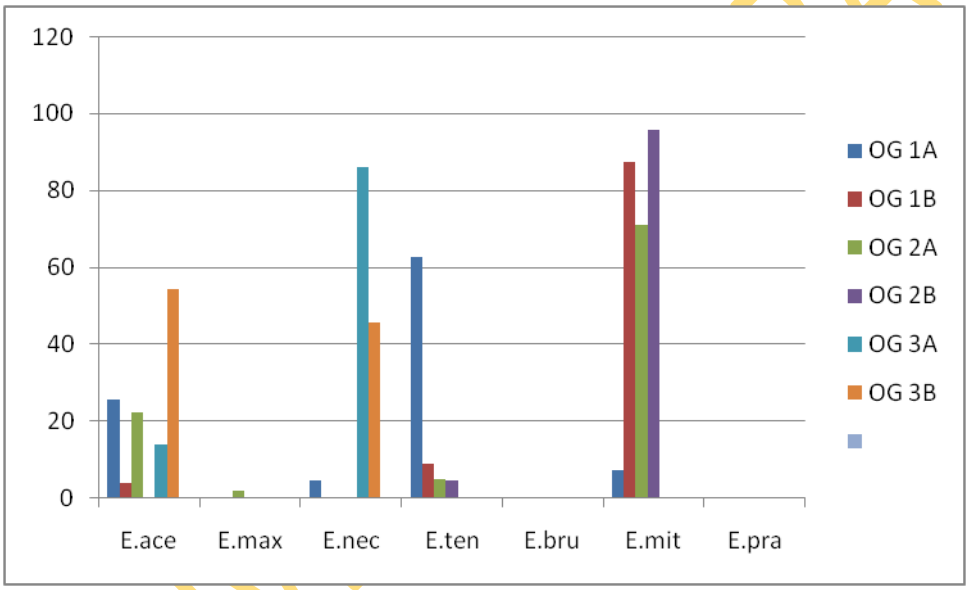


Figure 5.28 Relative Abundance of *Eimeria* Species in Ogun State

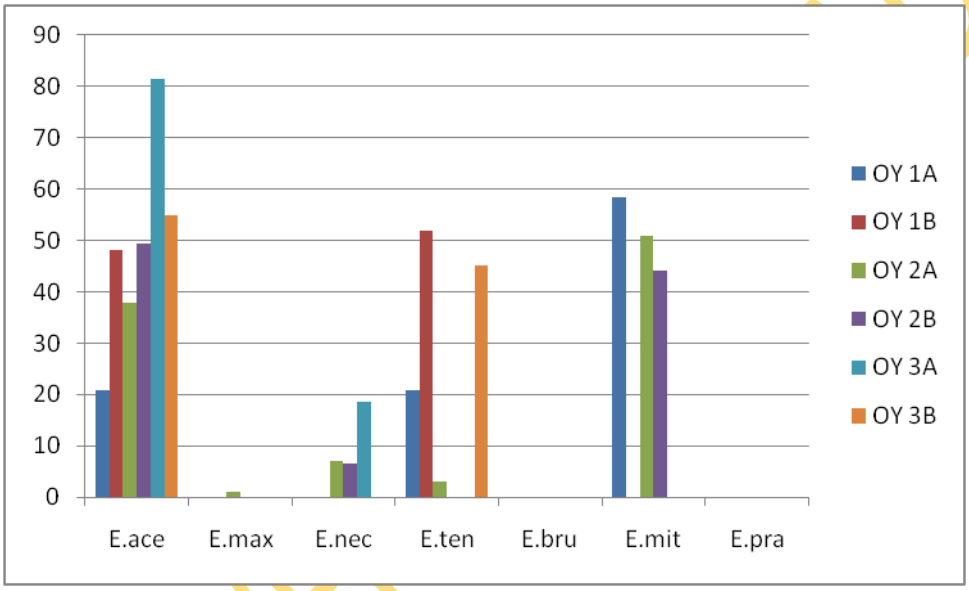


Figure 5.29 Relative Abundance of *Eimeria* Species in Oyo State

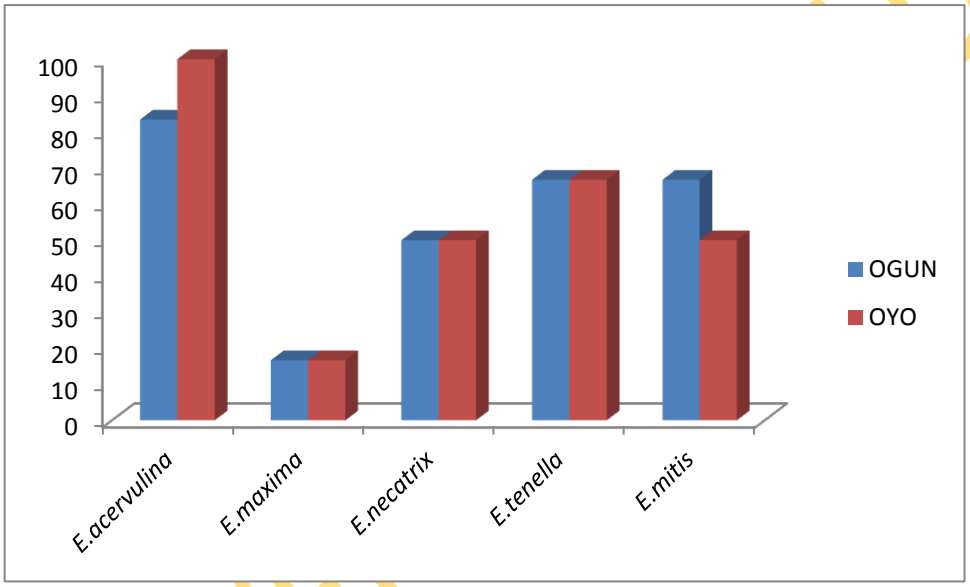


Figure 5.30 Comparism of *Eimeria* Species detected in Oyo and Ogun States

5.4.5 Comparism between Morphometric and Molecular Methods

Comparison of QPCR results with the tentative identification based on oocyst length measurements (i.e. belonging to category AM, NTP or BM) using Kappa test showed complete agreement in 77.8% (28 of 36) studied. The detailed result of the analysis is as shown in table 5.4.

5.5 Hypotheses Testing

The result of the RTQPCR plots showed that 5 species of *Eimeria species* were detected in all the pooled samples from Oyo State. They are *Eimeria acervulina, Eimeria maxima, Eimeria necatrix, Eimeria tenella* and *Eimeria mitis.* These five *Eimeria* species were also the ones detected in Ogun State. There is therefore no difference in the *Eimeria* species causing coccidial infection in Oyo and Ogun States.

Therefore the null hypothesis (H3) which states that the *Eimeria* species involved in coccidial infection in Oyo and Ogun States are the same is not rejected.

Statistical analysis gave a 77.8% agreement between morphometric and molecular method of *Eimeria* species identification. Therefore, the null hypothesis (H5) which states that there is a low level of agreement between molecular and morphometric method of *Eimeria* species identification is rejected. Statistical analysis also showed that there was a

significant difference ($P < 0.001$) between the mean OPG for chickens on vaccine and chemo prophylactic groups (Appendix xviii)

Table 5.4 Statistical Analysis of the Level of Agreement between Morphometric and Molecular Methods

Epizootiological categories	Complete Agreement	Disparity
OY 1A	2	1
OY 1B	3	0
OY 2A	3	0
OY 2B	3	0
OY 3A	2	1
OY 3B	2	1
OG 1A	2	1
OG 1B	1	2
OG 2A	3	0
OG 2B	3	0
OG 3A	2	1
OG 3B	2	1
Cummulative	28 (77.8%)	8 (22.2%)

Key: OY = Oyo state; OG = Ogun state; 1A = 1 to 8 weeks old birds on vaccine prophylaxis; 1B = 1 to 8 weeks old birds on chemoprophylaxis; 2A = 9 to 19 weeks old birds on vaccine prophylaxis; 2B = 9 to 18 weeks old birds on chemoprophylaxis; 3A = Above 19 weeks old birds in cages; 3B = Above 19 weeks old birds on deep litter.

5.6 Discussion

Precise identification and genetic characterisation of different species of *Eimeria* are central to prevention, surveillance, and control of coccidial infection. The development of molecular tools has allowed not only the diagnosis but also the study on genetic variability of pathogens based on small quantities of oocysts through molecular markers (Schnitzler *et al*., 1998; Costa *et al*., 2001). The result of the quantitative polymerase chain reaction analysis showed the presence of *Eimeria acervulina, E. maxima, E. necatrix, E. tenella* and *E. mitis.* This result agreed with those of Ramzi *et al*., (2000), Ahmed *et al* (2003), El Behairy, (2005), *Hamdinejat et al*., (2010), and Ahmed *et al.*, (2012) who also reported the same five species without *E. praecox* and *E. brunetti*. The result of this study is however at variance with that of Carvalho *et al.*, 2010, Sun *et al.*, (2008) and Al-Natour *et al.*, (2001) who all reported the presence of the seven *Eimeria* species. Although Schwarz *et al.*, (1998) reported six *Eimeria* species but *E. brunetti* and *E.praecox* were present.The non-detection of *E. brunetti* and *E. praecox* in this study contradicted the assertion of Majaro 1980, 1981and 1983 that the species of *Eimeria* causing coccidial infection in Nigeria were *E. tenella, E. necatrix, E. brunetti* and *E. acervulina*. However, the unreliability of morphological diagnosis of *Eimeria species* used in that study could be responsible for this discrepancy.

The detection of *Eimeria mitis* in 58.3% of the sample by RTQPCR is of immense epizootiological importance because apart from being the first time the presence will be definitively confirmed in Nigeria, it is not presently included in the different types of coccidiosis vaccine presently being marketed in Nigeria. In view of the lack of cross immunity among the various *Eimeria* species, the non inclusion of *E. mitis* may result in

possible vaccine failure. The Latin name, "*mitis*," means mild, because investigators of this pathogen have always considered it to be of minor significance in poultry. Although it is known to parasitise the lower part of the small intestine, no gross lesion has been ascribed to it, but it causes marked depression of weight gain in infected chicken (Shirley M.W., 2003). Experimental studies carried out at Veterinary Laboratories Agency in Weybridge (UK) showed that at equal infective doses, their strains of *E. mitis* were more pathogenic than those of *E. acervulina*.

The proliferation of *E. mitis* in those birds that received vaccine prophylaxis is possibly due to the presence of wild field species since it was not included in the vaccine formulation administered. However for those on chemoprophylaxis the widespread presence of *E. mitis* is of great concern as it might signify the emergence of resistance strains of the specie to frequently used anticoccidials. This is more so when the relative abundance of the specie in some of the epizootiological categories that received chemoprophylaxis (OG 2B, OG 1B and OY 3B) was as high as 95.6%, 87.3%, and 44.2% respectively. *E .mitis* was thought to be of low pathogenicity, but studies have shown that it impacts negatively on the growth and overall health of chickens (Gore and Long, 1982; Fitz-Coy and Edgar, 1992; Williams, 1998). In addition, some studies indicated that *E. mitis* was more pathogenic when it occurs concomitantly with *E. acervulina* (Gobbi *et al*. 2006) as recorded in this study. The negative effect of *E .mitis* on production (especially broiler production) may therefore be more than previously imagined.

Immunity to coccidiosis is known to increase with age but the 75% detection of *E. necatrix* in birds above 19 weeks as against 25% detection in younger birds recorded in this study raises fundamental epizootiological questions more so when the younger birds were those that received vaccines containing *E. necatrix* and is therefore likely to be of vaccinal origin. However, *E. necatrix* is known to be a poor oocyst producer, it may take a longer time for

an infective dose to be established in a susceptible flock. This finding corroborates the observation of Shirley M. W., (2003) who reported low detection of *E. necatrix* in commercial broiler farms. This might be responsible for its exclusion from coccidiosis vaccine meant for use in commercial broilers while being included in those for breeders. Shirley, (2003) further reported that whereas *E. mitis* is found commonly in both broiler and egg laying flocks, *E. brunetti* and *E. necatrix* tend to be found predominantly in egg laying flock.

Presence of low level of *E. necatrix* in poultry facilities may be beneficial because Majaro (1984) observed that chickens given small doses of *E. necatrix* oocyst had better weight gain than the unexposed groups. This stimulated growth was thought to be due to the fact that mild infection stimulates the functional activity of the intestinal crypts where digestive juices and enzyme occur which probably improved the appetite of the infected chickens. The stimulated growth may be due to improved efficiency of digestive processes rather than improvement in the appetite of the chickens. The preponderance of *E. acervulina* in this study (prevalence of 100% for Oyo and 83.3% for Ogun) is not unexpected considering its high fecundity in oocyst production in a condusive environment. This result confirms the findings of Mc Dougald *et al.*, (1997), Razmi *et al*., (2000) and Haug *et al*., (2008) which showed *E. acervulina* as the most prevalent *Eimeria* species in their respective studies. *E. tenella* was the second most prevalent in this study as shown by a prevalence of 66.7% each for Ogun and Oyo state respectively. *E. tenella* is also a prolific oocyst producer. This observation corroborates the report of Al Natour *et al.*, (2001), Hamedinejat *et al*., (2010) and Bachaya *et al*., (2012) in which *E. tenella* was widely distributed. This result is however at variance with that of Ahmed *et al*., (2012) in which *E. necatrix* with a prevalence of 58.27% was the most preponderant.

While *E. acervulina* is moderately pathogenic, *E. tenella*, and *E. necatrix* are the most pathogenic (Nowan *et al*., 2005) of the five *Eimeria species* identified. The presence of *E. tenella*, and *E. necatrix*, especially in those flocks on chemoprophylaxis is of great epizootiological importance not only because of the widespread damage caused while developing in the depth of the mucosa which causes high mortality as well severe weight loss but because of the possible development of multi-drug resistance strains. The low prevalence of *E. maxima* recorded in this study (16.7% each in Oyo and Ogun State respectively) coupled with the non-detection in flocks on chemoprophylaxis may be indicative of the absence of multi drug resistance strains culminating in the high sensitivity of this specie to commonly used anticoccidials. It might also indicate that the environmental factors in the study area do not favour the proliferation of this specie.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The finding in this study has provided current information on the prevalence of coccidial infection in Oyo and Ogun States. It also provided information about the *Eimeria* species and their relative abundance in the 2 states using both the traditional morphometric and the more recent molecular techniques. This study also provided information on the effect of age, prophylactic and management practices on the incidence and prevalence of coccidial infections.

Data obtained indicated a higher incidence of coccidial infection in chicks below 8 weeks and in birds kept on deep litter. This may be due to the low level of immunity of young chicks to coccidial infection and the increased proximity between sporulated oocysts and birds raised on deep litter system. The reported outbreak of Coccidiosis in 30% of the flock in spite of prophylactic measures indicates the need to review and improve current measures to enhance efficacy and engender protection from infection.

The usage of molecular method to characterise the *Eimeria* species enumerated revealed the presence of *Eimeria acervulina*, *E. maxima*, *E*, *necatrix*, *E. tenella* and *E. mitis* in both States. This is one of the few reports of the usage of molecular method to characterise *Eimeria* species in Nigeria. This method has a greater precision over the traditional morphometric method of identification hitherto in use because the latter

method adopt the measurement of oocyst sizes as part of the identification criteria whereas oocyst sizes has been shown to overlap among different species.

The five identified *Eimeria* species should be incorporated into a coccidiosis vaccine meant for use in the area especially for Layers and Breeder birds while *Eimeria necatrix* which is a poor oocyst producer and for which the possibility of sporulated oocysts accumulating enough to cause an infection in birds below 8 weeks could be lefi out of the formulation for commercial broilers in order to minimise cost. The positive characterization of Eimeria mitis is of epizootiological significance not only because it is the first time its presence will be unequivocally confirmed in Nigeria but because all coccidiosis presently being marketed in Nigeria do not contain this specie. The implication of that is obvious since there is no cross immunity among different *Eimeria* species. Although *E. mitis* is of moderate pathogenicity, it has been shown to cause poor weight gain in commercial broilers. Although *E. brunetti* and *E. praecox* were not positively characterised in this study, it does not mean that they are not present in other parts of the country.

In Nigeria presently, only live vaccines such as Livacox® and Immucox® are currently included in the program for coccidiosis control in the poultry industry. There remains a heavy reliance on anticoccidial chemotherapeutics, predominantly ionophore drugs. With increasing drug resistance and the negativity associated with drug residues in poultry meat and eggs, the dependency on chemotherapeutics will inevitably be altered in the near future and, thus, the prospect of vaccines becoming the future of coccidiosis control is likely

6.2 Contributions to Knowledge

(a) The molecular characterisation of five *Eimeria* species; *E. acervulina*, *E. maxima*, *E. necatrix*, *E. tenella* and *E. mitis* done in this study is a major contribution to knowledge on the prevention and control of coccidiosis because of the increasing adoption of vaccine prophylaxis by poultry farms in Nigeria and especially in the study area. In Vaccine prophylaxis unlike in chemoprophylaxis, each of the pathogenic local *Eimeria* species must be included in the vaccine formulation to ensure maximum protection and efficacy due to the absence of cross immunity among different species.

(b) The study reported a prevalence of coccidial infections of 65% and 61.2% for Oyo and Ogun States respectively and this provides vital current key information in respect of the epizootiology of *Eimeria* infections in the areas studied.

(c) The classification of sample collected into 6 epizootiological categories is novel and allowed for a more critical analysis of the empirical data collected. It is expected to be adopted in future epizootiological studies not only in coccidiosis research but in order areas where age, management and prophylactic measures may exert significant effect on the results obtained.

(d) The determination of the relative abundance of the different *Eimeria* species in this study can be used as a tool to determine the efficiency of any anticoccidial strategy on any farm in Nigeria.

(e) The molecular characterisation of *Eimeria mitis* in this study is perhaps the first confirmation of its presence in the study area.

6.3 Recommendations

The five of *Eimeria* species identified in this study; *Eimeria* acervulina, *E*. maxima, *E*. necatrix, *E*. tenella, and *E*. mitis should be included in any future coccidiosis vaccine for use in the study area to enhance vaccine efficacy and optimal protection.

The traditional morphometric methods could still continue to be used for routine tentative diagnosis of coccidiosis but Real-Time Quantitative PCR method with its higher precision should be used for definitive identification of *Eimeria* species especially for the purpose of incorporation into a coccidiosis vaccine formulation.

The Federal Government of Nigeria through the Federal Department of Livestock should provide resources for the carrying out of a nationwide molecular epizootiological survey to identify the *Eimeria* species in other parts of the country. This will provide a holistic picture of the circulating *Eimeria* species in Nigeria.

The classification of samples obtained for this study into different epizootiological categories which is novel is recommended for future epizootiological studies in which factors like age, management and prophylactic measures adopted can have a decisive effect on the obtained result.

In consideration of the statistically significant superior knowledge of coccidiosis by farmers that consulted Veterinary doctors, Veterinarians should be fully involved in the formulation and dissemination of technical information for the prevention and control of coccidiosis and coccidial infections in Nigeria.

REFERENCES

Adene, D. F. and Oluleye O.B. (2004). Coccidiosis of Poultry: The Biology, Diagnosis, Treatment and Control. In: D.F. Adene Poultry Health and Production principles and practices, Stirling-Horden Publishers (Nig) Ltd. Ibadan, Nigeria PP. 129-164.

Ahmed, N.E., Negm Eldin, M.M., El Akabawy. L.M.and El.Medawy, R.S. (2003): Incidences of some protozoan parasites in Birds. *Kafr El sheikh Vet. Med. J.* vol **1**, No 1:235 251.

Ahmed A. Al-Gawad1; Olfat A. Mahdy1; Aida A. N. El-Massry and Mohamed S. A. Al-Aziz (2012). Studies on Coccidia of Egyptian Balady Breed Chickens. *Life Science Journal* **9** (3)

Albanese A. and Smetana H. (1937). Studies on the effect of X-rays on the pathogenicity of *Eimeria tenella*. *Am J Hyg* **26**: 27–39.

Allen P. C. and Fetterer R. H. (2002). Recent Advances in Biology and Immunobiology of *Eimeria* Species and in Diagnosis and Control of Infection with These Coccidian Parasites of Poultry. *Clinical Microbiology Reviews*, p. 58–65 Vol.

Allocco, J. J., Profous-juchelka, R. W., Myers, B. Nare Schmatz, D. M. (1999): Biosynthesis and catabolism of mannitol is developmentally regulated in the protozoan parasite *Eimeria tenella*. *J. Parasitol.* **85**, 167–173.

Al Natour, M.Q., Suleiman, M.M. and Abo-Shehada, M.N. (2002). Flock-level prevalence of *Eimeria* species among broiler chicks in northern Jordan. *Preventive Veterinary Medicine*, **53**, 305-310.

Anderson, G. and Jorgensen, W. (2001). Development of Australian live vaccines against coccidiosis: characterisation of attenuated lines using standardised trials. *Proceedings of the VIIIth International Coccidiosis Conference*, Palm Cove Sydney, Australia (pp.70–71).

Andrews S.J., Crouch, C.F. and Francis, M.J. (2001). Administration of an attenuated *Eimeria* vaccine to newly hatched chicks by spray within the hatchery. *Proceedings of the VIIIth International Coccidiosis Conference*, Palm Cove, Sydney, Australia, (p. 71).

Avakian, A.P., Poston, R.M., Ard, B.B., Schaeffer, J.L., Redfern, T.R., Hutchins, J.E., Whitfill, C.E., Weber, F.H., Evans, N.A. & Doelling, V.W. (2001). Commercial field trials testing a live coccidiosis vaccine administered *in ovo*. *Proceedings of the VIIIth International Coccidiosis Conference*, Palm Cove Sydney, Australia (pp. 71–72).

Bafundo, K.W. (1989). Protective responses produced by immunization of day-old chicks with either *Eimeria maxima*, *E. acervulina* or *E. tenella* live oocyst vaccines. In P. Yvor´e (Ed.), *Coccidia and Intestinal Coccidiomorphs* Paris: INRA (pp. 395–400).

Bafundo, K.W. and Donovan, D.J. (1988). Predicting effects of coccidial lesions in broilers *Poultry*, **4** *(4)*, 36– 37.

Bafundo K.W. and Jeffers T.K. (1988). Method of improving the quality of animals. European Patent, 87307537.8.

Bafundo, K.W. and Jeffers, T.K. (1990). Anticoccidial method. *United States Patent*, 4,935,007.

Ball, S.J. (1966). The development of resistance to glycarbylamide and 2-chloro-4 nitrobenzamide in *Eimeria tenella* in chicks. *Parasitology*, **56**, 25– 37.

Bedrn´õ k, P. (1989). The role of different *Eimeria* species in a prospective coccidiosis vaccine. In P. Yvor´e (Ed.), *Coccidia and Intestinal Coccidiomorphs* Paris: INRA (pp. 666–670).

Bedrn´õ k, P. (1998). Comparison of efficacy of attenuated coccidiosis vaccines Livacox® and Paracox® in broiler fattening. In *COST 820* – Vaccines against Animal Coccidioses – Activity Report 1996 Luxembourg: European Commission (pp. 67–68)..

Bedrn´õ k, P., Provazn´õ kov´a, M. and Firmanov´a, A. (1993). Experimental and commercial use of Livacox®, a live attenuated vaccine against coccidiosis of domestic fowl. In J.R. Barta and M.A. Fernando (Eds.), *Proceedings VIth International Coccidiosis Conference,* Guelph, Canada(p. 165).

Bedrn´õ k, P., Hiepe, T., Mielke, D. and Dr¨ossigk, U. (1995). Antigens and immunisation procedures in the development of vaccines against poultry coccidiosis. In J. Eckert, R. Braun, M.W. Shirley and P. Coudert (Eds.), *COST 89/820: Biotechnology: Guidelines on Techniques in Coccidiosis Research* Luxembourg: European Commission (pp. 176–189).

Barksh, T., (1989). Poultry parasites and diseases. *African farming and food processing*. Jan/Feb.Edition. Allen Charles Publishing Ltd. London, p: 13

Biggs, P.M. (1982). The world of poultry disease. *Avian Pathology*, **11**, 281– 300.

Bishu G. (1982). Isolation and identification of two pathogenic *Eimeria* Species in chicken in Nigeria. MSc. Thesis, Ahmadu Bello University, Zaria, Nigeria pp 52-61

Blake, D.P. Qin, Z. Cai, J. Smith A.L. (2008). Development and validation of real-time polymerase chain reaction assays specific to four species of *Eimeria Avian Pathology*, **37** pp. 89–94

Brake, D. A., Strang, G., Lineberger, J. E. (1997): Immunogenic characterisation of a tissue culture-derived vaccine that affords partial protection against avian coccidiosis. *Poult. Sci.* **76**, 974–983.

Cantacessi, C. Riddell, S. Morris, G.M. Doran, T. Woods, W.G. Otranto, D. Gasser R.B. (2008). Genetic characterisation of three unique operational taxonomic units of *Eimeria* from chickens in Australia based on nuclear spacer ribosomal DNA *Vet. Parasitol.*, **152,** pp. 226–234

Callow, L.L. (1984). Animal health in Australia Protozoal and rickettsial diseases, vol. **5** Australian Government Publishing Service, Canberra Australia.

Carvalho F. S., Wenceslau A. A., Teixeira M, Matos J. A., Carneiro, Brandão Melo, Albuquerque R. G., (2011). Diagnosis of Eimeria species using traditional and molecular methods in field studies. *Veterinary Parasitology* **176,** 95–100

Caldwell, D.Y., Caldwell, D.J., Moore, R.W. and Hargis, B.M. (2001a). Effect of selected photo intensity regimes on chick preening behavior and competitive exclusion culture efficacy. *Journal of Applied Poultry Research,* **10**, 107–111.

Caldwell, D.Y., Moore, R.W., Caldwell, D.J. and Hargis, B.M. (2001b). Effect of photo intensity, sound intensity, and ambient temperature on preening behavior and

ingestion of spray-applied biologics. *Journal of Applied Poultry Research,* **10**, 99–106.

Caldwell, D.Y., Young, S.D., Caldwell, D.J., Moore, R.W. and Hargis, B.M. (2001c). Interaction of color and photo intensity on preening behavior and ingestion of spray-applied biologics. *Journal of Applied Poultry Research,* **10**, 112–116.

Chapman, H.D. (1976). *Eimeria tenella* in chickens: studies on resistance to the anticoccidial drugs monensin and lasalocid. *Veterinary Parasitology,* **2**, 187–196.

Chapman, H.D. (1978). The effect of monensin on the immunity arising from repeated low-level infections with *Eimeria maxima, E. brunetti* and *E. tenella. Avian Pathology,* **7,** 269–277.

Chapman, H.D. (1982). The use of enzyme electrophoresis for the identification of the species of *Eimeria* present in field isolates of coccidia. *Parasitology* **85**, 437– 442.

Chapman, H.D. (1986). *Eimeria tenella*: stability of resistance to halofuginone, decoquinate and arprinocid in the chicken. *Research in Veterinary Science,* **40**, 139–140.

Chapman, H.D. (1993). Twenty-one years of monensin for the control of coccidiosis – a review. *Proceedings of the VIth International Coccidiosis Conference*, Guelph, Canada, (pp. 37– 44).

Chapman, H.D. (1994). Sensitivity of field isolates of *Eimeria* to monensin following the use of a coccidiosis vaccine in broiler chickens. *Poultry Science,* **73**, 476–478.

Chapman, H.D. (1996a). Restoration of drug sensitivity following the use of live coccidiosis vaccines. *World Poultry, Special Supplement Coccidiosis* **2**, pp. 20–21.

Chapman, H.D. (1996b). Effects of different types of anticoccidial drug programmes upon the development of immunity to *Eimeria* species in the fowl. *Proceedings of the 45th Western Disease Conference,* Cancun, Mexico, pp. 115–117

Chapman, H.D. (1997a). Biochemical, genetic and applied aspects of drug resistance in *Eimeria* parasites of the fowl. *Avian Pathology*, **26**, 221– 244.

Chapman, H.D. (1997b). Immunity development in broilers given anticoccidial drugs and reared on new or used litter. *Proceedings of the VIIth International Coccidiosis Conference, Oxford* (p. 42). Oxford, UK.

Chapman, H.D. (1999a). Anticoccidial drugs and their effects upon the development of immunity to *Eimeria* infections in poultry. *Avian Pathology*, **28**, 521– 535.

Chapman, H.D. (1999b). The development of immunity to *Eimeria* species in broilers given anticoccidial drugs. *Avian Pathology*, **28**, 155–162.

Chapman, H.D. (2000a). Practical use of vaccines for the control of coccidiosis in the chicken. *World's Poultry Science Journal,* **56**, 7–20.

Chapman, H.D. (2000b). Long-term anticoccidial strategy: chemotherapy plus vaccination. *Feed International*, **March 2000**, 18–19.

Chapman, H.D. and Cherry, T.E. (1997a). Eye spray vaccination: infectivity and development of immunity to *Eimeria acervulina* and *Eimeria tenella*. *Journal of Applied Poultry Research*, **6**, 274– 278.

Chapman, H.D. and Cherry, T.E. (1997b). Comparison of two methods of administering live coccidiosis vaccines to newly hatched chicks: infectivity and development of

immunity to *Eimeria* species. *Proceedings of the VIIth International Coccidiosis Conference,* Oxford, UK, (p. 133).

Chapman, H.D. and Hacker, A.B. (1993). The effects of shuttle programmes upon the growth of broilers and the development of immunity to *Eimeria* species. *Poultry Science*, **72,** 658– 663.

Chapman, H.D., Cherry, T.E., Danforth, H.D., Richards, G., Shirley, M.W. and Williams, R.B. (2002). Sustainable coccidiosis control in poultry production: the role of live vaccines. *International Journal for Parasitology*, **32**, 617– 629.

Chapman, J. (1999). Chicken firm bans antibiotics over superbug fears. *The Daily Mail, London, 2 September*, p. 7. Cheng, S.E. and Edgar, S.A. (1979). Effect of genetic selection on pathogenicity and immunogenicity of *Eimeria tenella* and *Eimeria maxima*. *Poultry Science*, **58**, 1043.

Cheng S and Edgar S. 1979. Effect of genetic selection on the pathogenicity and immunogenicity of *Eimeria tenella* and *Eimeria maxima*. *Poult Sci*., **58**, 1043.

Chookymix L., Stella U., Sandy O., 2009. Coccidiosis in backyard Poultry Information Centres Php BB 2004-2009// backyard poultry.com

Christensen, N. (1999). Coccidiosis vaccination in New Zealand. *Proceedings of the Queensland Poultry Science Symposium*, **8**, 23 (1–4).

Christmas, R.B. and Harms, R.H. (1984). Performance of Leghorn and broiler day-old male chicks as affected by dietary monensin level. *Nutrition Reports International*, **30**, 1431–1434.

Colnago, G.L., Gore, T., Jensen, L.S. & Long, P.L. (1983). Amelioration of pale bird syndrome in chicks by vitamin E and selenium. *Avian Diseases*, **27**, 312– 316.

Corpet, D.E. (2000). M´ecanismes de la promotion de croissance des animaux par les additifs alimentaires antibiotiques. *Revue de M´edecine V´et´erinaire*, **151**, 99–104.

Crane, M.S.J., Goggin, B., Pellegrino, R.M., Ravino, O.J., Lange, C., Karkhanis, Y.D., Kirk, K.E. and Chakraborty, P.R. (1991). Cross protection against four species of chicken coccidia with a single recombinant antigen. *Infection and Immunity*, **59,** 1271–1277.

Chapman, H. D. (1998). Evaluation of the efficacy of anticoccidial drugs against Eimeria species in the fowl. *Int. J. Parasitol.* **28**,1141–1144

Conway, D.P., Sasai, K., Gaafar, S.M., Smothers, C.D., (1993). Effects of different levels of oocyst inocula of *Eimeria acervulina, E. tenella,* and *E. maxima* on plasma constituents, packed cell volume, lesion score, and performance in chickens. *Avian Dis.* **37**, 118–123.

Castanon, C.A.B., Fernandez, S., Fraga, J.S., Fontoura, L.F. and Gruber, A. (2007). Coccimorph: a real-time diagnostic tool based on automatic image recognition of protozoan parasites of the genus *Eimeria*. *Proceedings of the World Association for the Advancement of Veterinary Parasitology*, Gent, Belgium, 19-23

Danforth, H. D. (1998). Use of live oocyst vaccines in the control of avian coccidiosis: experimental studies and field trials. *Int. J. Parasitol*. **28**:1099–1109.

Dalloul, R. A. and Lillehoj, H. S. (2005). Recent advances in immunomodulation and vaccination strategies against coccidiosis. *Avian Disease*, **49**: 1-8.

Davies, S. F. M., Joyner L. P., and Kendall S. B. (1963). Coccidiosis. Oliver and Boyd Ltd.,

London, U.K.

Danforth, H.D., Lee, E.-H., Martin, A. and Dekich, M. (1997a). Evaluation of a gel-immunisation technique used with two different Immucox vaccine formulations in battery and floor-pen trials with broiler chickens. *Parasitology Research,* **83**, 445–451.

Danforth, H.D., Watkins, K., Martin, A. and Dekich, M. (1997b). Evaluation of the efficacy of *Eimeria maxima* oocyst immunization with different strains of day-old broiler and roaster chickens. *Avian Diseases*, **41**, 792– 801.

David S. (2000). Coccidiosis in poultry health and management 4th edition ELST with Blackwell Publishers pp 105-107

Davies, S.F.M. (1958). Value of interrupted treatments and preventive medication in the control of intestinal coccidiosis caused by *Eimeria necatrix. Journal of Comparative Pathology and Therapeutics*, **68**, 363–373.

Davies, S.F.M. and Joyner, L.P. (1955). Observations on the parasitology of deep litter in poultry houses. *Veterinary Record, 67*, 193–199. Davies, S.F.M. and Kendall, S.B. (1954). The practical application of sulphamezathine therapy for caecal coccidiosis. *Veterinary Record*, ***66,*** 19–21.

Davis, P.J. (1981). Immunity to coccidia. In M.E. Rose, L.N. Payne & B.M. Freeman (Eds.), *Avian Immunology* Edinburgh: British Poultry Science Ltd (pp. 361–385).

Davis, P.J. and Porter, P. (1983). *Eimeria tenella*: control of parasitic behaviour through mucosal immunity and in-feed immunization. *Annals of the New York Academy of Sciences*, **409**, 810– 811.

Davis, P.J., Barratt, M.E.J., Morgan, M. and Parry, S.H. (1986). Immune response of chickens to oral immunization by 'trickle' infections with *Eimeria*. In L.R. McDougald, L.P. Joyner and P.L. Long (Eds.), *Research in Avian Coccidiosis, Proceedings of the Georgia Coccidiosis Conference,* **1985**, University of Georgia, Athens, Georgia (pp. 618–633).

Delaplane, J.P., Batchelder, R.M. and Higgins, T.C. (1947). Sulfaquinoxaline in the prevention of *Eimeria tenella* infections in chickens. *North American Veterinarian,* **28**, 19–24.

Devriese, L.A., Daube, G., Hommez, J. and Haesebrouck, F. (1993). *In vitro* susceptibility of *Clostridium perfringens* isolated from farm animals to growth-enhancing antibiotics. *Journal of Applied Bacteriology*, **75**, 55– 57.

Dhama, K., Basaraddi, M. S., Ruchi T., and Shantaveer S. B., (2012). Coccidiosis: An Overview. Avian Diseases Section Division of Pathology, Indian Veterinary Research Institute, Izatnagar, 243 122 (U.P.)

Dibner, J., Ivey, F.J. and Knight, C.D. (1999). Direct delivery of live coccidiosis vaccine into the hatchling yolk sac. *World Poultry, Special Supplement Coccidiosis* **3**, pp. 28–29.

Dickinson, E.M., Babcock, W.E. and Kilian, J.G. (1959). A program of immunisation against avian coccidia. *Proceedings of the United States Livestock Sanitary Association,* **63**, 223–225.

Digby, M. R., and J. W. Lowenthal. 1995. Cloning and expression of the chicken interferon-gamma gene. J. Interferon Cytokine Res. 15:939–945.Doelling, V.W., Martin, A., Hutchins, J.E. and Tyczkowski, J.K. (2001). Infectivity of *E. acervulina* oocysts,

sporocysts and sporozoites with *in ovo* delivery. *Proceedings of the VIIIth International Coccidiosis Conference, Palm Cove* Sydney, Australia, (pp. 163–164).

Doran, D.J. (1966). Location and time of penetration of duodenal epithelial cells by *Eimeria acervulina* sporozoites. *Proceedings of the Helminthological Society of Washington,* **33**, 43–46.

Doran, D.J. (1973). Cultivation of coccidia in avian embryos and cell culture. In D.M. Hammond and P.L. Long (Eds.), *The Coccidia: Eimeria, Isospora, Toxoplasma, and Related Genera* Baltimore: University Park Press, pp. 183–252.

Doran, D.J. and Farr, M.M. (1965). Susceptibility of 1- and 3-day-old chicks to infection with the coccidium, *Eimeria acervulina. Journal of Protozoology*, **12**, 160–166.

Duan, J.-S. and Liu, S.-M. (1994). Studies on the action of Baycox on *E. tenella* and its influence on the development of coccidial immunity in chickens. *Proceedings of the Second Asian Conference on Coccidiosis,* Guangzhou, China, pp. 69–74.

Eckert, J., Braun, R Shirley, M.W., Coudert P. (1995). Biotechnology Guidelines on techniques in coccidiosis research, Office for Official Publications of the European Communities, Luxembourg, pp. 103–119

Edgar, S.A., Seibold, C.T., (1964). A new coccidium of chickens, *Eimeria mivati* sp. n. (protozoa: *eimeriidae*) with details of its life history. *J. Parasitol.* **50**, 193–204.

Edgar, S.A. (1955). Sporulation of oocysts at specific temperatures and notes on the prepatent period of several species of avian coccidia. *Journal of Parasitology*, **41**, 214– 216.

Edgar, S.A. (1954). Effect of temperature on the sporulation of oocysts of the protozoan *Eimeria tenella. Transactions of the American Microscopical Society,* **73**, 237– 242.

Edgar, S.A. (1955). Sporulation of oocysts at specific temperatures and notes on the prepatent period of several species of avian coccidia. *Journal of Parasitology,* **41**, 214– 216.

Edgar, S.A. (1958a). Control of coccidiosis in chickens and turkeys by immunization. *Proceedings of the Association of Southern Agricultural Workers,* Little Rock, Arkansas, USA, (pp. 203–204).

Edgar, S.A. (1958b). Control of coccidiosis of chickens and turkeys by immunization. *Poultry Science,* **37**, 1200.

Edgar, S.A. (1964). Stable coccidiosis immunization. *United States Patent,* **3**,147,186.

Edgar, S.A. and Fitz-Coy, S.H. (1985). Efficacy of CocciVac-D plus three anticoccidial drugs versus monensin in broiler chickens. *Poultry Science,* **64** *(Suppl. 1)*, 18.

Eduvie L. O. (2002). Poultry production in Nigeria; a training manual. National Animal Production Research Institute. Federal Ministry of Agriculture and Water Resources, Ahmadu Bello University, Zaria, Nigeria.

Elwinger, K., Berndtson, E., Engstr¨om, B., Fossum, O. and Waldenstedt, L. (1998). Effect of antibiotic growth promoters and anticoccidials on growth of *Clostridium perfringens* in the caeca and on performance of broiler chickens. *Acta Veterinaria Scandinavica,* **39**, 433–441.

Elwinger, K., Engstr¨om, B., Berndtson, E., Fossum, O. and Teglof, B. (1992). The effects of narasin on *Clostridium perfringens* in caecae and the occurrence of necrotic

enteritis in broiler chickens. In *Proceedings XIX World's Poultry Congress,* Amsterdam, The Netherlands, **3**, pp. (580–584).

Elwinger, K., Engstr¨om, B., Fossum, O., Hassan, S. and Tegl¨of B. (1994). Effect of coccidiostats on necrotic enteritis and performance in broiler chickens. *Swedish Journal of Agricultural Research,* **24**, 39– 44.

Ern´õ k, F. and Bedrn´õ k, P. (2001). Controlling coccidiosis in broiler growing. *Poultry International,* **40 (4),** 36– 42.

Etuk, E.B., Okoli I.C., Uko M.U. (2004). Prevalence and Management Issues Associated with

Poultry Coccidiosis in Abak Agricultural Zone of Akwa Ibom State, Nigeria International *Journal of Poultry Science,* **3 (2):** 135-139.

EU Commission (2003). Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003; on additives for use in animal nutrition. http://eur-lex.europa.eu

Fasanmi, F., (1990). Nigerian livestock industry and prospects for the 1990s. Nig. Inst. Soc. Econ. Res./Federal Livestock Production Comm. Seminar. Kaduna, Nigeria

Fernandez, S. Pagotto, A.H. Furtado, M.M. Katsuyama, A.M. Madeira, A.M.B.N. Gruber A. (2003). A multiplex PCR assay for the simultaneous detection and discrimination of the seven *Eimeria* species that infect domestic fowl, *Parasitology*, **127**, pp. 317–325.

Fernandez, S., Costa, A.C., Katsuyama, A.M., Madeira, A.M.B.N., Gruber A. (2003).

A survey of the inter and intra specific RAPD markers of *Eimeria* spp. of the domestic fowl and the development of reliable diagnostic tools *Parasitol. Res.*, pp. 437–445

Farrant, J. (2001). Ministers knock out six anticoccidials. *Poultry World*, *155*, **11**, 5.

Fischer, G.J. (1975). The behaviour of chickens. In E.S.E. Hafez (Ed.), The Behaviour of Domestic Animals 3rd edn (pp. 454–489). London: Bailli`ere Tindall.

Fitz-Coy, S.H. (1992). Antigenic variation among strains of *Eimeria maxima* and *E. tenella* of the chicken. *Avian Diseases*, **36**, 40–43.

Fitz-Coy, S.H. and Edgar, S.A. (1989). *Eimeria mitis*: immunogenicity and cross immunity of two isolates. *Avian Diseases*, **33**, 236– 237.

Fitz-Coy, S.H. and Edgar, S.A. (1992). Pathogenicity and control of *Eimeria mitis* infections in broiler chickens. *Avian Diseases*, **36,** 44– 48.

Fredericksen, T.L., Thaxton, J.P., Gildersleeve, R.P., Rowe, D.G., Ruff, M.D., Strohlein, D.A. and Danforth, H.D. (1989). *In ovo* administration of a potential recombinant coccidial antigen vaccine in poultry. In P. Yvor´e (Ed.), *Coccidia and Intestinal Coccidiomorphs* Paris: INRA pp. 655–660.

Fry, M. and Williams, R.B. (1984). Effects of decoquinate and clopidol on electron transport in mitochondria of *Eimeria tenella* (Apicomplexa: Coccidia). *Biochemical Pharmacology*, **33**, 229–240.

Gasser, R.B., Woods, W.G., Wood, J.M., Ashdown, L., Richards, G. & Whithear, K.G. (2001). Automated, fluorescence-based approach for the specific diagnosis of chicken coccidiosis. Electrophoresis, **22**, 3546-3550.

Gasser, R.B., Skinner, R., Fadavi, R., Richards, G. and Morris, G. (2005). High-throughput capillary electrophoresis for the identification and differentiation of seven species of *Eimeria* from chickens. Electrophoresis, **26**, 3479-3485.

Gasser R. B., Zhu, X. Q., (1999). Sequence based analysisof enzymatically amplified DNA fragments by mutation scanningdetection techniques. Parasitology today 1999; **15**; 462-465

Genteman, K.C., LeMay, M.A., Weber, F.H., Evans, N.A., Carter, A., Poston, R. & Doelling, V. (2001). Manual versus automated *in ovo* vaccination of broiler chicks against coccidiosis. *Proceedings of the VIIIth International Coccidiosis Conference, Palm Cove* Sydney, Australia, (p. 90).

Giambrone, J.J. and Klesius, P.H. (1985). Effect of levamisole on the response of broilers to coccidiosis vaccination. *Poultry Science*, **64**, 1083–1089.

Gobbi, C.L. and Williams, R.B. (1990). The use of a live attenuated vaccine to control coccidiosis in chickens. *Proceedings VIII Conferencia Europea de Avicultura, Barcelona* Barcelona, Spain, (Memoria **2**, pp. 579–582).

Goodrich, H.P. (1944). Coccidian oocysts. *Parasitology,* **36**, 72–79.

Gore, T.C. and Long, P.L. (1982). The biology and pathogenicity of a recent field isolate of *Eimeria praecox* Johnson, 1930. *Journal of Protozoology,* **29**, 82–85.

Govoni, S., Maestrini, N., Gobbi, L. and Tonelli, A. (1987). Prove di campo di un vaccino attenuato contro le coccidiosi del pollo. *Atti della Societ`a Italiana delle Scienze Veterinarie,* **41**, 1187–1192.

Graat, E.A.M., Henken, A.M., Ploeger, H.W., Noordhuizen, J.P.T.M. and Vertommen, M.H. (1994). Rate and course of sporulation of oocysts of *Eimeria acervulina* under different environmental conditions. *Parasitology,* **108**, 497– 502.

Greif, G. (2000). Immunity to coccidiosis after treatment with toltrazuril. *Parasitology Research,* **86**, 787–790.

Greif, G. and Haberkorn, A. (1997). Enhancement of immunity and protection against coccidiosis therapeutic medication with toltrazuril. *Proceedings of the VIIth International Coccidiosis Conference, Oxford* Oxford, UK pp. 42–43.

Gross, W.B. (1985). Effect of social environment and oocyst dose on resistance and immunity to *Eimeria tenella* challenge, *Avian Diseases*, **29**, 1018–1029.

Grumbles, L.C., Delaplane, J.P. and Higgins, T.C. (1948). Immunity studies on *Eimeria tenella* infection in chickens in relation to sulfaquinoxaline therapy. *Poultry Science,* **27**, 169–171.

Goto M, Honda E, Ogura A, Nomoto A, Hanaki K (2009). Colorimetric detection of loop-mediated isothermal amplification reaction by usinghydroxy naphthol blue. Biotechniques, **46(3),** 167-172.

Gore, T. C., and P. L. Long 1982. The biology and pathogenicity of a recent field isolate of *Eimeria praecox* Johnson, 1930. *J. Protozool.*, **29**:82–85, 1982.

Guillot, J.F. (1998). Les probiotiques en alimentation animale. *Cahiers Agricultures*, **7**, 49-54.

Haberkorn, A. (1986). Intermittent therapy: an alternative to permanent administration of anticoccidials. In L.R. McDougald, L.P. Joyner and P.L. Long (eds), *Research in*

Avian Coccidiosis, Proceedings of the Georgia Coccidiosis Conference, 1985 University of Georgia, Athens, Georgia, pp. 302–307.

Halle, P.D., J.U. Umoh, L. Saidu and P.A. Abdu, (1998). Disease of poultry in Zaria, Nigeria: a ten-year analysis of clinical records, *Nig. J. Anim. Prod.*, **25**: 88-92.

Hamidinejat H. Seifiabad, M., Shapouri, R., Mayahi, M., Pourmehdi Borujeni, M. (2010). Characterization of *Eimeria* Species in Commercial Broilers by PCR Based on ITS1 Regions of rDNA *Iranian J Parasitol*: **Vol. 5, No.4**, pp. 48-54

Haug A., Thebo, P., Mattsson, J.G., Kaldhusal, M., (2008). Coccidial infections in commercial broilers: epidemiological aspects and comparison of *Eimeria* species identification by morphometric and polymerase chain reaction techniques. *Avian Pathol.* **37**, 161–170. 2008.

Haug, A., Thebo, P., Mattsson, J.G. (2007). A simplified protocol for molecular identification of *Eimeria* species in field samples *Vet. Parasitol.*, **146**, pp. 35–45

He L, Zhou YQ, Oosthuizen MC, Zhao JL (2009). Loop-mediated isothermal amplification (LAMP) detection of *Babesia orientalis* in water buffalo (Bubalus babalis, Linnaeus, 1758) in China. *Vet Parasitol***, 165(1-2),** 36-40.

Hinshaw W. (1952). Coccidiosis vaccine. *Vet Med* **47**: 429–430.

Horton-Smith, C. and Long, P.L. (1954). Preliminary observations on the physical conditions of built-up litter and their possible effects on the parasite populations. In *Proceedings of the Tenth World's Poultry Congress, Edinburgh* Edinburgh, UK, **Section C**, pp. 266–272.

Hodgson J.N. (1970). **Coccidiosis: oocyst counting technique for coccidiostat evaluation.**

Exp Parasitol. **28(1):**99-102.

Huchzermeyer, F.W. (1976). Immunization against coccidiosis in fowls. *Journal of the South African Veterinary Association,* **47**, 253–254.

Idris A.B., Bounous D.I., Goodwin M.A., Brown J., Krushinskie E.A., (1997). Lack of correlation between microscopic lesion scores and gross lesion scores in commercially grown broilers examined for small intestinal *Eimeria* spp. coccidiosis. *Avian Diseases* **41(2):**388-91

Innes EA and Vermeulen A.N. (2006). Vaccination as a control strategy against the coccidial parasites *Eimeria*, Toxoplasma and Neospora. *Parasitology* **133**: S145–S168

Itagaki, K., Tsubokura, M. and Otsuki, K. (1974). Studies on methods for evaluation of anticoccidial drugs *in vitro*. *Japanese Journal of Veterinary Science,* **36**, 195– 202.

Jankievicz H and Schofield R. (1934). The administration of heated oocysts of *Eimeria tenella* as a means of establishing resistance and immunity to cecal coccidiosis. *J Am Vet Med Assoc*; **37**: 507–526.

Jenkins, M. C., Allen, P. Danforth, H., Augustine, P. (1998): Immunization of chickens with plasmid DNA encoding recombinant *Eimeria acervulina* antigen via jet-gun injection elicits immunity against coccidiosis, In: Proceedings 43rd Annual Meeting AAVP, p 47.

Jenkins, M. C., Augustine, P. C., Danforth, H. D., Barta, J. R. (1991): X-irradiation of Eimeria tenella oocysts provides direct evidence that sporozoite invasion and early

schizont development induce protective immune response. *Infect. Immun.* **59**, 4042–4048.

Jenkins, M. C., Sefarian, P. G., Augustine, P. C., Danforth, H. D. (1993): Protective immunity against coccidiosis elicited by radiation-attenuated Eimeria maxima sporozoites that are incapable of asexual development. *Avian Dis.* **37**, 74–82.

Jenkins M.C., (2001). Advances and prospects for subunit vaccines against protozoa of veterinary importance. *Vet Parasitol*., **101**: 291–310.

Jenkins, M., Allen, P., Wilkins, G., Klopp, S., Miska, K., (2008). *Eimeria praecox* infection ameliorates effects of *Eimeria maxima* infection in chickens.*Vet. Parasitol.* **155**, 10–14.

Jeffers, T.K. (1974). Immunisation against *Eimeria tenella* using an attenuated strain. In *Proceedings of the 15th World's Poultry Congress*, New Orleans, USA. (pp. 105–107).

Jeffers, T.K. (1975). Attenuation of *Eimeria tenella* through selection for precociousness. *Journal of Parasitology*, **61**, 1083–1090.

Jeffers, T.K. (1976a). Reduction of anticoccidial drug resistance by massive introduction of drug sensitive coccidia. *Avian Diseases*, **20**, 649–653.

Jeffers, T.K. (1976b). Genetic recombination of precociousness and anticoccidial drug-resistance in *Eimeria tenella. Zeitschrift f˙ur Parasitenkunde*, **50**, 251– 255.

Jeffers, T.K. (1978). Genetics of coccidia and the host response. In P.L. Long, K.N. Boorman and B.M. Freeman (Eds.), *Avian Coccidiosis* (pp. 51–125). Edinburgh: British Poultry Science Ltd.,

Jeffers, T.K. (1986). Attenuation of coccidia – a review. In L.R. McDougald, L.P. Joyner & P.L. Long (Eds.), *Research in Avian Coccidiosis, Proceedings of the Georgia Coccidiosis Conference, 1985* (pp. 482–501). University of Georgia, Athens, Georgia.

Jeffers, T.K. (1989). Anticoccidial drug resistance: a review with emphasis on the polyether ionophores. In P. Yvor´e (Ed.), *Coccidia and Intestinal Coccidiomorphs* (pp. 295–308). Paris: INRA.

Jeffers, T.K. and Long, P.L. (1985). *Eimeria tenella*: immunogenicity of arrested sporozoites in chickens. *Experimental Parasitology*, **60**, 175–180.

Johnson W. (1932). Immunity to coccidiosis in chickens produced by innoculation through the ration. *J Parasitol*; **19**: 106–161.

Johnson, C.A., Kennedy, T.J. and Moeller, M.W. (1986). Immunization of chickens against coccidiosis by termination of infections with BAY VI 9142. In L.R. McDougald, L.P. Joyner and P.L. Long (Eds.), *Research in Avian Coccidiosis, Proceedings of the Georgia Coccidiosis Conference, 1985* University of Georgia, Athens, Georgia. pp. 253–262.

Johnson, J.K. and Long, P.L. (1985). The pathogenicity and immunogenicity of a precocious strain of *Eimeria brunetti. Poultry Science,* **64 (suppl 1),** 123.

Johnson, J and Reid, W.M. (1970). Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens.*Experimental Parasitology,* **28**, 30– 36.

Johnson, J.K., Long, P.L. and McKenzie, M.E. (1986a). The pathogenicity, immunogenicity and endogenous development of a precocious line of *Eimeria brunetti*. *Avian Pathology,* **15**, 697–704.

Johnson, J., Reid, W.M., (1970). Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. *Exp. Parasitol.* **28**, 30–38.

Johnson, J., Reid, W.M. and Jeffers, T.K. (1979). Practical immunization of chickens against coccidiosis using an attenuated strain of *Eimeria tenella*. *Poultry Science*, **58**, 37– 41.

Johnson, J.K., McKenzie, M.E., Perry, E. and Long, P.L. (1986b). The immune response of young chickens given 'graded' or 'trickle' infections with coccidia. In L.R. McDougald, L.P. Joyner and P.L. Long (Eds.), *Research in Avian Coccidiosis, Proceedings of the Georgia Coccidiosis Conference, 1985* University of Georgia, Athens, Georgia. pp. 634–641.

Jorgensen, W. and Anderson, G., (2001). Development of Australian live vaccines against coccidiosis: selection, isolation and attenuation. *Proceedings of the VIIIth International Coccidiosis Conference*, Palm Cove Sydney, Australia, pp. 101–102.

Jorgensen, W.K., Stewart, N.P., Jeston, P.J., Molloy, J.B., Blight, G.W. and Dalgliesh, R.J. (1997). Isolation and pathogenicity of Australian strains of *Eimeria praecox* and *Eimeria mitis*. *Australian Veterinary Journal*, **75**, 592–595.

Jorgensen W.K, Anderson G.R, Jeston P.J, Blight G.W and Molloy J.B. (2006). Selection and characterisation of two attenuated vaccine lines of *Eimeria tenella* in Australia. *Aust Vet J 2006;* **84**: 89–94.

Jorgensen W.K, Richards G. and Anderson G.R. (2006). Evaluation of the most effective measures of infectivity of a precocious line of *Eimeria acervulina* in poultry. *Aust Vet J 2006;* **84**: 428–430.

Joyner, L.P. (1958). Experimental *Eimeria mitis* infections in chickens. *Parasitology*, **48**, 101–112.

Joyner, L.P. (1969). Immunological variation between two strains of *Eimeria acervulina*. *Parasitology*, **59**, 725–732.

Joyner, L.P. & Norton, C.C. (1969). A comparison of two laboratory strains of *Eimeria tenella*. *Parasitology*, **59**, 907– 913.

Joyner, L.P. & Norton, C.C. (1973). The immunity arising from continuous low-level infection with *Eimeria tenella*. *Parasitology*, **67**, 333– 340.

Joyner, L.P. & Norton, C.C. (1976). The immunity arising from continuous low-level infection with *Eimeria maxima* and *E. acervulina*. *Parasitology*, **72**, 115–125.

Jorgensen, W.K., Stewart, N.P., Jeston, P.J., Molloy, J.B., Blight, G.W., Dalgliesh R.J., (1997). Isolation and pathogenicity of Australian strains of *Eimeria praecox* and *Eimeria mitis Aust Vet J*, **75** pp. 592–595.

Joyner, L.P., Long, P.L. (1974). The specific characters of the *Eimeria* with special reference to the coccidia of the fowl. *Avian Pathology*, **3**, 145-157.

Kopko, S. H., Martin, D. S., Barta, J. R. (2000): Responses of chickens to a recombinant refractile body antigen of *Eimeria tenella* administered using various immunizing strategies. *Poult. Sci*. **79**, 36–342.

Kaldhusdal, M.I. (2000). Necrotic enteritis as affected by dietary ingredients. *World Poultry*, **16 (6),** 42– 43.

Karim, M.J. and Trees, A.J. (1989). Immunogenicity of a hybrid line of *Eimeria acervulina*. In P. Yvor´e (Ed.), *Coccidia and Intestinal Coccidiomorphs* Paris: INRA, pp. 639–643.

Karim, M.J., Hoare, J.A. and Trees, A.J., (1991). Limited heterogeneit ybetween strains of *Eimeria tenella* isolated from Britain and Bangladesh. *Research in Veterinary Science*, **51**, 29–33.

Karkhanis, Y.D., Nollstadt, K.A., Bhogal, B.S., Ravino, O., Pellegrino, R., Crane, M.S., Murray, P.K. and Turner, M.J. (1991). Purification and characterisation of a protective antigen from *Eimeria tenella*. *Infection and Immunity*, **59**, 983–989.

Kassid, J.F.H., Majeid, L.A. and Juma, F.T. (1988). Effect of dietary salinomycin on growth performance of broilers. *Iraqi Journal of Agricultural Sciences* **6**, 7–19.

Kawaguchi, H., Konishi, T. and Nakamura, T. (1988). Characteristics of a precocious line of *Eimeria tenella*: pathogenicity and endogenous development. *Japanese Journal of Veterinary Science*, **50,** 445–452.

Kawazoe, U. and Manarini, C.A. de L. (2001). Development and characterization of *Eimeria acervulina* Brazilian precocious strain. *Proceedings of the VIIIth International Coccidiosis Conference*, Palm Cove, Sydney, Australia, p. 172.

Kheysin, Y.M. (1972). *Life Cycles of Coccidia of Domestic Animals*. London: William Heinemann Medical Books Ltd.

Klasing, K.C. (1991). Interaction between nutrition and infectious disease in broiler chickens. *Proceedings of the 26th National Meeting on Poultry Health and Condemnations* pp. 97–106.

Klesius, P.H. and Giambrone, J.J. (1986). Modulation of the immune responses to coccidia. In L.R. McDougald, L.P. Joyner & P.L. Long (Eds.), *Research in Avian Coccidiosis, Proceedings of the Georgia Coccidiosis Conference, 1985* University of Georgia, Athens, Georgia. pp. 555–563.

Kogut M.H., Gore T.C. and Long P.L. (1983). Serial passage of *Eimeria tenella* and *E. necatrix* in turkey embryos. *Parasitology* **86** (**Pt 2**): 199–209.

K¨ohler, B. (2000). *Clostridium perfringens* intoxication affects bird performance. *World Poultry*, **16** (**5**), 57– 59

Krassner, S.M. (1963). Factors in host susceptibility and oocyst infectivity in *Eimeria acervulina* infections. *Journal of Protozoology*, **10,** 327– 333.

Kucera, J. (1991). Enzyme variants of *Eimeria* parasitising the domestic fowl and possibilities of species diagnostics. *Folia Parasitologia*, **38**, 193–199.

Kucera, J. & Bedrn´õ k, P. (1987). Pokus o oslaben´õ patogenity kokcidie *Eimeria maxima* metodou postupn´eho zkracov´an´õ v´yvojov´eho cyklu. *Biologizace a Chemizace Veterinaria*, **23,** 353–359.

Lamorde, A.G. (1998): Scenario building for the Nigerian Livestock Industry in the 21st century. A paper presented at the Silver Anniversary Conference of the Nigerian Society for Animal Production-Gateway Hotel, Abeokuta, Nigeria. March 21-26, 1998.

Laseinde, E.A.O., 2002. Poultry: God's goldmine in the livestock industry. An inaugural lecture, Federal University of Technology Akure. Classic Educational Publishers, Akure, Nigeria, pp: 48.

Lew, A.E. Anderson, G.R. Minchin, C.M. Jeston, P.J. Jorgensen W.K. (2003). Inter- and intra-strain variation and PCR detection of the internal transcribed spacer 1 (ITS-1) sequences of Australian isolates of Eimeria species from chickens. *Vet. Parasitol*., **112**, pp. 33–50

Lee, E. H. (1987). Vaccination against coccidiosis in commercial roaster chickens. *Canadian Veterinary Journal*, **28**, 434–436.

Lee, E.-H. (1989). Control of coccidiosis in broiler chickens by vaccination. Field trial comparison between Immucox (coccidiosis vaccine) and halofuginone, salinomycin program in Texas, USA. In P. Yvor´e (Ed.), *Coccidia and Intestinal Coccidiomorphs* Paris: INRA pp. 661– 665.

Lee, E.-H. (1993). Live coccidiosis vaccines and a field immune variant of *Eimeria maxima*: a case report. In J.R. Barta & M.A. Fernando (Eds.), *Proceedings VIth International Coccidiosis Conference*, Guelph, Canada, pp. 118–121.

LeMay, M.A., Genteman, K.C., Weber, F.H., Lewis, D.O. & Evans, N.A. (2001). Efficacy of *in ovo* vaccination with single and mixed species of live *Eimeria* oocysts. *Proceedings of the VIIIth International Coccidiosis Conference*, Palm Cove Sydney, Australia, p. 107.

Levine, N.D. (1961). *Protozoan Parasites of Domestic Animals and Man*. Minneapolis: Burgess Publishing Company.

Levine, P.P. (1942). Excystation of coccidial oocysts of the chicken, *Journal of Parasitology*, **28**, 426– 428.

Lewis, N.J. and Hurnik, J.F. (1990). Locomotion of broiler chickens in floor pens. *Poultry Science*, **69**, 1087–1093.

Lillehoj, H.S. (1988). Influence of inoculation dose, inoculation schedule, chicken age, and host genetics on disease susceptibility and development of resistance to *Eimeria tenella* infection. *Avian Diseases,* **32**, 437– 444.

Lillehoj, H.S. and Trout, J.M. (1993). Coccidia: a review of recent advances on immunity and vaccine development. *Avian Pathology,* **22**, 3–31

Liu, C.M. (1982). Microbial aspects of polyether antibiotics: activity, production, and biosynthesis. In J.W. Westley (Ed.), *Polyether Antibiotics, Naturally Occurring Acid Ionophores*,. New York: Marcel Dekker. *Biology* **1**, pp. 43–102.

Lien, Y.Y., Sheu, S.C., Liu, H.J., Chen, S.C., Tsai, M.Y., Luo, S.C., Wu, K.C., Liu, S.S. and Su, H.Y. (2007). Cloning and nucleotide sequencing of the second internal transcribed spacer of ribosomal DNA for three species of Eimeria from chickens in Taiwan. *Veterinary Journal,* **173**, 186-191.

Lillehoj HS. (1989). Intestinal intra-epithelial and splenic natural killer cell responses to eimerian infections in inbred chickens. *Infect Immun* **57**: 1879–1884.

Lillehoj, H. S and Choi K. D., (1998). Recombinant chicken interferon gamma inhibits in vitro *Eimeria tenella* development, and reduces in vivo oocyst production and body weight loss following challenge infection with *Eimeria acervulina*. *Avian Dis*. **42**:307–314.

Lillehoj, H. S., Chai., K. D., Heller, D., Jenkins, M. C., Vakharia, V. N. (1997): Naked DNA immunization with a plasmid encoding an *Eimeria* surface sporozoite protein and oral feeding of baculovirus-expressed *Eimeria* protein elicit serum antibodies and reduced oocyst output, In: *Proc. VII. Int. Coccidiosis Conf*., p. 105.

Lillehoj H. S., Choi K.D. (1998). Recombinant chicken interferon gamma-mediated inhibition of *Eimeria tenella* development in vitro and reduction of oocyst production and body weight loss following *Eimeria acervulina* challenge infection. *Avian Dis*, **42**: 307–314.

Lillehoj, H.S., Lillehoj, E. P. (2000). Avian coccidiosis: A review of acquired intestinal immunity and vaccination strategies. *Avian Diseases*, **44**: 408-425.

Long, P.L. and Joyner, L.P. (1984). Problems in identification of species of *Eimeria*. *The Journal of Parasitology*, **31**, 535-541.

Long, P.L., Millard, B.J., Joyner, L.P. and Norton, C.C. (1976). A guide to laboratorytechniques used in the study and diagnosis of avian coccidiosis. *Folia Veterinaria Latina*, **6**, 201-217

Long, P.L. (1965). Development of *Eimeria tenella* in avian embryos. *Nature, London*, ***208***, 509–510.

Long, P.L. (1966). The growth of some species of *Eimeria* in avian embryos. *Journal of Parasitology*, **56,** 575–581.

Long, P.L. (1968a). The pathogenic effects of *Eimeria praecox* and *E. acervulina* in the chicken. *Parasitology*, **58**, 691–700.

Long, P.L. (1968b). The effect of breed of chickens on resistance to *Eimeria* sp. infections. *British Poultry Science*, **9**, 71–78.

Long, P.L. (1971). Maintenance of intestinal protozoa *in vivo* with particular reference to *Eimeria* and *Histomonas*. In A.E.R. Taylor and R. Muller (Eds.), *Isolation and*

Maintenance of Parasites in Vivo Oxford: Blackwell Scientific Publications Ltd. pp. 65–75.

Long, P.L. (1972). *Eimeria tenella*: reproduction, pathogenicity and immunogenicity of a strain maintained in chick embryos by serial passage. *Journal of Comparative Pathology*, ***82,*** 429–437.

Long, P.L. (1973). Endogenous stages of a "chick embryo-adapted " strain of *Eimeria tenella*. *Parasitology,* **66**, 55– 62.

Long, P.L. (1974). Further studies on the pathogenicity and immunogenicity of an embryo-adapted strain of *Eimeria tenella*. *Avian Pathology,* **3**, 255–268.

Long, P.L. and Jeffers, T.K. (1982). Studies on the stage of action of ionophorous antibiotics against *Eimeria*. *Journal of Parasitology,* **68**, 363– 371.

Long, P.L. and Johnson, J. (1981). Coccidial infections acquired by sentinel birds exposed to litter from experimental and commercial broiler chickens. *Avian Pathology,* **10**, 387– 394.

Long, P.L. and Johnson, J.K. (1988). *Eimeria* of American chickens: characteristics of six attenuated strains produced by selection for precocious development. *Avian Pathology,* **17**, 305– 314.

Long, P.L. and Millard, B.J. (1979a). *Eimeria*: further studies on the immunisation of young chickens kept in litter pens. *Avian Pathology*, **8**, 213–228.

Long, P.L. and Millard, B.J. (1979b). Immunological differences in *Eimeria maxima*: effect of a mixed immunizing inoculum on heterologous challenge. *Parasitology,* **79**, 451–457.

Long, P.L. and Rowell, J.G. (1975). Sampling broiler house litter for coccidial oocysts. *British Poultry Science*, **16**, 583– 592.

Long, P.L., Johnson, J. and Baxter, S. (1985). *Eimeria tenella*: relative survival of drug-resistant and drug-sensitive populations in floor pen chickens. *Poultry Science*, **64**, 2403–2405.

Long, P.L., Tompkins, R.V. and Millard, B.J. (1975). Coccidiosis in broilers: evaluation of infection by the examination of broiler house litter for oocysts. *Avian Pathology*, **4**, 287–294.

Long, P.L., Johnson, J., McKenzie, M.E., Perry, E., Crane, M.S.J. and Murray, P.K. (1986). Immunization of young broiler chickens with low level infections of *Eimeria tenella*, *Eimeria acervulina* or *Eimeria maxima*. *Avian Pathology*, **15**, 271– 278.

Lotze, J.C. and Leek, R.G. (1969). Observations on *Eimeria tenella* in feces of inoculated chickens. *Journal of Protozoology*, **16**, 496–498.

Lowenthal, J. W., York, J. J. O'Neil, T. E. Rhodes, S. Prowse, S. J.D. G. Strom, and M. R. Digby **(**1997). In-vivo effects of chicken interferon-gamma during infection with *Eimeria*. *J. Interferon Cytokine Res*. **17:**551–558.

Lowenthal, J.W., Bean, A.G.D. and Johnson, M.A. (2001). New strategies for enhancing immunity: therapeutic delivery of vaccines and cytokines via fowl adenovirus. *Proceedings of the VIIIth International Coccidiosis Conference, Palm Cove* (p. 111). Sydney, Australia. Lowenthal, J. W., M. R. Digby, and J. J. York. 1995. Production of interferon- gamma by chicken T-cells. *J. Interferon Cytokine Res*. **15**:933–938.

Lowenthal, J. W., O'Neil, T. E. David, A. Strom, G and. Andrew M. E. (1999). Cytokine therapy: a natural alternative for disease control. *Vet Immunol. Immunopathol.* **72:**183–188.

Lowenthal, J.W., Richards, G.G., Bean, A.G.D. and Johnson, M.A. (2000). New strategies for enhancing immunity to *Eimeria*: interferon gamma is the natural approach to disease control. In *Proceedings of the COST 820 Annual Workshop; Immunity to Coccidial Parasites: from Natural Infections to Molecular Vaccination*, Dublin, Eire, pp. 36–37.

Majaro, O.M., (1980). The epidemiology and economic importance of poultry coccidiosis in Oyo State, Nigeria. *Rev. Elev. Med. Vet. Pays Trop*., **33**, 377- 379.

Majaro, O.M., (1983). Poultry coccidiosis: Evaluation of management systems on the incidence of coccidian infection in Nigeria. *Rev. Elev. Med. Vet. Pays Trop*., **36**, 343-346.

McDougald, L.R., Fuller, L., Mattiello, R. (1997). A survey of Coccidia on 43 poultry farms in Argentina. *Avian Diseases*, **41**, 923-929.

McDonald, V. and Ballingall, S. (1983a). Further investigation of the pathogenicity, immunogenicity and stability of precocious *Eimeria acervulina*. *Parasitology, **86***, 361– 369.

McDonald V, Wisher MH, Rose ME and Jeffers T.K. (1988). *Eimeria tenella*: immunological diversity between asexual generations. *Parasite Immunol*., **10**: 649–660

McDonald, V. and Ballingall, S. (1983b). Attenuation of *Eimeria mivati* (= *mitis*) by selection for precocious development. *Parasitology*, **86**, 371– 379.

McDonald, V. and Shirley, M. W. (1984). *Eimeria mitis*: a comparison of the endogenous developmental stages of a line selected for early maturation and the parent strain. *Parasitology*, **88**, 37–44.

McDonald, V., Ballingall, S. and Shirley, M.W. (1982). A preliminary study of the nature of infection and immunity in chickens given an attenuated line of *Eimeria acervulina*. *Parasitology*, **84**, 21– 30.

McDonald, V., Shirley, M.W. and Bellatti, M.A. (1986a). *Eimeria maxima*: characteristics of attenuated lines obtained by selection for precocious development in the chicken. *Experimental Parasitology*, **61**, 192– 200.

McDonald, V., Shirley, M.W. and Chapman, H.D. (1985). Attenuation of *Eimeria* species: further characterisation of two lines of *E. mitis*. *Research in Veterinary Science*, **39**, 328– 332.

McDonald, V., Shirley, M.W. and Millard, B.J. (1986b). A comparative study of two lines of *Eimeria tenella* attenuated either by selection for precocious development in the chicken or by growth in chicken embryos. *Avian Pathology*, **15**, 323–335.

McDonald V and Shirley M.W. (2009). Past and future: vaccination against *Eimeria*. *Parasitology*, **136**: 1477–1489.

McDougald, L.R. (1980). Compensatory growth in broiler chickens as related to the use of ionophorous antibiotic anticoccidial drugs. *Proceedings of the Georgia Nutrition Conference for the Feed Industry*, Georgia, USA pp. 11–16.

McDougald, L.R. (1982). Chemotherapy of coccidiosis. In P.L. Long (Ed.), *The Biology of the Coccidia* London: Edward Arnold, pp. 373–427.

McDougald, L.R. (1993). Chemotherapy of coccidiosis. *Proceedings of the VIth International Coccidiosis Conference, Guelph*. Guelph, Canada, pp. 45– 47.

McDougald, L.R., Fuller, L. and Mattiello, R. (1997). A survey of coccidia on 43 poultry farms in Argentina. *Avian Diseases*, **41**, 923–929.

McDougald, L.R., Da Silva, J.M.L., Solis, J. and Braga, M. (1987). A survey of sensitivity to anticoccidial drugs in 60 isolates of coccidian from broiler chickens in Brazil and Argentina. *Avian Diseases,* **31**, 287– 292.

McEvoy, J. (2001). Safe limits for veterinary drug residues: what do they mean? *Northern Ireland Veterinary Today*, *Spring*, 37– 40.

Martin, A.G., Danforth, H.D., Barta, J.R. and Fernando, M.A. (1997). Analysis of immunological cross-protection and sensitivities to anticoccidial drugs among five geographical and temporal strains of *Eimeria maxima. International Journal for Parasitology*, **27**, 527– 533.

Marusich, W.L., Ogrinz, E.F., Camerlengo, N. and Mitrovic, M. (1977). Effect of diet on the performance of chickens fed lasalocid in combination with growth promotants. *Poultry Science*, **56**, 1297–1304.

Mathis, G.F. (1999a). The influence of coccidiosis vaccine, Coccivac- B, on compensatory weight gain in broiler chickens in comparison with the anticoccidial, salinomycin. *Proceedings of the Southern Poultry Science Society Conference, Atlanta* (unpaginated).

Mathis, G.F. (1999b). The influence of the coccidiosis vaccine, Coccivac-B, on compensatory weight gain of broiler chickens in comparison with the anticoccidial, salinomycin. *Poultry Science,* **78 (suppl 1),** 117.

Mathis, G.F. and Lang, M. (2001a). Comparative efficacy of Coccivac-B in broiler chickens. *Proceedings of the VIIIth International Coccidiosis Conference, Palm* Sydney, Australia, *Cove* pp. 112–113.

Mathis, G.F. and Lang, M. (2001b). The influence of the coccidiosis vaccine, Coccivac-B®, on compensatory weight gain of broiler chickens in comparison with the anticoccidial, salinomycin. *Proceedings of the VIIIth International Coccidiosis Conference, Palm Cove* Sydney, Australia, p. 113.

Michael, A. (2001). Clinical trials with CoxAbic® against coccidiosis in chickens. *Proceedings of the VIIIth International Coccidiosis Conference, Palm Cove* Sydney, Australia, p. 118.

Mundt, H.C. and Haberkorn, A. (1989). New experiences with toltrazuril in the control of poultry coccidiosis. In P. Yvor´e (Ed.), *Coccidia and Intestinal Coccidiomorphs,* Paris: INRA pp. 401–406.

Murray, P.K, Bhogal, B.S., Crane, M.S.J. and MacDonald, T.T. (1986). *Eimeria tenella – in vivo* immunization studies with sporozoite antigen. In L.R. McDougald, L.P. Joyner and P.L. Long (Eds.), *Research in Avian Coccidiosis, Proceedings of the Georgia Coccidiosis Conference, 1985* University of Georgia, Athens, Georgia, pp. 564–573.

McDougald, L.R. Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, Swayne D. E. (2003). Diseases of Poultry, **11th** edn pp. es: Iowa State Press, 974-1023

Meireles MV, Roberto LO, Riera RF. (2004). Identification of *Eimeria mitis* and *Eimeria praecox* in Broiler Feces Using Polymerase Chain Reaction. *Brazilian J Poult Sci.*, **6(4):** 249–252

Mohammad Q., (2002). Al-Natoura, Maysoon M. Suleimana, Mahmoud N.Abo-Shehada,. Flock-level prevalence of *Eimeria* species among broiler chicks in northern Jordan. *Preventive Veterinary Medicine***, 53**, 305–310

Morgan, J.A.T, G.M. Morris, B.M. Wlodek, R. Byrnes, M. Jenner, C.C. Constantinoiu, G.R. Anderson, A.E. Lew-Tabor, J.B. Molloy, R.B. Gasser, W.K. Jorgensen (2009). Real-time polymerase chain reaction (PCR) assays for the specific detection and quantification of seven Eimeria species that cause coccidiosis in chickens. *Mol. Cell. Probes*, **23**, pp. 83–89

Morris, G.M., Gasser, R.B., (2006). Biotechnological advances in the diagnosis of avian coccidiosis and the analysis of genetic variation in *Eimeria*. *Biotechnol. Adv.* **24**, 590–603.

Morris, G.M., Wood, J.M., Richards, D.G. and Gasser, R.B. (2007a). The application of a polymerase chain reaction (PCR)-based capillary electrophoretic technique provides detailed insights into Eimeria populations in intensive poultry establishments. *Molecular and Cellular Probes*, **21**, 288-294.

Musa W., Saidu L., Jatau I.D., Adamu J., Otu M.O., Adu P. A (2010). Outbreak of Coccidiosis in 5 day-old commercial broiler breeder flock in Zaria, Nigeria. *International Journal of Poultry Science* **(12),** 1112-1115.

Muazu, A.A. Masdooq, J. Ngbede, A.E. Salihu, G. Haruna, A.K. Habu, M.N. Sati and H. Jamilu (2008). Prevalence and Identification of Species of *Eimeria* Causing Coccidiosis in Poultry within Vom, Plateau State, Nigeria. *International Journal of Poultry Science* **7 (9),** 917-918.

Nagamine K, Hase T, Notomi T (2002). Accelerated reaction by loop-mediated isothermal amplification using loop primers. Molecular and Cellular Probes. **16**: 223-229.

Nakai, Y., Uchida, T. and Kanazawa, K. (1992). Immunization of young chickens by trickle infection with *Eimeria tenella. Avian Diseases,* **36**, 1034–1036.

Newberry, R.C. and Hall, J.W. (1988). Space utilization by broiler chickens in floor pens. *Proceedings of the International Congress on Applied Ethology in Farm Animals*, Skara, Sweden, pp. 305–309.

Newberry, R.C. and Hall, J.W. (1990). Use of pen space by broiler chickens: effects of age and pen size. *Applied Animal Behaviour Science*, **25**, 125–136.

Newman, L.J. (1999). Coccidiosis control with vaccine: been there, done that. Or have we? *Proceedings of the Arkansas Poultry Symposium, Springdale* Arkansas, USA, (pp. 52–55).

Norton C.C. & Joyner L.P. (1981). *Eimeria acervulina* and *E. mivati*: oocysts, life-cycle and ability to develop in the chicken embryo. *Parasitology,* **83**, 269-279

Norton, C.C. and Joyner, L.P. (1986). Avian coccidiosis: the administration of encapsulated oocysts. *Parasitology*, **92,** 499–510.

Oliveira, C. de (2001). Latest thinking on avian coccidiosis. In *Vaccination at Work in Commercial Broilers* Driffield, UK: Positive Action Publications Ltd., **part 2**, p. 9.

Onaga, H., Kitamura, T., Nunoya, T. and Ishii, T. (1992a). Immunogenicity and pathogenicity of precocious lines of *Eimeria acervulina* and *E. maxima* for chickens. *Proceedings of the First Asian Conference on Avian Coccidiosis, Otsu*. Osaka, Japan, pp. 154–162.

Onaga, H., Nunoya, T., Togo, M., Kitamura, T., and Ishii, T. (1992b). Application of precocious lines of *Eimeria tenella* and *E. necatrix* to chickens reared in cages with litter on the floor. *Proceedings of the First Asian Conference on Avian Coccidiosis*, Otsu, Osaka, Japan, pp. 163–170.

Ovington, K.S., Alleva, L.M. and Kerr, E.A. (1995). Cytokines and immunological control of *Eimeria* spp. *International Journal for Parasitology*, **25**, 1331–1351.

Oladoja M.A. and Olusanya T.P., (2007). Adoption of Coccidiosis Vaccines by Poultry Farmers in Ijebu Area of Ogun State, *Nigeria International Journal of Poultry Science* **6 (12),** 883-887.

Oluyemi, J.A. and F.A. Roberts, (1979). Poultry production in warm wet climate. Low cost edition, Macmillan publishers Ltd. London, pp: 141-147.

Owoade A A, Oluwayelu D.O, Fagbohun O.A, Ammerlaan W, Mulders M.N, Muller C P. (2004) Serologic evidence of chicken infectious anaemia in commercial chicken flocks in South West Nigeria. *Avian Dis.* **48**, 202-205.

Oyagbemi T. O. and Adejinmi J. O. (2012). Supplementation of broiler feed with leaves of *Vernonia amygdalina* and *Azadirachta indica* protected birds naturally infected with *Eimeria* species. *African Journal of Biotechnology* Vol. **11**(33), pp. 8407-8413.

Pelle´rdy, L.P. (1974). Genus: Gallus. In L.P. Pelle´rdy (Ed.), Coccidia and coccidiosis 2nd edn Verlag Paul Parey, Berlin and Hamburg, Germany, pp. 220-261.

Parry, S.H. and Davis, P.J. (1986). A review of the characteristics of the immune system of the chicken which affect prospective vaccine development for coccidiosis. In L.R. McDougald, L.P. Joyner and P.L. Long (Eds.), *Research in Avian Coccidiosis*,

Proceedings of the Georgia Coccidiosis Conference, 1985 University of Georgia, Athens, Georgia, p.642.

Parry, S.H., Barratt, M.E.J., Davis, P.J. and Jones, S. (1989). Theoretical and practical aspects of vaccination against coccidiosis. In P. Yvor´e (Ed.), *Coccidia and Intestinal Coccidiomorphs* Paris: INRA, pp. 617–632.

Parry, S., Barratt, M.E.J., Jones, S., McKee, S. and Murray, J.D. (1992). Modelling coccidial infection in chickens: emphasis on vaccination by in-feed delivery of oocysts. *Journal of Theoretical Biology*, **157**, 407– 425.

Patterson, L.T., Johnson, L.W. and Edgar, S.A. (1961). The comparative resistance of several inbred lines of S.C. White Leghorns to certain infectious diseases. *Poultry Science*, **40**, 1442.

Philps, B.R. (1994). Programme planning for coccidiosis control in broilers. *Proceedings of the Second Asian Conference on Coccidiosis, Guangzhou* Guangzhou, China, (pp. 42– 50).

Philps, B.R. (1997). The art and science of productive coccidiosis control in broilers. *Proceedings of the VIIth International Coccidiosis Conference*, Oxford, UK, p. 42.

Pierson, F.W., Larsen, C.T. and Gross, W.B. (1997). The effect of stress on the response of chickens to coccidiosis vaccination. *Veterinary Parasitology*, **73**, 177–180.

Poston, R.M., Carter, A.L., Martin, A., Hutchins, J.E., Avakian, A.P., LeMay, M.A., Genteman, K.C., Weber, F.H. and Doelling, V.W. (2001). Efficacy of a coccidiosis vaccine delivered *in ovo* to commercial broilers. *Proceedings of the VIIIth International Coccidiosis Conference*, Palm Cove, Sydney, Australia, pp. 128–129.

Razmi, G.R., Kalideri, G.A., (2000). Prevalence of subclinical coccidiosis in broiler-chicken farms in the municipality of Mashhad, Khorasan, *Iran. Prev. Vet. Med.* **44**, 247–253.

Raines, T.V. (1968). Buquinolate – a review. *Poultry Science*, **47**, 1425–1432.

Rebollo, M., Lauro, T., S´anchez, H.F., Irion, T. and Greif, G. (2001). Evaluation of anticoccidial immunity conferred by toltrazuril treatment after coccidia vaccine application. *Proceedings of the VIIIth International Coccidiosis Conference, Palm Cove* Sydney, Australia, pp. 131–132.

Reid, W.M. (1960). The relationship between coccidiostats and poultry flock immunity in coccidiosis control programs. *Poultry Science*, ***39,*** 1431–1437.

Richards, G. and Woods, W. (2001). The use of four species live *Eimeria* vaccines in broilers and layers in Australia. *Proceedings of the VIIIth International Coccidiosis Conference,* Palm Cove, Sydney, Australia p. 133.

Riddell, C. and Kong, X.M. (1992). The influence of diet on necrotic enteritis in broiler chickens. *Avian Diseases*, **36,** 499– 503.

Roper, T.J. and Marples, N.M. (1997). Colour preferences of domestic chicks in relation to food and water presentation. *Applied Animal Behaviour Science,* **54**, 207– 213.

Rose, M.E. (1967). The influence of age of host on infection with *Eimeria tenella. Journal of Parasitology,* **53**, 924–929.

Rose, M.E. (1973). Immunity. In D.M. Hammond and P.L. Long (Eds.), *The Coccidia: Eimeria, Isospora, Toxoplasma, and Related Genera* Baltimore: University Park Press, pp. 295–341.

Rose, M.E. (1978). Immune responses of chickens to coccidia and coccidiosis. In P.L. Long, K.N. Boorman and B.M. Freeman (Eds.), *Avian Coccidiosis* Edinburgh: British Poultry Science Ltd. pp. 297–337.

Rose, M.E. (1982). Host immune responses. In P.L. Long (Ed.), *The Biology of the Coccidia* London: Edward Arnold, pp.329–371.

Rose, M.E. (1986). Immune responses to *Eimeria* infections. In L.R. McDougald, L.P. Joyner & P.L. Long (Eds.), *Research in Avian Coccidiosis, Proceedings of the Georgia Coccidiosis Conference, 1985* University of Georgia, Athens, Georgia pp. 449–469.

Rose, M.E. (1987). *Eimeria, Isospora* and *Cryptosporidium*. In E.J.L. Soulsby (Ed.), *Immunology, Immunopathology and Immunoprophy - laxis of Parasitic Infections* Boca Raton: CRC Press, **3**, pp. 275–312.

Ross, P.A. and Hurnick, J.F. (1983). Drinking behaviour of broiler chicks. *Applied Animal Ethology*, **11**, 25– 31.

Ruff, M.D. (1989). Interaction of avian coccidiosis with other diseases: a review. In P. Yvor´e (Ed.), *Coccidia and Intestinal Coccidiomorphs* Paris: INRA, pp. 173–181.

Ruff, M.D. (1991). An overview of control measures for coccidiosis ; present and future. In *Proceedings of the Seventh International Poultry Breeders' Conference,* Auchincruive, UK, pp. 29–38.

Ruff, M.D., Fredericksen, T.L., Thaxton, J.P., Strohlein, D.A., Danforth, H.D. & Gildersleeve, R.G. (1988). *In ovo* vaccination against *Eimeria tenella*. *Poultry Science*, **67 (Suppl 1),** 147.

Ryley, J.F. (1980). Drug resistance in coccidia. *Advances in Veterinary Science and Comparative Medicine*, **24,** 99–120.

Rose, M. E., Hesketh P., (1987). Eimeria tenella: effects of immunity of sporozoites within the lumen of the small intestine. *Exp. Parasitol*. **63**, 337-344

Ruff, M. D., H. D. Danforth. (1996): Resistance of coccidia to medications, In: *Proc. XXth World's Poult. Congr.*, **vol. II**., 427–430.

Schmatz, D. M. (1997): Anticoccidial drug discovery and design, In: Shirley, M. W., Tomley, F. M., Freeman, B. M. Control of coccidiosis into the next millennium. *Proc. VII Int. Coccidiosis Conf*., p.20–21.

Schnitzler, B. E., Thebo, P. L. Tomley F. M., Uggla, A., Shirley M.W., (1999). PCR identification of chicken Eimeria: a simplified read-out *Avian Pathol*., **28**, pp. 89–93

Seifert, H., (1996). Tropical Animal Health. Kluwer Academic Publishers, Boston,London.

Sainsbury, D.W.B. (1988). Coccidiosis control with a new disinfectant. *Clinical Insight*, ***3,*** 247– 248.

Sanda, A. (1977). Development of immunity to coccidiosis caused by *Eimeria tenella* in one-day-old chickens. *Acta Veterinaria, Brno*, ***46,*** 311– 314.

Schetters, T.P.M., Janssen, H.A.J.M. and Vermeulen, A.N. (1999). A new vaccination concept against coccidiosis in poultry. *World Poultry, Special Supplement Coccidiosis (3)*, pp. 26–27.

Schnitzler, B.E. and Shirley, M.W. (1999). Immunological aspects of infections with *Eimeria maxima*: a short review. *Avian Pathology*, **28**, 537– 543.

Seeger, K.C. (1947). Flock *Eimeria tenella* inoculation followed by sulfaguanidine or sulfamethazine treatment as a method of controlling caecal coccidiosis. *Poultry Science*, **26,** 554–555.

Shane, S.M., Koetting, D.G. and Harrington, K.S. (1984). The occurrence of *Clostridium perfringens* in the intestine of chicks. *Avian Diseases,* **28**, 1120–1124.

Sharma, J.M. and Burmester, B.R. (1982). Resistance to Marek's disease at hatching in chickens vaccinated as embryos with the turkey herpes virus. *Avian Diseases,* **26**, 134–149.

Sharma, V.D., Fernando, M.A. and Summers, J.D. (1973). The effect of dietary crude protein level on intestinal and cecal coccidiosis in chickens. *Canadian Journal of Comparative Medicine*, **37**, 195–199.

Shibalova, T.A. (1970). Cultivation of the endogenous stages of chicken coccidia in embryos and tissue cultures. *Journal of Parasitology,* **56 (4: Sect II, Part I),** 315–316.

Shibalova, T.A., Korolev, A.M. and Sobchak, I.A. (1969). Cultivation of chicken coccidia in chick embryos. *Veterinariya, Moskva,* **11**, 68–71.

Shimura, K. and Isobe, T. (1994). Pathogenicity and drug resistance of a recombinant line between a precocious line and a drug resistant field isolate of *Eimeria tenella. Proceedings of the Second Asian Conference on Coccidiosis,* Guangzhou, China, (pp. 119–123).

Shiotani, N., Baba, E., Fukata, T., Arakawa, A. and Nakanishi, T. (1992). Distribution of oocysts, sporocysts and sporozoites of *Eimeria tenella* and *Eimeria maxima* in the digestive tract of chicken. *Veterinary Parasitology,* **41**, 17–22.

Shirley M.W. (1980). *Eimeria necatrix:* the development and characteristics of an egg-adapted (attenuated) line. *Parasitology*; **81**: 525–535.

Shirley, M.W. (1988a). Control of coccidiosis with vaccines. In *Proceedings of the Second Asian/Pacific Poultry Health Conference*, Surfers' Paradise, Australia, (pp. 129–157).

Shirley, M.W. (1988b). Coccidiosis: interaction of immunity and disease with management and environment. In *Proceedings of the Second Asian/Pacific Poultry Health Conference* Surfers' Paradise, Australia, pp. 107–126.

Shirley, M.W. (1989). Development of a live attenuated vaccine against coccidiosis of poultry. *Parasite Immunology*, **11**, 117–124.

Shirley, M.W. (1993). Live vaccines for the control of coccidiosis. In J.R. Barta and M.A. Fernando (Eds.), *Proceedings VIth International Coccidiosis Conference*, Guelph, Canada, pp. 61–72.

Shirley, M.W. and Bedrn´õ k, P. (1997). Live attenuated vaccines against avian coccidiosis: success with precocious and egg-adapted lines of *Eimeria. Parasitology Today*, **13**, 481– 484.

Shirley, M.W. and Bellatti, M.A. (1984). *Eimeria necatrix*: selection of characteristics of a precocious (and attenuated) line. *Avian Pathology*, **13**, 657– 668.

Shirley, M.W. and Bellatti, M.A. (1988). Live attenuated coccidiosis vaccine: selection of a second precocious line of *Eimeria maxima. Research in Veterinary Science*, **44**, 25–28.

Shirley, M.W. and Harvey, D.A. (1996). *Eimeria tenella*: genetic recombination of markers for precocious development and arprinocid resistance. *Applied Parasitology,* **37**, 293– 299.

Shirley, M.W. and Hoyle, S.R. (1981). The antigenicity of *Eimeria maxima* populations obtained from commercial farms. *Journal of Parasitology*, **67**, 587– 588.

Shirley MW, McDonald V and Ballingall S. (1981). *Eimeria* spp. From the chicken: from merozoites to oocysts in embryonated eggs. *Parasitology*; **83**: 259–267.

Shirley, M.W. and Jeffers, T.K. (1990). Genetics of coccidia and genetic manipulation. In P.L. Long (Ed.), *Coccidiosis of Man and Domestic Animals* Boca Raton: CRC Press, pp. 205–220.

Shirley, M.W. and Long, P.L. (1990). Control of coccidiosis in chickens: immunization with live vaccines. In P.L. Long (Ed.), *Coccidiosis of Man and Domestic Animals* Boca Raton: CRC Press, pp. 321–341.

Shirley, M.W. and Millard, B.J. (1986). Studies on the immunogenicit y of seven attenuated lines of *Eimeria* given as a mixture to chickens. *Avian Pathology*, **15**, 629–638.

Shirley, M.W., Jeffers, T.K. and Long, P.L. (1983). Studies to determine the taxonomic status of *Eimeria mitis*, Tyzzer 1929 and *E. mivati*, Edgar and Seibold 1964. *Parasitology*, **87**, 185–198.

Shirley, M.W., McDonald, V. and Bellatti, M.A. (1986). *Eimeria brunetti*: selection and characteristics of a precocious (and attenuated) line. *Avian Pathology,* **15**, 705–717.

Shirley, M.W., McDonald, V., Chapman, H.D. and Millard, B.J. (1984). *Eimeria praecox*: selection and characteristics of precocious lines. *Avian Pathology*, **13**, 669–682.

Shirley, M.W., Bushell, A.C., Bushell, J.E., McDonald, V. and Roberts, B. (1995). A live attenuated vaccine for the control of avian coccidiosis: trials in broiler breeders and replacement layer flocks in the United Kingdom. *Veterinary Record,* **137**, 453– 457.

Shirley MW, Smith AL and Blake D.P., (2007). Challenges in the successful control of the avian coccidia. *Vaccine,* **25**, 5540–5547.

Shirley MW, Smith AL and Tomley F.M.(2005). The biology of avian *Eimeria* with an emphasis on their control by vaccination. *Adv Parasitol* **60**, 285–330.

Shumard, R.F. and Callender, M.E. (1970). Anticoccidial drugs: screening methods. *Experimental Parasitology,* **28**, 13– 24.

Siegel, H.S. (1995). Stress, strains and resistance. *British Poultry Science,* **36**, 3–22.

Sklan, D. (2001). Development of the digestive tract of poultry. *World's Poultry Science Journal,* **57,** 415–428.

Sluis, W. van der (2000). Clostridial enteritis is an often underestimated problem. *World Poultry,* **16 (7),** 42– 43.

Smith, C.K. and Strout, R.G. (1979). *Eimeria tenella*: accumulation and retention of anticoccidial ionophores by extracellular sporozoites. *Experimental Parasitology,* **48**, 325–330.

Smith NC, Wallach M, Miller CM, Morgenstern R, Braun R and Eckert J. (1994). Maternal transmission of immunity to *Eimeria maxima*: enzyme-linked immunosorbent assay analysis of protective antibodies induced by infection. *Infect Immun* **62**: 1348–1357.

Song, K. D., H. S. Lillehoj, K. D. Choi, D. Zarlenga, and J. Y. Han. **(**1997). Expression and functional characterization of recombinant chicken interferon- gamma. *Vet. Immunol. Immunopathol.* **58,** 321–333.

Stewart, N.P. and Jorgensen, W.K. (1997). The isolation, attenuation and characterisation of an Australian strain of *Eimeria acervulina. Proceedings of the VIIth International Coccidiosis Conference,* Oxford, UK, pp. 97–98.

Stiff, M.I. and Bafundo, K.W. (1993). Development of immunity in broilers continuously exposed to *Eimeria* spp. *Avian Diseases,* **37**, 295– 301.

Stuart, E.E., Bruins, H.W. and Keenum, R.D. (1963). The immunogenicity of a commercial coccidiosis vaccine in conjunction with trithiadol and zoalene. *Avian Diseases,* **7**, 12–18.

Sutton, C.A., Shirley, M.W. and McDonald, V. (1986). Genetic recombination of markers for precocious development, arprinocid resistance, and isoenzymes of glucose phosphate isomerase in *Eimeria acervulina. Journal of Parasitology,* **72**, 965– 967.

Shirley, M.W.. Smith, A.L Blake D.P., (2007). Challenges in the successful control of the avian coccidian. *Vaccine,* **25**, pp. 5540–5547

Shirley, M. Smith, A. Tomley F. (2005). The Biology of Avian *Eimeria* with an Emphasis on their Control by Vaccination. *Adv. Parasitol.*, **60**, pp. 285–330

Soulsby, E.J.L., 1973. Helminth Arthropods and Protozoa of Domesticated Anim., pp: 695.

Stucki, U., Braun, R. and Roditi, I. (1993). *Eimeria tenella*: characterization of a 5S ribosomal RNA repeat unit and its use as a speciesspecific probe. *Experimental Parasitology*, **76**, 68-75.

Su, Y.C., Fei, A.C. and Tsai, F.M. (2003). Differential diagnosis of five avian Eimeria species by polymerase chain reaction using primers derived from the internal transcribed spacer 1 (ITS-1) sequence. *Veterinary Parasitology*, **117**, 221-227.

Sun, X.M., Pang, W., Jia, T., Yan, W.C., He, G., Hao, L.L., Bentué, M., Suo, X., (2009). Prevalence of Eimeria species in broilers with subclinical signs from fifty farms *Avian Dis.* **53**, 301-305.

Thebo, P., Lund´en, A., Uggla, A. and Hooshmand-Rad, P. (1998). Identification of seven *Eimeria* species in Swedish domestic fowl. *Avian Pathology*, **27**, 613–617.

Tolman, C.W. (1964). Social facilitation of feeding behaviour in the domestic chick. *Animal Behaviour*, **12**, 245– 251.

Tolman, C.W. (1965). Emotional behaviour and social facilitation of feeding in domestic chicks. *Behaviour*, **13**, 493–496.

Traore, I.M., Lin, H. and Weng, Y. (1994). Selection of a precocious strain of *Eimeria maxima. Proceedings of the Second Asian Conference on Coccidiosis,* Guangzhou, China pp. 116–118.

Thomke, S., Elwinger, K. (1998). Growth promotants in feeding pigs and poultry. III.Alternatives to antibiotic growth promotants. *Ann. Zootech.*, **47**, 245-271.

Tomley, F. M. (1994): Characterization of rhoptry proteins of *Eimeria tenella* sporozoites: antigenic diversity of rhoptry epitopes within species of the genus *Eimeria* and among three asexual generations of a single species, *E. tenella*. *Infect. Immun.*, **62**, 4656–4658.

Tomley, F. M., Bumstead, J. M., Billington, K. J., and Dunn, P. P. J. (1996): Molecular cloning and characterization of a novel acidic microneme protein (Etmic-2) from the apicomplexan protozoan parasite *E. tenella*. *Mol. Biochem. Parasitol*. **79**, 195–206.

Tomley, F. M., Clark, L. E Kawazoe, U. Dijkema, R., and Kok, J. J, (1991). Sequence of gene encoding an immunodominant microneme protein of *E. tenella*. *Mol. Biochem. Parasitol*. **49,** 277–288.

Tsuji, N., Kawazu, S., Ohta, M., Kamio, T., Isobe, T., Shimura, K. and Fujisaki, K. (1997). Discrimination of eight chicken *Eimeria species* using the two-step polymerase chain reaction. *The Journal of Parasitology*, **83**, 966-970.

Trout J. M., and Lillehoj H. S., (1996). T lymphocyte roles during *Eimeria acervulina* and *Eimeria tenella* infections. *Vet Immunol Immunopathol.*, **53**, 163–172.

Tyzzer E. (1929). Coccidiosis in gallinaceous birds. *Am J Hyg*; **10**, 269–383.

Tyzzer E, Theiler H and Jones E (1932). Coccidiosis in gallinaceous birds II. A comparative study of species of *Eimeria* of the chicken. *Am J Hyg*; **15**, 319–393.

United Nations Development Programme (2006). Socio-economic Impact of Avian Influenza in Nigeria, Abuja.

Vermeulen, A.N., Schaap, D.C., and Schetters, T.P.M. (2001). Control of coccidiosis in chickens by vaccination. *Veterinary Parasitology*, **100**, 13– 20.

Vermeulen, A.N., Schetters, T.P.M., and Schaap, D.C. (2000a). Control of coccidiosis by vaccination. Paper no. S1.10.03 in CD-rom version of *Proceedings XXI World's Poultry Congress, Montreal*. Montreal, Canada.

Vermeulen, A.N., Schetters, T.P.M., Janssen, H.A.J.M., Hanenberg, E.R.O.M. and Donkers, A.M.J. (2000b). A new vaccination/control concept against coccidiosis in poultry: combining a vaccine with ionophore treatment. In *Proceedings of the COST 820 Annual Workshop; Immunity to Coccidial Parasites: from Natural Infections to Molecular Vaccination,* Dublin. Eire, p. 53.

Vertommen, M., (1996). Coccidiosis control methods. In *Proceedings of the Paracox European Symposium, Annecy* Annecy, France, pp. 11–20.

Victoria, G., Pavon, E., Rebollo, M., Torres, C.A. de and Vazquez, R., (1996). A comparison of toltrazuril vs. amprolium as coccidial postvaccination treatments and their effects on immunity in broilers. In *Proceedings of the 45th Western Poultry Disease Conference, Cancun* Cancun. Mexico, pp. 112–115.

Vermeulen, A. N. (1998): Progress in recombinant vaccine development against coccidiosis. A review and prospects into the next millenium. *Int J. Parasitol.* **28**, 1121–1130.

Vermeulen, A., Dijkema, R., Kok, J. J., (1994): Coccidiosis Vaccine European patent 0349071B1.

Vermeulen, A. N., Kok, J. J., Van Den Boogart, P., Dijkema, R., and Claessens, J. A. J. (1993): *Eimeria* refractile body proteins contain two potentially functional characteristice-transhydrogenase and carbohydrate transport. *FEMS Microbiol. Lett.*, 223–230.

Vladmir Vrba, Damer P. Blakeb and Martin Poplsteina, (2010). Quantitative real-time PCR assays for detection and quantification of all seven *Eimeria species* that infect the chicken *Veterinary Parasitology* **174**, 183–190.

Vladimir Vrba, Poplstein M., and Pakandl M., (2011). The discovery of the 2 types of small subunit ribosomal RNA gene in *Eimeria mitis* contests the existence of *E. mivati* as an independent species. *Vet. Parasitology* **183**, 47-53

Waldenstedt, L. (2000). Rearing broilers without growth promoters and coccidiostats. Paper no. W2.07 in CD-rom version of: *Proceedings XXI World's Poultry Congress, Montreal, Canada*. Montreal, Canada.

Waldenstedt, L. and Elwinger, K. (1995). Effects of the coccidiostat narasin (Monteban) on growth of broiler chickens in an *Eimeria*-free environment. *Archiv f¨ur Gefl¨ugelkunde*, **59 (Sonderheft 1),** 61–62.

Waldenstedt, L., Elwinger, K., Lund´en, A., Thebo, P. and Uggla, A. (2001). Sporulation of *Eimeria maxima* oocysts in litter with different moisture contents. *Poultry Science*, **80**, 1412–1415.

Waldenstedt, L., Lund´en, A., Elwinger, K., Thebo, P. and Uggla, A. (1999). Comparison between a live, attenuated anticoccidial vaccine and an anticoccidial ionophore, on performance of broilers raised with or without a growth promoter, in an initially *Eimeria* free environment. *Acta Veterinaria Scandinavica*, **40**, 11– 21.

Waletzky, E. and Hughes, C.O. (1949). Factors involved in tests for acquired immunity in *Eimeria tenella* infections of the chicken. *Annals of the New York Academy of Sciences*, **52**, 478–495.

Wallach, M. (2001). The development of a maternally-based, subunit vaccine, CoxAbic®, against coccidiosis in chickens. *Proceedings of the VIIIth International Coccidiosis Conference*, Palm Cove, Sydney, Australia, (p. 148).

Wallach, M., Smith, N.C., Petracca, M., Miller, C.M.D., Eckert, J. and Braun, R. (1995). *Eimeria maxima* gametocyte antigens: potential use in a subunit maternal vaccine against coccidiosis in chickens. *Vaccine,* **13**, 347– 354.

Wallach, M., Halabi, A., Pillemer, G., Sar-Shalom, O., Mencher, D., Gilad, M., Bendheim, U., Danforth, H.D. and Augustine, P.C. (1992). Maternal immunization with gametocyte antigens as a means of providing protective immunity against *Eimeria maxima* in chickens. *Infection and Immunity,* **60**, 2036–2039.

Wang, C.C. (1975). Studies of the mitochondria from *Eimeria tenella* and inhibition of electron transport by quinolone coccidiostats. *Biochimica et Biophysica Acta,* **396,** 210–219.

Wang, C.C. (1976). Inhibition of the respiration of *Eimeria tenella* by quinolone coccidiostats. *Biochemical Pharmacology,* **25,** 343– 349.

Wang, G.T. and Davidson, J. (1992). Comparative efficacy of ionophore/ growth promoter combinations for the control of necrotic enteritis in chickens. In *Proceedings XIX World's Poultry Congress*, Amsterdam, The Netherlands **Volume 3**, pp. 585–588.

Wastling SL, Picozzi K, Kakembo AS, Welburn SC (2010). LAMP for human African trypanosomiasis: a comparative study of detection formats. PLoS Negl. Trop. Dis. 4(11): e865.

Watkins, K.L., Bafundo, K.W. and Donovan, D.J. (1990). Anticoccidial effect of monensin against *Eimeria mitis* and *Eimeria dispersa. Poultry Science,* **69**, 1009–1011.

Watkins, K.L., Shryock, T.R., Dearth, R.N. and Saif, Y.M. (1997). Invitro antimicrobial susceptibility of *Clostridium perfringens* from commercial turkey and broiler chicken origin. *Veterinary Microbiology,* **54**, 195– 200.

Watkins, K.L., Brooks, M.A., Jeffers, T.K., Phelps, P.V. and Ricks, C.A., (1995). The effect of *in ovo* oocyst or sporocyst inoculation on response to subsequent coccidial challenge. *Poultry Science,* **74**, 1597–1602.

Weber, F.H., Farrand, M., LeMay, M.A., Lewis, D.O., Genteman, K.C. and Evans, N.A. (2001). Movement of oocysts within chicken embryos after *in ovo* vaccination with *Eimeria maxima. Proceedings of the VIIIth International Coccidiosis Conference,* Palm Cove, Sydney, Australia. p. 184.

Weng, Y., Lin, H., Shi, M. and Chen, S. (1994). Selection of a precocious and attenuated strain of *Eimeria tenella. Proceedings of the Second Asian Conference on Coccidiosis*, Guangzhou, China. pp. 200–204.

Weppelman, R.M., Olson, G., Smith, D.A., Tamas, T. and Iderstine, A. van, (1977). Comparison of anticoccidial efficacy, resistance and tolerance of narasin, monensin and lasalocid in chicken battery trials. *Poultry Science,* **56**, 1550–1559.

Wheelhouse, R.K., Groves, B.I., Hammant, C.A., Dijk, C. van and Radu, J. (1985). Effects of coccidiostats and dietary protein on performance and water consumption in broiler chickens. *Poultry Science,* **64**, 979–985.

White, G. and Williams, R.B. (1983). Evaluation of a mixture of trimethoprim and sulphaquinoxaline for the treatment of bacterial and coccidial diseases of poultry. *Veterinary Record*, **113**, 608– 612.

Williams, R.B. (1969). The persistence of drug resistance in strains of *Eimeria* species in broiler chickens following a change of coccidiostat. *Research in Veterinary Science,* **10**, 490–492.

Williams, R.B. (1978). Notes on some coccidia of peafowl, pheasants and chickens. *Veterinary Parasitology,* **4**, 193–197.

Williams, R.B. (1992a). *The Development, Efficacy and Epidemiological Aspects of Paracox, a New Coccidiosis Vaccine for Chickens*. Harefield, UK: Pitman-Moore Europe.

Williams, R.B. (1992b). Differences between the anticoccidial potencies of monensin in maize-based or wheat-based chicken diets. *Veterinary Research Communications,* **16,** 147–152.

Williams, R.B. (1994). Safety of the attenuated anticoccidial vaccine "Paracox" in broiler chickens isolated from extraneous coccidial infection. *Veterinary Research Communications,* **18**, 189–198.

Williams, R.B. (1995a). Epidemiological studies of coccidiosis in the domesticated fowl (*Gallus gallus*): IV. Reciprocity between the immune status of floor-reared chickens and their excretion of oocysts. *Applied Parasitology*, **36**, 290–298.

Williams, R.B. (1995b). Epidemiological studies of coccidiosis in the domesticated fowl (*Gallus gallus*): II. Physical condition and survival of *Eimeria acervulina* oocysts in poultry-house litter. *Applied Parasitology, 36*, 90–96.

Williams, R.B. (1995c). Epidemiological studies of coccidiosis in the domesticated fowl (*Gallus gallus*): I. The fate of ingested oocysts of *Eimeria tenella* during the prepatent period in susceptible chicks. *Applied Parasitology,* **36**, 83–89.

Williams, R.B. (1996). The epidemiology of coccidioses of chickens. *World Poultry, Special Supplement Coccidiosis* **2,** pp. 9–11.

Williams, R.B. (1997a). Economic importance of *Eimeria mitis*, *E. praecox* and *E. acervulina* infections in chickens. *Proceedings of the VIIth International Coccidiosis Conference,* Oxford, UK p. 39.

Williams, R.B. (1997b). Etude d'une m´ethode d'´epreuve pour ´evaluer l'efficacit´e d'un vaccin anticoccidien pour le poulet. *Deuxi`emes Journ´ees de la Recherche Avicole, Tours* Tours, France, **Tome II**, pp. 183–186.

Williams, R.B. (1997c). The mode of action of anticoccidial quinolones (6-decyloxy-4-hydroxyquinoline-3-carboxylates) in chickens. *International Journal for Parasitology*, **27**, 101-107.

Watkins, K.L., Bafundo, K.W. and Donovan, D.J. (1990). Anticoccidial effect of monensin against *Eimeria mitis* and *Eimeria dispersa*. *Poultry Science*, ***69***, 1009–1011.

Watkins, K.L., Shryock, T.R., Dearth, R.N. & Saif, Y.M. (1997). Invitro antimicrobial susceptibility of *Clostridium perfringens* from commercial turkey and broiler chicken origin. *Veterinary Microbiology*, **54**, 195– 200.

Watkins, K.L., Brooks, M.A., Jeffers, T.K., Phelps, P.V. and Ricks, C.A. (1995). The effect of *in ovo* oocyst or sporocyst inoculation on response to subsequent coccidial challenge. *Poultry Science,* **74**, 1597–1602.

Weber, F.H., Farrand, M., LeMay, M.A., Lewis, D.O., Genteman, K.C. and Evans, N.A. (2001). Movement of oocysts within chicken embryos after *in ovo* vaccination with *Eimeria maxima. Proceedings of the VIIIth International Coccidiosis Conference*, Palm Cove Sydney, Australia, p. 184.

Weng, Y., Lin, H., Shi, M. and Chen, S., (1994). Selection of a precocious and attenuated strain of *Eimeria tenella. Proceedings of the Second Asian Conference on Coccidiosis* Guangzhou, China, pp. 200–204.

Weppelman, R.M., Olson, G., Smith, D.A., Tamas, T. and Iderstine, A. van, (1977). Comparison of anticoccidial efficacy, resistance and tolerance of narasin, monensin and lasalocid in chicken battery trials. *Poultry Science*, **56**, 1550–1559.

Wheelhouse, R.K., Groves, B.I., Hammant, C.A., Dijk, C. van and Radu, J. (1985). Effects of coccidiostats and dietary protein on performance and water consumption in broiler chickens. *Poultry Science*, **64**, 979–985.

White, G. and Williams, R.B. (1983). Evaluation of a mixture of trimethoprim and sulphaquinoxaline for the treatment of bacterial and coccidial diseases of poultry. *Veterinary Record*, **113**, 608– 612.

Williams, R.B. (1969). The persistence of drug resistance in strains of *Eimeria* species in broiler chickens following a change of coccidiostat. *Research in Veterinary Science*, **10**, 490–492.

Williams, R.B. (1978). Notes on some coccidia of peafowl, pheasants and chickens. *Veterinary Parasitology*, **4**, 193–197.

Williams, R.B. (1992a). *The Development, Efficacy and Epidemiological Aspects of Paracox, a New Coccidiosis Vaccine for Chickens*. Harefield, UK: Pitman-Moore Europe.

Williams, R.B. (1992b). Differences between the anticoccidial potencies of monensin in maize-based or wheat-based chicken diets. *Veterinary Research Communications*, ***16***, 147–152.

Williams, R.B. (1994). Safety of the attenuated anticoccidial vaccine "Paracox" in broiler chickens isolated from extraneous coccidial infection. *Veterinary Research Communications,* **18**, 189–198.

Williams, R.B. (1995a). Epidemiological studies of coccidiosis in the domesticated fowl (*Gallus gallus*): IV. Reciprocity between the immune status of floor-reared chickens and their excretion of oocysts. *Applied Parasitology,* **36**, 290–298.

Williams, R.B. (1995b). Epidemiological studies of coccidiosis in the domesticated fowl (*Gallus gallus*): II. Physical condition and survival of *Eimeria acervulina* oocysts in poultry-house litter. *Applied Parasitology,* **36**, 90–96.

Williams, R.B. (1995c). Epidemiological studies of coccidiosis in the domesticated fowl (*Gallus gallus*): I. The fate of ingested oocysts of *Eimeria tenella* during the prepatent period in susceptible chicks. *Applied Parasitology,* **36,** 83–89.

Williams, R.B. (1996). The epidemiology of coccidioses of chickens. *World Poultry, Special Supplement Coccidiosis* **2,** pp. 9–11.

Williams, R.B. (1997a). Economic importance of *Eimeria mitis, E. praecox* and *E. acervulina* infections in chickens. *Proceedings of the VIIth International Coccidiosis Conference, Oxford* Oxford, UK, p. 39.

Williams, R.B. (1997b). Etude d'une m´ethode d'´epreuve pour ´evaluer l'efficacit´e d'un vaccin anticoccidien pour le poulet. *Deuxi`emes Journ´ees de la Recherche Avicole,* Tours, France **Tome II,** pp. 183–186.

Williams, R.B. (1997c). The mode of action of anticoccidial quinolones (6-decyloxy-4-hydroxyquinoline-3-carboxylates) in chickens. *International Journal for Parasitology*, **27**, 101–111.

Williams, R.B. (1998a). Epidemiological aspects of the use of live anticoccidial vaccines for chickens. *International Journal for Parasitology,* **28**, 1089–1098.

Williams, R.B. (1998b). Analysis of the phenotypes in field populations of *Eimeria acervulina* expressing dual drug-resistance to decoquinate and clopidol. *Avian Pathology,* **27**, 67–73.

Williams, R.B. (1998c). A laboratory method for the single-passage selection of drug-resistant mutants from populations of *Eimeria* species in chickens and its potential value in the choice of field use concentrations for new anticoccidial drugs. *Avian Pathology,* **27**, 366–372.

Williams, R.B. (1999a). A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. *International Journal for Parasitology,* **29**, 1209–1229.

Williams, R.B. (1999b). Anticoccidial vaccines: the story so far. *World Poultry, Special Supplement Coccidiosis* **3,** pp. 23–25.

Williams, R.B. (2001). Quantification of the crowding effect during infections with the seven *Eimeria* species of the domesticated fowl: its importance for experimental designs and the production of oocyst stocks. *International Journal for Parasitology,* **31**, 1056–1069.

Williams, R.B. and Andrews, S.J. (2001). The origins and biological significance of the coccidial lesions that occur in chickens vaccinated with a live attenuated anticoccidial vaccine. *Avian Pathology,* **30**, 215– 220.

Williams, R.B. and Catchpole, J. (2000). A new protocol for a challenge test to assess the efficacy of live anticoccidial vaccines for chickens. *Vaccine,* **18**, 1178–1185.

Williams, R.B. and Gobbi, L. (2002). Comparison of an attenuated anticoccidial vaccine and an anticoccidial drug programme in commercial broiler chickens in Italy. *Avian Pathology*, **31**, 253–265.

Williams, R.B., Johnson, J.D. and Andrews, S.J. (2000). Anticoccidial vaccination of broiler chickens in various management programmes: relationship between oocyst accumulation in litter and the development of protective immunity.*Veterinary Research Communications*, **24**, 309– 325.

Williams, R.B., Marshall, J.A. and Catchpole, J. (2001). A novel method for counting eimerian oocysts at very low concentrations in aqueous suspensions. *Research in Veterinary Science*, **71**, 67–71.

Williams, R.B., Carlyle, W.W.H., Bond, D.R. and Brown, I.A.G. (1999). The efficacy and economic benefits of Paracox, a live attenuated anticoccidial vaccine, in commercial trials with standard broiler chickens in the United Kingdom. *International Journal for Parasitology*, **29**, 341– 355.

Williams, R.B., Bushell, A.C., R´ep´erant, J.-M., Doy, T.G., Morgan, J.H., Shirley, M.W., Yvor´e, P., Carr, M.M. and Fr´emont, Y. (1996). A survey of *Eimeria* species in commercially-reared chickens in France during 1994. *Avian Pathology*, **25**, 113–130.

Wood-Gush, D.G.M. (1971). *The Behaviour of the Domestic Fowl*. London: Heinemann Educational Books Ltd.

Wallach, M., Halabi, A., Pillemer, G., Shalom, O., Mencher, D., Gilad, M., Bendheim U., Danforth, H. D., and Augustine, P. C. (1992). Maternal immunization with

gametocyte antigens as a means of providing immunity against *Eimeria maxima* in chickens. *Infect. Immun,*. **60**, 2036– 2039.

Williams, R.B., (1999). A compartmentalised model for the estimation of the cost of coccidiosis to the worlds chicken production industry. *J. Parasitol*., **29**, 1209–1229.

Williams, R.B. (1997a). Economic importance of *Eimeria mitis*, *E. praecox* and E. acervulina infections in chickens. *Proceedings of the VIIth International Coccidiosis Conference*, Oxford, UK, p. 39.

Williams, R.B. (1994). Safety of the attenuated anticoccidial vaccine 'Paracox' in broiler chickens isolated from extraneous coccidial infection. *Vet. Res. Commun,*. **18**,189-198

Xie, M., Cai, J., Li, A. and Peng, X. (2001). Coccidiosis of domestic fowl in China. *Proceedings of the VIIIth International Coccidiosis Conference*, Palm Cove Sydney, Australia. pp. 153–154.

Young, R. and Craig, A. (2001). The Use and Misuse of Antibiotics in UK Agriculture. *The Soil Association*. Bristol, UK. **Part 3**

Yun, C., Lillehoj, H., Zhu, J. (2000). Kinetic differences in intestinal and systemic interferon-gamma and antigen-specific antibodies in chickens experimentally infected with *Eimeria maxima*. *Avian Dis*, **44**: 305–312.

Yvor´e, P., Pery, P., Laurent, F. and Bessay, M. (1993). Vaccins anticoccidiens. Bilan et perspectives. *Veterinary Research*, **24**, 229–250.

APPENDICES

Appendix 1: QUESTIONNAIRE ON PREVALENCE OF COCCIDIOSIS IN OYO AND OGUN STATES

UNIVERSITY OF IBADAN DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

Dear Respondents,

This questionnaire survey is an academic exercise on the prevalence of Coccidiosis of poultry in Oyo and Ogun states. All information supplied will be treated with utmost confidentially and used for research only.

Thank you for your time and cooperation.

Dr. S. O. Adeyemi

(A) POULTRY OWNERS' BACKGROUND INFORMATION

1. Age: 10-30 yrs ( ) 31-50 yrs ( ) 51 yrs and above ( )
2. Educational level: Primary ( ) Secondary ( ) Tertiary ( )
3. Religious affiliation: Christianity ( ) Islam ( ) Traditional ( )
4. Marital status: Single ( ) Married ( ) Widowed ( ) Divorced ( )

(B) GENERAL FARM AND MANAGEMENT INFORMATION

1 Farm's name _____

2 Farm's location _____

3 How old is the farm? (a) Less than 1 year (b) 1-5 years (c) Above 5 years

4 What type of breed do you keep? (a) Broiler (b) Layers (c) Breeders

5 What is/are the age(s) of your birds? Tick as many as applicable (a) 0-8wk (b) 9-18 wks (c) 19 wks and above

6 Number of poultry pens at this location._____

7. What is the poultry population per pen (use attached paper if necessary)

8. What type of rearing pens do you have? (a) Open sided (b) Controlled environment

9. What type of rearing style do you practice? (a) Deep Litter. (b) Cage system. (c) both.

10. If answer to 8 is both, specify which applies to each breed. ----------

11 How is your feed produced? (a) On the farm. (b) By local suppliers..

12. What watering system do you have? (a) Manual (b) Automatic

13. Do you normally have spilled water on the litter? (a) Yes (b) No

14. If you have a deep liter system when water accidentally spill on the litter, what do you do?

(a) Allow the litter to dry on its own

(b) Pack out the wet litter and replace with dry ones

(c) Mix the wet litter with other spots that are dry

(d) Others, specify. _____

15. Do you notice caked litter? (a)Yes (b) No

16. How often do you replace your house liter? (a) Monthly (b) Every 3 months (c) Yearly (d) Others, specify _____

17. Do you have foot dip with disinfectants at the entrance to poultry pens? (a) Yes (b) No.

SPECIFIC INFORMATION ON COCCIDIOSIS

1. Have you heard of a disease called Coccidiosis? (a)Yes (b) No.

2. If yes, what was your source of information? (a) radio/tv/public communication (b) vet/ vet clinic (c) school (d) friend (e) others, specify _____

3. Has it occurred on your farm before? Yes or No.

4. If yes, at what age do your chicks often have coccidiosis? (a) Below 8 weeks (b) Above 8 weeks.

5. What season of the year do you normally have coccidiosis on your farm? (a) Dry season (b) Rainy season (c) Any time.

6. From your experience, is the disease of serious economic importance? (a) Yes (b) No.

7. What signs will you see to suspect an imminent outbreak of coccidiosis.? (a) Bloody dropping (b) Ruffled feathers (c) Dropping wings (d) In appetence and dosing

8. Which method of Coccidiosis prevention do you use? (a) Coccidiostat in feed or water (b) Coccidiosis vaccines (c) An alternation between a & b.

9. Who influenced the choice in 8 above? (a)Yours or that of internal staff (b) External Consultant (c) Fellow farmer's testimony.

10. How long have you been using the chosen method above? (a) Below 2 years (b) Between 2-5 years (c) Over 5 years.

11. How effective has the method been? (a) Very effective (b) Barely effective (c) Ineffective

12. Has there been any history of outbreak despite preventive measures? (a)Yes (b) No.

13. Have you ever changed the method or even contemplate changing it? (a)Yes (b) No.

14. If yes, why? _____

15. Was there an evaluation of the cost effectiveness of the chosen method? (a) Yes (b) No.

16. In your opinion, do you think Coccidiosis is still of economic significance in the Nigerian poultry industry? (a)Yes (b) No.

Appendix 2: ***Eimeria*** **Oocyst Burden for Oyo State.**

SERIAL NO	NAME OF FARM	EPIZOOTIOLOGICAL CATEGORIES	OOCYST PER GRAM OF FAECES (OPG)
1	OBASANJO 1	OY 1A	668
2	OBASANJO 2	OY 1A	1336
3	TERUDEE 1	OY 1A	6670
4	TERUDEE 2	OY 1A	10337
5	BAMFORT	OY 1A	1001
6	AJANLA 1	OY 1A	1001
7	AJANLA 2	OY 1A	1001
8	FIMHAB 1	OY 1A	58363
9	FIMHAB 2	OY 1A	8671
10	ZARTECH 1	OY 1B	8671
11	ZARTECH 2	OY 1B	2668
12	ZARTECH 3	OY 1B	1001
13	OBASANJO 1	OY 1B	2668
14	OBASANJO 2	OY 1B	668
15	AVIAN 1	OY 1B	8671
16	AVIAN 2	OY 1B	2668
17	KARMADEX 1	OY 1B	2668
18	KARMADEX 2	OY 1B	1001
19	SURULERE 1	OY 2A	26680
20	SURULERE 2	OY 2A	26680
21	FALANA 1	OY 2A	2001

22	FALANA 2	OY 2A	2001
23	AJANLA 1	OY 2A	1668
24	AJANLA 2	OY 2A	2001
25	AJANLA 3	OY 2A	5062
26	BELAPOP	OY 2A	2001
27	AJANLA 4	OY 2A	6670
28	SOKS 1	OY 2B	6336
29	SOKS 2	OY 2B	5670
30	G TADEK 1	OY 2B	0
31	G. TADEK 2	OY 2B	0
32	G. TADEK 3	OY 2B	0
33	OLULANA 1	OY 2B	5670
34	OLULANA 2	OY 2B	6336
35	FALANA 1	OY 2B	2174420
36	FALANA 2	OY 2B	1247290
37	NIYI 1	OY 2B	677956
38	NIYI 2	OY 2B	30349
39	OYEWONUOLA 1	OY 2B	60033
40	OYEWONUOLA 2	OY 2B	108386
41	AGTHAM 1	OY 2B	60033
42	AGTHAM 2	OY 2B	7004
43	GTHAM 1	OY 2B	5003
44	GTHAM 2	OY 2B	1710
45	KARMADEX 1	OY 2B	0

46	KARMADEX 2	OY 2B	0
47	KARMADEX 3	OY 2B	668
48	GREEN FIELD	OY 3A	668
49	GREEN FIELD	OY 3A	1001
50	BTA 1	OY 3A	334
51	SUSSEX 1	OY 3A	0
52	SUSSEX 2	OY 3A	0
53	BTA 2	OY 3A	0
54	PETER 1	OY 3A	60033
55	PETER 2	OY 3A	60033
56	GOD'S MERCY 1	OY 3A	0
57	GOD'S MERCY 2	OY 3A	668
58	SIAD 1	OY 3A	1247290
59	SIAD 2	OY 3A	1254288
60	DOMINIC 1	OY 3A	0
61	DOMINIC 2	OY 3A	334
62	OYEWONUOLA 1	OY3A	668
63	OYEWONUOLA 2	OY 3A	2668
64	OSINALD 1	OY 3A	0
65	OSINALD 2	OY 3A	0
66	OSINALD 3	OY 3A	0
67	OLUWOLE 1	OY 3A	0
68	OLUWOLE 2	OY 3A	0
69	F&B 1	OY 3A	0

70	F&B 2	OY 3A	0
71	OLANIPEKUN 1	OY 3A	0
72	OLANIPEKUN 2	OY 3A	0
73	WURAOLA 1	OY 3A	0
74	WURAOLA 2	OY 3A	0
75	HENRY T 1	OY 3A	7004
76	HENRY T 2	OY 3A	0
77	FALANA 1	OY 3A	2335
78	FALANA 2	OY 3A	6336
79	DOROLA 1	OY 3A	5670
80	DOROLA 2	OY 3A	1001
81	ABOF 1	OY 3A	2668
82	ABOF 2	OY 3A	6336
83	ADEWOYE 1	OY 3A	0
84	ADEWOYE 2	OY 3A	0
85	VIC THOMAS 1	OY 3A	0
86	VIC THOMAS 2	OY 3A	0
87	ALAYANDE 1	OY 3A	2335
88	ALAYANDE 2	OY 3A	2668
89	AGTHAM 1	OY 3A	0
90	AGTHAM 2	OY 3A	0
91	BAMFORT 1	OY 3A	0
92	BAMFORT 2	OY 3A	0
93	GTHAM 1	OY 3A	0

94	GTHAM 2	OY 3A	0
95	SAVENGER 1	OY 3A	7337
96	SAVENGER 2	OY 3A	1001
97	AFUN 1	OY 3A	0
98	AFUN 2	OY 3A	0
99	REHOBOTH 1	OY 3A	0
100	REHOBOTH 2	OY 3A	0
101	SUSSEX 2	OY 3A	0
102	TERUDEE 1	OY 3B	1336
103	TERUDEE 2	OY 3B	4669
104	AJILA	OY 3B	0
105	NIYI	OY 3B	5003
106	G.TADEK	OY 3B	2668
107	ADEDEJI	OY 3B	2668
108	OPE OLUWA	OY 3B	677956
109	LAWAL	OY 3B	690862
110	AJAO	OY 3B	30349
111	GREEN FIELD	OY 3B	0
112	FABJET	OY 3B	4669
113	OLADEWA	OY 3B	668
114	YINKA	OY 3B	0
115	KARMADEX	OY 3B	0
116	AJANLA 1	OY 3B	1334
117	AJANLA 2	OY 3B	2668

118	AJANLA 3	OY 3B	1334
119	AVIAN 1	OY 3B	1334
120	AVIAN 2	OY 3B	1001

OY 1A = 1-8 Week-old Chickens on Vaccine prophylaxis

OY 1B = 1-8 Week-old Chickens on Chemoprophylaxis

OY 2A = 9-18 Week-old Chickens on Vaccine Prophylaxis

OY 2B = 9-18 Week-old Chickens on Chemoprophylaxis

OY 3A = Chickens aged 19 Weeks and above in cages

OY 3B = Chickens aged above 19 Weeks on Deep Litter.

Appendix 3: *Eimeria* Oocyst Burden for Ogun State

SERIAL NUMBER	NAME OF THE FARM	EPIZOOTIOLOGICAL CATEGORY	OOCYST PER GRAM OF FAECES
1	AJIBOYE 1	OG 1A	334
2	AJIBOYE 2	OG 1A	668
3	F. A. 1	OG 1A	1001
4	F.A 2	OG 1A	141404
5	ANIMAL CARE 1	OG 1A	2668
6	ANIMAL CARE 2	OG 1A	13,007
7	ANIMAL CARE 3	OG 1A	2668
8	ODUNSI	OG 1A	6336
9	EL-SHADAI	OG 1A	5670
10	ALE 1	OG 1B	1001
11	ALE 2	OG 1B	1001
12	TAIYE 1	OG 1B	2668
13	TAIYE 2	OG 1B	2668
14	TAIYE 3	OG 1B	1001
15	OLUBAMISE 1	OG 1B	0
16	OLUBAMISE 2	OG 1B	0
17	EL-SHADAI 1	OG 1B	1001
18	EL-SHADAI 2	OG 1B	1001
19	SOPTIMAL 1	OG 1B	2668
20	SOPTIMAL 2	OG 1B	1001

21	SOPTIMAL 3	OG IB	1001
22	LIZPAD 1	OG 1B	0
23	LIZPAD 2	OG 1B	0
24	BAMISE 1	OG 1B	2668
25	BAMISE 2	OG 1B	1001
26	SOPTIMAL 1	OG 2A	334
27	SOPTIMAL 2	OG 2A	5670
28	SOPTIMAL 3	OG 2A	2668
29	BOSS 1	OG 2A	1267300
30	BOSS 2	OG 2A	108386
31	F. A. 1	OG 2A	60033
32	F A 2	OG 2A	108386
33	GAFO 1	OG 2A	5003
34	GAFO 2	OG 2A	0
35	GAFO 3	OG 2A	0
36	OWONOKO 1	OG 2A	2001
37	OWONOKO 2	OG 2A	668
37	OWONOKO 3	OG 2A	0
38	ANIMAL CARE 1	OG 2A	0
39	ANIMAL CARE 2	OG 2A	2668
40	ANIMAL CARE 3	OG 2A	2001
41	RABIU 1	OG 2A	2668
42	RABIU 2	OG 2A	2668
43	STELLAN	OG 2A	668

44	ODUNSI 1	OG 2B	668
45	ODUNSI 2	OG 2B	668
46	TAIYE 1	OG 2B	0
47	TAIYE 2	OG 2B	0
48	AKIN SOTUBO 1	OG 2B	0
49	AKIN SOTUBO 2	OG 2B	0
50	AROWOLO 1	OG 2B	0
51	AROWOLO 2	OG 2B	0
52	AKIN 1	OG 2B	1001
53	AKIN 2	OG 2B	334
54	GOLDEN YOLK 1	OG 2B	0
55	GOLDEN YOLK 2	OG 2B	0
56	SASBROTH 1	OG 2B	2668
57	SASBROTH 2	OG 2B	2001
58	SEGUN 1	OG 2B	0
59	SEGUN 2	OG 2B	0
60	LAMINA 1	OG 2B	2668
61	LAMINA 2	OG 2B	1001
62	FEEDWELL 1	OG 2B	668
63	FEEDWELL 2	OG 2B	0
64	FOLAKEMI 1	OG 2B	0
65	FOLAKEMI 2	OG 2B	0
66	GORI	OG 2B	0
67	MURPHY	OG 2B	0

68	SEBUM 1	OG 2B	0
69	SEBUM 2	OG 2B	0
70	MOLAYE	OG 2B	0
71	AROWOLO	OG 3A	2668
72	FOLAKEMI	OG 3A	2001
73	SASBROTH	OG 3A	0
74	SOPTIMAL	OG 3A	11673
75	GOLDEN YOLK	OG 3A	1668
76	AKIN	OG 3A	1336
77	SEGUN	OG 3A	2335
78	ALE	OG 3A	0
79	LAMINA	OG 3A	0
80	FEEDWELL	OG 3A	4336
81	GORI	OG 3A	2001
82	MURPHY	OG 3A	0
83	OLUBAMISE	OG 3A	0
84	KEN AYO 1	OG 3A	0
85	OLUWASEUN	OG 3A	0
86	TAIYE	OG 3A	334
87	KEN AYO 2	OG 3A	0
88	K. ABIOLA	OG 3A	0
89	OGUNMOLA	OG 3A	334
90	SEBUM 1	OG 3A	1670
91	SEBUM 2	OG 3A	668

92	AKIN SOTUBO	OG 3A	668
93	LIZPAD 1	OG 3A	0
94	LIZPAD 2	OG 3A	0
95	MOLAYE	OG 3A	12673
96	GAFO	OG 3A	0
97	WHITE COAT	OG 3A	0
98	AJIBOYE	OG 3A	334
99	OWONOKO	OG 3A	0
100	OSTAN	OG 3A	0
101	F A	OG 3A	54694
102	RABIU	OG 3A	2001
103	STELLAN	OG 3A	3335
104	ANIMAL CARE	OG 3A	0
105	COMFORT	OG 3A	0
106	MIDE	OG 3A	667
107	OROPO	OG 3A	0
108	EAT HEALTHY	OG 3A	0
109	AYOADE 2	OG 3A	5003
110	OLA QUADRI	OG 3B	0
111	FIKUN	OG 3B	668
112	AYOADE 1	OG 3B	668
113	NON NOMECLATURE	OG 3B	1001
114	REAL	OG 3B	1336
115	KAY	OG 3B	2001

116	OGIS	OG 3B	2668
117	OLUYEMI	OG 3B	60033
118	OROPO	OG 3B	0
119	GORIOLA	OG 3B	334
120	EAT HEALTHY	OG 3B	0
121	AYOADE 2	OG 3B	30349

OG 1A = 1-8 Week-old Chickens on Vaccine Prophylaxis

OG 1B = 1-8 Week-old Chickens on Chemoprophylaxis

OG 2A = 9-18 Week-old Chickens on Vaccine Prophylaxis

OG 2B = 9-18 Week-old Chickens on Chemoprophylaxis

OG 3A = Chickens aged 19 Weeks and above in cages

OG 3B = Chickens aged above 19 Weeks on Deep Litter

Appendix 4: Calculation of Infection Levels for Oyo and Ogun State

OY 3A	**OY 3B**	**OG 3A**	**OG 3B**
Mean OPG was **49494.** Calculation= 2672676/54 = 49494	Mean OPG wa**75185** Calculation = 1428519/19 =75185	Mean OPG was **2830** Calculation = 110399/39= 2830	Mean OPG was **8255** Calculation = 99058/12 = 8255
Mean OPG for OY 3A and 3B was **56181**. Calculation = 4101195/73 = 56181		Mean OPG for OG 3A and 3B was **4107** Calculation = 110399+99058/51= 4107.	

Appendix 5: Statistical Analysis of Oocyst Burden for Oyo and Ogun States

Results compared	P value obtained from Student T test.	Comment
OG 3A and OG 3B	0.342	Not significant
OG 3A and OY 3A	0.321	Not significant
OY 3A and OY 3B	0.705	Not significant
OY 3B and OG 3B	0.193	Not significant
OY 3A and OG 3B	0.382	Not significant

OG 3A = Birds above 19 weeks in cages in Ogun state; OG 3B = Birds above 19 weeks on deep litter in Ogun state; OY 3A = Birds above 19 weeks in cages in Oyo state; OY 3B = Birds above 19 weeks on deep litter in Oyo state

Appendix 6: Relative Abundance of *Eimeria* Species in 1-8 Week-old Chickens on Vaccination in Oyo State.

FARMS' NAME	EPICAT	OPG	BM N (%)	AM N (%)	NTP N (%)
OBASANJO 1	1A	668	668 (100)	0	0
OBASANJO 2	1A	1336	334 (25)	0	1002 (75)
TERUDEE 1	1A	6670	0	3001(45)	3669 (55)
TERUDEE 2	1A	10337	0	165 (1.6)	10172 (98.4)
BAMFORT	1A	1001	1001(100)	0	0
AJANLA 1	1A	1001	333 (33.2)	334 (33.4)	334 (33.4)
AJANLA 2	1A	1001	333 (33.2)	334 (33.4)	334 (33.4)
FIMHAB 1	1A	58363	0	37509 (64.3)	20854 (35.7)
FIMHAB 2	1A	8671	0	5575 (64.3)	3095 (35.7)
Cumulative		90049	2336	41180	46533
Relative Abundance			2.6%	45.7%	51.7%

BM = *E. brunetti* and *E. acervulina*, AM = *E. acervulina* and *E. mitis*, NTP = *E. necatrix*, *E. tenella* and *E. praecox*.

Appendix 7: Relative Abundance of *Eimeria* Species in 1-8 Week-Old Chickens on Chemoprophylaxis in Oyo State.

FARMS' NAME	EPI. CAT.	OPG	BM N (%)	AM N (%)	NTP N (%)
ZARTECH 1	1B	8671	0	2668 (30.8)	6003 (69.2)
ZARTECH 2	1B	2668	0	667 (25)	2001 (75)
ZARTECH 3	1B	1001	0	334(33)	667(67)
OBASANJO 1	1B	2668	0	667(25)	2001(75)
OBASANJO 2	1B	668	0	334 (50)	334 (50)
AVIAN 1	1B	8671	0	5546(64)	3125(36)
AVIAN 2	1B	2668	0	1334 (50)	1334 (50)
KARMADEX1	1B	2668	0	667 (25)	2001 (75)
KARMADEX 2	1B	1001	0	334(33)	667(67)
Cummulative		30684	0	12551	18133
Relative Abundance		100%	0	29.4%	70.6%

BM = *E. brunetti* and *E. maxima*, AM = *E. acervulina* and *E. mitis*, NTP = *E. necatrix*, *E .tenella* and *E. praecox*.

Appendix 8: Relative Abundance of *Eimeria* Species in 9-18 Week-old Chickens on Vaccination in Oyo State.

FARMS' NAME	EPICAT.	OPG	BM N (%)	AM N (%)	NTP N (%)
SURULERE 1	2A	26680	2001(7.5)	19338 (72.5)	5341(20)
SURULERE 2	2A	26680	2001(7.5)	19338(72.5)	5341(20)
FALANA 1	2A	2001	0	334(16.7)	1667 (83.3)
FALANA 2	2A	2001	0	1001(50)	1000(50)
AJANLA 1	2A	1668	0	1001(60)	667(40)
AJANLA 2	2A	2001	0	1001(50)	1000(50)
AJANLA 3	2A	5062	0	1001(15)	4061(85)
AJANLA 4	2A	2001	0	1335 (66.7)	666 (33.35)
BELAPOP	2A	6670	0	2335 (35)	4335 (35)
Cummulative		74764	4002	46684	24078
Relative Abundance		100%	5.4%	62.4%	32.2%

Appendix 9: Relative Abundance of *Eimeria* Species in 9-18 Week-old Chickens on Chemoprophylaxis in Oyo State

FARMS' NAME	EPIZOOTIOLOGAL CATEGORIES	OPG	BM N (%)	AM N (%)	NTP N (%)
SOKS 1	2B	6336	0	665 (10.5)	5671(89.5)
SOKS 2	2B	5670	0	998(17.6)	4672(82.4)
G. TADEK 1	2B	0	0	0	0
G.TADEK 2	2B	0	0	0	0
G.TADEK 3	2B	0	0	0	0
OLULANA 1	2B	5670	0	998 (17.6)	4672 (82.4)
OLULANA 2	2B	6336	0	3168(50)	3168(50)
FALANA 1	2B	2174420	0	1141571(52.5)	1032849(47.5)
FALANA 2	2B	1247290	0	769536(61.7)	477754(38.3)
NIYI 1	2B	677956	0	338978(50)	338978(50)
NIYI 2	2B	30349	0	3035(10)	27314(90)
OYEWONUOLA 1	2B	60033	0	18010(30)	42023(70)
OYEWONUOLA 2	2B	108386	0	21677(20)	86709(80)
AGTHAM 1	2B	60033	0	18010(30)	42023(70)
AGTHAM 2	2B	7004	0	4230(60.4)	2774(39.6)
GTHAM 1	2B	5003	0	0	5003(100)

GTHAM 2	2B	1710	0	334(19.5)	1376(80.5)
KARMADEX 1	2B	0	0	0	0
KARMADEX 2	2B	0	0	0	0
KARMADEX 3	2B	668	0	334(50)	334(50)
Cummulative		4396864	0	2321544	2075320
Relative Abundance		100%	0	52.8%	47.2%

Appendix 9: Relative Abundance of *Eimeria* Species in Chickens aged above 19 Weeks in Cages in Oyo State.

FARMS' NAME	EPIZOOTIOLOGICAL CATEGORIES	OPG	BM (%)	AM (%)	NTP (%)
GREEN FIELD	OY 3A	668	0	334(50)	334(50)
GREEN FIELD	OY 3A	1001	334(33.3)	333(33.3)	334(33.3)
BTA 1	OY 3A	334	0	0	334(100)
SUSSEX 1	OY 3A	0	0	0	0
SUSSEX 2	OY 3A	0	0	0	0
BTA 2	OY 3A	0	0	0	0
PETER 1	OY 3A	60033	0	9435(15.7)	50598(84.3)
PETER 2	OY 3A	60033	6336(10.6)	26680(44.4)	27017(45)
GOD'S MERCY 1	OY 3A	0	0	0	0
GOD'S MERCY 2	OY 3A	668	0	334(50)	334(50)
SIAD 1	OY 3A	1247290	67354(5.4%)	756942(60.7%)	422994(33.9%)
SIAD 2	OY 3A	1254288	62391(5)	741067(59.1)	450830(35.9)
DOMINIC 1	OY 3A	0	0	0	0
DOMINIC 2	OY 3A	334	0	334(100)	0
OYEWONUOLA 1	OY 3A	668	0	668(100%)	0
OYEWONUOLA 2	OY 3A	2668	0	1001(37.5)	1667(62.5)

OSINALD 1	OY 3A	0	0	0	0
OSINALD 2	OY 3A	0	0	0	0
OLUWOLE 1	OY 3A	0	0	0	0
OLUWOLE 2	OY 3A	0	0	0	0
F&B 1	OY 3A	0	0	0	0
F&B 2	OY 3A	0	0	0	0
OLANIPEKUN 1	OY 3A	0	0	0	0
OLANIPEKUN 2	OY 3A	0	0	0	0
WURAOLA 1	OY 3A	0	0	0	0
WURAOLA 2	OY 3A	0	0	0	0
HENRY T 1	OY 3A	7004	329(4.7)	2003(28.6)	4672(66.7)
HENRY T 2	OY 3A	0	0	0	0
FALANA 1	OY 3A	2335	0	0	2335(100)
FALANA 2	OY 3A	6336	0	0	6336(100)
DOROLA 1	OY 3A	5670	0	0	5670(100)
DOROLA 2	OY 3A	1001	0	0	1001(100)
ABOF 1	OY 3A	2668	0	0	2668(100)
ABOF 2	OY 3A	6336	0	0	6336(100)
ADEWOYE 1	OY 3A	0	0	0	0
ADEWOYE 2	OY 3A	0	0	0	0

VIC THOMAS 1	OY 3A	0	0	0	0
VIC THOMAS 2	OY 3A	0	0	0	0
ALAYANDE 1	OY 3A	2335	0	0	2335(100)
ALAYANDE 2	OY 3A	2668	900(33.7)	0	1768(66.3)
AGTHAM 1	OY 3A	0	0	0	0
AGTHAM 2	OY 3A	0	0	0	0
BAMFORT 1	OY 3A	0	0	0	0
BAMFORT 2	OY 3A	0	0	0	0
GTHAM 1	OY 3A	0	0	0	0
GTHAM 2	OY 3A	0	0	0	0
SAVENGER 1	OY 3A	7337	1335(18.2)	330(4.5)	5672(77.3)
SAVENGER 2	OY 3A	1001	0	0	1001(100)
AFUN 1	OY 3A	0	0	0	0
AFUN 2	OY 3A	0	0	0	0
REHOBOTH 1	OY 3A	0	0	0	0
REHOBOTH 2	OY 3A	0	0	0	0
Cummulative		2672676	138979	1539461	994236
RelativeAbundance		100%	5.2%	57.6%	37.2%

Appendix 10: Relative Abundance of *Eimeria* Species in Chickens above 19 Weeks on Deep-Litter in Oyo State.

FARMS' NAME	EPI. CAT	OPG	BM (%)	AM (%)	NTP (%)
TERUDEE 1	**3B**	1336	0	0	1336(100)
TERUDEE 2	**3B**	4669	333(7.1)	1001(21.4)	3335(71.5)
AJILA	**3B**	0	0	0	0
NIYI	**3B**	5003	0	2326(46.5%)	2677(53.5%)
G.TADEK	**3B**	2668	1000(37.5)	334(12.5)	1334(50)
ADEDEJI	**3B**	2668	2001(75)	0	667(25)
OPE OLUWA	**3B**	677956	33898(5)	404089(59.6)	239969(35.4)
LAWAL	**3B**	690862	34543(5)	241802(35)	414517(60)
AJAO	**3B**	30349	9014(29.7)	3005(9.9)	18330(60.4)
GREEN FIELD	**3B**	0	0	0	0
FABJET	**3B**	4669	0	1335(28.6)	3334(71.4)
OLADEWA	**3B**	668	0	334(50)	334(50)
YINKA	**3B**	0	0	0	0
KARMADEX	**3B**	0	0	0	0
AJANLA 2	**3B**	0	0	0	0
AJANLA 1	**3B**	2668	0	334(12.5)	2334(87.5)
AVIAN 1	**3B**	1334	0	333(25)	1001(75)

AVIAN 2	**3B**	1001	0	333(33.3)	668(66.7)
AJANLA 3	**3B**	1334	333(25)	0	1001(75)
Cummulative		1427185	81122	655226	690837
Relative Abundance		100%	5.7%	45.9%	48.4%

Appendix 11: Relative Abundance of *Eimeria* Species in 1-8 Week-old Chickens on Vaccination in Ogun State

FARMS' NAME	EPI. CAT	OPG	BM	AM	NTP
AJIBOYE 1	1A	334	0	0	334(100)
AJIBOYE 2	1A	668	334(50)	0	334(50)
F.A 1	1A	141404	28281(20)	59491(42.1)	53632(37.9)
F.A 2	1A	1001	0	0	1001(100)
ANIMAL CARE 1	1A	13007	4331(33.3)	4063(31.2)	4613(35.5)
ANIMAL CARE 2	1A	2668	1001(37.5)	1001(37.5)	666(25)
ANIMAL CARE 3	1A	2668	668(25)	1001(37.5)	999(37.5)
ODUNSI	1A	6336	1048(16.5)	2668(42.1)	2620(41.4)
EL-SHADAI	1A	5670	1001(17.7)	2668(47)	2001(35.3)
Cummulative		173756	36664	70892	66200
Relative Abundance		100%	21.1%	40.8%	38.1%

Appendix 12: Relative Abundance of *Eimeria* Species in 1-8 Week-old Chickens on Chemoprophylaxis in Ogun State

FARMS' NAME	EPI. CAT	OPG	BM (%)	AM (%)	NTP (%)
ALE 1	1B	1001	668(66.7)	0	333(33.3)
ALE 2	1B	1001	334(33.4)	0	667(66.6)
TAIYE 1	1B	2668	1000(37.5)	0	1668(62.5)
TAIYE 2	1B	2668	661(24.8)	0	2007(75.2)
TAIYE 3	1B	1001	0	0	1001(100)
OLUBAMISE 1	1B	0	0	0	0
OLUBAMISE 2	1B	0	0	0	0
EL-SHADAI 1	1B	1001	0	0	1001(100)
EL SHADAI 2	1B	1001	0	0	1001(100)
SOPTIMAL 1	1B	2668	1001(37.5)	0	1667(62.5)
SOPTIMAL 2	1B	1001	668(66.7)	0	333(33.3)
SOPTIMAL 3	1B	1001	668(66.7)	0	333(33.3)
LIZPAD 1	1B	0	0	0	0
LIZPAD 2	1B	0	0	0	0
BAMISE 1	1B	2668	1001(37.5)	0	1667(62.5)
BAMISE 2	1B	1001	668(66.7)	0	333(33.3)
Cummulative		18680	6669	0	12011
Relative Abundance		100%	35.7%	0	64.3%

Appendix 13: Relative Abundance of *Eimeria* Species in 9-18 Week-old Chickens on Vaccination in Ogun State

FARMS' NAME	EPIZOOTIOLOGICAL CATEGORY	OPG	BM (%)	AM (%)	NTP (%)
SOPTIMAL 1	2A	334	334(100)	0	0
SOPTIMAL 2	2A	5670	0	2268(40)	3402(60)
SOPTIMAL 3	2A	2668	0	1001(37.5)	1667(62.5)
BOSS 1	2A	1267300	0	1060380(83.7)	206920(16.3)
BOSS 2	2A	108386	0	79648(73.5)	28738(26.5)
F.A 1	2A	108386	27097(25)	37935(35)	43354(40)
F.A 2	2A	60033	0	60033(100)	0
GAFO 1	2A	5003	665(13.3)	0	4338(86.7)
GAFO 2	2A	0	0	0	0
GAFO 3	2A	0	0	0	0
OWONOKO 1	2A	2001	0	2001(100)	0
OWONOKO 2	2A	668	0	0	668(100)
OWONOKO 3	2A	0	0	0	0
ANIMAL CARE 1	2A	0	0	0	0
ANIMAL CARE 2	2A	2668	1001(37.5)	1667(62.5)	0
ANIMAL CARE 3	2A	2001	1001(50)	1000(50)	0
RABIU 1	2A	2668	333(12.5)	2335(87.5)	0
RABIU 2	2A	2668	991(37.1)	1677(62.9)	0
STELLAN	2A	668	0	668(100)	0

Cummulative	1571122	31422	1250613	289087
Relative Abundance	100%	2%	79.6%	18.4%

Appendix 14: Relative Abundance of *Eimeria* species in 9-18 Weeks Chickens on Chemoprophylaxis in Ogun State.

FARMS' NAME	EPI. CAT	OPG	BM (%)	AM (%)	NTP (%)
ODUNSI 1	OG 2B	668	0	334(50)	334(50)
ODUNSI 2	OG 2B	668	0	668(100)	0
TAIYE 1	OG 2B	0	0	0	0
TAIYE 2	OG 2B	0	0	0	0
AKIN SOTUBO 1	OG 2B	0	0	0	0
AKIN SOTUBO 2	OG 2B	0	0	0	0
AROWOLO 1	OG 2B	0	0	0	0
AROWOLO 2	OG 2B	0	0	0	0
AKIN 1	OG 2B	1001	0	334(33.4)	667(66.6)
AKIN 2	OG 2B	334	0	334(100)	0
GOLDEN YOLK 1	OG 2B	0	0	0	0
GOLDEN YOLK 2	OG 2B	0	0	0	0
SASBROTH 1	OG 2B	2668	0	1668(62.5)	1000(37.5)
SASBROTH 2	OG 2B	2001	0	1001(50)	1000(50)
SEGUN 1	OG 2B	0	0	0	0
SEGUN 2	OG 2B	0	0	0	0
LAMINA 1	OG 2B	2668	0	832(31.2)	1836(68.2)

LAMINA 2	OG 2B	1001	0	334(33.4)	667(66.6)
FEEDWELL 1	OG 2B	668	0	334(50)	334(50)
FEEDWELL 2	OG 2B	0	0	0	0
FOLAKEMI 1	OG 2B	0	0	0	0
FOLAKEMI 2	OG 2B	0	0	0	0
GORI	OG 2B	0	0	0	0
MURPHY	OG 2B	0	0	0	0
SEBUM 1	OG 2B	0	0	0	0
SEBUM 2	OG 2B	0	0	0	0
MOLAYE	OG 2B	0	0	0	0
Cummulative		11677	0	5839	5838
Relative Abundance		100%	0	50%	50%

Appendix 15: Relative Abundance of *Eimeria* species in Chickens above 19 weeks in Cages in Ogun State.

FARMS' NAME	EPI CAT	OPG	BM (%)	AM (%)	NTP (%)
AROWOLO	OG 3A	2668	1000(37.5)	1000(37.5)	668(25)
FOLAKEMI	OG 3A	2001	1335(66.7)	0	666(33.3)
SASBROTH	OG 3A	0	0	0	0
SOPTIMAL	OG 3A	11673	2668(22.9)	4336(37.1)	4669(40)
GOLDEN YOLK	OG 3A	1668	334(20)	667(40)	667(40)
AKIN	OG 3A	1336	0	668(50)	668(50)
SEGUN	OG 3A	2335	0	334(14.3)	2001(85.7)
ALE	OG 3A	0	0	0	0
LAMINA	OG 3A	0	0	0	0
FEEDWELL	OG 3A	4336	672(15.5)	997(23)	2667(61.5)
GORI	OG 3A	2001	334(16.7)	1334(66.7)	333(16.6)
MURPHY	OG 3A	0	0	0	0
OLUBAMISE	OG 3A	0	0	0	0
KEN AYO 1	OG 3A	0	0	0	0
OLUWASEUN	OG 3A	0	0	0	0
TAIYE	OG 3A	334	0	0	334(100)
KEN AYO 2	OG 3A	0	0	0	0
K. ABIOLA	OG 3A	0	0	0	0
OGUNMOLA	OG 3A	334	0	0	334(100)
SEBUM 1	OG 3A	1670	0	668(40)	1002(60)

SEBUM 2	OG 3A	668	334(50)	0	334(50)
AKIN SOTUBO	OG 3A	668	0	0	668(100)
LIZPAD 1	OG 3A	0	0	0	0
LIZPAD 2	OG 3A	0	0	0	0
MOLAYE	OG 3A	12673	0	1001(7.9)	11672(92.1)
GAFO	OG 3A	0	0	0	0
WHITE COAT	OG 3A	0	0	0	0
AJIBOYE	OG 3A	334	0	0	334(100)
OWONOKO	OG 3A	0	0	0	0
OSTAN	OG 3A	0	0	0	0
F A	OG 3A	54694	0	54694(100)	0
RABIU	OG 3A	2001	0	666(33.3)	1335(66.7)
STELLAN	OG 3A	3335	0	0	3335(100)
ANIMAL CARE	OG 3A	0	0	0	0
COMFORT	OG 3A	0	0	0	0
MIDE	OG 3A	667	0	0	667(100)
OROPO	OG 3A	0	0	0	0
EAT HEALTHY	OG 3A	0	0	0	0
GORIOLA	OG 3A	334	0	0	334(100)
Cummulative		105730	6677	66365	32688
Relative Abundance		100%	6.3%	62.8%	30.9%

Appendix 16: Relative Abundance of *Eimeria* species in Chickens Aged above 19 Weeks on Deep Litter in Ogun State

FARMS' NAME	EPI CAT	OPG	BM (%)	AM (%)	NTP (%)
OLA QUADRI	OG 3B	0	0	0	0
FIKUN	OG 3B	668	0	334(50)	334(50)
AYOADE	OG 3B	668	668(100)	0	0
NON NOMECLATURE	OG 3B	1001	333(33.3)	334(33.3)	334(33.4)
REAL	OG 3B	1336	334(25)	0	1002(75)
KAY	OG 3B	2001	0	334(16.7)	1667(83.3)
OGIS	OG 3B	2668	1000(37.5)	334(12.5)	1334(50)
OLUYEMI	OG 3B	60033	3002(5)	15008(25)	42023(70)
OROPO	OG 3B	0	0	0	0
GORIOLA	OG 3B	334	0	0	334(100)
EAT HEALTHY	OG 3B	00	0	0	0
AYOADE 2	OG 3B	30349	0	14249(47)	16100(53)
Cummulative		99058	5337	30593	63128
Relative abundance		100%	5.4%	30.9%	63.7%

Appendix 17: Comparism of Coccidial Oocyst Burden for Cage and Deep Litter System in Oyo and Ogun States

Epizootiological Category	Oyo state	Ogun state
Above 19 weeks old birds in cages	49494	2830
Above 19 weeks old birds on deep litter	71585	8255

3A = Birds above 19 weeks of age kept in cages; 3B = Birds above 19 weeks of age kept on deep litter; opg = Oocyst per gram of faeces.

Appendix 18: Statistical analysis of OPG for vaccinated and un-vaccinated Chickens in Oyo and Ogun States. One-way analysis of variance (ANOVA)

P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	8
F	13970000
R squared	1.000

Bartlett's test for equal variances

Bartlett's statistic (corrected)	230.6
P value	P<0.0001
P value summary	***
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	757200000000	7	108200000000
Residual (within columns)	844300	109	7746
Total	757200000000	116	

Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Oy 1A vs Oy1B	6485	221.1	P < 0.001	6356 to 6613
Oy 1A vs Oy2A	1587	54.10	P < 0.001	1459 to 1716
Oy 1A vs Oy2B	-209900	8405	P < 0.001	-210100 to -209800
Oy 1A vs Og1A	-9412	320.8	P < 0.001	-9540 to -9284
Oy 1A vs Og1B	8727	336.5	P < 0.001	8613 to 8840

Oy 1A vs Og2A	-72800	2865	P < 0.001	-72910 to -72690
Oy 1A vs Og2B	9462	395.0	P < 0.001	9357 to 9567
Oy1B vs Oy2A	-4898	167.0	P < 0.001	-5026 to -4769
Oy1B vs Oy2B	-216400	8665	P < 0.001	-216500 to -216300
Oy1B vs Og1A	-15900	541.9	P < 0.001	-16030 to -15770
Oy1B vs Og1B	2242	86.46	P < 0.001	2128 to 2355
Oy1B vs Og2A	-79280	3121	P < 0.001	-79390 to -79170
Oy1B vs Og2B	2977	124.3	P < 0.001	2872 to 3082
Oy2A vs Oy2B	-211500	8469	P < 0.001	-211600 to -211400
Oy2A vs Og1A	-11000	374.9	P < 0.001	-11130 to -10870
Oy2A vs Og1B	7140	275.3	P < 0.001	7026 to 7253
Oy2A vs Og2A	-74380	2928	P < 0.001	-74490 to -74270
Oy2A vs Og2B	7875	328.8	P < 0.001	7770 to 7979
Oy2B vs Og1A	200500	8028	P < 0.001	200400 to 200600
Oy2B vs Og1B	218700	10480	P < 0.001	218600 to 218800
Oy2B vs Og2A	137200	6783	P < 0.001	137100 to 137200
Oy2B vs Og2B	219400	11950	P < 0.001	219300 to 219500
Og1A vs Og1B	18140	699.5	P < 0.001	18030 to 18250
Og1A vs Og2A	-63380	2495	P < 0.001	-63500 to -63270
Og1A vs Og2B	18870	787.9	P < 0.001	18770 to 18980
Og1B vs Og2A	-81520	3813	P < 0.001	-81620 to -81430
Og1B vs Og2B	735.0	37.44	P < 0.001	649.1 to 821.0
Og2A vs Og2B	82260	4344	P < 0.001	82180 to 82340