EVALUATING THE EFFICACY OF SOME UNCONVENTIONAL REMEDIES USED FOR THE TREATMENT OF INFECTIOUS BURSAL DISEASE IN NIGERIA

BY

Johnson Joshua SHALLMIZHILI, DVM (ABU) 1995; MCVSN (Med) 2011; FCVSN (Med) 2015
(P13VTVM8005)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN AVIAN MEDICINE

DEPARTMENT OF VETERINARY MEDICINE

AHMADU BELLO UNIVERSITY ZARIA,

NIGERIA

APRIL 2018

DECLARATION

I declare that the work in this Dissertation entitled "**EVALUATING THE EFFICACY OF SOME UNCONVENTIONAL REMEDIES USED FOR THE TREATMENT OF INFECTIOUS BURSAL DISEASE IN NIGERIA**" has been carried out by me in the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University under the supervision of Prof. P. A. Abdu, Prof. S. B. Oladele and Dr. A. M. Wakawa. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree, diploma or certificate at any institution.

.....	.....	.....
Name of student	**Signature**	**Date**

CERTIFICATION

This Dissertation titled **EVALUATING THE EFFICACY OF SOME UNCONVENTIONAL REMEDIES USED FOR THE TREATMENT OF INFECTIOUS BURSAL DISEASE IN NIGERIA** by Johnson Joshua SHALLMIZHILI meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Professor P. A. Abdu Chairman, Supervisory Committee	_____ Signature	_____ Date
Professor S. B. Oladele Member, Supervisory Committee	_____ Signature	_____ Date
Dr. A. M. Wakawa Member, Supervisory Committee	_____ Signature	_____ Date
Professor P. A. Abdu Head, Department of Veterinary Medicine Ahmadu Bello University Zaria	_____ Signature	_____ Date
Professor A. Z. Abubakar Dean, School of Postgraduate Studies Ahmadu Bello University Zaria	_____ Signature	_____ Date

DEDICATION

This work is dedicated to God Almighty, and in loving memory of my beloved father, late Mr Barnabas Hyelngunasunyi Deburrh Gwani Shallmizhili who saw the beginning of this journey but did not live to see the end. I will also like to dedicate this work to my uncle late Mr Joseph Tagwi Kwala, my aunty Mrs Rifkatu J. Dibal and late Mrs Mairam Robert whose contributions at the most critical point in my educational life made this happen without knowing.

ACKNOWLEDGEMENTS

I will like to express my profound gratitude to those that contributed in making this work a reality. Many thanks to the Chairman of my supervisory committee, Professor P. A. Abdu who have been more than just a supervisor to me, he is my mentor, his guidance and devotion of time in thoroughly and painstakingly scrutinizing the entire work and provision of essential materials that were useful in the course of the work is most appreciated. The interest shown by Prof. S. B. Oladele my second supervisor is highly appreciated. His personal supervision of the research birds and the time taken to personally view the histopathology slides, advices, directions given and many more are most appreciated. To my third supervisor, Dr. A. M. Wakawa who has been a brother and a source of encouragement to me, I am most greatful. I am gtaeful to Prof. M. M. Suleiman of the Department of Veterinary Pharmacology, who also provided technical assistance to this work. I appreciate all my postgraduate lecturers for imparting knowledge on me.

My unalloyed devotion and thanks goes to those who sponsored this research work through donations of research materials, they include- 1. Grand cereals and oil meals limited (GCOML) for donating chicken feeds used in this research. 2. Animal care services Konsult for donating Polidine and V-Ox used in this research. 3. Turnerwright Limited for donating Heparenol used in this research. 4. Professor Ezeibe for donating the Aluminium magnesium silicate (AMS) used in this work and 5. Prof. Sa'idu Lawal for his contributions in making this research work a success. I urge that they continue the work of assisting/sponsorship of research works in Ahmadu Bello University and other institutions in Nigeria.

My thanks goes to Mr. David Leo, Mr. Musa Adejo of the Department of Veterinary Medicine, Mr. Edima Obaja, Mal. Sale Iliya of the Veterinary Teaching hospital, Mr Bitrus Bako of the Department of Veterinary Pathology, Mr. Ibrahim Kwano of Animal Science Department for their assistance during the course of sampling and the laboratory work together with others not mentioned, they made my work easier. I also appreciate the Head (Prof. P. A Abdu) and staff of the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. Lest I forget, I also appreciate all my postgraduate colleagues for their support.

To my wife who has been devoted and hardworking, special thanks for all the support. To the beautiful children God gave me- Ashilti, Avida, Hyelchiya and Wovinahyel, they are the most precious gift ever in my life, they have made me a proud dad and I will always be there to encourage and push them into greatness by the grace of God, I am full of gratitude. My mother, though aging, but surprisingly looking strong and young, she has been a mother indeed. Her love and believe in me are always appreciated. To my siblings, whose encouragement, support and love have made me persistent in my work, I am thankful. I am most thankful to my cousin, Mr. Ayuba Gwani for his love and interest. I must also thank all staff of Unity Veterinary Clinic for their dedication and commitment to duty.

Now unto the King Eternal, the only Living God, the God of all grace, who hath called me to his eternal glory by Christ Jesus, after I have suffered a while, having gone through the rigours of this study, will make me perfect, establish, strengthen and settle me. To him be glory and dominion forever and ever. Amen.

ABSTRACT

Infectious bursal disease (IBD) is endemic in Nigeria despite vaccination. Due to absence of effective chemotherapy against IBD, farmers and practitioners use remedies which they claim are effective. The study evaluated the efficacy of some of the remedies in mitigating the effects of very virulent IBD virus (vvIBDV) using 360 day-old ISA brown pullets divided into groups A to I of 40 chicks each. Twenty chicks from each group were bled at 2, 7, 14, 21, 24, 28, 31 and 35 days of age to determine the IBD antibody (Ab) titre levels using enzyme linked immune-sorbent assay (ELISA). Chicks in all groups, except negative control were innoculated with vvIBDV at 21 days of age. At 21 days of age, group A was treated with *Khaya senegalensis* extract, group (B) was treated with vitamin E, group (C) heparenol®, group (D) aluminium magnesium silicate, group (E) polidine®, group (F) V-ox® 1 and group (I) V-ox® 2, while groups (H) positive control and (G) negative control were not treated. Clinical signs, morbidity and mortality rates in inoculated groups were observed for 10 days post inoculation (dpi). Five chicks from 20 tagged birds in each group were selected and euthanized on 0, 3, 7 and 10 days post-inoculation (dpi). Euthanized chicks were examined and proportion of chicks with lesions calculated. The bursa of Fabricius (BF), spleen (SP) and thymus (TH) were weighed. The organs were processed for histopathology. Clinical signs were less severe in *Khaya senegalensis* (15.60%), Vitamin E (24.70%) and V-Ox® 2 (14.10%) groups when compared with positive group (50.70%). Morbidity rates were lower in *Khaya senegalensis* (18.18%), Vitamin E (37.50%), heparenol® (27.27) and V-Ox® 2 (25.00%) when compared with positive control (54.54%). Similarly, mortality was lower in *Khaya senegalensis*, vitamin E, and V-Ox® 2 groups (27.27%, 27.27% and 16.67%, respectively) compared to positive control (59.09%). There was reduction in the rate of atrophy of BF in *Khaya*

senegalensis (2.7%), heparenol® (1.5%), polidine® (16.8%) and V-Ox® 2 (18.4%) groups at 7 dpi compared with an increase in positive control (34.9%). There was higher splenic weight increase in vitamin E (68.3%), aluminium magnesium silicate (41.2%), V-Ox® 1 (54.8%) and V-Ox® 2 (51.3%) than positive control group (21.7%). By 7 dpi, thymic weight increased in aluminium magnesium silicate (25.5%) and V-Ox® 1 (8.5%) groups but decreased in positive control (-8.1%), polidine® (-32.2%) and V-Ox® 2 (48.6%) groups. The BF of *Khaya senegalensis*, vitamin E and positive control groups showed depletion of lymphocytes and severe necrosis, vacuolation and haemorrhages in *Khaya senegalensis* group and atrophy in vitamin E and positive control groups. The SP of vitamin E and V-Ox® 2 groups showed depletion of lymphocytes and haemorrhages in vitamin E group. TH of *Khaya senegalensis*, vitamin E and V-Ox® 2 groups also showed depletion of lymphocytes in medulla, necrosis and haemorrhages in *Khaya senegalensis* and positive control groups. Chicks in all groups had protective Ab titre level of ≥ 1,000 ELISA units at days 1 and 7 of age but, following vvIBDV inoculation, 28.57% of chicks in only vitamin E group had Ab titre level of ≥ 1,000 ELISA units by day 28 (7 dpi). In conclusion KS, vitamin E and V-ox® 2 reduced severity of clinical signs, morbidity and mortality, while vitamin E, polidine® and V-ox® 1 reduced gross lesions due to IBD. *Khaya senegalensis*, vitamin E, polidine® and V-ox® 2 reduced BF atrophy. It was recommended that Veterinarians could administer KS or vitamin E to mitigate the effects of IBD. Chicks derived from the breeders used in this study should be vaccinated against IBD by 17 days of age. Further research should be conducted to evaluate the safety of *K. senegalensis*.

TABLE OF CONTENTS

CONTENTS **PAGE**

TITLE PAGE……i

DECLARATION ii

CERTIFICATION iii

DEDICATION iv

ACKNOWLEDGEMENTS v

ABSTRACT vi

TABLE OF CONTENTS....Viii

LIST OF TABLES....xii

LIST OF PLATES xiiiii

LIST OF FIGURES....xiv

LIST OF APPENDICES....xv

LIST OF ABBREVIATIONS AND SYMBOLS....xvii

CHAPTER 1....1

1.0 INTRODUCTION 1

1.1 Introduction 1

1.2 Background Information 3

1.3 Statement of Research Problems 4

1.4 Justification of the Study 5

1.5 Aim of the Study 5

1.6 Objectives of the Study 5

1.7 Research Questions 6

CHAPTER 2....7

2.0 LITERATURE REVIEW 7

2.1 Infectious Bursal Disease 7

2.1.1 Definition and synonyms....7

2.1.2 History of infectious bursal disease....8

2.1.3 Aetiology of infectious bursal disease 8

2.1.4 Morphology of infectious bursal disease virus....10

2.1.5 Structure and genome composition of infectious bursal disease virus 10

2.1.6 Biological properties of infectious bursal disease virus 14

2.1.7 Infectious bursal disease virus replication 14

2.1.8 Resistance and susceptibility of infectious bursa disease virus to physical and chemical agents 20

2.1.9 Epidemiology of infectious bursal disease 21
2.1.10 Pathogenesis of infectious bursal disease ..... Error! Bookmark not defined.6
2.1.11 Clinical signs of infectious bursal disease 269
2.1.12 Pathology due to infectious bursal disease 29
2.1.13 Immunity to infectious bursal disease 32
2.1.15 Differential diagnosis of infectious bursal disease 49
2.1.16 Treatment of infectious bursal disease 49
2.1.17 Prevention and control of infectious bursal disease 50
2.1.18 Economic impact of infectious bursal disease 58
2.2 Phytochemical agents used in infectious bursal disease treatment 59
2.2.1 Medicinal plants 59
2.2.2 *Khaya senegalensis* 60
2.3 Chemicals agents used in the treatment of infectious bursal disease 61
2.3.1 Vitamin E 61
2.3.2 Heparinol® 64
2.3.3 Aluminum magnesium silicate 68
2.3.4 Polidine® 70
2.3.5 V-ox® 71
CHAPTER 3 74
3.0 MATERIALS AND METHODS 74
3.1 Study Location 74
3.2 Experimental Chicks 74
3.3 Management of Chicks 75
3.4 Infectious Bursal Disease Virus 75
3.5 Unconcventional Agents Used for the Treatment of Infectious Bursal Disease 755
3.5.1 *Khaya senegalensis* 755
3.5.2 Vitamin E 766
3.5.3 Chemicals 77
3.6 Enzyme Linked Immunosorbent Assay Kit 777
3.7 Proximate and Phytochemical Analyses of Feed and Medicinal Plant Leaves. 78
3.7.1 Proximate analysis of feed and medicinal plant leaves 78
3.7.2 Phytochemical screening of plant leaves 78
3.8 Inoculation of ISA Brown chicks with Infectious Bursal Disease Virus 788
3.9 Collection of Blood Sample 79
3.10 Detection of Infective Infectious Bursal Disease Virus in the Bursa of Inoculated ISA Brown Chicks Using Agar Gel Precipitin Test 79

3.10.1 Agar gel preparation 79

3.10.2 Agar gel precipitin test 80

3.11 Clinical Studies of ISA brown chicks Inoculated With Very Virulent Infectious Bursal Disease Virus 80

3.11.1 Clinical signs 80

3.12 Pathological Studies of ISA Brown Pullets Inoculated With Very Virulent Infectious Bursal Disease Virus 81

3.12.1 Gross pathology 81

3.12.2 Histopathology 81

3.13 Enzyme-Linked Immunosorbent Assay 82

3.14 Experimental Design 83

3.15 Data Analyses 83

CHAPTER 4 87

4.0 RESULTS 87

4.1 Nutrient Composition of Feed and *Khaya senegalensis* leaves 87

4.2 Phytochemical Constituents of *Khaya senegalensis* leaves 87

4.3 Clinical Signs in ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies 87

4.4 Morbidity, Mortality and Case Fatality Rates in ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies 88

4.5 Presence of Infectious Bursal Disease Virus Precipitin Antigens 89

4.6 Gross Pathological Lesions Score in ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies 89

4.7 Body weights of ISA brown chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies 90

4.8 Mean Weights of Bursa of Fabricius of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies 96

4.9 Mean Weights of Spleen Weight of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies 96

4.10 Mean Weights of Thymus of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies 97

4.11 Bursa of Fabricius to Body Weight Ratio of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies 98

4.12 Spleen to Body Weight Ratio of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies 99

4.13 Thymic to Body Weight Ratio of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies.....99

4.14 Organ to Body Weight Index in ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies.....100

4.15 Percent (%) of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies having Antibody Titre Level of 1,000 ELISA Titre and Above.....104

4.16 Microscopic Lesions in ISA brown chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies 106

4.16.1 Histopathology of the bursa of fabricius 106

4.16.2 Histopathology of the thymus.....106

4.16.3 Histopathology of spleen 106

CHAPTER 5.....116

5.0 DISCUSSION.....116

CHAPTER 6.....124

6.0 CONCLUSION AND RECOMMENDATIONS 124

6.1 Conclusion.....124

6.2 Recommendations.....125

6.3 Limitations of the Study.....125

6.4 Contribution to Knowledge 126

REFERENCES 127

APPENDICES.....154

LIST OF TABLES

Table **Title** **Page**

3.1 Experimental design for testing the efficacy of some unconventional remedies used for the treatment of infectious bursal disease in Nigeria. 855

4.1 Severity of clinical signs in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.911

4.2 Morbidity rates in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies. 922

4.3 Mortality rates in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies. 933

4.4 Morbidity, mortality and case fatality rates in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.....944

4.5 Summary of gross pathological lesion score in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.....955

4.6 Organ and body weights of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.1011

4.7 Organ/body weight ratios of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies. 1022

4.8 Organ/body weight indexes of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.....1033

4.9 Percent (%) of chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies that have antibody titre levels of 1,000 enzyme linked immunosorbent assay units and above.....1055

LIST OF PLATES

Plate **Title** **Page**

I Photomicrograph of bursa of Fabricius from chick seven days postinoculation (pi) with vvIBDV and oral administration of vitamin E showing depletion And necrosis (a) and atrophy (b) of the bursal follicles.....107

II Photomicrograph of bursa of Fabricius from a positive control chick at day seven post inoculation with vvIBDV showing depletion and necrosis (a) and atrophy (b) of the follicles.....108

III Photomicrograph of bursa of Fabricius from a chick seven days post inoculation with vvIBDV and oral administration of *Kaya senegalensis*, showing depletion (a), severe necrosis (b), vacuolation (c) and haemorrhages (d) of the follicles.....109

IV Photomicrograph of thymus of group H (positive control) from a chick at day seven pi showing haemorrhages (a) and depletion of lymphoid cells in the medullary portion (b).....110

V Photomicrograph of thymus from a chick seven days postinoculation (pi) with vvIBDV and oral administration of vitamin E (group B) at seven days pi showing depletion (a), necrosis (b) and haemorrhages (c).....111

VI Photomicrograph of thymus from a chick at seven days post inoculation with vvIBDV and oral administration of *Kaya senegalensis* (group A) showing depletion of lymphoid cells (a) in the medullary portion.....112

VII Photomicrograph of section of spleen of group G (negative control) chicks at day seven showing mild depletion of lymphoid cells (a).....113

VIII Photomicrograph of spleen from a chick seven days postinoculation (pi) with vvIBDV and oral administration of V-Ox®2 (group I) showing mild depletion of lymphoid cells (a).....114

IX Photomicrograph of spleen from a chick ten days post inoculation (pi) with vvIBDV and oral administration of vitamin E (Group B) showing depletion (a) and haemorrhages (b).....115

LIST OF FIGURES

Figure	Title	Page
2.1:	Diagrammatic representation of infectious bursal disease virion Indicating A: VP 1, 2 and 3 viral proteins B: T=13….....……	12
2.2:	A diagrammatic representation of infectious bursal disease virus viral genome showing viral genome and gene expression (segments A and B)…….	13
2.3:	A schematic representation of viral transcription and translation of reproductive cycle (a and b)……..……	17
2.4:	Schematic representation of Infectious bursal disease viral multiplication cycle.....	18

LIST OF APPENDICES

Appendix	Title	Page
1	Photograph of *Khaya senegalensis* tree – (A) and leaves – (B)	154
2	Proximate composition of *Khaya senegalensis* leaves	155
3	Concentration of phytochemicals in *Khaya senegalensis* leaves	156
4	Phytochemical test on in *Khaya senegalensis* leaves	157
5	Summary of phytochemicals in *Khaya senegalensis* leaves	158
6	Morbidity and mortality rates in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies	159
7	Severity of clinical signs, morbidity and mortality in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies	160
8	Body weight of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies	162
9	Gross lesions score in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies	163
10	Mean weight of bursa of Fabricius of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies	164
11	Mean weight of spleen of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies	165
12	Mean weight of thymus of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies	166
13	Bursa of Fabricius/body weight ratio of Isa brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies	167

14 Spleen/body weight ratio of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.....168

15 Thymus/body weight ratio of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.....169

16 Geometric mean titre of antibody titre levels of Isa brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.....170

17 Mean antibody titre level of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.....171

18 Percent coefficient of variation (%CV) of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.....172

19 Clinical signs data generation sheet for ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.....173

20 Postmortem data generation sheet for ISA brown chicks inoculated with very virulent infectious bursal diseasevirus and treated with some unconventional remedies.....174

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage (s)
µg	Microgram
µl	Microlitre
µm	Microns
AA	Amino acids
Ab	Antibody
ABU	Ahmadu Bello University
AC-ELISA	Antigen capture- enzyme linked immunosorbent assay
Ad-lib	*ad libitum*
AGID	Agar gel immunodifussion
ALT	Alanine aminotransferase
Am	Ante-meridian
AMS	Aluminium magnesium silicate
ANOVA	Analyses of variance
ANVISA	Agência Nacional De Vigilância Sanitária
AOAC	Association of Official Analytical Chemist
APS	Ammonium per sulphate
AVA(S)	Antiviral agent (s)

BBI	Bursa/body weight index
BBWR	Bursa to body weight ratio
B-cells	B-lymphocytes
BF	Bursa of Fabricius
Bp	Base pair
C_4	Carbon 4
CALT	Conjunctiva associated lymphoid tissue
CAM	Chorio-allantoic membrane
CAV	Chicken anaemia virus
CBN	Central Bank of Nigeria
cDNA	Complementary DNA
CEF	Chicken embryo fibroblast
CFEDUSAHA	Committee on Foreign and Emerging Diseases of the US Animal Health Association
CFR	Case fatality rate
CIA	Chicken infectious anaemia
CID_{50}	Chicken mean infectious dose
cm^2	Centimetre square
CO_2	Carbon dioxide
CPE	Cytopathic effect

CV	Coefficient of variation
DAHSE	Dupont Animal Health Solutions Europe
DNA	Dioxyribonucleic acid
DOC	Day Old Chicks
dpi	Days post-inoculation
dsRNA	Double stranded riboxyribonucleic acid
E	East of Greenwich Meridian
EFA	Essential fatty acids
ELD_{50}	Embryo mean lethal dose
ELISA	Enzyme linked immunosorbent assay
et al	And others
FAT	Florescent antibody test
FDA	Food and Drug Administration
FNBIM	Food and Nutrition Board, Institute of Medicine
g	Grams
GALT	Gut associated lymphoid tissues
GCOML	Grand Cereals and Oil Meals Limited
GDP	Gross domestic product
GLP	Good laboratory practice

GSH-PX	Glutathione peroxide
H & E	Hematoxylin and Eosin
h	Hour (s)
HALT	Head associated lymphoid tissues
HMPC	Herbal Medical Products Committee
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
IBP	ISA brown pullets
IBS	Irritable bowel syndrome
kb	Kilobyte
kbp	Kilobase pair
KDa	Kilo daltons
LAH	Lohmans Animal Health
M	Matrix
m^2	Metre squared
MAb	Monoclonal antibody
MALT	Mesentery associated lymphoid tissues
MARFA	Ministry of Agriculture, Rural Development, Forestry and Aquaculture

MDA	Maternally derived antibody
MGLS	Minimal gross lesions seen
ml	Millilitre (s)
mm	Millimetre (s)
N	North of the Greenwich Meridian
NACL	Sodium chloride
NAS	National Academy of Science
NBSCS	Number of birds showing clinical signs
NCSO	Number of clinical signs observed
NBD	Number of birds dead
NGLS	No gross lesions seen
nm	Nanometre
NBS	Number of birds sick
^{o}C	Degree Celsius
OH	Hydroxyl group
OIE	Office Internationale des Epizootics
OMFRA	Ontario Ministry of Agriculture Food and Rural Affairs
ORF	Open frame reading
PBS	Phosphate buffered saline

PHS	Physiologic saline
PI	Postinoculation
RNA(S)	Ribonulceic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SAN	Specific antigen negative
SBWI	Spleen/body weight index
SBWR	Spleen/body weight ratio
SDCN	Subcommittee on Dairy Cattle Nutrition
SEM	Standard error of mean
SGL	Severe gross lesions
SI	Severity of illness
SN	Serum neutralisation
SP	Spleen
SPF	Specific pathogen free
SPSS	Statistical Package for Social Sciences
TBWI	Thymus/body weight index
TBWR	Thymus/body weight ratio
TH	Thymus
TMG	Trimethyl glycerine

TMVM	The Merck Veterinary Manual
TNB	Total number of birds
TNCS	Total number of clinical signs
USA	United States of America
UV	Ultra Violet
V/W	Volume by weight
VN	Virus Neutralisation
VP1	Viral protein 1
VP2	Viral protein 2
VP4	Viral protein 4
VSGL	Very severe gross lesions
VTH	Veterinary Teaching Hospital
vvIBDV	Very virulent infectious bursal disease virus
w/v	Weight by volume
WHO	World Health Organization
χ^2	Chi squared

CHAPTER ONE

INTRODUCTION

1.1 Introduction

In Nigeria, poultry occupies a pivotal position among livestock–based vocations, because of its enormous potential to bring about rapid economic growth. The importance of the poultry sub-sector is chiefly in the provision of meat and eggs, employment either directly or indirectly and the contribution to the revenue (gross domestic product) of the country (Afolabi *et al.,* 2013). The poultry sector accounts for about 58.2% of the total livestock production (Amos, 2006). The poultry industry in Nigeria currently is responsible for less than 15% to 18% employment opportunities, due to the fact that the industry is mainly subsistent (Afolabi *et al.,* 2011). The poultry sub-sector offers the quickest returns to investment outlays in livestock enterprise by virtue of its short gestation period, high feed conversion ratio alongside being one of the cheapest, commonest and best sources of animal protein in the country (Ojo, 2003). Poultry production is the most efficient and cost-effective way of increasing the availability of high-protein food, as eggs are known to provide the most perfectly balanced food containing all the essential amino acids, minerals and vitamins (Branckeart *et al.,* 2000). Production of eggs and poultry occupies a prime position for improving animal protein consumption of both rural and urban households (Aihonsu and Sunmola, 1999). In Nigeria, poultry contributes about 15% of the total annual protein intake with approximately 1.3 kg of poultry products consumed per head per annum (Ologbon and Ambali, 2012). The poultry industry in Nigeria has greatly expanded over the years holding the greatest promise in affordable animal protein supply, food security and poverty alleviation in both rural and urban populace (Musa *et al.,* 2012).

The importance of poultry to the national economy cannot be overemphasized, as it has become a popular industry for the smallholders that make great contribution to the economy of the country.

In the past decades, there has been improvement in poultry production in Nigeria with its share of the gross domestic product (GDP) increasing in absolute terms. It was reported that the contribution of poultry egg and meat to the livestock share of the GDP increased from 26% in 1995 to 27% in 1999 with an increase in egg production alone accounting for about 13% during the period (Ojo, 2003). It contributed approximately 4.45% of the total livestock contribution to the agricultural GDP in 2004 (CBN, 2004). In spite of the significance of the poultry industry to the national economy, poultry farms face challenges inimical to the growth of the industry. Poultry production in general is facing low capital base, inefficient management, disease and parasite, housing and marketing problems, etc (Alabi *et al.,* 2000). Constraints especially of diseases and in particular, Gumboro disease have rendered this investment fearful and unrealistic to both organizations and individuals (Okoye, 1983; Abdu, 1986; Lukert and Saif, 1997; Islam and Samad, 2004; Musa *et al*., 2010). Diseases remain one of the major threats to boosting the poultry production in Nigeria (Adewole, 2012). The major diseases are Newcastle disease, avian influenza, avian pox, IBD, colisepticeamia, coccidiosis and worm infestation (Usman and Diarra, 2008). Also, economic losses experienced by poultry farmers for the years 2009-2011 was estimated to amount to over three billion Nigerian Naira due to IBD outbreaks alone (Musa *et al*., 2012). Infectious bursal disease is a major setback to productivity and profitability in the poultry industries of both developing and industrialized nations (Shane *et al*., 1994).

To this end, an up-to-date knowledge of the diseases, particularly IBD affecting the industry will go a long way in bridging some knowledge gap and help in formulating policies aimed at ensuring increased and more profitable poultry production in the country.

1.2 Background Information

Infectious bursal disease (also known as Gumboro disease) has been reported throughout the world, and the socio-economic significance of the disease is considerable world-wide. For many years, IBD has constituted a serious problem for the poultry industry and the 're-emergence' of the infectious bursal disease virus (IBDV) in the form of antigenic variants and hypervirulent strains has been the cause of significant losses (van den Berg *et al.,* 2000). In Nigeria, IBD has continued to be a serious challenge to the commercial poultry industry with the occurrence of outbreaks in both vaccinated and non-vaccinated flocks (Abdu, 1997; Ezeokonkwo, 1997; Oyekunle and Adeniji, 2008). The disease affects young chickens and is characterized by high morbidity, mortality, atrophy of the bursa of Fabricius (BF) and immunosuppression. It is a highly contagious and acute viral disease that is characterized by destruction of lymphoid cells in the BF (Ashraf, 2005). Direct losses are linked to specific mortality, and depend on the dose and virulence of the strain, the age and breed of the chickens, and the presence or absence of passive immunity (van den Berg *et al*, 2000). The indirect economic impact of the disease is also considerable, due to virus-induced immunosuppression and/or potential interactions between IBDV and other viruses, bacteria, fungi or parasites. These indirect losses are due to secondary infections, growth retardation and condemnation of carcasses at the slaughterhouse.

Moreover, the increased use of antibiotics against secondary infections constitutes a growing public health concern.

Gumboro disease is of socio-economic importance causing loses due to high mortality, poor feed conversion and growth with subsequent poor egg production (van den Berg, 2001). It also affects negatively, response to treatment and vaccination against other diseases (Giambrone *et al*., 1976; Ivanyi and Moris, 1976; van den Berg, 2001; Paul *et al.*, 2004). Moreover, the infection, when not fatal, leads to a degree of immunosuppression which is often difficult to measure (van den Berg, 2001). Finally, the control measures used are subject to variations and seldom follow a specific or standardized plan.

1.3 Statement of Research Problems

The emergence of IBDV in the form of antigenic variants and hypervirulent strains has been the cause of significant economic losses due to IBD (van den Berg *et al*., 2000; Luque *et al*., 2007). Infectious bursal disease outbreaks globally and in Nigeria results in high mortality and immunosupression (Abdu *et al*., 2001). To date, there is no known chemotherapeutic agent that is effective in the control of IBD (OMFRA, 2015). However, some farmers and Veterinarians have administered different chemicals which may be ineffective and harmful but they claimed cure without empirical evidence.

1.4 Justification of the Study

Despite biosecurity and vaccination, outbreaks of IBD still occur causing anxiety among poultry farmers and animal health workers (Ivanyi and Morris, 1976; Okoye and Uzoukwu, 1981; Abdu, 1988; Abdu *et al.,* 2001; Luque *et al*., 2007; Michell *et al.,* 2009) hence the need to find other forms of remedy. Although some farmers and animal health workers have claimed to cure IBD using chemicals as remedies, research have not been conducted to validate such claims. It is on this premise that six IBD remedies used in the field were selected and tested for their efficacy. Any of the remedies found to be effective, would be recommended for inclusion in the treatment of IBD in Nigeria.

1.5 Aim of the Study

The aim of the study was to evaluate the efficacy of some unconventional remedies used in the field for the treatment of IBD in Nigeria.

1.6 Objectives of the Study

The objectives of the study were; to determine the ability of some unconventional remedies to:

1. Mitigate the severity of clinical signs, morbidity and mortality due to vvIBDV in chicks.
2. Mitigate the pathology due to vvIBDV in chicks.
3. Potentiate IBD antibody response in chicks.

1.7 Research Questions

The research questions were; does the administration of some unconventional remedies to chicks:

1. Mitigate the severity of clinical signs, morbidity and mortality due to vvIBDV?

2. Mitigate the pathology due to vvIBDV?

3. Potentiate IBD antibody response in chicks?

CHAPTER TWO

LITERATURE REVIEW

2.1 Infectious Bursal Disease

2.1.1 Definition and synonyms

2.1.1.1 Definition

Infectious bursal disease is an infectious and highly contagious acute viral disease of 3-6 weeks old chickens (Ashraf, 2005). The disease occurs globally affecting the immune system of poultry, and is characterized by high morbidity, mortality and the destruction of the lymphoid organs, in particular the BF, where B lymphocytes mature and differentiate (Abdu *et al*., 1987). The target cell of the virus is the B lymphocyte in an immature stage, and the infection, when not fatal, causes an immunosuppression, in most cases temporary, the degree of which is often difficult to determine (van den Berg *et al*., 2000; Mittal *et al*., 2005). The disease in young chicks of 3-6 weeks is characterised by high mortality, but a less acute or subclinical disease is common in 0-3-week-old chicks. Subclinical IBD can cause secondary problems due to the effect of the virus on the BF (Mittal *et al*., 2005; OIE, 2008). There are two distinct serotypes of the IBDV; serotype 1 and 2. Serotype 1 IBDV causes clinical disease in chickens younger than 10 weeks. Older chickens rarely show clinical signs (Mittal *et al.,* 2005; OIE, 2008). Serotype 2 antibodies are very widespread in turkeys and are sometimes found in chickens and ducks. There are no reports of clinical disease caused by infection with serotype 2 IBDV (OIE, 2008).

2.1.1.2 Synonyms

The synonyms of IBD are: Gumboro disease, infectious bursitis and infectious avian nephrosis (Cosgrove, 1962; Alexander, 1999; LAH, 2004).

2.1.2 History of infectious bursal disease

The first report of a specific disease affecting the BF in chickens was made by Cosgrove in 1962 (Cosgrove, 1962). The first cases were observed in the area of Gumboro, in Delaware (United States of America [USA]), which is the origin of the name of the disease; although the terms 'IBD' or 'infectious bursitis' are more accurate descriptions (van den Berg, 2000). Between 1960 and 1964, the disease affected most regions of the USA and reached Europe in the years 1962 to 1971. From 1966 to 1974, the disease was identified in the Middle East, southern and western Africa, India, the Far East and Australia (van den Berg, 2000). Infectious bursal disease is currently an international problem: including New Zealand which had been free of the disease until 1993 (van den Berg, 2000). The disease was first reported in Nigeria by Ojo *et al.*, in 1973, since then however, IBD has become endemic causing high economic losses due to mortality and immunosupression (Ojo *et al*., 1973; Onunkwo, 1975; Nawathe *et al*., 1978; Abdu, 1988; van den Berg *et al.*, 2000; Abdu *et al*., 2001; Ibrahim *et al*., 2003; Oladele *et al*., 2004; Mbuko *et al.*, 2010; Abdu and Musa, 2014).

2.1.3 Aetiology of infectious bursal disease

Infectious bursal disease is caused by a non-enveloped, single capsid of icosahedral symmetry, bi-segmented genome, double stranded RNA virus that belongs to the genus

Avibirnavirus of family *Birnaviridae* with a diameter of about 55-60 nm (Muller *et al*., 1979; Ismail and Saif, 1990; van den Berg, 2000; Caston *et al*., 2001; Mittal *et al*., 2005; Luque *et al*., 2007; Eterradossi and Saif, 2008; Abdu and Musa, 2014). This relatively simple structure renders the virus very resistant to the outside environment (van den Berg, 2000). Serotype 2 though apathogenic, have been isolated from chickens and turkeys (van den Berg *et al.*, 1991; van den Berg, 2000; Ahad, 2002; Ashraf, 2005; Mittal *et al*., 2005; TMVM, 2006; Luque *et al*., 2007). Within serotype 1, many subtypes or pathotypes have evolved (Ahad, 2002). The two serotypes are differentiated *in vitro* by the absence of cross-neutralisation, and *in-vivo*, by the absence of cross-protection (McFerran *et al*., 1980; Jackwood *et al*., 1982; Jackwood *et al*., 1984; Ismail *et al*., 1988).

2.1.3.1 Classification of infectious bursal disease virus

Viruses of both serotypes 1 and 2 IBDV share common group antigens that can be detected by fluorescent antibody test (FAT) and by ELISA (Jackwood *et al*., 1982). The common (group) antigens for both serotypes have serotype-specific group antigens that induce VN antibodies (Azad *et al*., 1987; Becht *et al*., 1988). Pathogenic serotype 1 IBDV field strains can be grouped into classical, antigenic variant and very virulent (vv) strains (Brown *et al.*, 1994). In addition to serological classification, the viral strains may be classified according to virulence (mortality and bursal lesions). Thus, strains of IBDV may be considered apathogenic, attenuated (vaccines), classical virulent, variant, or hypervirulent (vvIBDV).

2.1.4 Morphology of infectious bursal disease virus

Infectious bursal disease virus is non-enveloped and has a single capsid shell of icosahendral symmetry composing of 32 isomers (van den Berg and Meulemans, 1991). The genome of IBDV is composed of two segments of double stranded RNA, hence the name *Birnavirus* (Muller and Becht, 1982), it consists of a larger and a smaller particle. The larger particle measures up to 55-60 nm in diameter and are hexagonal in outline while the smaller particle measures up to 18-22 in diameter and is smaller in size (Ahad, 2002; Mittal *et al*., 2005). The longer segment A is 3.2 Kb in length while the shorter B segment is 2.8 Kb. Segment A is 3261 base pair (bp) in serotype 1 and 3264 in serotype 2 while segment B is 2827 bp (Muller *et al*., 1979).

2.1.5 Structure and genome composition of infectious bursal disease virus

2.1.5.1 Infectious bursal disease viral structure

The IBD virion is spherical (Figure 2.1), with a diameter of ≈650-700 Å, and consists of segments A and B and 5 encoded mature proteins (VP1 to VP5) (Figure 2.2) (Mittal *et al.*, 2005; Luque *et al*., 2007). Most of these proteins are encoded by segment A, which has 2 partially overlapping open reading frames; the first one encodes a dispensable non-structural protein, VP5 (17 kDa), and the second encodes three proteins synthesized as a polyprotein (110 kDa). Segment B has a single open reading frame encoding VP1 (98 kDa), the RNA-dependent RNA polymerase (Luque *et al.,* 2007). Whereas the VP3 is considered as a group specific antigen as it is recognized by monoclonal antibodies directed against VP3 from strains of both serotypes 1 and 2 (van den Berg *et al.,* 2000; Mittal *et al*., 2005), VP4 is involved in the processing of 110 kDa precursor polyprotein

(Alexander, 2003). VP2 and VP3 are the major structural proteins of the virion. VP2 is major host protective antigen of IBDV and contains the antigenic region responsible for the induction of antibodies (Eterradossi *et al.*, 1997), VP2 is the major structural protein involved in viral capsid formation, cell entry and induction of protective immune responses (Azad *et al*., 1985; Hudson *et al*., 1986; Becht *et al*., 1988; Jagadish *et al*., 1988; Murphy *et al*., 1995; Boot *et al*., 2000; Lombardo *et al*., 2000; Caston *et al*., 2001).

2.1.5.2 Infectious bursal disease virus genome composition

Among the viral proteins, VP2 is the major structural protein involved in viral capsid formation, cell entry and induction of protective immune responses, VP2 protein contains important neutralizing antigenic sites and elicits protective immune response and most of the amino acids (AA) changes between antigenically different IBDVs are clustered in the hypervariable region of VP2. Thus this hypervariable region of VP2 is the obvious target for the molecular techniques applied for IBDV detection and strain variation studies (Caston *et al*., 2001; Caston *et al*., 2008). The IBDV capsid protein exhibits structural domains that show homology to those of the capsid proteins of some positive-sense single-stranded RNA viruses, such as the nodaviruses and tetra viruses, as well as the T = 13 capsid shell protein of the Reoviridae (Caston *et al*., 2001; Caston *et al*., 2008) (Figure 2.2). The IBDV capsid (65–70 nm in diameter) is a single shell built by 260 trimers of the VP2 polypeptide arranged in an icosahedral lattice with T = 13 symmetry (Delgui *et al*., 2009). VP2 is produced by proteolytic maturation of a precursor polypeptide, known as pVP2. VP2 is the only component of the virus capsid, and thus responsible to the interaction with host cell receptors. The T=13 shell of the IBDV capsid is formed by

trimers of VP2, a protein generated by removal of the C terminal domain from its precursor, pVP2 (Delgui *et al*., 2009). The trimming of pVP2 is performed on immature particles as part of the maturation process. The other major structural protein, VP3, is a multifunctional component lying under the T = 13 shell that influences the inherent structural polymorphism of pVP2. The virus-encoded RNA-dependent RNA polymerase, VP1, is incorporated into the capsid through its association with VP3. VP3 also interacts extensively with the viral dsRNA genome (Caston *et al*., 2008) (Figure 2.2).

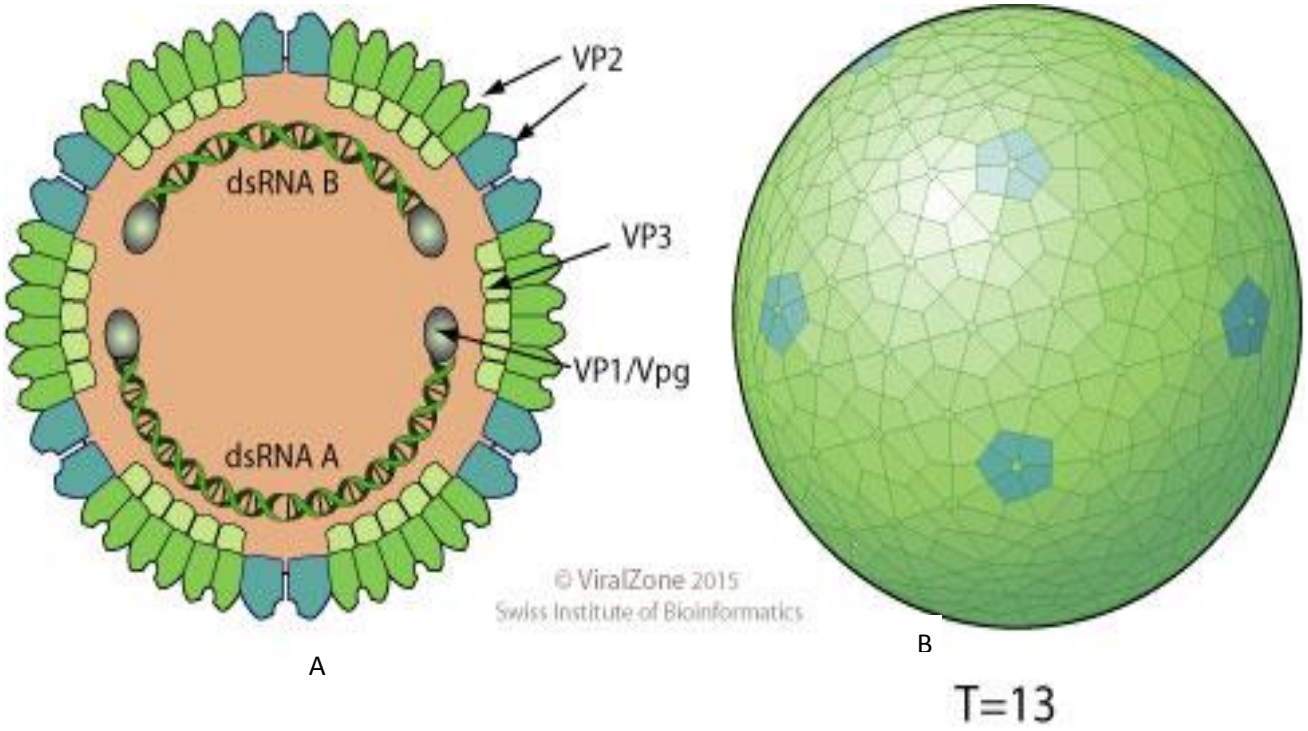
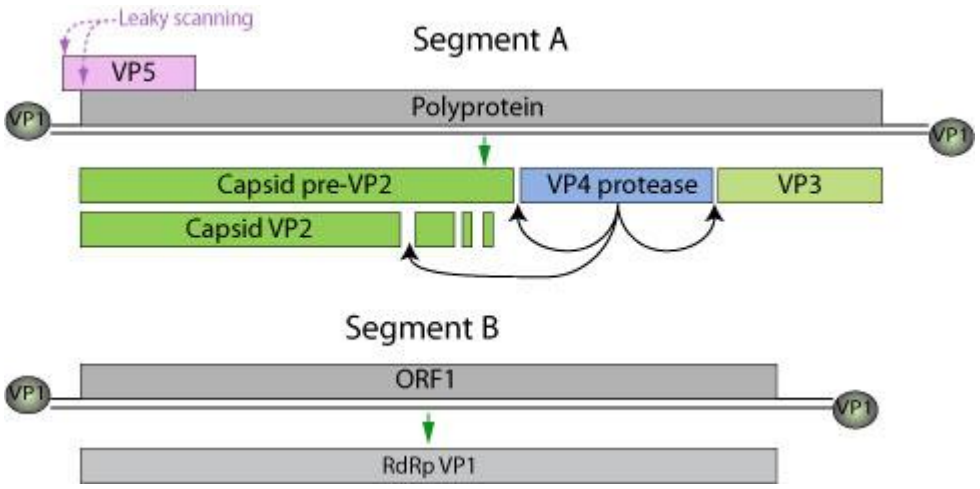


Figure 2.1: Diagrammatic representation of infectious bursal disease virion indicating A: VP 1, 2 and 3 viral proteins, B: T=13. (Viral zone, 2015).

Key: VP 1, 2, 3 = viral proteins

Non enveloped, single-shelled T=13 icosahedral symmetry capsid of about 70 nm in diameter, composed of 260 trimers of VP2 that form spikes projecting radially from the capsid. The peptides derived from pre-VP2 C-terminal cleavages remain associated within virion. VP3 forms a ribonucleoprotein complex with the genomic RNA. Minor amounts of VP1 are also incorporated in the virion.



Viral genome

Figure 2.2: A diagrammatic representation of infectious bursal disease virus viral genome showing viral genome and gene expression (Segments A and B) (Viral zone, 2009).

Key: VP 1, 2, 3 = viral protein; ORF = open reading frame.

Segmented linear dsRNA genome: 2 segments (A, B) encode for 5-6 proteins. VP1 is found in a free form and covalently attached at the 5' genomic RNA end (VPg). Segments size is about 2.3-3 kb. Genome total size is about 6 kb.

Gene expression

Genomic segment A encodes for a structural polyprotein which is matured in *cis* by VP4. It also encodes an alternative ORF translated possibly by leaky scanning (VP5). Genomic segment B encodes for VP1 *Swiss Institute of Bioinformatics* (Viral zone, 2015).

2.1.6 Biological properties of infectious bursal disease virus

2.1.6.1 Antibody neutralisation activity of infectious bursal disease virus

Glycoprotein VP2 trimers from IBDV constitute the external surface of the mature virus capsid, containing the antigenic regions responsible for elicitation of neutralizing antibodies (Fahey *et al.*,1989; Zhu *et al.,* 2008). This property (cross virus neutralisation test) is used for detecting various serotypes and for viral virulence (Zhu *et al.*, 2008).

2.1.7 Infectious bursal disease virus replication

2.1.7.1 Virus attachment and penetration

Virus attachment is the first step in virus infection. The distribution of a virus receptor mainly determines the cell and tissue tropism of the virus and the site of pathology associated with infection (Ta-Wei *et al*., 2007). Virus receptors are cell surface molecules that mediate binding (attachment and adsorption) and/or trigger membrane fusion or entry through other processes. Infectious bursal disease virus entry into host cells occurs by recognition of specific cellular receptors with viral envelope glycoprotein which comprises the initial and key step of infection and infection can be inhibited by blockage of this process. Glycoprotein VP2 trimmers from IBDV constitute the external surface of the mature virus capsid, containing the antigenic regions responsible for elicitation of neutralising antibodies (Zhu *et al.*, 2008).

Based on the atomic structure of the viral particles the external domain of the VP2 trimmers exists as ‘protrusions’ on the capsid surface and is believed to be responsible for receptor binding (Zhu *et al*., 2008). That glycoprotein VP2, responsible for the recognition

of corresponding receptor, has been certificated in Vero cells on the molecular level (Zhu *et al*., 2008 citing Yip *et al.,* 2007). Immature surface immunoglobulin M (SIgM)-bearing B lymphocytes are the target cells of infection by the virus (Zhu *et al.,* 2008). SIgM is necessary as a signal molecule for the processes that occur after virus attachment, such as penetration uncoating and replication. It is certified that chick embryo fibroblasts (CEF), B lymphocytes and other permissive cells have the same binding sites for both vvIBDV and attenuated IBDV while certain IBDV can only replicate in a specific kind of cells (Zhu *et al*., 2008). More than one molecule is involved in the process of virus attachment (Zhu *et al*., 2008). As a result of attachment and/or membrane penetration IBDV induces k^+ current changes with respect to both the kinetics of activation and inactivation and the ca^{2+} dependence of the activation. These changes are time dependent (Repp *et al.,* 1998) (Figure 2.3).

2.1.7.2 Infectious bursal disease virus transcription, penetration and replication

After attachment to the cellular receptor and penetration, the initiation of viral RNA synthesis occurs through a semi-conservative strand displacement mechanism and involves the VP1 molecule serving as primer and polymerase for chain elongation (van den Berg, 2000). Regulated expression of viral genes is essential as a precursor-product relationship demonstrated for the synthesis of VP2, VP3 and VP4 proteins. Several specific sequences, distributed throughout the genome is suspected to be playing a crucial role in the signalling of the viral cycle by the constitution of primary (promoters, enhancers, binders sites) or secondary (stem and loops) recognition structures (van den Berg, 2001). The 32-nucleotide consensus sequence at each 5' end, the conserved pentamer at 3', the direct terminal and inverted repeats and several palinromic sequences have been identified (van den Berg,

2001). The viral polyprotein cotranslationally processed by the viral protease VP4 rendering the VP2 precursor termed pVP2 (512 residues 54.4 kDa, VP3 (29 kDa), and VP4 (27 kDa) (Luque *et al.,* 2007). Most of the pVP2 C-terminal region is further processed at three Ala-Ala bonds, which are secondary VP4 targets (positions 487, 494 and 503) the resulting intermediate pVP2 is again cleaved, by an unknown mechanism, between residues 441 and 442, giving the mature VP2 polypeptide (47 kDa) (Luque *et al.,* 2007). The released C-terminal peptides remain inside the capsid. VP2 (and a residual amount of pVP2) representing about 50% of the total protein, is the structural component of the capsid, and VP3 which is about 40% is a multifunctional protein that interacts with pVP2, dsRNA, VP1 and itself (Luque *et al*., 2007). Each modification in these structures could influence the viral cycle, the host specificity and the pathogenicity of the strain (van den Berg, 2001).

2.1.7.3 Replication of infectious bursal disease virus in the cytoplasm

Infectious bursal disease virus replicates in the cytoplasm without being exposed to the cytoplasm (Figure 2.4). This is summarized as follows:

1. Virus penetrates into the cytoplasm.
2. Transcription of the dsRNA genome by viral polymerase occurs inside the virion, so that dsRNA is never exposed to the cytoplasm. This plus-strand transcript is used as template for translation.
3. (+) RNAs are encapsulated in virion particle, inside which they are translated to give RNA (-) molecules with which they become base-paired to produce dsRNA genomes.
4. Mature virions are released by budding.

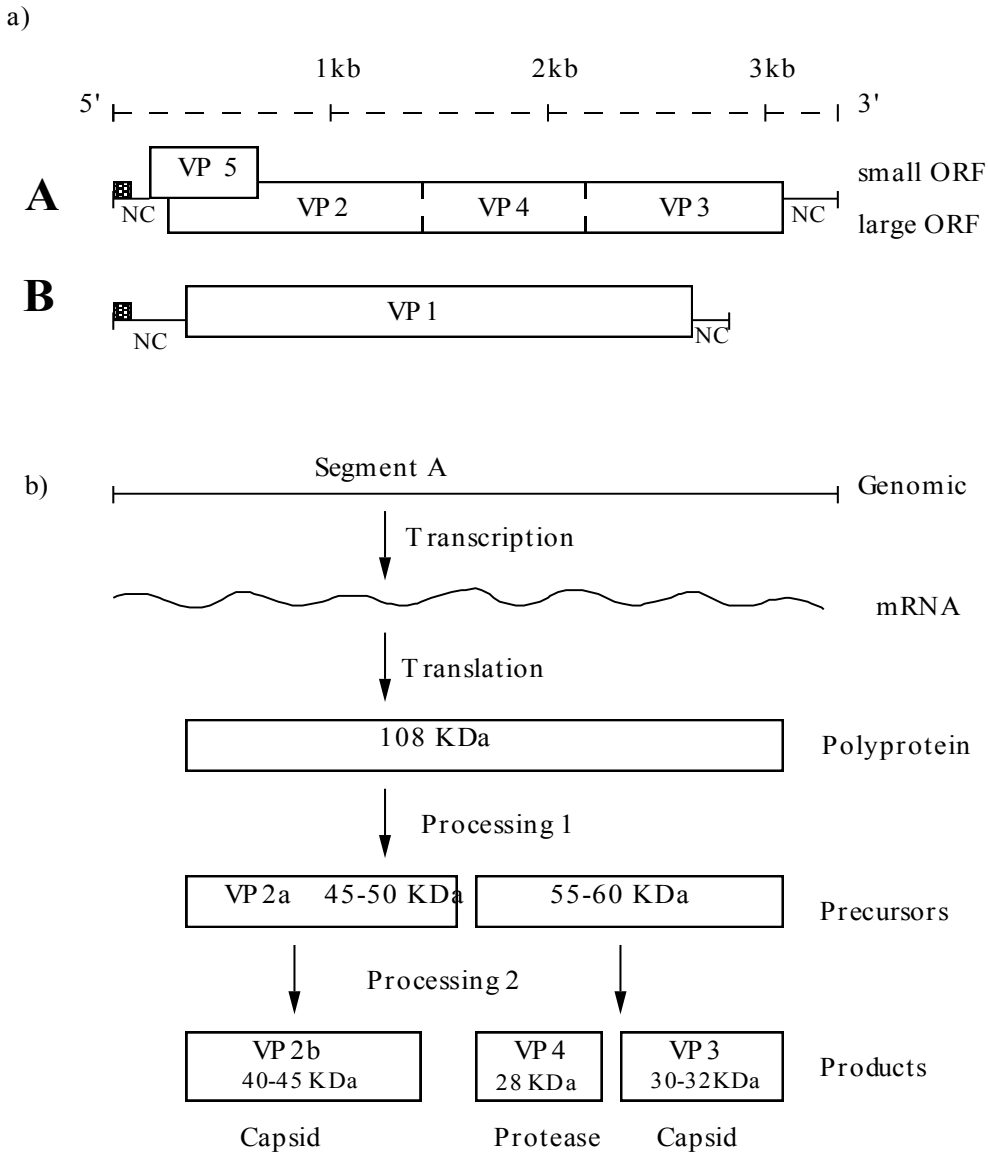


Figure 2.3: A schematic representation of viral transcription and translation of reproductive cycle (a and b) (van den Berg, 2000).

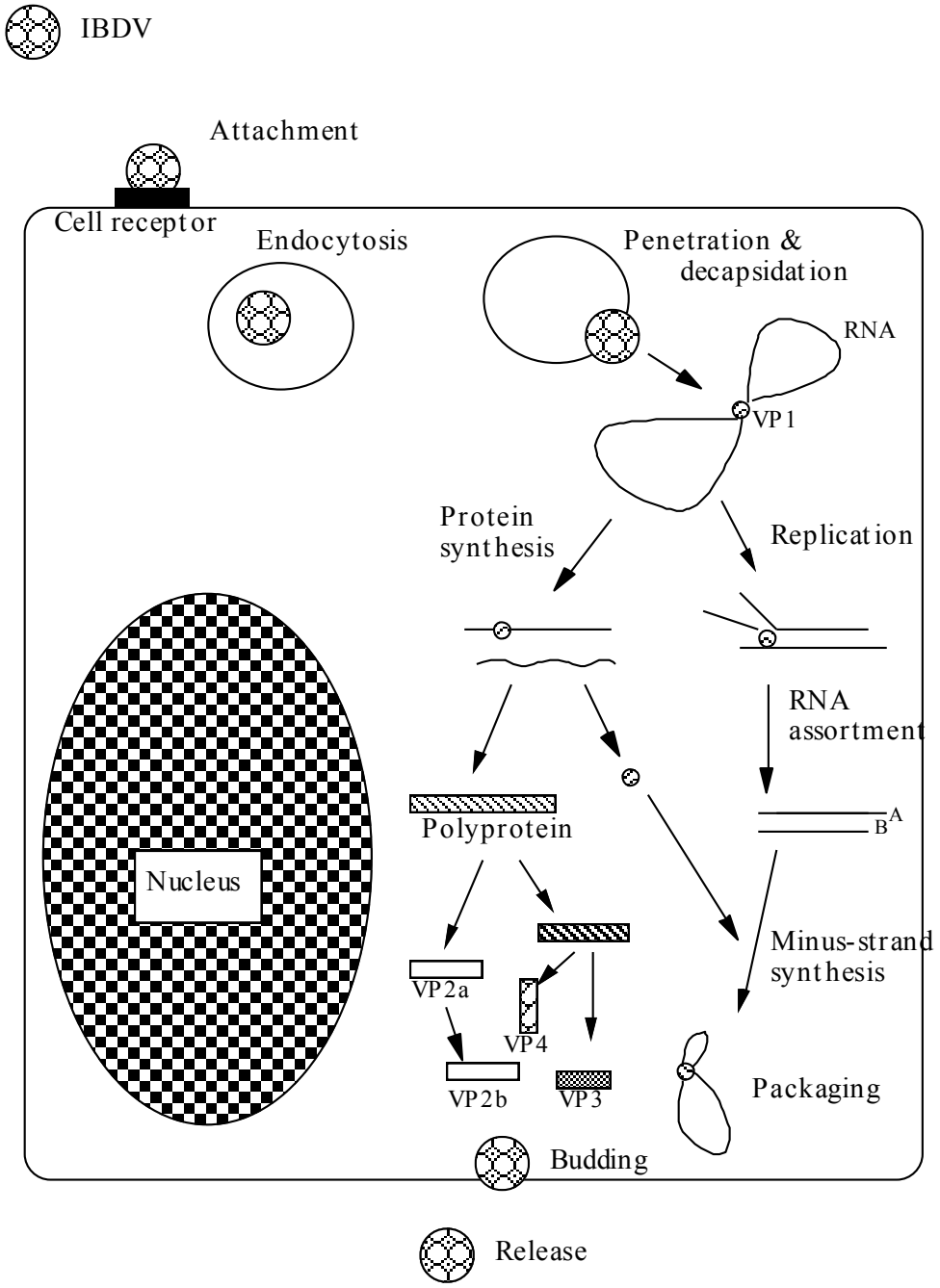


Figure 2.4: Schematic representation of Infectious bursal disease viral multiplication cycle (van den Berg *et al.*, 2000).

2.1.7.4 Virus assembly, maturation and release

Complex capsids require one or more auxiliary proteins (scaffold, accessory, and proteolytic proteins) acting as morphogenic factors to trigger structural changes (Luque *et al.,* 2007). During capsid assembly, VP2 is synthesised as a protein precursor, called pVP2, whose 71-residue C-terminal is proteolitically processed. VP3 plays numerous roles during the viral cycle and acts as a scaffolding protein required for assembly control (Luque *et al.,* 2007). Expression of mature VP2 results in the spontaneous assembly of icosahedral T-1 subviral particles (SVP) with an all-pentamer capsid, where as pVP2 or intermediate pVP2 variant expression leads to the assembly of tubular structure with a hexagonal lattice (Luque *et al.,* 2007). The pVP2 C-terminal domain, a 71 residue sequence is responsible for allowing the formation of multiple VP2 conformations; specifically, this ability resides within the amphipathic α-helix at residue 443 to 445. VP3 also participates in the inherent polymorphism of pVP2 through the interaction with the pVP2 C-terminal end working as a canonical scaffolding protein. In the absence of VP3, efficient T=13 capsid-like assembly can be achieved but requires a His tag bound covalently at the VP2 N-terminus (chimeric protein His-VP2-466; 46 residues), which probably mimics VP3 function during viron assembly (Luque *et al*., 2007). During assembly, the basic structure of the virus particle is formed in the cytoplasm of the infected host cell. The viral glycoproteins are translated as membrane proteins and transported to the cell membrane. M (matrix) protein enables nucleocapsids to interact with the regions of the plasma membrane which have the glycoproteins inserted. The virus buds out through membrane (Alexander, 2003) (Figure 2.4). The neuraminidase may facilitate release; sialic acid appears to be an important part of the receptor. This is removed by neuraminidase from the cell surface. Since sialic acid

would have been largely removed from the cell surface and the progeny virons, neither would have functional receptors, so progeny virons will not stick to each other or to the cell they have just budded out from or any other infected cell). They will therefore be able to diffuse away until they meet an uninfected cell (Alexander, 2003).

2.1.8 Resistance and susceptibility of infectious bursa disease virus to physical and chemical agents

The IBDV is non-enveloped and quite resistant to physical and chemical agents. Due to the stability and hardiness of the virus, it persists in poultry premises even after cleaning and disinfection (Benton *et al*., 1967; Lukert and Saif, 2003). The virus is inactivated at a pH of 12.0 but not at pH 2.0 (Ashraf, 2005). Benton *et al*. (1967) reported that IBDV survived a temperature of 37 ^{o}C for 90 min and 56 ^{o}C for 5 hours (Ashraf, 2005). A marked reduction in infectivity of the virus was observed after treatment with 0.5% formalin for 6 h. The virus remained unaffected by ether, choloroform, phenol, thiomaesal, Staphene and Hyamine 2389 treatments (Ashraf, 2005). The virus survived treatments with various concentrations of three disinfectants (an iodine complex, a phenolic derivative and a quaternary ammonium compound) for a period of 2 min. At 23 ^{o}C, only the iodine complex had any deleterious effects (Ashraf, 2005).

Cho and Edgar (1969) reported that the IBDV was inactivated by exposure for 1 hour to 1% formalin, 1% cresol and 1% phenol. It remained stable at 60 ^{o}C for 90 min and was still infectious at room temperature (25°C) for 21 days. Petek *et al*. (1973) observed that IBDV was more resistant than Reovirus to heat, ultraviolet irradiation and photodynamic

inactivation. The hardiness of the virus makes it difficult to eradicate it from poultry houses after outbreaks of IBD (Alexander and Chettle, 1998). It has been shown that IBDV survived at 60 ^{o}C but did not survive at 70 ^{o}C for 30 min and 0.5% chloramines killed the virus after 10 min. Innert soaps with 0.05% sodium hydroxide either inactivated or had a strong inhibitory effect on the virus (Ashraf, 2005). The virus is unusually resistant to inactivation by cooking so there is a risk of introduction to the backyard flocks through uncooked chicken meat products since viable virus might be present in meat from apparently healthy chickens (Ashraf, 2005).

Infectious bursal disease virus was completely dissociated into subunits at a high hydrostatic pressure of 240 MPa at 0 ^{o}C as revealed by the change in intrinsic fluorescence spectrum and light scattering. Electron microscopy showed that virus morphology had an obvious change after pressure treatment. Elevating pressure also destroyed the infectivity of the virus while retaining its immunogenicity (Ashraf, 2005).

2.1.9 Epidemiology of infectious bursal disease

2.1.9.1 Evolution of infectious bursal disease virus

The evolution of the virus since 1984 has been marked by events. The first was the discovery of antigenic drift in serotype 1 viruses. Commencing in 1984, several strains of the serotype were isolated in the USA from broiler flocks that have been properly vaccinated (van den Berg *et al.*, 2000). The new virus did not cause the characteristic clinical signs of the infection, but had a major immunosuppressive potential. These strains were termed ‘variant since they were capable of infecting chicks that possessed an antibody titre considered protective in normal circumstances. The variant viruses have

since been found to carry modified neutralizing epitopes, and several successive generations of these viruses, which gradually have accumulated antigenic mutations, have been found in the USA (van den Berg *et al*., 2000). Thus, six sub-groups have been described among thirteen strains tested by serum neutralisation (SN). The second major epidemiological event was the emergence, in 1987, of 'hypervirulent, viruses (vvIBDV) in Europe, particularly on farms that were well managed and on which all hygiene and sanitary control measures have been implemented (van den Berg *et al.*, 2000). These viruses were found to be significantly more pathogenic than the classical strains, and also capable of infecting chicks with normal protective antibody titres. Mutations in the RNA genome of the virus have resulted in the appearance of variant strains in the US and very virulent strains all over the world (Ashraf, 2005).

2.1.9.2 Factors responsible for susceptibility to infectious bursal disease

The age of maximum susceptibility is between three and six weeks, corresponding to the period of maximum bursa development, during which the acute clinical signs are observed. Infections occurring prior to the age of three weeks are generally subclinical and immunosuppressive. Clinical cases may be observed up to the age of fifteen to twenty weeks (Ley *et al*., 1979; Okoye and Uzoukwu, 1981). Light strains of laying stock are more susceptible to disease than the heavy broiler strains (van den Berg and Meulemans, 1991; Bumstead *et al*., 1993; Hassan and Saif, 1996).

2.1.9.3 Natural and experimental hosts of infectious bursal disease virus

Only chickens *(Gallus gallus)* develop cloacal IBD after infection by serotype 1 viruses. Turkeys (*Meleagris gallopavo)* may be asymptomatic carriers of serotype 2 (McFerran *et al*., 1980; Ismail *et al.,* 1988), and at times, of serotype 1 viruses whose pathogenicity for turkeys is ill-defined (Owoade and Durojaiye, 1995). The Pekin duck *(Cairina moschata)* can also be an asymptomatic carrier of serotype 1 viruses (McFerran *et al*., 1980). Antibodies to IBDV have been detected in guinea fowls *(Numida meleagris)* (Adewuyi *et al*., 1989), common pheasants *(Phasianus colchicus)* (Louzis *et al*., 1979) and ostriches *(Struthio camelus)* (Cadman *et al.,* 1994), have also been demonstrated to carry serotype 2 viruses (Guittet *et al*., 1992). Neutralizing or precipitating antibodies have been detected, *inter alia*, in various species of wild duck, goose, tern, puffin, crow and penguin, which means that wild birds act as reservoirs or vectors of IBDV (Wilcox *et al.,* 1983; Gardner *et al*., 1997; Ogawa *et al*., 1998).

Infectious bursal disease virus is host specific. IBDV has been reported in Ostriches (Ashraf, 2005), in Baltic ducks and herring Gulls (Ashraf, 2005), various raptor and passerine species in Japan (Ashraf, 2005). Anti-IBDV antibodies have been reported from Antarctic penguins (Ashraf, 2005). The virus has also been documented from the lesser mealworm (*Alphitobius sp*) fed on contaminated IBDV feed. Experimental inoculation of pheasants, partridges, guinea fowls and quails showed no signs of disease (Ashraf, 2005). Japanese quails are refractory to IBDV infection (Ashraf, 2005). They tested negative for virus replication, detection of precipitating or virus neutralizing antibodies and gross as well as microscopic changes in BF associated with infection (Ashraf, 2005) However, quail-chicken hybrids can be infected with IBDV (Ashraf, 2005). In one study, dogs were

evaluated as a potential carrier of the virus since viable virus persisted in the faeces two days after initial ingestion and maintained its original characteristics (Ashraf, 2005).

2.1.9.4 Incidence and distribution of infectious bursal disease

Infections with serotype 1 IBDV are of worldwide distribution, occurring in all major poultry producing areas of the world (Lukert and Saif, 1997). Since the first report in Delaware (United States of America [USA]) between 1960 and 1964, the disease affected most regions of the USA (Lasher and Davis, 1997), and reached Europe in the years 1962 to 1971 (Faragher, 1972). From 1966 to 1974, the disease was identified in the Middle East, southern and western Africa, India, the Far East and Australia (Faragher, 1972; Provost *et al.*, 1972; Firth, 1974; Jones, 1986; Lasher and Shane, 1994; van der Sluis, 1999; van den Berg, 2000). Infectious bursal disease is currently an international problem. Since its first report in Nigeria by Ojo *et al.*, in 1973, the disease has acquired endemic status among the Nigerian poultry populations (Nawathe *et al*., 1978; Durojaiye *et al*., 1984; Abdu, 1988).

2.1.9.5 Transmission of infectious bursal disease

Infectious bursal disease infection is transmitted horizontally via the oro-faecal route, but may be via the conjunctiva and respiratory tract, with affected bird excreting high levels of the virus for approximately two weeks after infection (Butcher and Miles, 2011). Infected subjects excrete the virus in faeces as early as 48 h after infection, and may transmit the disease by contact over a sixteen-day period (Vindevogel *et al.*, 1976). The disease is transmitted by direct contact with excreting subjects, or by indirect contact with any

inanimate or animate (farm staff, animals) contaminated vectors. Some researchers have suggested that insects may also act as vectors (Howie and Thorsen, 1981). The extreme resistance of the virus to the outside environment enhances the potential for indirect transmission. The virus can survive for four months in contaminated bedding and premises (Benton *et al*., 1967), and up to fifty-six days in lesser mealworms *(Alphitobius* sp) taken from an IBDV contaminated building (McAllister *et al.*, 1995). In the absence of effective cleaning, disinfection and insect control, the resistance of the IBDV leads to perennial contamination of infected farm buildings.

2.1.9.6 Potential risk of spreading infectious bursal disease virus through trade

Vertical transmission of the disease has not been reported. Horizontal transmission due to external contamination of egg shells has not been documented (but fertile eggs to be incubated can be disinfected by fumigation). As a result, the most likely sources of contamination during commercial trade of poultry products are live poultry and poultry meat. Infectious bursal disease is an OIE List B disease (OIE, 1999).

The IBD-free status of imported live animals can only be established by a negative serological test, repeated after sufficiently long quarantine to allow for the eventuality of seroconversion (at least three weeks) (OIE, 1999). Although imported meat has not been demonstrated to be responsible for the spread of IBDV, this remains a theoretical possibility. Contaminated meat may be produced, either by the slaughter of viraemic asymptomatic chickens (Winterfield *et al*., 1972; Vindevogel *et al.*, 1976), or by the slaughter of convalescent chickens, ten to sixteen days after infection, are no longer

symptomatic, but continue to carry pathogenic virus in the digestive tract, and thus may constitute a viral source of cross-contamination along the slaughter line. The resistance of infectivity of IBDV to temperatures below freezing (at least three years at -20°C) (Cho and Edger, 1969), and to heat (Benton *et al*., 1967; Alexander, and Chettle, 1998), is another factor in the spread of IBDV through trade in poultry meat derivatives.

2.1.10 Pathogenesis of infectious bursal disease

After oral infection or inhalation, the virus replicates primarily in the lymphocytes and macrophages of the gut-associated tissue. Then virus travels to the BF via the blood stream, where replication occurs. By 13 h postinoculation (pi), most BF follicles are positive for virus and by 16 hpi, a second and pronounced viraemia occurs with secondary replication in other organs leading to disease and death (Müller *et al*., 1979). Helmboldt and Garner (1964) detected histological evidence of infection in BF within 24 h. Müller *et al*. (1979), using immunofluorescence techniques, observed infected gut-associated macrophages and lymphoid cells within 4-5 h after oral exposure to IBDV.

2.1.11 Clinical signs of infectious bursal disease

2.1.11.1 Incubation period of infectious bursal disease

The incubation period of IBD is short ranging from 2-4 days with an average of 3 days, following natural exposure. This depends on the strain, the susceptibility of the population, age and breed of the birds, intercurrent primary and secondary pathogens, the presence or absence of neutralising antibodies, environmental conditions, the route of exposure, the

dose of the virus, immune status and management factors (van den Berg *et al.*, 2000; OIE, 2008; Mbuko *et al.*, 2010; Butcher and Miles, 2011).

2.1.11.2 Morbidity and mortality due to infectious bursal disease

Infectious bursal disease is extremely contagious. In infected flocks, morbidity is high, with up to 100% serological conversion, after infection, whilst mortality is variable. Hypervirulent field strains may cause high mortality rates of up to 50% to 60% in pullets and 25% to 30% in broilers. These hypervirulent field strains may cause up to 100% mortality in specific-pathogen-free (SPF) chickens (van den Berg, *et al*., 1991; Nunoya *et al*., 1992). All breeds are affected but severe reactions with highest mortality rate observed in White Leghorn (Lukert and Saif, 1997). Mortality due to IBD on various farms ranged from 1 to 40% in broilers and from 2 to 40% in layers (Kurade *et al*., 2000) and from 1.5 to 30% in native and broiler flocks respectively (Saif *et al*., 2000). However, Meroz (1966) found that there was no difference in mortality between heavy or light breeds.

2.1.11.3 Pathogenicity and epizootiology of infectious bursal disease

An early subclinical infection before three weeks of age (Lukert and Saif, 1997), even in newly hatched chicks (Fadley and Nazerian, 1983), may occur. Natural infections of turkeys and ducks have been reported (McFerran *et al*., 1980). The disease spreads rapidly by direct contact because of its highly contagious nature (Benton *et al*., 1967). There is no report of egg transmission of IBDV. Infected birds will excret the virus in their droppings for at least 14 days (Baxendale, 2002). Fish meal in the feed contaminated with IBDV may act as a transmitter of the disease (Yongshan *et al.,* 1994), while lesser mealworm as well

as mosquito may act as a reservoir of IBDV (Howie and Thorson, 1981; McAllister *et al*., 1995).

Typical clinical signs of infectious bursal disease may include depression, inappetance, unsteady gait, huddling under equipment, vent pecking, diarrhoea with urates in mucus (Anonymous, 2014). In acute cases, the birds are depressed, exhausted, prostrated, dehydrated, suffer from watery diarrhoea, and feathers are ruffled (van den Berg *et al*., 2000). Birds are disinclined to move and peck at their vents (Cosgrove, 1962) and pericloacal feathers are stained with urates (Landgraf *et al*., 1967). The initial outbreaks may be characterized by high morbidity (80%) and correspondingly significant mortality, attaining 25% in broilers and 60% in pullets over a 7-day period (Chettle *et al*., 1989; van den Berg *et al*., 1991; Nunoya *et al*., 1992).

Mortality commences on the third day of infection, reaches a peak by day four, then drops rapidly, and the surviving chickens recover a state of apparent health after five to seven days (van den Berg *et al*., 2000). Disease severity depends on the age and breed sensitivity of the infected birds, the virulence of the strain, and the degree of passive immunity (van den Berg *et al*., 2000). Initial infection on a given farm is generally very acute, with very high mortality rates if a very virulent strain is involved. If the virus persists on the farm and is transmitted to successive flocks, the clinical forms of the disease appear earlier and are gradually replaced by subclinical forms. Nonetheless, acute episodes may still occur. Moreover, a primary infection may also be in apparent when the viral strain is of low pathogenicity or if maternal antibodies are present (van den Berg *et al*., 2000).

The clinical signs of IBD vary considerably from one farm, region, country or even continent to another. The global situation can be divided into three principal clinical forms, as follows:

i. The classical form; is caused by the classical virulent strains of IBDV. Specific mortality is relatively low, and the disease is most often subclinical, occurring after a decline in the level of passive antibodies (Faragher, 1972).

ii. The subclinical disease or the immunosuppressive form is caused by low-pathogenic strains of IBDV, as well as by variant strains, which partially resist neutralisation by antibodies against the so-called 'classical' viruses (Jackwood and Saif, 1987; Snyder, 1990).

iii. The severe acute disease form, is caused by 'hypervirulent' strains of IBDV, and is characterized by an acute progressive clinical disease, leading to high mortality rates on affected farms (Stuart, 1989; Chettle *et al*., 1989; van den berg *et al*., 1991).

2.1.12 Pathology due to infectious bursal disease

2.1.12.1 Gross pathology of infectious bursal disease

The principal target of the IBDV is the BF (Kaufer and Weiss, 1980) but in the acute form of the disease caused by hypervirulent strains, macroscopic lesions may also be observed in other lymphoid organs (thymus (THY), spleen (SP), caecal tonsils, Harderian glands, Peyer's patches and bone marrow) (Hiraga *et al.,* 1994; Inoue *et al.*, 1994; Tsukamoto *et al*., 1995; Tanimura *et al*., 1995; Tanimura and Sharma 1997; Inoue *et al.,* 1999). Macroscopic lesions are observed principally in the BF which presents all stages of inflammation following acute infection (McFerran, 1993; van den Berg, 2000). Autopsies

performed on birds that died during the acute phase (three to four days following infection) reveal hypertrophic, hyperaemic and oedematous BF and by the 5th day after infection, the bursa has returned to normal size and by the 8th day it has atrophied to about one third of its original weight (Baxendale, 2002; The poultry site, 2014). The most severe cases are characterized by a major infection of the mucous membrane and a serous transudate, giving the bursal surface a yellowish colour. Chickens which die acutely of primary IBD infection show dehydration of the subcuatneous fascia and pectoral musculature (Cosgrove, 1962), haemorrhages in skeletal muscle (especially pectoral and thighs) and petechial hemorrhages are observed in the mucosa at the junction of the proventriculus and gizzard (Lukert and Saif, 1997). There is also, increased mucus in the intestine (Cosgrove, 1962; Baxendale, 2002). There may be hypertrophy and white appearances of the kidneys and associated dilatation of the tubules containing deposits of urate crystals and cell debris (Skeels *et al*., 1979; Anonymous, 2014). The splenic enlargement was documented by Morales and Boclair (1993) who showed highly significant differences in bursa: spleen weight ratio of 2.4 for controls compared with 0.9 in chicks seven days after challenge. Very often small grey foci are uniformly dispersed on the surface of the spleen (Rianldi *et al*., 1965).

2.1.12.2. Microscopic pathology of infectious bursal disease

All lymphoid follicles are affected by 3 or 4 days post infection. Lymphocytes are replaced by heterophils, pyknotic debris and hyperplastic reticuloendothelial cells. Hemorrhages often appear but are not consistent lesions (Helmbodt and Garner, 1964; Cheville, 1967; Mandelli *et al*., 1967; Peters, 1967). The B lymphocytes are destroyed in the follicles of the bursa as well as in the germinal centres of the spleen. The bursa is infiltrated by heterophils

and undergoes hyperplasia of the reticulo-endothelial cells and of the interfollicular tissue. As the disease progresses, the surface epithelium disappears and cystic cavities develop in the follicles. Severe panleukopenia is also observed. These microscopic lesions are exacerbated in the acute forms of the disease (Cheville, 1967).

Following lytic changes, follicles are replaced by cysts lined by columnar epithelium surrounded by a fibroblastic interfollicular stroma (Okoye and Uzoukwu, 1990). Cystic cavity develops after subsiding the inflammatory reaction and there is fibroplasia in interfollicular connective tissue (Cheville, 1967; Lukert and Saif, 1997). One of the recent isolates (variant A) of IBDV was reported to cause extensive lesions in BF but the inflammatory response was lacking (Sharma *et al*., 1989). In spleen following initial perivascular reticuloendothelial hyperplasia, lymphoid necrosis was observed in the germinal centres by the 3rd day after infection (Helmbodt and Garner, 1964). Repopulation commences by the fifth day and is complete in eight days (Okoye and Uzoukwu, 1990). Type 1 IBDV infection in 1-day-old broilers devoid of maternal IBD antibody was investigated by Dohms *et al*. (1981) who showed that plasma cells, which normally populated the Harderian gland by 3 weeks of age, were significantly reduced in numbers compared with non-infected controls. Histologic lesions of the kidney are nonspecific (Peters, 1967) and probably occur because of severe dehydration of affected chickens. Helmbodt and Garner (1964) found kidney lesions in less than 5% of birds examined. The liver may have slight perivascular infiltration of monocytes (Peters, 1967).

2.1.13 Immunity to infectious bursal disease

Prophylaxis of IBD in chickens is based on two approaches. The first consists of protecting young birds by the passive immunity transmitted by the hen and the second is vaccination using live vaccines (van den Berg and Meulemans, 1991). Majority of field infections are subclinical (Butcher and Miles, 2011). Recovery from disease or subclinical infection will lead to immunosupression. Generally birds that survive infection with virulent strain of IBDV or had suffered from subclinical infection develop a long lasting immunity to further infection with IBD virus. The bases of this immunity are circulating antibodies (Butcher and Miles, 2011).

2.1.13.1 Active immunity to infectious bursal disease.

The immune response to IBD is generally grouped as active and passive. Passive immunity appears to be more useful as active immunity causes some degree of damage to the lymphoid organs which could result in immune-suppression (Ahad, 2002 citing Lukert and Saif, 1997). Active immunity can either be cell-mediated or humoral.

i. Cell-mediated Immunity of infectious bursal disease

Cell mediated immunity appears to be the first immunological response that can be demonstrated after IBD vaccination. This could be detected as early as 2-3 days, reaching a peak during the first or second week gradually decreasing during the third and fourth week after IBD vaccination. Although the immunosupression caused by IBDV is principally directed towards B lymphocytes, an effect on cell-mediated immunity has been demonstrated (van den Berg and Meulemans, 1991).

ii. Humoral immunity of infectious bursal disease

Protective antibody titre levels against IBD are conferred by vaccination and recovery from infection. Multiple vaccinations with both live and killed IBDV have been found to confer significant and long lasting protective antibody on flocks. Live IBD vaccines are administered either *in ovo* or at hatching, and in the field through booster vaccines (van den Berg and Meulemans, 1991). Vaccinated chicks will have concentration of neutralizing antibodies which could persist to twenty four weeks and be transferred to egg yolk (van den Berg and Meulemans, 1991; Fussel, 1998). Neutralizing antibodies to IBDV are usually seen in recovered, infected and vaccinated birds. Birds exposed to IBDV at three weeks of age may not develop high neutralizing antibody titre as those exposed at four weeks of age (van den Berg and Meulemans, 1991). Such vaccinated chicks are resistant to challenge with virulent IBD virus regardless of the age of vaccination. Both precipitating and neutralizing antibodies can appear 10-15 days after infection and can persist for up to 90 days post infection (van den Berg and Meulemans, 1991). The neutralizing antibodies can be detected by the plaque neutralizing test for post vaccination control test and evaluation of passively acquired immunity in chicks (van den Berg and Meulemans, 1991).

2.1.13.2 Passive immunity (hen hyperimmunization) to infectious bursal disease

Passive immunity may protect against challenge with homologous IBDV, as does previous infection with homologous avirulent strains. Breeder flocks may be immunised against IBD so that they would transfer protective antibodies to their progenies, such as broiler and

pullet chicks. Low-attenuated vaccine strains may cause damage to the BF and immunosuppression in susceptible chicks (Caston *et al*., 2008).

The most popular strategy for IBD control is hen hyperimmunization (Fussel, 1998). Live IBDV vaccine and two or more inactivated vaccines are used in order to hyperimmunize hens. Passive immunity to IBD is then conferred on chicks providing some level of early protection against field challenge (Fussel, 1998).

Passive immunity is usually transferred from dams with significant antibody levels through the egg yolk. The presence of maternal antibody will modify the way the disease progresses (Butcher and Miles, 2011). Antibodies produced by the hen are passed through the egg to the chicks (Butcher and Miles, 2011). These antibodies, if present in adequate levels, protect the chicks against subclinical IBD (Butcher and Miles, 2011). Such chicks may be protected at eight days with some becoming susceptible at 14 days old and all become susceptible at 17 days of age (Baxendale, 1999). Vaccination of breeder hens with IBD vaccine has become a widespread practice throughout the world. This is done because of the ability of the hen to transfer antibodies against IBD from her blood stream to the chick's yolk sac (Fussell, 1998; LAH, 2004). The passive immunity provided by maternal antibodies, prevents bursal atrophy and immunosupression but bursal atrophy may be seen without immunosupression (Fussell, 1998; LAH, 2004). The levels of Mab necessary to neutralize IBDV vary with the invasiveness and pathogenicity of the virus strain. If a ''hot'' (invasive, pathogenic) IBD challenge is present, higher Mab levels and/or an effective progeny vaccination programme will provide the desired protection (LAH, 2004).

2.1.13.3 Immunosupression due to infectious bursal disease

The destruction of B lymphocytes in the bursa creates an immunosupression which will be more severe in younger birds. Recovery from disease or subclinical infection will lead to immunosupression with heavier consequences if infection occurs early in life. The most severe and longest-lasting immunosuppression occurs when day-old chicks are infected by IBDV (Allan *et al*., 1972; Faragher *et al*., 1974; Sharma *et al*. 1989; Sharma *et al*., 1994). In field conditions, this rarely occurs since chickens tend to become infected at approximately two to three weeks, when maternal antibodies decline. Evidence suggests that the virus has an immunosuppressive effect at least up to the age of six weeks (Wyeth, 1975; Giambrone, 1979; Lucio and Hitchner, 1980). Although the immunosupression caused by IBDV is principally directed towards B lymphocytes, an effect on cell-mediated immunity has been demonstrated (van den Berg and Meulemans, 1991). Chicks affected early in life are more susceptible to infections by other disease causing organisms (Ahad, 2002). In addition, it will affect the immune response of the chicken to subsequent vaccinations which are essential in all types of intensive animal production (van den Berg *et al*., 2000).

2.1.14 Diagnosis of infectious bursal disease

2.1.14.1 Field diagnosis of infectious bursal disease

Field diagnosis relies on clinical findings which include ruffled feathers, slimy watery diarrhoea, huddling together, inappetance, severe weakness, morbidity and mortality rates (van den Berg *et al.,* 2000).

2.1.14.2 Diagnosis of infectious bursal disease by bursal body weight ratio

The BF functions are half of the bird's immune system and the size of the BF reflects the bird's overall health status. Sick or stressed birds have small BF while healthy, productive birds have large BF. Bursal size is a biological measure of how well flocks are managed and protected from disease (Bolis *et al*., 2003). The different aggressions of the environment such as stress, poor hygiene, vaccination and pathologies influenced the anatomical and physiological development of BF (Tabeekh and Al-Maya, 2009). The measures of immunity that have been commonly used and assessed in poultry are lymphoid organ weights (Alloui *et al*., 2005). Lymphoid organ weights are easily measured and they reflect the body's ability to provide lymphoid cells during an immune response (Alloui *et al*., 2005).

The evaluation of bursal weight and the determination of bursal index (BI) (or bursal:body weight ratio) is the most used model to estimate protection rate given by vaccines against IBD (Bolis *et al*., 2003). Bursal weight was the best indicator of the stress that was related to housing density in which BI gave the most consistent and reliable indication of stress. As density increased, bursal weight and BI decreased significantly. Problems in the barn environment may be the cause of the small bursa since stress can also decrease bursa size (Bolis *et al*., 2003; Alloui *et al.*, 2005). Alloui *et al.* (2005) found that the evolution of the bursal weight with regard to the body weight gives a positive and significant correlation, which explains that the weight of the bursa increased effectively according to the body weight, but without reaching some compliant values (Alloui *et al*., 2005).

Bursal index (BI) is one of the most important parameters for evaluation of immunosuppression caused by IBDV and by IBD vaccine (Bolis *et al*., 2003). The normal weight of the BF in broilers is about 0.3% of body weight, weights below 0.1% are highly suggestive of disease condition (De wit, 2014; The Poultry Site, 2014). Sahar *et al.* (2004) reported that the BF enlargement is more obvious in chicks vaccinated with the hot strain followed by vaccination with the intermediate strain and least in those vaccinated with the intermediate and boostered with the hot one. Kulikova *et al*. (2004) indicated that the intermediate vaccine in the group of Leghorn chickens produced the lowest average post vaccination antibodies titers and statistically insignificant BF index. The most virulent vaccines demonstrated an almost, high post-vaccination antibodies titres and statistically significant bursal atrophy. Cookson and Giambrone (2004) referred that the bursal to the body weight ratio are the standard method for calculating percent protection to IBDV. Bolis *et al.* (2003) concluded that bursa weight: body weight ratio evaluated before and after challenge with vvIBDV was not enough to consistently and conclusively differentiate or estimate the protection given by the vaccination.

According to Ahad (2000), bursal: body weight ratios calculated after infection with local isolate in rural chicks in Bangladesh resulted in the swelling and the hypertrophy of the BF in the challenged groups at day 3 pi. Atrophy of BF was observed at day 10 pi. The virulence of a local isolate of vvIBDV in vaccinated and non-vaccinated local Sonali chickens, in Bangladesh, against IBD was also evaluated. The ratio between the BF weight and body weight was calculated using the following formula: bursal weight divided by body weight multiplied by 1,000 (BW/ BBW x 1,000).

2.1.14.3 Histological diagnosis of infectious bursal disease

Histological diagnosis of IBD is based on the detection of modifications (depletion of lymphocytes, necrosis, atrophy, vacuolation and haemorhages of the follicles) occurring in the BF. The ability to cause histological changes in the non-bursal lymphoid organs such as the THY, the SP or bone marrow has been reported as potential characteristics of hypervirulent IBDV strains (van den Berg *et al*., 2000). The histological approach has the advantage of allowing for diagnosis of both the acute and immunosuppressive or subclinical forms of IBD (van den Berg *et al.*, 2000).

2.1.14.4 Virological diagnosis of infectious bursal disease

Infectious bursal disease virus may be detected in the BF of chickens in the acute phase of the infection, ideally within the first three days following the appearance of clinical signs (Müller *et al*., 1979).

i. Sample collection and processing for virological diagnosis of IBD

a. Sample collection: Samples should be collected from recently dead or moribund birds that have been killed humanely.

b. Sample processing: Aseptically remove the BF from approximately five affected chickens in the early stages of the disease. Chop the BF using two scalpels, and add a small amount of peptone broth containing penicillin and streptomycin (1,000 μg/ml each), and homogenise in a tissue blender. Centrifuge the homogenate at 3,000 g for 10 min. Harvest the supernatant fluid for use in the laboratory (OIE, 2008). Filtration through a 0.22 μ filter may prove necessary to further control bacterial contamination, although this may cause a

reduction in virus titre (OIE, 2008). This sample must be kept cool during transportation to the laboratory where they should be stored at 4^{o}C if they are to be processed within 24 hours or frozen at -20^{o}C until the isolation is attempted (OIE, 2008).

ii. Infectious bursal disease virus isolation and detection

a. Infectious bursal disease virus isolation

1. Isolation of infectious bursal disease virus in chicken embryo

Inoculate 0.2 ml of infected BF homogenate sample into the yolk sac of five 6-8 day-old specific antigen negative (SAN) chicken embryos and on to the chorioallantoic membrane of five 9-11-day-old SAN chicken embryos. Specific antigen negative embryos are derived from flocks shown to be serologically negative to IBDV. The eggs are candled daily and dead embryos are discarded up to 48 h post- inoculation (OIE, 2008). Embryos that die after this time are examined for lesions. Serotype 1 IBDV produces dwarfing of the embryo, subcutaneous oedema, congestion and subcutaneous or intracranial haemorrhages. The liver is usually swollen, with patchy congestion producing a mottled effect. If lesions are observed, the virus should then be tested against a monospecific anti-IBDV serum in an embryo-revealed virus neutralisation assay (OIE, 2008). Serotype 1 IBDV usually causes death in at least some of the embryos on primary isolation, serotype 2 IBDV does not induce subcutaneous oedema or haemorrhages in the infected embryos, but embryos are of smaller size with a pale yellowish discoloration (OIE, 2008). For the preparation of embryo-propagated stock virus or subsequent passaging, embryos with lesions or embryos suspected to be infected, respectively are harvested aseptically. Their head and limbs are discarded and the main body is minced for preparation as virus suspension (OIE, 2008).

2. Isolation of infectious bursal disease virus in cell culture

A 0.5 ml of infected BF homogenate sample is inoculated into each of four freshly confluent chicken embryo fibroblast (CEF) cultures from specific pathogen free (SPF) source in 25 cm^2 flasks. This is adsorbed at 37^oC for 30-60 min. and is washed twice with Earle's balanced salt solution and adding maintenance medium to each flask. The cultures are incubated at 37^oC and observed daily for evidence of cytopathic effect (CPE). This is characterised by small round refractive cells. If no CPE is observed after 6 days, the medium is discarded, then freeze and thaw the cultures and inoculate the resulting lysate into fresh cultures. This procedure may need to be repeated at least 3 times. If CPE is observed the virus should be tested against IBDV antiserum in a tissue culture virus neutralisation (VN) test. The more pathogenic IBDV strains usually cannot be adapted to grow in CEF unless the virus has first been submitted to extensive serial passage in embryo (OIE, 2008). Most of the strains isolated in the field, and in particular, the hypervirulent strains, cannot be multiplied in cell cultures, since they require either previous passages on embryonated eggs or several blind passages in cell cultures before a cytopathogenic effect is obtained. This adaptation is accompanied by an attenuation of the strain. For this reason, no satisfactory alternative to the use of three- to six-week-old SPF chickens has been found for the preparation of challenge viruses or the characterization of these strains according to virulence. However, the continuous LSCC-BK3 line has been used for propagation of hypervirulent strains without any visible cytopathic effect (Tsukamoto *et al.,* 1995). Primary bursa cells could also be useful, although to date their use has been limited (Shakya *et al.*, 1999).

3. Isolation of infectious bursal disease virus in chickens

Five susceptible and five IBDV-immune chickens (3-7 weeks of age) are inoculated by the eye-drop route with 0.05 ml of infected BF homogenate sample. The chickens are killed 72-80 h after inoculation, and their BF examined. The bursae of chickens infected with virulent serotype 1 IBDV appear yellowish (sometimes haemorrhagic) and turgid, with prominent striations (OIE, 2008). Peribursal oedema is sometimes present, and plugs of caseous material are occasionally found. The plicae may be petechiated (OIE, 2008).

The presence of lesions in the bursae of susceptible chickens along with the absence of lesions in immune chickens is diagnostic of IBD (OIE, 2008). The bursae from both groups may be used as antigen in an agar gel immunodifussion (AGID) test against known positive IBD antiserum (OIE, 2008). The extent of bursal damage may vary considerably with the pathogenicity of the studied IBDV strain. However, as the samples submitted for virus isolation may vary in virus content, the extent of bursal damage observed in susceptible chickens at the isolation stage gives a limited indication on strain pathogenicity (OIE, 2008). The bursae of chickens infected with serotype 2 IBDV do not exhibit any gross lesions (OIE, 2008).

b. Infectious bursal disease virus detection

1. Detection of infectious bursal disease virus by immunofluorescence

The viral antigens specific to IBDV may be detected by direct and indirect immunofluorescence (Meulemans *et al*., 1977; Allan *et al*., 1984) or by immunoperoxidase staining (Cho *et al.,* 1987) in the bursal follicles of infected chickens between the fourth

and sixth day after inoculation. No viral antigen is detectable after the tenth day. The use of monoclonal antibodies for detection of the virus enhances the specificity of the test (Winterfield *et al.,* 1972; Vindevogel *et al*., 1976; Cho *et al*., 1987; van den Berg *et al*., 2000).

Sections of bursa are prepared using microtome cryostat, dried at room temperature and then fixed in cold acetone. Fluorescent-labelled IBDV-specific antisera are applied to the sections, which are then incubated at 37^{o}C for 1 h in a humid atmosphere. At the end of the incubation period, they are washed for 30 minutes using phosphate buffered saline (PBS) (pH 7.2) and then rinsed in distilled water. The sections are mounted using buffered glycerol (pH 7.6) and examined by UV microscopy for IBDV- specific fluorescence (OIE, 2008).

2. Detection of infectious bursal disease by antigen-capture enzyme-linked immunosorbent assay

Enzyme linked immunosorbent assay plates are coated with IBDV-specific antibodies. The capture antibody may be a mouse anti-IBDV monoclonal antibody (MAb), or a mix of such MAbs, or a chicken post infectious anti-IBDV polyclonal serum. It has been suggested that AC-ELISAs using polyclonal antibodies may have a higher sensitivity. Samples of bursal homogenates diluted to 1/10 to 1/25 (w/v) in a suitable dilution buffer are incubated in the coated wells. Unbound antigens are discarded at the end of the incubation period by washing with a suitable washing buffer (e.g. PBS, pH 7.2 + 0.2% Tween 20). The captured antigens are then revealed, as in an indirect ELISA, with a detection antibody (which must have been developed from a different animal species than the capture antibody), followed by an enzyme conjugate that binds to the detection

antibody only followed by the enzyme substrate. Finally, optical densities, which parallel the amount of captured IBDV antigens, are read with an ELISA reader (OIE, 2008).

3. Detection of infectious bursal disease virus by molecular techniques

The most frequently used molecular method is the detection of IBDV genome by reverse-transcription polymerase chain reaction (RT-PCR). This method can detect the genome of IBDV, which is unable to grow in cell culture, because it is not necessary to grow the virus before amplification (OIE, 2008). Molecular identification is focused on the characterisation of the larger segment of IBDV (segment A) and especially of the vVP2 encoding region. The RT-PCR is performed in three steps: extraction of nucleic acid from the studied sample, reverse transcription (RT) of IBDV RNA into cDNA, and amplification of the resulting cDNA by PCR. The two later steps require that the user selects oligonucleotidic primers that are short sequences complementary to the virus-specific nucleotidic sequence. Nucleotide sequencing of RT-PCR products provides an approach to assessing more precisely the genetic relatedness among IBDV strains. Deferent areas of the genome will be amplified depending on the location from which the primers have been selected (OIE, 2008). In most very virulent strains, four typical amino acids are present (222 A, 256 I, 294 I and 299 S) (OIE, 2008). However, it is not clear whether these amino acids play a role in virulence or if they are merely an indication of clonal origin of most vvIBDV isolates (OIE, 2008).

iii. Infectious bursal disease virus strain differentiation

a. Strain differentiation of infectious bursal disease viral genome by deoxyribonucleic acid probes

Deoxyribonucleic acid (DNA) probes labelled with ^{32}P, biotin (Davis and Boyles, 1990; Jackwood, 1990; Kibenge, 1992), biotin (Jackwood, 1990) or digoxigenin (Hatchcock and Giambrone, 1992) have been used on prints of infected tissues to detect the multiple virus strains of serotype 1 and 2. No genomic probe enabling differentiation between variant viruses or vvIBDVs has yet been described, undoubtedly owing to the degree of genetic resemblance between serotype 1 strains of the virus (van den Berg *et al.,* 2000).

b. Strain differentiation of infectious bursal disease by pathogenicity testing

Infectious bursal disease virus strains can be further identified by testing their pathogenicity in specific antigen negative (SAN) chickens, by investigating their antigenic reactivity in cross VN tests or using MAbs, by determining the nucleotide sequence of RT-PCR amplification products derived from IBDV genome, or by studying the number and sequence of restriction fragment obtained following digestion of such RT-PCR products with restriction endonucleases (OIE, 2008).

1. Pathogenicity testing of infectious bursal disease virus

Studies to compare the pathogenicity of IBDV strains must be carried out in secure biocontainment facilities to avoid dissemination of the virus. SAN birds with a known microbial status (ideally SPF chickens) must be used to avoid interference by contaminating agents (OIE, 2008). The main variable when comparing the results of pathogenicity trials are the breed, age and immune status of the challenged chickens, the

dose and route of inoculation of the challenge virus, and the possible presence of contaminating agents in the inoculums. Light layer breeds have been reported to be more susceptible to IBDV than heavy broilers (van den Berg and Meulemans, 1991). Differences in susceptibility may also occur between different SPF chicken lines. The highest susceptibility to acute IBD occurs in chickens between 3 and 6 weeks of age (Lukert and Saif, 1997). A higher dose of challenge virus is necessary so that all inoculated chickens become infected at once without requiring bird-to-bird transmission of the inoculated virus. The presence in the inoculums of contaminating agents, such as adenovirus or chicken infectious anaemia virus, may modify the severity of IBD and signs observed after challenge (OIE, 2008). The term 'variant', 'classical' and 'very virulent' have been used to describe IBDV strains that exhibit a different pathogenicity.

iv. Detection of infectious bursal disease viral antigen

a. Detection of infectious bursal disease viral antigen by enzyme-linked immuno-sorbent assay

ELISA is the most rapid and sensitive method, and presents the fewest variations due to the viral strain used as an antigen (Roney and Freund, 1988). Considerable inter-and intra-laboratory variability can occur with certain commercial kits (Kreider *et al.,* 1991). Although the correlation between results obtained using SN and ELISA is high, ELISA remains less sensitive, and does not detect low neutralizing titres which are sufficient to block vaccine administration (residual maternal antibodies). Enzyme-linked immunosorbent assays which use a recombinant VP2 protein as the sole antigen may be better correlated with protection (van den Berg *et al.,* 1997; Jackwood, 1990).

Antigenic capture as revealed by ELISA (AC-ELISA) consists of capturing the viral antigens present in the suspensions, using anti-IBDV antibodies and a polystyrene support. The viral antigens captured are detected through a sandwich ELISA with an anti-IBDV antibody conjugated with peroxidase (Tsukamoto *et al.,* 1992) or with an anti-IBDV antibody followed by an adapted anti-species conjugate (Kwang *et al*., 1987; Snyder *et al*., 1988; Kumar and Rao, 1989; van der Marel *et al*., 1990; Hassan *et al*., 1996; Eterradossi *et al*., 1997). The use of a polyclonal serum for capture enhances the sensitivity of the test. The use of monoclonal antibodies in the capture or detection stages allows for more precise antigenic characterization of the captured viruses. Different batteries of monoclonal antibodies enable a tentative identification of the variants (Snyder *et al*., 1988) or vvBDVs (Eterradossi *et al.,* 1997; Eterradossi *et al*., 1998; Eterradossi *et al.,* 1999).

b. Detection of infectious bursal disease viral antigen agglutination test technique

Agglutination tests, using latex beads coated with an anti-IBDV monoclonal antibody (Nakamura *et al*., 1993) or sheep red blood cells coupled with anti-IBDV immunoglobulins are also possible (Nachimutu, 1995).

v. Antigenicity testing of infectious bursal disease virus

Antigenicity relatedness among IBDV strains may be assayed in cross VN tests, which correlate best with cross protection. Such tests have to be performed in SAN embryonated eggs when the studied virus does not grow in CEF. Differences in cross VN results among serotype 1 IBDV strains have led to the definition of serotype 1 ‘subtypes’. Another

approach to the study of genetic relatedness is the use of mouse MAbs that bind to IBDV neutralising epitopes (OIE, 2008).

2.1.14.5 Serological diagnosis of infectious bursal disease

i. Antibody detection of infectious bursal disease virus by serological tests

a. Sample collection and processing for serological tests; Clotted blood samples and/or serum samples collection. Blood is aseptically collected from live birds with 22-25 gauge needles (1-1.5 ml/bird) drawn directly into a syringe, or collected into a tube from the jugular or brachial vein without anticoagulants, carefully to avoid damage to the veins. In both cases, either syringe and tube, the sample is subsequently placed nearly horizontally to allow clotting and to permit separation of the serum sample, which should be straw coloured. The serum sample should be kept cool during transport, until it can be frozen in the laboratory prior to analyses if not immediate (Grimmes, 2002).

Current serological tests cannot distinguish between the antibodies induced by pathogenic IBDV and those induced by attenuated vaccine viruses, so serological diagnosis is of little help in endemic zones. Nonetheless, the quantification of IBDV-induced antibodies is important for the medical prophylaxis of the disease in young chickens, in order to measure the titre of passive antibodies and determine the appropriate date for vaccination (Kouwenhoven and van den Bos, 1994; deWit, 1999) or in laying hens to verify success of vaccination (Lucio, 1987; Meulemans *et al*., 1987). Serology is likewise essential to confirm the disease-free status of SPF flocks. Each serological analysis must include a sufficient number (at least twenty) of individual serum samples representative of the flock under study. A kinetic study requires at least two serological analyses separated by an

interval of three weeks (paired sera). The most widely used quantitative tests are the detection of precipitating antibodies by AGID (Hirai *et al.*, 1972; Cullen and Wyeth, 1975), ELISA (Meulemans *et al.*, 1987) and SN in cell culture (Weismann and Hitchner, 1978).

ii. Infectious bursal disease virus neutralisation tests

Serum neutralization presents the disadvantages that specialized equipment and five days incubation are required. The technique is much more sensitive than AGID and correlates better with the level of protection of the subjects tested (Weismann and Hitchner, 1978; Roney and Freund, 1988; Jackwood, 1990). Virus neutralisation tests are carried out in cell culture but are more sensitive for detection of antibody and this may be useful for evaluating vaccine responses and for differentiating between IBDV 1 and 2 serotypes (OIE, 2008).

iii. Detection of infectious bursal disease viral antigen by agar gel immunodifussion test

The AGID test is the most useful of the serological tests for the detection of specific antibodies in serum, or for detecting viral antigens or antibodies in bursal tissue (OIE, 2008). Blood samples should be taken early in the course of the disease, and repeat samples should be taken 3 weeks later. For detection of antigens in the BF, the organ should be removed aseptically from about ten chickens at the acute stage of infection. The bursae are minced using two scalpels in a scissor movement, and then small pieces are placed in the wells of the AGID plate against known positive serum. Freeze-thaw cycles of the minced tissue may improve the release of IBDV antigens from the infected bursal

tissue (OIE, 2008). Agar gel immunodiffusion is the simplest, but least sensitive technique. Results are obtained after an incubation period of 48 h. Variability in results may be due to the investigator, as well as the nature of the viral strain used as an antigen (Weismann and Hitchner, 1978; Wood *et al*., 1979; Wood *et al*., 1984; Nicholas *et al*., 1985; van den Berg, *et al*., 1991). The AGID technique is based on a comparison between the suspension to be tested and a specific antiserum or a monoclonal antibody. The appearance of precipitation lines signals the presence of viral antigens (Hirai *et al*., 1974; Snyder *et al*., 1992; Takase *et al*., 1996).

2.1.15 Differential diagnosis of infectious bursal disease

The conditions most liable to be clinically mistaken for IBD are avian coccidiosis, Newcastle disease in some visceral forms, stunting syndrome, haemorrhagic syndrome, chicken infectious anemia, mycotoxicoses, nephropathogenic forms of infectious bronchitis, cryptosporidiosis of the bursa (rare) (Lukert and Saif, 1997). In all acute cases, the presence of BF lesions allows for a diagnosis of IBD. In subclinical cases, an atrophy of the bursa may be confused with other diseases such as Marek's disease or infectious anaemia. A histological examination of the bursa will allow differentiation between these diseases (Lukert and Saif, 1997; van den Berg *et al*., 2000).

2.1.16 Treatment of infectious bursal disease

No known chemotherapeutic or antibiotic agent is effective in the treatment or control of infectious bursal disease as an *in vivo* disease (MAFRA, 2014). Drug therapy is often

inadvisable in the presence of severe kidney damage (MAFRA, 2014). Hygienic measures may however reduce spread, especially in endemic situation and multi age sites. Electrolyte and/or multivitamin supplement administration may be helpful in flocks where the disease is of relatively long standing and appetites are poor (MAFRA, 2014). Good ventilation, warm temperatures and fresh water will help to reduce mortality. Antibiotic medication may be indicated if secondary bacterial infection occurs but this should be kept to a minimum (MAFRA, 2014; Anonymous, 2014). Formalin and iodophores can be effective in the decontamination of poultry premises (Abdu and Musa, 2014).

2.1.17 Prevention and control of infectious bursal disease

Block *et al.* (2007) reported that the right strategy for IBD control and its success rate under field conditions depends on hygiene management, IBDV field pressure, level and variation in IBDV maternally derived antibodies, and the IBD vaccine strain to be used. It is also dependent on effective vaccination of parents and progeny (Lukert and Saif, 1997). Usually, standard vaccination programmes are used, which are not always adapted to the specific conditions on the farm and to the immune status of chickens. Therefore it is necessary to estimate the optimal time for vaccination for a specific flock based on the maternally derived antibody level, its variation, the genetic background of the chickens, and the IBD vaccine strain (Block *et al.,* 2007). When outbreaks do occur, biosecurity measures may be helpful in limiting the spread between sites, and tracing of contacts may indicate sites on which a more rebust vaccination programme is indicated (Anonymous, 2014).

Prevention of IBD necessitates hygiene measures and medical prophylaxis. No vaccine can solve the problem if major sanitary precautions are not taken. These precautions include 'all-in/all-out' farming methods, cleaning and disinfection of premises and observance of a 'down time' (a period of rest between depopulation and restocking) (Ivanyi and Morris, 1976). Given the very contagious nature of the disease and the resistance of the virus, certain essential steps in the cleaning/disinfection process should be adhered to. Prior to cleaning, all insects and pests (e.g. rats and mice) must be eliminated as soon as the farm premises are empty. Old bedding and dung must be eliminated and composted. All farm equipment must be disassembled and stored in cleaning rooms located outside the farm buildings. The buildings, immediate surroundings and farm equipment must be dry-cleaned first, in order to eliminate all dust, and then washed using hot water (60°C) with a detergent, at a pressure of 80 bar to 150 bar. A second disinfection of the full premises must be performed before the introduction of the chicks. Feed silos must be emptied completely and cleaned inside and outside. Under no circumstances may feed remains from previous flocks be reused. Disinfection is to be undertaken only after all the buildings have been cleaned. All disinfectants are more active at a temperature above 20°C; however, chlorinated and iodinated disinfectants cannot be heated above 43°C. The quantity of disinfectant solution to be used is approximately 4 litres per 15 m^2. Infectious bursal disease is controlled through biosecurity with adequate restriction to farm visitation and distancing from other flocks. Post outbreak hygiene measures may not be effective as the virus can survive for long periods in both housing and water (Caston *et al*., 2008).

Gumboro is controlled by a combination of biosecurity and vaccination of chicks and breeders (Lukert and Saif, 1997; Giambrone, 2008; Anon, 2009). Breeder vaccination is conducted in order to transfer antibodies known as maternally derived antibodies (MDA) to their offspring for protection against immunosuppression and clinical IBD within the first few weeks of age. To protect chicks for the rest of their life they are vaccinated with live vaccines. Live vaccines are however susceptible to neutralization by MDA and vaccinated chicks become susceptible to infection earlier than they would be and respond poorly to vaccination. It is therefore important to determine the most appropriate age to vaccinate chicks to avoid interference by MDA. Vaccination failures have been reported in Nigeria and neutralization of vaccine virus by MDA has been attributed as one of the causes of the failures (Okoye, 1984; Abdu, 1986; Onunkwo and Okoye, 1991; El Yuguda and Baba, 2004). The success of any IBD vaccination programme depends on the knowledge of breeder vaccination and the level of antibodies in breeders and MDA in chicks (Lukert and Saif, 1997). If chicks are vaccinated when MDA levels are still high, the vaccine may be neutralized by MDA, resulting in a reduced immune response. Conversely, if vaccinations are delayed and MDA levels drops, a severe vaccine reaction may result and the chick will be left vulnerable to the disease prior to vaccination (Anon, 2009).

2.1.17.1 Exclusion/eradication of infectious bursal disease

The very high resistance of IBDV to physical and chemical agents (Benton *et al.,* 1967) accounts for persistence of the virus in the outside environment, particularly on contaminated farms, despite disinfection. Eradication in affected countries therefore seems

unrealistic. Control of the disease relies mainly on vaccination, but its protective effect is compromised by the apoptotic effect of live vaccines on the bursa of vaccinated chickens and sequence mutations of IBDV isolates (Snyder, 1990; Sharma *et al*., 2000).

2.1.17.2 Genetic selection for resistance to infectious bursal disease

Different consanguineous lines of poultry show highly variable susceptibility to experimental infection with the same strain of IBDV. The results of crosses between resistant and susceptible lines show that resistance is a dominant hereditary characteristic. However, the genes responsible for resistance have not been identified, and genetic selection for resistance has not yet been practiced (Bumstead, 1998; Bumstead *et al.,* 1993).

2.1.17.3 Vaccination against infectious bursal disease

Specific and sensitive diagnostic tools are now available and effective vaccines have been prepared for combating the disease (Ashraf, 2005) but peri-focal vaccination may not be effective for the combat of an outbreak, due to the rapidity of wild-IBDV spreading (Caston *et al.,* 2008). Vaccination, including passive protection via breeders, vaccination of progeny depends on virulence and age of challenge. In most countries breeders are immunised with a live vaccine at 6-8 weeks of age and then re-vaccinated with an oil-based inactivated vaccine at 18 weeks. A strong immunity follows field challenge. Immunity after a live vaccine can be poor if maternal antibody was still high at the time of vaccination (Anonymous, 2014).

The principal method of IBD control is by vaccination using attenuated or inactivated virus. Of the two serotypes of infectious bursal disease virus (IBDV) only serotype 1 viruses are used in commercial vaccines, because recognized serotype 2 viruses are not pathogenic (Jackwood *et al*., 1984; Ismail *et al.,* 1988). There have been reports on isolation of serotype 1 IBDV from commercial chickens that had bursal lesions and high levels of maternal antibodies (Saif, 1984; Rosenberger and Cloud, 1986; Brown 1986; Saif *et al*., 1987; Ismail *et al*., 1990). It was speculated that the presence of antigenically different strains of IBDV might provide an explanation for the failure of some vaccination programs. In addition to strict compliance with rules of hygiene and disinfection, the success of vaccination depends on the choice of the vaccine strain and on the vaccination schedule. These must take account of the existence of certain pathotypes and the presence of antigenic variants in certain regions.

i. Types of vaccines used for the control infectious bursal disease

a. Live virus vaccines used for the control of infectious bursal disease

Live virus vaccines are very widely used. These are made from strains of IBDV that have been attenuated by serial passages in embryonated eggs. Depending on the degree of attenuation, the vaccine strains cause histological lesions of varying severity to the BF of SPF chickens and are classified as mild, intermediate or hot (OIE, 2000). The hot strains induce histological lesions in SPF chickens which are comparable to those caused by pathogenic strains, the only difference being that the hot strains do not cause mortality. The mild strains are used chiefly for the vaccination of breeder flocks. These are very sensitive to interference by homologous maternal antibodies and are administered when these

antibodies have disappeared, i.e. between the fourth and eighth week of age, depending on whether the grandparent flocks have or have not been vaccinated with an oil-emulsion inactivated vaccine before lay (OIE, 2000).

Intermediate vaccines are used for vaccinating broilers and pullets (Mazariegos *et al*., 1990). These are administered to chicks in breeder flocks which are at risk of challenge by highly pathogenic strains at an early age. Although intermediate vaccines are also sensitive to neutralization by passive antibodies, they may be administered at day-old by nebulisation in order to protect a chick that may not have a sufficient level of specific antibodies. Another reason for such early vaccination is to bring about replication of the vaccine virus in the chicks, and the dissemination of the virus within the farm. This would partially, provide indirect vaccination to the other chicks at a time when they become sensitive to the infection. In high-risk farms, two vaccinations are generally performed. The age at vaccination depends on the maternal antibody titres present in the chicks at hatch. Vaccines are usually administered through drinking water, although nebulisation is also possible (Mazariegos *et al*., 1990).

The vaccine is a mixture of virus and specific antibody and is injected into eighteen-day-old embryos. Broiler chicks hatched from these eggs are immunized against IBDV throughout the growing period. This method avoids interference by parental antibodies (Heine and Boyle, 1993). Various vaccines using recombinant viruses expressing the VP2 protein of IBDV have been described, and have proven efficacy in laboratory tests. The advantages of these vaccines are the absence of residual pathogenicity, sensitivity to

maternal antibodies and risk of selection of mutants, as well as the possibility of use *in ova* and of differentiation between infected and vaccinated animals (Heine and Boyle, 1993; Thiry *et al*., 1994; Darteil *et al*., 1995). No commercial version of these vaccines is currently available.

b. Inactivated vaccines used for the control of infectious bursal disease

Inactivated vaccines are essentially used to produce high, uniform and persistent antibody titres in hens prior to lay that have been vaccinated with a live virus or have been naturally infected through exposure to the virus on the farm (Cullen and Wyeth, 1976; Wyeth and Cullen, 1978; Wyeth and Cullen, 1979; Guittete *et al.*, 1992). These vaccines are administered by subcutaneous or intramuscular route at the age of sixteen to twenty weeks. Progeny of hens that have been vaccinated in this way have protective antibodies until the age of approximately thirty days (Wyeth and Cullen 1976; Box, 1989; Wyeth and Chettle, 1990; van den Berg and Meulemans, 1991; Wyeth *et al*., 1992). The chicks are thus protected during the period of susceptibility to the IBDV strains that only provoke immunosuppression. However, the chicks are not protected from other highly pathogenic strains that may inflict high mortality rates at later stages (Wyeth and Cullen, 1979; van den Berg and Meulemans, 1991). The decision to use an inactivated vaccine will thus depend on the epidemiological context, namely: presence or absence of highly pathogenic strains requiring vaccination of broilers with live virus vaccines. Where no risk of infection with vvIBDVs exists, boosting of laying hens with an inactivated vaccine just before lay is fully justified. However, the duration and uniformity of the immunity thus conferred upon chicks will, to a great extent, depend on the concentration and the antigenic specificity of the virus present in the vaccine. These vaccines are obtained either from bursal

homogenates of infected chicks, or from viral cultures on embryonated eggs or fibroblasts, which are then inactivated by formaldehyde and presented as oil emulsions. Sub-unit vaccines produced in yeast (Fahey *et al*., 1989) or insect cell cultures (Vakharia *et al*., 1993) have also been described, but are not currently in use.

1. Causes of vaccination failure in infectious bursal disease

The causes of failure of live-virus vaccinations are numerous; the most trivial causes are non-observance of the expiry date, inappropriate storage, non-observance of recommended doses, and incorrect or deficient vaccination techniques (van den Berg *et al.,* 2000). Freeze-dried live vaccines must be rehydrated immediately before use in distilled water. The use of distilled water to dilute the vaccines is compulsory when the spray technique is used (van den Berg *et al*., 2000). When the vaccine is administered in drinking water, it is particularly important to deprive the birds of water for two to three hours before distributing the vaccine solution. Only fresh water, with no organic matter, chlorine or heavy metals, may be used. Adding powdered milk at a concentration of 2 g per litre helps to stabilize the vaccinal virus (van den Berg *et al*., 2000). Interference from parental antibodies is one of the most frequent causes of failure (van den berg *et al.,* 2000). The date of vaccination of the offspring must therefore be determined on the basis of the immune status of the chicks and thus of the vaccination protocol used for the parents. Vaccination failure with inactivated vaccines is rare, but may occur, either due to the absence of previous contact of some of the birds with a live virus (a vaccine or field virus), or to the existence of antigenic variants not present in the vaccine (van den Berg *et al*., 2000).

2. Differentiation between infectious bursal disease vaccine and wild virus strains

No markers are currently available to differentiate between vaccine and wild IBDV strains. A simple discrimination method might be to cultivate field strains on chicken embryo fibroblasts. The vaccine strains, with the exception of the hot strains, are cytopathic, whereas the highly pathogenic strains are not (van den Berg *et al.*, 2000). Moreover, the amplification and sequencing of the gene coding for the VP2 protein after reverse transcription would allow differentiation between all the vaccine strains, and may be a preferred method to differentiate between vaccinal and wild IBDV (OIE, 1995; van den Berg, *et al*., 2000). No serological test is able to distinguish between the response to a pathogenic or a vaccine IBDV strain at the flock level.

2.1.18 Economic impact of infectious bursal disease

Apart from direct losses due to specific mortality, indirect losses also occur, due to acquired immunodeficiency or potential interactions between IBDV and other viruses, bacteria or parasites. Further losses may occur as a result of growth retardation or the rejection of carcasses showing signs of haemorrhages (van den Berg, 2000). The emergence of hypervirulent strains has further increased the financial impact of IBD. Substantial economic losses result from both the clinical and subclinical (or immunosuppressive) forms of the disease, but also from interactions between IBDV and other viruses such as CAV and MDV. The economic impacts of IBD include losses due to morbidity and mortality, immunosuppression in the surviving chickens since IBDV infection exacerbates infections with other disease agents, reduction in the chicken's ability to respond to vaccination and risk of introduction to exotic places from importing infected

poultry products. The economic impact of the disease is influenced by pathogenicity of the strain of IBDV, susceptibility and breed of flock, other prevalent pathogens and environmental and management practices (Ashraf, 2005).

2.2 Phytochemical agents used in infectious bursal disease treatment

2.2.1 Medicinal plants

For centuries, plants are the basic therapeutic option available to mankind and herbal formulations make up to 40% of total prescriptions. Plant kingdom is a great source of various chemical substances, with potential therapeutic properties (Ghani, 1986). A significant rise in the practice of phytotherapeutical agents is observed over the past few years due to minimal side effects. Due to their properties, medicinal plants are used as primary health care aid among 80% of the world's population in the form of plant extracts or their active components (Shahid and Hussain, 2012).

Several published reports have shown the effectiveness of traditional herbs against microorganisms (Ghani *et al*., 1989; Iwu, 1993; Shajahan and Ramesh, 2004; Obafemi *et al*., 2006). Medicinal plants contain physiologically active principles which, over the years, have been exploited in traditional medical practice for the treatment of various ailments (Adebanjo *et al*., 1985). As a result, plants are one of the bedrocks of modern medicine. The screening of plant extracts and natural products for antimicrobial activity has shown that higher plants represent a potential source of new, anti-microbial agents, as well as serving drug discovery from natural products for primary lead compounds. According to the World Health Organization (WHO, 2001), phytomedicines are herbal preparations,

produced by subjecting plant materials to extraction, fractionation, purification, concentration, or other physical or biological processes which may be produced for immediate consumption, or as the basis for herbal products. Phytomedicines can also be naturally-occurring substances, usually of plant origin, used in the prevention and treatment of diseases. African plants constitute a rich untapped pool of natural products (Adebanjo *et al*., 1985). The therapeutic value of medicinal plants has long been exploited for the management of various disease conditions in traditional practice (Onu *et al*., 2013). This practice has gained appreciable acceptance in health care delivery in developing and developed nations with the notion that they are relatively harmless (Onu *et al*., 2013).

2.2.2 *Khaya senegalensis*

Khaya senegalensis, commonly known as African mahogany, is a species of plant in the *Meliaceae* family. There are about five species and four of which (*K. anthoteia, K. senegalensis, K. ivoriensis* and *K. grandifolia*) are found in West Africa (Keay *et al*., 1989; Iwu, 1993) and are known as African mahogany. *Khaya senegalensis* is found in many African countries is distributed in the sub-Saharan savannah from Senegal to Sudan and Uganda including Nigeria, being native to tropical Africa (Ijeoma *et al.*, 1997). It is a round evergreen crown of dark shiny foliage, pinnate leaves and characteristic round capsules that grows up to 40 m high (Keay *et al*., 1989). It is an important multipurpose tree in its natural range within sub-Saharan Africa, being particularly valued for timber, fuel wood and medicinal purposes (Anon, 2000). It has attracted world-wide attention for its high quality timber production (Kubmarawa *et al*., 2008). The therapeutic value of KS

has been recognized in traditional medicine for the treatment of various conditions. The decoction of the stem bark extract is used for treating jaundice, dermatoses, malaria, fever, mucous diarrhoea, and venereal diseases as well as for hookworm infection and a taeniacide remedy (Ijeoma *et al*., 1997; Umeh *et al*., 2005; Kubmarawa *et al*., 2008; Ali *et al*., 2011; Idu and Igeleke, 2012). In Cameroon, the bark is in demand as an additive in local beer brewing. Roots are applied against jaundice, stomach-ache, oedema and amenorrhoea, lumbago, cough, rheumatism, stomach ache and gastric pain (Dalziel, 1937). The roots and/or bark are an ingredient of complex arrow poisons. *K. senegalensis* extracts have been reported to exhibit anti-inflammatory effects (Lompo *et al*., 1998) as well as anti-bacterial (Kone *et al*., 2004), anthelmintic (Ademola *et al*., 2004), antitumour, antioxidant (Androulakis *et al*., 2006), and antiplasmodial activities (El-Tahir *et al*., 1998). The wood of the plant has oleo-resin in their vessels and this makes it resistant to insect attack. It is also used in the treatment of worm infestation, ulcer and mucous diarrhoea in horses and camels (Dalziel, 1937).

2.3 Chemicals agents used in the treatment of infectious bursal disease

2.3.1 Vitamin E

2.3.1.1 Composition of vitamin E

Vitamin E is a fat soluble vitamin that acts as a biological antioxidant and is required for normal reproduction (Chukwu, 2010). Vitamin E is a mixture of nine tocopherols, the most active of which is alpha-tocopherol (Radostitis *et al*., 2006). There are four naturally occurring tocopherols; of these, alpha-tocopherols is the most active form of vitamin E and is the compound most commonly included in foods (Chow, 2001).

2.3.1.2 Sources of vitamin E

Major vitamin E sources are vegetable oils and some other plant-derived foods. Food sources rich in vitamin E include wheat germ, corn oil, cottonseed, soybean oil, and sunflower oil, whole grains, and alfalfa meal. Synthetic tocopherols are available, usually added to chick starter and breeder ration and egg yolk can provide some vitamin E as well (Schenk, 2010). In general, the vitamin E content of oil increases according to its linoleic acid concentration. Most animal food sources supply only limited amounts of vitamin E. Egg yolk can contain a moderate amount of vitamin E depending on the diet of the hen, but milk and dairy products are very poor sources. Cereal grains, green pasture, and well-cured fresh hay contain adequate amounts of the vitamin. A-Tocopherol levels are high in green grasses and clovers, but there are wide variations in the concentrations from one area to another. The level of a-tocopherol in pasture declines by up to 90% as it matures (Radostitis *et al.,* 2006).

2.3.1.3 Functions of vitamin E in the body

Vitamin E is essential for good muscle tone and has been shown to affect the condition of the entire body and particularly of the heart muscle. It promotes the health of sebaceous glands, improves the coat condition and restores normal breeding function in some cases to bitches that refuse to come on heat and to males that lack libido (Radostitis *et al*., 2006). It is a natural antioxidant for cellular membrane, modulate inflammatory response, regulate production of prostaglandin, and minimize the damage resulting from cytotoxic action in organs (Pugh, 2002). It also improves phagocytic activity of macrophages in chicks. As an antioxidant it prevents oxidative damage to sensitive membrane lipids by decreasing hydroperoxide formation. The vitamin has a central role in protection of cellular

membranes from lipoperoxidation, especially membranes rich in unsaturated lipids, such as mitochondria, endoplasmic reticulum, and plasma membranes (Case *et al*., 2011). The vitamin maintains cellular integrity and normal immune function. Vitamin E's chief function in the diet and in the body is as a potent antioxidant. Polyunsaturated fatty acids (PUFAs) that are present in foods and in the lipid membranes of the body's cells are very vulnerable to oxidative damage (Case *et al*., 2011). The vitamin interrupts the oxidation of these fats by donating electrons to the free radicals that induce lipid peroxidation. Peroxidation of the body's lipids can destroy the structural integrity of cell membranes, resulting in impairment of normal cellular functioning. Peroxidation of fats in foods causes rancidity and a loss of the nutritional value of the food's essential fatty acids (EFAs). In addition to its action on PUFAs, vitamin E also protects vitamin A and the sulfur-containing amino acids from oxidative damage (Radostitis *et al.*, 2006). As a direct result of these functions, an animal's requirement for dietary vitamin E depends on the level of PUFAs in its diet and the degree to which dietary fatty acids have undergone peroxidation during processing and storage.

2.3.1.4 Uses of vitamin E

Vitamin E is one of the most important *in vivo* antioxidants; the intake of which has been linked to the prevention of several cancers and coronary disease (Radostitis *et al.,* 2006). In addition, vitamin E is used widely to improve oxidative stability. Vitamin E is closely associated with selenium in its mode of action and a deficiency of either can lead to white muscle disease, depressed immune function and depressed fertility in animals. Vitamin E is poorly stored in the body, so daily intake is crucial (Pugh, 2002).

2.3.1.5 Deficiency of vitamin E

A deficiency of vitamin E can lead to decreased reproductive performance, retinal degeneration, and impairment of the immune system. Vitamin E deficiency is associated with the development of a range of specific diseases involving major tissues of the organism, including immune system incompetence, impairment of lipid metabolism, fertility problems, and increased susceptibility to common and specific diseases (Collins and Norman, 1991). Deficiency will lead to degeneration of muscle, lack of tone and hypersensitivity to pain. Hair may fall out, particularly around the head, on the legs, and the skin may be wrinkled with sores. Muscular dystrophy, exudative diathesis, skin depigmentation, erythrocyte 25 -50 ppm haemolysis, splenic and pancreatic hemosiderosis, fatty liver, ceroid deposition (Van Fleet and Kennedy, 1989).

2.3.1.6 Safety of vitamin E

There is no known toxicity with this vitamin (Schenk, 2010).

2.3.2 Heparinol®

2.3.2.1 Composition of heparenol®

Heparenol® contains lipotropic factors: choline chloride, betain, methionine, choleretic, diuretics and digestion stimulants, sorbitol, lysine and plants extracts (Artichoke, Gentian, Boldo) (Anonymous, 2016; Detopoulou *et al.*, 2008).

2.3.2.2 Functions of heparenol® in the body

Heparenol® has a quadruple action, it supports digestion and lipid assimilation. A simultaneous contribution of methionine, betain and choline, allows an immediate use of

choline by the liver for lipid circulation, a delayed synthesis of new choline molecules starting from contribution of betain and methionine and immediate and long acting hepatic protector. Methionine; 1 methyl group and choline are precursors of lipid conveyors (lipoproteins) to the liver. Betain; 3 methyl group aid in synthesis and transport by the liver, while, lysine makes digestion easier through synthesis and excretion of biliary salts from the gallbladder and sorbitol helps energy contribution. Plant extracts (choleretic effect) release of biliary salts in the digestive tract in the (cholagogue effect) makes digestion easier, sorbitol stimulates digestive transit and activates hormones, which induces excretion of biliary salt, induce lipase release by the pancreas and aid in villikines release which activates the local mixing of the digestive content (Anonymous, 2016; Detopoulou *et al.*, 2008).

Betain is essential for osmoregulation in stress situation this prevents a decrease of intracellular enzyme activity in cases of dehydration or overheating. Betain enhance the growth, activity and vitality of cells and thereby general animal health. Betain is frequently used to reduce the percentage of fat and raise the percentage of lean meat. Heparenol® is a product that improves the normal hepatic functions thus obtaining a maximum assimilation of the feed. It also support and stimulates an intense renal activity and improve the assimilation of the fat nutrient and liposoluble vitamins (ADEK). Heparenol® also stimulates appetite for feed and water. It prevents and treats heat stress, ascites and other metabolic dysfunctions even as it facilitate elimination of mycotoxins and other toxic substances. Choline plays an important role in reducing the risk of neural tube defects, fatty liver disease, and other pathologies (Anonymous, 2016; Detopoulou *et al.*, 2008).

2.3.2.3 Uses of heparenol®

Heparenol® helps in unfavourable circumstances such as stress which decrease the production yield and general body resistance. The liver must perform more metabolic work due to its general adaptation syndrome which affects the organism. Heparenol® revitalizes the liver and therefore helps to reduce the consequences of stress (Anonymous, 2016). In cases of colibacillosis: heparenol® contributes to the neutralization of the hepatic toxic action of *Escherichia coli* endotoxins. In cases of some parasitic enterohepatitis when heparenol® is combined with a specific antiparasitic treatment, it achieves a beneficial effect on the liver. Heparenol® acts as hepatic regenerator thus producing a large recovery in cases of parasitic enterohepatitis caused by *hexamitus.* It also helps in cases of intoxication produced by drugs or bacterial toxic metabolites. Heparenol® activates the elimination of toxins through the bile due to the action of cynarine which is provided from the plant extract (Artichoke). Heparenol® aids in its detoxification as well as in the regeneration of the hepatic tissues (Anonymous, 2016; Detopoulou *et al.,* 2008).

2.3.2.4 Deficiencies of heparenol®

Choline deficiency may play a role in liver disease, atherosclerosis and neurological disorders. One sign of choline deficiency is an elevated level of the liver enzyme ALT (Das *et al.,* 2008). Choline supplementation can be used in the treatment of liver disorders, hepatitis, glaucoma (Das *et al.,* 2008), atherosclerosis, Alzheimer's disease (Bjelland *et al*., 2009), and possibly other neurological disorders (Glier *et al*., 2014). Choline plays a role in the synthesis of phospholipids, phosphatidylcholine and sphinogomyelin, the key structural components of the human cell membrane (Detopoulou *et al*., 2008). Choline is needed to properly transport fat from the liver to cells throughout the body. It plays a part in

transporting cholesterol and triglycerides, two forms of important fats, from the liver to other parts of the body where they are needed (Detopoulou *et al*., 2008). In people who have low levels of choline, some studies have found that they are more at risk for experiencing liver damage and even liver failure (Detopoulou *et al.*, 2008).

Choline also helps in the synthesis of LDL cholesterol within the liver for healthy functioning — without enough, the body will suffer by storing fat in the liver (Zeisel *et al*., 2003). Research has shown that choline deficiency quickly results in metabolic syndrome, a condition involving insulin resistance, elevated serum triglycerides, increased serum cholesterol and obesity. In combination with a low-protein diet, choline deficiency also contributes to fatty liver disease (Zeisel *et al*., 2003). Studies consistently found that people with diets rich in choline have the lowest levels of inflammatory markers such as IL-6, TNF-alpha and C-reactive protein. Additionally, people that eat a choline-deficient diet for as short as a month have been found to have a significant increase in DNA damage and cancer risk (Zeisel *et al*., 2003).

2.3.2.5 Safety of heparenol®

Heparenol® is safe and it is the therapy of choice for detoxification of the organisms of birds affected by mycotoxins. Choline chloride is considered to be a safe nutrient and rarely causes negative side effects. However, like all nutrients, when too much is taken, it can become toxic and cause diarrhoea, nausea, fatigue, higher blood pressure, excessive perspiration and a fishy odour of the skin. The most common lesions of choline deficiencies are fatty liver and haemorrhagic kidney necrosis. Consuming a choline-rich diet will relieve the deficiency symptoms.

A study of this on animals has created some controversy due to the inconsistency in dietary modifying factors (Bjelland *et al*., 2009).

2.3.3 Aluminum magnesium silicate

2.3.3.1 Composition of Aluminium magnesium silicate

Aluminium magnesium silicate (AMS) consists of Aluminium silicate and magnesium silicate which are reacted to get a synthetic form of AMS {Al_4 $(SiO_4)_3$ + $3Mg_2SiO_4$ → $2Al_2Mg_3$ $(SiO_4)_3$ (Ezeibe *et al*., 2009a).

2.3.3.2 Functions of aluminium magnesium silicate

Genome of every virus is made of positively charged ions, such as, Na^+, Mg^{++} and K^+ while the phosphate component is negatively charged thus, some viruses have negative electrical charges while others are positively charged (Ezeibe *et al*., 2009a; Ezeibe *et al*., 2009b). Opposite electrical charges on AMS molecules and on viruses make viral particles to adsorb onto AMS molecules, thus blocking viral adsorption onto cells which is first stage in viral replication. In patients, AMS adsorbs onto viral particles released from infected cells so new foci of infections are minimized. Reduction of the number of foci of infections gives the body immune systems advantage over viral infections so that synergy between the patients` immune responses and antiviral effects of the AMS could lead to recovery from the infections.

Ezeibe *et al*. (2011a) demonstrated through an *in vitro* study that the AMS inhibited viruses of four viral families (*Paramyxoviridae*, *Orthomyxoviridae*, *Birnarviridae* and *Parvorviridae*) comprising 5 RNA viruses and 3 DNA viruses. This led to the cure of

animals experimentally challenged with viruses of *Paramyxoviridae*, *Birnarviridae* and *Parvoviridae* families which also included both RNA and DNA viruses. Antiviral agents are those that inhibit either attachment of viruses to cells, virus-cell fusion, viral uncoding, and/or reverse transcription of viral genomic RNAs (Ezeibe *et al*., 2011a; Ezeibe *et al*., 2011b). Aluminium-Magnesium Silicate is safe to normal cells (Ezeibe *et al*., 2009a).

2.3.3.3 Uses of Aluminium magnesium silicate

The two opposite electrical charges on molecules of AMS make them, when in solution, to hydrate to form three dimensional colloidal structures. The structures stabilize medicines that are in formulations with AMS. So AMS protects medicines from being rapidly degraded by metabolic processes so that bioavailability of the medicines is prolonged. When bioavailability is prolonged, effects of antimicrobials improve (Ezeibe *et al*., 2010b). It is already in use as antidiarrhoea therapy, as binder to make tablet dosage forms, to aid disintegration of capsules when swallowed and to stabilize emulsions (Ezeibe *et al*., 2009a). The AMS molecules are only 0.96 nm thick (Ezeibe *et al*., 2009a) = Nanoparticles (Ezeibe *et al*., 2011a). Nanoparticles cross physiological barriers to reach viruses and abnormal cells in all organs. Aluminium magnesium silicate disintegrates capsules, so it may also lyse virus-infected cells and cancer cells. Destroying infected cells makes intracellular viruses extracellular. It then adsorbs onto the extracellular viruses to prevent establishment of new foci of infection in treated patients. It also stabilizes chemotherapeutics to prolong their bioavailability thus leading to improved effects of antimicrobials against secondary infections. It is therefore concluded that AMS is not only

antiviral agent but it is also an adjuvant and a potential anticancer agent (Ezeibe *et al*., 2011a).

2.3.3.4 Safety of Aluminium magnesium silicate

Aluminium Magnesium Silicate is not harmful to normal cells. Each molecule of the AMS is composed of platelets that are only 0.96 nm-thick (Nanoparticles).

2.3.4 Polidine®

2.3.4.1 History of Polidine®

Povidine iodine complex was discovered in 1955 at the Industrial Toxicology Laboratories in Philadelphia by H. A. Shelanski and M. V. Shelanski (Sneader, 2005). They carried out *in vitro* tests to demonstrate anti-bacterial activity, and found that the complex was less toxic in mice than tincture of iodine (Sneader, 2005).

2.3.4.2 Functions of Polidine®

Povidone-iodine is a broad spectrum antiseptic for topical application in the treatment and prevention of infection in wounds, using the antiseptic properties of iodine. It may be used in first aid for minor cuts, burns, abrasions and blisters. Povidone-iodine exhibits longer lasting antiseptic effects than tincture of iodine, due to its slow absorption via soft tissue, making it the choice for longer surgeries (Fleischer and Reimer, 1997).

2.3.4.3 Uses of Polidine®

Following the discovery of iodine by Bernard Courtois in 1811, it has been broadly used for the prevention and treatment of skin infections, as well as the treatment of wounds.

Iodine has been recognized as an effective broad-spectrum bactericide and is also effective against yeasts, molds, fungi, viruses and protozoans (Simmons *et al.,* 2009). Povidone-iodine has found application in the field of nanomaterials. A wound-healing application has been developed which employs a mat of single wall carbon nanotubes (SWNTs) coated in a monolayer of povidone-iodine (Simmons *et al*., 2009).

2.3.4.4 Safety of Polidine®

Drawbacks to the use of polidine in the form of aqueous solutions include irritation at the site of application, toxicity when swallowed or inhaled and the staining of surrounding tissues (Simmons *et al*., 2009).

2.3.5 V-ox®

2.3.5.1 Composition of V-ox®

V-ox® contains ammonium persulphate, potassium persulphate, sulphonic acid, malic acid, sodium hexometophosphate and sodium chloride. Ammonium persulfate is a white, odorless single crystal and the formula is $(NH_4)_2S_2O_8$ (O'Neil, 2006).

2.3.5.2. Functions of V-ox®

Ammonium persulfate (APS) is a strong oxidizing agent that is used in polymer chemistry as an etchant and as a cleaning and bleaching agent, often used with tetramethyl-ethylene - diamine to catalyze the polymerization of acrylamide and bisacrylamide to prepare polyacrylamide gels for electrophoresis (O'Neil, 2006). It can hydrolyze into ammonium

hydrogen sulfate and hydrogen peroxide in an aqueous solution. The dry product has good stability, storage is easy and it has the advantage of convenience and safety. When heated to 120 °C, it can decompose, easily damped and cakes in moist air (O'Neil, 2006).

2.3.5.3 Uses of V-ox®

Ammonium persulfate forms oxygen free radicals in aqueous solution by a base-catalyzed mechanism (O'Neil, 2006). As an oxidizing agent and a source of radicals, APS finds many commercial applications. Salts of sulfate are mainly used as radical initiators in the polymerization of certain alkenes. Commercially important polymers prepared using persulfates include styrene-butadiene rubber and polytetrafluorethylene. The sulfate radical adds to the alkene to give a sulfate ester radical. It is also used along with tetramethylenediamine to catalyze the polymerization of acrylamide in making a polyacrilamide gel, hence being important for SDS-PAGE and western blot (Jakob *et al*., 2005). Illustrative of its powerful oxidizing properties, it is used to etch copper on printed circuit boards as an alternative to ferric chloride solution (Jakob *et al*., 2005). Ammonium persulfate is a standard ingredient in hair bleach. Persulfates are used as oxidants in organic chemistry. For example in the Minisci reaction and can be used as analytical reagents, photographic fixing agent and reducing agent. It can also be used as food preservative, oxidizing agent and initiator of high-molecular polymer and as raw material of producing persulfate and hydrogen peroxide in chemical industry, inhibitor of polymerization organic polymer, initiator during the polymerization of vinyl chloride monomer. It can be used as bleaching agent in grease, soap industry and as corrodent in plate metals cutting eclipse and oil extraction in oil industry. For food-grade, it can be used as modifier of wheat,

brewer's yeast mildew (Turrentine, 1908). Ammonium persulfate is non-flammable, but it can release oxygen, so it supports combustion (Turrentine, 1908).

2.3.5.4 Safety of V-ox®

Ammonium persulfate airborne dust may be irritating to eye, nose, throat, lungs and skin upon contact. Exposure to high levels of APS dust may cause difficulty in breathing. It has been noted that persulfate salts are a major cause of asthmatic effects in women (De Vooght *et al.,* 2010). Furthermore, it has been suggested that exposure to APS can cause asthmatic effects in hair dressers and receptionists working in the hair dressing industry. These asthmatic effects are proposed to be caused by the oxidation of cysteine residues, as well as methionine residues (De Vooght *et al.,* 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Location

The study was carried out at the Poultry Research Pens of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Zaria is in the Northern Guinea Savannah, between Latitude 11^{o} and 12^{o} N and Longitude 7^{o} and 8^{o} E and stands at an elevation of 650 m above sea level (Sawa and Buhari, 2011). The climate in the area is divided into two: dry and wet seasons. The wet season (May-October) characterized by convectional rainfall and a dry (November-April) season characterized by low temperatures (December-February)-the harmattan; and hot dry (March-April) with temperatures reaching 32ºC (Iloeje, 2004). Zaria has high relative humidity during the rainy season which drops in the dry season. It has monthly temperatures ranging from 13.8ºC to 36.7°C (Sawa and Buhari, 2011). The area has a tropical savannah climate with annual total rainfall of about 1,099 mm (Iloeje, 2004).

3.2 Experimental Chicks

Three hundred and sixty (360) day-old ISA brown pullets (IBP) were purchased from a reputable hatchery in Ibadan, Nigeria. Ethical clearance to conduct the study was sought from the ABU Committee on Animal Use and Care. The protocol employed met the guidelines of Good Laboratory Practice (GLP) regulations of World Health Organisation and the guidelines governing handling of laboratory animals as stipulated by the Ahmadu Bello University Animal Research Ethics Committee. The chicks were kept in well ventilated poultry research pens with 12 h day/night cycles.

3.3 Management of Chicks

The chicks were fed pelletized feed containing the following nutriments; crude protein (17%), crude fibre (12%), fat (8%), calcium (1.0%), available phosphorus (0.40%) and metabolisable energy (2,600 Kcal/kg) and provided water *ad libitum*. The chicks were housed on deep litter in pens with a floor space of 0.14 m^2 per bird. The pens were washed with detergent and then disinfected with Diskol® (5% benzalkonium chloride, 7.5% glutaraldehyde, 7.5% formaldehyde, stabilizers and antioxidants 9.5) at recommended rate of 40 ml per 10 litres of water. Sodium hypochloride (bleach) and 10% formaldehyde were also used at recommended doses at intervals of 7 days before the arrival of the chicks and subsequently sanitized weekly.

3.4 Infectious Bursal Disease Virus

A vvIBDV was obtained from the Department of Veterinary Medicine, ABU Zaria. The IBDV was obtained from previously vaccinated commercial layers that died of a natural outbreak of IBD. Sixty five per cent (65%) of commercial cockerels inoculated with the IBDV at 30 days of age with 50 µL of bursal suspension (v/w) in PHS (pH 7.4) died. One millilitre of bursal suspension (v/w) in PBS (pH 7.4) contained $10^{-9.76}$ CID_{50} of IBDV.

3.5 Unconcventional Agents Used for the Treatment of Infectious Bursal Disease

3.5.1 *Khaya senegalensis*

3.5.1.1 Collection and identification of Khaya senegalensis

Fresh leaves of KS (Appendix 1a and b) used in this study were collected in December, 2015 in the morning hours in a forest at Anchau village, Kubau Local Government Area

(LGA) of Kaduna State, Nigeria. The plant was validated to specie level by a Plant Taxonomist in the Herbarium Unit of the Department of Biological Sciences, Faculty of Life Sciences, ABU Zaria and the specimen voucher number; 900181 for KS was deposited with the Herbarium for reference purposes. Photographs of the plants were also taken and kept for documentation.

3.5.1.2 Extraction of Khaya senegalensis leaves powder

The fresh leaves of KS were washed, cut into small parts and allowed to dry at room temperature until a constant weight of the dried leaves was obtained. Dried leaves were pulverized into powder using Lab mill machine (type 8 serial number 50157 model 8000 RPM made by Christy and Norris Limited, Process Engineers, Chelmsford-England) and sieved through a 0.25 mm sieve (Okwu and Josiah, 2006). One hundred grams of powdered leaves were added to 300 ml of water and extracted by maceration at a ratio of 1 g of plant material to 3 ml of water (1:3). The mixture was allowed to stand for 24 h and supernatant decanted, the mag pressed to further extract liquid. The extract was filtered using a piece of clean sterile Muslin cloth to remove debris and then filtered using two different grades and millimetre sizes of commercial kunu (local brew) sieves to further remove debris. The extract was administered *ad libitum* as the only source of drinking water.

3.5.2 Vitamin E

Vitamin E was obtained from Agricare STD Limited, Nigeria and administered at 1 ml per litre of drinking water for 7 days.

3.5.3 Chemicals

3.5.3.1 Heparenol®

Heparenol® was obtained from Merial, France through a Nigerian agent and administered at 1 ml per 1 litre of drinking water for 7 days.

3.5.3.2 Aluminium magnesium silicate

Aluminium magnesium silicate was obtained from the University of Agriculture Umudike, Abia State, Nigeria and administered at 1 g per 1 litre of drinking water for 7 days.

3.5.3.3 Polidine®

Polidine® was obtained from Polchem Hygiene Laboratories in India through a Nigerian agent and administered at 4 ml per litre of drinking water for 7 days.

3.5.3.4 V-Ox®

V-Ox® was obtained from Polchem Hygiene laboratories in India through a Nigerian agent and administered at 1 g or 2 g per 1 litre of drinking water for 7 days.

3.6 Enzyme Linked Immunosorbent Assay Kit

The ELISA Kit, used for this study was obtained from IDEXX Laboratories Incorporation, USA.

3.7 Proximate and Phytochemical Analyses of Feed and Medicinal Plant Leaves

3.7.1 Proximate analysis of feed and medicinal plant leaves

The feed (Vital Feeds®, Jos, Nigeria) was analysed for dry matter, crude protein, crude fibre, oil, ash and nitrogen free extracts contents, while the KS leaves was analysed for carbohydrates, fats, crude protein, moisture, dry matter, crude fibre, nitrogen free extract and ash content in the laboratory of the Department of Animal Science, Faculty of Agriculture, ABU Zaria according to the methods of the Association of Official Analytical Chemists (AOAC) (Kapadia *et al.,* 1978; AOAC, 2003; David and Bender, 2005).

3.7.2 Phytochemical screening of plant leaves

Phytochemical analyses of KS leaves was conducted according to the methods described by Trease and Evans (1996) at the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The KS leaves powder was tested for the presence of the following metabolites: alkaloids (Dragendoff's test), steroids (Sakowski test), tannins (lead subacetate test), anthraquinones (Bontrager test), cardiac glycosides (Keller-kiliani test), flavonoids (sodium hydroxide test) and saponins (frothing test).

3.8 Inoculation of ISA Brown chicks with Infectious Bursal Disease Virus

At week three (21 days) of age, the chicks in the test groups and the positive control group were inoculated with 0.05 ml of vvIBDV via conjunctiva (0.025 ml) and intra nasal (0.025

ml) instillation based on the methods of Okoye and Uzoukwu (1981) while chicks in the negative control group were not inoculated.

3.9 Collection of Blood Sample

About 0.5 to 2 ml of blood was collected from twenty already tagged chicks per group at day-old and the same number at 7, 14, 21, 28, 31 and 35 days of age via the heart for the determination of IBD antibody titre levels. The chicks were selected randomly from the twenty and restrained between the index finger and the thumb. Blood was collected via the heart at 9:00 am on collection days using 2 ml syringe and 23 guage needle. The blood collected was poured into a screw capped container and allowed to stand on a table at room temperature to allow for serum formation. After 4 h, serum was decanted into a bujou bottle and stored in a deep freezer until tested for antibodies.

3.10 Detection of Infective Infectious Bursal Disease Virus in the Bursa of Inoculated ISA Brown Chicks Using Agar Gel Precipitin Test

3.10.1 Agar gel preparation

Agar gel was prepared by weighing out 1.3 g of agarose (Life Tecnologies, USA), 80 g of sodium chloride, 0.2 g of sodium azide and these were reconstituted in 100 ml of distilled water. The mixture was solubilised by boiling for 2 min and later autoclaved at 115 ^{o}C for 15 min. According to the modified method of Abdu (1997), the agar plate was made by dispensing 25.7 ml of agar into 9 x 9.5 cm petri dishes to give a depth of 3 mm. Wells of 6 mm width with 3 mm separation were cut into already set agar. A parallel 6 vertical rows

with 6 wells with a middle row having 3 wells which are at the centre of 4 wells were created on the agar gel plate using a template.

3.10.2 Agar gel precipitin test

The BF of the chicks inoculated that died of IBD was harvested and the IBDV antigen detected using agar gel precipitin test (AGPT) as described by OIE (2008). The central wells were filled with 0.02 ml of positive serum and 0.02 ml of the test BF homogenate was dispensed into the wells in the parallel vertical columns. The Petri dishes were incubated in a humid chamber at room temperature and observed after 24 and 48 h for precipitin lines

.

3.11 Clinical Studies of ISA brown chicks Inoculated With Very Virulent Infectious Bursal Disease Virus

3.11.1 Clinical signs

From 21 days of age (0 dpi) to 35 days of age (day 14 pi), clinical signs and mortalities due to IBD including sudden death, depression, somnolence, anorexia, diarrhoea, soiled vent, prostration, huddling together, weakness, dehydration, vent pecking, in-coordination, prostration, recombency, trembling and mortality were noted, scored and recorded (Appendix 19).

3.12 Pathological Studies of ISA Brown Pullets Inoculated With Very Virulent Infectious Bursal Disease Virus

3.12.1 Gross pathology

Five chicks from each group were euthanized on days 21, 24, 28 and 31 (0, 3, 7, and 10 dpi) and postmortem (pm) examination was carried out to identify gross lesions like enlargement, atrophy, oedema, haemorrhages and creamy exudates in the BF; haemorrhages in the body skeletal muscles, proventriculus, liver, kidneys and intestinal mucosa; enlargement of the liver, kidneys, spleen; whitish kidneys with urates and debris; cheesy core, grey foci in spleen and mucus in intestinal mucosa were noted, scored and recorded (Appendix 20). At days 0, 3, 7 and 10 pi, euthanized chicks were weighed, BF, spleen and thymus removed and weighed using a digital weighing balance (Harris, England, model number, YP-602N and serial number, CS 12A 0806).

3.12.2 Histopathology

Specimens of the BF, SP and THY were fixed in 10% buffered neutral formalin for histopathology using the method of Bancroft and Cook (1994). The tissues were trimmed and identified and were then dehydrated through a series of graded ethanol (70%, 80%, 90%, 95% and 100%). The blocks were cleared in xylene and then infiltrated with molten paraffin wax. Sections of 5 microns (μm) were cut from embedded tissue using Jung Rotary Microtome (model 42339). The tissues were mounted on grease free clean glass slides, kept at room temperature and later stained with Hematoxylin and Eosin (H & E). The permanent slides prepared were examined using a light microscope (Olympus

binocular microscope) at x4, x10 and x40 objectives for histopathological changes (Hair-Bejo *et al*., 2000; Babiker *et al*., 2008). Photomicrographs of the tissues were taken using a digital microscopic objective camera. The photographs were transferred to a computer and detailed studies carried out to ascertain histopathological lesions (Babiker and Tawfeeq, 2008).

3.13 Enzyme-Linked Immunosorbent Assay

Enzyme linked immunosorbent assay was carried out at the Animal Care Services Konsult Laboratories, Ogere, Ogun State as described by IDEXX Laboratories Incorporation, USA. Briefly, the antigen coated plates and the ELISA kit reagents were adjusted at room temperature prior to test. The test sample was diluted to five hundred folds (1:500) with sample diluents prior to the assay. A 100 µl of diluted sample was then poured into each well of the plate. This was followed by 100 µl of undiluted negative control into the well A1 and A2, 100 µl of undiluted positive control into well A3 and A4. The plate was incubated for 30 min at room temperature. Each well was then washed with approximately 350 µl of distilled water 3 times. Goat anti-chicken conjugate (100 µl) was dispensed into each well. The plate was incubated at room temperature for 30 min, followed by washing each well with 350 µl of distilled water 3 times. Tetramethylebenzidine (TBD) solution (100 µl) was dispensed into each well. The plate was then incubated at room temperature for 15 min. Finally 100 µl of stop solution was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 650 nm. Infectious bursal disease antibody titre level was calculated automatically using soft ware by Blankfard and Silk (Blankfard and Silk, 1989).

3.14 Experimental Design

The chicks were randomly selected into 9 groups, A-I of 40 each, 20 birds from each group were tagged and given identification numbers. Twenty chicks from each group were bled at 2, 7, 14, 21, 24, 28, 31 and 35 days of age to determine the IBD antibody titre levels. The remaining chicks were inoculated with vvIBDV in 0.05 ml/bird at 21 days of age while group G (negative control) was not inoculated. At 21 days of age, group A was treated with KS and immediately clinical signs were observed (2 dpi), the remaining chicks were treated with vitamin E (group B), heparenol (C), aluminium magnesium silicate (D), polidine (E), V-ox® 1 (F) and V-ox® 2 (I), while H (positive control) and G (negative control) were not treated. At 21, 24, 28, 31, 35 days of age, inoculated birds were observed for clinical signs, morbidity, mortality, gross and microscopic lesions (Table 3.1).

3.15 Data Analyses

The clinical signs observed in each group were calculated as a percentage of the initial number of chicks as described by Babiker *et al*. (2008). The clinical signs were graded and scored as; normal (0%), low (1-30%), moderate (31-50%), high (51-60%), and very high (61-100%), respectively. Severity of illness was obtained by dividing the number of clinical signs observed by the expected number of clinical signs of IBD multiplied by number of chicks sick divided by number of chicks in that group multiplied by 100.

$$SI = \frac{NCSS \times NBS}{TNCS \times TNB} \times 100$$

Where SI = severity of illness; NCSS = Number of clinical signs observed; TNCS = Total number of clinical signs due to IBD; NBS = number of chicks showing clinical signs; TNB = Total number of chicks in that group. Morbidity rates were obtained by grading and

scoring the clinical signs as; normal (0%), low (1-30%), moderate (31-50%), high (51-60%) and very high (61-100%), respectively. Mortality rate was calculated as number of chicks dead divided by number challenged multiplied by 100 (Babiker *et al*., 2008). Also, case fatality rate was calculated by dividing the number of chicks that died by the number of sick chicks multiplied by 100.

$$CFR = \frac{ND}{NS} X\ 100;$$

Where CFR = case fatality rate; ND = Number of chicks that died in the group; NS = Number of chicks that were sick in the group. Dead chicks were examined and the proportions of dead chicks showing specific gross lesions were noted, scored and graded as 0 = normal [(no gross lesions (NGLS)], 0.1-2.0 = moderately gross lesions (MGLS), 2.1-5.0 = moderately severe gross lesions (MSGL), 5.1-9.0 = severe gross lesions (SGL) and (9.1-12 = very severe lesions (VSGL), based on the presence and severity of lesions on BF, skeletal muscles, kidneys, liver, spleen, thymus, coecal tonsils, gall bladder, pericardium, skin, kidneys, erosions and haemorrhages of mucosa of the proventriculus (Hair-Bejo *et al*., 2000; OIE, 2004; Babiker *et al*., 2008).

Table 3.1: Experimental design for testing the efficacy of some unconventional remedies used for the treatment of infectious bursal disease in Nigeria.

	Age of birds in days (days post inoculation with vvIBDV)							
	2	7	14	21 (0)	24 (3)	28(7)	31(10)	35 (14)
	Number of birds bled							
	20	20	20	20		20	20	20
	Number of birds euthanized							
Group				5	5	5	5	5
A (*Khaya senegalensis)*	Bled	Bled	Bled	Bleed, IBDV, *K. seegalensis* Euthanized, Observed	Euthanized, Observed	bled , euthanized observed	Bled, euthanized observed	Bled, euthanized Observed
B (Vitamin E)	Bled	Bled	Bled	Bled, IBDV, euthanized Observed	VitaminE, euthanized Observed	bled , euthanized observed	Bled, euthanized observed	Bled, euthanized Observed
C(Heparenol®)	Bled	Bled	Bled	Bled, IBDV ,euthanized Observe	heparenol® Euthanized, Observed	bled , euthanized observed	Bled, euthanized observed	Bled, euthanized Observed
D (Aluminium magnesiul silicate)	Bled	Bled	Bled	Bled, IBDV, euthanized Observed	Ammonium magnesium silicate Euthanized, Observed	bled , euthanized observed	Bled , euthanized observed	Bled, euthanized Observed
E (Polidine®)	Bled	Bled	Bled	Bled, IBDV, euthanized Observed	polidine® Euthanized, Observed	bled , euthanized observed	Bled , euthanized observed	Bled, euthanized Observed
F (V-ox® 1)	Bled	Bled	Bled	Bled, IBDV euthanized Observed	V-ox® 1Euthanized, Observed	bled , euthanized observed	Bled, euthanized observed	Bled, euthanized
G (Negative control)	Bled	Bled	Bled	Bled, No IBDV euthanized, Observed	No treatment Euthanized, Observed	bled euthanized observed	Bled, euthanized observed	Bled euthanized Observed
H (Positive Control)	Bled	Bled	Bled	Bled, IBDV euthanized Observed	No treatment Euthanized, Observed	bled , euthanized observed	Bled, euthanized observed	Bled, euthanized Observed
I (V-ox® 2)	Bled	Bled	Bled	Bled, IBDV euthanized Observed	V-ox® 2 Euthanized Observed	bled , euthanized observed	Bled , euthanized Observed	Bled , euthanized Observed

KEY: Bled - Collection of blood for serum; IBDV – Inoculation with IBDV; Observed – Observe for clinical signs and gross lesion; vvIBDV- very virulent infectious bursal disease virus; Euthanized – To sacrifice the chickens for observations

Weight of lymphoid organs were obtained and scored in relation to total carcass weight (organo-somatic index). Individual bursal to body weight ratio (BBWR) were calculated by dividing bursal weight by body weight and multiplying by 10^3 (Giambrone and Closser, 1990; Eterradossi *et al*., 2004).

Organ: body weight ratio was calculated using the formula:

$$\text{Organ: body weight ratio} = \frac{\text{Organ weight in grams}}{\text{Body weight in grams}} \times 1{,}000$$

The organ: body weight index was obtained by employing the formula:

$$\text{Organ: body weight index} = \frac{\text{Mean organ: body weight ratio of test groups}}{\text{Mean organ: body weight ratio of negative control group}}$$

Clinical signs, morbidity, mortality, gross pathological changes and antibody titre level data obtained were entered into Microsoft Excel 2007 and exported to Statistical Package for Social Sciences (SPSS) version 20.0 database where descriptive statistics were carried out. The data were presented in graphs and tables. Morbidity and mortality patterns were analysed using graphs and chi square (χ^2). The body weight, organ weight and organ body weight ratio were expressed as mean ± SEM. With the aid of Graph Pad Prism 6, body weight, organ weight, organ body weight ratio and index data were subjected to a one-way analyses of variance (ANOVA) to compare the means of various groups while Tukey posthoc test was used to compare variations within and in between groups (Myra, 1989). The antibody titre level was expressed as mean ± SEM and subjected to two-way ANOVA. Coefficient of variation (CV) in Ab titre was calculated in percentages. Ninety five (95) % confidence interval and odds ratio was calculated and values of $P < 0.05$ were considered significant.

CHAPTER FOUR

RESULTS

4.1 Nutrient Composition of Feed and *Khaya senegalensis* leaves

The feed fed to the chicks contained 93.67% dry matter, 17.02% crude protein, 6.38% crude fibre, 4.24% oil, 7.20% ash and 65.16% nitrogen free extracts. Proximate analyses of medicinal plant leaves indicated that KS contained 93.88% dry matter, 11.81% crude protein, 10.15% crude fibre, 0.98% oil, 10.98% ash and 66.08% nitrogen free extract (Appendix 2).

4.2 Phytochemical Constituents of *Khaya senegalensis* leaves

Phytochemical analyses of KS leaves extract indicated the presence of tannin, phytate, oxalate, saponin and alkaloids in the plant leaves tested (Appendix 3, 4 and 5). The quantity of tannin in KS was 18.64 mg/100 g, phytate 0.03 mg/100 g, oxalate 0.17 mg/100 g, saponin 0.68 mg/100 g and alkaloid 0.80 mg/100 g (Appendix 3).

4.3 Clinical Signs in ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

The clinical signs noticed in chicks inoculated with vvIBDV included; sudden death, depression, somnolence, anorexia, diarrhoea, soiled vent, prostration, huddling together, weakness, dehydration, weakness, vent pecking, incoordination, recumbency, trembling and mortality (Appendix 19). Clinical signs initially appeared on 2 dpi in all groups except group C where there was delay until 3 dpi. Clinical signs disappeared at 6 dpi in groups A, I, C and F at 7 dpi in groups B, D, E and H. In all inoculated groups, clinical signs commenced at 2 dpi and peaked on day 4 pi and declined at 6 or 7 dpi when the clinical

signs disappeared. In all groups, the clinical signs were more severe at 4 dpi (Appendix 7). Clinical signs were severe 4 dpi in groups H (positive control) (50.7%), D (49.0%), F (42.3%), E (38.4%) and C (28.6%) but it was less severe in groups A (15.6%), B (24.7%) and I (14.1%), (Table 4.1).

4.4 Morbidity, Mortality and Case Fatality Rates in ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

Morbidity rate was highest on day 4 pi in groups B, D, E, F, H and I, high on day 5 pi in group C and day 3 pi in group A. Group H (positive control) had the highest morbidity of 54.54% followed by groups D, E and F with 50.00% each. The lowest rates were recorded in groups A (18.18%), B (37.50%), C (27.27%) and I (25.0%) (Table 4.2). Morbidity between groups were significantly different ($P \leq 0.05$) at $p = 0.002$ (Appendix 6). High mortality rates were seen in groups C (31.81%), D (45.45%), F (36.36%) and H (27.27%). Low mortality rates were recorded in groups A, B, E and I with 13.60%, 18.18%, 18.18% and 8.33%, respectively (Table 4.3). Total mortality were higher in group C, 19 (86.36%), followed by group D, 16 (72.73%) and group H, 13 (59.09%). Lower overall mortality rates were recorded in groups I, (16.67%) followed by groups A and B (27.27%). Mortality between groups were significantly different ($P \leq 0.05$) at $p = 0.000$ (Appendix 6). Case fatality rates were higher in group C (317.0%) followed by group D (145.45%) and group H (108.33%). Lower case fatality rates were recorded in group I (66.67%) followed by group E (81.82%) and groups A, B and F (100.0%) each (Table 4.4).

4.5 Presence of Infectious Bursal Disease Virus Precipitin Antigens

Precipitin antigens were only detected in the BF of chicks in groups I and B at day 7 pi and in group H (positive control) at 10 dpi.

4.6 Gross Pathological Lesions Score in ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

At day 3 pi, all groups except group G (negative control) had high gross lesion score, but the score was higher in groups F (10.4) and B (9.4), and lower in groups C (4.6) and D (4.6) (Appendix 9). On day 7 pi, there was significant reduction in lesions in all groups with the highest percent reduction seen in groups E (80.0%), B (78.7%) and F (65.4%). Reduction of lesion was lower in groups A (27.7%), D (29.2%) and H (30.0%). By day 10 pi, all groups had low lesion score. Reductions in lesions were seen in all the groups except in group E that had an increased lesion by +16.7% while all birds in groups C and D died. The per cent reduction in lesions were higher in groups I (86.4%), H (75.0%), B (70.0%) and F (66.7%), while it was lower in group A (48.0%) by 10 dpi (Table 4.6). On day 3 pi, treatment groups B (9.4) and F (10.4) had very severe lesions seen, followed by groups A (6.8), E (6.0) and H (8.0) with severe gross lesions. The moderately severe lesion score were noticed in groups C (4.6) and D (4.8), while no lesions were seen in groups G (negative control). On day 7 pi, group H (5.6) had severe lesions followed by groups A (5.0), D (3.2), F (3.6) and I (4.4) with moderately severe lesions. Groups B (2.0), C (2.2) and E (1.2) had moderately severe lesions while no lesions were seen in group G (negative control). On day 10 pi, groups A (2.6), B (0.6), E (1.4), F (1.2), H (1.4) and I (0.6) had

moderate lesions seen, there were no lesions seen in group G (negative control) while all the chickens in groups C and D died before day 10 pi (Table 4.5).

4.7 Body weights of ISA brown chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

Generally there was an increase in body weight as the birds aged (Appendix 8). There was however, an initial decrease in body weight noticed 3 days pi in groups A (11.5%), D (10.04%) and F (3.2%) and an increase in body weight in the other groups, which was significant in groups G (negative control) (21.7%) and B (21.3%). On day 7 pi, significant increase in body weights were seen in G (negative control) (39.0%), F (31.9%) and D (27.5%). On day 10 pi, higher increase in body weights were noticed in groups F (40.7%), B (30.1%), E (23.2%) and A (19.2%) (Table 4.6). On day 3 pi the body weights between groups A (140 ± 6.06 g) and B (188.8 ± 9.94 g) were significantly different ($p = 0.0093$). On day 7 pi, the body weights in groups A (162.8 ± 13.54 g), D (178.2 ± 9.46 g), E (178.4 ± 14.46 g), G (245.2 ± 15.24 g) and I (191 ± 4.54 g) were significantly different ($P = 0.0013$). Body weights in groups C (193.3 ± 7.51g), E (178.4 ± 14.46 g), and I (191 ± 4.54 g) at $P = 0.0013$ were significantly different. On day 10 pi the body weights were 185 ± 27 g, 201.4 ± 5.97 g, 204.7 ± 9.21 g, 219.8 ± 13.51 g, 249.4 ± 13.98 g and 251.8 ± 6.09 g in groups I, A, H, E, G and B respectively, which are significantly different ($P < 0.005$) (Table 4.6).

Table 4.1: Severity of clinical signs in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Days postinoculation						
	2	3	4	5	6	7	
Group	% severity of clinical signs						Total
A (*Khaya Senegalensis*)	6.60	14.30	15.60	13.30	12.20	0.00	62.00
B (Vitamin E)	0.60	3.20	24.70	6.90	2.80	0.50	38.70
C (Heparenol®)	0.00	9.40	28.60	30.00	3.80	0.00	71.80
D(Aluminium magnesium silicate)	5.13	21.70	49.00	10.30	20.50	2.00	108.60
E (Pololidine®)	4.20	9.40	38.40	21.20	14.80	6.00	94.00
F (V-ox®1)	4.20	19.30	42.30	15.60	5.30	0.00	86.70
G (Negative control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H (Positive control)	4.10	5.50	50.70	12.00	5.30	6.00	83.60
I (V-ox® 2)	1.40	4.80	14.10	5.20	4.60	0.00	30.10

Table 4.2: Morbidity rates in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

		Days postinoculation				
		2	3	4	5	6
Group	n	Number of sick birds (%)				
A(*Khaya senegalensis*)	22	3 (13.60)	6 (27.27)	4 (18.18)	4 (18.18)	3 (13.63)
B (Vitamin E)	22	1 (4.50)	2 (9.10)	6 (37.50)	2 (9.10)	1 (4.50)
C (Heparenol®)	22	0 (0.00)	1 (4.50)	2 (9.10)	6 (27.27)	5 (22.72)
D (Aluminium) magnesium sislicate	22	2 (9.10)	9 (41.00)	11 (50.00)	4 (18.18)	3 (13.63)
E (Polidine®)	22	2 (9.10)	5 (22.72)	11 (50.00)	6 (27.27)	4 (18.18)
F (V-Ox® 1)	22	2 (9.10)	8 (36.40)	11 (50.00)	6 (27.27)	1 (4.50)
G (Negative control)	22	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
H(Positive control)	22	3 (13.60)	5 (22.72)	12 (54.54)	4 (18.18)	1 (4.50)
I (V-Ox® 2)	12	1 (8.33)	2 (16.66)	3 (25.00)	1 (8.33)	1 (8.33)

Table 4.3: Mortality rates in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

		Days postinoculation					
		3	4	5	6	7	
Group	n	Number of birds dead (%)					Total (%)
A (*Khaya senegalensis*)	22	0 (0.00)	1 (4.50)	3 (13.60)	2 (9.09)	0 (0.00)	6 (27.27)
B (Vitamin E)	22	1 (4.50)	0 (0.00)	4 (18.18)	0 (0.00)	1(4.50)	6 (27.27)
C (Heparenol®)	22	0 (0.00)	7 (31.81)	7 (31.81)	5 (22.27)	0 (0.00)	19 (86.36)
D (Aluminium magnesium silicate)	22	1 (4.50)	1 (4.50)	10 (45.45)	3 (13.60)	1 (4.50)	16 (72.72)
E (Polidine®)	22	0 (0.00)	2 (9.09)	4 (18.18)	2 (9.09)	1 (4.50)	9 (40.90)
F (V-Ox® 1)	22	0 (0.00)	1 (4.50)	8 (36.36)	2 (9.09)	0 (0.00)	11 (50.00)
G (Negative control)	22	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
H (Positive control)	22	3 (13.60)	0 (0.00)	6 (27.27)	3 (13.60)	1 (4.50)	13 (59.09)
I (V-Ox® 2)	12	0 (0.00)	1 (8.33)	1 (8.33)	0 (0.00)	0 (0.00)	2 (16.67)

Table 4.4: Morbidity, mortality and case fatality rates in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A (*Khaya senegalensis*)	B (Vitamin E)	C (Heparenol®)	D (Aluminium magnesium silicate)	E (Polidine®)	F (V-ox® 1)	G (Negative control)	H (Positive control)	I (V-ox® 2)
Rate	Number of birds challenged								
	22	22	22	22	22	22	22	22	22
Morbidity n (%)	6(27.27)	6(27.27)	6(27.27)	11(50.00)	11(50.00)	11(50.00)	0(0.00)	12(54.55)	3 (25.00)
Mortality n (%)	6(27.27)	6 (27.27)	19(86.36)	16(72.73)	9(40.91)	11(50.00)	0(0.00)	13(59.09)	2(16.67)
Case fatality (%)	100.00	100.00	317.00	145.45	81.82	100.00	0.00	108.33	66.67

Table 4.5: Summary of gross pathological lesion score in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A (*Khaya senegalensis*)	B (Vitamin E)	C (Heparenol®)	D (Aluminium magnesium silicate)	E (Polidine®)	F (V-ox® 1)	G (Negative control)	H (Positive control)	I (V-Ox® 2)
	Gross pathological score (% reduction)								
Days postinoculation									
3	6.8	9.4	4.6	4.8	6.0	10.4	NGP	8.0	NE
7	5.0 (-7.70)	2.0 (-78.70)	2.2(-52.20)	3.2(-29.20)	1.2 (-80.00)	3.6 (-65.40)	NGP	5.6 (-30.00)	4.4 (NA)
10	2.6 (-8.00)	0.6 (-70.00)	ABD	ABD	1.4 (-16.70)	1.2 (-66.70)	NGP	1.4 (-75.00)	0.6 (-86.40)

Key: ABD = All birds died; NGP = No gross pathology; NE = Not euthanized, NA = not applicable.

4.8 Mean Weights of Bursa of Fabricius of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

On day 3 pi, there was an increase in the weight of BF in groups E (10.3%), C (9.0%), F (8.6%) and D (6.8%), while other groups had a decrease in the weight of BF. On day 7 pi a decrease in the weight of BF was noticed in all the groups except group G (negative control) in which the weight of BF increased by 10.5%. There was reduction in the rate of atrophy of BF in groups A (2.7%), B (1.5%), E (16.8%) and I (18.4%) at 7 dpi compared with an increase in group H (34.9%). Decreases in the weight of BF were noticed in all groups on day 10 pi but a more significant decrease was recorded in group H (positive control) (29.0%) (Table 4.6). On day 7 pi there was significant difference ($P < 0.05$ at $P = 0.0063$) in weight of BF between group A (0.85 ± 12 g) and G (1.434 ± 0.15 g), and between groups D (0.852 ± 0.07 g), E (0.892 ± 0.08 g), F (0.79 ± 0.09 g), H (0.784 ± 0.09 g) and group G (1.434 ± 0.15 g) at $P = 0.0063$. There was no significant difference ($P < 0.05$) in weight of BF between the groups on day 10 pi (Table 4.6) (Appendix 10).

4.9 Mean Weights of Spleen Weight of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

On 3 dpi, there was increase in the weight of spleen in all the groups, but the increase was more significant ($P < 0.05$) in groups C (69.1%), F (50.7%), and A (47.1%) while there was a decrease in the weight of SP in group H (8.0%) (positive control). On 7 dpi, the weight of SP decreased in groups A (5.2%), while there was an increase in each group but significant ($P < 0.05$) increases were noticed in groups B (58.3%), F (54.8%), I (51.3%) and D (41.2%). At 10 dpi, groups A, B, F and I had a decrease in the weight of SP by

13.6%, 5.5%, 14.8% and 17.0%, respectively while there was an increase in groups H (17.7%) and E (14.6%) (Table 4.6). On day7 dpi, the weight of SP was 0.616 ± 0.06 g in group A and 1.068 ± 0.19 g in group F. There was significant difference ($P < 0.05$ at $P = 0.060$) in the weight of SP (Appendix 11) between group F (1.068 ± 0.19 g) and groups A D E G and H (0.616 ± 0.06 g, 0.672 ± 0.09 g, 0.666 ± 0.09 g, 0.676 ± 0.04 g and 0.728 ± 0.08 g) respectively. On day10 dpi, there was significant difference ($P < 0.05$) in the weight of SP between group A (0.532 ± 0.05 g) and groups B, F and H with 0.824 ± 0.11 g, 0.91 ± 0 g and 0.8567 ± 0.15 g respectively at $P = 0.0601$ (Table 4.6).

4.10 Mean Weights of Thymus of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

The weights of Thymus (THY) decreased in all groups except in groups C and E which increased by 17.4% and 9.7%, respectively on day 3 pi. By 7 dpi, the thymic weight increased in groups D, F and G by 25.5%, 8.5% and 36.6%, respectively while other groups had decreases which were higher in group E (-32.2%) and I (-48.6%). On day 10 pi, the weight of THY in group I decreased by 4.7% while there were increases in other groups with significant increase in groups G (62.5%), B (58.4%) and F (44.0%) (Table 4.6). The thymic weights were significantly different ($P < 0.05$) between groups A and E, (1.23 ± 0.08 g and 1.094 ± 0.09 g) and groups B, C, D, F, G and H, (1.462 ± 0.07 g, 1.334 ± 0.14 g, 1.358 ± 0.22 g, 1.41 ± 0.11 g, 1.604 ± 0.05 g and 1.39 ± 0.15 g respectively (at $P = 0.2127$). There was significant difference ($P < 0.05$) between groups C and G, (1.497 ± 0.30 g) and (1.322 ± 0.21 g) and A, B, D, E, F, G and I (0.73 ± 0.09 g, 1.01 ± 0.16 g, 1.094 ± 0.18 g, 0.814 ± 0.23 g, 0.986 ± 0.10 g, 0.958 ± 0.12 g, 0.848 ± 0.13 g) respectively. On day 10 pi the thymic weight were significantly different ($P \leq 0.005$) between groups B and

G, (1.6 ± 0.11 g and 2.148 ± 0.32 g and groups A, E, F, H and I (0.762 ± 0.05 g, 1.043 ± 0.26 g, 2.148 ± 0.32 g, 0.913 ± 0.15 g and 0.915 ± 0.24 g respectively) (Table 4.6) (Appendix 13).

4.11 Bursa of Fabricius to Body Weight Ratio of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

The bursa body weight ratios (BBWR) increased on day 3 pi in groups D, E, and F by +9.5%, +8.3%, and +14.9%, respectively. By day 7 pi all groups had decreased BBWR but groups A, B, C, D, and E with -2.9%, -23.1%, -32.8%, -43.7%, and -27.0%, respectively had lower BBWR compared to a higher decrease in group H (-46.1%). By day 10 pi, the decrease in BBWR were higher in groups A, F, E and B by -2.0%, -41.2%, -39.2%, and -9.1%, respectively (Table 4.7). The BBWR were significantly different ($P \leq 0.05$) between group G (8.577 ± 1.18 g) and groups A and B (6.185 ± 0.57 g and 6.572 ± 0.50 g respectively at P = 0.5152) on day 3 pi. On day 7 pi, the BBWR was significantly different ($P \leq 0.05$) between groups H and F (3.773 ± 60 g) and (4.112 ± 0.41 g) respectively and groups A, B, C, D, E, G and I (5.386 ± 0.85 g, 5.056 ± 0.67 g, 4.765 ± 0.09 g, 4.825 ± 0.41 g, 5.246 ± 0.78 g, 5.875 ± 0.51 g and 5.012 ± 0.81 g) respectively (at P = 0.5152). On day 10 pi BBWR was significantly different ($P \leq 0.05$) between group G (5.452 ± 1.04 g) and groups A (3.124 ± 1.20 g), B (3.077 ± 0.41 g, E (3.192 ± 0.43 g), F (2.416 ± 0 g), H (2.692 ± 0.31 g), and I (4.55 ± 0.31 g) at P = 0.5152 (Table 4.7). Within groups, BBWR decreased from 0 dpi to 10 dpi except in groups D, E and F where there was an initial increase by 3 dpi from 7.264 ± 0.57 g to 8.577 ± 1.18 g in group D, 6.646 ± 0.73 g to 5.246 ± 0.78 g in group E and 6.935 ± 0.45 g to 4.112 ± 0.41 in group F (Table 4.7) (Appendix 13).

4.12 Spleen to Body Weight Ratio of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

The SBWR increased in all groups by 3 dpi except group B which decreased by -5.6%. The increase was higher in group F (+62.7%). By 7 dpi SBWR significantly ($P < 0.05$) increased in groups B (+62.7%), C (+35.7%), I (+28.6%), F (+13.1%), D (+11.8%) and E (+3.2%) but decreased in groups A (-18.5%) and G (-8.3%). By 10 dpi all groups showed decrease in SBWR except in group H (positive control) which had an increase of +22.6% (Table 4.7). On 7 dpi, there was significant difference ($P < 0.05$) between SBWR groups F and G with 5.681 ± 1.13 g and 2.818(-8.3), respectively at $P = 0.846$. With in groups, there was initial increase in SBWR in all groups before a reduction except group H (positive control) where increases continued up to 10 dpi (Table 4.7) (Appendix 14).

4.13 Thymic to Body Weight Ratio of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

There was an increase in TBWR on 3 dpi in groups C (+3.1%) and E (+8.0%) each. On 7 dpi there was also an increase in TBWR +4.0% in group B. By 10 dpi, there were increases in TBWR of +23.5% group B, +57.2% G, +18.0% I and +8.8% in group E (Table 4.7). There was significant difference ($P \leq 0.05$) in TBWR on 10 dpi between group A (3.784 ± 0.23g) and groups B, E, F, G, H and I (6.511 ± 0.68 g, 4.789 ± 1.30 g, 4.796 ± 0 g, 8.481 ± 0.88 g, 4.416 ± 0.53 g and 5.243 ± 2.04 g) respectively (at $P = 0.0663$) (Table 4.7) (Appendix 15).

4.14 Organ to Body Weight Index in ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

There was an increase in BBWI on 3 dpi in groups C, D, E and F. The increases were however higher in groups D (+27.8%) and F (+24.3%). There was an increase in BBWI on 7 dpi in groups A (+9.4%) and I (+14.0%) (Table 4.8). On day 3 pi, the SBWI increased in groups A (+52.2%) and F (+48.1%), but there was a decrease of -14.2% in group B. On 7 dpi, there was an increase in BBWI in groups B (+77.4%), C (+48.0%) and F (+23.3%) but a decrease in groups A (-12.0%) and I (-7.6%). On 10 dpi the SBWI of groups A and B increased by +14.1% and +11.4%, respectively but it decreased by -14.7% and -50.1% in groups E and H, respectively (Table 4.8). Thymus/body weight index increased in all groups on 3 dpi with the highest increase recorded in groups C (+102.1%), D (+44.0%) and E (+111.4%) on 3 dpi. On 7 dpi, there was an increase in TBWI in groups B, D and I of +10.0%, +4.2% and 24.8%, respectively while in other groups there was a decrease. On 10 dpi all groups had a decrease in TBWI (Table 4.8).

Table 4.6: Organ and body weights of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies

	Group								
	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Aluminium magnesium silicate)	E(Polidine®)	F(V-Ox® 1)	G(Negative control)	H(Positive control)	I(V-Ox® 2)
	Number of birds sampled								
	5	5	5	5	5	5	5	5	5
	Body weight in grams (% + increase or - reduction)								
Age in days (days postinoculation)									
21 (0)	158.2	155	145.8	155.4	146.2	149.8	145	153.4	NE
24 (3)	140(-11.5)	188 (+21.3)	169.8(+16.5)	139.8 10.04)	148.0(+1.8)	145(3.2)	176.4 (+21.7)	171.6 (+11.9)	162.5 (NA)
28 (7)	162.8 (+16.3)	193.6 (+3.0)	193.3(+13.8)	178.2 +27.5)	178.4 (+19.8)	191.2 (+31.9)	245.2 (+39.0)	213 (+24.1)	191 (+17.5)
31 (10)	201.4 (+19.2)	251.8 (+30.1)	ABD	ABD	219.8 (+23.2)	269 (+40.7)	249.4 (+1.7)	204.7 (3.9)	185(3.1)
	Weight(g) of Bursa of Fabricius (% + increase or - reduction)								
21 (0)	1.104	1.804	1.098	1.11	0.972	1.048	1.158	1.26	NE
24 (3)	0.874(-20.8)	1.228(-31.9)	1.196(+9.0)	1.186(+6.8)	1.072(+10.3)	1.138(+8.6)	1.298(+12.1)	1.204(-4.4)	1.155 (NA)
28 (7)	0.85(-2.7)	0.964(-21.5)	0.92(-51.3)	0.582(-51.8)	0.892(-16.8)	0.79(-30.6)	1.434(+10.5)	0.784(-34.9)	0.942(-18.4)
31 (10)	0.632(-25.6)	0.762(-21.0)	ABD	ABD	0.695(-22.1)	0.65(-17.7)	1.358(-5.3)	0.557(-29.0)	0.85(-9.8)
	Weight(g) of Spleen (% + increase - reduction)								
21 (0)	0.442	0.452	0.446	0.438	0.436	0.458	0.402	0.65	NE
24 (3)	0.65(-47.1)	0.518(-14.6)	0.754(-69.1)	0.476(-8.7)	0.528(-21.1)	0.69(-50.7)	0.54(-34.3)	0.598(8.0)	0.55 (NA)
28 (7)	0.616(5.2)	0.872(-68.3)	0.89(-18.0)	0.672(-41.2)	0.666(-26.1)	1.068(-54.8)	0.676(-25.2)	0.728(-21.7)	0.832(-51.3)
31 (10)	0.532(13.6)	0.824(5.5)	ABD	ABD	0.763(-14.6)	0.91(14.8)	0.572(15)	0.857(-17.7)	0.685(17)
	Weight(g) Thymus (% + increase - reduction)								
21 (0)	1.23	1.462	1.334	1.358	1.094	1.41	1.604	1.39	NE
24 (3)	0.738(-40.0)	1.11(-24.1)	1.566(+17.4)	0.874(-35.6)	1.2(-9.7)	0.826(-41.4)	0.968(-40.0)	1.042(-25.0)	1.65 (NA)
28 (7)	0.73(-1.1)	1.01(-9.0)	1.497(-4.4)	1.094(+25.2)	0.814(-32.2)	0.896(+8.5)	1.322(+36.6)	0.958(-8.1)	0.848(-48.6)
31 (10)	0.762(+4.4)	1.6(+58.4)	ABD	ABD	1.043(+28.1)	1.29(+44.0)	2.148(+62.5)	0.913(-4.7)	0.915(+7.9)

Key: ABD = All birds died; NE = Not euthanized

Table 4.7: Organ/body weight ratios of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A(*Khaya senegalensis*)	B (Vitamin E)	C (Heparenol®)	D(Aluminium magnesium silicate)	E(Polidine®)	F(V-Ox® 1)	G(Negative control)	H(Positive control)	I(V-Ox® 2)
	Number of birds sampled								
	5	5	5	5	5	5	5	5	
Age in days (days postinoculation)									
	Bursa of Fabricius/body weight ratio (% + increase or - reduction)								
21 (0)	7.004	6.953	7.479	7.264	6.646	6.935	7.994	8.203	NE
24 (3)	6.185(-1.7)	6.572(-2.7)	7.09(-5.2)	8.577(+9.5)	7.181(+8.3)	7.965(+14.9)	7.382(-7.7)	7.001(-14.7)	7.187 (NA)
28 (7)	5.386(-2.9)	5.056(-23.1)	4.765(-32.8)	4.825(-43.7)	5.246(-27.0)	4.112(-48.4)	5.875(-20.4)	3.773(-46.1)	5.012(-43.4)
31(10)	3.124(-2.0)	3.077(-9.1)	ABD	ABD	3.192(-39.2)	2.416(-41.2)	5.452(-7.2)	2.692(-28.7)	4.55(-9.2)
	Spleen/body weight ratio (% + increase - reduction)								
21 (0)	2.799	2,929	3.073	2.875	2.988	3.082	2.791	2.954	NE
24 (3)	4.64(+65.8)	2.766(-5.6)	3.391(+10.3)	3.419(+18.9)	3.552(+18.9)	5.025(+64.9)	3.073(+10.)	3.53(+19.5)	3.408 (NA)
28 (7)	3.79(-18.3)	4.501(+62.7)	4.6(+35.7)	3.821(+11.8)	3.666(+3.2)	5.681(+13.1)	2.818(-8.3)	3.395(-3.8)	4.381(+28.6)
31 10)	2.659(-9.8)	3.257(-27.6)	ABD	ABD	3.433(6.4)	3.383(40.5)	2.301(-18.3)	4.163(+22.6)	3.715(-15.2)
	Thymus/body weight ratio (% + increase - reduction)								
21 (0)	7.83	9.422	9.095	8.604	7.455	9.519	11.191	9.263	NE
24 (3)	5.27(-32.7)	5.074(-46.1)	9.379(+3.1)	6.315(-26.6)	8.048(+8.0)	5.77(-39.4)	5.707(-49.0)	6.055(-34.6)	10.318 (NA)
28 (7)	4.47(-15.2)	5.274(+4.0)	7.803(-16.8)	6.217(-1.6)	4.401(-45.3)	5.142(-10.9)	5.394(-5.5)	4.479(-26.0)	4.445(-57.0)
31 10)	3.784(-5.3)	6.511(+23.5)	ABD	ABD	4.789(+8.8)	4.796(-6.7)	8.481(+57.2)	4.416(-1.4)	5.243(+18.0)

Key: ABD = All birds died; NE = Not euthanized

Table 4.8: Organ/body weight index of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group							
	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Aluminium magenesium silicate)	E(Polidine®)	F(V-Ox®1)	H(Positive control)	I(V-Ox® 2)
	Number of birds sampled							
	5	5	5	5	5	5	5	5
Age day (days post inoculation)								
	Bursa of Fabricius/body weight index (% + increase - reduction)							
21 (0)	0.876	0.87	0.936	0.909	0.831	0.868	1.026	NE
24 (3)	0.838(-4.3)	0.89(+2.3)	0.96(+2.6)	1.162(+27.8)	0.973(+17.1)	1.079(+24.3)	0.948(-7.6)	0.679 (NA)
28 (7)	0.917(+9.4)	0.861(-3.3)	0.811(-15.5)	0.821(-29.3)	0.893(-8.2)	0.7(-35.1)	0.642(-32.3)	0.774(+14.0)
31 (10)	0.573(-37.5)	0.564(-34.5)	ABD	ABD	0.585(-34.5)	0.00(0.00)	0.494(-23.1)	0.00(0.00)
	Spleen/body weight index (% + increase - reduction)							
21 (0)	1.003	1.049	1.101	1.03	1.071	1.104	1.058	NE
24 (3)	1.527(+52.2)	0.9(-14.2)	1.103(+0.2)	1.113(+8.1)	1.156(+8.0)	1.635(+48.1)	1.149(+8.6)	1.426 (NA)
28 (7)	1.345(-12.0)	1.597(+77.4)	1.632(+48.0)	1.356(+21.8)	1.301(+12.5)	2.016(+23.3)	1.205(+4.9)	1.318(-7.6)
31 (10)	1.156(-14.1)	1.415(-11.4)	ABD	ABD	1.492(+14.7)	0.00(0.00)	1.809(+50.1)	0.00(0.00)
	Thymus/body weight index (% + increase - reduction)							
21 (0)	0.642	0.842	0.813	0.769	0.667	0.85	0.83	NE
24 (3)	0.923(+43.8)	0.889(+5.6)	1.643(+102.1)	1.107(+44.0)	1.41(+111.4)	1.011(+19.0)	1.061(+27.8)	0.779 (NA)
28 (7)	0.829(-10.2)	0.978(+10.0)	1.447(-12.0)	1.153(+4.2)	0.816(-42.1)	0.953(-5.7)	0.83(-21.8)	0.972(+24.8)
31 (10)	0.446(-46.2)	0.768(-21.5)	ABD	ABD	0.565(-30.8)	0.00(0.00)	0.521(-37.2)	0.00(0.00)

Key: ABD = All birds died; NE = Not euthanized

4.15 Percent (%) of ISA brown Chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies having Antibody Titre Level of 1,000 ELISA Titre and Above

On day 1 of age, 100% of chicks in all groups had antibody titre levels of 1,000 ELISA units and above except groups C, F and G among which 91.67% had 1,000 ELISA units and above. On day 7 of age, 100% of chicks in group A, G (negative control) and H (positive control) had antibody titre level of 1,000 ELISA units and above, while group C had 91.67%, groups B and E had 90.9% each, group F had 88.89% while group D had the lowest at 87.5% of chicks that had antibody titre level of 1,000 ELISA units and above. At 14 days of age, only groups D, F and G had 16.67%, 12.5% and 15.38% of chicks with antibody titre level of 1,000 ELISA units and above. The other groups, A, B, C and E, had no chick with antibody titre level of 1,000 ELISA units and above. By day 21 of age, no group had antibody titre level of 1,000 ELISA units and above and on day 28, only group B had 28.57% of the chicks with antibody titre level of 1,000 ELISA units and above. By day 35, 100% of chicks in groups A, E, F, H and I had antibody titre level of 1,000 ELISA units and above, while group B had 83.33% of chicks with antibody titre level of 1,000 ELISA titre and above. All chicks in groups C and D died before day 35 of age (Table 4.9).

Table 4.9: Percent (%) of chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies having antibody titre of 1,000 enzyme linked immunosorbent assay units and above.

	Group								
	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Aluminium magnesium silicate)	E(Polidine®)	I(V-Ox® 1)	G(Negative control)	H(Positive control)	I(V-Ox® 2)
	% chicks with titre ≥ 1,000								
Age in days									
1	100.00	100.00	91.67	100.00	100.00	91.67	91.67	100.00	NB
7	100.00	90.90	91.67	87.50	90.90	88.89	100.00	100.00	NB
14	0	0	0	16.67	0	12.50	15.38	10.00	NB
21	0	0	0	0	0	0	0	0	NB
28	0	28.57	0	0	0	0	0	0	0
35	100	83.33	ABD	ABD	100	100	0	100	100

4.16 Microscopic Lesions in ISA brown chicks Inoculated with Very Virulent Infectious Bursal Disease Virus and Treated with some Unconventional Remedies

4.16.1 Histopathology of the bursa of fabricius

Histopathology revealed depletion of lymphocytes, severe necrosis, vacuolation and haemorrhages in bursal follicles (Plate I) among chicks treated with *Khaya senegalensis* extract (group A) at 7 days pi. The bursa of Fabricius from the chicks in the positive control group (H) showed similar depletion of lymphocytes, necrosis and atrophy of follicles (Plate II) during the same period. Depletion of lymphoid cells and atrophy of bursal follicles (Plate III) were also observed in the BF from chicks in vitamin E treated group (B) on day 7 pi.

4.16.2 Histopathology of the thymus

The thymus from chicks in positive control group (H) showed significant extravasation of erythrocytes and depletion of lymphoid cells in the medullary portion (plate IV) at 7 days pi. Similar depletion and haemorrhages occurred with necrosis (Plate V) in the thymus of chicks from the vitamin E treated group (B) while lymphoid depletion in the medullary portion (Plate VI) was observed in thymus from chicks treated with extracts of *Khaya senegalensis*.

4.16.3 Histopathology of spleen

Splenic sections from chicks in the negative control group (G) and group I (V-Ox 2) at day 7 pi showed mild depletion of lymphoid cells, Plate VII and VIII respectively. At day 10 pi, there were lymphoid depletion and haemorrhages (Plate IX) observed among chicks in group B (treated with vitamin E).

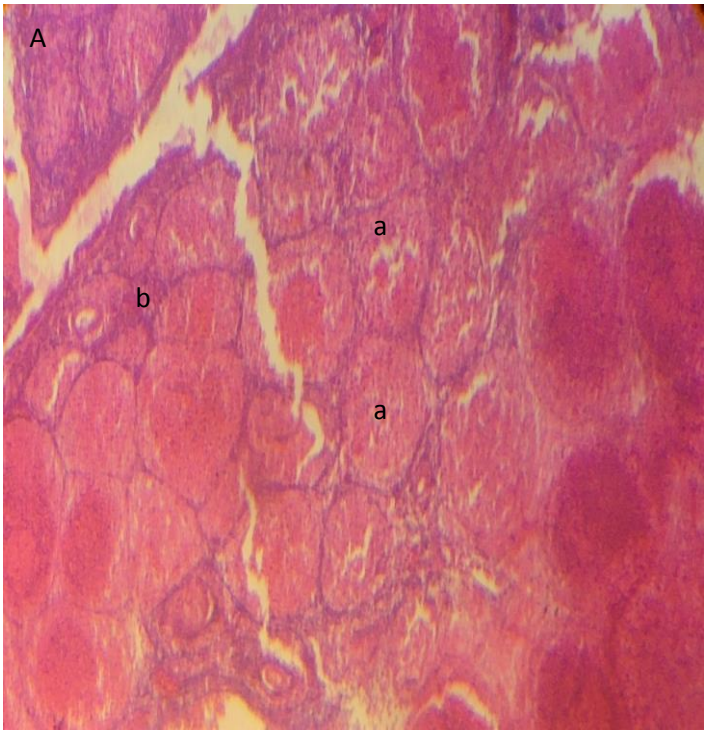


Plate I: Photomicrograph of bursa of Fabricius from chick seven days postinoculation (pi) with vvIBDV and oral administration of vitamin E showing depletion and necrosis (a) and atrophy (b) of the bursal follicles. H&E x 100.



Plate II: Photomicrograph of bursa of Fabricius from a positive control chick at day seven post inoculation with vvIBDV showing depletion and necrosis (a) and atrophy (b) of the follicles. H&E x 100.

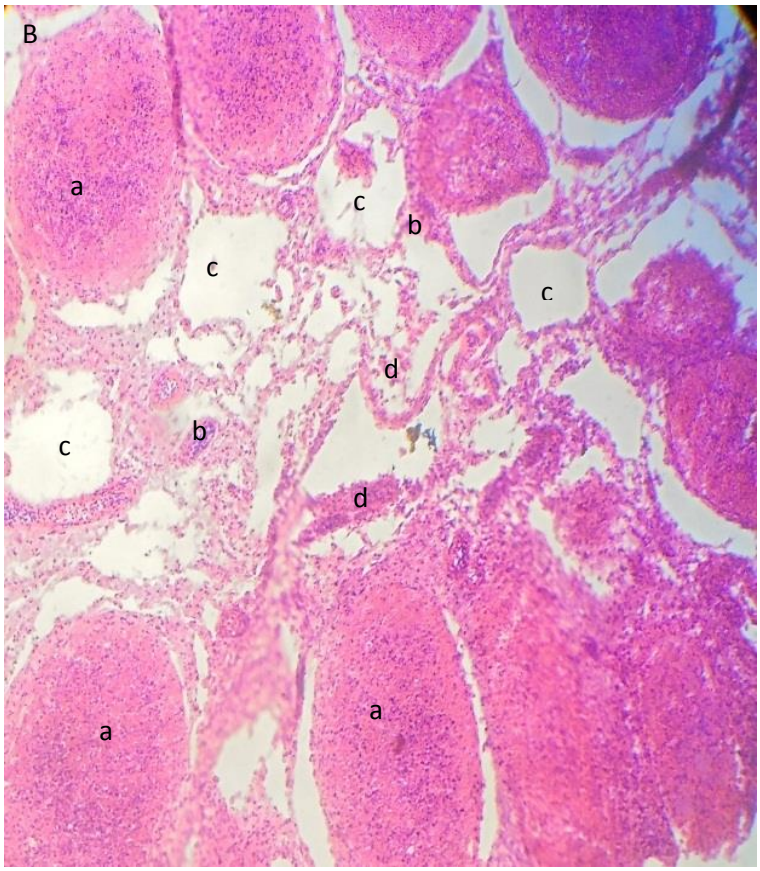


Plate III: Photomicrograph of bursa of Fabricius from a chick seven days post inoculation with vvIBDV and oral administration of *Kaya senegalensis,* showing depletion (a), severe necrosis (b), vacuolation (c) and haemorrhages (d) of the follicles. H&E x 200.

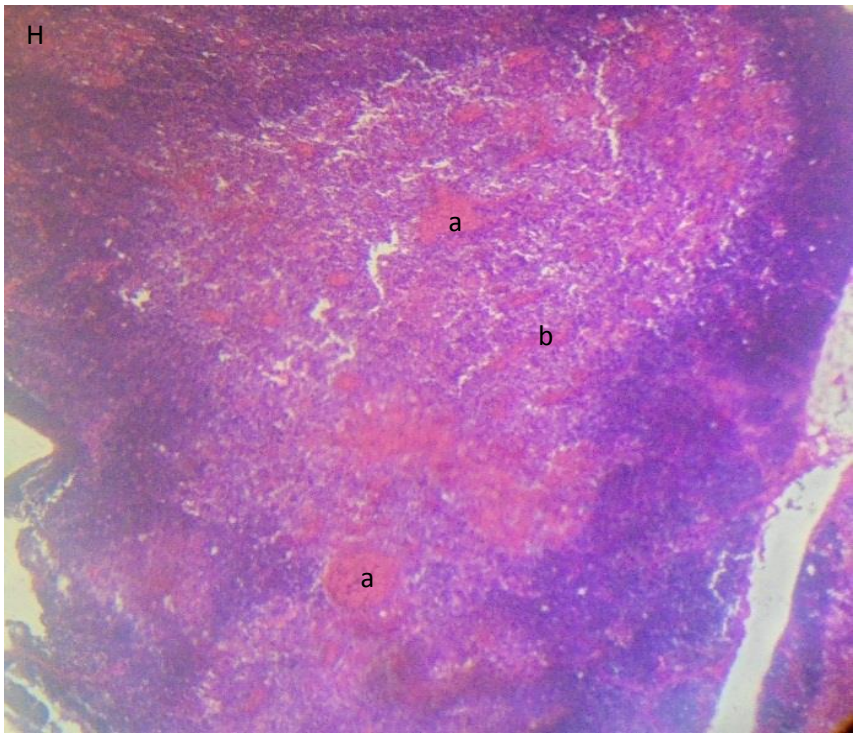


Plate IV: Photomicrograph of thymus of group H (positive control) from a chick at day seven pi showing haemorrhages (a) and depletion of lymphoid cells in the medullary portion (b). H&E x 100.

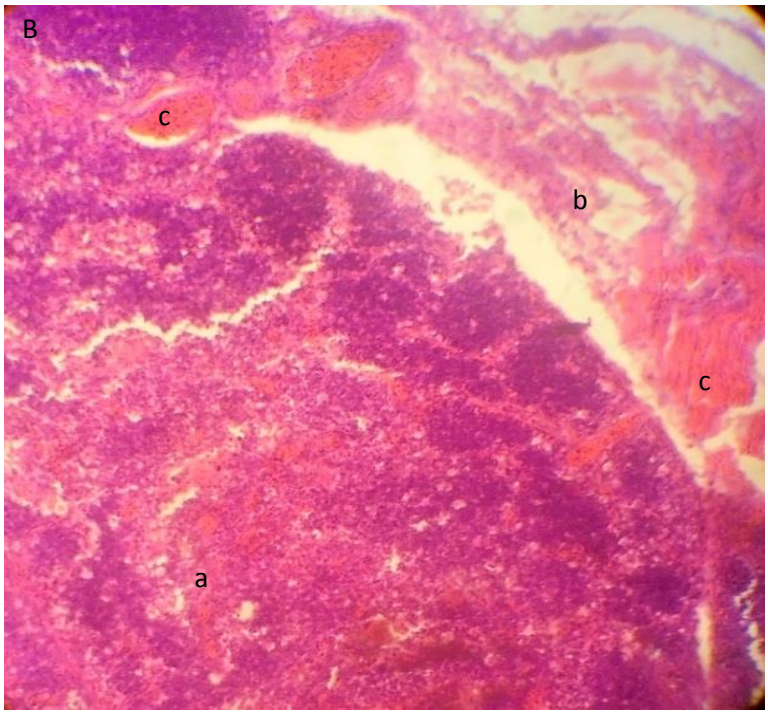


Plate V: Photomicrograph of thymus from a chick seven days postinoculation (pi) with vvIBDV and oral administration of vitamin E (group B) showing depletion (a), necrosis (b) and haemorrhages (c). H&E x 100.

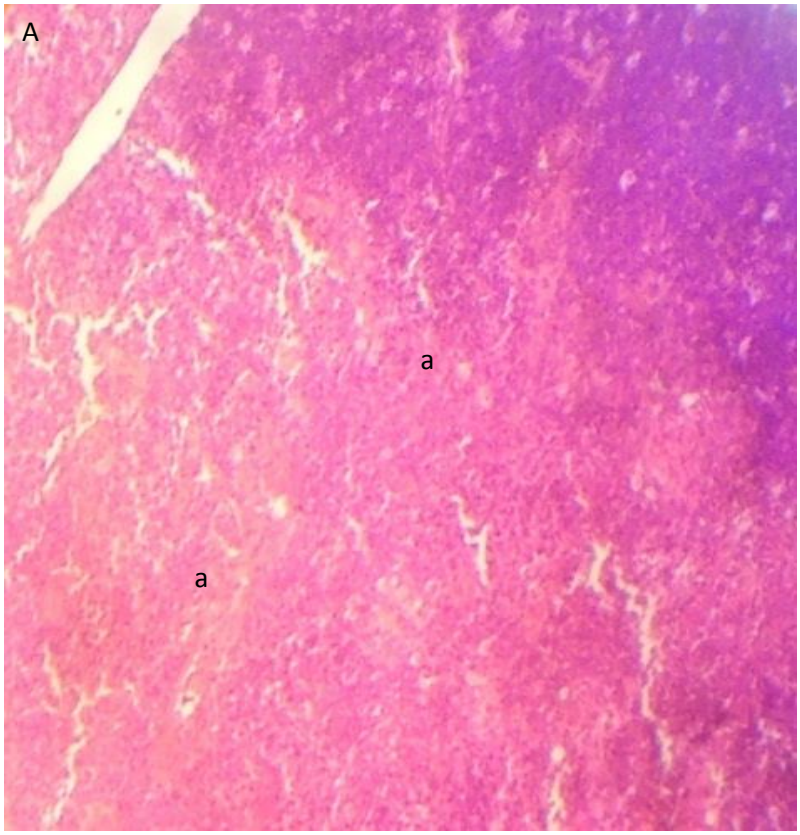


Plate VI: Photomicrograph of thymus from a chick at seven days post inoculation with vvIBDV and oral administration of *Kaya senegalensis* (group A) showing depletion of lymphoid cells (a) in the medullary portion. H&E x 200.

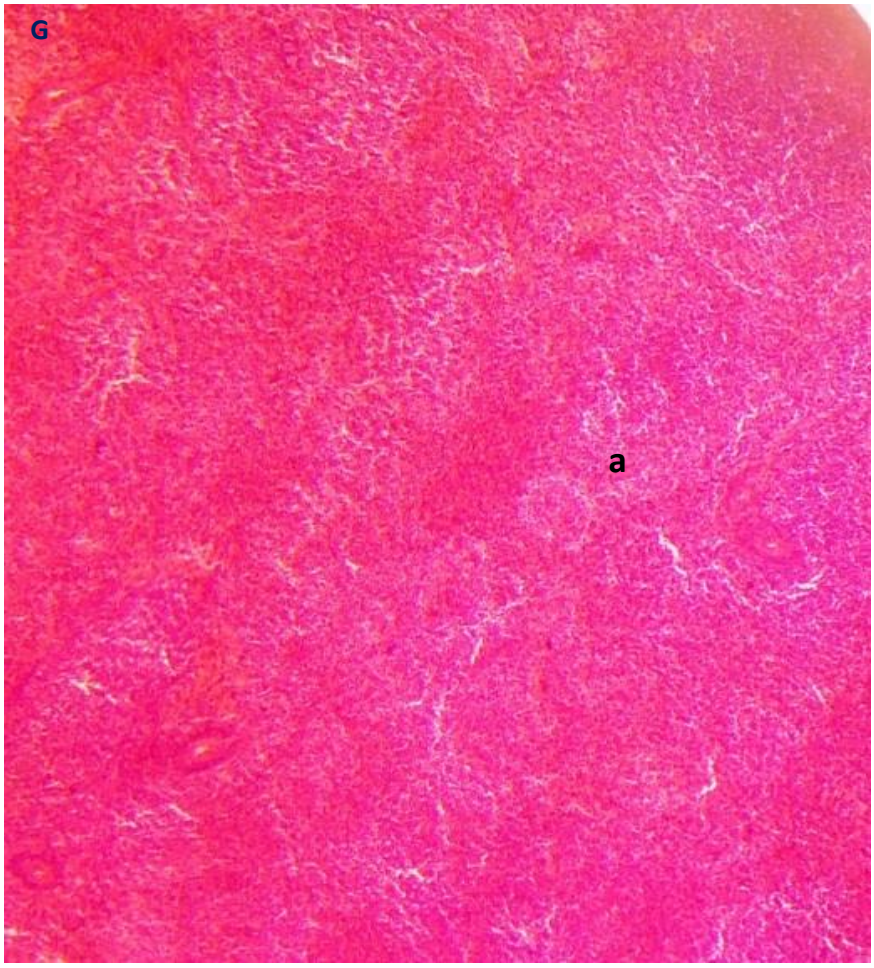


Plate VII: Photomicrograph of spleen of group G (negative control) chicks at day seven showing mild depletion of lymphoid cells (a). H&E x 100.

\

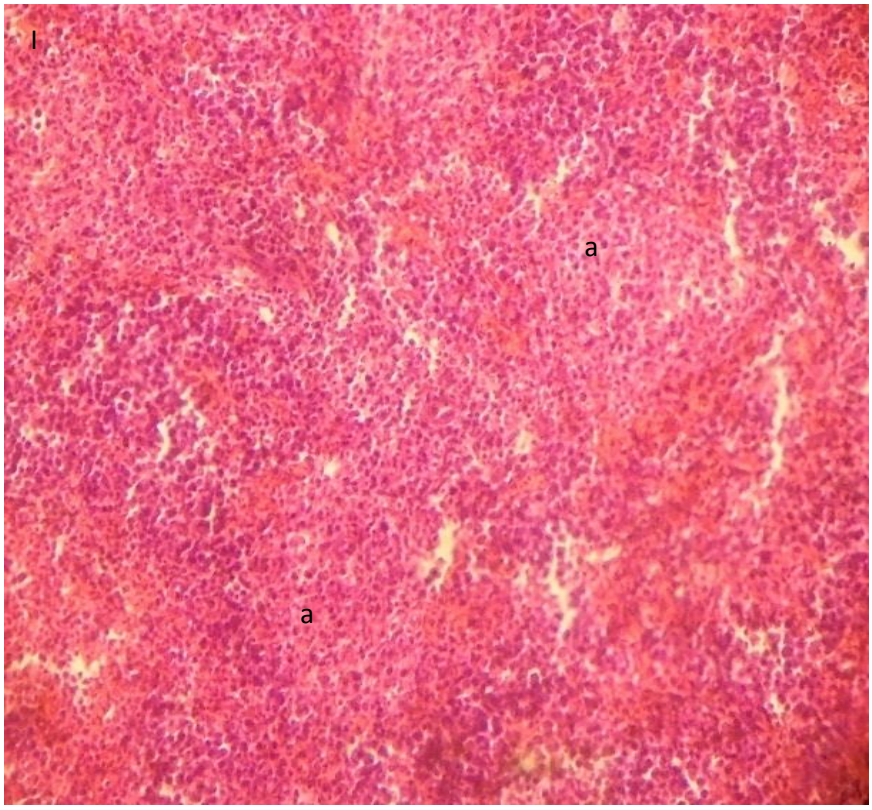


Plate VIII: Photomicrograph of spleen from a chick seven days postinoculation (pi) with vvIBDV and oral administration of V-Ox®2 (group I) showing mild depletion of lymphoid cells (a). H&E x 100.

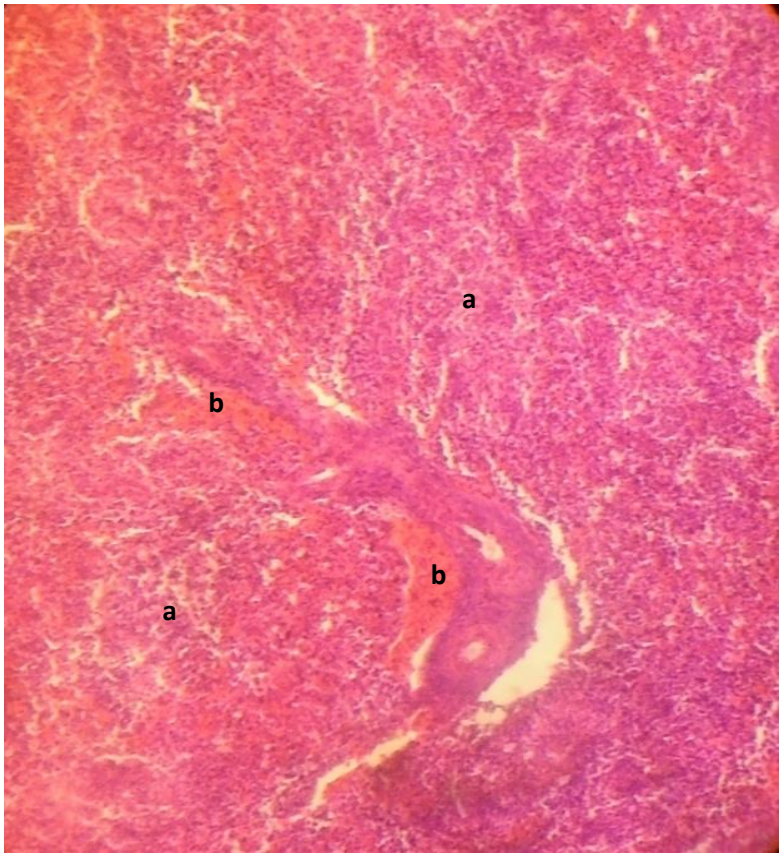


Plate IX: Photomicrograph of spleen from a chick ten days post inoculation (pi) with vvIBDV and oral administration of vitamin E (Group B) showing depletion (a) and haemorrhages (b). H&E x 100.

CHAPTER FIVE

DISCUSSION

Clinical signs were more severe in positive control when compared to those in groups A (*Khaya senegalensis)*, B (Vitamin E), C (Heparenol®), D (Aluminium magnesium silicate), E (Polidine®), F (V-Ox® 1) and I (V-Ox® 2). The onset of clinical signs was 2 dpi in all groups except in group C (Heparenol®) which is in agreement with the incubation period of 2-4 days for IBD as earlier reported by Lukert and Saif (1997); Mbuko *et al*. (2010); Butcher and Miles (2011). The three-day delay of onset of clinical signs in group C (Heparenol®) could be due to the fact that choline chloride cleanses the liver and aid detoxification as well as regenerate hepatic cells, thus maintaining the integrity of the organ as reported by (Detopoulou *et al*., 2008). The pattern (commencing at 2 dpi, peaking at 4 dpi and declining 6 or 7 dpi) of morbidity was consistent with previous observations in natural outbreaks of vvIBD (van den Berg *et al.,* 2000). The clinical signs of IBD were more severe 4 dpi in group H (positive control), followed by group D (Aluminium magnesium silicate), E (Polidine®) and F (V-Ox® 1), while they were less severe in groups A (*Khaya senegalsensis),* B (Vtamin E), C (Heparenol®) and I (V-Ox® 2), indicating the ability of the remedies administered to mitigate clinical signs due to IBD.

Although morbidity could be up to 100% in natural outbreaks of vvIBD as reported previously (van den Berg *et al.,* 1991; Nunoya *et al*., 1992), in this study, the overall morbidity rate was highest in groups H (positive control) 54.54%, B (Aluminium magnesium silicate), E (polidine®) and F (V-Ox® 1) with 50.00% each and lowest in group A (*Khaya senegalensis)* 27.27%, B (Vitamin E) 37.50% and I (V-Ox® 2) 25.00%. The lower morbidity rate recorded even in positive control could be due to pathogenicity of the

strain of vvIBDV used in this study (Angani *et al*., 2014), absence of concurrent infections, enhanced biosecurity level and presence of residual MDA detected at 28 days of age. The lowest overall mortality rates were recorded in group A (*Khaya senegalensis)*, B (Vitamin E) and I (V-Ox® 2) indicating that the remedies used in these groups were able to reduce mortality due to vvIBDV.

At 3 dpi, all IBDV inoculated groups had high gross lesions score which is in accordance with the findings of Ahad (2000). This was followed by significant reduction of lesions by 7 and 10 dpi in all groups but the per cent reduction of gross lesions were higher in groups A (*Khaya senegalensis)*, B (Vitamin E), F (V-Ox® 1), H (Positive control) and I (V-Ox® 2) by 10 dpi. The anti-viral (Aluminium magnesium silicate, Polidine®, V-Ox® 1 and V-Ox® 2), anti-bacterial (*Khaya senegalensis*, Polidine®, V-Ox® 1 and V-Ox® 2), anti-inflammatory (*Khaya senegalensis* and VitaminE) and anti-oxidant (*Khaya senegalensis*, Vitamin E, Heparenol®, V-Ox® 1 and V-Ox® 2) substances contained in the remedies administered could have been responsible for the reduction in the severity of clinical signs, morbidity and mortality rates and gross pathology as reported by previous workers (Lompo *et al*., 1998; Antec, 2003; Kone *et al*., 2004; Sneader, 2005; Umeh *et al*., 2005; Androulakis *et al*., 2006; Ditopoulou *et al*., 2008; Ezeibe *et al*., 2009a; Cheah *et al*., 2009; Simmons *et al*., 2009; Case *et al*., 2011).

Antigens to IBDV were detected in groups B (Vitamin E) and I (V-Ox® 2) on day 7 pi and group H (positive control) on day 10 pi, an indication that the vvIBDV infected and multiplied in the BF of the chicks in these groups. The vvIBDV could not be detected in group A (*Khaya senegalensis)*, C (Heparenol®), D (aluminnium mmagnesium silicate), E

(Polidine®) and F (V-Ox® 1) at 7 and 10 dpi possibly because the remedies administered to the chicks in the five groups prevented the vvIBDV to persist for long time in the BF.

The increase in body weights in all groups up to 28 days of age as the chicks aged in this study could be as a result of normal weight gain due to growth, as observed by Angani *et al*. (2014) who reported increase in the body weights with age. However, the decrease in body weight noticed 3 dpi in groups A (*Khaya senegalensis)* and D (Aluminium magnesium silicate) could be due to the negative effect of IBD as it has been reported by Alloui *et al*. 2005) that it causes weight loss. The significant increase in body weight seen in group B (Vitamin E) could indicate vitamin E might have helped to enhanced weight gain. Vitamin E is an antioxidant that prevent oxidative damage to sensitive membrane as reported by Case *et al.* (2011), thus promoting normal organ function resulting in increased feed consumption, absorption of nutrients from the gut and metabolism in the liver, where as in negative control group, the increase in body weight gain (negative control) could be because the chicks were not inoculated with vvIBDV and therefore not affected by negative effects of vvIBDV which include weight loss.

There were initial increases in BF weight in groups C (Heparenol®), D (Aluminium magnesium silicate), E (Polidine®) and F (V-Ox® 1) on 3 dpi followed by decreases by 10 dpi in groups A (*Khaya senegalsensis),* B (Vitamin E), E (Polidine®) and F (V-Ox® 1), in contrast to groups A (*Khaya senegalensis),* H (Postive control) and I (V-Ox® 2) which showed progressive decreases in BF weight at 3, 7 and 10 dpi. This indicates initial enlargement of the BF in Heparenol®, Aluminium magnesium silicate, Polidine® and V-

Ox® 1 groups due to hyperaemia and oedema followed by atrophy of BF which is in agreement with the findings of Ahad (2000) and Lukert and Saif (1997) who reported swelling and hypertrophy of the BF followed by atrophy in IBD. Lukert and Saif (1997) reported that there could be initial enlargement of BF before atrophy in IBD, while in some cases there may be only atrophy due to massive destruction of the B-lymphocytes in the BF by the vvIBDV. Evaluating bursal size to determine IBD involvement is however, not absolute, as various other factors, including environmental stress and diseases, play a significant role in determining bursal weight according to Bolis *et al*. (2003).

The increase in BBWR noticed on 3 dpi in group D (Aluminium magnesium silicate), E (Polidine®), and F (V-Ox® 1) before decreases could indicate enlargement of the BF while the other groups showed atrophy. It has been reported by Eterradossi *et al.* (2004) that the bursa may initially enlarge before atrophying in IBD. There was however, severe atrophy of BF on 7 and 10 dpi. Krishnamohan and Koteeswaran (2002) investigating IBD outbreaks in Tamil Nadu reported reduction in BBWR after outbreak on days 7, 10 and 14 indicating bursal atrophy where as it was higher in the acute phase of the disease (3 dpi). Ahad (2002) also reported swelling and hypertrophy of the BF in IBDV challenged groups at 3 dpi and atrophy of the BF at 10 dpi. In this study, the increases in BBWI on 3 dpi in groups C (Heparenol®), D (Aluminium magnesium silicate), E (Polidine®) and F (V-Ox® 1) could indicate enlargement of the BF due to hyperaemia and oedema. Generally, BF atrophied immediately in most groups A (*Khaya senegalensis)*, B (Vitamin E), C (Heparenol®), H (Positive control) and I (V-Ox® 2) while in group B (Aluminium magnesium silicate), E (Polidine®) and F (V-Ox® 1), there was initial enlargement of the

BF before atrophy. There were increases in BBWI on day 7 pi in groups A (*Khaya senegalensis)* and I (V-Ox® 2) suggestive of healing and a return to normal structure in these groups. At day 10 pi there was a decrease in BBWI in group A (*Khaya senegalensis),* B (Vitamin E), E (Polidine®), and H (positive control). It is important to note that infectious bursal disease is usually characterized by the destruction of the BF where B lymphocytes mature and differentiate as previously reported by Abdu *et al*. (1987). Bursal atrophy observed at 7 dpi in group A (vitamin E) and H (Positive control) could be due to proliferation of the vvIBDV in the BF (Hair-Bejo *et al*., 2004). Groups A (vitamin E) and H (positive control), so the lesions observed suggest that vitamin E were unable to prevent the proliferation of the vvIBDV, and group A (*Khaya senegalensis)* has antiviral property (Androulakis *et al*., 2006) thus preventing the proliferation of vvIBDV.

The increase in spleen weight noticed from 3 dpi in all the groups in this study could be as a result of response to the vvIBDV. This is in agreement with the findings of Morales and Boclair (1993) who recorded splenic enlargement in IBD. The enlargement was due to massive mobilization of lymphocytes to counter IBDV, resulting in hypertrophy of the spleen as earlier reported by Morales and Boclair (1993). The decrease in spleen weight seen on 10 dpi in groups A (*Khaya senegalensis),* B (Vitamin E), F (V-Ox® 1) and I (V-Ox® 2) indicates healing in these groups when the spleenic cells are recovering and regeneration taking place as against the increase in positive control group. Except in group B (Vitamin E), all other groups including group A (*Khaya senegalsensis),* C (Heparenol®), D (Aluminium magnesium silicate), E (Polidine®), F (V-Ox® 1), G (Negative) and H (positive controls) had increases in SBWR 3 dpi indicating that the remedy administered to

group B (Vitamin E) could have anti-inflammatory properties. Within groups, initial increases in SBWR in all groups before reduction indicate initial inflammation in response to vvIBDV infection before possible healing in some groups. On day 3 pi, the SBWI increased in groups A (*Khaya senegalensis)* and F (V-Ox® 1) which could be as a result of enlargement, but there was a decrease in group B (Vitamin E). On 7 dpi, there were increases in groups B (Vitamin E), C (Heparenol®) and F (V-Ox® 1) but decreases in group A (*Khaya senegalensis)* and I (V-Ox® 2). On 10 dpi SBWI of group A (*Khaya senegalensis)* and B (Vitamin E) increased by 14.1% and 11.4%, respectively but decreased by 14.7% and 50.1% in groups E (Polidine®) and H (Positive control). On day 3 pi, the SBWI increased in groups A (*Khaya senegalensis)* and F (V-Ox® 1) which could be as a result of enlargement, but there was a decrease in group B (Vitamin E). On 7 dpi, there were increases in groups B (Vitamin E), C (Heparenol®) and (V-Ox® 1) but decreases in group A (*Khaya senegalensis)* and I (V-Ox® 2). On 10 dpi SBWI of groups A (*Khaya senegalensis)* and B (Vitamin E) increased by 14.1% and 11.4%, respectively but decreased by 14.7% and 50.1% in groups E (Polidine®) and H (Positive control). On 7 dpi the spleen showed mild depletion of lymphoid cells in groups B (Vitamin E) and I (V-Ox® 2). The spleen has both B and T-lymphocytes (Cheville, 1967) and so the observation herein being reported might indicate that the vvIBDV possibly attacked both cells (Cheville, 1967).

There was initial atrophy of the thymus in all groups which could be as a result of the vvIBDV infection. It has been observed that the IBDV also destroyed T-lymphocytes in the thymus resulting in atrophy (Tanimura and Sharma, 1997). This was subsequently

followed by enlargements indicative of a return to normal function due to thymic cell recovery and regeneration.

Increases in TBWR on 3 dpi were followed by slight decrease in group B (Vitamin E) on day 7 pi. The increase in TBWR observed in groups B (Vitamin E), (Polidine®), and (V-Ox® 2) on day 10 pi could be an indication of healing. Increase in TBWR in groups C (Heparenol®) and E (Polidine®) could indicate enlargement of this organ on 3 dpi but the increase in size on day 7 pi in group B (Vitamin E) could be suggestive of healing. This was also observed in groups F (V-Ox® 1) and I (V-Ox® 2) on 10 dpi. The TBWI increased in all the groups on 3 dpi with higher increase noticed in groups C (Heparenol®), D (Aluminium magnesium silicate) and E (Polidine®) indicating enlargement of the thymus in these groups on 3 dpi. On 7 dpi, increases were seen in groups B (Vitamin E), D (Aluminium magnesium silicate) and (V-Ox® 2) while there were decreases observed in other groups indicating atrophy. On 10 dpi all groups showed decreases in TBWI indicating atrophy.

On 7 dpi the thymus showed depletion of lymphoid cells in the medullar in groups A (*Khaya senegalensis)*, B (Vitamin E) and H (Positive control) indicating that, although the B-lymphocytes are the target cells of IBDV, T-lymphocytes could also be destroyed by IBDV. This is similar to the report of (Cheville, 1967). There was necrosis and haemorrhages in the thymus of chicks in group B while group H had only haemorrhages on 7 dpi.

All chicks used in this study had protective antibody titre level of 1,000 ELISA units and above at day 1 to day 7 of age after which the level declined to below 1,000 units. This finding is in agreement with that of Abdu (1986) and Hair-Bejo *et al*. (2004) who reported a drop in MDA after 17 and 14 days of age, respectively. The decline in ELISA units of MDA at 14 days of age signifies that the chicks need to be vaccinated before 14 days of age to boost their antibody titre levels. This is in agreement with the recommendations of Abdu and Ibe (2011) that chicks should be prevented from exposure to a vvIBDV within the first 7-13 days of life before vaccination. By the 14th day of age they have no protective MDA titre and would be vaccinated only after 14 to 31 days of age (depending on the source and type of bird) with intermediate IBD vaccine to avoid neutralization by MDA. The increase in antibody titre level on day 28 in 28.57% of the chicks in group B (Vitamin E) could be due to the immunopotentiating effects of vitamin E, while the increase in antibody titre level in all groups on day 35 of age was a result of seroconversion following inoculation with vvIBDV as reported by Abdu and Ibe (2011).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study, the following conclusions were made:

1. Vitamin E (group B), *K. Senegalensis* (group A) and V-ox® 2 (group I) reduced the severity of clinical signs (24.7%, 15.6% and 14.1%,), morbidity (37.5%, 27.3% and 30.0%) and mortality (25%, 18.8% and 11.1%) due to vvIBDV compared to those of positive control (50.7%, 85.7% and 50%), respectively.

2. Polidine® (group E), vitamin E (group B) and V-ox® 1 (group F), significantly ($P \leq 0.05$) reduced gross lesions due to vvIBDV by 80.0%, 78.7% and 65.4% respectively, on 7 dpi compared to those of positive control (30.0%).

3. Vatamin E (group B), increased body weight gain by 30.1% on 10 dpi compared to a loss of 3.9% in positive control.

4. *K. Senegalensis* (group A) and V-ox® 2 (group I) increased BBWI by 9.4% and 14.0% respectively, compared to a decrease of 32.3% in positive control.

5. *K. Senegalensis* (group A) and V-ox® 2 (group I) decreased SBWI by 12.0% and 7.6% respectively compared to an increase in positive control (4.9%) dpi.

6. Vitamin E (group B), aluminium magnesium silicate (group D) and V-ox® 2 (group I) increased TBWI by 10.0%, 4.2% and 24.8% respectively compared to a reduction of 21.8% in positive control at 7 dpi.

7. Chicks in all groups had protective maternal Ab titre level of 1,000 ELISA units and above at days 1 and 7 of age, following inoculation with vvIBDV, only 28.57% of the chicks treated with viamin E had Ab titre level of 1,000 ELISA units and above at 7 dpi.

6.2 Recommendations

Based on the findings of the study, it is recommended that:

1. Veterinarians and animal health workers could consider administering extract of *K. senegalensis* or vitamin E to mitigate the severity of signs, morbidity, mortality and pathology due to IBD.

2. Veterinarians could vaccinate pullet chicks derived from the breeder stock used in this study against IBD by 17 days of age.

3. Further research should be conducted to evaluate the safety of *K. senegalensis* and V-ox® 2.

6.3 Limitations of the Study

This study is limited in scope due to the fact that the:

1. Acute and chronic toxicity of the remedies used in this study were not determined.

2. Extract of *K. senegalensis* was not tested for palatability.

3. Markers of oxidative stress in tissues were not assayed.

4. *In vitro* antiviral activities of the remedies used in this research were not tested.

6.4 Contribution to Knowledge

This research has shown that in the face of an outbreak, treatment with *K. Senegalensis* extract, vitamin E or V-ox® 2 could mitigate the severity of clinical signs, morbidity, mortality and pathology due to IBD.

REFERENCES

Abdu, P.A., (1986). An outbreak of Gumboro disease in vaccinated flock in Zaria Nigeria. *Zariya Veterinarian,* 1: 40-41.

Abdu, P. A. (1988). Case report; Infectious bursal disease in a flock of broilers and local chicken in Nigeria. *Bulletin of Animal Health and Production in Africa,* 18: 269-271.

Abdu, P.A. (1997). Studies on the problems associated with vaccination against infectious bursal disease in Nigeria. *PhD Thesis,* Ahmadu Bello University Zaria, Pp. 129.

Abdu, P.A and Ibe, C. (2011). Vaccination strategies in breeder and commercial farms and infectious bursal disease maternally derived antibodies in day old chicks in Nigeria. *NVRI Seminar Series,* National Veterinary Research Institute, Vom, Pp. 23-32

Abdu, P.A. and Musa, U. (2014). Infectious bursal disease. In: *Textbook of Avian Medicine* First Edition. P.A Ndahi Printing, S/Gari Zaria, Nigeria, Pp. 92-94.

Abdu, P. A., Abdullahi, S. U., Ezekoli, C. D. and Adesiyun, A. A. (1987). Vaccination against infectious bursal disease in the presence of maternally derived antibodies. *Zariya Veterinarian,* 2 (1): 58-61.

Abdu, P.A., Umoh, J.U., Abdullahi, S.U. and Sa'idu, L. (2001). Infectious bursal disease (Gumboro) of chickens in Nigeria. *Tropical Veterinarian,* 19: 216-236.

Adebanjo, A.O., Adewumi, C.O. and Essien, E.E. (1985). Anti-infective agents in higher plants. *5th International Symposium of Medical Plants. University of Ife, Nigeria*. Pp. 152 – 158.

Ademola, I.O., Fagbemi, B.O. and Idowu, S.O. (2004). Evaluation of the anthelmintic activity of *Khaya senegalensis* extract against gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Veterinary Parasitology*, 122(2): 151–164.

Adewole, S.O. (2012). The efficacy of drugs in the treatment of coccidiosis in chicken in selected poultries. *Academic Research International*, 2: 20-24.

Adewuyi, O.A., Durojaiye, O.A. and Adene, D.F. (1989). The status of guinea fowls (*Numida meleagris)* in the epidemiology of infectious bursal disease of poultry in Nigeria. *Journal* of *Veterinary Medicine,* 36: 43-48.

Afolabi, C.A., Adebayo, K., Afolabi, O.I. and Odutola, O.I. (2011). Economics of poultry egg marketing in Mushin Local Government Area, Lagos State. In: Odedina SA *Proceedings of the 25th Farm Management Association of Nigeria (FAMAN) Conference,* Pp. 526-533.

Afolabi, O.I., Adegbite, D.A., Ashaolu, O.F. and Akinbode, S.O. (2013). Profitability and resource-use efficiency in poultry egg farming in Ogun State, Nigeria. *African Journal of Business Management,* 7(16): 1536-1540.

Ahad, A. (2002). Isolation and pathogenic characterisations of IBDV isolation from an outbreak of IBD in a rural poultry unit in Bangladesh. *M.Sc Thesis.* Department of Veterinary Microbiology and Network for Small Holder Poultry Development. The Royal Veterinary and Agricultural University. Dyrlageveg 2, 1870 Frederiksberg C., Denmark and Sylhet Govt. Veterinary College, Tilagorh Sylhet-3100, Bangladesh, Pp. 6-66.

Ahad, A. (2000). Isolation and pathogenic characterization of IBDV isolate from an outbreak of IBD in rural poultry unit, In: *Bangladesh Agricultural University*, Pp. 25-30.

Aihonsu, J.O.Y. and Sunmola, M.A. (1999). Optimal laying period for profitable and sustainable egg production. *Ife Journal of Agriculture*, 20: 67-80.

Alabi, R.A., Tariuwa, I.O., Onemolease, P.E.A., Mafimisebi, A., Isah, T.A., Esobhawan, A.O., and Oviasogie, D.I. (2000). Risk management in poultry enterprises in Edo State through insurance scheme. *Proceedings of the 5th Annual Conference of Animal Science Association of Nigeria,* September 19-22, 2000, Port Harcourt, Nigeria, Pp 182-184.

Alexander, D.J. (1999), Paramyxoviridae (Newcastle disease and others), In: *Poultry Diseases,* Fourth Edition, edited by Jordan, F.T.W. and Pattison, M., Pp. 139-155.

Alexander, D.J. (2003): Newcastle disease, Other Avian Paramyxoviruses and Pneumovirus infections: Newcastle disease. In: *Diseases of Poultry*. Saif, Y.M. (Ed in chief) Iowa state University Press USA Pp. 64-87.

Alexander, D.J. and Chettle, N.J. (1998). Heat inactivation of serotype 1 infectious bursal disease virus. *Avian Pathology,* 27(1): 97-99.

Ali, S. A. M., ElBadwi, S. A. M., Idris, T. M. and Osman, K. M. (2011). Hepatoprotectective activity of aqueous extract of *Khaya senegalensis* bark in rats. *Journal of Medicinal Plants Research,* 5(24): 5863–5866.

Allan, W.H., Faragher, J.T. and Cullen, G.A. (1972). Immunosuppression by the infectious bursal agent in chickens immunized against Newcastle disease. *Veterinary Records*, 90: 511-512.

Allan, G.M., McNulty, M.S., Connor, T.J., McCracken, R.M. and McFerran, J.B. (1984). Rapid diagnosis of infectious bursal disease infection by immunofluorescence on clinical material. *Avian Patholology*, 13: 419-427.

Alloui, M. N., Sellaouil, S. and Djaaba, S. (2005). Morphometrical and anatomopathological survey of the bursa of Fabricius in broiler chickens. *Indian Society of Animal Health*, 26: 52-55.

Amos, T.T. (2006). Analysis of backyard poultry production in Ondo state, Nigeria. *International Journal of Poultry Science*, 5: 247-250.

Androulakis, X. M., Muga, S. J., Chen, F., Koita, Y., Toure, B. and Wargovich, M. J. (2006). Chemopreventive effects of *Khaya senegalensis* bark extract on human colorectal cancer, *Anticancer Research*, 26(3): 2397–2405.

Angani, M.T., Abdu, P. A., Sa'idu, L. and Kazeem, H. M. (2014). Comparison of bursal and body weights in cockerels vaccinated with a live and two killed vaccines against infectious bursal disease virus. *Journal of Veterinary Advances*, 4(6): 576-581.

Anonymous (2009). Immune system. Retrieved July 18, 2009 from *www.poultryhub.org.*

Anonymous (2000). Forestry Compendium – A Silvicultural Reference Global Module. *CAB International*, Wallingford, UK, CD-ROM.

Anonymous *(*The Poultry Site). (2014). Infectious bursal disease, IBD, Gumboro. *Retrieved 20 January, 2016*

Anonymous (2016). Heparenol. Merial France

Antec International (2003). Virkon S® Antec International LTD by *Antec International Limited*, Pp. 1-11.

Ashraf, S. (2005). Studies on infectious bursal disease virus. *A Dissertation Presented in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of the Ohio State University*, Pp. 10-11.

Association of Official Analytical Chemists (AOAC). (2003). *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17th edn. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Azad, A.A., Barett, S.A. and Fahey, K.J. (1985). Characterization and molecular cloning of the double stranded genome of an Australian strain of infectious bursal disease virus. *Virology*, 143: 35-44.

Azad, A. A., Jagadish, M. N., Brown, M. A. and Hudson, P. J. (1987). Deletion mapping and expression in *E. coli* of the large genomic segment of a birnavirus. *Virology*, 161: 145-152.

Babiker, M.A.A. and Tawfeeq, E. (2008). Role of administration routes of anti-infectious bursal disease virus (Gumboro) vaccine on immunization of chicken. *International Journal of Poultry Science*, 7(3): 279-282.

Babiker, M.A.A., Yahia, I.E., Noura, K. and Manal, M.E. (2008). Evaluation of four commercial anti-infectious bursal disease vaccines under Sudan condition. *International Journal of Poultry Science*, 7(6): 560-573.

Bancroft, J.D. and Cook, H.C. (1994). *Manual of Histological Techniques and Their Diagnostic Application.* Churchill Livingstone, Edinburgh, UK.

Baxendale, W. (2002). Birnaviridae. In: *Poultry Diseases*, edited by Frank Jordan, Mark Pattison, Dennis Alexander & Trevor Faragher 5th edition, W.B. Saunders, Pp. 319-323.

Baxandale, W. (1999). Infectious bursal disease (Gumboro disease*);* In: *Poultry Diseases*, Fourth edition, Edited by Jordan, F.T.W. and Pattison, M., Pp. 199-203.

Becht, H., Muller H. and Muller, H.K. (1988). Comparative study on structural and antigenic properties of two serotypes of infectious bursal disease virus. *Journal of General Virology*, 69: 631-640.

Benton, W.J., Cover, M.S., Rosenberger, J.K. and Lake, R.S. (1967). Physicochemical properties of the infectious bursal agent (IBA). *Avian Diseases*, 11: 430-438.

Bjelland, I., Tell, G.S., Vollset, S.E., Konstantinova, S. and Ueland, P.M. (2009). Choline in anxiety and depression: the Hordaland Health Study. *The American Journal of Clinical Nutrition*, 90(4): 1056–1060.

Blankfard, M. and Silk, B. C. (1989). Enzyme-Linked Immunosorbent Assay software, KPL, R. Gaithesburg, Md., USA.

Block, H. K., Meyer-Block, K., Rebeski, D. E., Scharr, H., de Wit, S., Rohn K. and Rautenschlein, S. (2007). A field study on the significance of vaccination against infectious bursal disease virus (IBDV) at the optimal time point in broiler flocks with maternally derived IBDV anti-bodies. *Avian Pathology,* 36(5): 401-409.

Bolis, D.A., Paganini, F.J., Simon, V.A., Zuanaze, M., Scanavini, N.H., Correa, A. and Ito, N. (2003). Gumboro disease; Evaluation of serological and anatomopathological response of vaccinated broiler chickens challenged with very virulent virus strain. *Brazil Journal of Poultry Science,* 5(2): 137-146.

Boot, H.J., Huurne, A.H. and Peeters, B.P. (2000). Generation of full-length cDNA of the two genomic dsRNA segments of infectious bursal disease virus. *Journal of Virology Methods,* 84: 49–58.

Box, P. (1989). High maternal antibodies help chickens beat virulent virus. *World Poultry,* 53: 17-19.

Branckeart, R.D.S., Gavira, L.. Jallade J. and Seiders, R.W. (2000). Transfer of technology in poultry production for developing countries. *FAO Sustainable Development Dimensions, October 2000 and Proceedings* WPC2000 Montreal, Canada.

Brown, M.D., Green, P. and Skinner, M.A. (1994). VP2 sequences of recent European 'very virulent' isolates of infectious bursal disease virus are closely related to each other but are distinct from those of 'classical' strains. *Journal of General Virology*, 75: 675-680.

Brown, R.H. (1986). Researchers isolate new strains of infectious bursal disease virus. *Feedstuff,* p. 30.

Bumstead, N. (1998). Genetic resistance to avian viruses. In: Genetic Resistance to Animal Diseases (M. Müller and G. Brem, eds). *Review Science Technical Office International des Epizootics,* 17**(**1): 249-255.

Bumstead, N., Reece, R.L. and Cook, J.K.A. (1993). Genetic differences in susceptibility of chicken lines to infection with infectious bursal disease virus. *Poultry Science*, 72(3): 403-410.

Burkhardt, E. and Müller, H. (1987). Susceptibility of chicken blood lymphoblasts and monocytes to IBDV. *Archives of Virology,* 94: 297-303.

Butcher, G.D. and Miles, R.D. (2011). Infectious bursal disease (Gumboro) in commercial broilers. *University of Florida IFAS Extension.* Retrieved June 11, 2011 from: http://edis.ifas.ufl.edu.

Cadman, H.F., Kelly, P.J., Zhou, R., Davelaar, F. and Mason, P.R. (1994). A serosurvey using enzyme-linked immunosorbent assay for antibodies against poultry pathogens in ostriches *{Struthio camelus)* from Zimbabwe. *Avian Diseases*, 38(3): 621-625.

Case, L.P., Daristotle, L. Hayek, M.G. and Raasch, M.F. (2011). Vitamins. In: *Canine and Feline Nutrition.* Third edition. Elsevier 3251 Riverport Lane Maryland Heights, Missouri 63043 Elsevier. Retrieved 2011 from: http://www.elsevier.com.

Caston, J. R., Martinez, J. L. and Lombardo, E. (2001). C-terminus of infectious bursal disease virus major capsid protein VP2 is involved in definition of the T number for capsid assembly. *Journal of Virology,* 75: 10815 –10828.

Caston, J.R., Martinez, J.L. and Lombardo, E. (2008). Infectious bursal disease virus (IBDV). Segmented double-stranded RNA viruses: *Structure and Molecular Biology*. Caister Academic Press. ISBN 978-1-904455-21-9. Retrieved on 01 March, 2015 at 6:38 pm from http://.horizonpress.com/rnav.

Central Bank of Nigeria (CBN). (2004). Annual report and statement of account. *Central Bank of Nigeria (CBN),* Abuja, Nigeria.

Cheah, L-H., Marsh, A.T., Mcneill, M.R. and Hedderley, D.I. (2009). Evaluation of disinfectant products for microbial decontamination of imported used footwear. *The New Zealand Institute for Plant and Food Research Limited, Private Bag. New Zealand Plant Protection,*62: 130-135.

Chettle, N., Stuart, J.C. and Wyeth, P.J. (1989). Outbreak of virulent infectious bursal disease in East Anglia. *Veterinary Records,* 125: 271-272.

Cheville, N.F. (1967). Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen and thymus of chicken. *American Journal of Pathology*, 51: 527.

Cho, Y. and Edgar, S.A. (1969). Characterization of the infectious bursal agent. *Poultry Science*, 48: 2102-2109.

Cho, B.R., Snyder, D.B., Lana, D.P. and Marquardt, W.W. (1987). Infectious bursal disease: rapid diagnosis by immunoperoxydase monoclonal antibody stain. In: *Proceedings of the 36th Western Poultry Disease Conference,* 3-5 March, Davis, California. University of California, Davis, Pp. 112.

Chow, C.K. (2001). Vitamin E. In: Rucker, R.B, Suttie, J.W, McCormick, D.B, Machlin, L.J, editors: *Handbook of Vitamins*, New York, Marcel Dekker, Pp. 165–197.

Collins, E. D., and Norman, A. W. (1991). *Handbook of Vitamins* (2nd edn). (Ed.) L. J. Machlin, Marcel Decker; New York. Pp. 59–99.

Chukwu, N. (2010). Vitamin deficiencies, fluid and electrolyte therapy. A *Membership Lecture Notes, College of Veterinary Surgeons Nigeria.* Ibadan Study Centre, University of Ibadan, Nigeria.

Committee on Foreign and Emerging Disease of the United States Animal Health Association (CFEDUSAHA) (2008). Animal pathogen disinfectants. In: *Foreign Animal Diseases* Seventh edition Pp. 60.

Cookson, K.C. and Giambrone, J.J. (2004). The use of spleen to body weight adjunct to bursa to body weight ratio analysis to estimate ratios as an IBD virus protection. *Forum*, 2004: 1794.

Cosgrove, A.S. (1962). An apparently new disease of chickens. Avian nephrosis. *Avian Diseases*, 6: 385-389.

Cowen, B.S. and Braune, M.O. (1988). The propagation of avian viruses in a continuous cell-line (QT-35) of Japanese quail origin. *Avian Diseases*, 32: 282-297.

Cullen, G.A. and Wyeth, P.J. (1975). Quantitation of antibodies to infectious bursal disease. *Veterinary Records*, 97: 315.

Cullen, G.A. and Wyeth, P.J. (1976). Response of growing chickens to an inactivated IBD antigen in oil emulsion. *Veterinary Records*, 99: 418.

Dalziel, J. M. (1937). The useful plants of west tropical Africa. *Crown Agents, London*, Pp. 612.

Darteil, R., Bublot, M., Laplace, E., Bouquet, J.F., Audonnet, J.C. and Riviere, M. (1995). Herpesvirus of turkey recombinant viruses expressing infectious bursal disease virus (IBDV) VP2 immunogen induces protection against an IBDV virulent challenge in chickens. *Virology*, 211: 481-490.

Das, S.K., Saha, S.K., Das, A., Halder, A.K. and Banerjee, S.N., Chakraborty, M. (2008). A study of comparison of efficacy and safety of talc and povidone iodine for pleurodesis of malignant pleural effusions. *Journal of Indian Medical Association,* 106(9): 589–592.

David, A. and Bender, D.A. (2005). Proximate analysis. *A Dictionary of Food and Nutrition. Encyclopedia.com.* Retrieved on 14 July. 2015 from <http://www.encyclopedia.com>.

Davis, V. and Boyle, J.A. (1990). Random cDNA probes to infectious bursal disease virus. *Avian Diseases*, 34: 329-335.

De Vooght, V., Jesus Cruz, M., Haenen, S., Wijnhoven, K., Munoz, X., Cruz, M.; Munoz, X., Morell, F. and Nemery, B. (2010). "Ammonium persulfate can initiate an asthmatic response in mice." *Thorax*. 65: 252–257. Doi: 10.1136?thx.2009.121293

Delgui, L., Oña, A., Gutérrez, S., Luque, D., Navarro, A., Caston, J. R. and Rodriguez, J. F. (2009). The capsid protein of infectious bursal disease virus contains a functional α4β1 integrin ligand motif. *Virology*, 386(2): 360-372.

Detopoulou, P., Panagiotakos, D, B., Antonopoulou, S., Pitsavos, C. and Stefanadis, C. (2008). Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. *The American Journal of Clinical Nutrition*, 87(2): 424–430.

de Wit, J.J. (1999). Gumboro disease: optimizing vaccination. *International Poultry Production*, 7(5): 19-21.

de Wit, J.J. (2014). Safety and efficacy of different Gumboro disease vaccines. de Wit, J. J, Estimation of optimal time of vaccination by the Deventer formula. *Animal Health Service*, A.A. Deventer, The Netherlands.

Dohms, J.E., Lee, K.P. and Rosenberger, J.K. (1981). Plasma cell changes in the gland of Harder following infectious bursal disease virus infection of the chicken. *Avian Diseases*, 25: 683 695.

Durojaiye, O.A., Ajibade, H.A. and Olafimihan, G.O. (1984). An outbreak of infectious bursal disease and prevention in 20 weeks old birds. *Tropical Veterinarian*, 2: 175-176.

El-Tahir, A., Ibrahim, A.M., Satti, G.M.H., Theander, T.G., Kharazmi, A. and Khalid, S.A. (1998). The potential antileishmanial activity of some Sudanese medicinal plants. *Phytotherapy Research*, 12: 576–579.

El Yuguda, A.D. and Baba, S.S. (2004). An outbreak of infectious bursal disease in eight weeks old IBD vaccinated commercial poultry flock in Maiduguri, Nigeria. *Tropical Veterinarian*, 22(3 and 4): 93-98.

Etteradossi, N. and Saif, Y.M. (2008). Infectious bursal disease. In: *Diseases of Poultry*, 12th Edition. Edtr Saif, Y.M., Pp. 185-208.

Eterradossi, N., Toquin, D., Rivallan, G. and Guittet, M. (1997). Modified activity of a VP2-located neutralizing epitope on various vaccine, pathogenic and hypervirulent strains of infectious bursal disease virus. *Archives of Virology*, 142: 255-270.

Eterradossi, N., Arnauld, C., Toquin, D. and Rivallan, G. (1998). Critical amino acid changes in VP2 variable domain are associated with typical and atypical antigenicity in very virulent infectious bursal disease viruses. *Archives of Virology*, 143(8): 1627-1636.

Eterradossi, N., Arnaud, C, Tekaia, F., Toquin, D., Le Coq, H., Rivallan, G., Guittet, M., Domenech, J., Van den Berg, T.P. and Skinner, M.A. (1999). Antigenic and genetic elationship between European very virulent infectious bursal disease viruses and an early West African isolate. *Avian Pathology*, 28: 36-46.

Eterradossi, N., Gauthier, C., Reda, I., Comte, S., Rivallan, G., Toquin, D., de Boisséson, C., Lamandé, J., Jestin, V., Morin, Y., Cazaban, C. and Borne, P. M. (2004). Extensive antigenic changes in an atypical isolate of infectious bursal disease virus and experimental clinical control of this virus with an antigenically classical live vaccine. *Avian Pathology*, 33: 423-431.

Ezeibe, M.C.O., Mbuko, I.J., Okoroafor, O.N., Okonkwo, A.C., Animoke, P.C., Orajaka, L.J.E and Ngene, A.A. (2009a). *In vitro* and *in vivo* effects of aluminium-magnesium silicate on infectious bursal disease virus in chikens. *Animal Science and Reproduction*, 3(4): 132-137.

Ezeibe, M.C.O., Ijabo, O., Okoroafor, O.N., Orajaka, L.J.E., Ukomadu, N.M. Chukwu, O.S. and Ngene, A.A. (2009b). Antiviral effects of Aluminium-Magnesium Silicate on Peste des Petit Ruminants Virus. *Animal Science and Reproduction*, 3(4): 141-147.

Ezeibe, M.C.O., Okafor, U.C., Okoroafor, O.N., Eze, J.I., Ngene, A.A., Animoke, P.C. and Mbuko, I.J.(2011a). Effect of Aluminium–magnesium silicate on anticcocidial activity of sulphadimidine. *Tropical Veterinaria*, 29: 41-44.

Ezeibe, M.C.O., Ijabo, O., Uzopuo, C., Okoroafor, O.N., Eze, J.I. Mbuko, I.J., Sanda, M.E., Animoke, P.C. and Ngene, A.A. (2011b). Effects of Aluminium–magnesium silicate on Newcastle disease virus and on recovery of infected chicks. *International Journal of Biology and Chemistry*, 5(2): 825 – 829.

Ezeokonkwo, R.C. (1997). Infectious bursal disease outbreak in a flock of 4-week old pullets despite repeat vaccination with IBD vaccine. *Nigeria Veterinary Journal*, 18: 253-256.

Fadley, A.M., and Nazerian, K. (1983). Pathogenesis of infectious bursal disease in chickens infected with virus at various ages. *Avian Diseases*, 27: 714-723.

Fahey, K.J., Erny, K. and Crooks, J. (1989). A conformational immunogen on VP2 of infectious bursal disease virus that induces virus-neutralizing antibodies that passively protect chickens. *Journal of General Virology*, 70: 1473-1481.

Faragher, J.T. (1972). Infectious bursal disease of chicken. *Veterinary Bulletin,* 42: 361-369.

Faragher, J.T., Allan, W.H. and Wyeth, C.J. (1974). Immunosuppressive effect of infectious bursal agent on vaccination against Newcastle disease. *Veterinary Record,* 95: 385-388.

Firth, G.A. (1974). Occurrence of an infectious bursal syndrome within an Australian poultry flock. *Australian Veterinary Journal*, 50: 128-130.

Fleischer, W. and Reimer, K. (1997). Povidone-iodine in antisepsis—State of the art. *Dermatology,* 195 (Suppl 2): 3–9.

Fussel, L.W. (1998). Poultry industry strategy for the control of immunosuppressive disease. *Poultry Science*, 17: 1193-1196.

Gardner, H., Kerry, K., Riddle, M., Brouwer S. and Gleeson, L. (1997). Poultry virus infection in Antarctic penguins. *Nature*, 387:245.

Ghani, A. (1986). Medicinal plants and traditional medicinal position: Problems and prospects. *Journal of Pharmacognosy and Drug Research,* 4: 78-78.

Ghani, A., Abdulrahman, E.M. and Onaolapo, J.A. (1989). Chemical and microbiological evaluation of some Nigerian traditional preparations. *The Report,* October 7, 1989.

Giambrone, J.J. (2008). Variants strain of infectious bursal disease: Epidemiology and control. Retrieved June 25, 2008 from: *www.auburn.edu/giambjj/.*

Giambrone, J.J. (1979). Effect of early infectious bursal disease virus in immunity to Newcastle disease in adult chickens. *Poultry Science,* 58: 794-798.

Giambrone, J.J. and Closser, J. (1990). Efficacy of live vaccines against serologic subtypes of infectious bursal disease virus. *Avian Diseases*, 34(1): 7-11.

Giambrone, J.J., Edison, C.S., Paige, R.K., Fletcher, O.J., Berger B.O. and Kleven, S.H. (1976). Effect of infectious bursal agents on the response of chickens to Newcastle disease and Marek's disease vaccination. *Avian Diseases*, 20: 534-544.

Glier, M.B., Green, T, J. and Devlin, A.M. (2014). Methyl nutrients, DNA methylation, and cardiovascular disease. *Molecular Nutrition & Food Research*, 58: 172–182.

Grimmes, S.E. (2002). *A Basic Laboratory Manual for the Small-scale Production and Testing of 2 Newcastle Disease Vaccine*. Food and Agriculture Organisation of the United Nations (FAO) - Animal Production and Health Commission for Asia and the Pacific (APHCA).

Guittete, M., Le Coq, H., Picault, J.P., Eterradossi, N. and Bennejean, G. (1992). Safety of infectious bursal disease vaccines: assessment of an acceptability threshold. *Development Biology Standards*, 79: 147-152.

Hair-Bejo, M., Ng, M.K. and Ng, H.Y. (2004). Day-old vaccination against IBD in broiler chickens. *International Journal of Poultry Science*, 2004(3): 124-128.

Hair-Bejo, M., Salina, S., Hafiza, H. and Julaida, S. (2000). *In ovo* vaccination against infectious bursal disease in broiler chickens. *Journal of Veterinary Science, Malaysia*, 2: 63-69.

Hassan, M.K. and Saif, Y.M. (1996). Influence of the host system on the pathogenicity, immunogenicity, and antigenicity of infectious bursal disease virus. *Avian Diseases*, 40: 553-561.

Hassan, M.K., Al-Natour, M.Q., Ward, L.A. and Saif, Y.M. (1996). Pathogenicity, attenuation and immunogenicity of infectious bursal disease virus. *Avian Diseases*, 40: 567-571.

Hatchcock, T.L. and Giambrone, J.J. (1992). Tissue-print hybridization using a non-radioactive probe for the detection of infectious bursal disease virus. *Avian Diseases*, 36: 202-205.

Hedayati, A., Nili, H. and Bahonar, A. (2005). Comparison of pathogenicity and serologicresponse of four commercial infectious bursal disease live vaccines. *Archives Razi Institute*, 59: 65-73.

Heine, H.G. and Boyle, D.B. (1993). Infectious bursal disease virus structural protein VP2 expressed by a fowl pox virus recombinant confers protection against disease in chickens. *Archives of Virology*, 131(3-4): 277-292.

Helmboldt, C.F. and Garner, E. (1964). Experimentally induced gumboro disease. *Avian Diseases*, 8: 561-575.

Hiraga, M., Nunoya, T., Otaki, Y., Tajima, M., Saito, T. and Nakamura, T. (1994). Pathogenesis of highly virulent infectious bursal disease virus infection in intact and bursectomized chickens. *Journal of Veterinary Medical Science*, 56: 1057-1063.

Hirai, K., Shimakura, S. and Hirose, M. (1972). Immunodiffusion reaction to avian infectious bursal virus. *Avian Diseases*, 16: 961-964.

Hirai, K., Kawamoto, E. and Shimakura, S. (1974). Some properties of precipitating antigens associated with infectious bursal disease virus. *Infectious Immunology*, 10: 1235-1240.

Howie, R.I. and Thorsen, J. (1981). Identification of a strain of infectious bursal disease virus isolated from mosquitoes. *Canadian Journal of Comparative Medicine*, 45: 315-320.

Hudson, P.J., McKeru, N.M., Power, B.E. and Azad, A.A. (1986). Genomic structure of the large segment of infectious bursal disease virus. *Nucleic Acid Research*, 14: 5001-5012.

Ibrahim, U.I., Ambali, A.G., Geidam, Y.A. and Gulani, I.A. (2003). Outbreak of infectious bursal disease (IBD) in a vaccinated flock of chickens in Maiduguri, Nigeria. *Sahel Journal of Veterinary Science*, 2: 35-37.

Idu, M. C. and Igeleke, L. (2012). Antimicrobial activity and phytochemistry of *Khaya senegalensis* roots. *International Journal of Ayurvedic and Herbal Medicine,* 2(3): 416: 422.

Ijeoma, J.U., Idu, M. and Umar, A.H. (1997). Medicinal plant in use by the Fulani traditional herbalist in Yola North and Yola South L. G. A. of Adamawa State. *Journal of Applied Science Management*, 1(1): 59–63.

Iloeje, M.P. (2004). *A New Geography of Nigeria*. Published by Longman Nigeria PLC 52 Oba Akran, Avene, P.M.B 21036, Ikeja, Lagos, Nigeria.

Inoue, M., Fukuda, M. and Miyano, K. (1994). Thymic lesions in chicken infected with infectious bursal disease virus. *Avian Diseases,* 38(4): 839-846.

Inoue, M., Fujita, A. and Maeda, K. (1999). Lysis of myelocytes in chickens infected with infectious bursal disease virus. *Veterinary Pathology,* 36(2): 146-151.

Islam, M.T. and Samad, M.A. (2004). Mortality in chicks associated with economic impact and prospect of layer chick rearer package programme of the participatory livestock development project in Bangladesh. *International Journal of Poultry Science,* 3: 119-123.

Ismail, N. M. and Saif, Y. M. (1990*).* Differentiation between antibodies to serotype 1 and 2 infectious busal disease in chicken sera. *Avian Diseases,* 34: 1002-1004.

Ismail, N.M., Saif, Y.M. and Moorhead, P.D. (1988). Lack of pathogenicity of five serotype 2 infectious bursal disease viruses in chickens. *Avian Diseases,* 32: 757-759.

Ismail, N.M., Saif, Y.M., Wigle, W.L., Havenstein, G.B. and Jackson. C. (1990). Infectious bursal disease virus variant from commercial leghorn pullets. *Avian Diseases,* 34: 141-145.

Ivanyi, J. and Morris, R. (1976). Immunodeficiency in chicken IV. An immunological study of infectious bursal disease. *Clinical and Experimental Immunology*, 23: 154-165.

Iwu, M. (1993). Pharmacognostical profile of selected medicinal plants. *Hankbook of African Medicinal Plants.* Boca Raton Press CRC Press Inc. Free Enterprise Publishers, Lagos, Pp. 435.

Jackwood, D.J. (1990). Development and characterization of nucleic acid probes to infectious bursal disease viruses. *Veterinary Microbiology,* 24: 253-260.

Jackwood, D.J. and Saif, Y.M. (1987). Antigenic diversity of infectious bursal disease viruses. *Avian Diseases,* 31: 766-770.

Jackwood, D.J., Saif, Y.M. and Hughes, J.H. (1982). Characteristics and serologic studies of two serotypes of infectious bursal disease virus in turkeys. *Avian Diseases,* 26: 871-882.

Jackwood, D.J., Saif, Y.M., Moorhead, P.D. and Bishop, G. (1984). Failure of two serotype II infectious bursal disease viruses to affect the humoral immune response of turkeys. *Avian Diseases,* 28: 100-116.

Jackwood, D.H., Saif, Y.M. and Hughes, J.H. (1987). Replication of infectious bursal disease virus in continuous cell lines. *Avian Diseases,* 31: 370-375.

Jagadish, M.N., Staton, V.J., Hudson P.J. and Azad, A.A. (1988). Birnavirus precursor polyprotein is processed in *Escherichia coli* by its own virus encoded polypeptide. *Journal of Virology,* 62: 1084-1087.

Jakob. H., Leininger, S., Lehmann, T., Jacobi, S. and Gutewort, S. (2005), Peroxo compounds, inorganic, *Ullman's Encyclopaedia of Industrial Chemistry,* Weinheim: Wiley-VCH, Doi: 10.1002/14356007.a19 177.pub2.

Jones, B.A.H. (1986). Infectious bursal disease serology in New Zealand poultry flocks. *New Zealand* V*eterinary Journal,* 34: 36.

Kapadia, G., Chung, E., Ghosh, B., Shukla, V., Basak, S., Morton, J. and Pradhan, S. (1978). Carcinogenicity of some folk medicinal herbs in rats. *Journal of National Cancer Institue,* 60: 683-686.

Kaufer, I. and Weiss, E. (1980). Significance of bursa of Fabricius as target organ in infectious bursal disease. *Infection and Immunology,* 27: 364-367.

Keay, R.W.J., Onochie, C.F.F. and Stanfield, J. (1989). *A Revised Version of Trees of Nigeria,* Clarendon Press, New York, NY, USA.

Kibenge, F.S.B. (1992). Differential detection of infectious bursal disease virus serotypes using cDNA probes to VP2 coding region. *American Journal of Veterinary Research,* 53: 1337- 1342.

Koné, W.M., Kamanzi, A.K., Terreaux, C., Hostettmann, K., Traoré, D. and Dosso, M. (2004). Traditional medicine in North Cote-d'Ivoire: screening of 50 medicinal plants for antibacterial activity, *Journal of Ethnopharmacology*, 93(1): 43–49.

Kouwenhoven, B. and van den Bos, J. (1994). Control of very virulent infectious bursal disease (Gumboro disease) in the Netherlands with more virulent vaccines. In: *Proceedings of First International Symposium on Infectious Bursal Disease and Chicken Infectious Anaemia,* 21-24 June, Rauischholzhausen (E. Kaleta, ed.). World Veterinary Poultry Association, Giessen, Pp. 262-271.

Kreider, D.L., Skeeles, J.K., Parsley, M., Newberry, L.A. and Story, J.D. (1991). Variability in a commercially available enzyme-linked immunosorbent assay system. II. Laboratory variability. *Avian Diseases,* 35: 288-293.

Krishnamohan, Y.R. and Koteeswaran, A. (2002). B/BW ratio for IBD diagnosis: usefulness of bursa/body weight ratio for field level diagnosis of infectious bursal disease. *The Indian Journal of Animal Science,* 72(5): 70-73.

Kubmarawa, D., Khan, M.E., Punah, A.M. and Hassan, M. (2008). Phytochemical screening and antimicrobial efficacy of extracts from *Khaya senegalensis* against human pathogenic bacteria. *African Journal of Biotechnology*, 7(24): 4563–4566.

Kulikova, L., Jurajda, V. and Juranova, R. (2004). Effect of IBD vaccination strains on the immune system of leghorn chickens. *Acta Vet Brno*, 73: 205-209.

Kumar, A. and Rao, A.T. (1989). Double-antibody sandwich ELISA for detection of infectious bursal disease virus. In: *Proceedings of IXth International World Veterinary Poultry Association Congress,* 13-17 August, Brighton. World Veterinary Poultry Association, Positive Action Publications, London, Pp. 25-26.

Kurade, N.P., Bhat, T.K. and Jithendran, K.P. (2000). Occurrence of infectious bursal disease and its pathology in the birds of Himachal Pradesh. *Indian Journal of Veterinary Patholology*, 24(2): 133-134.

Kwang, M.J., Lu, Y.S., Lee, L.H., Lin, D.F., Liao, Y.K., Lee, C. and Lee, Y.L. (1987). Detection of infectious bursal disease viral antigen prepared from the cloacal bursa by ELISA. *Journal of Chinese Society of Veterinary Science*, 13: 265-269.

Landgraf, H., Vielitz, E. and Kirsch, R. (1967). Untersuchugen über das auftreten einer infecktiösen erkrankung mit beteligung der bursa Fabricii (gumboro disease) *Deutsche Tierärztl.Wochenschr*, 74: 6-10.

Lasher, H.N. and Davis, V.S. (1997). History of infectious bursal disease in the USA. The first two decades. *Avian Diseases*, 41: 11-19.

Lasher, H.N. and Shane, S.M. (1994). Infectious bursal disease. *World's Poultry Science Journal*, 50: 133-166.

Ley, D.H., Storm, N., Bickford, A.A. and Yumamoto, R. (1979). An infectious bursal disease virus outbreak in 14- to 15-week-old chickens. *Avian Diseases*, 23: 235-240.

Lohman Animal Health International (LAH). (2004). Infectious bursal disease. Retrieved 10 October, 2013 from www.LAH.com by 11:00 pm.

Lombardo, E., Maravet, A., Espinosa, I., Fernandez Arias, A. and Rodriguez, J.F. (2000). VP5, the nonstructural polypeptide of infectious bursal disease virus, accumulates within the host plasma membrane and induces cell lysis. *Virology*, 277: 345- 357.

Lompo, M., Nikiema, J. B. Guissou, I. P., Moes, A. J. and Fontaine, J. (1998). The topical anti-inflammatory effect of chloroform extracts from *Khaya senegalensis* stem barks. *Phytotherapy Research*, 12: 448–450.

Louzis, C., Gillet, J.P., Irgens, K., Jeannin, A. and Picault, J.P. (1979). La maladie de Gumboro: apparition chez le faisan d'élevage. *Bulletin of Mens Society du Véterinaire Pratice France*, 63: 3-7.

Lucio, B. and Hitchner, S.B. (1980). Immunosuppression and active response induced by infectious bursal disease virus in chickens with passive antibody. *Avian Diseases*, 24: 189-196.

Lucio, B. (1987). Quantitative agar gel precipitation test: an alternative for monitoring infectious bursal disease vaccination programs. In: *Proceedings of 36th Western Poultry Disease Conference,* 3-5 March, Davis, California, University of California, Davis, Pp. 116-119.

Lukert, P.D. and Saif, Y.M. (2003). Infectious bursal disease. In: *Diseases of Poultry*, 11th edition, Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R. and Swayne, D.E. (eds), Iowa State Press, Ames, Iowa, Pp. 161–179.

Lukert, P.D. and Saif, Y.M. (1997). Infectious bursal disease. In: *Diseases of Poultry*, 10th Ed. (B.W. Calnek with H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif, eds), Iowa State University Press, Ames, Pp. 721-738.

Luque, D., Saugar, I., Rodrı´guez, J. F., Verdaguer, N., Garriga, D., Martı´n, C. S., Vela´zquez-Muriel, J. A., Trus, B. L., Carrascosa, J. L. and Casto´n, J. R. (2007). Infectious bursal disease virus capsid assembly and maturation by structural rearrangements of a transient molecular switch. *Journal of Virology*, 81(13): 6869-6878.

Mandelli, G., Rinaldi, A., Cerioli, A. and Cervio, G. (1967). Aspetti ultrastrutturali della borsa di Fabrizio nella malattia di Gumboro de pollo. *Society for Italia Science Veterinaire*, 21: 615-619.

Mazariegos, L. A., Lukert, P. D. and Brown, J. (1990). Pathogenicity and immunesuppressive properties of infectious bursal disease 'intermediate' strains. *Avian Diseases*, 34: 203-208.

Mbuko, I. J., Musa, W. I., Ibrahim, S., Sa'idu, L., Abdu, P. A., Oladele, S. B. and Kazeem, H.M. (2010). A retrospective analysis of infectious bursal disease diagnosed at Poultry Unit of Ahmadu Bello University, Nigeria. *International Journal of Poultry Science*, (8): 784-790.

McAllister, J.C., Steelman, C.D., Newberry, L.A. and Skeeles, J.K. (1995). Isolation of infectious bursal disease virus from the lesser mealworm, *Alphitobius diaperinus* (Panzer). *Poultry Science,* 74: 45-49.

McFerran, J. B. (1993): Infectious bursal disease. In: *Virus Infections of Birds* (Eds McFerran, J.B. and McNulty, M. S.), Elsevier Science Publishers, Amsterdam, Pp. 213-228.

McFerran, J. B., McNulty, M. S., McKillop, E. R., Conner, T. J., McCracken, R. M., Collins, D. S. and Allan, G. M. (1980). Isolation and serological studies with infectious bursal disease viruses from fowl, turkey and duck: demonstration of a second type. *Avian Pathology*, 9: 395-404.

Meroz, M. (1966). An epidemiological survey of Gumboro disease. *Refuah Veterinarian,* 23: 227-235.

Meulemans, G., Antoine, O. and Halen, P. (1977). Application de l'immunofluorescence au diagnostic de la maladie de Gumboro. *Bulletin of Office International des Epizootics,* 88: 225-229.

Meulemans, G., Decaesstecker, M., Halen, P. and Froyman, R. (1987). Comparaison des tests ELISA et de séroneutralisation pour la recherche des anticorps contre le virus de la maladie de Gumboro. Applications pratiques du test ELISA. *Record Médicines Véterinaire,* 163: 561-565.

Michell, B.C., Gomes, A.D., Baiao, N.C., Resende, M., Lara, L.G.C. and Martins, N.R.S. (2009). Effect of maternally-derived antibodies on the performance and immunity of broilers induced by *in ovo* or post-hatching immunizations with a live vaccine against infectious bursal disease. *Brazilian Journal of Poultry Science,* 11(1): 57-63.

Ministry of Agriculture, Food and Rural Affairs, (MAFRA) Canada (2014). Infectious bursal disease of chickens (Gumboro Disease). *Ministry of Agriculture, Food and Rural Affairs,* Canada.

Mittal, M.D., Jindal, N., Gupta, S.L., Kataria, R.S. and Tiwari, A. K. (2005). Detection of infectious bursal disease virus in field outbreaks in broiler chickens by reverse transcription-polymerase chain reaction. *International Journal of Poultry Science,* 4(4): 239-243.

Morales, O. E. and Boclair, W. (1993). Morphometric relations of bursa/spleen in infectious bursaldisease. *Proceedings of the 42nd Western Poultry Disease Conference,* Sacramento, California, Pp. 91-92.

Muller, H. and Becht, H. (1982). Biosynthesis of virus-specific proteins in cells infected with infectious bursal disease virus and their significance as structural elements for infectious virus and incomplete particles. *Journal of Virology,* 44: 384-392.

Muller, H., Scholtissek, C. and Betch, H. (1979). Genome of infectious bursal disease virus consists of two segments of double stranded RNA. *Journal of Virology*, 31: 584-589.

Murphy, F. A., Fauquet, C. M. and Bishop, D. H. (1995). Virus taxonomy, classification and nomenclature of viruses. *Sixth Report of the International Committee on Taxonomy of Viruses.* New York, Springer Verlag, Pp. 253-264.

Musa, I.W., Saidu, L. and Abalaka, E.S. (2012). Economic impact of recurrent outbreaks of gumboro disease in a commercial poultry farm in Kano, Nigeria. *Asian Journal of Poultry Science,* 6: 152-159.

Myra, L. S. (1989). *Statistics for Life Sciences*. Dellen Publishing Company, Riverside, N.J., Pp. 203-210.

Nachimutu, K., Dhinakar, R. G., Thangavelu, A. and Venkatesan, R.A. (1995). Reverse passive haemagglutination test in the diagnosis of infectious bursal disease. *Tropical Animal Health and Production,* 27: 43-46.

Nakamura, T., Kato, A., Lin, Z., Hiraga, M., Nunoya, T., Otaki, Y. and Ueda, S. (1993). A rapid quantitative method for detecting infectious bursal disease virus using polystyrene latex microspheres. *Journal of Virology Methods*, 43: 123-130.

Nawathe, D. R., Onunkwo, O. and Smith, I. M. (1978). Serological evidence of infection with the virus of infectious bursal disease in wild and domestic birds in Nigeria. *Veterinary Records*, 102: 444.

Nicholas, R.A.J., Reed, N.E., Wood, G.W., Hebert, C.N. Muskett, J.C. and Thornton, D.H. (1985). Detection of antibodies against infectious bursal disease: a comparison of three serological methods. *Research in Veterinary Science*, 38: 189-192.

Nunoya, T., Otaki, Y., Tajima, M., Hiraga, M. and Saito, T. (1992). Occurrence of acute infectious bursal disease with high mortality in Japan and pathogenicity of field isolates in specific pathogen free chickens. *Avian Diseases,* 36: 597-609.

Obafemi, C. A., Akinpelu, D. A., Taiwo, O. O. and Adeloye, A. (2006). Antimicrobial activity of solvent extracts of *Terminalia catappa* Lin leaves. *Ife Journal of Science,* 8(1): 29–33.

Organization de Internationale des Epizootics (OIE). (2008). Infectious bursal disease (Gumboro disease) *OIE Terrestrial Manual*. World Organisation for Animal Health. Retrieved June, 2011 from www.oie.int/eng/normes/mcode/en chapter 2.3.12 Pp. 549-565.

Organisation des Epizootics Internationale (OIE), (2004). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals,* 5th Edn., Chapter 2-7-1, part 2, section 2-7.

Office Internationale des Epizooties (OIE) (1995). Resolution No. XVIII. Progress in the diagnosis and control of serious poultry diseases: salmonellosis and Gumboro disease. *Bulletin of Office Internationale des Epizooties*, 107(5): 363-364.

Office Internationale des Epizooties (OIE) (1999). Infectious bursal disease (Gumboro disease). In: *International Animal Health Code: Mammals, Birds and Bees*, OIE, Paris, Pp. 247-248.

Office Internationale des Epizooties (OIE) (2000). *Manual of Standards for Diagnostic Tests and Vaccines, OIE,* Paris.

Ogawa, M., Wakuda, T., Yamaguchi, T., Murata, K., Setiyono, A., Fukushi, H. and Hirai K. (1998). Seroprevalence of infectious bursal disease virus in free-living wild birds in Japan. *Journal of* V*eterinary Medical Science,* 60(11): 1277-1279.

Ojo, S.O. (2003). Productivity and technical efficiency of poultry egg production in Nigeria. *International Journal of Poultry Science,* 2(3): 459-464.

Ojo, M.O., Oduye, O.O., Noibi, L.M. and Idowu, A.L. (1973). Gumboro-like disease in Nigeria. *Tropical Animal Health and Production*, 5: 52-56.

Okoye, J. O. A. (1984). Infectious bursal disease in a vaccinated flock in Nigeria. *Bullentin of Animal Health and Production in Africa,* 32(2): 194-196.

Okoye, J.O.A., (1983). The effect of late infectious bursal disease on the severity of naturally occurring *Eimeria necatrix* infection in chickens. *Bulletin of Animal Health and Production in Africa,* 31: 263-267.

Okoye, J. O. A. and Uzoukwu, M. (1990): Pathogenesis of infectious bursal disease in embryonally bursectomised chickens. *Avian Pathology*. 19: 555-569.

Okoye, J.O.A. and Uzoukwu, M. (1981). An outbreak of infectious bursal disease amongst chickens between 16 and 20 weeks old. *Avian Diseases*, 25: 1034-1038.

Okwu, D. E. and Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology*, 5(4): 357-361.

Oladele, O.A., Emikpe, B.O., Oluwayelu, O.D. and Ohore, O.G. (2004). Comparison of agal gel precipitation test (AGPT) and enzyme linked immunosorbent assay (ELISA) in the detection of infectious bursal disease virus (IBDV) antibody in village chickens in Oyo State, Nigeria. *Nigeria Veterinary Journal*, 25(1): 26-29.

Ologbon, O.A.C. and Ambali, O.I. (2012). Poultry enterprise combination among small-scale farmers in Ogun State, Nigeria: A technical efficiency approach. *Journal of Agriculture and Veterinary Science*, 4: 7-15.

O'Neil, M.J. (ed.). (2006). The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ, Merck and Co., Inc., 2006, Pp. 88.

Ontario Ministry of Agriculture Food and Rural Affairs (OMFRA) (2015). Infectious bursal disease of chicken (Gumboro Disease*). Queens Printer for Ontario Retrieved on 26/8/15 by 6:38 pm.*

Onu, A., Saidu, Y., Ladan, M. J., Bilbis, L. S., Aliero, A. A. and Sahabi, S. M. (2013). Effect of aqueous stem bark extract of *Khaya senegalensis* on some biochemical, haematological, and histopathological parameters of rats. *Journal of Toxicology*. Volume 2013 (2013), Article ID 803835, 9 pages Research Article http://dx.doi.org/10.1155/2013/803835.

Onunkwo, O. (1975). An outbreak of infectious bursal disease of chicken in Nigeria. *Veterinary Records*, 97: 433-435.

Onunkwo, O. and Okoye, J. O. (1991). First report of an infectious bursal disease in a vaccinated chicken flock in Anambra State, Nigeria. *Revue Scientifique Et Technique Office International Des Epizooties,* 44(4): 411-414.

Owoade, A.A. and Durojaiye, O.A. (1995). Infectious bursal disease in 14-week-old turkeys in Nigeria. *Tropical Animal Health and Production,* 27: 47-49.

Oyekunle, M.A. and Adeniji, O.O. (2008). On farm investigation to determine appropriate age to vaccinate chicks with the Nigerian infectious bursal disease vaccine, Gumbova®). Retrieved from: www.ajol.info/view article. 21/05/09 11:30 a.m.

Paul, B. K., Das, S. K., Badhy, S. C., Amin, M. R. Amin, K. M. R. and Banik, S. C. (2004). Effect of existing and imposed vaccination on body weight against Gumboro disease in broilers under farm condition. *International Journal of Poultry Science,* 3(10): 655-657.

Petek, M., D'Aprile, P. N. and Cancelloti, F. (1973). Biological and physiochemical properties of infectious bursal disease virus. *Avian Pathology*, 2: 135-152.

Peters, G. (1967). Histology of Gumboro disease. *Berl Muench Tierärztl Wochenschr,* 80: 394-396.

Provost, A., Borredon. C. and Bocquet, P. (1972). Deux maladies aviaires nouvelles au Tchad : la laryngotrachéite infectieuse et la maladie de Gumboro. *Review Eleve Médicines. Véterinaire Pays Tropica*, 25(3): 347-356.

Pugh, D.G. (2002). *Sheep and Goat Medicine*. (edited by). Pugh, D.G 1st edition. Pp. 26-27.

Radostitis, O. M., Gay, C.C., Hinchliff, K.W. and Constable, P.D. (2006). *Veterinary Medicine*. A text book of the diseases of cattle, sheep, goats, pigs and horses.10th edition publishers- Sanders, Elsevier. Pp. 743-755.

Repp, H., Nieper, H., Draheim, H.J., Koschinski, A., Muller, A. and Dreyer, F. (1998). Infectious bursal disease virus changes the potassium current properties of chicken embryo fibroblasts. *Virology*, 246(2): 362-369.

Rianldi, A., Cervio, G. and Mandelli, G. (1965). Aspetti epidemiologici, anatomo-clinici ed istologici di una nuova forma morbosa dei polli verosimilmente identificable con la considetta Malattia di Gumboro. *Societa Italiena de Patologia Aviar, Convocation Patologia Aviare,* Pp. 77-83.

Roney, C.S. and Freund, R.C. (1988). A comparison of infectious bursal disease antibody titers using different antigens in the serum neutralization and enzyme-linked immunosorbent assay tests. In: *Proceedings of 37th Western Poultry Disease Conference,* 29 February-2 March, Davis, California, University of California, Davis, Pp. 17-20.

Rosenberger, J.K. (1989). *A Laboratory Manual for the Isolation and Identification of Avian Pathogens.* American Association of Avian Pathologists, Kendall-Hunt, Dubuque, Iowa, Pp. 165-166.

Rosenberger, J. K., and Cloud, S. S. (1986). Isolation and characterization of variant infectious bursal disease viruses. *Proceedings of Annual Meeting of the American Veterinary Medical Association,* Atlanta, Ga. Abstract Number 181.

Sahar, M.O., Ali, A.S. and Rahman, E.A. (2004). Residual pathogenic effect of IBD vaccine containing intermediate and hot strains of the virus in broiler chickens. *International Journal of Poultry Science*, 3: 415-418.

Saif, Y. M. (1984). Infectious bursal disease virus type. *Proceedings of the 19th National Meeting on Poultry Health and Condemnations,* Ocean City, Md, Pp. 105-107.

Saif, E. M., Aly, M., Aziz, E. and Mohamed, F. M. (2000). Epidemiological studies on Gumboro diseases in Upper Egypt. *Assiut Veterinary Medical Journal,* 42(84): 223-241.

Sawa, B. A. and Buhari, B (2011). Temperature variability and outbreak of meningitis and measles in Zaria, Northern Nigeria. *Research Journal of Applied Sciences, Engineering and Technology,* 3(5): 399-402.

Schenk, P. (2010). Nutrients. In: *Home-Prepared Dog and Cat Diets,* Second Edition. Wiley- Blackwell. A John Wiley & Sons, Ltd., Publication. 2121 State Avenue, Ames, Iowa 50014-8300, USA, 2: 23.

Shahid, M. and Hussain, F. (2012). Chemical composition and mineral contents of *Zingiber officinale* and *Alpinia allughas* (Zingiberaceae) rhizomes. *International Journal of Chemical and Biochemical Sciences,* 2: 101-104.

Shajahan, A. and Ramesh, S. (2004). Antimicrobial activity of crude ectocarp extract of *Punica granatum* against some selected enteropathogenic bacteria. *Association Journal of Microbiology, Biotechnology and Environmental Science,* 6: 647 – 648.

Shakya, S., Joshi, R.K., Gupta, N. and Chauhan, H.V. (1999). Organ culture of chicken bursa as a model to study the pathogenicity of infectious bursal disease virus isolates. *Avian Diseases,* 43(2): 167-171.

Shane, S., Lasher, H., Paxton, K.W. (1994). Economic impact of infectious bursal disease. In: Proceedings of the 2nd International Symposium of Infectious Bursal Disease and Chicken Anaemia. Rauischholzhausen, Germany.

Sharma, J.M., Dohms, J.E. and Metz, A.L. (1989). Comparative pathogenesis of serotype 1 and variant serotype 1 isolates of infectious bursal disease and their effect on humoral and cellular immune competence of SPF chickens. *Avian Diseases,* 33: 112-124.

Sharma, J.M., Karaca, K. and Pertile, T. (1994). Virus-induced immunosuppression in chickens. *Poultry Science,* 73: 1082-1086.

Sharma, J.M., Kim, I.J., Rautenschlein, S. and Yeh, H.Y. (2000). Infectious bursal disease virus of chicken: pathogenesis and immunosuppression. *Developmental Comprehension Immunology,* 24: 223-235.

Simmons, T., Lee, S.H., Park, T.J., Hashim, D.P., Ajayan, P.M., Linhardt, R.J. (2009). Antiseptic single wall carbon nanotube bandages. *Carbon,* 47(6): 1561–1564. *doi*:*10.1016/j.carbon.2009.02.005*.

Sneader, W. (2005). *Drug Discovery*: *A History*. New York. John Wiley and Sons, p. 68. ISBN 0-471-89979-8.

Snyder, D.B. (1990). Changes in the field status of infectious bursal disease virus - Guest Editorial. *Avian Pathology,* 19: 419-423.

Snyder, D.B., Lana, D.P., Savage, P.K., Yancey, F.S., Mengel, S.A. and Marquardt, W.W. (1988). Differentiation of infectious bursal disease viruses directly from infected tissues with neutralizing monoclonal antibodies: evidence for a major antigenic shift in recent field isolates. *Avian Diseases*, 32: 535-539.

Snyder, D.B., Yancey, F.S. and Savage, P.K. (1992). A monoclonal antibody-based agar gel precipitin test for antigenic assessment of infectious bursal disease viruses. *Avian Patholology,* 21: 153-157.

Stuart, J.C. (1989). Acute infectious bursal disease in poultry. *Veterinary Records*, 125(10): 281.

Tabeekh, M.A.S. and AL- Mayah, A.A.S. (2009). Morphological investigation of bursa of Fabricius of imported broilers and local chicks vaccinated with two types of IBD vaccines. *Proceedings of the 5th Scientific Conference*, College of Veterinary Medicine, University of Mosul. *Iraqi Journal of Veterinary Sciences,* 23 (II): 201-206.

Takase, K., Baba, G.M., Ariyoshi, R. and Fujikawa, H. (1996). Susceptibility of chicken embryos to highly virulent infectious bursal disease virus. *Journal of Veterinary Medical Science,* 58(11): 1129-1131.

Tanimura, N. and Sharma, J.M. (1997). Appearance of T-cells in the bursa of Fabricius and cecal tonsils during the acute phase of infectious bursal disease virus infection in chickens. *Avian Diseases,* 41 (3): 638-645.

Tanimura, N., Tsukamoto, K., Nakamura, K., Narita, M. and Maeda, M. (1995). Association between pathogenicity of infectious bursal disease virus and viral antigen distribution detected by immunochemistry. *Avian Diseases,* 39: 9-20.

Ta-Wei L., Chi-Wen L., Su-Yuan L., Ruey-Jane F., Chao-Jung L., Yu-mei C., Rekha T., Andrew H., Wang, J. and Min-Ying W. (2007). Chicken heat shock protein 90 is a component of the putative cellular receptor complex of infectious bursal disease virus. *Journal of Virology*, 81(16): 8730-8741.

The Merck Veterinary Manual (TMVM), (2006). Infectious bursal disease review. Merck's Veterinary Manual 8th Edn. Editor Aiello, S.E. Retrieved June 26, 2007 from: *http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/203100.htm*.

Thiry, G., Paami, G., Nordgren, R. and Colau, D. (1994). Evaluation of safety and efficacy of vaccination of chickens with live recombinant fowlpox expressing infectious bursal disease virus antigens, In: *Proceedings of First International Symposium on Infectious Bursal Disease and Chicken Infectious Anaemia* (E. Kaleta, ed.), 21-24 June, Rauischholzhausen, Germany, World Veterinary Poultry Association, Giessen, Pp. 336-339.

Trease, G. E. and Evans, W. C. (1996). Phenols and phenolic glycosides. In: *Trease and Evans Pharmacognosy*, Biliere and Tindall, London, Pp. 832.

Tsukamoto, K., Tanimura, N., Hihara, H., Shirai, J., Imai, K., Nakamura, K. and Maeda, M. (1992). Isolation of virulent infectious bursal disease virus from field outbreaks with high mortality in Japan. *Journal* of V*eterinary Medical Science*, 54(1): 153-155.

Tsukamoto, K., Tanimura, N., Mase, M. and Imai, K. (1995). Comparison of virus replication efficiency in lymphoid tissues among three infectious bursal disease virus strains. *Avian Diseases*, 39 (4): 844-852.

Turrentine, J. W. (1908). Action of ammonium persulphate on metals. *Journal of Physical Chemistry*, 11: 623–631.

Umeh, E. U., Oluma, H. O. A. and Igoli, J. O. (2005). Antibacterial screening of four local plants using an indicator-based microdilution technique. *African Journal of Traditional, Complementary and Alternative Medicines*, 2(3): 238–243.

Usman, B.A. and Diarra, S.S. (2008). Prevalent diseases and mortality in egg type layers: An overview. *International Journal of Poultry Science*, 7: 304-310.

Vakharia, V.N., Snyder, D.B., He, J., Edwards, G.E., Savage, P.K. and Mengel-Whereat, S.A. (1993). Infectious bursal disease virus structural proteins expressed in a baculovirus recombinant confer protection in chickens. *Journal of General Virology*, 74: 1201-1206.

van den Berg, T. P. (2000). Acute infectious bursal disease in poultry: a review. *Avian Pathology*, 29: 175-194.

van den Berg, T. P. (2001). Introduction to COST 839. In: *Proceedings of the 2nd International Symposium on Infectious Bursal Disease and Chicken Infectious Anaemia*, Pp. 61-65.

van den Berg, T.P. and Meulemans, G. (1991). Acute infectious bursal disease in poultry: protection afforded by maternally derived antibodies and interference with live vaccination. *Avian Pathology*, 20(3): 409-421.

van den Berg, T.P., Gonze, M. and Meulemans, G. (1991). Acute infectious bursal disease in poultry: isolation and characterisation of a highly virulent strain. *Avian Pathology*, 20(1): 133-143.

van den Berg, T.P., Morales, D., Lambrecht, B. and Meulemans, G. (1997). Use of a baculo derived VP2 protein for diagnosis and control of infectious bursal disease. In: *Proceedings of Xlth International Congress of the World Veterinary Poultry Association*, 18-22 August, Budapest (N. Dren, ed.). World Veterinary Poultry Association, Budapest, Pp. 57.

van den Berg, T.P., Eterradossi, N., Toquin, N.D. and Meulemans, G. (2000). Infectious bursal disease (Gumboro disease). *Review Science Technical Office International des Epizootics*, 19(2): 527-543.

van der Sluis, W. (1999). 1999 world poultry diseases update. *World Poultry*, 15: 30-32.

van Fleet, J.F. and Kennedy, S. (1989). *Compendium on Continuing Education for the Practicing Veterinarian*, 11: 662.

Vindevogel, H., Gouffaux, M., Meulemans, G., Duchatel, J.P. and Halen, P. (1976). Maladie de Gumboro: distribution et persistance du virus chez le poussin inoculé. Études sur la transmission de la maladie. *Avian Pathology*, 5: 31-38.

Viral zone (2015). Diagrmmatic representation of infectious bursal disease viron indicating VP 1, 2 and 3. Swiss Institute of Bioinformatics

Viral zone (2009). Digrammatic representation of infectious bursal disease viral genome and gene expression. Swiss Institute of Bioinformatics

Weisman, J. and Hitchner, S.B. (1978). Virus neutralization versus agar-gel precipitin tests for detecting serological response to infectious bursal disease virus. *Avian Diseases*, 22: 598-603.

World Health Organisation (WHO). (2001). Legal status of traditional medicines and complementary/alternative medicine. A worldwide review, *WHO publishing* 1.

Wilcox, G.E., Flower, R.L.P., Baxendale, W. and Mackenzie, J.S. (1983). Serological survey of wild birds in Australia for the prevalence of antibodies to EDS-76 and infectious bursal disease viruses. *Avian Pathology*, 12: 135-139.

Winterfield, R.W., Adly, A.M. and Bickford, A. (1972). Infectivity and distribution of infectious bursal disease virus in the chicken. Persistence of the virus and lesions. *Avian Diseases*, 16: 622-632.

Wood, G.W., Muskett, J. C., Hebert, C.N. and Thornton, D.H. (1979). Standardization of the quantitative agar gel precipitin test for antibodies to infectious bursal disease. *Journal of Biological Standards*, 7: 89-96.

Wood, G.W., Muskett, J. C, Reed, N.E. and Thornton, D.H. (1984). The effect of antigen variation on the quantitative agar gel precipitin test for antibodies to infectious bursal disease virus. *Journal of Biological Standards*, 12: 311-314,

Wyeth, P.J. (1975). Effect of infectious bursal disease on the response of chickens to *Salmonella typhimurium* and *Escherichia coli* infection. *Veterinary Records*, 96: 238-243.

Wyeth, P. J. and Chettle, N.J. (1990). Use of infectious bursal disease vaccines in chicks with maternally derived antibodies. *Veterinary Records*, 126: 577-578.

Wyeth, P. J. and Cullen, G. A. (1976). Maternally derived antibody-effect on susceptibility of chicks to infectious bursal disease. *Avian Pathology*, 5: 253-260.

Wyeth, P.J. and Cullen, G.A. (1978). Transmission of immunity from inactivated infectious bursal disease oil emulsion vaccinated parent chickens to their chicks. *Veterinary Records*, 103: 36-38.

Wyeth, P. J. and Cullen, G.A. (1979). The use of an inactivated infectious bursal disease oil emulsion vaccine in commercial broiler parent chickens. *Veterinary Records*, 104: 188-193.

Wyeth, P.J., Chettle, N.J. and Mohepat, A.R. (1992). Use of an inactivated infectious bursal disease oil emulsion vaccine in commercial layer chicks. *Veterinary Records*, 130: 30-32.

Yongshan, W., Zong'an, Z., Chunsheng, Z. and Xiangbai, H. (1994). Studies on the transmission of infectious bursal disease virus (IBDV) by feed. *Animal Husbandry and Veterinary Medicine*, 26: 150-152.

Zeisel, S., Mar, M.H., Howe, J. and Holden, J. (2003). Concentrations of choline-containing compounds and betaine in common foods. *Journal of Nutrition*, 133: 1302–1307.

Zhu, L. Q., Wu, S.L., Zhang, G.P. and Zhu, G.Q. (2008). The cellular receptors for infectious bursal disease virus. *African Journal of Biotechnology*, 7(25): 4832-4835.

APPENDICES

Appendix 1: Photograph of *Khaya senegalensis* tree – (A) and leaves – (B).





Appendix 2: Proximate composition of *Khaya senegalensis* leaves.

Proximate content (%)					
DryMatter	Crude protein	crude fibre	Oil	Ash	Nitrogen free extract
93.88	11.81	10.15	0.98	10.98	66.08

Appendix 3: Concentration of phytochemicals in *Khaya senegalensis* leaves.

Concentration of Bioactive substances in *Khaya senegalensis* (mg/100g)				
Tannin	Phytate	Oxalate	Saponin	Alkaloid
18.64	0.03	0.17	0.68	0.80

Appendix 4: Phytochemical test on in *Khaya senegalensis* leaves.

Bioactive substances	Quantity	Colour change/reactions
Mlisch's test (Carbohydrates)	++	Reddish coloured ring
Fehling's test (Reducing sugar)	+	Brick red precipitate
Cardiac glycosides		
A (Kella-Killiani test)	++	Purple-brown ring
Kedde's test(cardenolides)	+	Purple blue colour
Salkwoski's test (Steroids)	++	Cherry red colour
Anthraquinones derivatives		
A Free anthraquinones (Borntrager's test)	+	Bright pink colour
Combined Anthracene (Mordified Borntrager's test)	++	Rose pink to cherry red
Test for saponins		
a. Frothing test	++	A honeycomb froth 10-15min
b. Hemolysis test	+	Settling down of rbc
Test for steroid and Triterpenes (Lieberman-Burchards test)	+++	Blue-blue green upper layer& reddish, pink or purple colour
Test for flavonoids		
a. Shinoda test		
b. Sodium hydroxide test	+	Yellow colour
Test for Tannins		
a. Lead sub-acetate test	+	Coloured precipitate
b. Ferric chloride test	++	Greenish-black ppt
c. Bromine water test	+	Buffy colouer precipitate
Test for alkaloids		
a. Mayer's test	+	Cream precipitate
b. Dragendorff's	+	Reddish brown precipitate

Key: + scanty, ++ moderate, +++ high.

Appendix 5: Summary of phytochemicals in *Khaya senegalensis* leaves.

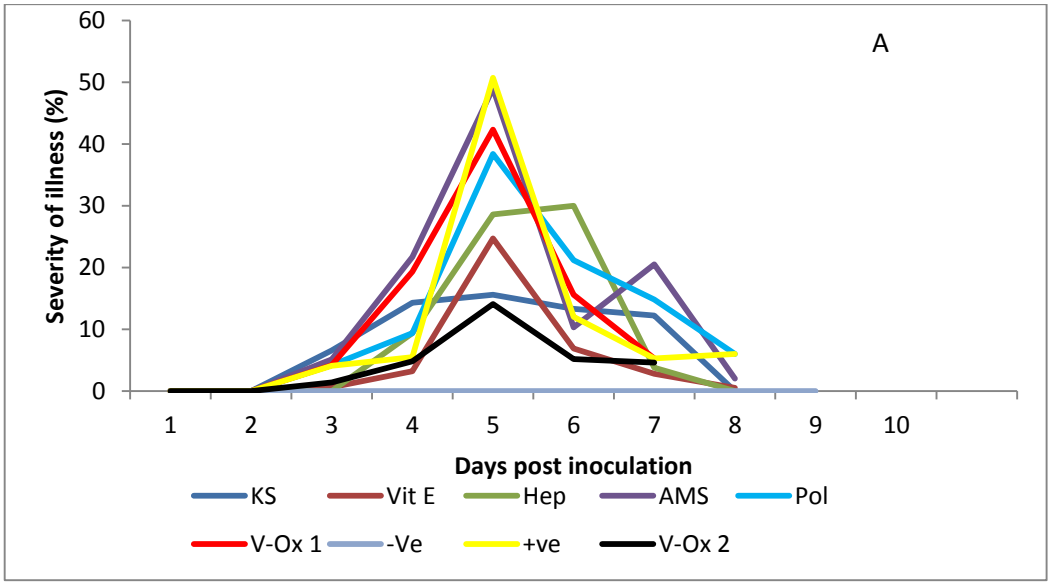
Bioactive substances	Test result
Carbohydrates	++
Cardiac glycosides	++
Anthraquinones	++
Saponins	++
Steroid	+++
Flavonoids	+
Tannins	++
Alkaloids	+

Key: + scanty, ++ moderate quantity, +++ High quantity

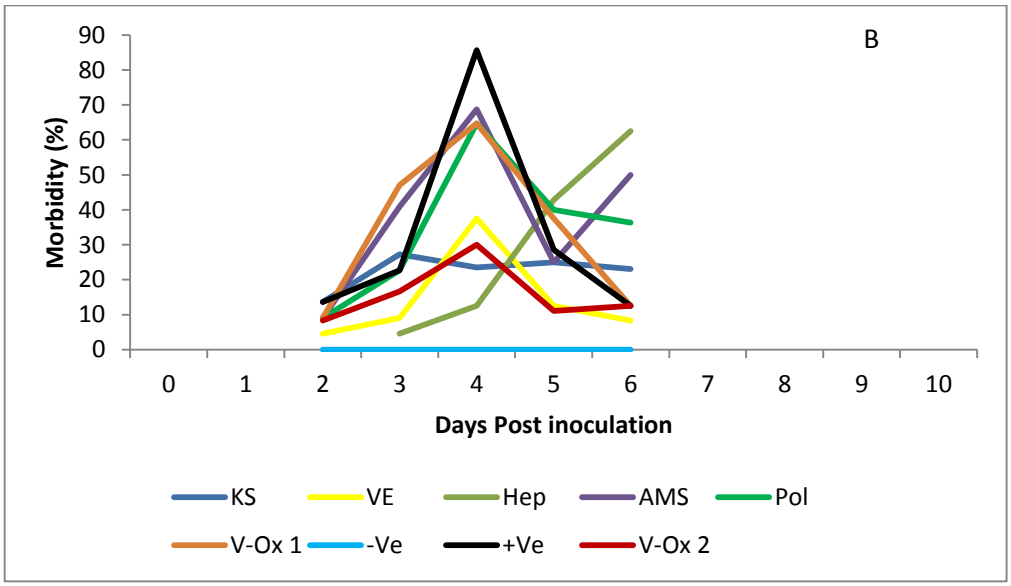
Appendix 6: Morbidity and mortality rates in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

Group	Total no. of chicks	Morbidity n (%)	Chi-square	P-value	Mortality n (%)	Chi-square	P-value
A(*Khaya senegalensis)*	22	6 (27.3)	24.293	0.002	6 (27.3)	51.842	0.000
B(Vitamin E)	22	6 (27.3)			6 (27.3)		
C(Heparenol®)	22	6 (27.3)			19 (86.4)		
D(Aluminum magnesium silicate)	22	11 (50.0)			16 (72.7)		
E(Polidine®)	22	11 (50.0)			9 (40.9)		
F(V-Ox® 1)	22	11 (50.0)			11 (50.0)		
G(Negative control)	22	0 (0.0)			0(0.0)		
H(Positive control)	22	12 (54.5)			13 (59.1)		
I(V-ox® 2)	12	3 (25.0)			2 (16.7)		

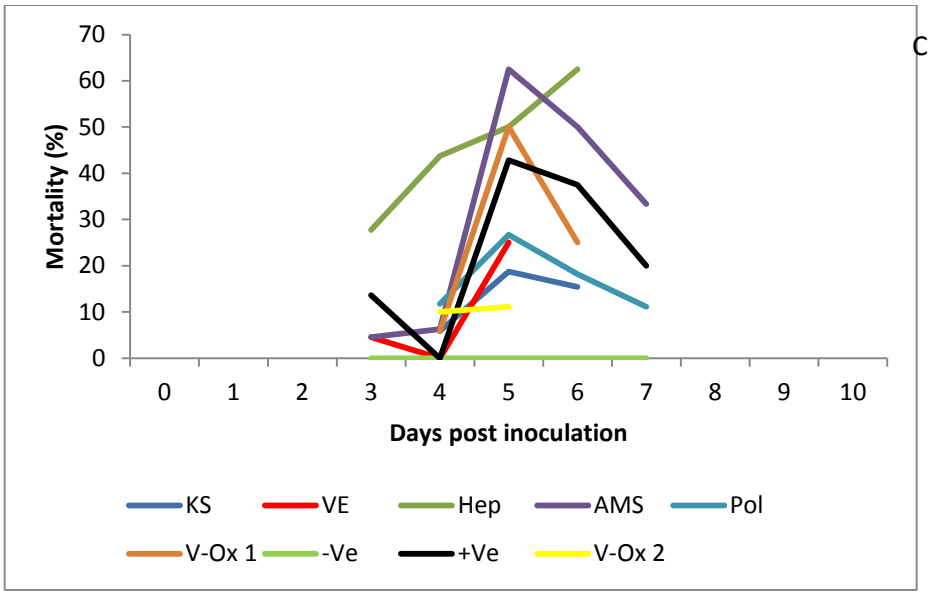
Appendix 7: Severity of clinical signs, morbidity and mortality in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.



Key: KS= *Khaya senegalensis*, Vit E = vitamin E, Hep = heparenol®, AMS = Aluminium magnesium silicate, Pol = polidine®, -Ve = negative control, +Ve = Positive control.
G = negative control, H = positive control, I = V-ox® 2.



Key: KS= *Khaya senegalensis*, Vit E = vitamin E, Hep = heparenol®, AMS = Aluminium magnesium silicate, Pol = polidine®, -Ve = negative control, +Ve = Positive control.
G = negative control, H = positive control, I = V-ox® 2.



Key: KS = *Khaya senegalensis*, Vit E = vitamin E, Hep = heparenol®, AMS = Aluminium magnesium silicate, Pol = polidine®, -Ve = negative control, +Ve = Positive control.
G = negative control, H = positive control, I = V-ox® 2.

Appendix 8: Body weight of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Aluminium magnesium silicate)	E(Polidine®)	F(V-Ox®1)	G(Negative Control)	H(Positive Control)	I(V-Ox® 2)
	Body weight in grams								
Days Postinoculation									
0	158.2 ± .33	155 ± 4.05	145.8 ± 4.39	155.4 ± 15.15	146.2 ± 3.86	149.8 ± 8.43	145 ± 7.11	153.4 ± 8.86	NE
3	140 ± .06a	188.8 ± .94b	169.8 ± 6.81	139.8 ± 4.11c	148.8 ± 6.90	145 ± 3.13	176.4 ± 16.79	171.6 ± 8.59	162.5 ± 20.5
7	162.8 ± 13.54a	193.6 ± 2.94	193.3 ±7.51	178.2 ± 9.46ac	178.4± 14.46acd	191.2± 6.97acde	245.2 ± 15.24b	213 ± 12.18	191± 4.54acdef
10	201.4 ± .97	251.8 ± .09	ABD	ABD	219.8 ± 13.51	269 ± 0	249.4 ± 13.98	204.7 ± 9.21	185 ± 27

Key: Superscripts with different alphabets are significantly different $P \leq 0.05$.
ABD = all birds died; NE = not euthanized.

Appendix 9: Gross pathological lesions score in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

Days postinoculation															
	3					7					10				
						Range of lesion score									
	0	0.1-2	2.1-5	5.1-9	9.1-12	0	0.1-2	2.1-5	5.1-9	9.1-12	0	0.1-2	2.1-5	5.1-9	9.1-12
						Lesion score									
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Group															
A(*Khaya senegalensis*)				+				+				+			
B(Vitamin E)					+		+					+			
C(Heparenol®)			+				+								
D(Aluminium magnesium silicate)			+					+							
E(Polidine®)				+			+					+			
F(V-Ox® 1)					+			+				+			
G(Negative control)	Ngl	ngl	ngl	ngl	ngl	Ngl	ngl	ngl	ngl	ngl	ngl	Ngl	ngl	ngl	ngl
H(Positive control)				+					+			+			
I(V-Ox® 2)								+				+			

Key: ngl = no gross lesions seen, 0 No gross lesions seen (NGLS), 0.1– 2.0 – minimal gross lesions seen (MGLS), 2.1 – 5.0 moderately severe gross lesions seen (MSGLS), 5.1 – 9 severe gross lesions seen (SGLS), 9.1 – 12 very severe gross lesions seen (VSGLS), + = lesion score, empty spaces = no lesion score.

Appendix 10: Mean weight of bursa of Fabricius of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Alumiium magnesium silicate)	E(Polidine®)	F(V-Ox® 1)	G(Negative control)	H(Positive control)	I(V-Ox® 2)
	Bursa of Fabricius weight in grams								
Days Postinoculation									
0	1.104 ± 0.05	1.084 ± 0.09	1.098 ± 0.15	1.11 ± 0.10	0.972 ± 0.11	1.048 ± 0.12	1.158 ± 0.08	1.26 ± 0.11	NE
3	0.874 ± 0.11	1.228 ± 0.07	1.196 ± 0.14	1.186 ± 0.14	1.072 ± 0.10	1.138 ± 0.10	1.298 ± 0.17	1.204 ± 0.14	1.155 ± 0.05
7	0.85 ± 0.12a	0.964 ± 0.10	0.92 ± 0.02	0.852 ± 0.07ac	0.892 ± 0.08acd	0.79 ± 0.09acde	1.434 ± 0.15b	0.784 ± 0.09acdef	0.942 ± 0.15
10	0.632 ± 0.25	0.762 ± 0.10	ABD	ABD	0.695 ± 0.11	0.65 ± 0	1.358 ± 0.29	0.557 ± 0.09	0.85 ± 0.18

Key: Superscripts with different alphabets are significantly different $P \leq 0.05$, ABD = all birds died; NE = not euthanized, ABD = all birds died; NE = not euthanized.

Appendix 11: Mean weight of spleen of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Aluminium magnesium silicate)	E(Polidine®)	F(V-Ox® 1)	G(Negative Control)	H(Positive control)	I(V-Ox® 2)
	Spleen weight in grams								
Days Postinoculation									
0	0.442 ± 0.01	0.452 ± 0.02	0.446 ± 0.02	0.438 ± 0.02	0.436 ± 0.02	0.458 ± 0.02	0.402 ± 0.01	0.45 ± 0.03	NE
3	0.65 ± 0.21	0.518 ± 0.02	0.574 ± 0.04	0.476 ± 0.05	0.528 ± 0.02	0.69 ± 0.19	0.54 ± 0.06	0.598 ± 0.07	0.55 ± 0.04
7	0.616 ± 0.06b	0.872 ± 0.12	0.89 ± 0.09	0.672 ± 0.09b	0.666 ± 0.09b	1.068 ± 0.19a	0.676 ± 0.04b	0.728 ± 0.08b	0.832 ± 0.06
10	0.532 ± 0.05a	0.824 ± 0.11b	ABD	ABD	0.763 ± 0.26	0.91 ± 0b	0.572 ± 0.32	0.8567 ± 0.15b	0.685 ± 0.24

Superscripts with different alphabets are significantly different $P \leq 0.05$; Key: ABD = all birds died; NE = not euthanized.

Appendix 12: Mean weight of thymus of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Aluminum magnesium silicate)	E(Polidine®)	F(V-Ox® 1)	G(Negative control)	H(Positive Control)	I(V-Ox® 2)
	Thymus weight in grams								
Days Postinoculation									
0	1.23 ± 0.08a	1.462 ± 0.07b	1.334 ± 0.14b	1.358 ± 0.22b	1.094 ± 0.09a	1.41 ± 0.11b	1.604 ± 0.05b	1.39 ± 0.15b	NE
3	0.738 ± 0.04	1.11 ± 0.13	1.566 ± 0.32	0.874 ± 0.12	1.2 ± 0.30	0.826 ± 0.12	0.968 ± 0.10	1.042 ± 0.24	1.65 ± 0.0
7	0.73 ± 0.09b	1.01 ± 0.16b	1.497 ± 0.30a	1.094 ± 0.18b	0.814 ± 0.23b	0.986 ± 0.10b	1.322 ± 21a	0.958 ± 0.12b	0.848 ± 0.13b
10	0.762 ± 0.05a	1.6 ± 0.11	ABD	ABD	1.043 ± 0.26ab	1.29 ± 0	2.148 ± 0.32c	0.913 ± 0.15abd	0.91 5± 0.24abde

Appendix 13: Bursa of Fabricius/body weight ratio of Isa brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
Day	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Aluminium magnesium silicate)	E(Polidine®)	F(V-Ox® 1)	G(Negative control)	H(Positive control)	I(V-Ox® 2)
	Bursa of Fabricius/body weight ratio in grams								
Days Postinoculation									
0	7.004 ± 0.39	6.953 ± 0.41	7.479 ± 0.91	7.264 ± 0.57	6.646 ± 0.73	6.935 ± 0.45	7.994 ± 0.43	8.203 ± 0.52	NE
3	6.185 ± 0.57a	6.572 ± 0.50a	7.09 ± 0.87	8.577 ± 1.18	7.181 ± 0.50	7.965 ± 0.74	7.382 ± 0.0.65b	7.001 ± 0.69	7.187 ± 0.63
7	5.386 ± 0.85a	5.056 ± 0.67a	4.765 ± 0.09a	4.825 ± 0.41a	5.246 ± 0.78a	4.112 ± 0.41b	5.875 ± 0.51a	3.773 ± 60b	5.012 ± 0.81a
10	3.124 ± 1.20a	3.077 ± 0.41a	ABD	ABD	3.192 ± 0.43a	2.416 ± 0a	5.452 ± 1.04b	2.692 ± 0.31a	4.55 ± 0.31a

Key: Superscripts with different alphabets are significantly different $P \leq 0.05$; ABD = All birds died, NE = Not euthanized.

Appendix 14: Spleen/body weight ratio of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A(*Khaya senegalensis*)	B(Vitamin E)	C (Heparenol®)	D(Aluminium magnesium silicate)	E(Polidine®)	F(V-Ox® 1)	G(Negative control)	H(Positive control)	I(V-Ox® 2)
	Spleen/body weight ration in grams								
Days post inoculation									
0	2.80 ± 0.09	2.93 ± 0.20	3.07 ± 0.19	2.88 ± 0.15	2.99 ± 0.18	3.08 ± 0.14	2.79 ± 0.11	2.95 ± 0.19	NE
3	4.64 ± 1.48	2.77 ± 0.15	3.39 ± 0.26	3.42 ± 0.41	3.55 ± 0.04	5.03 ± 1.65	3.07 ± 0.17	3.53 ± 0.47	3.41 ± 0.18
7	3.79 ± 0.18	4.50 ± 0.55	4.60 ± 0.40	3.82 ± 0.55	3.67 ± 0.26	5.68 ± 1.13[a]	2.82 ± 0.29[b]	3.40 ± 0.27	4.38 ± 0.38
10	2.66 ± 0.40	3.26 ± 0.19	ABD	ABD	3.43 ± 0.31	3.38 ± 0	2.30 ± 0.22	4.16 ± 0.34	3.72 ± 0.08

Key: Superscripts with different alphabets are significantly different $P \leq 0.05$; ABD = all birds died; NE = not euthanized.

Appendix 15: Thymus/body weight ratio of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A (*Khaya senegalensis*)	B (Vitamin E)	C (Heparenol)	D(Aluminium magnesium silicate)	E (Polidine®)	F (V-Ox®1)	G (Negative control)	H (Positive control)	I (V-Ox® 2)
	Thymus/body weight ration in grams								
Days Postinoculation									
0	7.83 ± 0.69	9.42 ± 0.35	9.10 ± 0.78	8.60 ± 0.74	7.46 ± 0.53[a]	9.52 ± 0.88	11.19 ± 0.72b	9.26 ± 1.25	NE
3	5.27 ± 0.10	5.07 ± 1.43	9.38 ± 2.15	6.32 ± 0.96	8.05 ± 1.85	5.77 ± 0.74	5.71 ± 0.76	6.06 ± 1.30	10.32 ± 1.30
7	4.47 ± 0.32	5.27 ± 0.85	7.80 ± 1.72	6.22 ± 1.15	4.40 ± 0.97	5.142 ± 0.45	5.40 ± 0.90	4.48 ± 0.45	4.45 ± 0.67
10	3.78 ± 0.23[a]	6.51 ± 0.68[b]	ABD	ABD	4.79 ± 1.30[b]	4.80 ± 0[b]	8.48 ± 0.88[b]	4.42 ± 0.53[b]	5.24 ± 2.04[b]

Key: Superscripts with different alphabets are significantly different $P \leq 0.05$; ABD = all birds died; NE = not euthanized.

Appendix 16: Geometric mean titre of antibody titre levels of Isa brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Aluminium magnesium silicate)	E(Polidine®)	F(V-Ox® 1)	G(Negative control)	H(Positive control)	I(V-Ox®2)
Age in days	Geometric mean titre								
1	2247	2172	2186	2121	2332	1879	2135	2321	NB
7	1881	1912	1549	1544	1847	1544	1607	1740	NB
14	564	583	454	479	413	447	556	446	NB
21	120	120	287	226	231	185	183	197	NB
28	140	296	108	351	124	157	58	162	206
35	1505	1256	ABD	ABD	1476	1432	62	1679	1683

Appendix 17: Mean antibody titre level of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Aluminium magnesium silicate)	E(Polidine®)	F(V-Ox® 1)	G(Negative control)	H(Positive control)	I(V-Ox® 2)
Age in days					Mean titre				
1	231 ± 145.1	2253 ± 167.6	2376 ± 273.9	2215 ± 200.7	2407 ± 190.2	1962 ± 163.2	2253 ± 204.1	2407 ± 196.4	0.00
7	195 ± 151.6	2036 ± 189.5	1652 ± 166.7	1650 ± 199.9	1974 ± 219.9	1641 ± 183.3	1675 ± 125.8	1819 ± 163.2	0.00
14	586.5 ± 49.54	629.4 ± 98.96	517.8 ± 78.09	540.3 ± 124.1	504 ± 94.89	563.3 ± 139.9	619.9 ± 80.82	519.3 ± 91.03	0.00
21	133 ± 20.69	255.8 ± 35.25	304.9 ± 39.04	252.5 ± 64.59	255 ± 32.06	217.4 ± 50.78	189.8 ± 15.99	227.4 ± 46.46	0.00
28	151.6 ± 23.21	510 ± 205.3	131.7 ± 58.38^{ab}	357.7 ± 47.98^{bc}	172.8 ± 55.14	218.3 ± 98.68	61.43 ± 9.258	230.1 ± 77.26	313.1 ± 120.2
35	1529 ± 118.4^{af}	1312 ± 190.4^{aef}			1530 ± 231.1^{aef}	1432 ± 0^{aefg}	68.14 ± 12.99^{bd}	1725 ± 280.6^{aeh}	1718 ± 342.5^{aei}

Key: Superscripts with different alphabets are significantly different $P < 0.0001$.

Appendix 18: Percent coefficient of variation (%CV) of ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

	Group								
	A(*Khaya senegalensis*)	B(Vitamin E)	C(Heparenol®)	D(Aluminium magnesium silicate)	E(Polidine®)	F(V-Ox® 1)	G(Negative control)	H(Positive control)	I(V-Ox® 2)
Age in days	Coefficient of variation (%)								
1	20.9	24.7	38.2	30.1	26.2	27.6	30.0	27.1	00
7	28.0	29.4	33.5	32.0	35.2	31.6	28.1	29.7	00
14	25.3	31.4	39.9	51.4	56.5	65.7	45.2	52.6	00
21	41.2	41.2	33.9	44.3	39.8	61.8	26.6	54.1	00
28	37.5	98.6	62.7	19.0	71.3	78.3	36.9	82.2	94.0
35	17.3	32.4	00	00	26.2	0	46.7	23.0	19.9

Appendix 19: Clinical signs data generation sheet in ISA brown chicks inoculated with very virulent infectious bursal disease virus and treated with some unconventional remedies.

		Age in days postinoculation														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number of birds with clinical signs																
S/N	Type of clinical signs															
1	Mortality															
2	Morbidity															
3	Depression															
4	Dehydration															
5	Dilapidated															
6	Diarrhoea with urates															
7	Inappetance															
8	Swollen vent															
9	Recumbency															
10	Ruffled feathers															
11	Huddling															
12	Vent pecking															
13	Soiled vent															
14	Anorexia															
15	Trembling/unsteady gait															
16	Prostration															

Appendix 20: Postmortem data generation sheet in ISA brown chicks inoculated with very virulent infectious bursal diseasevirus and treated with some unconventional remedies.

		Age in days post inoculation														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
S/N	Type of gross pathological lesions	Numberof birds with gross pathological findings														
1	Enlarged bursa of Fabricius															
2	Atrophied bursa of Fabricius															
3	Oedema bursa of Fabricius															
4	Haemorrhagic bursa of Fabricius															
5	Creamy transudate in the kidney															
6	Haemorrhage muscles															
7	Hemrhages in the proventriculus															
8	Enlareged liver															
9	Hemrhage liver															
10	Swollen whitish kidney with urates and debris															
11	Enlarged kidneys															
12	Haemrhages in the kidneys															
13	Haerrhages in the intestine															
14	Cheesy core															
15	Increas mucus in intestine															
16	Enlarge spleen															
17	Spleen with grey foci															
18	Hemorrhages at ventriculoproventricular junction															