COMPARATIVE EFFICACIES OF DNA AND LIVE OOCYSTS VACCINES AGAINST EXPERIMENTAL INFECTION WITH LOCAL AND HOUGHTON STRAINS OF *EIMERIA TENELLA* IN BROILER CHICKENS

BY

ZAINAB AHUOYIZA SHUAIBU

DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA

APRIL, 2018

COMPARATIVE EFFICACIES OF DNA AND LIVE OOCYSTS VACCINES AGAINST EXPERIMENTAL INFECTION WITH LOCAL AND HOUGHTON STRAINS OF *EIMERIA TENELLA* IN BROILER CHICKENS

BY

Zainab Ahuoyiza SHUAIBU, DVM (ABU) 2013.
(P14VTPE8011)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA.
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF SCIENCE IN VETERINARY PROTOZOOLOGY

DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY
AHMADU BELLO UNIVERSITY,
ZARIA

APRIL, 2018

DECLARATION

I declare that the work in this Dissertation entitled ‘‘C**omparative Efficacies of DNA and Live Oocysts Vaccines Against Experimental Infection with Local and Houghton Strains of *Eimeria tenella* in Broiler Chickens’’** was carried out by me in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and in the list of references provided therein. No part of this work has been presented for another degree or diploma at any institution.

Zainab Ahuoyiza SHUAIBU _____ _____

Signature

Date

CERTIFICATION

This Dissertation entitled ‘‘**COMPARATIVE EFFICACIES OF DNA AND LIVE OOCYSTS VACCINES AGAINST EXPERIMENTAL INFECTION WITH LOCAL AND HOUGHTON STRAINS OF *EIMERIA TENELLA* IN BROILER CHICKENS’’ by** Zainab Ahuoyiza SHUAIBU meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for contributing to knowledge and literary presentation.

Dr. I. D. Jatau
Chairman, Supervisory Committee _____ _____
Signature Date

Dr. A.M. Wakawa _____ _____
Member, Supervisory Committee Signature Date

Prof. O. O. Okubanjo
Head of Department _____ _____
Signature Date

Prof. S.Z. Abubakar _____ _____
Dean, School of Postgraduate Studies
A. B. U., Zaria.Signature Date

DEDICATION

This dissertation is dedicated to Almighty Allah and in memory of my late Father.

ACKNOWLEDGEMENTS

All praise is due to Allah; the Lord of lords and creator of everything for His guidance, blessings and protection from the first day of my existence and in all my endeavours. Surely He is to be praised.

I wish to express my profound gratitude to my supervisors; Dr. I. D. Jatau and Dr. A.M. Wakawa for their patience, understandings, support and guidance towards making this work a success.

I wish to acknowledge Prof. D. P. Blake of Department of Pathology and Pathogen Biology Royal Veterinary College United Kingdom for offering the Houghton strain of the parasite as well as the DNA vaccines used in this study.

Also my gratitude goes to the entire lecturers and staff of Veterinary Parasitology and Entomology Department, most specially Mall. M. Yusuf for giving their support for the success of this project.

My special appreciation goes to Dr. Sani Nuhu Abdul, Prof. C. Njoku and Prof. Sani Adamu of Veterinary Pathology Department, Ahmadu Bello University, Zaria for viewing and interpreting the histopathological slides.

My appreciation also goes to Dr. Babashanni M. of Veterinary Teaching Hospital, Ahmadu Bello University, for assisting in the statistical analysis of my data.

To the entire staff of Veterinary Pathology Laboratory, most specially, Mall. Yunusa and Mall. Muhammed Ibrahim of Chemical Pathology Department, Ahmadu Bello University Teaching Hospital, Zaria. I say thank you for being part of this success.

My appreciation goes to Dr. Hayatu, Dr. Y. Y. Pala and to Mall. Yahuza Suleiman of Vet. Teaching Hospital and Dept. of Vet. Public Health respectively for their technical assistance

To my friend Engr. Kudirat Yusuf and Nimat Kutemi, Dr. A. Hakeem, and my course mates, thank you for your support, it's been wonderful having you around me.

I will forever remain indebted to my parents for their love, support and care. May Almighty Allah reward them abundantly.

I wish to express my profound gratitude to my siblings Abdullahi, Memunat, Fatimah, Mariam and Sefiya for their prayers and wishes towards this success.

Most of all my heartily appreciation to the love of my life (my Romeo) Dr. Hassan Otuoze Suleiman for your burden of love and labour will never be in vain. I love you most dearly. And to my precious children Azeezah, Adeelah and Fatimah, thanks for your understanding and patience throughout the course of this work. You mean the world to me.

ABSTRACT

The search for a sustainable cost effective vaccine against coccidiosis that will be attractive to all poultry sectors has so far yielded the identification of novel recombinant DNA vaccine candidates such as Apical Membrane Antigen-1 (AMA-1). This study was aimed at evaluating the efficacy of vaccination of broiler chickens against coccidiosis using *Eimeria tenella* AMA-1(*Et* AMA-1) DNA vaccine in comparison to live vaccine based on body weight, gross caecal lesion score, oocyts production, histopathology, haematology and changes in serum enzyme levels of the immunized and challenged broiler chickens. A total of 80-day old broiler chickens were assigned to eight groups (1, 2, 3, 4, 5, 6, 7 and 8) of 10 each. At 14 days of age, the birds in groups 1(unchallenged unvaccinated control group), 2, 3 (challenged control groups for Local (L) and Houghton (H) *E. tenella* strains respectively) and group 4 (vector control group) were given 0.1 ml Phosphate Buffered Saline (PBS) on thigh muscle. Chickens in groups 5 and 6 (DNA vaccine group challenged with L and H strains respectively) were vaccinated with 0.1 ml of 100 µg of *Et*AMA-1 vaccine plasmid in PBS PH (7.6) by intramuscular injection on the thigh muscle and a repeat dose was given a week later (at day 21). Birds in groups 7 and 8 (Live oocysts vaccine group challenged with L and H strains respectively) were administered 0.2 ml Livacox® in 200 ml chlorine free clean water. At 28 days old, all chickens except group 1 were inoculated orally with 1×10^4 sporulated oocysts of the local and Houghton strains of *E. tenella*. A day prior to the experimental inoculation and seven days later, 2 ml of blood was collected from each bird by cardiac puncture for haematological and serum biochemical analyses. The birds were weighed aday prior to and reweighed 7 days after

challenge to determine the weight gain after which they were sacrificed for postmortem examination, gross lesion scores, oocyst production and histopathology. There was a significant decrease ($p < 0.05$) in the mean body weight of infected unvaccinated birds (groups 2, 3 and 4) (15.4 %, 15.4 % and 19.2 %) respectively when compared with infected vaccinated groups (42.3 % and 50.0 %, for groups 5 and 6, 45.2 % and 46.2 % for groups 7 and 8 compared to the uninfected control group 1 (100%). Both vaccines were able to significantly ($p <0.05$) reduce the effects of *E. tenella* on gross lesion score (1.4 ± 0.86, 1.0 ± 0.92, 1.0 ± 0.92 and 1.0 ±0.62) for groups 5, 6, 7 and 8 compared to (4.0 and 3.4) of unvaccinated infected group 2 and 3 respectively. The relative oocyst production for the vaccinated groups 5, 6, 7 and 8 were 45 %, 44 %, 34 % and 29 % respectively compared to 100 % for unvaccinated groups 2 and 3 respectively. There was observable decrease in mean PCV values (24.6 % and 24.8 %) for unvaccinated groups 2 and 3 respectively compared to 27.4 %, 27.6 %, 27.2 % and 26.8 % for vaccinated groups 5, 6, 7 and 8 respectively. There was a significant decrease ($p < 0.05$) in mean total plasma protein and significant increase ($p < 0.05$) in AST and ALP values in unvaccinated groups compared to the vaccinated groups. There was an overall higher Anti Coccidial Index (ACI) of 177, 179, 180 and 179 for vaccinated groups 5, 6, 7 and 8 compared to 170 and170 of unvaccinated groups 2 and 3, respectively. In conclusion, *Et*AMA-1 DNA vaccination ameliorated the effects of *E. tenella* infection in broiler chicks comparable to live oocysts vaccination. Combinations of two or more antigens into a single DNA formulation is recommended with hope of achieving protection that is comparable to live oocyst vaccine.

TABLE OF CONTENT

TitlePage

Title Page…….…….....i

Declaration…….…….....ii

Certification…….…….....iii

Dedication……...…...……...…….....iv

Acknowledgements……...…….....….....v

Abstract ……...…….....….....vi

Table of Content ……...……...……...….....…….....viii

List of Tables……..…….…….....…...xiii

List of Plates ……...…..…….....xv

List of Appendices……..…….……...……..….....xvi

List of Abbreviations ……...…….....xvii

CHAPTER ONE

1.0 INTRODUCTION

1.1Background of Study……...……..…….....1

1.2 Statement of Research Problem…….……...….3

1.3 Justification of the Study…….…….…….....5

1.4 Aim of the Study.……..…..…..…….6

1.5 Objectives of the Study…….…….....….....6

1.6 Research Questions……..……..…...……...…….6

CHAPTER TWO

2.0 LITERATURE REVIEW……..…….....…..……8

2.1 Introduction……..…….…..…….....8

2.2 Etiology of Coccidiosis in Chickens……..……..……..……...9

2.3 Transmission of Coccidian Parasite……...……..……..…….11

2.4 Clinical Signs of Coccidiosis……..……....12

2.5 Life Cycle of *Eimeria* in Chickens…….……...…….12

2.6 Caecal Coccidiosis……..…..……..……...13

2.7 Diagnosis of Coccidiosis…….....…….....……..15

2.8 Haematological Changes in Coccidia Infection……...……...….....17

2.9 Serum Enzymes and Electrolyte Changes in Coccidiosis……..……18

2.10 Immune Response…….…...…….……...……..…….19

2.11 Gross and Histopathological Changes in Coccidiosis……..…...……...…..21

2.12 Prevention and Control of Coccidiosis.……..…..…….……..22

2.12.1 Immunoprophylaxis.....……....…..……..……...22

2.12.2 Anticoccidial vaccines……...…....……...…..…….…..…….24

2.12.3 Live *Eimeria* vaccines. ……..……...……..……..……...24

2.12.4 Sub-unit and recombinant *Eimeria* vaccines……....……...…….30

2.12.5 DNA vaccines against coccidiosis……..……..…….……...31

2.12.6 Use of natural products in controlling coccidiosis……...…..……...…….36

CHAPTER THREE

3.0 MATERIALS AND METHODS...……..38

3.1 Study Area……..……...…….…….……38

3.2 Experimental Chickens……...……..……...38

3.3 *Eimeria tenella* Isolate……..……..…….....38

3.4 Sources of the *Eimeria* Vaccine…….……40

3.5 DNA Vaccine Construct extraction…….…….....……...40

3.5.1 Culturing of *E. coli*……..…….40

3.5.2 Plasmids extraction…..……..……..…….....41

3.6 Experimental Grouping……...……42

3.7 Immunization and Challenge of Experimental bird……..……..……..42

3.8 Evaluation of Efficacy of the Vaccine Against Experimental Challenge…..…43

3.9 Histopathological Examination of the Tissue Samples……..…….....44

3.10 Haematological Analysis…….……...……...….....45

3.10.1 Determination of packed cell volumes…..…...……..……...……45

3.10.2 Determination of white blood cell values…..……...……....46

3.10.3 Determination of differential cell counts……..……46

3.10.4 Determination of haemoglobin concentration…….……....……...…47

3.11 Serum Biochemistry……...……47

3.11.1 Determination of total plasma protein……...……..…….…...48

3.11.2 Determination of serum albumin…….……48

3.11.3 Determination of serum alkaline phosphatase….……..…….48

3.11.4 Determination of serum aspartate amino transferase...…….……...….49

3.11.5 Determination of serum alanine amino transferase…...…….49

3.11.6 Determination of serum electrolyte values…...……..…….50

3.12 Data Analyses……....…….……...50

3.13 Ethical Statement…….....50

CHAPTER FOUR

4.0

RESULTS…..…...……..…….…….....51

4.1 Effects of Vaccination Against Experimental Challenge with Local and Houghton Strains of *Eimeria tenella* on Clinical Signs …..……..…..….51

4.2 Effects of the Vaccination on Body Weight Gain…….....…..…….....51

4.3 Gross Lesion Scores of Broilers……..…….…….....52

4.4 Oocysts Production...……...……...……...….54

4.5 Anti Coccidial Index…..……...…….....62

4.6 Histopathological Lesions in Broilers……...……..…...…....62

4.7 Effects of DNA and Live Vaccination on Local and Houghton Strains of *Eimeria tenella* on Pack Cell Volume……...…….....73

4.8 Effects of DNA and Live Vaccines on Local and Houghton Strains of *Eimeria tenella* on Haemoglobin concentration...…...….....….....73

4.9 Effects of DNA and Live Vaccines Against Local and Houghton Strains of *Eimeria tenella* Infection on White Blood Cell Count…….76

4.10 Effects of DNA and Live Vaccines on Heterophils value Against Local and Houghton Strains of *Eimeria tenella* Infections in Broiler...….....76

4.11 Effects of DNA and live vaccines against local and Houghton Strains of *Eimeria tenella* Infection on Lymphocytes Value…….....……...79

4.12 Effects of DNA and live vaccines against local and Houghton Strains of *Eimeria tenella* on Monocytes Value…….……....79

4.13 Effects of DNA and Live Vaccines Against Local and Houghton Strains of *Eimeria tenella* on Mean Total Plasma Protein……..…….82

4.14 Effects of DNA and Live Vaccines Against Local and Houghton Strain of *Eimeria tenella* on Aspartate Amino Transferase…….....…….82

4.15 Effects of DNA and Live Vaccines on Alkaline Phosphatase in Local and Houghton Strain of *Eimeria tenella* Infection in Broilers……....85

4.16 Effects of DNA and Live Vaccines Against Local and Houghton Strain of *Eimeria tenella* on Alanine Amino Transferase…….....……...85

4.17 Effects of DNA and Live Vaccines Against Local and Houghton strain of *Eimeria tenella* on Mean Albumin Values……..……88

4.18 Effects of DNA and Live Vaccines Against Local and Houghton Strains of *Eimeria tenella* on Mean Globulin Values……..…88

4.19 Effects of DNA and Live Vaccines Against Aocal and Houghton Strains of *Eimeria tenella* on Mean Sodium Values……...…91

4.20 Effects of DNA and Live Vaccines Against Local and Houghton Strains of *Eimeria tenella* on Mean Potassium Values…...…….91

4.21 Effects of DNA and Live Vaccines Against Local and Houghton Strains of *Eimeria tenella* on Mean Chloride Values…….…94

4.22 Effects of DNA and Live Vaccines Against Local and Houghton Strains of *Eimeria tenella* on Bicarbonate Value……....…….94

CHAPTER FIVE

5.0 DISCUSSION……...…….……...….97

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS…….....103

6.1 CONCLUSION…..……..…….....103

6.2 RECOMMENDATION…….....…….……..104

REFERENCES……..…… ….……105

LIST OF TABLE

Table **Title** **Page**

2.0Species names and Intestinal Segments ……..…....10

2.1 Live attenuated and none-attenuated live *Eimeria* Vaccines……...27

2.2 Recently studied DNA vaccines……....…….....34

4.1 Mean (± SEM) body weight of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*…….....53

4.2 Mean (± SEM) lesion score of broiler ckicks vaccinated with DNA and livacox® vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*……..…….…….....55

4.3 Mean (± SEM) of oocysts values of broiler chicks vaccinated with DNA and live coccidian vaccines and challenged with local and Houghton strains of *Eimeria tenella*……...……...…….61

4.4 Effects of DNA and live vaccines against local and Houghton strains of *Eimeria tenella* challenge on differentparameters…….....63

4.5 Mean (± SEM) of pack cell values of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*……...……...74

4.6 Mean (± SEM) of haemoglobin of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*……..…….…….…….…...75

4.7 Mean (± SEM) of white blood cells of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*…….…….……....…77

4.8 Mean (± SEM) of heterophils of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*…….……..…..…..78

4.9 Mean (± SEM) of lymphocytes of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*……..…..80

4.10 Mean (± SEM) of monocytes of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*…….....….81

4.11 Mean (± SEM) of total plasma protein of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*……..…….....…..83

4.12 Mean (± SEM) of aspartate amino transferase of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*……..….84

4.13 Mean (± SEM) of alkaline phosphatase of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*……..……..……..86

4.14 Mean (± SEM) of alanine amino transferase of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*.....87

4.15 Mean (± SEM) of albumin of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*.....89

4.16 Mean (± SEM) of globulin of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*…….....…..…..…….90

4.17 Mean (± SEM) of sodium ion of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella* 92

4.18 Mean (± SEM) of potassium ion of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*……..…….93

4.19 Mean (± SEM) of chloride ion of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*……...……..….……95

4.20 Mean (± SEM) of bicarbonate ion of broiler chicks vaccinated with DNA and live coccidian vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*..……..……..96

LIST OF PLATES

Plate	Title	Page
I	General life cycle of coccidian of the genus *Eimeria*……..…..…….....14	
II	Caecum of chicken showing normal appearance graded as score (0).……56	
III	Caecum of chicken experimentally infected with Houghton strain of *E. tenella* graded score (1)……..……..…….57	
IV	Caecum of chicken experimentally infected with Houghton strain of *E. tenella* graded score (2) …….....……...…58	
V	Caecum of chicken experimentally infected with Houghton strain of *E. tenella* graded score (3) …….59	
VI	Caecum of chickens experimentally infected with Local strain of *E. tenella* graded score (4) ……...….60	
X	Photomicrograph showing normal caecum of broiler chicken in group 1…..….65	
XI	Photomicrograph of the caecum of a broiler chicken experimentally infected with *E. tenella* (local strain).……...66	
XII	Photomicrograph of the caecum of broiler chicken experimentally infected with *E. tenella* (Houghton strain)..…….....67	
XIII	Photomicrograph of the caecum of Vector vaccinated chicken experimentally infected with *E. tenella* (local strain).…….....68	
XIV	Photomicrograph of the caecum of broiler chicken vaccinated with DNA vaccine and experimentally infected with *E. tenella* (local strain)..……..69	
XV	Photomicrograph of the caecum of broiler chicken vaccinated with DNA vaccine and experimentally infected with *E. tenella* (Houghton strain).....70	
XVI	Photomicrograph of the caecum of broiler chicken vaccinated with live oocysts vaccine and experimentally nfected with *E. tenella* (local strain) …….....71	
XVII	Photomicrograph of the caecum of broiler chicken vaccinated with live oocyst vaccine and experimentally infected with *E. tenella* (Houghton strain) …….....72	

LIST OF APPENDICES

Appendix **Title** **Page**

I Mean body weight of all groups (1-8) for pre infection and post infection...118
II Mean egg per gram of all groups (2-8) at 35 day post challenge.....……...119
III Changes in mean pack cell volume of all groups (1-8) for pre infection and post infection……..…….....120
IV Changes in mean haemoglobin values of all Groups (1-8) for pre infection and post infection…….…….....121
V Changes in mean white blood cell values of all groups (1-8) for pre infection and post infection……..……..….122
VI Changes in mean heterophils values of all groups (1-8) for pre infection and post infection..……..……..……...123
VII Changes in mean lymhpocytes values of all groups for pre infection and post infection……..……...124
VIII Changes in mean monocyte values of all groups for pre infection and post infection.....……..…….....125
IX Changes in mean total plasma protein values of all groups for pre infection and post infection…..……..……126
X Changes in mean aspartate amino transferase values of all groups for pre infection and post infection…..……..……127
XI Changes in mean alkaline phosphatase values of all groups (1-8) for pre infection and post infection…..……..……..…….128
XII Changes in mean alanine amino transferase values of all groups (1-8) for pre infection and post infection.……..…….……129
XIII Changes in mean albumin values of all groups (1-8) for pre infection and post infection…….……...130
XIV Changes in mean globulin values of all groups (1-8) for pre infection and post infection……...……..…..……131
XV Changes in mean sodium values for all groups (1-8) for pre infection and post infection..…….....……...….....132
XVI Changes in mean potassium values of for all groups (1-8) for pre infection and post infection…….....…..…….…...133
XVII Changes in mean chloride values for all groups (1-8) for pre infection and post infection..……..……..…..……134
XVIII Changes in mean bicarbonate values for all groups (1-8) for pre infection and post infection…….....……...……135
XIX Effects of DNA and live oocysts vaccines against local and Houghton strains of *Eimeria tenella* on red blood cell..……..136
XX Mean (± SEM) of red blood cell of broiler chicks vaccinated with DNA and live oocysts vaccines prior to challenge with local and Houghton strains of *Eimeria tenella*…..……..…..…137
XXI Changes in mean red blood cell values of all groups (1-8) for Pre-infection and post- infection…..……..…….……138

LIST OF ABBREVIATIONS

ACI	Anti coccidial index
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
AMA-1	Apical membrane antigen
AST	Aspatate amino transferase
ANON	Anonymous
Bp	Base pairs
BCG	Bromo cresol green
$CD4^+$	Cluster of differentiation (Helper T Cell)
$CD8^+$	Cluster of differentiation (T Lymphocytes)
ChIFN	Chimeric interferon gamma
DNA	Deoxyribonucleic acid
´E	East
EDTA	Ethylene diamin tetra acetate
Ebr-AMA-1	*Eimeria brunnetti* apical membrane antigen 1
Em-AMA-1	*Eimeria maxima* apical membrane antigen 1
Et-AMA-1	*Eimeria tenella* apical membrane antigen 1
G	Grams
g/L	Grams per litre
H & E	Hematoxyline and Eosin
HICN	Drabklin's reagent for measuring hemoglobin

H_2S	Hydrogen sulphide
IBD	Infectious bursal disease
KDa	Kilodalton
LB	Leuria Bertani medium
Min	Minutes
ml	Millilitre
´N	North
Nm	Nanometer
NVRI	National Veterinary Research Institute
O℃	Celcius
OPG	Oocysts per gram
OUT	Operational Taxonomic Units
PCR	Polymerase chain reaction
PCV	Packed cell volume
pH	Hydrogen ion concentration
RBC	Red blood cell
RNAS	Ribonucleic acids
RONs	Rhoptry neck proteins
S	Standard
SB	Standard blank
T	Test
TB	Test blank
TSI	Tripple suger iron agar
µg	Microgram

µL	Microliter
WBC	White blood cells
+	Positive sign
-	Negative sign

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Coccidiosis in chickens is caused by an apicomplexan parasite belonging to the genus *Eimeria* of which at least seven species are incriminated in the cause of the disease (Conway and Elizabeth, 2007; McDougald and Fitz-Coy, 2008). Infection with *Eimeria* occurs when a susceptible chicken ingests the abundant infective sporulated oocysts from the environment, contaminated feed or water (Majaro, 2001). This infectious disease causes economic losses worldwide estimated to be greater than $3 billion annually (Williams, 1999; Dalloul and Lillehoj, 2006). Approximately, 80% of these losses are due to mortality, inefficient feed utilization and impaired growth rate of broilers and or reduced egg production in layers. While the remaining 20% losses is due to the cost of chemoprophylaxis (Wiliams *et al*., 1999). *Eimeria tenella* is the commonest and most pathogenic of all the *Eimeria* species affecting chicken and is prevalent throughout the world. *Eimeria tenella* is the major cause of haemorrhagic coccidiosis in poultry establishment worldwide causing high morbidity and variable mortalities. (Taylor *et al*., 2007). Good animal husbandry such as maintaining litter quality, controlling house temperature, ventilation, moisture level and thorough cleaning between flocks plays a vital role in the prevention and control of coccidiosis by reducing oocysts sporulation and recycling (Blake and Tomley, 2014). However, good husbandry alone is not adequate for the control of the disease. Chemo-prophylactic and chemotherapeutic treatment so far remains the most practiced means of controlling coccidiosis. However, the increase in resistance of parasite population and increasing public pressure to limit the use of chemicals in animal feeds highlights the need to find

alternative methods (Blake *et al*., 2011). Vaccination against coccidiosis using live oocysts vaccine is the most effective way of control and prevention of the disease since vaccinated birds usually develop solid immunity against secondary homologous infection (Williams, 2006). However, production limitation including a requirement for *in vivo* passage has limited the production capacity and elevated the cost of live vaccines (Williams, 2006). Consequently, their use is limited to the breeding and egg-laying sectors of the poultry industry and rarely used in the broiler sector where economic margin per bird is much lower (Blake and Tomley, 2014). The quest for more cost-effective vaccine against coccidiosis for the mass broiler market has led to the discovery of various subunit vaccine candidates which include the apical membrane antigen-1 (AMA-1) (Santos *et al*., 2011).

Apical membrane antigen-1 (AMA-1) is a micronemal protein of apicomplexan parasites that appears to be essential during the entry into the host's cells by the invasive stages of the parasites (Besteiro *et al*., 2009; Santos *et al*., 2011; Lamarque *et al*., 2011; Curtidor *et al*., 2011; Jiang *et al.,* 2012). It was first identified as a conserved antigenic protein in the malaria parasite *Plasmodium knowlesi* (Thomas *et al*., 1984). Together with rhoptry neck proteins (RONs), AMA-1 is involved in the formation of the moving junction complex, which is a circumferential zone that moves backward and eventually pinches the PV from the host cell membrane (Besteiro *et al*., 2009; Curtidor *et al*., 2011; Lamarque *et al*., 2011; Santos *et al*., 2011). *Plasmodium falciparum* AMA-1 (PfAMA-1) has been demonstrated to induce protective immunity against the parasite challenge in animal models (Remarque *et al*., 2008). Similarly, preliminary reports have established that recombinant protein vaccines derived from AMA-1 of *E. brunetti* (EbrAMA-1), *E. maxima* (EmaAMA-1) and *E. tenella* (EtAMA-1) have conferred

significant immune protection against experimental challenge by the respective parasites (Blake *et al*., 2011; Jiang *et al*., 2012). Apical membrane antigen-1 has also been identified as an immune protective protein from other apicomplexan parasites, such as *Babesia*, *Toxoplasma*, *Neospora* and *Theileria* (Donahue *et al*., 2000; Gaffer, 2004; Zhang *et al*., 2007; Tonukari, 2010). Interestingly, the homologues of the AMA-1 antigen appear to be protective against a range of apicomplexan parasites including *Plasmodium* spp., which suggest that there may be some protective antigens shared across this diverse group of parasites (Blake *et al*., 2011). According to Jiang *et al.* (2012) EtAMA-1 has an open reading frame of 1608 bp encoding a protein of 535 amino acids and is expressed at higher level in sporozoites than in unsporulated oocysts.

1.2 Statement of Research Problem

Records have shown that poultry farming is one of the fastest growing sectors of animal production in many countries and expansion is predicted to continue for at least 30 years in Africa and Asia (Blake and Tomley, 2014). In Africa including Nigeria, many people depend on small scale poultry production systems for food and source of income but, diseases such as coccidiosis are a major limiting factor (Jatau *et al*., 2012). Avian coccidiosis is one of the major diseases of poultry world-wide, which result to great economic losses in excess of 3 billion US dollars annually (Shirley *et al*., 2005). It is a major recurring disease that exerts negative effect on profitable and sustainable small scale poultry enterprises around the world including in Africa (Fornace *et al*., 2013).

Through a combination of ubiquitous nature of *Eimeria* parasites, high fecundity rate and resistant to the extremes of environmental conditions, eradication of coccidiosis is not practically possible especially in birds kept under deep litter system. Vaccination

with wild-type or attenuated live parasites is the most effective way of controlling avian coccidiosis (Shirley *et al*., 2005). But, live vaccines are relatively expensive and very labor intensive to produce, since it is only possible to generate oocysts in live chickens (Allen and Fetterer, 2002). It also risks a negative effect on bird performance due to intestinal damage caused by the live parasite, most notably a risk with wild-type vaccines (Waldensted *et al*., 1999; Williams *et al*., 1999; Allen and Fetterer, 2002). For these reasons, live vaccine usage is mainly confined to the breeding and egg-laying sectors of poultry industry.

Recent efforts to develop an alternative strategy for the control of coccidiosis are directed towards cloning the genes from *Eimeria* spp. for potential recombinant vaccines (Dalloul and Lillehoj, 2006; Blake and Tomley, 2014), and indeed, many vaccine candidate genes have been identified (Subramanian *et al*., 2008; Blake *et al*., 2011; Jiang *et al*., 2012; Oakes *et al*., 2013; Yin *et al*., 2013).

Blake *et al.* (2011) and Jiang *et al.* (2012) had discovered that immunization of birds with the recombinant apical membrane antigen-1 (AMA-1) of *E. maxima, E. tenella* and *E. brunette* respectively; confer significant immune protection against homologous experimental challenges by the respective parasites. However, the established knowledge of the existence of antigenic variation among strains of *Eimeria* parasites from different geographical locations of the world (Fitz-Coy, 1992; Martin *et al*., 1997; Danforth, 1998), temper confidence whether the recombinant AMA-1 vaccine derived from foreign strains of these parasites will protect against challenge by local isolates.

1.3 Justification of the Research

With the widespread occurrence and the rate at which drug resistant *eimeria* parasites emerge as well as the difficulties associated with the production and use of live vaccines, new approaches of controlling coccidiosis are urgently needed (Innes and Vermeulen, 2006; Williams, 2006; Morris *et al*., 2007). The search for a sustainable cost effective vaccine against coccidiosis that will be attractive to all poultry sectors has so far yielded the identification of novel recombinant vaccine candidates of which AMA-1 has great potential of becoming a more realistic candidate. Under experimental conditions, studies with clonal reference strains of *Eimeria* have found AMA1 to induce good levels of immune protection in challenged broiler chickens (Blake *et al*., 2011; Jiang *et al*., 2012)., making the development of recombinant AMA-1 or vectored subunit anticoccidial vaccine technically more realistic.

Recent studies reporting low level of coding polymorphism for AMA1 across Nigerian and spatially diverse *Eimeria tenella* samples, combined with the cross-protection detected between genetically distinct isolates when tested in out bred broiler chickens, supports the validity of this antigen to confer universal protection against field strains of *Eimeria* species when used in future vaccine development (Jatau, 2014; Blake *et al*., 2015).

However, for any novel subunit anticoccidial vaccine to proceed beyond the experimental phase evidence of likely field efficacy against novel strains is essential. To best of our knowledge, there is no study describing the efficacy of the homolog of AMA-1 DNA vaccine derived from the reference laboratory strain of *E. tenella* against regional field strain in Nigeria.

1.4 Aim of the Study

The aim of this study was to evaluate the comparative efficacies of DNA and Live oocysts vaccines against experimental infection with Local and Houghton strains of *Eimaria tenella* in broilers chickens.

1.5 Objectives of the Study

The specific objectives of the study were to evaluate in comparison to live anticoccidial vaccination the:

1. Efficacy of EtAMA-1 DNA vaccine on the body weight and oocyst production in birds experimentally infected with Local isolate and Houghton strains of *E. tenella* in broiler chickens.
2. Effect of immunization with EtAMA-1 DNA vaccine on pathological changes in broiler chicks experimentally infected with Local isolate and Houghton strains of *E. tenella.*
3. Effect of immunization with EtAMA-1 DNA vaccine on changes in haematological parameters in broiler chicks experimentally infected with Local isolate and Houghton strains of *E. tenella.*
4. Effect of immunization with EtAMA-1 DNA vaccine on changes in serum biochemical parameters in broiler chicks experimentally infected with Local isolate and Houston strain of *E. tenella.*

1.6 Research Questions

i. Does immunization with EtAMA-1 DNA and live oocysts vaccines have effects on the body weight gain and oocyst production in broiler chickens

experimentally infected with Local isolate and Houghton strain of *Eimeria tenella*.

ii. Does immunization with EtAMA-1 DNA and live oocysts vaccines have effects on pathological changes in broiler chicks experimentally infected with Local isolate and Houghton strains of *Eimeria tenella*.

iii. What is the effect of immunization with EtAMA-1 DNA and Live oocysts vaccines on haematological parameters in broiler chicks experimentally infected with Local isolate and Houghton strains of *E. tenella.*

iv. What is the effect of immunization with EtAMA-1 DNA and Live oocysts vaccines on biochemical parameters in broiler chicks experimentally infected with Local isolate and Houghton strains of *E. tenella.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Coccidiosis is an important disease of poultry caused by an apicomplexan parasite belonging to the genus *Eimeria* of which at least seven species are known to cause the disease (Conway and Elizabeth, 2007; McDougald and Fitz-Coy, 2008). These infections lead to digestive disorders ensuing from damage to the intestinal epithelium, malabsorption of nutrients, changes in protein metabolism after absorption, reduced efficiency of feed conversion, and reduction in weight gain (Conway *et al*., 1993; Shirley *et al*., 2005). Mortality and economic losses, especially in cases of outbreaks, are common (Morris and Gasser, 2006).

Coccidiosis, one of the most widelyreported diseases of the world within the poultry sector from an economic point of view (Williams, 2002). Coccidia are present normally in almost all poultry raisingoperations throughout the world, though,disease occurs following the presence of significantnumber of oocysts inside the intestines. Coccidiosis are ubiquitous, they are everywhere chickens are reared traditional, industrial, or organic-bio farms. Their survival is secured by some highly resistant oocysts that can survive for several months in the environment. Coccidia are obligatory parasites (sporozoa) that are characterized by the presence of an apical complex in the free stages of the cycle (sporozoites and merozoites) which invade the epithelial cells. The disease is as a result of a collapse in the balance between: The parasites: their numbers, their pathogenicity and their ability to promote immunity in the host. The host: its susceptibility, including its protection by anticoccidials, and its ability to regenerate

from the damages caused by the parasite. The environment: intensive rearing particularly predisposes conditions for coccidiosis.

2.2 Etiology of Coccidiosis in Chickens

There are mainly seven species of *Eimeria* that causes chicken Coccidiosis namely;*Eimeria tenella*, *E. maxima*, *E. acervulina*, *E. brunetti*, *E. necatrix*, *E. praecox* and *E. mitis*. These species arevery specific and inhabit different parts ofintestine of its host Urquhart *et al*. (1996) (Table 1.0).

Although Eimeria species in Nigerian chickens were identified traditionally using morphological and pathological criteria in the diagnosis of coccidiosis that resulted in the identification of seven main species *E. tenella*, *E. brunetti*, *E. acervulina E. maxima*, *E. mitis*, *E. necatrix* and *E. praecox*. (Muazu *et al.*, 2008., Jatau *et al.*, 2012). Operattional Taxonomic Units (OTU) x, y and z has only been described in Australia (*Cantacessi et al., 2008., Fornace et al., 2013,* Godwin *et al.*, 2015). Recently, molecular tools for accurate species identification and characterization of a small number of field isolates clearly indicated for the first time that all three OTU genotypes are circulating within Nigerian poultry (Jatau *et al.,* 2016). Four of nine samples tested were found to contain at least one OTU genotype (44 %), which is comparable to the occurrence detected within Australian flocks (Godwin *et al.*, 2015). The risk posed by these genotypes is currently unclear.

Table 2.0 *Eimeria* species and intestinal segments found

Species name	Intestine segments
Eimeria. tenella	Caeca
E. acervulina	Duodenal loop and upper small intestine
E. necatrix	Upper small intestine
E. maxima	Small intestine
E. mitis	Duodenal loop and upper small intestine
E. mivati	Small intestine
E. brunetti	Small intestine and caeca
E. praecox	Small intestine

Urquhart *et al*. (1996)

2.3 Transmission of Coccidian Parasite

Coccidian oocysts are highly resistant to environmental stress and disinfectants (McDougal, 2003). Temperatures above 56°C and below 0°C are deadly but it seems very difficult to decontaminate a previously contaminated poultry house or environment (McDougal, 2003). Sporulated oocysts can be spread mechanically by wild birds, insects or rodents and via contaminated boots, clothing, equipment or dust. Although feco-oral route remains the natural mode of infection (McDougal, 2003). Under the right environmental conditions (i.e. increased moisture), the oocysts can contaminate all areas of the poultry environment including feed, litter and soil and can be viable for months in the environment. Therefore, controlling substrate and/or litter moisture levels are essential. The substrate or litter material should be "friable" meaning if you pick it up it should clump in your hand but also crumble relatively easily. If the litter is too dry it can be a particulate that can be inhaled by the chickens and by us. If it is too wet, it will clump and not come apart. In addition, if the litter material is too wet it may have a strong ammonia smell which can cause coccidial overgrowth and other flock management problems (McDougal, 2003). Birds are more susceptible to disease if they are immunosuppressed either by disease or stress. For example, sexually immature chickens that have the immunosuppressive disease infectious bursal disease (IBD) are more likely to become infected after being exposed to coccidia. Likewise, intestinal coccidiosis may predispose birds to other concurrent intestinal infections such as necrotic enteritis, salmonellosis, and some intestinal viral infections Therefore it is essential to create a healthy environment for chickens. (Maurice, 2012).

2.4 Clinical Signs of Coccidiosis

Coccidiosis in poultry is divided basically into caecal and intestinal. Caecal coccidiosis is known to produce bloody droppings and anaemia often resulting to death whereas intestinal coccidiosis is more chronic in nature (Muazu *et al*., 2008; Ali *et al*., 2014). Sub clinically; signs manifested are reduce feed intake, poor flock uniformity, poor growth, impaired feed conversion, general unthrifty appearance, and a watery diarrhea that may be greenish or bloody. Tremors, convulsions, and lameness are also occasionally seen Ali *et al*., 2014). Young birds that survive severe infections may suffer retardation of growth (Muazu *et al*., 2008). lnfected bird sheds non infective oocysts with faeces into the environment Oocysts sporulate within 48 hours and become infective to susceptible bird.

2.5 Life Cycle of *Eimeria* in Broiler Chicken

The life cycle of most caecal coccidia are complex but direct in which the infective stage of the parasite invades an appropriate animal host for development to sexual maturity (Morris and Gasser, 2006). Stages of coccidia in chickens appear both within the host as well as outside. The developmental stages in the chicken give rise to a microscopic egg (called an oocyst) that is passed out in the droppings. Under proper conditions of temperature and moisture, the oocyst develops within one to two days to form a sporulated oocyst, which is capable of infecting other chickens. At this stage, the oocyst contains eight bodies (called sporozoites), each of which is capable of entering a cell in the chicken's intestine after the oocyst is eaten. When sporozoites enter the cells, they divide many times producing either a few or many offspring (merozoites). The numbers produced depend on the species of coccidia involved. Each merozoite, in turn, may enter another intestinal cell. This cycle may be repeated several times. Because of

this cyclic multiplication, large numbers of intestinal cells are destroyed. Eventually, the cycle stops and sex cells (male and female) are produced. The male fertilizes the female to produce an oocyst, which ruptures from the intestinal cell and passes in the droppings. Thousands of oocysts may be passed in the droppings of an infected chicken. (Plate 1).

2.6 Caecal Coccidiosis

Caecal coccidiosis is caused by only one of the species (*Eimeria tenella*) of coccidia. This type of coccidiosis is frequently referred to as "brooder-house coccidiosis" or "bloody coccidiosis (Muazu *et al*., 2008). Caecal coccidiosis is characterized by a bloody diarrhea, which is due to the damage done by the coccidial parasite to the mucous membrane of the ceca and rectum (Muazu *et al*., 2008).

In the average commercial poultry flock, cecal coccidiosis is most likely to occur during the brooding period. It is observed most often in chicks between 4 and 10 weeks of age. Susceptible chickens younger and older than this, however, may become infected. In most outbreaks the condition appears quite suddenly, and blood in the droppings may be noted before many of the chicks show symptoms. Following the blood in the droppings, usually within a few hours, the infected chicks lose their appetite and become droopy. Death soon follows in most chickens that are severely infected (Muazu *et al*., 2008). The mortality following an outbreak of caecal coccidiosis may vary from a few birds to as much as 25 per cent or more. This variation depends upon several factors but especially upon the circumstances under which the chicks are being kept.

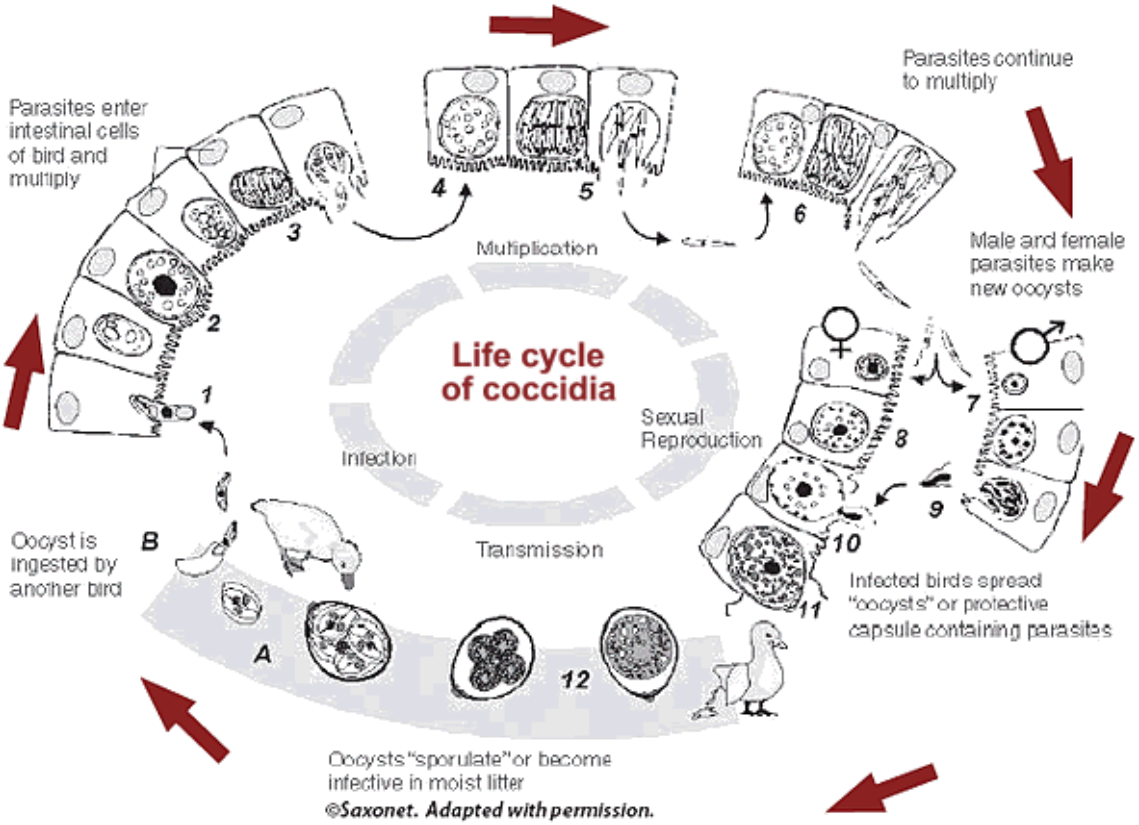


Plate 1: General life cycle of coccidia of the genus *Eimeria*

http://www.thepoultrysite.com/publications/2/Coccidiosis%20Management/41/life-cycle-and-types-of-coccidia/

The sudden onset of the disease with bloody diarrhea from the chicks is quite indicative of cecal coccidiosis. Post-mortem examination of sick or dead chicks that reveal the ceca distended with blood is practically certain to be cecal coccidiosis. Some chicks that have survived an infection for several days may have the ceca filled with a cheese like "plug." These "plugs" are organized blood clots and may have a pinkish color on the inside when cut open. The presence of reddish or orange-colored slimy droppings should not be confused with pure blood in the droppings because this type of dropping may be passed by chickens that are not infected with coccidia. Microscopic examination of material from suspected lesions for coccidial forms is the mostly dependable means of positively diagnosing caecal coccidiosis (Muazu *et al*., 2008).

2.7 Diagnosis of Coccidiosis

Coccidiosis is one of the major problems in poultry sector; this is so because of difficult in its diagnosis. The classical parasitological methods of diagnosis are labor intensive and thus costly. The impact of the parasite on the performance of a flock cannot solely rely on oocyst per gram (OPG) counts in faeces or litters. Identification of different species based on morphology of oocysts is very challenging and requires expertise. Lesionscoring is an explanation 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 (e.g. five) 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 personnel. Moreover, it has been demonstrated there can be a poor relation between macroscopic and microscopic lesions, emphasizing using

macroscopic lesion scoring alone is not suitable to detect all economical relevant coccidiosis infections (Idris *et al.*, 1997).

As coccidiosis is a disease that cannot currently be eradicated, the objective of coccidiosis prevention is finding the economical optimal balance between costs of diagnosis, prevention, treatment and development of host immunity while trying to keep the subclinical loss as low as possible. Necropsy sessions are performed on dead birds; this involved an evaluation of the lesion scores and gut health on poultry complex. Another method of diagnosis of coccidiosis is that given by Morris and Gasser (2006). It is analysis in genetic variation in *Eimeria specie*. This comprises of both biochemical and molecular methods such as southern blot analysis, multilocus enzyme electrophoresis, pulsed-field gel electrophoresis and several PCR techniques. These techniques made a major addition for scientific research and more practical applications in establishing vaccine quality control, but regrettably, the lack of a rapid, low-cost and especially quantitative test is preventing their broad scale use. Gruber *et al*. (2007) postulate an innovative technique called Coccimorph. It is a computational approach for parasite diagnosis, in this case *Eimeria* spp. Images from sporulated oocysts from a known species were assessed on different features: size and symmetry, curvature characterization and internal structure characterization. Users from any part of the world can upload their digital images from unidentified oocysts and have the program identify the species concerned. This is very accessible and the low cost is a major advantage. A disadvantage is only sporulated oocysts can be identified.

Anticoccidial sensitivity testing (AST) is a well-known technique to try to assess resistance of a certain coccidial isolate to different anticoccidial drugs (Chapman, 1998;

Naciri *et al*., 2003; Peek and Landman, 2003). Although a valid method for a certain isolate, this technique is not routinely used because of the long duration and very high cost associated with the complicated *in vivo* character of the test. AST results from strains with a known drug history, better information can be obtained on how fast resistance is induced, how long it remains established in a certain coccidial population and on whether there is cross-resistanceamongst drugs (Chapman, 1998; Naciri *et al*., 2003; Peek and Landman, 2003).

2.8 Haematological Changes in Coccidia Infection

Anemia, characterized by decreased PCV, RBCs, and/or Hb, is the most common erythrocyte abnormality seen in birds. Birds with a PCV less than 35% are generally considered anemic (Sirois, 1995). Coccidiosis causes loss of blood into the gastrointestinal tract, resulting in decrease in the numbers of circulating red blood cells (Irizaary-Rovira, 2004). Coccidiosis also causes increased numbers of lymphocytes, monocytes, eosinophils and heterophils (Irizaary-Rovira, 2004) and Wakenell (2010). In primary infections, the number of peripheral blood lymphocytes increase and these changes were found in the count of polymorphonuclear cells, lymphocytes and large mononuclear cells. Similar findings were also mentioned by Ricklefs and Sheldon (2007), who found the high counts of lymphocytes, heterophils and eosinophils in parasitic (malaria and haemosporidin) infected birds.

The increase in the lymphocyte is usually attributed to the effect of the inflammation of the caeca and intestine. Chronic antigenic stimulation may result in a greatly expanded circulating lymphocyte pool because the primary functions of the lymphocytes are

immunological response, humoral antibody formation and cell mediated immunity Irizaary-Rovira (2004). Eosinophilia in birds rarely occurs but may be associated with parasitism (mites, intestinal parasites, parasites with tissue migration) according to Irizaary-Rovira (2004). Acute or chronic inflammatory disease is the predominant cause of monocytosis or heterophilia in birds (Irizaary Rovira, 2004).

2.9 Serum Enzymes and Electrolyte Changes in Coccidiosis

Coccidiosis-related malabsorption was reviewed by Ruff (1986). Infections of the duodenum, jejunum, ileum or caecum cause reduced absorption of nutritional elements by the intestinal epithelium, Ruff (1986). A decrease in plasma enzyme levels is much less frequently used for clinical interpretation. However, there are a few specific cases where low plasma enzyme levels will indicate that the relevant organ is hyperplastic, atrophied or destroyed (Kerr, 2002). ALT and AST are the enzymes found in erythrocytes; therefore, the decrease in the activities of serum ALT and AST may be associated with the high reduction of erythrocytes because of the loss of blood into the gastrointestinal tract. Unfortunately, ALP, a serum enzyme associated with bile ductular epithelium and cholestasis in mammals, is not specifically associated with biliary epithelium in birds (Irizaary-Rovira, 2004). Alkaline phosphatase is found particularly in bone (osteoplasts), liver and the intestinal wall, with high levels being found in young animals with high osteoblastic activity (Kerr, 2002). Markedly increased plasma activities are particularly associated with parasite-induced damage to the intestinal wall in horses, though an elevation of this enzyme activity is normal in young birds (Kerr, 2002). Increased serum activities of ALP might be associated with the metabolic alteration and damage of the bone marrow as compensation for the blood losses; the bone marrow might be forced to produce excessive blood cellular components. Though

there is paucity of information on the effects of vaccination against coccidiosis and the electrolyte values, however, Parmer *et al.* (1987) reported lower serum calcium concentration and its effects on the birds' performance which resulted in lower weight at slaughter (Runho *et al.,* 2001). They found that birds with lower serum phosphorus concentrations presented hypocalcemia, hypophosphatemia and lower weight.

2.10 Immune Response to *Eimeria*

The life cycle of *Eimeria* parasites comprises both intracellular and extracellular stages in their infection of the host. The humoral component of the immune system plays a role which appears to be very important during the early stage of the infection. Specific antibodies for coccidial parasite in circulation can be detected within one-week post inoculation with oocysts. (Lillehoj, 1991). Host factors such as genetics composition of the host, age and the species of *Eimeria* involved affects thelevel and duration of antibody response. (Lillehoj, 1988). Though, (Giambrone *et al*., 1981; Lillehoj, 1987) showed in their studies on bursectomy or transfer of protection by using *Eimeria* immune antibodies, show that antibodies are not involved in host resistance to challenge by coccidial infection. However, according to (Wakelin and Rose, 1990; Lillehoj and Trout, 1993; Ovington *et al*., 1995). Whose studies showed extensive evidence those cell - mediated immune responses is essential in limiting oocyst production in primary and subsequent infections. Usually, different *Eimeria* species antigens vary in their immunogenecity, (Ovington *et al*., 1995). The antigenic determinants of the different species are not cross-reactive (Wallach *et al*., 1990). Thus Bumstead and Millard, (1992) showed that chickens from lines which produced the largest numbers of oocysts following infection with *E.tenella* produced the smallest number when infected with E *maxima*, E. *mitis*, or E. praecox. Even within *Eimeria* species, immunogenecity also

varies between stages of development, the asexual stage being more immunogenic than the sexual stage.

Even though coccidial merozoite antigens that induced T-cell proliferation were associated with resistance to infection (Jenkins *et al*., 1998), merozoite fractions that induced lymphocyte proliferation were different from those that induced interferon (IFN-() production, indicating that different antigens favor different cellular responses (Martin *et al.*, 1995). Previous studies have shown that interferons (IFN) play a role in resistance to primary infection that may be population dependent (Ovington *et al*., 1995). Now *Eimeria* reside outside the host for part of their life cycle but, the greater part of it is completed inside the host during asexual and sexual stages of development that occurrs outside or inside enteric tissues. Upon ingestion of viable oocyst(s), by birbds, a series of events occur involving specific and non-specific defense mechanisms of immunity (Lillehoj and Lillehoj, 2000). Even though series of research has been completed on immunity to *Eimeria*, there is still no clear picture as to how complete resistance is acquired and which mechanisms are sequentially involved in generation of immunity (Rose, 1979). In chickens that are previously unexposed to *Eimeria*, a variety of pathological and immunological responses are induced in such infection, this helps the host defend against the parasite and acquire protective immunity against future exposure. However, the level of protection each facade of the immune system provides varies with the stage of the parasite. Initially, before the generation of a specific immune response, the host tries to exclude the *Eimeria* through non-specific immune pathways such as competitive exclusion by normal flora, lysozymes, increased gastric secretions, and peristalsis to quickly flush parasites from the digestive tract (Lillehoj and Lillehoj, 2000; Yun *et al*., 2000). However, it has been reported that innate defenses

as well as specific immunologically mediated defenses play a vital role in the intestinal mucosal surface during *Eimeria* invasion (Lillehoj and Trout, 1993). So, the naive host probably does not eliminate the parasite utilizing only nonspecific pathways, but infection can be controlled to a certain degree prior to the completion of the *Eimeria* life cycle and generation of a specific immune response (Lillehoj and Trout, 1993).

Studies have shown that *Eimeria* parasites are vulnerable to the host immune system at three distinct phases of their development: A (the period between excystation and sporozoite penetration of epithelium,) B (once the sporozoite enters the host epithelium and is exposed to intraepithelial lymphocytes, and) C (during transport of the sporozoite from the surface enterocyte, through the lamina propria and into the crypt epithelium. After these stages in the life cycle, direct interaction between the parasite and cells of the host immune response are unlikely, and the probability of intervention is ceased (Jeurissen *et al*., 1996). Nevertheless, protective immunity hinders fecal oocyst production, and invasion of the mucosal epithelium is altered. Interestingly, in immune birds, sporozoites penetrate the villus epithelium but are incapable of reaching the crypt epithelium and prevented from further development (Jeurissen *et al*., 1996).

2.11 Gross and Histopathological changes in Coccidiosis

The crucial role that histopathology plays in diagnosis of avian pathology is undeniable, though histopathology of chicken intestine is an outdated method, it is still one of the test performed in the first instance that helps to indicate more specific diagnostic techniques to obtain a final diagnosis (Dolka *et al*., 2012).Histopathological lesions in case of caecal form revealed loss of epithelial tissue, congestion of blood vessels which indicated disruption followed by leakage of blood, severe muscular oedema, necrosis of

sub-mucosa, loss of villi, disruption of caecal mucosa, cluster of oocysts and marked haemorrhage, necrosis of caecal mucosa and lymphoid cells showing hyperplasia. In case of intestinal forms, lesions were found in the form of complete detachment of the mucosal layer from sub-mucosal layer accompanied with the accumulation of cell debris in the intestinal lumen, and the lymphoid cells showed hyperplasia.

2.12 Prevention and Control of Coccidiosis

According to David (2007), management of coccidiosis requires the adoption of sanitary and hygienic procedures to reduce the level of exposure to infection. Sporulation of oocysts is favored by moisture; therefore, it is important to maintain dry litter, especially around drinkers and feeding areas. As dry litter will reduce oocyst numbers and the possibility that birds could be exposed to a reasonable numbers of oocysts that can cause clinical coccidiosis. It has been shown (Blake *et al*., 2015) that different strains are capable of having antigenic diversity; therefore, immunity conferred by one species strain does not totally protect chickens against further challenge with a different strain of the same species. Talebi and Mulcahy, 1995, stated that *Eimeria* isolates from different geographical areas do not always show cross-protection following oral immunization. This may be a challenging factor in producing specific vaccines for coccidial control (Talebi and Mulcahy, 1995).

2.12.1 Immunoprophylaxis

A coccidian free environment is not likely achieved, most chicks that become infected usually develops acquired immunity or give way to diseases if the balance is in favour of the parasite (Chookyinox *et al*., 2009). The basic prophylactic method is to make it impossible for the coccidia oocysts to develop and spread. This may be obtained by

maintaining proper hygiene conditions on the farm and at the same time decreasing the oocysts' survival rate and their possibility to transform into invasive forms (Albert, 2013). Immunity to coccidiosis in chickens is *Eimeria* specie specific (Urquhart *et al*., 1996; David, 2000). Birds that survive severe coccidiosis will on no account be productive, while survivors of one strain of *Eimeria* may not necessary protect against being infected with another different strain, hence, the need for further treatment (Chookyonix *et al*., 2009). Resistance to the diseases more often than not increases as birds grow older (Chapman, 1997; Uza *et al*., 2001). David (2000) reported maternal immunity against coccidiosis occur at the height of serum antibody production, regrettably serum antibodies have been found to be very essential for prevention against coccidiosis.

The second extremely significant element of coccidiosis prophylaxis is the effective disinfection of farm buildings before placing the birds there. Dedicated products are available on the market but the preparation should be particularly considered. Coccidiosis chemoprophylaxis is almost exclusively related to the addition of chemical or ionophoric coccidiostat drugs to chicken feed. However, too long administration of these substances limits their therapeutic capabilities since the local resistance to the most common coccidiostat drugs is more and more often diagnosed. There are ways to limit the resistance by introducing a rotation program. It is based on the changes of coccidiostat drug from one group (chemical or ionophoric one) into another one, from other groups. What is more, one and the same product should not be administered too long, and upon the end of its readiness to be fed, a long enough break should be applied. Another possibility is vaccinating production flock hens. Live vaccines containing attenuated and non-attenuated strains or subunit vaccines are used. The vaccines may be

administered in the breeding unit (large drop method) or on the farm (with drinking water or in the feed spray). It is also possible to administer the vaccine to the eye on the 18th day of incubation. In case of prophylactic vaccination, infeed coccidiostat drug should not be administered, and the vaccinated birds must be in a good condition and have appropriate environmental conditions (Albert, 2013).

2.12.2 Anticoccidial vaccines

Several approaches to the prevention of coccidiosis involve immunization with live, potentially virulent parasites (Chapman, 2000). Although management of poultry droppings is important to reduce infection levels because live vaccines can cause the disease they are intended to prevent. Attenuated vaccines i.e. with reduced pathogenicity have been developed; however, they are expensive to produce. The first coccidiosis vaccine was introduced in the 1950s although were employed throughout the rearing phase of broiler breeder production, its use in broiler flocks has been limited. Recently, there has been an increased in the use of vaccines and several new products have emerged, with more expected in the near future. With the advent of spray techniques, vaccination in the hatchery has enhanced the efficacy of coccidiosis vaccines. (Chapman *et al*., 2005).

2.12.3 Live*Eimeria*vaccines

Immunological control of coccidiosis an alternative method to prophylactic medication in the prevention of the disease. The use of live vaccine against coccidiosis in large commercial chicken operations is dependent upon three main features of the disease (McDonald and Shirley, 2009). First of all, *Eimeria* species infection are self-limiting in that a distinct number of asexual cycles limits infectivity and only large numbers of

cycling oocysts can produce severe state of infection (McDonald and Shirley, 2009). On the second hand, age of chicks, very young chicks are less susceptible to infection therefore, as soon as they are hatched, the birds can be inoculated and development of immunity commences with less risk of severe pathogenesis (McDonald and Shirley, 2009). Lastly, acquired immunity can be generated for a homologous *Eimeria* species, similar to that elicited by a trickle infection, with limited-to-no pathogenesis (McDonald and Shirley, 2009). Correct vaccination might confer long-term protective immunity by three to four weeks of age which can persist until broiler slaughter or layer placement (Long and Millard, 1979). Live vaccine immunization against coccidiosis is a long-term solution that can be used on a wide commercial scale and is not restricted by drug-resistance. Though there is the risk of the emergence of antigenically distinct strains of *Eimeria* species that may not be controlled by the vaccine strains of these parasites, hence the need to modify wild type oocysts by incorporating any immunological variants into efficient vaccine formulations. Below is list of live vaccine present in commercial market (Vermeulen *et al.*, 2001; Williams, 2002; Anonymous, 2011, Shivaramaiah *et al.*, 2014).

2.12.3.1*Advantages of live Eimeria vaccines*

The use of coccidiostats in the prevention of coccidiosis may result in deposit of residues in poultry products that is detrimental to humans when consumed (Albert, 2013). Therefore, live *Eimeria* vaccines are used as an alternative method. Several studies have shown that an *Eimeria* vaccine provides a high level of immunity against coccidiosis in broilers. According to the findings of (Jenkins *et al.*, 2012), vaccine administration through a gel at 1 day of age proved to ensure a synchronous exposure and uniform protection among the broiler birds. Live vaccines administration also

assists in the replacement of drug resistant strains of coccidia in the broiler house with drug-susceptible vaccine strains, hence, increases the effectiveness of anticoccidial drugs that are used in rotation programs with vaccines (Williams, 1998). More so, strong immunity is build up with the use of live vaccines against future exposure and challenge by coccidian, particularly with the use of attenuated vaccines (Williams, 1994).

2.12.3 2Disadvantages of live Eimeria Vaccines

One of the major challenges with the use of live vaccine is the oocysts of *Eimeria* spp. that are included in live vaccines have a known tendency of losing their infectivity to the host with prolonged storage periods, hence compromising their efficacy in inducing an acquired immunity in the birds (Jeston *etal*., 2002). Also, the cost of producing live vaccine oocyst is huge, given that their propagation is constrained to live chickens, and no cell line is yet available for their multiplication. However, the antigenicity of the coccidial strains can vary according to geographical locations. Hence, it is imperative to characterize at the molecular level the local populations of the coccidial parasite, and to determine the ability of the available live vaccines to protect against the prevalent field strains (Danforth, 1998). Furthermore, there is a risk of introducing cocktail of *Eimeria* species present in the live vaccines into the farm surroundings. Hence requires monitoring of *Eimeria* spp. vaccines that contain oocysts of the species that are matching in its antigenicity to the ones that are prevalent in a certain ecological area. Also, the risk of economic losses seen in immune-compromised chickens (Anderson, 1977).

Table 2.1; Live attenuated and non-attenuated live *eimeria* vaccines developed for commercial broilers, layers and breeders (Vermeulen *et al.*, 2001).

Trade name	Bird type	Species	Attenuated	Route of administration	Age of chicks / Dose
Coccivac®-BA, B, C	Heavy broilers	*E. acervulina, E. maxima, E. mivati, E. tenella*	No	Hatchery spray, ocular, drinking water, feed spray	Single dose at 1 to 14 days
Coccivac®-DA, B, C	Breeders/ layers	*E. acervulina, E. brunetti, E. hagani, E. maxima, E. mivati, E. necatrix, E. praecox, E. tenella*	No	Hatchery spray, ocular, drinking water, feed spray	Single dose at 1 to 14 days
Coccivac®-D2A, B, C	Breeders/ Layers	*E. tenella, E. brunetti, E. maxima, E. mivati, E. necatrix,*	No	Hatchery spray, ocular, drinking water, feed spray	Single dose at 1 to 14 days
Immucox® C1A, B, C	Broilers, roasters	*E. acervulina, E. maxima, E. necatrix, E. tenella*	No	Drinking water, oral gel	Single dose 1 to 4 days

Immucox® C2A, B, C	Breeders, layers	*E. acervulina, E. brunetti, E. maxima, E. necatrix, E. tenella, E. mivati, E. praecox*	No	Drinking water, oral gel	Single dose 1 to 4 days
Inovocox®B	Broilers	*E. tenella, E. acervulina,* two strains of *E. maxima*	No	*in ovo*	Single dose *in ovo*
Inovocox® EM1B	Broilers	*E. tenella, E. acervulina,* one strain of *E. maxima*	No	*in ovo*	Single dose *in ovo*
Advent®B	Broilers	*E. acervulina, E. maxima, E. tenella*	No	Hatchery spray, feed spray	Single dose at day of hatch (for hatchery spray) or on first feed (for feed spray)
Livacox ® TC	Broilers, breeders	*E. acervulina, E. maxima, E. tenella*	Yes	Drinking water, ocular	Single dose at 1 to 10 days
Livacox® QC	Broilers	*E. acervulina, E. brunetti, E. maxima, E. tenella*	Yes	Drinking water, ocular	Single dose at 1 to 10 days

Paracox®C	Broilers, breeders, layers	*E. acervulina, E. brunetti,* two antigenically different strains of *E. maxima, E. mitis, E. necatrix, E. praecox, E. tenella*	Yes	Drinking water, feed spray	Single dose at 1 to 9 days
Paracox®-5C	Broilers	*E. acervulina, E. maxima* (two antigenically different strains), *E. mitis, E. tenella*	Yes	Hatchery spray, drinking water, feed spray	Single dose at 1 or 3 days
Hatchpak® Cocci IIIB	Broilers	*E. acervulina, E. tenella, E. maxima* and a live culture	Yes	Hatchery spray	Single dose at 1 day old
Hipracox®C	Broilers	*E. acervulina, E. maxima, E. mitis,*	Yes	Hatchery spray	Single dose at 1 day old

The improper administration of the live *Eimeria* spp. vaccines may result in asynchronous exposure to the birds, with significant variation in the number of ingested oocysts, resulting in unacceptable homogeneity of their immunities, and a likelihood of the disease outbreak in birds with low immune responses to protecting antigens of *Eimeria* (Chapman *et al*., 2002).

2.12.4 Subunit and recombinant *Eimeria* vaccines

Subunit vaccines, these are an engineered protein that is derived from the virulent species of *Eimeria*parasite (Ziomco *et al*., 2005). Different stages of the life cycle *Eimeria* spp have specific antigens that are capable of inducing protective immunity, an essential step in development of subunit vaccines (Ziomco *et al*., 2005). Recombinant proteins found in both the protozoan surface and internal antigens were recognized as candidates for such vaccines. Surfaceproteins focus mainly on the invasive stages of *Eimeria* parasite, that is merozoites, sporozoites and gametocytes (Ziomco *et al*., 2005).These recombinant proteins can induce a satisfactorylevel of cell mediated and humoral immunities. This is seen in one commercial product, CoxAbic®, recognized by Wallach (1997). CoxAbic® is an oil emulsion vaccine inducing maternally derived antibodies aimed at protecting the broiler progeny (Ziomko *et al*., 2005). This is the only available recombinant commercial coccidiosis vaccine that transmits passive immunity through the yolk of hatching eggs to the progeny (Ziomco *et al*., 2005). The acquired immunity in the chicken breeders is developed against purified gametocyte antigens 230kDa, 82KDa, and 56KDa of *E. maxima* that are present in this recombinant vaccine (Wallach, 1997). The immunization of the breeders by gametocyte antigens mentioned above is proven to protect their offspring from challenges with *E. tenella*, *E. acervulina* and *E. maxima*, reducing oocyst output by 60-

80%, when compared to similar challenged to offspring produced by breeders that were deprived of subunit vaccination (Wallach, 1997). However, pertinent limiting factor in the development of recombinant vaccines for protection against coccidiosis is paucity of information related to the nature of antigens responsible for the effective protective immunity against field strains, hence, the need for further study on the field *Eimeria* strains and its host interaction, at the cellular and molecular level, with the hope of the discovery of appropriate recombinant vaccines that can protect against the diversified prevalent field strains. The *E. tenella* genome project may be helpful in identifying the protective antigens in this parasite and their interaction with the host immune system (Shirley *et al*., 2005).

2.12.5 DNA vaccines against coccidiosis

Dna vaccines, one of the recent studied as a novel approach to elicit protection against Coccidiosis, it is capable of stimulating cellular immune response (Shah *et al*., 2011; Christian *et al*., 2007). Recently, immunological research of vaccines is aimed at achieving this goal. Natural modulators of the immune system such as the cytokines are being used as vaccine adjuvant to enhance host immune responses induced by DNA vaccines in Coccidiosis (Kim *et al*., 1999). Studies have shown that gamma interferon (chIFN gamma) is a strong adjuvant candidate for the general enhancement of immune response induced by vaccine antigen and modulation of the immune response generated (Kim *et al*., 1999). It can induce a microbicidal state that is involved in the resolution of the infection by intracellular parasites, thus enhancing vaccine efficacy (Takehara *et al*., 2003).

2.12.5.1*DNA vaccination, the fundamental principle*

DNA vaccines, produced using plasmids, include a gene encoding target antigen under the transcriptional control of an effective viral/eukaryotic promoter, along with a polyadenylation signal sequence (poly-A) and a bacterial origin of replication (Gurunathan *et al*., 2000). The poly-A provides stability and effective translation; and the antibiotic resistance gene facilitates selection of bacteria (Gurunathan *et al*., 2000). For complete optimization of DNA vaccine, the plasmid should have a Kozak sequence (GCCA/GCC) upstream of initiator codon and an enhancer, downstream of the poly-A signal (Gurunathan *et al*., 2000). But, to elicit an effective immune response, the protein ought to undergo post translational modification and retain their tertiary structure (Gurunathan et *al*., 2000). After *in vivo* generation, the antigenic peptides are processed and presented by professional antigen presenting cells (APCs), like dendritic cells, by getting primed through direct or by obtaining proteins from myocytes (Gurunathan *et al*., 2000).

2.12.5.2 *DNA Vaccination with reference to Eimeria*

Immunity against *Eimeria* depends on the cellular arm of the immune response including CD4+ and CD8+ T cells with antibodies playing only a minor role in protection (Yun *et al.,* 2000). Dna vaccination has been broadly studied as a novel approach to elicit protection against Coccidiosis for its ability to stimulate cellular immune response (Ding *et al.*, 2005; Shah *et al.*, 2011). The administration of a simple plasmid can induce a broad spectrum of immune responses against *Eimeria* parasites (Shah *et al.,* 2011). Vaccination of Coccidiosis with DNA is one promising novel immunization techniques against *Eimeria* pathogens. A lot of useful efforts have been put forward to identify methods of enhancing the immune

response of plasmid DNA, to enable its practical implementation. The most significant has been the development of vaccines which can elicit both Humoral and cellular immune responses. The use of different types of immune modulators, cytokines and co-stimulatory molecules in DNA coccidiosis vaccination reveal a positive approach to enhancing the immunity level against *Eimeria* (Shah *et al.*, 2011). If the potency is improved, plasmid DNA vaccines, having numerous advantages, can be useful for the active immunization against coccidiosis. Considering the present status and many other possibilities, efforts should be targeted towards improving their delivery or to increase their immunogenic potential. Poor cellular uptake and rapid *in vivo* degradation of plasmid DNA has to be taken into account and novel delivery systems has to be developed along with the optimization of the plasmid vector. Some of the recently studied *Eimeria* DNA vaccines are tabulated in Table 2.2

Table 2.2 Recently studied DNA vaccines

DNA vaccine	*S*pecies studied	Reference
pVAX1-cSZ-2-IL-2	*Eimeria tenella*	
	Eimeria necatrix	
	Eimeria maxima	
	Eimeria acervulina	Shah *et al*., 2011, Shah *et al* 2010.
pcDNA-TA4-IL-2	*Eimeria tenella*	
	Eimeria necatrix	
	Eimeria maxima	
	Eimeria acervulina	Song *et al.*, 2009, Xu Q *et al.*, 2008.
pcDNA3.1-Et1A-TA4	*Eimeria tenella*	Wu *et al*., 2004.
3-1E cDNA-pBK-CMV	*Eimeria acervulina*	Song *et al*., 2000.
pcDNA3.1-3-1E-ChIL-15	*Eimeria acervulina*	Ma *et al*., 2011.

2.12.5.3Factors affecting success of DNA vaccination

Several factors affect successes of DNA immunization, these includes: structure of the plasmid backbone, quantity of plasmid delivered, route and times of immunization, age of animals and so on (Leiner *et al*., 2000). Several routes have been used for DNA vaccine delivery, intravenous, intranasal intramuscular, intradermal, and epidermal (Fynan *et al*., 1995). DNA Studies in mammalian systems clearly recognized that route and dose of DNA immunization influenced the types of immunity elicited. In general, intramuscular and intradermal inoculations induced T helper (Th1) responses while epidermal injection elicited T helper 2 (Th2) responses (Robinson and Torres, (1997), (Donnelly *et al*., 1997). Xu *et al*. (2008) studied some of these factors which are known to influence the efficacy of immunization, for instance, the routes, doses, and time. These factors such as immunization dose, immunization route, primary immunization age, immunization time, affected the magnitude and type of immune response induced by plasmid DNA, of which of all these factors, for any chosen route of delivery, immunization dose was the most important. This is as reported by (Wu, *et al*., 2004, Lillehoj *et al*., 2005) that chickens of 3 days old immunized with 50 μg of pcDNA3.1-Et1A-TA4 intramuscularly showed the highest ACI in different doses immunized chickens with *E. acervulina* gene 3-1E.

The anti-3-1E antibody titers were enhanced in a dose dependent manner and decreased the number of oocysts and increased the weight gain at relatively high-dose (Lillehoj *et al*., 2005). More plasmid correlated with stronger response, but not necessarily linearly. Chickens immunized intramuscularly with a range of doses of pMP13 expression vector

ranging from 5 μg, to 100 μg, showed that both 5 μg and 50 μg DNA group presented more effective in reducing the oocyst production after challenge with *E. acervulina*. However, the highest dose group of 100 μg was the worst one. Immunization route plays an important role in the induction of protective immune responses to DNA immunization. Intravenous, intramuscular and mucosal administration of DNA provided some protective immunity against a lethal challenge with avian influenza virus (Fynan *et al*., 1995). Studies have shown that intramuscular injection provides higher levels of serum antibody as compared to subcutaneous injection in chickens immunized with the 3-1E gene of *E. acervulina* (Fynan *et al*., 1995). Also, the primary immunization age and immunization times are significant in DNA immunization of coccidiosis. Immunization at an early age has obvious effect on the efficacy of intramuscular injection, because the immature muscle cells of young animals are beneficial to be transformed (Fynan *et al*., 1995).

2.12.6 Use of natural products in controlling coccidiosis

This is an alternative approach that involves the use of natural products such as varied collection of plant, bacterial and other substances claimed to alleviate coccidiosis either directly or indirectly by improving "gut health" or the chicken immune system (Chapman, 2005). This advance would be useful in organic poultry production where use of chemical agents and antibiotic growth promoters is restricted. Allen *et al*. (1998) investigated ways to boost the innate resistance of poultry to *Eimeria* infection by identifying natural substances capable of modifying the intestinal micro environment. *Pediococcus*, *Saccharomyces* and *Lactobacillus* based probiotics have been used to enhance the local cell mediated immune response in poultry. A variety of substances, as well as many plant based extracts, such as menhaden oil, flaxseed oil, artemisinin, betaine, and pro anthocyanidin,

have been claimed to have such an effect because of their antioxidant properties (Chapman, 2005). However, studies intended to demonstrate the efficacy of natural products are not well designed, lack suitable control, and have insufficient reproduction (Chapman, 2005). Hence, Holdsworth *et al.* (2004) provided guidelines to aid investigators in the design, implementation, and interpretation of studies for the assessment of product efficacy. As for vaccines there is a need for floor-pen experiments as well as field trials. Frequently inclusion levels are unrealistically high and therefore would be difficult to accommodate in commercial feed formulations. Therefore, efficacies are often based upon marginal reductions in oocyst count and lesion scores. Such claims would not be acceptable if an anticoccidial drug were under investigation (Chapman, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This research was carried out in the Department of Veterinary Parasitology and Entomology Ahmadu Bello University, Zaria (Latitude 11°10′N and Longitude 7°38′E). Located in the Northern Guinea Savannah zone of Nigeria (Anonymous, 2013). It has an annual ambient temperature that ranges between 18.0 ± 3.7℃ and 31.8 ± 3.2℃. The cold dry period of the year (harmattan season) occurs from November to February, whereas the hot-dry season lasts from March to May and the rainy season from June to October (Anonymous, 2013).

3.2 Experimental Chickens

Eighty day-old broiler chicks of Mashal breed were purchased from from Elim poultry, Kwangila, Zaria and transported in clean brooder cages to Animal Research Unit of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The chicks were brooded in a brooder pen for two weeks and fed on coccidiostat-free commercial broiler starter feed as well as providing clean water. The faeces of the chicks were screened daily to ascertain they are free from *Eimeria* infection prior to vaccination and experimental challenge with the *Eimeria* parasites.

3.3 The *Eimeria tenella* Isolate

The *Eimeria tenella* isolate used in this study was obtained from the National Veterinary Research Institute (NVRI), Vom, Jos, Platue State Nigeria. *E. tenella* Houghton strain was

obtained from the Parasite Laboratory of the Department of Pathology and Pathogen Biology of the Royal Veterinary College, United Kingdom. Sporulated oocysts of each strain (Local and Houghton) were propagated *in vivo* by oral inoculation of 1, 000 sporulated oocysts to five 2-weeks old coccidia free broiler chicks. Progeny oocysts were recovered from caecal tissue and contents collected during post-mortem, 7 days' post infection, sporulated and purified as described by Shirley (1995) as follows:

The harvested caeca were carefully cut open longitudinally and placed on a glass plate to minimise the likelihood of losing any material. By holding the edge of each caecum a glass microscope slide was used to carefully scrape the caecal contents, plus the mucosal and the deeper muscle layers, away from the remaining tissue and placed into a beaker containing phosphate buffered saline (PBS). The scrapings were homogenized using a glass rod in the beaker. 1.5% (w/v) trypsin was added to the homogenate (to break down any connective tissue) and incubated in a water bath at 41^{o}C for between 30 to 90 minutes. The beaker was swirled occasionally during the course of incubation. The trypsin treated homogenate was strained through a double layer of muslin into a centrifuge tubes and centrifuged at ~750 × *g* for 12 minutes. The supernatant was poured off and replaced with distilled water and centrifuged at ~750 × *g* for 12 minutes. The washing was repeated three times using distilled water. The sediment containing the oocysts was transferred into Petri-dishes, mixed with 2.5% (w/v) potassium dichromate solution and allowed to sporulate at room temperature for 7 days. After sporulation the potassium dichromate solution was washed off by centrifugation at ~750 × *g* for 12 min three times with distilled water. The washed sporulated oocysts were then transferred into a clean 20 ml screw capped tubes; fresh potassium dichromate solution was added to the tubes, stored at 4^{0}C until when needed.

3.4 Sources of the *Eimeria* Vaccines

The DNA vaccine used in this study was a DNA vaccine construct of *E. tenella* AMA-1 developed from a laboratory strain (Houghton strain) in the Department of Pathology and Pathogen Biology of the Royal Veterinary College, UK. Live oocyst vaccine (Livacox^{R}) a polyvalent vaccine against *E. tenella, E. maxima, E. necatrix and E. acervulina* was produced by Research Institute of Biopharmacy and Veterinary drugs, Czech Republic was purchased from a Pierrodex, a reputable veterinary product distributor in Jos, Pleatue State Nigeria.

3.5 DNA Vaccine Constructs Extraction

3.5.1 Culturing of *E. coli* transformed with *Et*AMA-1 DNA

The AMA-1 construct was extracted from the transformed *E. coli* according to the methods described by Robert (2008) as follows: In other to obtain sufficient transformed *E. coli* cells containing the construct, the isolate was suspended on brain hearth infusion broth, incubated at 37℃ for 24 hours. It was then inoculated on eosin methylene blue agar incorporated with ampicillin supplement and incubated at 37℃ for 24 hours. The colonies were identified morphologically by the presence of colonies that are small and pink on EMB agar with a green metallic sheen, it was then followed by a biochemical test for confirmation (Mohammed *et al*. 2014). Similarly, control *E. coli* without DNA vaccine was cultured according to the methods described above for the purpose of harvesting the plasmids that served as vector control.

LB medium was used to grow the respective *E. coli* for plasmids extraction as described by He (2011) as follows: 1.5 g of peptone water, 0.5 g of sodium chloride and 0.5 g of yeast extract were dissolved in 100 ml of distilled water; 5 ml of Lb. medium was dispensed into a universal bottle. Inoculum of respective *E. coli* colony containing the plasmids was placed into the LB medium and incubated for 24 hours.

3.5.2 Plasmids extraction

Plamids extraction was performed according to the method of Alkaline Lysis as described by He (2011). As follows;

1 ml of the respective *E. coli* broth was put into 1.5 Eppendorf tube, centrifuged at 10,000 *g* for 1 minute, supernatant was discarded and 250 µl of resuspension solution was added and was then vortexed to completely dissolve. 10 µl of RNAS was added to resuspension medium, 250 µl of lysis buffered was added and thoroughly mixed by inverting the tube 6 times. 350 µl of the neutralization solution was added and thoroughly mixed by inverting the tube 6 times; it was then centrifuged at 10,000 *g* for 5 minutes. The supernatant was transferred to Gene JET spin column where it was centrifuged for 1 minute. Flow through discarded column was placed back into the same collection tube and 500 µl of washed buffer was added. It was then centrifuge at 10,000 *g* for 1minute. The column was placed back to the collection tube, flow-through discarded. The spin column was transferred to 1.5 ml micro centrifuge tube and 50 µl of Elution buffer was added and allowed to stand at room temperature for 2 minutes. It was then centrifuged at 10,000 *g* for 2 minutes. The spin column was discarded and the resultant plasmids were quantified and stored in the freezer until when needed.

3.6 Experimental Grouping

At 14 days of age, the chickens were weighed and randomly distributed into 8 groups of 10 chicks each as follows:

Group 1 Non-vaccinated and non-challenged control group.

Group 2 Non-vaccinated and challenged with *E. tenella* Local isolate control group.

Group 3 Non-vaccinated and challenged with *E. tenella* Houghton strain control group.

Group 4 Vector (*E. coli* plamid DNA without EtAMA-1) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with Livacox® prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with Livacox® prior to challenge with Houghton strain of *E. tenella*.

3.7 Immunization and Challenge of Experimental Birds

Chickens groups 1 (unchallenged unvaccinated control group), 2, 3 (challenged control groups for Local isolate and Houghton strains respectively) and group 4 (vector control) were given 0.1 ml sterile PBS on thigh muscle as a placebo. Chickens in groups 5 and 6 (DNA Vaccine for Local and Houghton strains respectively) were vaccinated with 100 µg *Et*AMA-1 vaccine plasmid in phosphate buffered saline (PBS) PH (7.6) by thigh

intramuscular injection at day 14 and a booster dose at day 21 of age, respectively. Birds in groups 7 and 8 (Live oocysts vaccine for Local and Houghton strains respectively) were administered oral commercial live oocyst vaccine in chlorine free clean water according to the manufacturer's instruction. At 28 days of age, all chickens except the unchallenged control group were inoculated orally with 1×10^4 sporulated oocysts of the respective *E. tenella* isolates. A day prior to the experimental infection and seven days later, 2 mls of blood was collected from five birds from each group by cardiac puncture for haematological and serum biochemical analyses. Seven days after challenge, five chickens from each group were re-weighed and sacrificed for postmortem analysis and lesion scoring. Caecal samples were also collected for histopathology.

3.8 Evaluation of Efficacy of the Vaccines against the Experimental Challenge

Efficacy of protection by the vaccine was evaluated on the basis of the following parameters:

3.8.1 Survival rate

This was estimated by the number of surviving chickens divided by the number of initial chickens per group at the time of challenge (Shah *et al*., 2011).

3.8.2 Body weight gain

This was determined by subtracting the body weight at the time of challenge in each group from the body weight of the chickens at the end of the experiments day 7 post challenged (Shah *et al*., 2011).

3.8.3Lesion score

Caecal lesion score of the chickens from each group were determined according to the method of (Johnson and Reid, 1970). Briefly each score 0–4 covers a range of gross lesions

which are species specific. Generally, a score of 0 = gross lesions absent; 1 = a few scattered petechiae are found on the caecal wall. There is no thickening of the caecal walls or blood present. Caecal contents appear normal; 2 = Lesions more numerous with noticeable blood in the caecal contents; caecal wall is somewhat thickened, normal caecal contents present.; 3 = Large amounts of blood or caecal cores present; caecal walls greatly thickened; little if any fecal contents in the caeca and 4 = Caecal wall greatly distended with blood or large caseous cores; fecal debris lacking or included in cores.

3.8.4 Oocyst decrease ratio

2 g of caecal content for each sacrificed bird in each group were collected separately by opening the caecum longitudinally placed on a clean petridish and carefully scraped the content using scapel blade into the pre-weighed beaker until about 2 g of the content was obtained. Oocyst per gram of content (OPG) was determined using the McMaster's counting technique (Subramanian *et al*., 2008). Oocyst decrease ratio was calculated as the (mean number of oocysts from (positive control chickens) – (vaccinated chickens)/ (positive control chickens) × 100% (Shah *et al*., 2011).

3.8.5 Anti-coccidial index (ACI)

The anti-coccidial index is a criterion for assessing the protective effect of a medicine or vaccine against coccidiosis was calculated as follows: (Relative weight gain (%) + % Survival rate) − (Mean lesion value + Mean oocyst value). Anti coccidial index usually ranges from zero to 200 Graham *et al.* (1978). Anti coccidial index numbers above 175 are indicative of good anticoccidial efficacy.

3.9 Histopathology Examination of the Tissue Samples

Histopathological studies of the caeca tissues were carried out in the Histopathology Laboratory of, Faculty of Veterinary Medicine, ABU, Zaria according to the method described by*Mitchell et al. (2009)* as follows; tissue samples (Section of caeca) from the sacrificed chickens were fixed in buffered neutral 10% formalin for at least 48 hours. The fixed tissues were dehydrated in graded concentrations of alcohol (70%, 80%, 90%, 95% and 100%) at 1 hour intervals. The tissues were cleared with xylene for about 2 hours, infiltrated with molten paraffin wax at 50℃ to 60℃ for 2 hours, embedded in the molten paraffin wax and labeled appropriately. Sections 5µ thick were cut from the embedded tissues using a microtome knife attached to a microtome. The sectioned tissues were mounted on grease free, clean glass slides; they were then dried at room temperature and stained with Haematoxylin and Eosin (H & E) stain. The tissue sections on slides were studied using Olympus light microscope at x200 and x400 magnifications. Photomicrographs of the slides were taken using a digital camera, transferred to a computer and labeled appropriately.

3.10 Haematological Analysis

Two mililitre of blood collected from each bird by cardiac puncture was placed into a sample bottle containing EDTA transported to the Clinical Pathology Laboratory of the Department of Veterinary Pathology, Ahmadu Bello University Zaria, Nigeria for the determination of packed cell volume (PCV), using the microhaematocrit method (Sirois, 1995) and total white blood cell count (WBC) was determined using the method described by Samour (2001).

3.10.1 Determination of packed cell volume

Packed cell volume (%) was determined in the Clinical Pathology Laboratory of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. PCV (%) was determined using the microhaematocrit method (Sirois, 1995). Microhaematocrit capillary tubes were filled with blood by capillary action to about 75% of their length, and outside of the tubes were carefully dried with cotton wool after filling each of the test tubes with blood. The opposite ends were then sealed with flame from a Bunsen burner. The sealed tubes were then packed in a haematocrit centrifuge with their sealed ends placed near the outside rim of the centrifuge. Blood samples were centrifuged in heparinized capillary tubes for 5 minutes at 4,383 *xg* using the Saitexiangyi TG12MX® micro-haematocrit centrifuge machine. Then the proportion of cells in the total volume of blood was measured and recorded as a percentage using the Hawksley® microhaematocrit reader.

3.10.2 Determination of total white blood cell counts

Absolute WBCwas determined using the method described by Clark *et al.* (2009) as follows; Sample tubes were labeled using a permanent marker, 1.9 ml of 1% ammonium oxalate solution was dispensed into sample tubes using automatic dispenser, and was allowed to stay for 5 minutes to reach room temperature. 100 µl of whole blood was dispensed using micro pipette into the side of the sample tube. Sample tube was then placed on a roller mixer for 3 minutes, a cover slip was placed onto hemocytometer and slide gently over it, a filter paper was placed at the bottom of the petri dish and was covered and left for 5 minutes to settle down. Cells contained in the four center large squares of the

hemocytometer was counted for total white blood cell count using N/20 = WBC × 10^9/ L where N = number of cells counted in 64 n small squares.

3.10.3 Determination of differential cell counts

Differential leucocyte counts were determined using method described by Clark *et al.* (2009)**.** A thin blood smear was prepared, air dried, labeled and fixed in methanol for 3-5 minutes. Air dried and stained with Giemsa solution for 25-30 minutes. It was then rinsed with tap water and air dried; a drop of oil immersion was added on the slide, mounted under light microscope at x100 magnification for view of white blood cells including lymphocytes, heterophils, basophils, eosinophils, monocytes and bandcells.

3.10.4 Determination of haemoglobin concentration

Blood haemoglobin concentration was assayed colorimetrically as cyanomethhaemoglobin (Drabkin, 1945). Five millilitres of HICN (Drabkin) solution were measured using a 5 ml syringe into plastic test tubes. Twenty microlitres of blood was measured using a micropipette and then added to the Drabkin solution in the test tube and properly mixed by gently shaking the test tube. It was centrifuged at 1,509 xg for 15 minutes to separate the empty RBC from interfering with the reading. The supernatant was separated into a sample bottle. The mixture was absorbed into the haemoglobin meter (XF-C, China). After the wiggling pump stops working, the value displayed on the screen was recorded in g/dl as the haemoglobin concentration.

3.11 Serum Biochemistry

1ml of blood samples collected were placed in dry, clean tubes for serum extraction as follows; serum was separated carefully and the extracted serum was analysed for the following biochemical constituents: Total Protein, Albumin, and Globulins as well as the activities of these liver enzymes; Alanine amino transferase (ALT), Aspartate amino transferase (AST) and alkaline phosphatase (ALP) and some Electrolytes (sodium, potassium, chloride and bicarbonate) activities, as described below.

3.11.1 Determination of total plasma protein

Total plasma protein was determined using Laxco™ hand refractometer method as described by Rodica (2013).A drop of plasma is placed in the optical surfaces of hand refractometer, covered and viewed on the central calibrated scale; readings were expressed in g/dl.

3.11.2 Determination of serum albumin

Serum albumin was determined colorimetrically using bromocresol green dye as described by Alberto (1996).Three test tubes were arranged on a test tube rack, 4 ml of Bromocresol green (BCG) was added to all the test tubes, 0.02 ml of distilled water was added to one of the tubes as blank and 0.02 ml of serum was added to both test and standard tubes. The mixtures were mixed well and absorbence was read at 628 nm zeroing the instrument with the blank. Values of albumin was calculated as:

$$\text{Albumin (g/L)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard (Alberto, 1996).}$$

3.11.3 Determination of serum alkaline phosphatase

one ml of alkaline buffer was placed into a test tube. One ml of phenol phosphate substrate was added to the tube. The test tube was placed in a water bath (37℃) for 5 minutes and

thereafter; 0.1 ml of serum was added into the tube in an incubator. The solution was mixed and incubated at 37℃ for 30 minutes. After the tube was removed from the incubator, 0.8 ml of NaOH, 1.2 ml of $NaHCO_{3,}$ 1.0 ml of 4-amino antipyrine and 1.0 ml of potassium ferric cyanide were added to it while 1.2 ml of buffer was added to 1.0 ml of phenol and treated as test after incubation. Absorbance was read at 510 nm. Serum alkaline phosphatase activity was read as

$$\text{Serum ALP activity (µmol/min/Litre)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}}$$ (Alberto, 1996).

3.11.4 Determination of serum aspartate amino transferase

Serum AST was determined according to the methods described by Najih (1998).Three test tubes were arranged on a test tube rack labeled: Test (T), Test blank (TB), Standard (S) and Standard blank (SB) respectively. 0.5 ml buffered substrate was added to all the tubes and incubated for 5 minutes. Afterward, 0.1 ml of serum was added to the test (T), and then 0.1 ml of pyruvate was added to the standard (S). The solutions were mixed and incubated at 37℃ for 60 minutes, then 0.5 ml dinitrophenylhydrazine (DNPH) was added to all four test tubes while 0.1ml of serum was added to test blank (TB), the solutions were mixed and allowed to stand at room temperature for 20 minutes, then 5 ml of 0.4 NaOH and solutions were allowed to stand for further 5 minutes. Absorbance was read at 510 nm. Value of AST were read as

$$\text{Serum AST activity (µmol/min/Litre)}. = \frac{T - TB}{S - SB} \times \frac{\text{Concentration of standard}}{\text{Incubation time}} \times \frac{1000}{\text{volume of sample used}}$$

3.11.5 Determination of serum alanine amino transferase

The serum ALT was also determined using the methods described by Najih (1998).

The procedure used here was the same as that used for AST above except for:

the substrate used here was alanine α - ketoglutarate solution and the incubation time is 30 minutes and not 60 minutes as for AST. Value of ALT were read as

$$\text{Serum ALT activity } (\mu\text{mol/min/Litre}). = \frac{\text{T} - \text{TB} \times \text{Concentration of standard} \times 1,000}{\text{SSB}}$$

Incubation time volume of sample used.

3.11.6 Determination of serum electrolyte concnetration

The serum electrolyte was determined using Jiangsu® Automatic electrolyte analyser (Audicom) as follows. 0.5 mlof serum sample was put into a small rubber cup numbered 1-20. The rubber cups containing the serum was placed into a plate numbered 1-20, the audicom machine was switched on. Appropriate key was pressed and the readings of the various electrolyte values were displaced on the screen of the machine and noted in μmol/L.

3.12 Data Analyses

Data collected (Body weight, Oocyst count, Haematological and Biochemical parameters) were expressed as mean ± SD and statistically analyzed by one-way analysis of variance (ANOVA), complimented by post hoc analysis using the Tukey's HSD test. Statistical analyses were processed by the SPSS 13.0 Data Editor software (SPSS Inc., Chicago, IL). The differences between groups were considered to be significant at p values less than 0.05.

3.13 Ethical Statement

The research was approved by the Ahmadu Bello University Committee on Animal Use and Care with approval number ABUCAUC/2017/015.

CHAPTER FOUR

RESULTS

4.1 Effects of Vaccination on Clinical Signs

The clinical signs observed in all infected unvaccinated chickens (groups 2, 3, 4) were reduced activity, reduced feed intake and bloody diarrhea observed on day6. While signs exhibited by birds in all experimental and infected groups (5, 6, 7 and 8) were very mild and included reduce activity, reduced feed intake and mild diarrhea observed on day 6. Clinical signs were not observed in the unvaccinated uninfected control group (group 1).

4.2 Effects of Vaccination on Body Weight Gain

The mean body weight values of group1 (unvaccinated uninfected group) increased from the pre-infection (4 weeks of age) of 930 ± 39.91 g, to 1190 ± 40.0 g one week later (7 days' post infection) with a net weight gain of 260 ± 64.03 g. Similarly, the mean weight values of groups 2, 3 and 4 (unvaccinated infected and vector vaccinated infected groups) increased from pre infection (4 weeks of age) values of 870 ± 25.49 g, 860 ± 36.74 g and

900 ± 36.36 g respectively to 910 ± 24.49 g, 900 ± 35.36 g and 950 ± 36.36g respectively at 7 days post infection (5 weeks of age), (Table 4.1). The mean weight gains for the groups were 40 ± 12.25 g, 40±43.59 g and 50 ± 11.36 g for groups 2, 3 and 4 birds respectively (Table 4.1).

The mean weight values of groups 5, 6, 7 and 8 (vaccinated infected groups) increased from the pre infection values of 900 ± 35.36 g, 890 ± 33.17 g, 904 ± 16.31 g and 890 ± 29.15 g at 4weeks of age to 1010 ± 36.66 g, 1020 ± 47.37 g, 1020 ± 46.37 g and 1010 ± 50.99 g respectively 7 days post infection (5 weeks of age) with respective mean net weight gains of 110 ± 29.15 g, 130 ± 25.50 g, 116 ± 43.89 g and 120 ± 65.68 g (Table 4.1).

There was no statistical significant difference ($p > 0.005$) recorded in all groups for pre infection. However, there was a statistical significant difference ($p < 0.005$) recorded in mean body weights of group1compared to all other groups 2-8 for post infection (Table 4.1). Similarly, amongst the challenged groups, there was a statistical significant difference ($p < 0.005$) recorded in mean body weights of unvaccinated groups (2, 3 and 4) when compared to the vaccinated groups (5, 6, 7 and 8). Although, both vaccines were able to offer protection to the vaccinated birds in relative to the non-vaccinated groups, protection was more pronounced against the Houghton strain of the parasite for both vaccines when compared to the Local strain, though, not statistically significant.

4.3 Gross Lesions Scores of Caeca of Broilers Chickens of all Challenged Groups

The birds in group 2 (unvaccinated challenged with Local strain) had a mean lesion score of 4.0 ± 0.0 with complete ballooning and distension of the caecum packed with caeceous cores (Table 4.2).

Some of the caeca had brownish, watery and slimy contents with thickened caeca mucosa. The broilers in group 3 (unvaccinated challenged with Houghton strain) had mean lesion score of 3.4 ± 0.82 (Table 4.2). The caeca were moderately ballooned, there was presence of moderately thickened caecal mucosa and presence of caecal core. While broilers in group 4 (Vector vaccination challenged with Local strain) had a mean lesion score of 3.0 ± 0.6 (Table 4.2).

Table 4.1: Mean (± SEM) body weights (g) of broiler chicks vaccinated with DNA and Live oocyst vaccines against experimental challenge with local and Houghton strain of *E. tenella*.

Group	Pre Infection	Post Infection	Net weight increase	Relative weight gain
1	930 ± 39.91[a]	1190 ± 40.0[a]	260 ± 40.62[a]	100 %
2	870 ± 25.49[a]	910 ± 24.49[b]	40 ± 12.25[b]	15.4 %
3	860 ± 36.74[a]	900 ± 35.36[b]	40 ± 43.59[b]	15.4 %
4	900 ± 35.36[a]	950 ± 36.36[b]	50 ± 11.36[b]	19.2 %
5	900 ± 35.36[a]	1010 ± 36.66[b]	110 ± 29.15[c]	42.3 %
6	890 ± 33.17[a]	1020 ± 47.37[b]	130 ± 25.50[c]	50.0 %
7	904 ± 16.31[a]	1020 ± 46.37[b]	116 ± 43.89[c]	45.2 %
8	890 ± 29.15[a]	1010 ± 50.99[b]	120 ± 65.68[c]	46.2%

Means in the same column with different superscript are significantly different ($P < 0.05$).

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella.*

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

There was mild ballooning of the caecum with moderately thickened caecal wall (mucosa) and bloody caecal content. Broilers in group 5 (DNA vaccination challenged with Local strain) had mean lesion score of 1.4 ± 0.6, with mild ballooning of the caecum, mild petechial hemorrhage in caecal mucosa (Table 4.2). The broilers in group 6 (DNA vaccination challenged with Houghton strain) had mean lesion score of 1.0 ± 0.9 with mild ballooning of the caecum, there was mild bloody caecal mucosa (Table 4.2). The broilers in group 7 (Livacox® vaccination challenged with Local stain) had mean lesion score of 1.0 ± 0.95, there were few scattered lesions in the caecum, petechial hemorrhage in the caecal mucosa. The caeca of the broilers in group 8 (Livacox® vaccination challenged with Houghton strain) had a mean lesion score of 1.0 ± 0.84, with mild scattered lesions, mild petechial hemorrhage in the caecal walls with normal caecal content present (Table 4.2).

4.4 Oocyts Count

Mean oocyst per gram of caecal content were significantly higher in the unvaccinated infected control groups (groups 2 and 3) with mean OPG values of 1.3 $x10^5 \pm 11.00$ and 1.1 $x10^5 \pm 18.00$ respectively followed by the vector vaccinated group (group 4) values of 9.8 $x10^4 \pm 22.7$

The mean oocyst per gram of the vaccinated groups was however lower with values of 5.8 $x10^4 \pm 18.00$, 4.8 $x10^4 \pm 25.9$, 4.4 $x10^4 \pm 42.36$ and 3.2 $x10^4 \pm 20.48$ respectively for groups 5, 6, 7 and 8 (Table 4.3). The percentage oocyst decrease of these vaccinated groups were 45 %, 44 %, 34 % and 29 %, respectively (Table 4.3). There was statistical significant difference $P < 0.005$ recorded in all groups 2-5 (Table 4.3).

Table 4.2: Mean (± SEM) lesion score of broiler chicks vaccinated with DNA and live oocyst vaccines against Local and Houghton strain of *E. tenella*

Group	Mean lesion score (± SEM)
1	0.00^a
2	4 ± 0.00^b
3	3.4 ± 0.82^b
4	3.0 ± 0.60^b
5	1.4 ± 0.86^a
6	1.0 ± 0.92^a
7	1.0 ± 0.62^a
8	1.0 ± 0.5^a

Means in the same column with different superscript are significantly different ($P<0.05$).

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.



Plate II: Caecum of broiler chicken graded score (0) showing normal appearance of the caecum.



Plate III: Score (1). Caecum of chicken experimentally infected with Houghton strain of *E. tenella* showing a few petechiae haemorrhage (arrow) found on the caecal wall.



Plate IV: Score (2).Caecum of chicken experimentally infected with Houghton strain of *E. tenella*. Lesions more numerous with noticeable blood in the caecal, thickened caecal wall (arrow).



Plate V: Score (3). Caecum of chicken experimentally infected with Houghton strain of *E. tenella*. Large amount of blood or caecal cores (arrows) present.



Plate VI: Score (4): Caecum of chicken experimentally infected with local strain of *E. tenella* Caecal wall greatly distended with blood or large caseous cores (arrows)

Table 4.3: Mean (± SEM) of oocyst value of broiler chicks vaccinated with DNA and live oocystvaccines post challenge with local and Houghton strains of *E. tenella*

Group	oocyst value	Relative oocyst production
2	$1.3 \times 10^5 \pm 11.00^a$	100.0
3	$1.1 \times 10^5 \pm 18.00^b$	100.0
4	$0.98 \times 10^5 \pm 22.7^c$	80.0
5	$0.58 \times 10^5 \pm 18.00^d$	45.0
6	$0.48 \times 10^5 \pm 25.9^e$	44.0

7	$0.44 \times 10^5 \pm 42.36^f$	34.0
8	$0.32 \times 10^5 \pm 20.48^g$	29.0

*Group 1omitted because no oocyst was produced.

Key:

Group 1	Non-vaccinated/non-challenged control group.
Group 2	Non-vaccinated/challenged with *E. tenella* local isolate control group.
Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella.*
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to isolate of *E. tenella.*
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella.*

4.5 Anti-Coccidial Index

Anti coccidial index of unvaccinated challenged and vector vaccinated groups for local and Houghton strains were 170, 170 and 167 (groups 2, 3 and 4) respectively with group 4 having the least ACI, While ACI of the vaccinated groups were 177, 179, 180 and 179 for (groups 5, 6, 7 and 8) respectively (Table 4.4).

Although, vaccinated groups had ACI higher than the unvaccinated infected groups, live vaccine produced a slightly higher ACI values when compared to that of the Dna vaccine. Similarly, birds challenged with local strain of *E. tenella* had ACI higher than the Houghton strain (Table 4.4).

4.6 Histopathological Lesions in Broilers

Sections of the caeca of the broilers from group 1 (unvaccinated uninfected group) showed normal histopathology of the caeca of broiler chickens (Plate X). Sections of the caeca of the broilers from group 2 (unvaccinated infected with local strain of *E. tenella*) showed various developmental stages of *Eimeria tenella*, there was infiltration of inflammatory cells into mucosal and sub mucosal surfaces (Plate XI). The epithelial surfaces had hemorrhages as well as edema at the sub mucosa. While those infected with Houghton strain of *E. tenella* (group 3) showed moderate infiltration of inflammatory cells (lymphocytes, plasma cells, eosinophils, and heterophils) (Plate XII). They were presence of schizonts, microgamete and macrogamete's in the mucosa.

Table 4.4 Anticoccidial index (ACI) of *Et AMA-1* DNA and live oocyst vaccines in broiler chickens challenged with Local and Houghton strain of *E. tenella* on different parameters.

Group	Survival rate (%)	Relative body weight gain (%)	Mean lesion score	Mean Oocyst score ($\times 10^4$)	ACI
1	100%	100	0	0	200
2	100%	76	4.0	1.34	170
3	100%	75	3.4	1.13	170
4	100%	80	3.0	9.8	167

5	100%	84	1.4	5.8	177
6	100%	85	1.0	4.8	179
7	100%	85	1.0	4.4	180
8	100%	84	1.0	3.2	179

Key:

Group 1	Non-vaccinated/non-challenged control group.
Group 2	Non-vaccinated/challenged with *E. tenella* local isolate control group.
Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

There was congestion of the blood vessels. However, the histopathology observed in the vector vaccinated group with local strain of *E. tenella* (group 4) were mild infiltration of inflammatory cells, desquamation of surface epithelium and high numbers of developmental stages of *E. tenella* (Plate XIII). In the group vaccinated with DNA vaccine and infected with local strain of *E. tenella* (group 5) there was mild infiltration of inflammatory cells (lymphocytes, heterophils, plasma cells, eosinophils) (Plate XIV). More so, moderate numbers of developmental stages of *E. tenella* were observed.

In the group vaccinated with DNA vaccine and infected with Houghton strain of *E. tenella* (group 6) showed similar pathology as in group 5, in addition, there were presence of hemorrhagic and edematous areas in the mucosa (Plate XV). In the group vaccinated with Liva cox® and infected with local strain of *E. tenella* (group 7) the histopathology observed were massive infiltration of inflammatory cells (lymphocytes, plasma cells, eosinophils) (Plate XVI). There was presence of developmental stages of *E. tenella* and mild petechial hemorrhage in the mucosa. While the group vaccinated with Liva cox® and infected with Houghton strain of *E. tenella* (group 8)showed mild lymphocytic infiltration, mild desquamation of the villi as well as developmental stages of *E. tenella* within the mucosa (Plate XVII).

Although both vaccinated and unvaccinated groups had pathologies in their caeca, pathologies in vaccinated groups were mild compared to the unvaccinated groups. However, among the vaccinated groups, live oocyst vaccination (Plate XVI and Plate XVII) had less pathology when compared to the DNA vaccination (Plate XIV and Plate XV).

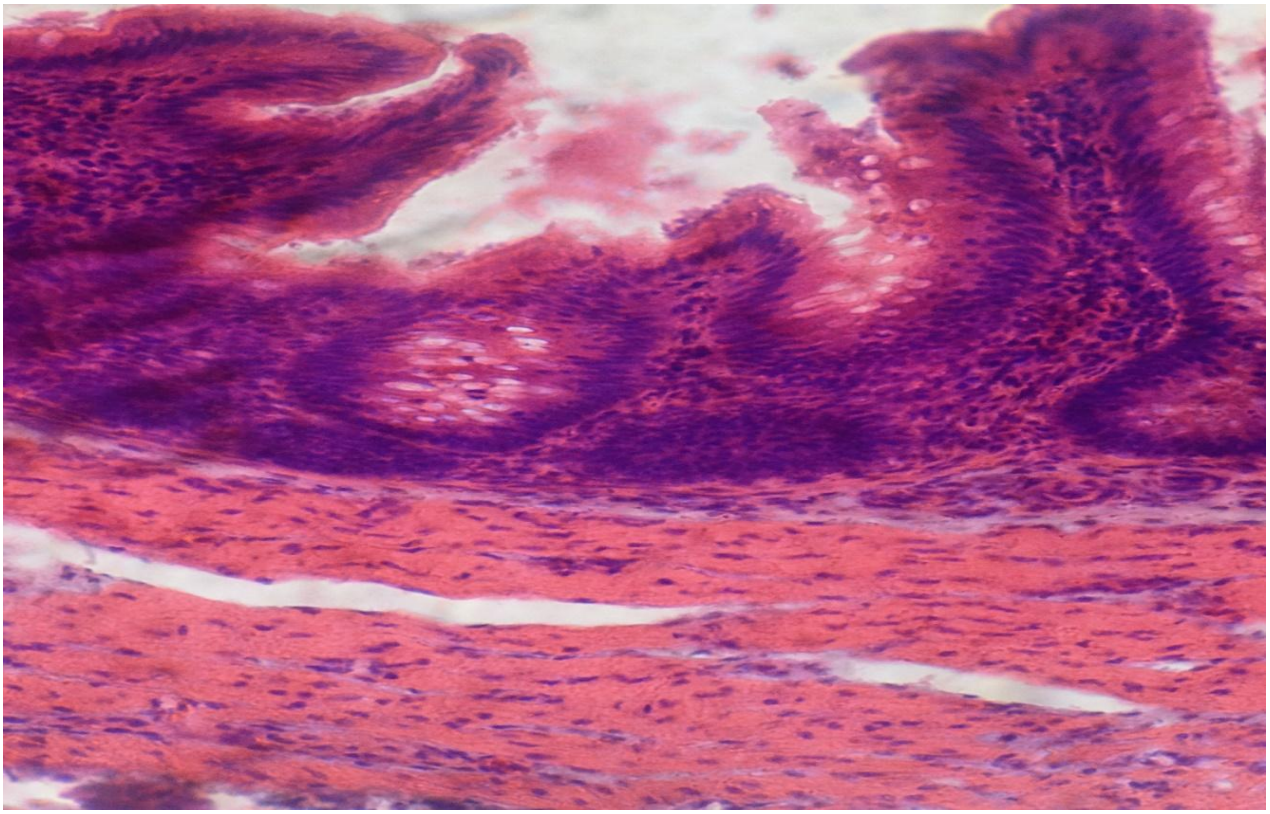


Plate X: A photomicrograph showing normal caecum of broiler chicken in the uninfected Group 1 (H&E x200).

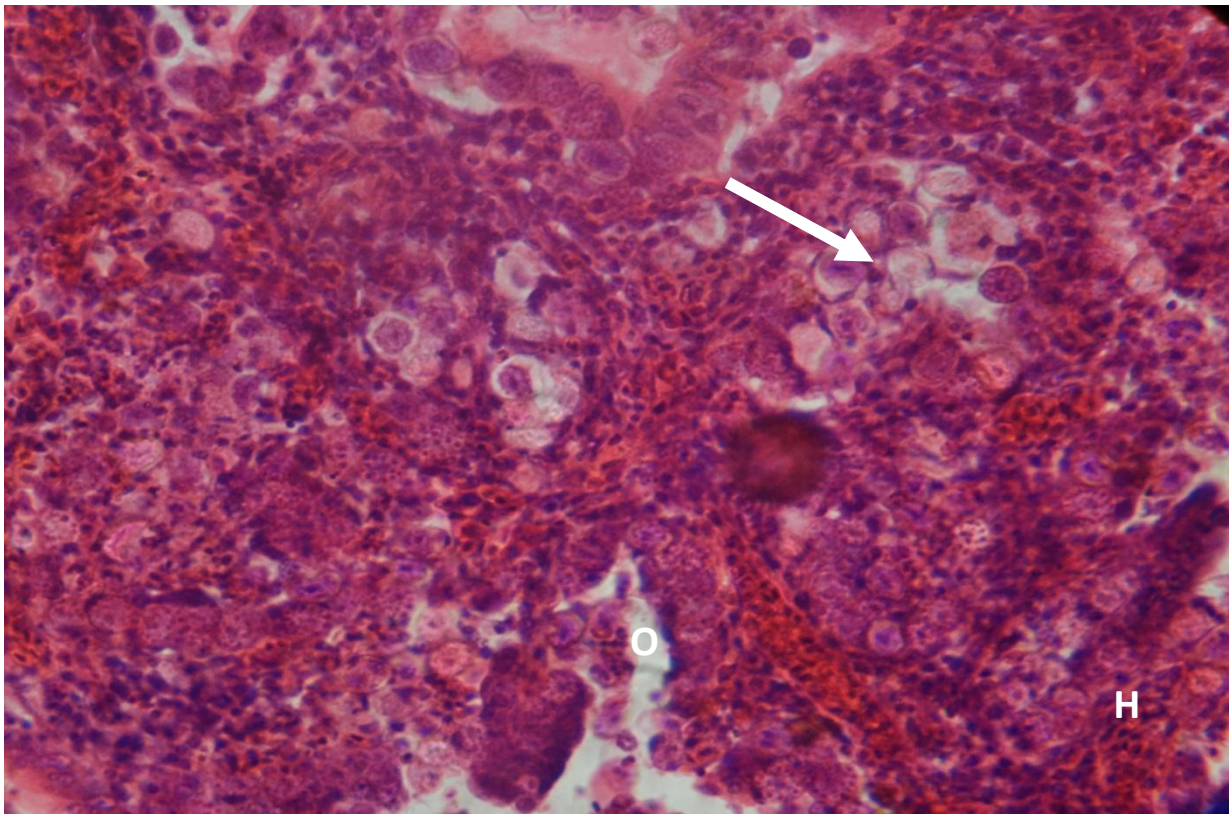


Plate XI: Photomicrograph of the caecum of a broiler chicken experimentally Infected with *E. tenella* (local strain). Group 2. Note the developmental stages of *Eimeria tenella* (arrows), oedema (O) and haemorrhage (H). (H&E x400).

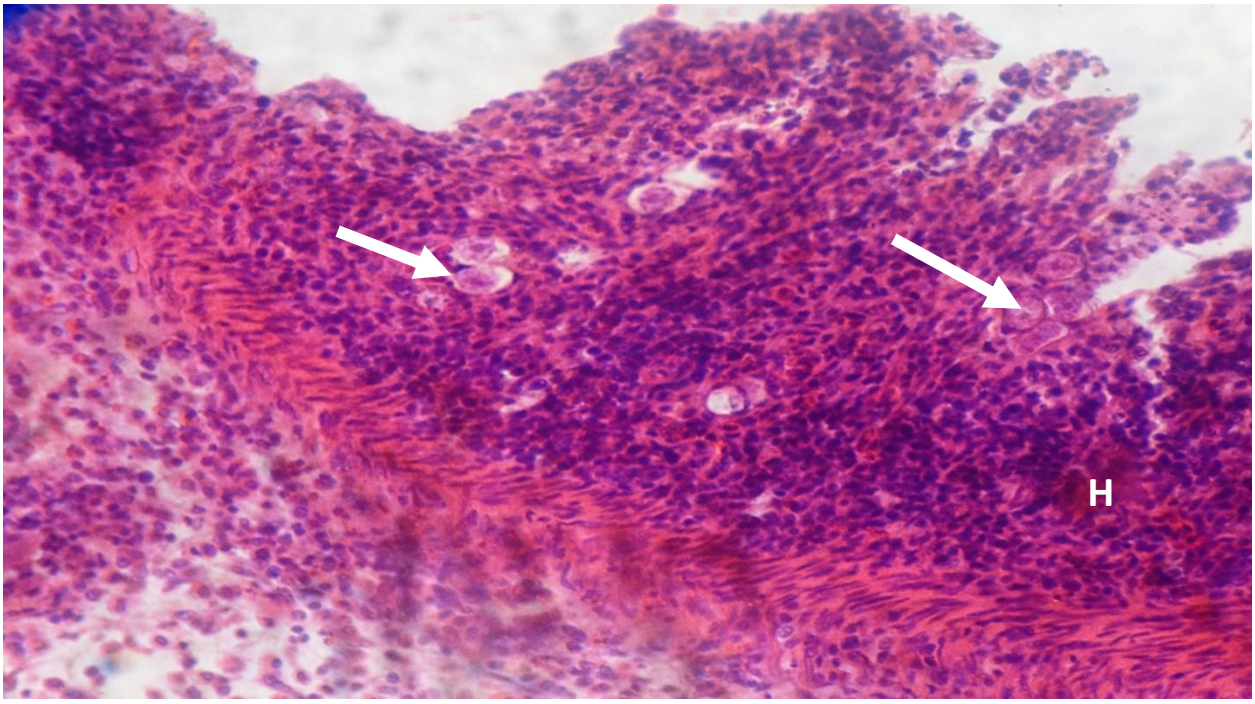


Plate XII: Aphotomicrograph of the caecum of a broiler chicken experimentally infected with *E. tenella* (Houghton strain). Group 3. Note the developmental stages of *Eimeria tenella* (arrows), and haemorrhagic mucosa (H). (H&E x200).

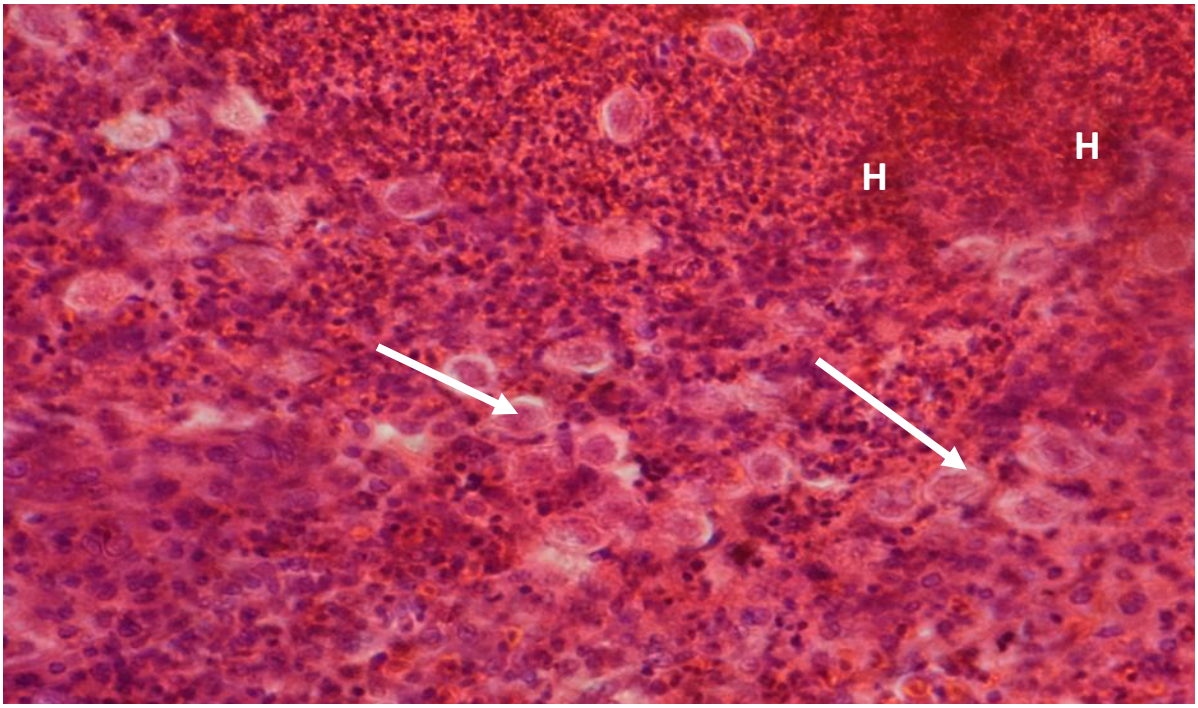


Plate XIII: Aphotomicrograph of the caecum of a vector vaccinated broiler chicken experimentally infected with *E. tenella* local strain (Group 4). Note the developmental stages of *Eimeria tenella* (arrows), and haemorrhages (H) in the mucosa. (H&E x400).

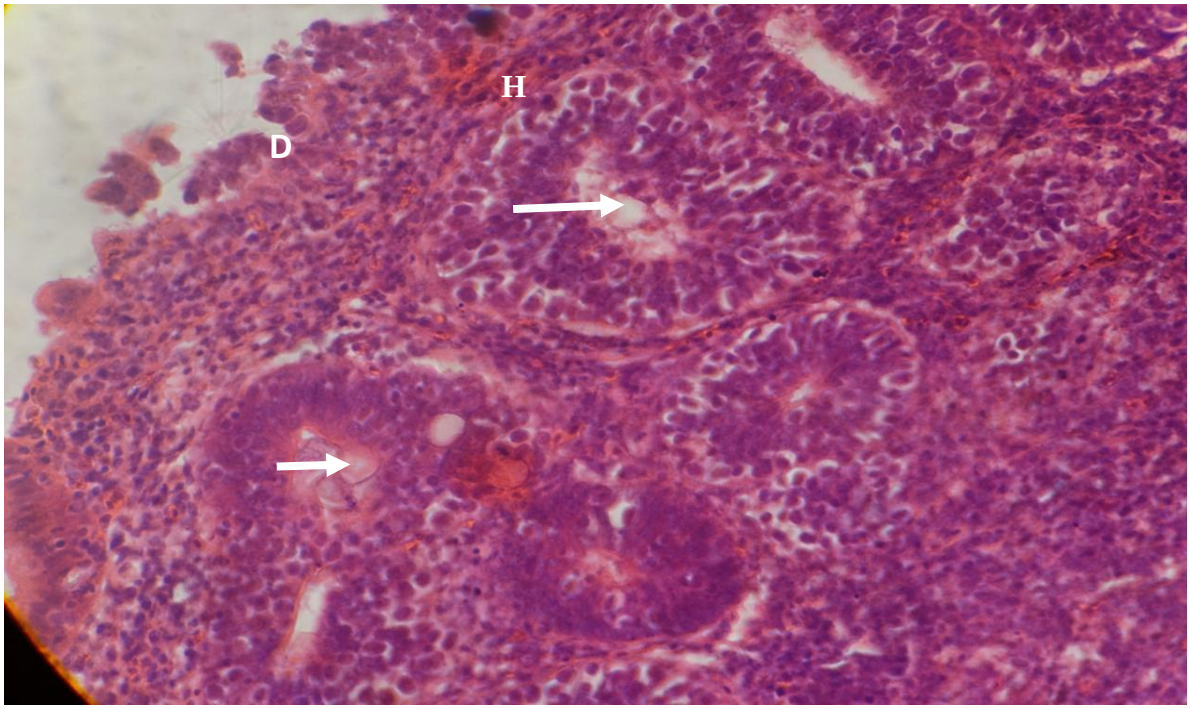


Plate XIV: A photomicrograph of the caecum of a broiler chicken vaccinated with DNA vaccine and experimentally infected with local isolate of *E. tenella* local (Group 5). Note the developmental stages of the parasite (arrrow), desquamation of the villi (D) and haemorrhage (H) in the submucosa. (H&E x200).

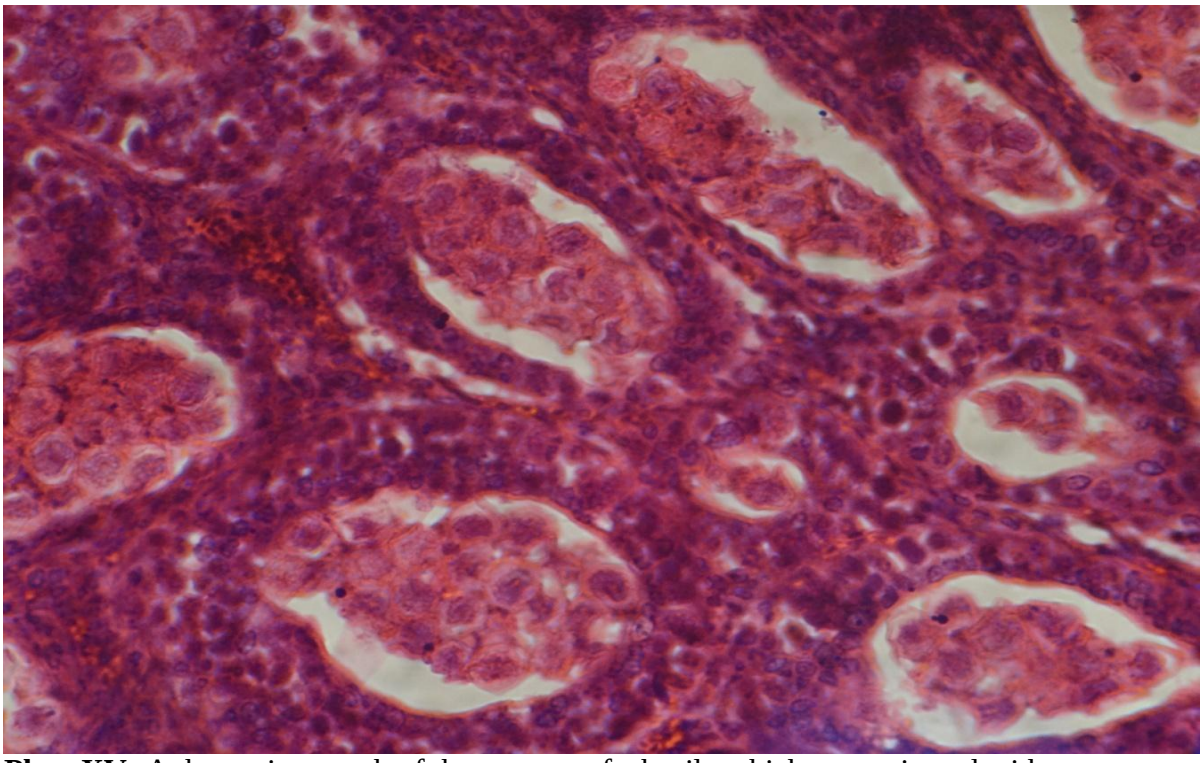


Plate XV: Aphotomicrograph of the caecum of a broiler chicken vaccinated with (*Et*AMA1) DNA vaccine and experimentally infected with Houghton strain*E. tenella* (Group 6). Note the aggregates of *Eimeria tenella*, (arrows) and haemorrhages (H) in the mucosa. (H&E x200).

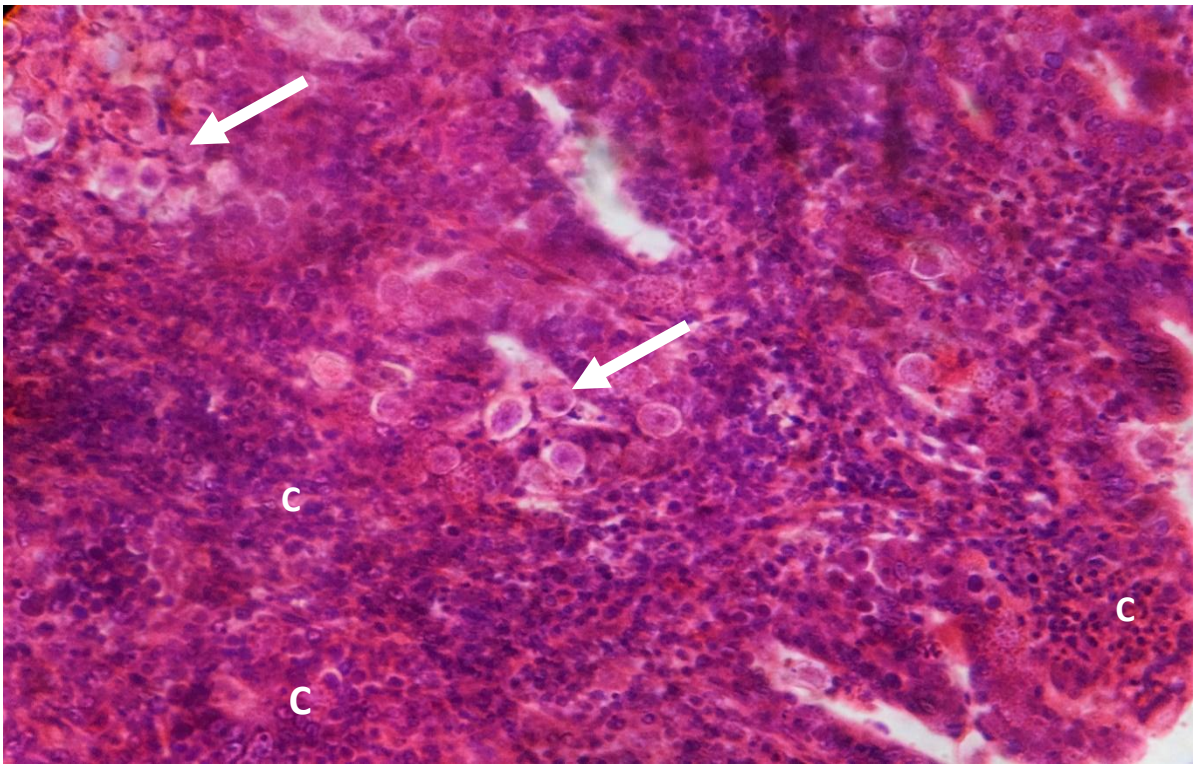


Plate XVI: Aphotomicrograph of the caecum of a broiler chicken vaccinated with live oocyst vaccine and experimentally infected with local isolate of *E. tenella* (Group 7). Note the developmental stages of the parasites (arrows) and diffused infiltration of mononuclear cells (**C**). (H&E x400).

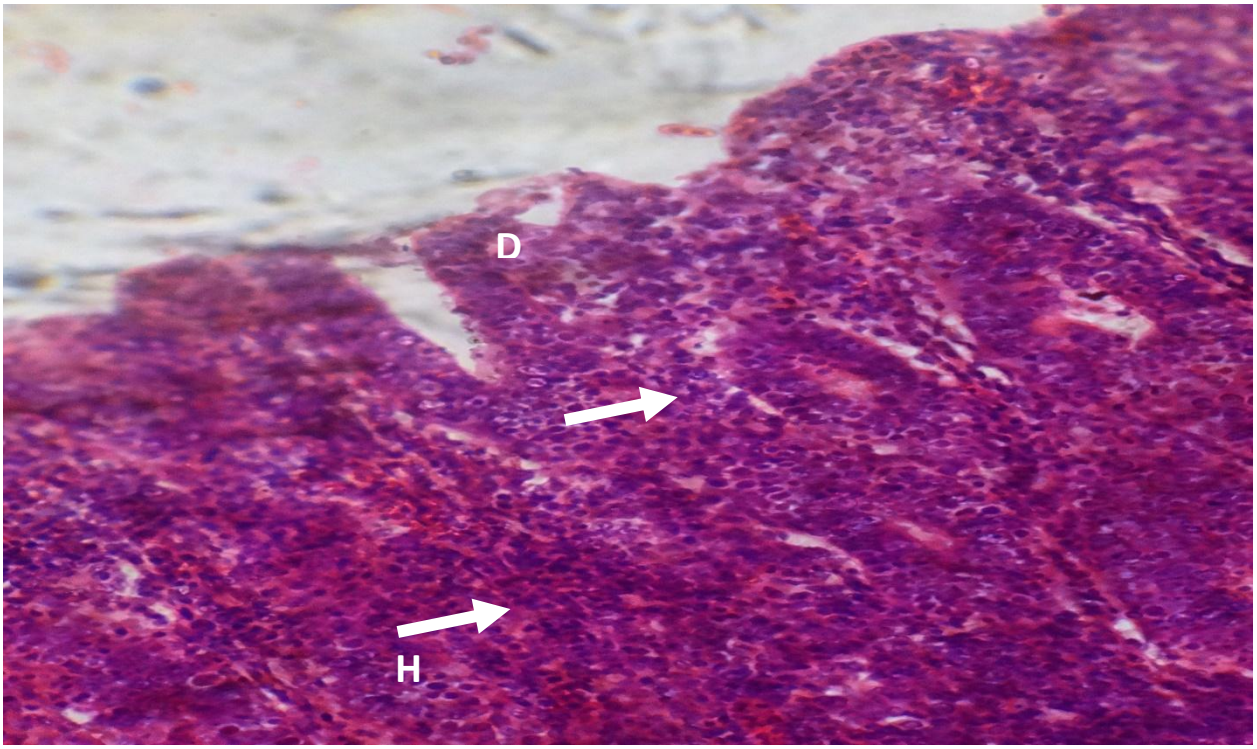


Plate XVII: Aphotomicrograph of the caecum of a broiler chicken vaccinated with live oocyst vaccine and experimentally infected with Houghton strain of *E. tenella* (Group 8). Note the haemorrhages in the submucosa (H), mononuclear cellular infiltration (arrow) and desquamation of the villi(D), (H & E x200).

4.7: Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Pack Cell Volume (%)

The mean PCV (%) values for groups 2, 3 and 4 decreased from pre infection values of 26.4 ± 0.51, 26.6 ± 2.16 and 26.2 ± 1.20 on a day prior to infection to 24.6 ± 1.40, 24.6 ± 2.16 and 24.8 ± 0.58 on day 7 post infection (Table 4.5). The mean PCV values of vaccinated groups 5, 6, 7 and 8 also decreased but slightly when compared to the challenged groups from pre infection values of 28.2 ± 2.15, 28.2 ± 2.35, 28.6 ± 1.08 and 27.6 ± 1.25 to 27.4 ± 0.93, 26.6 ± 1.12, 27.2 ± 0.86 and 26.8 ± 2.22 on day 7 post infection (Table 4.5). The mean PCV values for group 1 increased significantly from pre infection values of 27.2 ± 2.06 on a day prior to infection to 29.8 ± 2.24 on day 7 post infection (Table 4.5).

There was no statistical significant difference recorded between all the groups of the experimental birds ($P > 0.05$).

4.8 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Haemoglobin Concentration (g/dL)

The mean Hb values for groups 2, 3 and 4 decreased from pre infection values of 8.82 ± 0.59, 8.82 ± 0.71 and 9.42 ± 0.60 on a day prior to infection to 8.24 ± 0.47, 8.24 ± 0.40 and 8.5 ± 0.23 on day 7 post infection (Table 4.6). The mean Hb values for groups 5, 6, 7 and 8 also decreased but slightly from pre infection values of 8.8 ± 0.86, 9.96 ± 0.33, 9.6 ± 0.56

and 9.6 ± 0.51 on a day prior to infection to 8.6 ± 0.51, 8.82 ± 0.37, 8.8 ± 0.81 and 8.84 ± 0.72 on day 7 post infection (Table 4.6).

The mean Hb values for group1 increased slightly from pre infection values of 8.84 ± 0.24 to 9.88 ± 0.75 (Table 4.6).

There was no statistical significant difference recorded between all the groups of the experimental birds (P > 0.05).

Table 4.5: Mean (± SEM) of pack cell volume (%) of broiler chicks vaccinated with DNA and live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	27.2 ± 2.06[a]	29.8 ± 2.24[b]
2	26.4 ± 0.51[a]	24.6 ± 1.40[a]
3	26.6 ± 2.16[a]	24.6 ± 2.16[a]
4	26.2 ± 1.20[a]	24.8 ± 0.58[a]
5	28.2 ± 2.15[a]	27.4 ± 0.93[a]
6	28.2 ± 2.35[a]	26.6 ± 1.12[a]
7	28.6 ± 1.08[a]	27.2 ± 0.86[a]
8	27.6 ± 1.25[a]	26.8 ± 2.22[a]

a, b means in the same column with different superscript are significantly different (P=0.001).

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge

with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Table 4.6: Mean (± SEM) of haemoglobin (g/dL) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	8.84 ± 0.24 [a]	9.88 ± 0.75 [a]
2	8.82 ± 0.59 [a]	8.24 ± 0.47 [a]
3	8.82 ± 0.71 [a]	8.24 ± 0.40 [a]
4	9.42 ± 0.60[a]	8.5 ± 0.23 [a]
5	8.8 ± 0.86 [a]	8.6 ± 0.51 [a]
6	9.96 ± 0.33[a]	8.82 ± 0.37 [a]
7	9.6 ± 0.56[a]	8.8 ± 0.81 [a]
8	9.6 ± 0.51[a]	8.84 ± 0.72 [a]

a, b means in the same column with different superscript are significantly different ($p > 0.05$).

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

4.9 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on White Blood Cell count ($\times 10^9$/L)

The mean WBC values for groups 2, 3 and 4 increased slightly from pre infection values of 10.8 ± 0.55, 9.6 ± 0.70 and 9.66 ± 1.32 on a day prior to infection to 11.8 ± 0.37,10.84 ± 1.28and 10.2 ± 2.05 on day 7 post infection (Table 4.7). The mean WBC values for groups 5, 6, 7 and 8 increased from pre infection values of 11.6 ± 1.14, 10.6 ± 1.10, 10.2 ± 0.61 and 9.48 ± 1.56 on a day prior to infection to 11.98 ± 1.82, 10.94 ± 0.50, 10.86 ± 1.66 and 10.2 ± 0.64 on day 7 post infection (Table 4.7).

The mean WBC values for group 1 remained relatively within the same range of pre infection of 10.61 ± 0.65 on a day prior to infection to 10.6 ± 0.94 on day 7 post infection (Table 4.7). There was no statistical significant difference recorded between all the groups of the experimental birds ($P > 0.05$).

4.10 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Heterophils (%) values

The mean heterophil values for groups 2, 3 and 4 increased from pre infection values of 11.6 ± 1.03, 10 ± 1.30 and 12.2 ± 0.46 on a day prior to infection to 12.2 ± 0.61, 12.4 ± 0.50and 12.6 ± 1.61 on day 7 post infection (Table 4.8). The mean heterophil values for groups 5, 6, 7 and 8 also increased but slightly from pre infection values of 10.6 ± 2.36, 12 ± 1.45, 9.8 ± 0.97 and 8.0 ± 1.41 on a day prior to infection to 11.4 ± 0.75, 12.5 ± 0.32, 12.2 ± 0.73 and 10.8 ± 1.69on day 7 post infection (Table 4.8). The mean heterophil values for group 1 remained relatively within the same range of 10.6 ± 1.63 on a day prior to infection to 10.5 ± 0.87 on day 7 post infection (Table 4.8). There was no statistical significant difference recorded between all the groups of the experimental birds ($p > 0.05$).

Table 4.7: Mean (± SEM) of white blood cell ($\times 10^9$/L) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	10.61± 0.65[a]	10.6 ± 0.94[a]
2	10.8 ± 0.55[a]	11.8 ± 0.37[a]
3	9.6 ± 0.70[a]	10.84 ± 1.28[a]
4	9.66 ± 1.32[a]	10.2 ± 2.05[a]
5	11.6 ± 1.14[a]	11.98 ± 1.82[a]
6	10.6 ± 1.10[a]	10.94 ± 0.50[a]
7	10.2 ± 0.61[a]	10.86 ± 1.66[a]
8	9.48 ± 1.56[a]	10.2 ± 0.64[a]

a, b means in the same column with different superscript are significantly different ($p > 0.005$).

Key:

Group 1	Non-vaccinated/non-challenged control group.
Group 2	Non-vaccinated/challenged with *E. tenella* local isolate control group.
Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella.*
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Table 4.8: Mean (± SEM) of Heterophils (%) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	10.6 ± 1.63^{a}	10.5 ± 0.87^{a}
2	11.6 ± 1.03^{a}	12.2 ± 0.61^{a}
3	10 ± 1.30^{a}	12.4 ± 0.50^{a}
4	12.2 ± 0.46^{a}	12.6 ± 1.6^{a}
5	10.6 ± 2.36^{a}	11.4 ± 0.75^{a}
6	12 ± 1.45^{a}	12.5 ± 0.32^{a}

7	9.8 ± 0.97[a]	12.2 ± 0.73 [a]
8	8.0 ± 1.41[a]	10.8 ± 1.69 [a]

a, b means in the same colume with different superscript are significantly different (P >0.05).

Key:

Group 1	Non-vaccinated/non-challenged control group.
Group 2	Non-vaccinated/challenged with *E. tenella* local isolate control group.
Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

4.11 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Lymphocyte (%)

The mean lymphocyte values for groups 2, 3 and 4 increased slightly from pre infection values of 87 ± 1.95, 82 ± 2.66 and 84 ± 0.71 on day prior to infection to 90.2 ± 0.66, 89.2 ± 2.65 and 88 ± 1.67 on day 7 post infection. The mean lymphocyte values for groups 5, 6, 7 and 8 increased from the pre infection values of 84.2 ± 4.13, 86.8 ± 2.08, 86.8 ± 3.43 and 87.4 ± 2.68 on a day prior to infection to

97.6 ± 0.98, 94.2 ± 2.80, 94.2 ± 2.91 and 91.8 ± 1.88 on day 7 post infection. The mean lymphocyte values for group 1 remained relatively within the same range of pre infection values of 86 ± 2.47 on a day prior to infection to 85.2 ± 1.39 on day 7 post infection (Table 4.9). There was no statistical significant difference recorded between all the groups of the experimental birds ($p > 0.05$).

4.12 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Monocyte (%)

The mean monocyte values for groups 2, 3 and 4 increased slightly from pre infection values of 0.60 ± 0.60, 0.80 ± 0.40 and 0.60 ± 0.60 on a day prior to infection to 0.9 ± 0.00, 1.00 ± 0.00 and 1.00 ± 0.92 on day 7 post infection. The mean monocyte values for groups 5, 6, 7, and 8 also increased from pre infection values of 0.80 ± 0.80, 1.00 ± 1.17, 0.80 ± 0.24 and 0.88 ± 0.80 on a day prior to infection to 1.00 ± 0.80, 1.10 ± 0.40, 0.96 ± 0.40 and 0.98 ± 0.58 on day 7 post infection. The mean monocyte values for group 1 remained relatively within the same range of pre infection value of 0.82 ± 0.40 on a day prior to infection to 0.80 ± 0.51 of day 7 post infection (Table 4.10). There was no statistical significant difference recorded between all the groups of the experimental birds ($p > 0.05$).

Table 4.9: Mean (± SEM) of lymphocytes (%) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella.*

Group	1 day pre infection	7 days post infection
1	86 ± 2.47 [a]	85.2 ± 1.39 [a]
2	87 ± 1.95 [a]	90.2 ± 0.66 [a]
3	82 ± 2.66 [a]	89.2 ± 2.65 [a]

4	84 ± 0.71 [a]	88 ± 1.67 [a]
5	84.2 ± 4.13 [a]	97.6 ± 0.98[a]
6	86.8 ± 2.08 [a]	94.2 ± 2.80[a]
7	86.8 ± 3.43 [a]	94.2 ± 2.91[a]
8	87.4 ± 2.68 [a]	91.8 ± 1.88 [a]

a, b means in the same column with different superscript are significantly different ($p>0.05$).

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Table 4.10: Mean (± SEM) of monocytes (%) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	0.82 ± 0.40 [a]	0.80 ± 0.51 [a]
2	0.60 ± 0.60 [a]	0.9 ± 000 [a]
3	0.80 ± 0.40 [a]	1.00 ± 0.00 [a]
4	0.60 ± 0.60 [a]	1.00 ± 0.92 [a]
5	0.80 ± 0.80 [a]	1.00 ± 0.80 [a]
6	1.00 ± 1.17 [a]	1.10 ± 0.40 [a]
7	0.80 ± 0.24 [a]	0.96 ± 0.40 [a]
8	0.88 ± 0.80 [a]	0.98 ± 0.58 [a]

a, b means in the same column with different superscript are significantly different (P >0.05).

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

4.13 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Mean Total Plasma Protein (mg/dl)

The mean total plasma protein values for groups 2, 3 and 4 decreased significantly from pre infection values of2.8 ± 0.14, 3.8 ± 0.26 and 3.8 ± 0.37 on a day prior to infection to 2.6 ± 0.20, 2.8 ± 0.13 and 2.8 ± 0.13 on day 7 post infection. The mean Total plasma protein values for groups 5, 6,7 and 8 also decreased but slightly from the pre infection values of 3.9 ± 0.33, 4.5 ± 0.27, 3.9 ± 0.33 and 4.6 ± 0.16 on a day prior to infection to 3.9 ± 0.33, 3.9 ± 0.33, 3.5 ± 0.12 and 3.5 ± 0.22 on day 7 post infection. The mean Total plasma protein values of group1 remained relatively within the normal range of the pre infection values of 3.7 ± 0.2 on a day prior to infection to 3.8 ± 0.37 on day 7 post infection (Table 4.11). There was no statistical significant difference recorded between all the groups of the experimental birds ($p > 0.05$).

4.14 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Serum Aspertate Amino Transferase (IU/L)

The mean AST values for groups 2, 3 and 4 increased from pre infection values of 69.40 ± 7.66, 63.20 ± 7.23 and 65.20 ± 4.97 on a day prior to infection to 90.40 ± 3.70, 86.80 ± 3.99 and 76.60 ± 3.04 on day 7 post infection. The mean AST values for groups 5, 6, 7 and 8 decreased from pre infection values of 75.60 ± 4.26, 69.20 ± 6.53, 76.80 ± 4.89 and 71.80 ± 3.34 on a day prior to infection to 66.40 ± 5.24, 63.40 ± 6.56, 66.60 ± 4.37 and 67 ± 5.95 on day 7 post infection. The mean AST values of group 1 remained relatively within the normal range of the pre infection values of 81 ± 3.90 on a day prior to infection to 80 ±

0.89 on day 7 post infection (Table 4.12). There was no statistical significant difference recorded in all the groups of the experimental birds ($p > 0.05$).

Table 4.11: Mean (± SEM) of total plasma protein (mg/dl) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	3.7 ± 0.2^{a}	3.8 ± 0.37^{a}
2	3.8 ± 0.14^{b}	2.6 ± 0.20^{b}
3	3.8 ± 0.26^{a}	2.8 ± 0.13^{b}
4	3.8 ± 0.37^{a}	2.8 ± 0.13^{b}
5	3.9 ± 0.33^{a}	3.9 ± 0.33^{a}
6	4.5 ± 0.27^{a}	3.9 ± 0.33^{a}
7	3.9 ± 0.33^{a}	3.5 ± 0.12^{a}
8	4.6 ± 0.16^{a}	3.5 ± 0.22^{a}

a, b means in the same column with different superscript are significantly different ($P < 0.05$).

Key:

Group 1	Non-vaccinated/non-challenged control group.
Group 2	Non-vaccinated/challenged with *E. tenella* local isolate control group.
Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Table 4.12: Mean (± SEM) of aspartate amino transferase (IU/L) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella.*

Group	1 day pre infection	7 days post infection
1	81 ± 3.90^{a}	80 ± 0.89^{a}
2	69.40 ± 7.66^{a}	90.40 ± 3.70^{b}
3	63.20 ± 7.23^{a}	86.80 ± 3.99^{a}
4	65.20 ± 4.97^{a}	76.60 ± 3.04^{a}
5	75.60 ± 4.26^{a}	66.40 ± 5.24^{a}
6	69.20 ± 6.53^{a}	63.40 ± 6.56^{a}
7	76.80 ± 4.89^{a}	66.60 ± 4.37^{a}
8	71.80 ± 3.34^{a}	67 ± 5.95^{a}

a, b means in the same column with different superscript are significantly different ($P < 0.05$).

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella.*

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

4.15 Effects of DNA and Live coccidian vaccination against Local and Houghton strains of *Eimeria tenella* on Serum Alkaline Phosphatase (IU/L)

The mean ALPvalues of groups 2, 3 and 4 increased from the pre infection values of 288 ± 13.93, 300 ± 4.74 and 295 ± 6.12 on a day prior to infection to 398 ± 6.41, 324 ± 12.88 and 325 ± 4.47on day 7 post infection respectively. The mean ALP values of groups 1, 5, 6, 7, and 8 remained relatively within the same range from pre infection values of 305 ± 9.06, 290 ± 6.52, 300 ± 11.14, 295 ± 19.25 and 314 ± 3.41 on a day prior to infection to 300 ± 6.39, 300 ± 5.23, 310 ± 12.8, 285 ± 33.76 and 317 ± 37.42on day 7 post infection respectively (Table 4.13). There was no statistical significant difference recorded between all the groups of the experimental birds ($p > 0.05$).

4.16 Effects of DNA and Live coccidian vaccination against Local and Houghton strains of *Eimeria tenella* on Serum Alanine Amino Transferase (IU/L)

The mean ALT values of groups 2, 3 and 4 decreased from pre infection values of 26 ± 1.76, 25 ± 1.73 and 28.80 ± 1.39 on a day prior to infection to 24.80 ± 5.03, 24 ± 1.70 and 27 ± 4.59 on day 7 post infection. Similarly, the mean ALT values of groups 5, 6, 7 and 8

decreased from pre infection values of 27.40 ± 1.54, 28.40 ± 1.21, 28.60 ± 1.36 and 29.20 ± 0.73 on a day prior to infection to 26.60 ± 1.08, 27.80 ± 1.50, 26.40 ± 1.0 and 26.80 ± 2.03 on day 7 post infection respectively. Mean ALT values of group 1 remains relatively within the normal range of the pre infection values of 28 ± 2.7 on a day prior to infection to 28 ± 1.41 on day 7 post infection (Table 4.14). There was no statistical significant difference recorded between all the groups of the experimental birds (p >0.05).

Table 4.13 Mean (± SEM) of alkaline phosphatase (IU/L) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	305 ± 9.06[a]	300 ± 6.39 [a]
2	288 ± 13.93[a]	398.8 ± 6.41[b]
3	300 ± 4.74[a]	324 ± 12.88 [a]
4	290 ± 6.12[a]	325 ± 4.47 [a]
5	290 ± 6.52[b]	300 ± 5.23[a]
6	300 ± 11.14[a]	310 ± 12.98 [a]
7	295 ± 19.25[a]	285 ± 33.76[a]
8	314 ± 35.41[a]	317 ± 37.42 [a]

a, b means in the same column with different superscript are significantly different (P >0.05).

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate

buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella.*

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Table 4.14 Mean (± SEM) of alanine amino transferase (IU/L) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1day pre infection	7 days post infection
1	28 ± 2.70 [a]	26 ± 1.41 [a]
2	26 ± 1.76 [a]	24.80 ± 5.03 [a]
3	25 ± 1.73 [a]	24 ± 1.70 [a]
4	28.80 ± 1.39 [a]	28 ± 4.59 [a]
5	27.40 ± 1.54 [a]	26.60 ± 1.08 [a]
6	28.40 ± 1.21 [a]	27.80 ± 1.50 [a]
7	28.60 ± 1.36 [a]	26.40 ± 1.03 [a]
8	28.20 ± 0.73	26.80 ± 2.03 [a]

a, means in the same column with same superscripts are not significantly different (P >0.05)

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

4.17 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Mean Albumin (mg/dl)

The mean albumin values for groups 2, 3 and 4, decreased from pre infection values of 2.2 ± 0.11, 2.9 ± 0.20 and 2.8 ± 0.37on a day prior to infection to 2.1 ± 0.32, 2.6 ± 0.34, and 2.2 ± 0.32 on day 7 post infection. The mean albumin values of vaccinated groups 5, 6, 7 and 8 also decreased from pre infection values of 3.0 ± 0.32, 3.4 ± 0.19, 2.9 ± 0.24 and 3.4 ± 0.13 37on a day prior to infection to 2.9 ± 0.31, 2.9 ± 0.33, 2.1 ± 0.08 and 2.8 ± 0.28 on day 7 post infection. The mean albumin values for group 1 remained relatively within the same range of pre infection values of 3.6 ± 0.32 on a day prior to infection to 3.5 ± 0.28on day 7 post infection. There was no statistical significant difference recorded between all the groups of the experimental birds

($p > 0.05$).

4.18 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Mean Globulin

The mean globulin values for groups 2, 3 and 4 increased from pre infection values of 0.6 ± 0.11, 0.9 ± 0.1, 0.6 ± 0.00 on a day prior to infection to1.0 ± 0.14, 1.0 ± 0.23 and 1.2 ± 0.35 on day 7 post infections. The mean globulin values for vaccinated groups, 5, 6, 7 and 8 remained relatively within the same range of pre infection values of 0.9 ± 0.1,1.1 ± 0.12, 1.0 ± 0.16 and 1.1 ± 0.07 on a day prior to infection to 1.0 ± 0.14, 1.0 ± 00, 0.9 ± 0.14 and 1.0 ± 0.17 on day 7 post infection. Similarly, the mean globulin values for group 1 remained relatively within the same range of pre infection values of 0.4 ± 00 on a day prior to infection to 0.3 ± 0.35 on day 7 post infection (Table 4.16).

There was no statistical significant difference recorded between all the groups of the experimental birds ($p > 0.05$).

Table 4.15: Mean (± SEM) of albumin of broiler chicks vaccinated with DNA and Live oocysts vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	3.6 ± 0.32^a	3.5 ± 0.28^a
2	2.2 ± 0.11^a	2.1 ± 0.32^a
3	2.9 ± 0.20^a	2.6 ± 0.34^a
4	2.8 ± 0.37^a	2.2 ± 0.32^a
5	3 ± 0.32^a	2.9 ± 0.31^a
6	3.4 ± 0.19^a	2.9 ± 0.33^a
7	2.9 ± 0.24^a	2.7 ± 0.08^a
8	3.4 ± 0.13^a	2.8 ± 0.28^a

a, b means in the same column with different superscript are significantly different ($P<0.05$).

Key:

Group 1	Non-vaccinated/non-challenged control group.
Group 2	Non-vaccinated/challenged with *E. tenella* local isolate control group.
Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Table 4.16: Mean (± SEM) of globulin of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7days post infection
1	0.4 ± 00^{a}	0.3 ± 0.35^{b}
2	0.6 ± 0.11^{a}	1.0 ± 0.14^{a}
3	0.9 ± 0.1^{a}	1.0 ± 0.23^{a}
4	0.6 ± 00^{a}	1.2 ± 0.35^{a}
5	0.9 ± 0.1^{a}	1.0 ± 0.14^{a}
6	1.1 ± 0.12^{a}	1.0 ± 00^{a}
7	1.0 ± 0.16^{a}	0.9 ± 0.14^{a}
8	1.1 ± 0.07^{a}	1.0 ± 0.17^{a}

a, b means in the same column with different superscript are significantly different (P =0.001).

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

4.19 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Mean Sodium (Mmol/L)

The mean sodium values of groups 2, 3, and 4 decreased from pre infection values of 145 ± 3.50, 148 ± 2.95, and 143 ± 1.20 on a day prior to infection to 137 ± 2.13, 139.40 ± 2.48 and 136 ± 1.92 on day 7 post infection respectively. However, the mean Sodium values of vaccinated groups 5, 6, 7 and 8 increased from the pre infection values of 138 ± 3.30, 141 ± 2.20, 137 ± 4.76 and 141 ± 4.70 on a day prior to infection to 142.0 ± 1.64, 141 ± 2.93, 140 ± 0.81 and 142 ± 3.39 on day 7 post infection respectively. Similarly, the mean Sodium values of group 1 increased from the pre infection values of 138.4 ± 4.07 on a day prior to infection to 140 ± 1.30 on day 7 post infection (Table 4.17). There was no statistical significant difference recorded between all the groups of the experimental birds ($p > 0.05$).

4.20 Effects of DNA and Live vaccination against Local and Houghton strains of *Eimeria tenella* on Mean Potassium (Mmol/L)

The mean Potassium values of groups 2,3 and 4 increased from the pre infection values of 4.80 ± 0.31, 4.58 ± 0.07and 4.26 ± 0.27on a dayprior to infection to 5.46 ± 0.55, 4.74 ± 0.04 and 4.40 ± 0.18on day 7 post infection respectively. However, the mean Potassium values of vaccinated groups 5, 6, 7 and 8 decreased from pre infection values of 4.72 ± 0.93,4.46 ± 0.27, 4.24 ± 0.64 and 4.48 ± 0.31 on a dayprior to infection to 4.46 ± 0.24, 3.92

± 0.33, 4.18 ± 0.46 and 4.10 ± 0.18 on day 7 post infection respectively (Table 4.18). The mean potassium values of group 1 remained relatively within the same range of pre infection values of 4.08 ± 0.57 on a dayprior to infection to 4.08± 0.25 on day 7 post infection (Table 4.18).

There was no statistical significant difference recorded in all the groups of the experimental birds ($p > 0.05$).

Table 4.17: Mean (± SEM) of sodium ion (Mmol/L) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strains of *E. tenella*

Group	1 day pre infection	7 days post infection
1	138.4 ± 4.07[a]	140 ± 1.30 [a]
2	145 ± 3.50 [a]	137 ± 2.13 [a]
3	148 ± 2.95 [a]	139 ± 2.48 [a]
4	143 ± 1.20 [a]	136 ± 1.92 [a]
5	138 ± 3.30 [a]	142 ± 1.64 [a]
6	141 ± 2.20 [a]	141 ± 2.93 [a]
7	137 ± 4.76 [a]	140 ± 0.81 [a]
8	141 ± 4.70 [a]	142 ± 3.39 [a]

a, means in the same column with same superscripts are not significantly different ($P > 0.05$).

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Table 4.18: Mean (± SEM) of potassium ion (Mmol/L) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	4.08 ± 0.57 [a]	4.08 ± 0.25[a]
2	4.80 ± 0.31 [a]	5.46 ± 0.55[a]
3	4.58 ± 0.07 [a]	4.74 ± 0.04[a]
4	4.26 ± 0.27 [a]	4.40 ± 0.18[a]
5	4.72 ± 0.93 [a]	4.46 ± 0.24[a]
6	4.46 ± 0.27 [a]	3.92 ± 0.33[a]
7	4.24 ± 0.64 [a]	4.18 ± 0.46[a]
8	4.48 ± 0.31 [a]	4.10 ± 0.18[a]

a, means in the same column with same superscripts are not significantly different (P >0.05)

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

4.21 Effects of DNA and Live coccidian vaccination against Local and Houghton strains of *Eimeria tenella* on Mean Chloride (Mmol/L)

The mean Chloride values of groups 2, 3 and 4 decreased from the pre infection values of 100 ± 2.00, 102 ± 20 and 102 ± 2.41 on a dayprior to infection to 96.60 ± 2.40, 100 ± 40 and 93.60 ± 2.64 on day 7 post infection respectively. The mean Chloride values for vaccinated groups 5, 6, 7 and 8 increased from pre infection values of 100 ± 3.42, 97.80 ± 2.73, 98.80 ± 4.92 and 99 ± 2.10 on a dayprior to infection to 102.20 ± 2.13, 102.6 ± 1.08, 102.8 ± 1.66 and 103.8 ± 39 on day 7 post infection respectively. However, the mean Chloride values of group 1 remained relatively within the same range of pre infection values of 99.20 ± 2.65 to 98.60 ± 2.04 on day 7 post infection (Table 4.19).

There was no statistical significant difference recorded between all the groups of the experimental birds ($p > 0.05$).

4.22 Effects of DNA and Live coccidian vaccination against Local and Houghton strains of *Eimeria tenella* on Mean Bicarbonate (Mmol/L)

The mean HCO_3 values of groups 2, 3 and 4 decreased from the pre infection values of 20.40 ± 1.50, 18.60 ± 1.86and 22 ± 2.30 on a dayprior to infection to 19.40 ± 1.29, 19.40 ± 1.69and19.40 ± 0.98on day 7 post infection respectively. The mean HCO_3 values for vaccinated groups 5, 6, 7 and 8 increased from pre infection values of 19.60 ± 2.11, 19 ± 1.10, 23.60 ± 4.72and 21.40 ± 2.82 to 21.60 ± 2.14,23.20 ± 2.01, 22.80 ± 0.97and 21.20 ± 4.97on day 7 post infection respectively. However, the mean HCO_3 values of group 1 remained relatively within the same range of pre infection values of 21.20 ± 4.63 to 22.40 ± 1.17on day 7 post infection (Table 4.20).

There was no statistical significant difference recorded between all the groups of the experimental birds ($p > 0.05$).

Table 4.19: Mean (± SEM) of chloride ion (Mmol/L) of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	99.20 ± 2.65 [a]	98.60 ± 2.04 [a]
2	100 ± 2.00 [a]	96.60 ± 2.40 [a]
3	102.20 ± 2.03 [a]	102.40 ± 3.61 [a]
4	102 ± 2.41 [a]	93.60 ± 2.64 [a]
5	100 ± 3.42 [a]	102.20 ± 2.13 [a]
6	97.80 ± 2.73 [a]	102.6 ± 1.08 [a]
7	98.80 ± 4.92 [a]	102.8 ± 1.66 [a]
8	99 ± 2.10 [a]	103.8 ± 3.89 [a]

a, means in the same column with same superscripts are not significantly different ($P > 0.05$)

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Table 4.20: Mean (± SEM) of bicarbonate ion of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella*

Group	1 day pre infection	7 days post infection
1	21.20 ± 4.63 [a]	22.40 ± 1.17 [a]
2	20.40 ± 1.50 [a]	19.40 ± 1.29 [a]
3	18.60 ± 1.86 [a]	19.40 ± 1.69 [a]
4	22 ± 2.30 [a]	19.40 ± 0.98 [a]
5	19.60 ± 2.11 [a]	21.60 ± 2.14 [a]
6	19 ± 1.10 [a]	23.20 ± 2.01 [a]
7	3.60 ± 4.72 [a]	22.80 ± 0.97 [a]
8	21.40 ± 2.82 [a]	21.20 ± 4.97 [a]

a, means in the same column with same superscripts are not significantly different (P >0.05)

Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

CHAPTER FIVE

DISCUSSION

In the present study, all birds challenged with either the Local or Houghton strain showed signs of clinical caecal coccidiosis such as droopiness, loss of appetite and bloody diarrhoea indicating that the two strains are pathogenic to the broiler chicks. However, there was no mortality recorded which could be due to the fact that the challenge dose might be low to cause mortality. Similar observation was made by Song *et al*. (2009), that no chicken died following homologous coccidiosis challenge.

Birds vaccinated with DNA and Oocyst vaccine had higher net body weight compared to the unvaccinated groups. However, among the vaccinated groups (4, 5, 6, 7 and 8), the vector vaccinated group (group 4) had lowest weight gain, which was close to that oberved for the unvaccinated infected groups (2 and 3), this suggests that the DNA vaccine is solely responsible in ameriorating the effects of the parasites on body weight gains. On the other hand, the decreased weight gain (weight loss) observed in the infected birds could be attributed to the changes in the intestinal villi as a result of damage caused by the invasion and replication of the parasite, thus impairing digestion and absorption of nutrients (Broussard *et al*., 2008, Shaban, 2012). Similarly, Fetterer *et al*. (2001) and Jatau *et al*. (2014) attributed decrease weight in caecal coccidiosis to the shared effects of muscle breakdown and anorexia that take place throughout the acute stage of *Eimeria* infection.Lillehoj *et al.*, (2005) and Song *et al* (2009) had similarly shown the ability of recombinant DNA vaccine in improving the weight gain of vaccinated birds.

The gross lesions observed in the present study were severe in the unvaccinated and vector vaccinated infected groups (2, 3, and 4), demonstrated by the high mean lesion scores recorded in those groups. However, mild lesions were observed in the vaccinated infected groups (5, 6, 7, and 8), this shows that the vaccines may have exacted some protective effects in reducing the severity of the lesions in the vaccinated groups. This is similar to the findings of Subramanian *et al.* (2008) who recorded a marginal reduction in the lesion score in vaccinated birds while evaluating potential of EtMIC1 invasion as a candidate for sub-unit vaccine for poultry against coccidiosis. Ayaz *et al*. (2003) who reported that mild to moderate lesions may be produced, despite high level of immunity conferred in chickens immunizedwith *E. tenella* gametocyte antigen vaccines.

In this study the oocyst output upon the experimental challenge was considerably reduced in the vaccinated chickens by up to 41%, 55%, 56%, 66% and 71%, in chickens vaccinated with Vector on local strain (group 4), DNA vaccine on local strain (group 5), DNA vaccine on Houghton strain (group 6), Live vaccine on local strain (group 7) and Live vaccine on Houghton strain (group 8) respectively. Unvaccinated infected groups 2 and 3, had the highest oocysts shed per gram of faeces. This is an indication that the vaccination has limiting effects on the proliferation of the parasites manifested as the reduced oocysts output in the vaccinated groups. Although both DNA and the live oocyst vaccines were able to reduce the mean oocyst output in the vaccinated birds, live vaccine exacted the highest percentage reduction in both strains of *E. tenella*. This could be due to the fact that in the live vaccine, the vaccinal parasite proliferate in the host expressing between 6,000 and 9,000 proteins during the course of its life cycle providing a complex portfolio of

antigens that stimulates strong immunoprotective response (Reid *et al*., 2014). AMA-1 is just one of such antigens and therefore is most likely going to produce lesser immunoprotection than the one produced by the live vaccine.

Anti-coccidial index (ACI) is a synthetic criterion for assessing the protective effect of a medicine or vaccine. In this study, unvaccinated and vector vaccinated infected groups 2, 3 and 4 had lower values of ACI when compared to vaccinated groups 5, 6, 7, and 8. This further confirms that the vaccinated birds acquired some level of protection against the infection. Muhammed *et al.* (2013), also reported high ACI of 192 in broilers vaccinated with construct of DNA encoding anti-3-1E gene of *Eimeria acervulina* with chicken interlukin-2 (IL-2) and gamma interferon (IFN-Y) against homologous infection. The fact that broiler chickens vaccinated with the DNA vaccine had relatively close values of ACI to those vaccinated with the live oocyst vaccine strongly suggest that the former could be an effective cheaper alternative to the later.

Histopathological changes observed in the caeca of the unvaccinated infected groups 2 and 3 is similar to findings reported by McDougald and Fitz-Coy (2008), Naphade *et al.* (2010), and Zulpo *et al.* (2007). They attributed these pathologies to the most pathogenic stage of the parasite, the second generation schizont that causes excessive tissue damage, haemorrhage, disruption of the caecal glands and destruction of the mucosa and muscularis layer. Schizonts, gamatocytes and immature oocyst were seen on the tissue section on day 7 post infections and are consistent with the reports of Getachew (2004), Mersha *et al*. (2009)

and Jatau *et al*. (2014). The oedema observed may be due to the inflammatory process and the dilation of the submucosa and proliferation of epithelium cells in the acute phase of the disease. The damages caused by the invasion of the second generation merozoites into the mucosal linings of the caecum affects the functional integrity of the cecum, which may lead to cellular infiltration (Rose *et al*., 1979 and Lillehoj *et al.,* 1989). This may perhaps explain the diffuse cellular infiltration observed in all groups of the infected chickens.

In this study, the slight decrease in the values of PCV, and Hb in unvaccinated infected and vaccinated infected groups post infection can be attributed to blood loss in the form of haemorrhagic diarrhoea as a result of caeca destruction commonly seen during the acute phase of infection with *E. tenella*. Vaccinated groups though, had higher mean PCV values when compared to unvaccinated infected groups. Ogbe *et al.* (2010) and Meskerem *et al.* (2013) also recorded drop in the PCV and Hb values in *E. tenella* infected broilers. The slight increase in mean values of lymphocytes and heterophils in the unvaccinated and vector vaccinated groups post infection may be attributed to the effects of the inflammation of the caeca caused by *Eimeria tenella*(Irizaary-Rovira, 2004). Also, chronic antigenic stimulation due to the infection with *Eimeria tenella*may result in a greatly expanded circulating lymphocyte pool.

This study showed increased level of AST and ALP values post infection in unvaccinated and vector vaccinated groups (2, 3 and 4). However, a decreased serum ALT was recorded in these groups. These results are similar to the findings of Biu *et al.* (2006), who also reported an increased ALP activity but a decreased serum ALT level in mixed (*Eimeria tenella, E. maxima and E. acervulina*) coccidian-infected chickens. Similarly, Meskerem *et*

al. (2013) reported an increase serum ALP values in broiler chickens infected with *E. tenella* and *E. brunetti.* This work however, disagrees with the results of Mondal *et al*. (2011) who reported a decreased ALT values and increased plasma AST activity in broiler chickens infected with a field isolate of *E. tenella*. Low plasma enzyme levels are less regularly used for clinical interpretation. Nevertheless, there are a few specific cases in which low plasma enzyme levels will indicate that the applicable organ is hyperplastic, atrophied or destroyed (Kerr, 2002). ALT is an enzyme found in erythrocytes; the decrease in the activities of serum ALT reported in this study can be attributed to the reduction of erythrocyte due to loss of blood into the caeca. The increased levels of AST and ALP activities in this study can be associated with the metabolic alteration of the bone marrow as compensation for the blood losses; the bone marrow is forced to produce excessive blood cellular components in response to anemia Maskere *et al*. (2013).

Reduced values of total serum protein and albumin recorded in both vaccinated and unvaccinated groups post infection may be attributed to nutrient malabsorption a consequence of damages done by the parasite. Jatau *et al. (*2014) made similar report of hypoproteinemia in two breeds of broiler chicks experimentally infected with *E. tenella*. The severity of the hypoproteinemia is however more in the unvaccinated groups than in the vaccinated birds which further suggest some level of protection by the vaccines.

Electrolytes are chemical substance that separates when dissolves in fluids, into electrically charged particles (ions) capable of conducting electric currents vital for the function of nerves and muscles. There was no statistical significant difference recorded in the mean electrolyte values (sodium, potassium, chloride and bicarbonate) ions for all groups (1-8) at

pre infection and post infection. The ranges of normal electrolytes recorded in this study may be attributed to mild diarrhoea and acuteness of the infection. Although there is paucity of information on the effects of vaccination against coccidiosis and its effects on electrolyte values, however, Parmer *et al.* (1987) reported lower serum calcium concentration in birds infected with coccidiosis. Similarly, Runho *et al.* (2001). Found that coccidiosis infested birds had lower serum phosphorus concentrations presented in the form of hypocalcemia and hypophosphatemia.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The present study demonstrated that vaccination with DNA and lives oocysts vaccines;

1. Ameliorated the effects of *E. tenella* on weight reduction by 84 % and 85 %, 85% and 84 %) for both DNA and Live oocysts vaccines against local and Houghton strains respectively.
2. Reduced the effectscaused by both strains of *E. tenella* on gross lesion score (1.4 ± 0.86, 1.0 ± 0.92, 1.0 ± 0.92 and 1.0 ± 0.62) for vaccinated groups (5, 6, 7, 8) compared to 4.0 and 3.4 of unvaccinated groups (2, 3) respectively.
3. Ameliorated the histopathologcal lesions associated with caecal coccidiosis.
4. Reduced the multiplication and proliferation of the *E. tenella* parasites evident by percentage reduction in oocyst shed (45%, 44%, 34% and 29%) for vaccinated groups (5, 6, 7, 8) compared to (100 % and 100 %) to unvaccinated groups (2, 3) respectively.
5. Presents observable higher mean PCV values (27.4 %, 27.6 %, 27.2 % and 26.8 %) for vaccinated groups (5, 6, 7, 8) compared to (24.6 % and 24.8 %) of unvaccinated groups respectively.
6. Presents observable higher mean Hb concentration (8.6, 8.82, 8.80 and 8.84 g/dl) for vaccinated groups (5, 6, 7, 8) compared to (8.24 and 8.24 g/dl) for unvaccinated groups (2, 3) respectively.
7. Showed significant increase ($p < 0.05$) in mean values of total plasma protein (3.9, 3.9, 3.5 and 3.5) g/L for vaccinated groups (5, 6, 7, 8) compared to 2.6 and 2.8 g/L of unvaccinated groups (2, 3). respectively.

8. Showed overall higher ACI of (177, 179, 180 and 179) for vaccinated groups (5, 6, 7 compare to 170 and170 of unvaccinated groups (2, 3), respectively.

6.2 Recommendations

1. While this experimental monovalent AMA-1 vaccination was able to induce some level of immune protection against wild strain of *E. tenella*, further studies should be conducted using combinations of two or more antigens (IMP1, MICs, RONs) into a single formulation with the hope of achieving broader *Eimeria* species protection that is comparable to live oocysts vaccine.
2. Detailed immunological studies should be conducted to determine the nature and level of immune response stimulated by the AMA-1 in birds under our local husbandry system comparing them with those stimulated by live oocysts vaccine.
3. Further studies on different routes of administration in order to ascertain the most efficient and convenient route of delivery since inoculation of individual bird may not be feasible.

REFERENCES

Abdu, P.A., Wakawa, A.M., Ibrahim, N. D. G. and Sanusi A. (2008). *Veterinary clinical Practice* Bulletin. Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria.1: 7-13.

Albert, N. (2013). Control and treatment of coccidiosis in the poultry sector. *International Poultry Production*, 21: 8-10.

Alberto, F., Charles, S. and Harry, G. (1996). An Improved method for determination of serum albumin and globulin. *Clinical Chemistry*, 12: 42-45.

Ali, H., Naqvi, F. and Tariq, N. (2014). Prevalence of coccidiosis and its association with risk factors in poultry of Quetta, Pakistan. *Asian Journal of Applied Sciences,* 2(4): 554-558.

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 Review*, 15: 58-65.

Anderson, W. I., Reid, W. M., Lukert, P. D. and Fletcher, O. J. Jr. (1977). Influence of infectious bursal disease on the development of immunity to *Eimeria tenella*. *Avian Diseases*, 21: 637-641.

Anonymous (2013). Retrieved from: http://Zaria, en. Wikipedia.Org/wiki/Zaria.

Anonymous (2011). Inovocox EM1®. Retrieved from: http://animalhealth. pfizer. com/sites/pahweb/US/EN/Products/Pages/InovocoxEM1. aspx (Pfizer Animal Health United States).

Ayaz, M. M. (2003). Development of egg adapted vaccine (local isolate) against coccidiosis in poultry. *PhD Thesis*. University of Agriculture, Faisalabad, Pakistan.

Besteiro, S., Michelin, A., Poncet, J., Dubremetz, J. F. and Lebrum, M. (2009). *Toxoplasma gondii* rhoptry neck protaines complex at the host cell membrane to form the moving junction during invasion. *PLoS Pathology*, 5(2) 000309.

Biu, A. A., Yusuf, S. D. and Rabo, J. S. (2006). Use of Neem (*Azadirachta indica*) aqueous extract as a treatment for poultry coccidiosis in Borno, Maiduguri, Nigeria. *African Science,* 7: 3-6.

Blake, D.P. and Tomley, F.M. (2014). Securing poultry production from the ever-present *Eimeria* challenges. *Trends in Parasitology*, 30(1): 12-19.

Blake, D. P., Clark, E.L., Macdonald, S.E., Thenmozhi, V., Kundu, K., Garg, R.,Jatau, I.D., Ayoade, S., Kawahara, F., Moftah, A., Reid, A.M., Adebambo, A.O., Zapata, R.A., Ani S.R., Rao, S., Thangaraj, K., Banerjee, P.S., Dhinakar-Raj, G., Raman, M. and

Tomley, F.M. (2015). Population, genetic and antigenic diversity of the apicomplexan *Eimeria tenella* and their relevance to vaccine development, *Proceedings of the National Academy of Sciences, USA* .www.pnas.org/cgi/doi/10.1073/pnas.

Blake, D.P., Billington, K. J., Copestake, S. L., Oakes, R. D., Quail, M. A., Wan, K. L., Shirly, M.W. and Smith, A. L., (2011). Genetic mapping identifies novel highly protective antigens for an apicomplexan parasite. *PLoS Pathology*, 7(2): 5-9.

Broussard, J.G., Hany, M., El-Sheikha, H.M. and. Morsy, T.A. (2009). Avian coccidiosis: The basic pathology to control. *Journal of Egyptian Social Parasitology*, 39: 85-98.

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

Chapman, H. D. (2005). Perspectives for the control of coccidiosis in poultry by chemotherapy and vaccination (pp. 99-104). In: *Proceedings of the Ninth International Coccidiosis Conference*, FACTA, Foz do Iguazu, Brazil.

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

Chapman, H.D. (1998). Evaluation of the efficacy of anticoccidial drugs against *Eimeria* species in the fowl. *International Journal for Parasitology* 28(7): 1141-1144.Review.

Chapman, H.D. (1997). Biochemical, genetic and applied aspects of drug resistance in *Eimeria* parasite of fowl. *Avian Pathology*, 26: 221-224.

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, H. D., Roberts, B., Shirley, M. W. and Williams, R. B. (2005). Guidelines for evaluating the efficacy and safety of live anticoccidial vaccines and obtaining approval for their use in chickens and turkeys. *Avian Pathology*, 34: 279-290.

Chookyinox, L.U, Stella, U. and Sandy, O. (2009). Coccidiosis. Backyard Poultry Information Centre Php BB 2004-2009/backyard poultry.com**.**

Christian, K., Florian, G., Richard, L. and Thomas, P. (2007). Identification of *Eimeria tenella* genes encoding for secretory proteins and evaluation of candidates by DNA immunization studies in chickens. *Vaccine*, 25: 6625-6634.

Conway, D. P., Sasai, K., Gaafar, S.M. and 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 scores, and performance in chickens. *Avian Diseases*, 37: 118-123.

Conway, Donald, P. and Elizabeth, M. M. (2007). *Poultry Coccidiosis, Diagnostic and Testing Procedures*. Blackwell Publishing, Iowa, USA.

Curtidor, H., Patino, L., Arevalo-Pinzo, G. and Patarroyo, M. (2011). Identification of the *Plasmodium falciparum* rhoptry neck protains5 (PfRON5). *Gene*, 474(1-2): 22-28.

Dalloul, R. A. and Lillehoj, H.S. (2006) Poultry coccidiosis: recent advancements in control measures and vaccine development. *Expert Review Vaccines*, 5(1): 143–163.

Danforth, H. D. (1998). Use of live oocyst vaccine in the control of avian coccidiosis. Experimental studies and field trials. *International Journal for Parasitology*, 28(7): 1099-1100.

David, H. C. (2007): Coccidiosis and the poultry industry. *Health and Welfare Featured Articles. (On line) Retrieved from: http://www.the poultry site.com/articles/502/coccidiosis-and the-poultry-industry*.

David, S. (2000). Coccidiosis. *Poultry Health and Management*. Fourth Edition, *ELST* with Blackwell Publishers. Pp, 105-107.

Ding, X., Lillehoj, H. S., Dalloul, R. A., Min, W., Sato, T., Yasuda, A. and Lillehoj, E. P. (2005). In ovo vaccination with the *Eimeria tenella* EtMIC2 gene induces protective immunity against coccidiosis. *Vaccine*, 23: 3733-3740.

Dolka, I., Sapierzyński, R., Bielecki, W., Malicka, E., Żbikowski, A. and Szeleszczuk, P. (2012). Histopathology in diagnosis of broiler chicken and layer diseases. *Polish Journal of Veterinary Sciences*, 4; 773-779

Donahue, C. G., Carruthers V. B., Gilk S. D. and Ward, G. E. (2000). The *Toxoplasma* homolog of *Plasmodium* apical membrane antigen-1 (AMA-1) is a micronemeprotein secreted in response to elevated intracellular calcium levels. *Moleuler Biochemical Parasitology*, 111(1): 15–30.

Donnelly, J.J., Ulmer, J. B., Shiver, J. W. and Liu, M. A. (1997). DNA vaccines. *Annual Revision Immunology*, 15: 617-648.

Drabkin, D.R. (1945). Crystallographic and optical properties of human haemoglobin. A proposal for standardization of haemoglobin. *American Journal of Medicine and Surgery*, 209: 268-270.

Fitz-Coy, S. H. (1992). Antigenic variation among stains of *Eimeria maxima* and *E. tenella* of chicken. *Avian Diseases*, 36(1): 40-43.

Fornace, K. M., Clark, E. L., Macdonald, S. E., Namangala, B., Karimuribo, E., Awuni, J. A., Thieme, O., Blake, D. P. and Rushton, J. (2013). Occurrence of *Eimeria* species parasites on small-scale commercial chicken farms in Africa and indication of economic profitability. *PLoS ONE*. 812: 84254.

Gaffer, F. R., Yatsuda, A. P., Franssen, F.F.J. and Vries, E. (2004). Erythrocyte invasion by *Babesia bovis* merozoites is inhibited by polyclonal antisera directed against peptidse derived from a homologue of *Plasmodium falciparum* apical membrane antigen 1. *Infection and Immunity*, 72(5): 2947-2955.

Getachew, G.J. (2004). Studies on poultry coccidiosis in Tiyo Wereda, Arsi Zone, Oromia Regional State. *MSc. Thesis*. AAU, FVM, Debre Zeit, Ethiopia.

Giambrone, J. J., Klesius, P. H., Eckamn, M. K. and Edgar, S. A. (1981). Influence of hormonal and chemical bursectomy on the development of acquired immunity to coccidia in broiler chickens. *Poultry Science,* 60: 2612-2618.

Godwin, R. M. and Morgan, J. A. (2015). A molecular survey of *Eimeria* in chickens across Australia. *Veterinary Parasitology*, 16–21.

Graham, W. Donald, Edward, F. Rogers and Wallace, T. A. (1978). 5 Deazariboflavin and its derivatives. *Avian Diseases,* 25: 18-20.

Gruber, A., Castanon, C. A. B., Fernandez, S., Fraga, J. S., and Fontoura, L. F. (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*, 19-23.

Gurunathan, S., Klinman, D. M. and Seder, R.A. (2000). DNA vaccines: immunology, application, and optimization. *Annual Revision Immunology*, 18: 927-974.

Haug, A., Gjevre, A. G., Skjerve, E. and Kaldhusdal, M. (2008). A survey of the economic impact of subclinical *Eimeria* infections in broiler chickens in Norway. *Avian Pathology*, 37: 333-341.

He, F. (2011). Plasmid DNA Extraction from *E. coli* using alkaline lysis method. *Biology Protocol, 101:* 25-30.

Holdsworth, P. A., Conway, D. P., Mckenzie, M. E., Dayton, A. D., Chapman, H. D., Mathis, G. F., Skinner, J. T., Mundt, H. C. and Williams, R. B. (2004). World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anticoccidial drugs in chickens and turkeys. *Veterinary Parasitolology*, 121: 189-212.

http://www.thepoultrysite.com/publications/2/Coccidiosis%20Management/41/life-cycle-and-types-of-coccidia/

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): 388-391.

Iheukwumere, F. C., Abu, A. H. and Ameh, M. (2006). Effect of human menopausal gonadotropin on haematological and serum biochemical parameters of Nigerian indigeneous chickens. *Proceedings of 20th Annual National Conference of Farm Management Association of Nigeria*, held 18th-21st September, 2006 at Forestry Research Institute, Jos, pp. 483-486.

Innes, E. A. and Vermeulen, A.N. (2006). Vaccination as a control strategy against the coccidial parasites *Eimeria, Toxoplasma* and *Neospora*. *Parasitology*, 133: 145-168.

Irizaary-Rovira, A.R. (2004). Avian and reptilian clinical pathology (Avian hematology & biochemical analysis). *Veterinary Clinical Pathology Secrets,* 282–313.

Islam, M.S., Lucky, N.S., Islam, M.R., Ahad. A., Das, B.R., Rahman, M.M. and Siddiu, M.S.I. (2004). Haematological parameters of Fayoumi, Assil and local chickens reared in Sylhet region in Bangladesh. *International Journal of Poultry Science*, 3(2): 144-147.

Jatau, D. I. (2014). Prevalence, identification and genetic diversity of *Eimeria* species in commercial poultry farms in Zaria and environs, Kaduna State, Nigeria. *PhD thesis*, Ahmadu Bello University, Zaria, Nigeria.

Jatau, D. I., Sulaiman, N., Musa, I., Lawal, A., Okubanjo, O., Isah, I. and Magaji, Y. (2012). Prevalence of coccidia infection and preponderance *Eimeria* species in free range indigenous and intensively managed exotic chickens during hot - wet season, in Zaria, Nigeria. *Asian Journal of Poultry Science*, 6: 79–88.

Jatau, D. I, Lawal, A. I., Kwaga, J. K. P., Fiona, M. T., Damer, P. B and Andrew, J. N. (2016). Three operational taxonomic units of Eimeria are common in Nigerian chickens and may undermine effective molecular diagnosis of coccidiosis. *Biomedical Central Veterinary Research*, 12: 86-90.

Jean, L. V. (2000). Electrophoresis of DNA and other polyelectrolytes: Physical mechanisms. Reviews of Modern Physics, 72: 813–872.

Jenkins, M. C. (1998). Progress on developing a recombinant coccidiosis vaccine. *International Journal for Parasitology*, 28: 1111–1119.

Jenkins, M. C., Parker C., Klopp S., O'Brien C., Miska K. and Fetterer R. (2012). Gel-Bead Delivery of *Eimeria* oocysts protects chickens against coccidiosis. *Avian Diseases*, 56: 306-309.

Jeston, P. J., Blight, G. W., Anderson, G. R., Molloy, J. B. and Jorgensen, W. K. (2002). Comparison of infectivity of *Eimeria tenella* oocysts maintained at 4, 12 or 28ºC up to 10 months. *Australian Veterinary Journal,* 80: 91-92.

Jeurissen, S. H. M., Janse, E. M., Vermeulen, A. N. and Vervelde, L. (1996). *Eimeria tenella* infections in chickens: aspects of host-parasite interaction. *Veterinary Immunology and Immunopathology,* 54: 231-238.

Jiang, L., Lin, J., Han, H., Zhao, Q., Zhu, S. and Huang, B. (2012). Identification and characterization of *Eimeria tenella* apical membrane antigen -1(AMA1). *PLOS ONE,* 7(7): 41115.

Jiao, H., Pan, Z., Yin, Y., Geng, S., Sun, L. and Jiao, X. (2011). Oral and nasal DNA vaccines delivered by attenuated Salmonella enterica serovar Typhimurium induce a protective immune response against infectious bronchitis in chickens. *Clinical Vaccine Immunology,* 18: 1041-1045.

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

Kerr, M. G. (2002). Clinical biochemistry and hematology. *Internal Veterinary Laboratory Medicine.* Oxford, UK. Blackwell Scientific Publications. pp. 1–30.

Kim, J. J., Simbiri, K. A., Sin, J. I., Dang, K., Oh, J. and Dentchev, T. (1999). Cytokine molecular adjuvants modulate immune responses induced by DNA vaccine constructs for HIV-1 and SIV. *Journal Interferon Cytokine Research,* 19: 77-84.

Lamarque, M., Besteiro, S., Papoin, J., Roques, M., Vulliez-Le Normand, B., Morlon, Guyot, J., Dubremetz, J. F., Fauquenoy, S., Tomavo, S., Faber, B, W., Knocken, C. H., Thomas, A. W., Boulanger, M. J., Bentley, G. A. and Lebrum, M. (2011). The RON2- AMA1 interaction is a critical step in moving junction dependent invasion by apicomplexan parasites. *PLoS Pathogen,* 7(2): 34-37.

Lee, B.H., Kim, W.H., Jeong, J., Yoo, J. and Know, Y. (2010). Prevalence and cross immunity of *Eimeria* species on Korean chicken farms. *Journal of Veterinary Medical Sciences,* 72: 985-989.

Leiner, W. W., Ying, H. and Restifo, N. P. (2000). DNA and RNA based vaccines: principles, progress and prospects. *Vaccine,* 18: 765-777.

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

Lillehoj, H. S. (1987): Effects of immunosuppression on avian coccidiosis: cyclosporin A but not hormonal bursectomy abrogates host protective immunity. *Infection Immunology,* 55: 1616-1621.

Lillehoj, H. S., Ruff, M. D., Bacon, L. D. and Lamont, J. S. (1989). Genetic control of immunity to *Eimeria tenella*. Interaction of MHC gene and non-MHC genes influence concentrations of disease susceptibility. *Veterinary Immunology*, 20: 135–148.

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

Lillehoj, H. S. and Okamura, M. (2003). Host immunity and vaccine development to coccidia and salmonella infections in chickens. *Poultry Science,* 40: 151-193.

Lillehoj, H. S., Ding X., Quiroz, M. A., Bevensee, E. and Lillehoj, E. P. (2005). Resistance to intestinal coccidiosis following DNA immunization with the cloned 3-1E *Eimeria* gene plus IL-2, IL-15, and IFN-gamma. *Avian Diseases,* 49: 112-117.

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

Ma, D., Ma, C., Pan, L., Li, G., Yang, J., Hong, J., Cai, H. and Ren, X. (2011). Vaccination of chickens with Dna vaccine encoding *Eimeria acervulina* 3-1E and chicken IL-15 offers protection against homologous challenge. *Experimental Parasitology*, 127: 208-214.

Majaro, O. M. (2001). New house syndrome: Investigation into the possible role of house fly (*Musca domestica*) in the epizootiology of coccidiosis in chickens. *Tropical Veterinarian*, 19: 237-242.

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 geographical and temporal strains of *Eimeria maxima. International Journal for Parasitology*, 27: 527-533.

Martin, A., S. A. and Lillehoj, H. S. (1995). Characterization of cell-mediated responses to *Eimeria acervulina* antigens. *AvianDiseases,* 39: 538-547.

Maurice, P. (2012). *Coccidiosis in Chickens*. University of California Cooperative Extension, UC Davis School of Veterinary Medicine, 23: 65-69.

McDougald, L. R. (2003). Coccidiosis. In: *Diseases of Poultry*. Iowa State Press, Ames, Iowa, USA, pp. 974-991.

McDougald, L.R. and Fitz-Coy, S. H. (2008). Protozoal infection. *Diseases of Poultry*; 23: 1068–1080.

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

Mersha, C., Tamiru, N. and Samuel, B. T. (2009). Occurrence of concurrent infectious diseases in broiler chickens is a threat to commercial poultry farms in central Ethiopia. *TropicalAnimal Health and Production*, 41: 1309–1317.

Meskerem, A., Chaiwat, B., Nirat, G. and Montakan, V. (2013). Hematological, biochemical and histopathological hanges caused by coccidiosis in chickens. *Kasetsart Journal of Natural Science*, 47: 238 – 246.

Mhatre, A.J. and Joshi, V.G. (1993). Effect of different temperature regimens on some haematological parameters in broilers. *Indian Veterinary Journal*, 70: 915-920.

Mitchell, R. S., Kumar, V., Abbas, A. K. and Fausto, N. (2009). *Robbins Basic Pathology*. Philadelphia: Saunders. ISBN 1-4160-2973-7. 8th edition.

Mohammed, Y., Shobrak, A. E. and Abo, A. (2014). Role of wild birds as carriers of multi drug resistant *Escherichia coli* and *Escherichia vulneris*. *Brazilian Journal of Microbiology*, 45: 15-17.

Mondal, D. K., S. Chattopadhyay, S., Batabyal, A. K. Bera and Bhattacharya, D. (2011). Plasma biochemical indices at various stages of infection with a field isolate of *Eimeria tenella* in broiler chicken. *Veterinary World*, 4: 404–409.

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

Morris, G. M., Woods, W.G., Richards, D. G. and Gasser, R. B. (2007). Investigating a persistent coccidiosis problem on a commercial broiler breeder farm utilizing PCR-coupled capillary electrophoresis. *Parasitology Research*, 101: 583-589.

Muazu, A., Masdooq, A. A., Ngbede, J., Salihu, A. F., Haruna, G., Habu, A. K., Sati, M. N. and Jamilu, H. (2008). Prevalence and identification of species of *Eimeria* causing coccidiosis in poultry within Vom, Plateau State, Nigeria. *International Journal of Poultry Sciences*, 7(9): 917-918.

Muhammad, A. and Shah, A. (2013). DNA vaccines as sustainable coccidiosis control strategies in chickens. *Science Letters*, 5: 1-4.

Naciri, M., D. E. Gussem, K., Fort, G., Bernardet, N., Nerat, F. and Chausse, A. M. (2003). Interest of anticoccidial sensitivity tests (ASTs) in the prévention of chicken coccidiosis. *British Poultry Science*, 44: 826-827.

Najih, N. and Saleh, A. N. (1998). *Clinical Chemistry Laboratory Manual*. Page 155-200.

Naphade, S.T., Hiware, C.J. and Desarda, S.M. (2010). Studies on the pathological changes during experimental caecal coccidiosis of broiler chicks treated with allopathic amprolium and homoeopathic medicine Mercurius corrosivus. *Trends Research Science and Technology;* 2: 49–55

Oakes, R. D., Kurian, D., Bromley, E., Ward, C., Lal, K., Blake, D. P., Reid, A. J., Pain, A., Sinden, R.E., Wasttling, J. M. and Tomley, F. M. (2013). The rhoptry proteome of *Eimeria tenella* sporozoites. *International Journal for Parasitology*, 43: 181-188.

Ogbe, A. O., Adeyefa, C.A.O. and Joshua, R. A. (2003). Growth rate and haematological parameters of broiler chickens vaccinated with IBD (Gumboro) vaccines exposed to different handling temperature. *Journal of Science Technology Resource,* 2(4): 36-38

Ogbe, A.O., Atawodi, S. E., Abdu, P. A, Oguntayo, B. O. and Noel, D. (2010). Oral treatment of *Eimeria tenella*-infected broilers using aqueous extract of wild mushroom (*Ganoderma* Sp): Effect on haematological parameters and histopathology lesions. *African Journal of Biotechnology*, 9: 8923–8927.

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.

Parmer, D. N., Mishra, A., Misra, S. C. and Misra, U. K. (1987). Comparative haematological and clinicopathological changes between *Eimeria tenella* infected and super cox treated broiler chicken. *Indian Veterinary Journal*, 74(1-4): 206-209.

Peek, H. and Landman, W. (2003). Resistance to anticoccidial drugs of Dutch avian *Eimeria* spp. field isolates originating from 1996, 1999 and 2001. *Avian Pathology,* 32(4): 391-401.

Perry, B., Randolph, T., McDermott, J., Sones, K. and Thornton, P. (2002). *Investing in Animal Health Research to Alleviate Poverty*. International Livestock Research Institute, Nairobi, Kenya.

Rasheda, M. and Bano, M. (1985). Histopathology of coccidiosis by *Eimeria garnhamiPakistani Veterinary Journal,* 1: 27-29.

Reid, J.S., Treeck, M. and Boothroyd, J. C. (2014). Focus on the ringleader: the role of AMA1 in apicomplexan invasion and replication. *Trends Parasitology*, 27(9): 410–420.

Remarque, E. J., B. W., Kocken, C. H. and Thomas, A. W. (2008). Apical membrane antigen 1: a malaria vaccine candidate in review. *Trends in Parasitology*, 24(2): 74-84.

Ricklefs, R. E. and Sheldon, K. S. (2007). Malaria prevalence and white-blood-cell response to infection in a tropical and in a temperate thrush**:** *The American Ornithologists Union*, 124: 1254–1266.

Robert Cruickshank (2008). *Volume 2 of Medical Microbiology: A Guide to the Laboratory Diagnosis andControl of Infection*, pp 58-65.

Robinson, H. L. and Torres, C. A. (1997). DNA vaccines. *Seminar Immunology*, 9: 271-283.

Rodica, C., Caprita, A., Iuliana, C. and Valeria, N. (2013). Spectrophotometric and refractometric determination of total protein in avian plasma. Animal Science and Biotechnologies, 46:(2): 162-165.

Rose, M. E., Hesketh, P. and Ogilvie, B. M. (1979). Peripheral blood leukocyte response to coccidial infection: A comparison of the response in rats and chickens and its correlation with resistance to reinfection. *Immunology*,36: 71–79.

Ruff, A. (1986). Total intestinal absorption of glucose and l-methionine in broilers infected with *Eimeria acervulina, E. mivati, E.maxima* or *E. brunetti*. *Parasitology*, (3): 555-569.

Runho, B., Padmavathi, P. and Muralidharan, S. R. G. (2001). Studies on the alteration in the serum metabolites during the *Eimeria tenella* infection in chicks. *Industrial. Veterinary Journal*, 74: 206-209.

Samour, M. (2001). Haematological indicators of inflammation exhibited by Austrailian falloniformes. *Clinical Pathology*, 18: 1-4.

Santos, J. M., Ferguson, D. J. P., Blackman, M. J., Soldati-Favre, D. (2011). Intramembrane cleavage of AMA1 triggers *Toxoplasma* to switch from an invasive to a replicative mode. *Science*, 331(6016): 473–477.

Shaban, K.S. (2012). Exacerbating effect of Newcastle disease virus (NDV) infection on subclinical caecal coccidiosis in broilers vaccinated against Newcastle disease virus (NDV). *Researcher*, 4: 55–59.

Shah, A., Muhammed, A., Xu, L., Yan, R., Song, X. and Li, X. (2010). A recombinant DNA vaccine encoding *Eimeria acervulina* cSZ-2 induces immunity against experimental *E. tenella* infection. *Veterinary Parasitolology*, 169: 185-189.

Shah, A., Muhammed, A., Xu, L., Yan, R., Song, X. and Li X. (2011). Construction of DNA vaccines encoding Eimeria acervulina cSZ-2 with chicken IL-2 and IFN-γ and their induced immunity against poultry Coccidiosis. *Research Veterinary Science*, 90: 72-77.

Shirley, M. W., Smith, A. L. and Tomley F. M. (2005). The biology of avian *Eimeria* with an emphasis on their control by vaccination. *Advance. Parasitolology*, 60: 285-330.

Shivaramaiah, C., Barta, J. R., Hernandez-Velasco, X., Téllez, G. and Hargis, B. M. (2014). Coccidiosis: Recent advancements in the immunobiology of *Eimeria* species, preventive measures, and the importance of vaccination as a control tool against these Apicomplexan parasites. *Veterinary Medicine: Research and Reports*, 4: 1-12.

Sirois, M. (1995). Blood chemistry. In: *Veterinary Clinical Laboratory Procedures,* Mosby's Fundamentals of Veterinary Technology, 6: 101-105.

Song, K. D., Lillehoj, H. S., Choi, K. D., Yun, C.H., Parcells, M. S., Huynh, J.T. (2000). A Dna vaccine encoding a conserved *Eimeria* protein induces protective immunity against live *Eimeria acervulina* challenge. *Vaccine*, 19: 243-52.

Song, X., Xu, L., Yan, R., Huang, X., Shah, M. A. A. and Li, X. (2009). The optimal immunization procedure of DNA vaccine pcDNATA4- IL-2 of *Eimeria tenella*, its cross immunity to *Eimeria necatrix and Eimeria acervulina. Veterinary Parasitology*, 159: 30-36.

Soomro, N.M., Rind, R., Arijo, A. G. and Soomro, S. A. (2001). Clinical, gross and histopathological studies of coccidial infection in chicken. *International Journal of Agriculture & Biology*, 4: 426–427.

Subramanian, B. M., Sriraman, R., Rao., N. H., Raghul, R., Thiagarajan, D. and Srinivasan, V. A. (2008). Cloning, expression and evaluation of the efficacy of a recombinant *Eimeria tenella* sporozoite antigen in birds. *Vaccine*, 26: 3489- 3496.

Takehara, K., Kobayashi K., Ruttanapumm, R., Kamikawa, M., Nagata, T., Yokomizo, Y. and Nakamura, M. (2003). Adjuvant effect of chicken interferon gamma for inactivated *Salmonella enteritidis* antigen. *Journal Veterinary Medical Sciences*, 65: 1337-1341.

Talebi, A. and Mulcahy, G. (1995). Correlation between immune responses and oocysts production in chicken's mono specifically infected with *E. maxima*. *Avian Pathology*, 24: 485-495.

Taylor, M. A., Coop, R. L. and Wall, R. L. (2007). Parasites of poultry and game birds. *Veterinary Parasitology*, 3rd edition. Iowa State, Blackwell Publishing, USA. Pp 459-557.

Thomas, A. W., Deans, J. A., Mitchell, G. H., Alderson, T. and Cohen, S. (1984). The Fab fragments of monoclonal IgG to a merozoite surface antigen inhibit *Plasmodium knowlesi* invasion of erythrocytes. *Molecular and Biochemical Parasitology*, 13(2): 187-199.

Tonukari, N.J. (2010). *Theileria parva* apical membrane antigen-1 (AMA-1) shares conserved sequences with apicomplexan homologs. *International Journal of Biotechnology and Moleculer Biololgy Research*, 1(3): 36–41.

Urquhart, G. M., Amour, J., Duncan, J. L., Dunn, J. N. and Jennings, F.W. (1996). *Coccidiosis of Poultry*. BlackwellPublishers, London, pp. 228-230.

Uza, D.V., Olarunju, S. A. S. and Orkpeh, J. M. T. (2001). An assessment of the disease and production status of indigenous poultry in Benue and Nassarawa states of

Nigeria. *Proceedings of the 26th Annual Conference ofNigerian Society of Animal Production,* Zaria, Nigeria, 26: 73-75.

Vermeulen, A. N., Schapp, D. C. and Schetters, T. P. (2001). Control of coccidiosis in chickens by vaccination. *Veterinary Parasitology*, 100: 13-20.

Wakelin, D., and Rose, M. E. (1990). Immunity to coccidiosis. Pages 282-301 In: Coccidiosis of Man and Domestic Animals. *P. L.Long, ed. CRC Press, Inc., Boca Raton, FL.*

Wakenell, P.S. (2010). Hematology of chickens and turkeys. *Veterinary Hematology*, 122: 957–967.

Waldenstedt, L., Lunden, A., Elwinger, D., Thebo, P. and Uggla, A. (1999). Comparison between a live, attenuated anticoccidial vaccine and an anticoccidial ionophore, on performance of broiler raised with or without a growth promoter, in an initially *Eimeria*-free environment. *Acta Veterinary Scandinavica*, 40: 11-21.

Wallach, M. P. (1997). The importance of transmission-blocking immunity in the control of infections by Apicomplexan parasites. *International Journal for Parasitology*, 27: 1159-1167.

Wallach, M., Pillemer, S. Yarus, A. Halabi, T. Pugasch, A. and Mencher, D. (1990). Passive immunization of chickens against *Eimeriamaxima* infection with monoclonal antibodydeveloped against a gametocyte antigen. *Infection and Immunity,* 58:557-562.

Williams, R. B. (2006). Tracing the emergence of drug-resistance in coccidian (*Eimeria* spp.) of commercial broiler flock medicated with decoquinate for the first time in the United Kingdom. *Veterinary Parasitology*, 135(1): 1-14.

Williams, R. B. (2002) Anticoccidial vaccines for broiler chickens: Pathways to success. *Avian Pathology,* 31: 317-353.

Williams, R. B. (1999). A compartmentalized 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. (1998). Epidemiological aspects of the use of live anticoccidial vaccines for chickens. *International Journal for Parasitology*, 28: 1089-1098.

Williams, R. B. (1994). Safety of the attenuated anticoccidial vaccine "Paracox" in broiler chickens isolated from extraneous coccidial infection. *Veterinary Research Communications*, 18: 189-219.

Wu, S. Q., Wu, M., Wang, Q., Liu, Y. J., Zhu, X. and Jiang, J. S. (2004) Construction of DNA vaccines and their induced protective immunity against experimental *Eimeria tenella* infection. *Parasitolology Research,* 94: 332-336.

Xu, Q., Song, X., Xu, L., Yan, R., Shah, M. A. A. and Li, X. (2008). Vaccination of chickens with a chimeric DNA vaccine encoding *Eimeria tenella* TA4 and chicken IL-2 induces protective immunity against coccidiosis. *Veterinary Parasitology*, 15: 319-323.

Yin, G., Qin, M., Liu, X., Suo, J., Tang, X., Tao, G., Han, Q., Suo, X. and WU, W. (2013). An *Eimeria* vaccine candidate based on *Eimeria tenella* immune mapped protein and the TLR-5 agonist *Salmonella typhimurium* Flic flagellin. *Biochemical and Biophysical Research Communications*, 440: 437-442.

Yun, C. H., Lillehoj, H. S. and Lillehoj, E. P. (2000). Intestinal immune responses to Coccidiosis *Development Complex Immunology*, 24: 303-324.

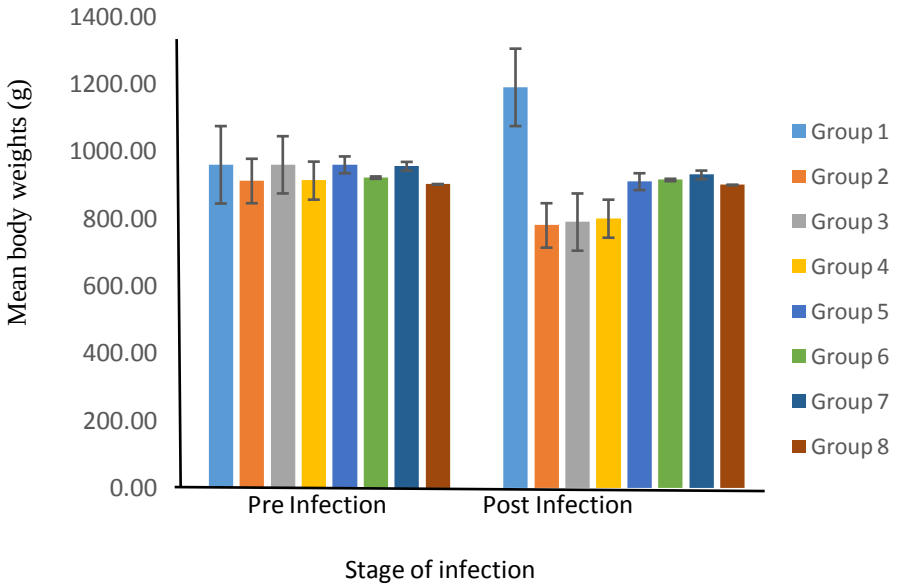
Zhang, H., Compaore, M. K., Lee, E. G., Liao, M., Zhang, G., Sugimoto, C., Fujisaki, K., Nishikawa, Y. and Xuan, X. (2007). Apical membrane antigen 1 is a cross-reactive antigen between *Neospora caninum* and *Toxoplasma gondii*, and the anti-NcAMA1 antibody inhibits host cell invasion by both parasites. *Molecular and Biochemical Parasitology*, 151: 205-212.

Ziomko, I., Karamon, J., Cencek, T., Gornowicz, E., Skoracki, A. and Ashash, U. (2005). Prevention of broiler chick coccidiosis using the inactivated subunit vaccine Coxabic®. *Bulletin of the Veterinary Institute in Pulawy*, 49: 299-302.

Zulpo, D. L, Peretti, J., Ono, L. M., Longhi, E., Oliveira, M. R., Guimarães, I. G., Headley, S. A., Guimarães, Junior, J.S. and Garcia, J.L. (2007). Pathogenicity and histopathological observations of commercial broiler chicks experimentally infected with isolates of *Eimeria tenella*, *E. acervulina* and *E. maxima*. *Ciências Agrárias, Londrina*, 28: 97–104.

APPENDICES

Appendix I: Mean body weights of all groups (1-8) for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

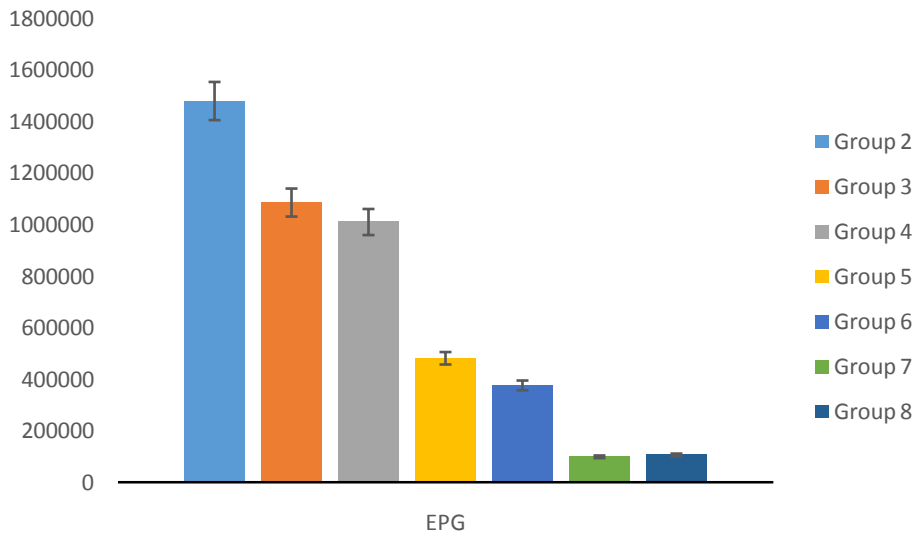
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix II: Mean Egg per gram(EPG) for groups (2-8) at days 35 post challenge.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

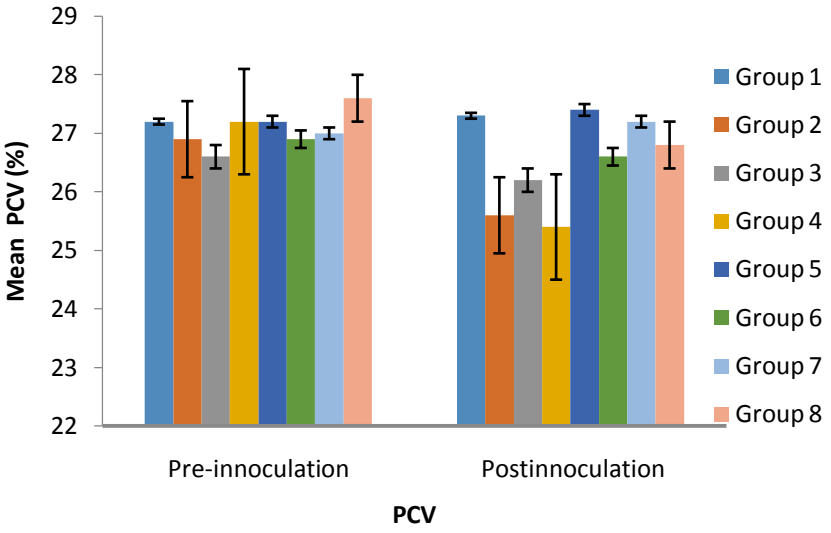
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix III: Changes in mean pack cell volume of all groups for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

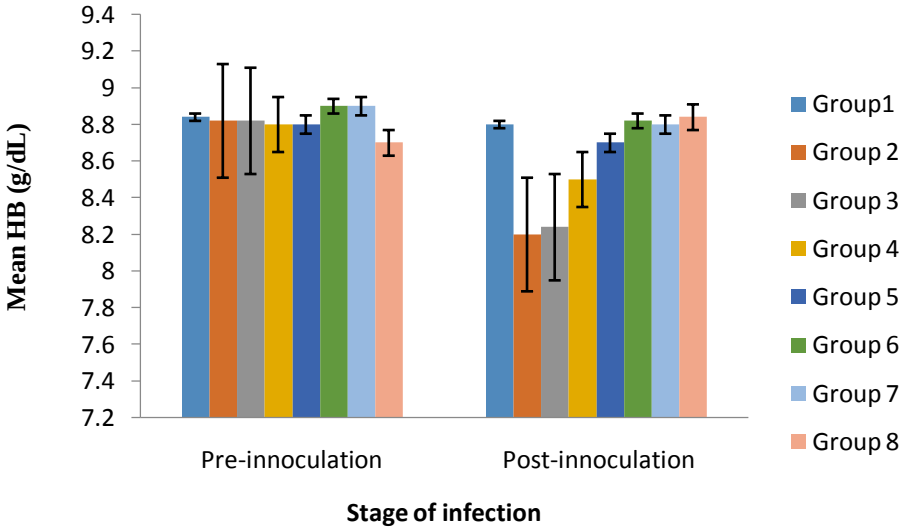
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix IV: Changes in mean haemoglobin values of all groups (1-8) for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

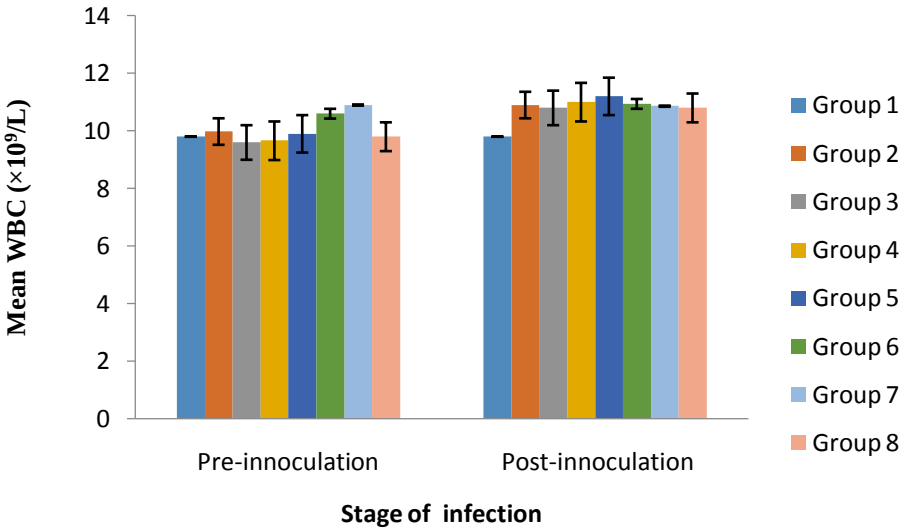
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella.*

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix V: Changes in mean white blood cell values of all groups (1-8) for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

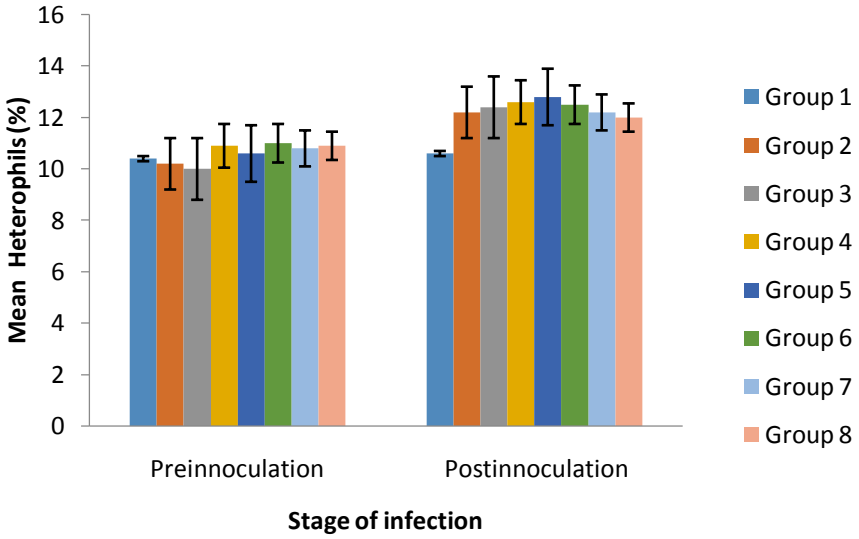
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix VI: Changes in mean heterophil values of all groups (1-8) for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

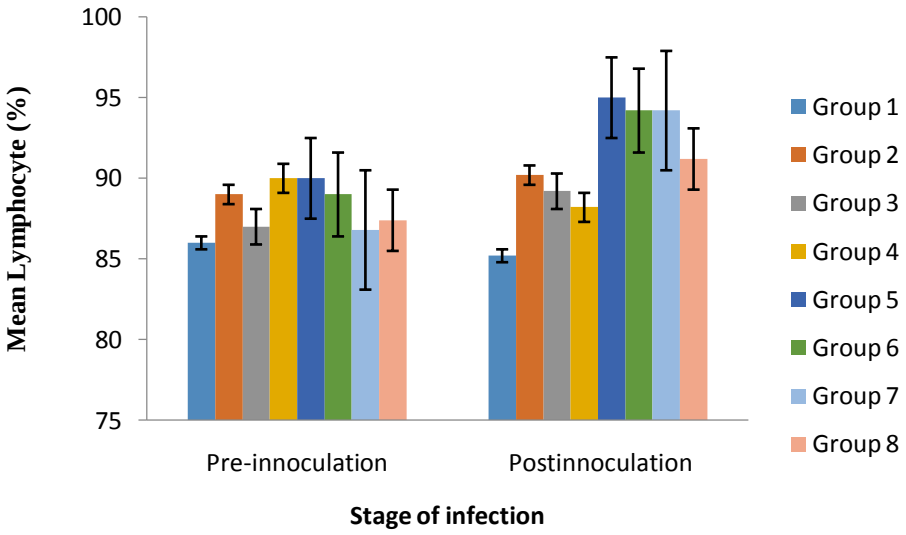
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella.*

Appendix VII: Changes in mean lymphocytymphocytes values of all groups (1-8) for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

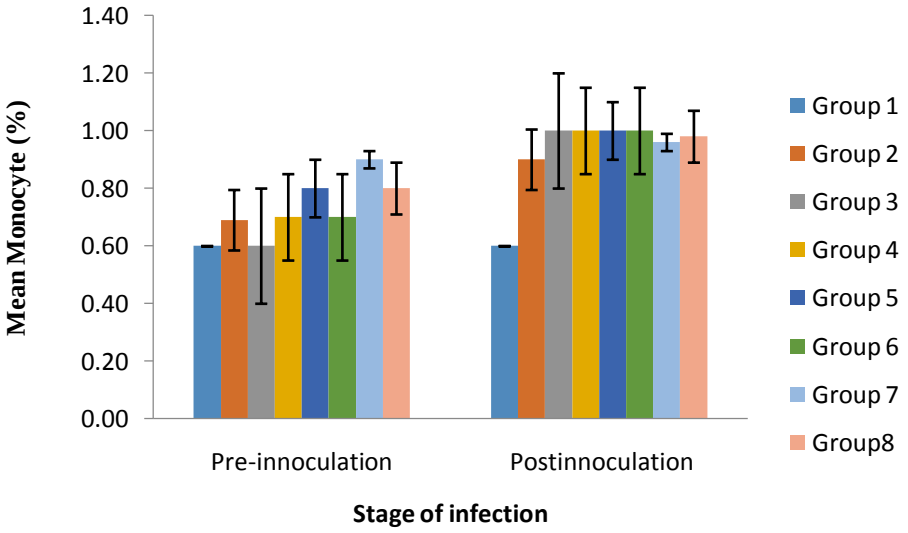
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix VIII: Changes in mean monocytes values of all groups (1-8) for pre infection and post infection.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

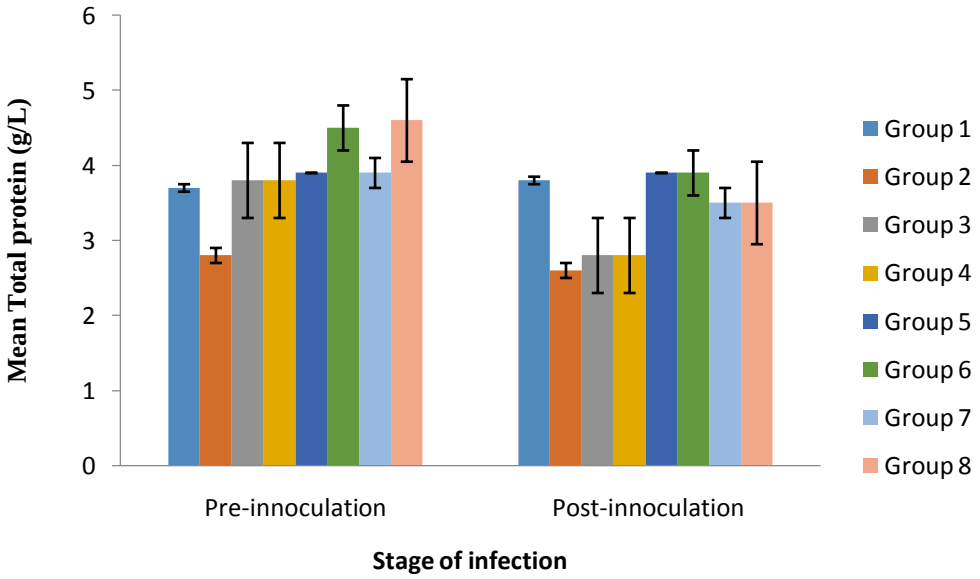
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

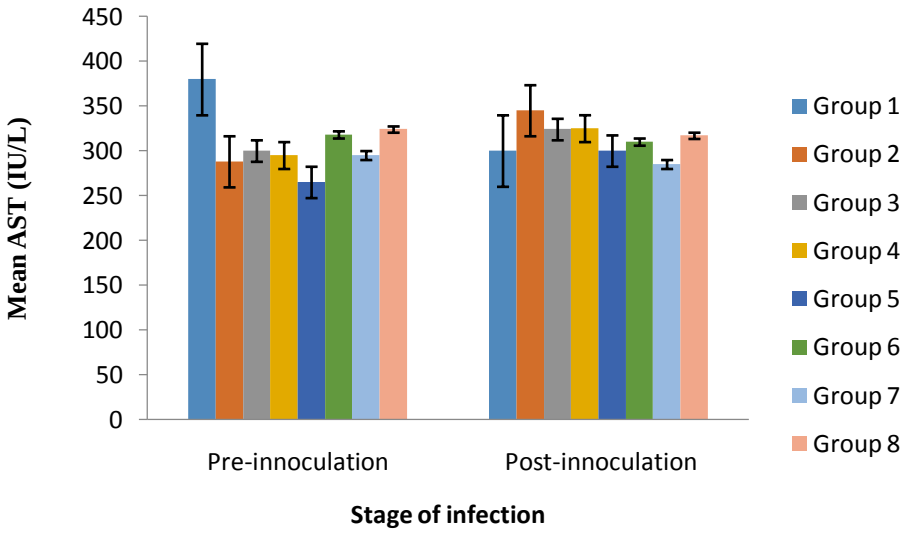
Appendix IX: Changes in mean total plasma protein values of all groups for pre infection and post infection with Local and Houghton strains of *E. tenella* in broilers chickens.



Key:

Group 1	Non-vaccinated/non-challenged control group.
Group 2	Non-vaccinated/challenged with *E. tenella* local isolate control group.
Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix X: Changes in mean serum aspertate amino transferase values of all groups for pre and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.

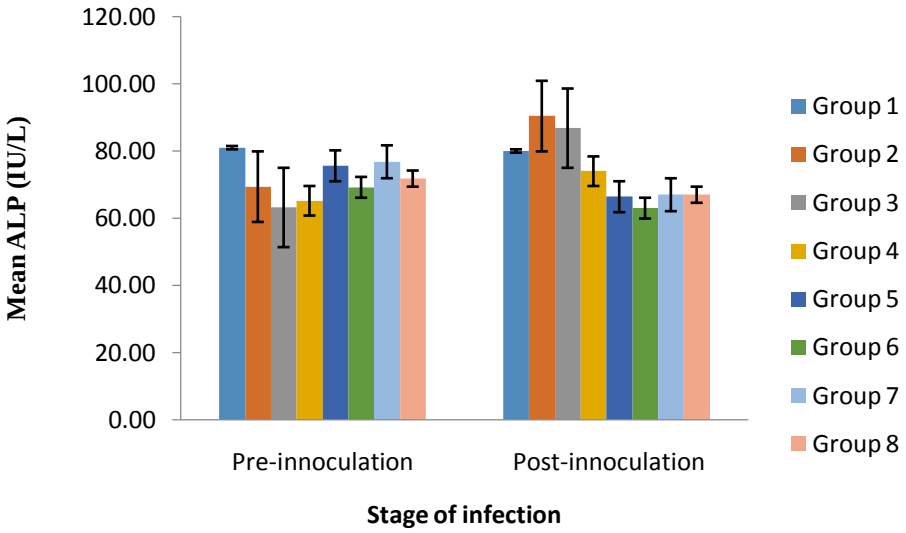


Key:

Group 1	Non-vaccinated/non-challenged control group.
Group 2	Non-vaccinated/challenged with *E. tenella* local isolate control group.
Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix XI: Changes in mean serum alkaline phosphatase values of all groups

for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

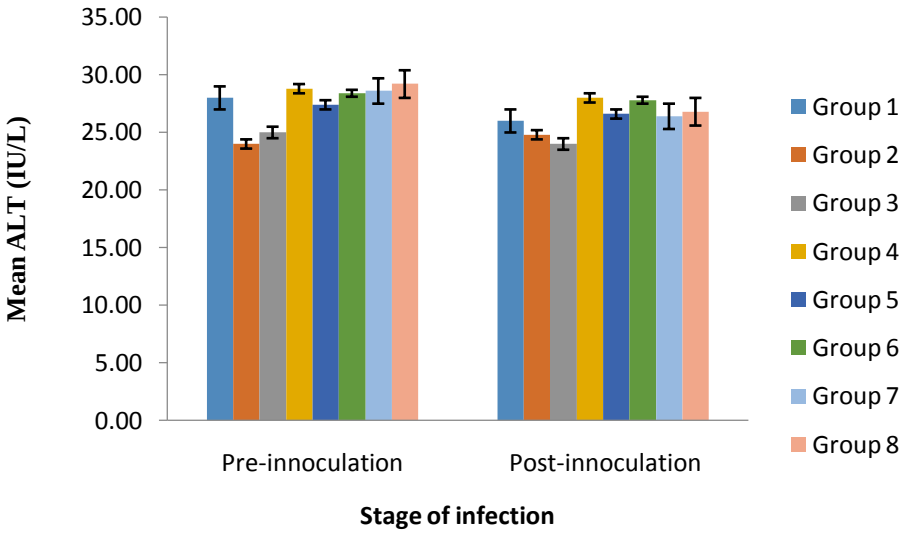
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella.*

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella.*

Appendix XII: Changes in mean serum alanin amino tranferase values of all groups for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

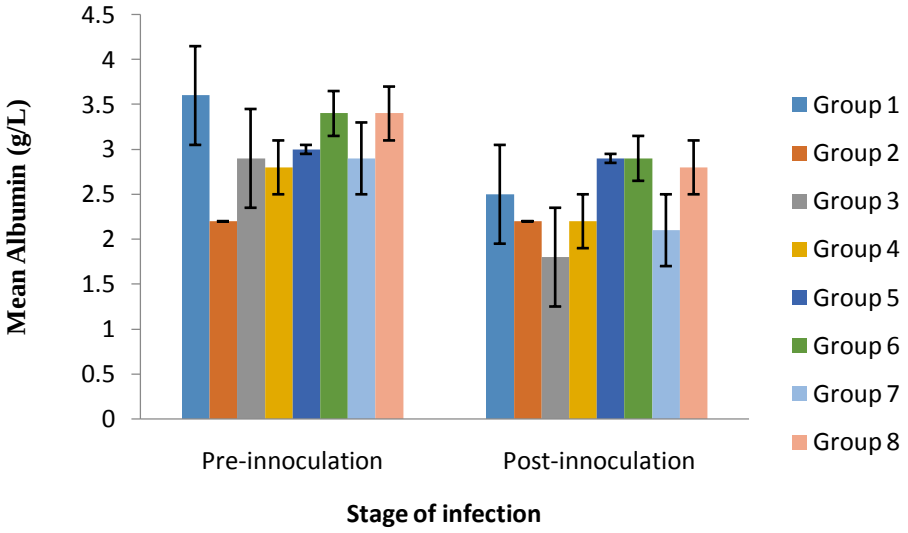
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix XIII: Changes in mean albumin values of all groups for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.

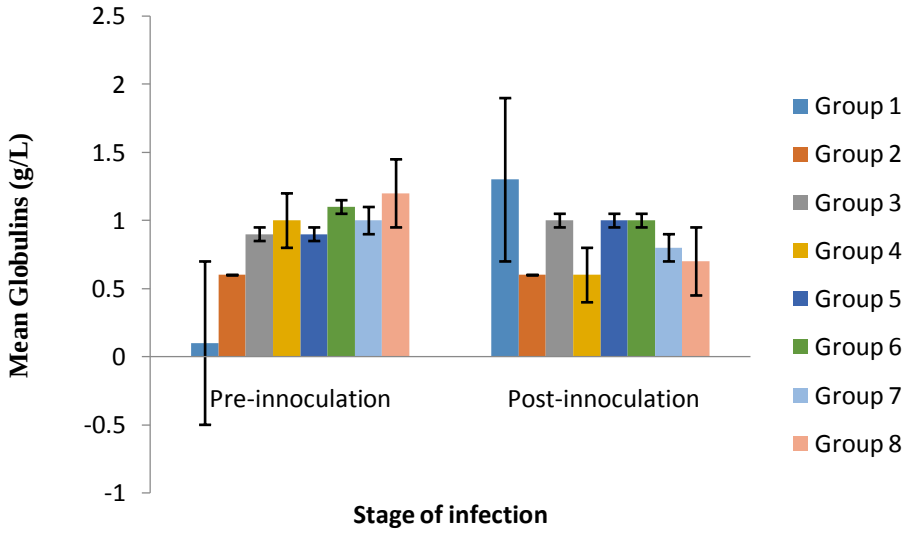


Key:

Group 1	Non-vaccinated/non-challenged control group.
Group 2	Non-vaccinated/challenged with *E. tenella* local isolate control group.
Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella.*
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix XIV: Changes in mean globulin values of all groups for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella.*

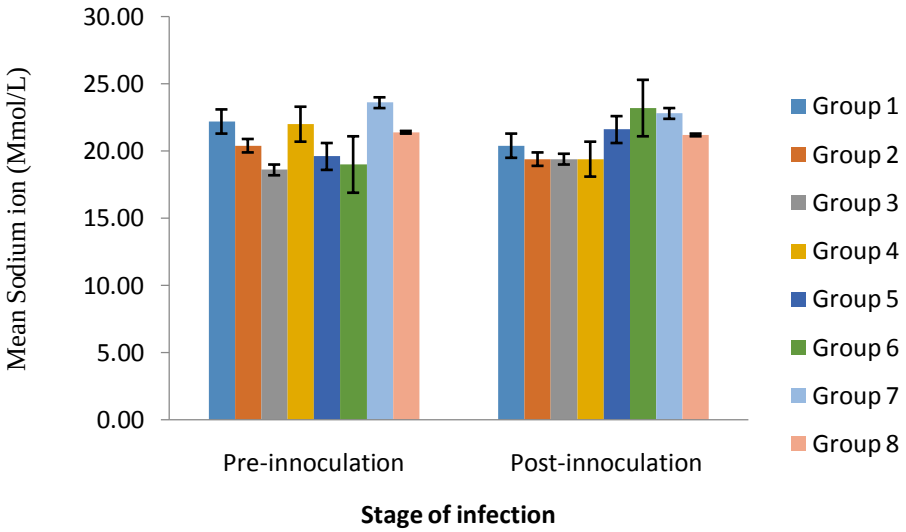
Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton

strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix XV: Changes in mean serum sodium values of all groups for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

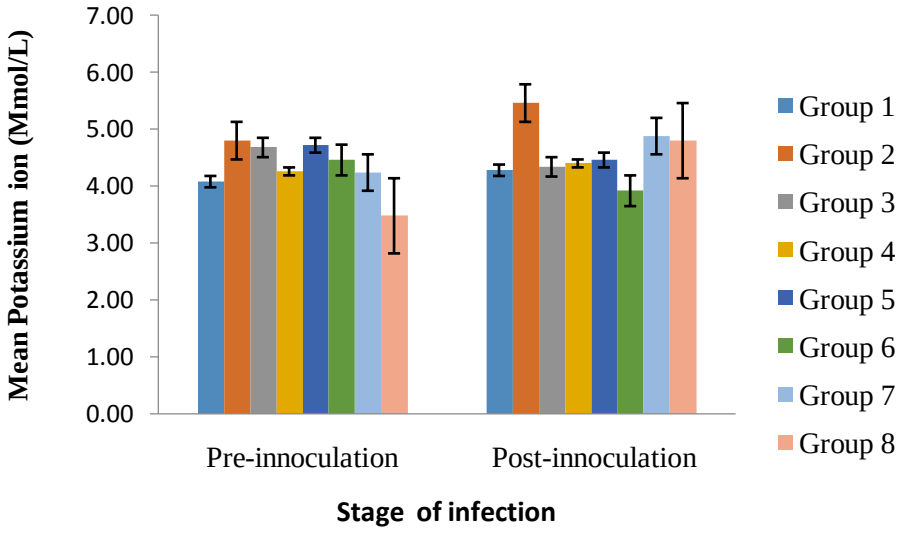
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix XVI: Changes in mean serum potassium values of all groups (1-8) for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

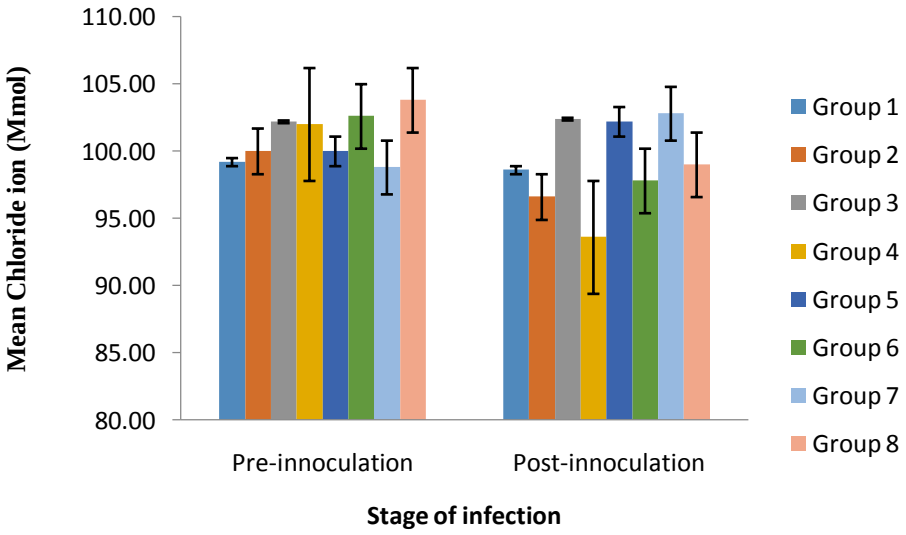
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix XVII: Changes in mean serum chloride values of all groups for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

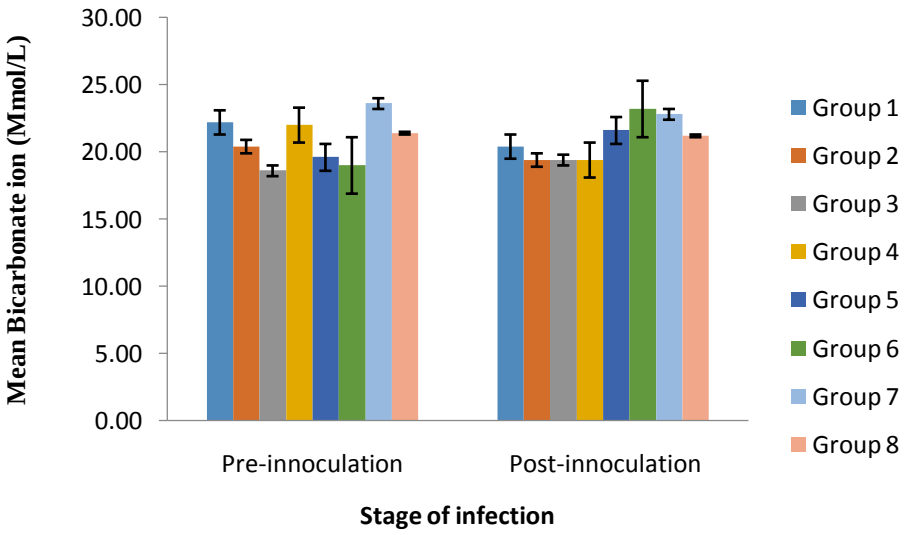
Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella.*

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix XVIII: Changes in mean serum bicarbonate values of all groups for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix XIX: Effects of DNA and Live vaccines against Local and Houghton strains of *Eimeria tenella* on Red Blood Cells

The mean RBC values for pre vaccination (day14) for vaccinated groups 5, 6, 7, 8 were 5.4±0.17, 4.9±0.13, 5.0±0.27, 4.96±0.23 while unvaccinated groups 2, 3, 4 and negative control were 4.9±0.10, 5.2±0.15, 5.48±0.13 and 5.7±2.13. On day 28 post vaccination, vaccinated groups 5, 6, 7, 8 had mean RBC values of 5.14±0.91, 4.98±0.28, 4.96±0.15, 5.1±0.25 while unvaccinated groups 2, 3, 4 and negative control group1 were 4.86±0.31, 4.82±0.38, 4.92±0.36 and 5.02±0.44. On day 35 post infection, vaccinated groups 5, 6, 7, 8 had mean RBC values of 4.9±0.14, 4.8±0.13, 4.82±0.42, 4.94±0.36 while unvaccinated

groups 2, 3, 4 and negative control group1 were 4.6±0.25, 4.64±0.23, 4.7±0.36 and 5.2±0.10 (Table 4.15). There was no statistical significant difference in all groups for pre vaccination, post vaccination and post infection ($p>0.05$). However, the mean RBC values of unvaccinated infected and vector vaccinated infected groups (2, 3 and 4) were slightly lower than the other groups post infection.

Appendix XX: Mean (± SEM) of red blood cell (10^{12}/L) **of broiler chicks vaccinated with DNA and Live oocyst vaccines prior to challenge with local and Houghton strain of *E. tenella***

Group	1 day Pre Infection	7 day Post Infection
1 (n=5)	5.02±0.44[a]	5.2±0.10[a]
2 (n=5)	4.86±0.31[a]	4.6±0.25[a]
3 (n=5)	4.82±0.38[a]	4.64±0.23[a]
4 (n=5)	4.92±0.36[a]	4.7±0.36[a]
5 (n=5)	5.14±0.91[a]	4.9±0.14[a]
6 (n=5)	4.98±0.28[a]	4.8±0.13[a]
7 (n=5)	4.96±0.15[a]	4.82±0.42[a]

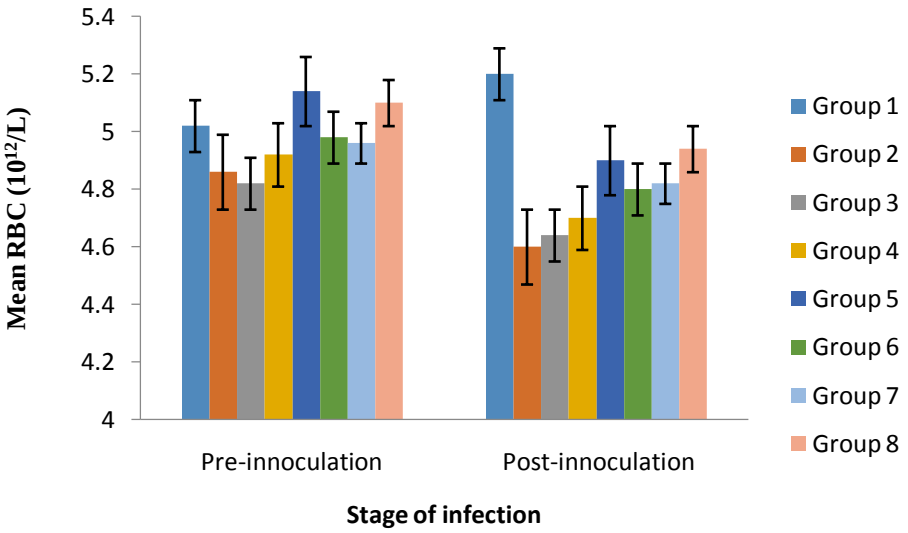
8 (n=5)	5.1±0.25[a]	4.94±0.36[a]

a, b means in the same column with different superscript are significantly different (p >0.05).

Key:

Group 1	Non-vaccinated/non-challenged control group.
Group 2	Non-vaccinated/challenged with *E. tenella* local isolate control group.
Group 3	Non-vaccinated/challenged with *E. tenella* Houghton strain control group.
Group 4	Vector only (pcDNA3.1 (+) vaccinated/challenged control group.
Group 5	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.
Group 6	Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.
Group 7	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella.*
Group 8	Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.

Appendix XXI: Changes in mean Red blood cell values of all groups for pre infection and post infection with Local and Houghton strains of *E. tenella* in broiler chickens.



Key:

Group 1 Non-vaccinated/non-challenged control group.

Group 2 Non-vaccinated/challenged with *E. tenella* local isolate control group.

Group 3 Non-vaccinated/challenged with *E. tenella* Houghton strain control group.

Group 4 Vector only (pcDNA3.1 (+) vaccinated/challenged control group.

Group 5 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with local isolate of *E. tenella*.

Group 6 Experimental group immunized with EtAMA-1 DNA vaccine plasmid in sterile phosphate buffered saline (pH 7.6) prior to challenge with *E. tenella* Houghton strain.

Group 7 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with local isolate of *E. tenella*.

Group 8 Experimental group immunized with commercial anti-coccidial live vaccine prior to challenge with Houghton strain of *E. tenella*.