PATHOLOGICAL, HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN COCKERELS EXPERIMENTALLY INFECTED AND VACCINATED AGAINST INFECTIOUS BURSAL DISEASE VIRUS

BY

IBRAHIM KASSIM

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

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER DEGREE IN VETERINARY PATHOLOGY

DEPARTMENT OF VETERINARY PATHOLOGY, FACULTY OF VETERINARY MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA.

AUGUST, 2014

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Ibrahim KASSIM
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NIGERIA

AUGUST, 2014

DECLARATION

I declare that the work in this Thesis entitled **"PATHOLOGICAL HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN COCKERELS EXPERIMENTALLY INFECTED AND VACCINATED AGAINST INFECTIOUS BURSAL DISEASE VIRUS"** has been carried out by me in the Department of Veterinary Pathology, Ahmadu Bello University Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Thesis was previously presented for another degree or diploma at this or any other institution.

Ibrahim KASSIM _____ _____

Signature Date

CERTIFICATION

This Thesis entitled **"PATHOLOGICAL, HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN COCKERELS EXPERIMENTALLY INFECTED AND VACCINATED AGAINST INFECTIOUS BURSAL DISEASE VIRUS"** by Ibrahim KASSIM meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

PROF. S.B. OLADELE Chairman, Supervisory Committee	_____ Signature	_____ Date
DR. N. M. USEH Member, Supervisory Committee	_____ Signature	_____ Date
PROF. L. SAIDU Member, Supervisory Committee	_____ Signature	_____ Date
DR. B. MOHAMMED Head of Department	_____ Signature	_____ Date
PROF. A. Z. HASSAN Dean, School of Postgraduate Studies, A. B. U. Zaria	_____ Signature	_____ Date

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Abstract

This study was conducted to determine pathological, haematological, and biochemical changes in cockerels experimentally challenged with very virulent infectious bursal disease virus (vvIBDV), and those vaccinated against Gumboro disease. Two hundred and fifty day-old cockerels (chicks) were randomly distributed into 5 groups (A, B, C, D and E) of 50 chicks per group. Group A chicks were vaccinated at 2 and 4 weeks of age with intermediate strain (Georgia) IBD vaccine orally at dose of 10 ml (equivalent of 10^3 plague-forming units) per bird. Each bird in groups B, C and D was inoculated with 0.4 ml of vvIBDV inoculum equivalent to 16 x10 $^{4.6}$ ELD_{50} of undiluted viral suspension via conjunctival instillation at 4, 6 and 8 weeks, respectively. Group E served as the control group. Chicks in this group were neither vaccinated against nor challenged with vvIBDV. Blood samples were collected from birds in each group and analysed for haematological parameters. Serum samples obtained from the birds were used to determine biochemical parameters, and antibody titres to IBDV, using an enzyme linked immunosorbent assay (ELISA) kit. Blood samples were collected one week after each vaccination in group A, first and second week after challenge in groups B, C, and D, and weekly (week 1 – 10) in the control group (E). Clinical signs exhibited by birds in the challenged groups include whitish-yellowish mucoid diarrhoea, recumbency, prostration, drooped wings, and reluctance to move. One hundred per cent morbidity rate was recorded in all the challenged groups (B, C, and D), while mortality rates of 54%, 72% and 50% were observed in groups B, C and D, respectively. The haematological parameters of the challenged groups revealed significant decrease ($p < 0.05$) in packed cell volume (PCV), total red blood cell count (TRBC) and haemoglobin concentration (Hb); and significant

decrease ($p < 0.05$) in lymphocyte and white blood cell count (WBC) values. On the other hand, there was a significant increase ($p < 0.05$) in heterophil value. In the vaccinated group, the haemogramme revealed significant decrease ($p < 0.05$) in lymphocyte and heterophil values. There were significant decreases ($p < 0.05$) in total protein (TP) and total cholesterol; as well as significant increase in urea ($p < 0.05$) in the challenged groups. In the vaccinated group potassium and total protein were significantly decreased ($p < 0.05$). Gross lesions, like enlarged, congested, haemorhagic and turgid bursa of Fabricius; enlarged and congested kidneys; and haemorhages in mucosae of the proventriculus were observed in the challenged groups. In the vaccinated group, no clinical signs were exhibited, but mild gross lesions were seen. Histopathological changes in the challenged groups revealed haemorhages, congestion, and necrosis of bursa of Fabricius, liver, kidney, spleen and muscle. In conclusion, the study revealed some pathological, haematological and biochemical differences in cockerels challenged with vvIBDV, and those vaccinated against IBDV.

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ABBREVIATIONS

% - Percentage

< - Less-than

> - Greater- than

µL- micro liter

µmol/L- micromole per liter

Abs- Antibodies

ABU- Ahmadu Bello University

AC-ELISA- Antigen capture- Enzyme-linked immunosorbent assay

AGID- Agar gel immunodiffusion

ATP- Adenosine tri-phosphate

BF-Bursa of Fabricius

Cr- Creatinine

CrCl- Creatinine clearance

EDTA- Ethylene diamine tetra acetic acid

eGFR- estimated Glomerular filtration rate

ELD_{50}- Effective lethal dose fifty

ELISA- Enzyme- linked immunosorbent assay

fL- Femtoliter

GFR- Glomerular filtration rate

H&E- Hematoxylene and eosine

Hb- Haemoglobin concentration

HDL- High- density lipoprotein

IB- infectious bronchitis

IBD- Infectious bursal disease

IBDV- Infectious bursal disease virus

IFCC- International federation of clinical chemistry

Ig- Immunoglobulin

LDL- Low- density lipoprotein

LYMP- Lymphocyte counts

MA- Maternal antibody

MCH- Mean corpuscular haemoglobin

MCHC- Mean corpuscular haemoglobin concentration

MCV- Mean corpuscular volume

mmol/L- millimole per liter

ND- Newcastle disease

NEUT- Neutrophils counts

OIE- Office international des ėpizooties

PCV- Packed cell volume

PFU- plaque forming unit

Pg- picogram

RBC- Red blood cell counts

RNA- Riboxy nucleic acid

SN- Serum neutralization

SPF- Specific pathogen free

USA- United State of America

VLDL- Very- low-density lipoprotein

vvIBDV- very virulent infectious bursal disease virus

WBC- White blood cell counts

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Infectious bursal disease (IBD) is one of the most important viral diseases of commercial chickens worldwide (Lukert and Saif, 2003). The disease is caused by infectious bursal disease virus (IBDV). This organism is a small, non-enveloped virus, belonging to the family *Birnaviridae*, which is characterized by a bisegmented double stranded RNA genome (Kibenge *et al.*, 1988). Infectious bursal disease also known as Gumboro disease is an acute highly contagious viral infection of 3 to 11 weeks old susceptible chickens (Parkhurst, 1964; Lukert and Saif, 1991; Mbuko *et al*., 2010). The disease was first reported in United States of America in 1957 (Cosgrove, 1962) and was relatively under control due to proper vaccination program both in the hens and chicks (Chettle *et al.,* 1989). However, in late 1980's, outbreak of the disease with high mortality due to very virulent IBD virus (vvIBDV) was reported in Europe (Chettle *et al.,* 1989). The disease is manifested either as immuno-depression or as a clinical illness, depending on the age of the bird (Bayliss *et al*., 1990). Of the two IBDV serotypes, only serotype I is pathogenic for chickens (Lukert and Saif, 2003). Until 1987, the strains of the virus were of low virulence, causing less than 2% mortality, and efficiently controlled by vaccination (Chettle *et al.,* 1989).

In 1986, vaccination failure was described in different parts of the world due to the re-emergence of the very virulent strains. Europe has experienced the emergence of "very virulent" (vv) strains of IBDV, which can cause up to 70% flock mortality in laying pullets (Chettle *et al*., 1989; van den Berg *et al.,* 2000). These strains can cause lesions typical of IBDV and are antigenically

similar to the "classical" strains, which were prevalent for some decades (Eterradossi *et al.*, 1992).

Clinical signs of IBD in chicken were sudden onset of sickness, including trembling, weakness, depression, somnolence, ruffled feathers, drooped wings, anorexia and prostration, yellowish or whitish diarrhoea (Bishu *et al*., 1977; Islam *et al.*, 2001). Gross and microscopic lesions associated with the disease include haemorhages in the proventriculus, duodenum, jejunum, ileum, liver, wings, leg and thigh muscles and caecal tonsil. There is also enlargement of the bursa, spleen and kidney. Urates are also found in the ureters (Abdu and George, 1986; Islam *et al.*, 2001). In chicken flocks, the clinical picture and the course of the disease usually are indicative of an IBDV infection (Eterradossi *et al.,* 1998). Pathological changes observed in the bursa of Fabricious are characteristic, and histopathological investigations combined with the demonstration of viral antigens by immuno-histochemistry confirm an IBDV infection (Eterradossi *et al.,* 1998; Islam *et al.*, 2001).

In veterinary medicine, blood examination is performed for several reasons, such as screening procedure to assess general health of animals and for disease follow-up (Oladele, 2009). Besides, hematological values are important for clinico-pathological diagnosis of several disease conditions, such as traumatic injury, parasitism, organic disease, bacterial septicaemia and nutritional deficiencies (Ifon and Basir, 1980; Campbell, 1995; Oladele, 2009). However, in veterinary medicine blood analyses have been performed much less often in avian medicine in comparison to their routine uses in large animal practice (Oladele, 2009).

Packed cell volume (PCV), haemoglobin concentration (Hb) and total protein (Tp) have been shown to be important indices of livestock health and production status (Oyewale and Fajimi, 1988; Oladele and Samuel, 2014). The PCV on its own, is a reliable indirect index of Hb and of the number of circulating erythrocytes (Benjamin, 1985).

It is known that values of some biochemical parameters in chickens are affected by diseases. Also, high degrees of correlation between serum concentration of biochemical parameters and humoral immunity against some poultry diseases have been reported (Talebi, 2006). Changes in plasma TP, and especially of plasma globulin may reflect the severity of disease processes in birds, and serve as basis of prognosis (Hawkey *et al.,* 1983, Oladele *et al*., 2005b). Serum biochemical parameters may also provide valuable information for differential diagnosis of nutritional disorders (Quintavalla *et al*., 2001; Oladele and Audu, 2010), anti-toxic effects of probiotic (Aqawane and Lonkar, 2004) and many poultry diseases (Panigrahy *et al*., 1986; Kral and Suchy, 2000; Koinarski *et al*., 2001; Kumar *et al*., 2003).

Biochemical values of chicken's serum may be influenced by several factors, such as feed additives (Oguz *et al*., 2000; Cetin *et al*., 2002), dietary nutrients (Odunsi *et al.*, 1999; Eroksuz *et al*., 2001; Al-Homidan *et al*., 2002; Kurtoglu *et al*., 2005), drugs (Zaman *et al*., 1995), housing systems (Gunes *et al*., 2002), environmental temperatures (Vecerek *et al*., 2002), and water restrictions (Iheukwumere and Herbert, 2003).

Management of abnormalities in birds requires an understanding of how diseases change the biochemical function and electrolyte of the bodies. Because the clinical signs of illness in birds

are frequently subtle, clinical chemistry is necessary to evaluate cellular changes (Ritchie *et al*., 1994). Serum biochemical profile is known to complement the haemogramme. It allows the assessment of tissue functions, glucose metabolism, and plasma ion concentration that permits the understanding of the effect of management and disease of animal (Polat *et al*., 2003).

1.2 Statement of Research Problems

Infectious bursal disease is a major problem in poultry production throughout the world; affected chickens have reduced antibody response to vaccination, strong post-vaccination reactions and increased susceptibility to concurrent or secondary infections (Gary and Richard, 2003). There is the emergence of "very virulent" (vv) strains of IBDV, which can cause up to 70% flock mortality in laying pullets (Chettle *et al*., 1989; van den Berg *et al.,* 2000; Ikuta *et al*., 2001).

This virus causes an acute, highly contagious and immuno-suppressive disease in chickens (Lukert and Saif, 2003). However, vvIBDV can establish infection in the face of levels of maternally derived antibodies that were previously protective against "classical" strains. Meanwhile, vvIBDV infections also have been observed in Africa, Asia and, South America (Ikuta *et al*., 2001). In Nigeria, IBD occurs throughout the year with high incidence from April to July and from November to December. Mortality due to IBD was observed to be highest during the dry season in Zaria (Abdu *et al.,* 1992; Mbuko *et al*., 2010).

Blood analyses have been performed much less often in avian medicine in comparison to their routine uses in large animal practice (Oladele, 2009), consequently vital information that could aid diagnosis from blood parameters are not routinely determined.

1.3 Justification of the Research

Poultry is an essential component of the Nigerian economy and a good source of high quality protein for the ever growing population of Nigeria. In livestock production, poultry occupies a prominent position of animal protein and this accounts for about 25% of local meat production in Nigeria (Ekeren *et al*., 1995).

Serum biochemical profile is known to complement the haemogramme. It allows for the assessment of tissue functions, glucose metabolism, and plasma ion concentration that permit the understanding of the effect of management and diseases of animals (Polat *et al.*, 2003). It has been established that in the avian species, knowledge of blood parameters is of value in diagnosis of certain diseases (Onyeyili *et al.,* 1991, Oladele *et al.,* 2005b). It is also speculated that changes in normal values of blood parameters are pointers to various disease conditions even at subclinical level in birds (Christie and Halliday, 1979; Oladele and Ayo, 1999; Oladele and Samuel, 2014).

As IBD is one of the most prevalent diseases in commercial poultry that causes serious economic losses in Nigeria, effort should not be spared in combating the disease in every way possible (Ojo *et al*., 1973), as well as exploring knowledge of diagnosing clinical and subclinical cases of the disease.

Since avian medicine is undergoing a new turn in the use of haematological and biochemical indices as tools for diagnosing avian diseases (Oladele, 2009), this study was designed to evaluate the effect of IBDV and vaccine on pathological, haematological and serum

biochemical parameters of infected birds which may provide further insights into the diagnosis of IBD.

1.4 Aim of the Study

1. The aim of the study was to evaluate the effects of infection with infectious bursal disease virus and vaccine on pathological, haematological and serum biochemical parameters of cockerels reared in Zaria, Nigeria.

1.5 Objectives of the Study

1. To evaluate changes in haematological parameters following experimentally infection of cockerels with vvIBDV or vaccinated against IBDV.
2. To evaluate some serum biochemical changes in cockerels vaccinated against IBDV or experimentally infected with vvIBDV.
3. To determined the IBD ELISA titres antibody in cockerels before vaccination or challenged and after vaccination or challenged.
4. To determine the gross pathological and histopathological changes in cockerels, vaccinated against IBD or experimentally infected with vvIBDV.

1.6 Hypotheses

1.6.1 Null hypotheses

1. Infectious bursal disease virus infection or vaccine does not affect haematological parameters of cockerels in Zaria.

2. Infectious bursal disease virus infection or vaccine does not affect serum biochemical parameters of cockerels in Zaria.

1.6.2 Alternate hypotheses

1. Infectious bursal disease virus infection or vaccine affects haematological parameters of cockerels in Zaria.
2. Infectious bursal disease virus infection or vaccine affects serum biochemical parameters of cockerels in Zaria.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of Infectious Bursal Disease in Nigeria

Infectious bursal disease was first observed in 1969 in Western and Midwestern States of Nigeria, and later reported by Ojo in 1973 (Okoye and Uzoukwu, 2001), eleven years after its initial appearance in Gumboro, Delaware, USA (Cosgrove, 1962). In 1975, Onunkwo further confirmed the presence of IBD at Vom, Plateau State of Nigeria. The first diagnosed outbreak of IBD occurred in Zaria in October 1972 (Ojo *et al*., 1973; Okoye and Uzoukwu, 2001). Previously, however a disease with similar clinical signs and postmortem lesions had occurred at the poultry farm of the School of Agriculture, Ahmadu Bello University (ABU), Zaria, Nigeria in 1970 (Agbede, 1975). In the south of the country, there was a reported of about 140 outbreaks of disease involving 105 million chickens annually (Nawathe and Lamorde, 1982). In 1977, it was included in the list of diseases to be compulsorily reported annually in Nigeria (Onukwo and Momoh, 1981).

2.2 Aetiology of Infectious Bursal Disease

The aetiologic agent is a bisegmented (segments A and B) double-stranded RNA avibirnavirus. Classical serotype 1 infectious bursal disease virus strains have been of great concern to the poultry industry for a long time, while all known serotype 2 IBDV strains are avirulent for chickens (Ismail *et al.,* 1988). In the late 1980s, classical serotype 1 IBDV evolved into variant forms, in the USA (Synder, 1990), or very virulent (vvIBDV) forms in Europe, Asia and other major parts of the world (van den Berg, 2000). However, all three major subtypes of IBDV are commonly found in a single country (Kim *et al.,* 2010). Segment A of the IBDV genome

encodes a polyprotein from its larger open reading frame, which is processed into two structural proteins (VP2, VP3) and a viral protease (VP4) (Hudson *et al.,* 1986). The smaller partially overlapping segment A open reading frame encodes a non-structural protein (VP5) (Mundt *et al.,* 1995). The IBDV segment B encodes VP1, which has RNA-dependent RNA polymerase activity (Spies *et al.,* 1987). The antigenic site responsible for the induction of neutralizing antibodies against IBDV is located on VP2 structural protein (Azad *et al.,* 1987). The genetic basis for the antigenicity and/or virulence of the VP2 protein of IBDV has been studied extensively (Schnitzler *et al.,* 1993; Brandt *et al*., 2001; van den Berg *et al.,* 2004; Letzel *et al.,* 2007; Durairaj *et al*., 2011).

2.3 Transmission of Infectious Bursal Disease

Only horizontal transmission has been described, with healthy chicken being infected by the oral or respiratory pathways. Infected chickenss 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 possibility of persistent infection in recovered birds has not been researched. The disease is transmitted by direct contact with excreting chickens, 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, 1967), and up to fifty-six days in lesser mealworms *(Alphitobius* spp.) taken from a contaminated building (McAllister *et al.,* 1995). In the absence of effective cleaning, disinfection

and insect control, the resistance of the virus leads to perennial contamination of infected farm buildings.

2.4 Pathogenesis of Infectious Bursal Disease

The selected host of the virus is young chickens where a clinical disease occurs, while in older birds the infection is essentially subclinical. Susceptibility of different breeds has been described with higher mortality rates in light than in heavier breeds (Bumstead *et al.*, 1993; Nielsen *et al.*, 1998). Inoculation of IBDV in other avian species fails to induce disease (McFerran, 1993). The target organ of IBDV is the bursa of Fabricius at its maximum development, which is a specific source for B lymphocytes in avian species. Bursectomy can prevent illness in chicks infected with virulent virus (Hiraga *et al.*, 1994). The severity of the disease is directly related to the number of susceptible cells present in the bursa of Fabricious; therefore, the highest age of susceptibility is between 3 and 6 weeks, when the bursa of Fabricius is at its maximum development. This age susceptibility is broader in the case of vvIBDV strains (van den Berg *et al.*, 1991; Nunoya *et al.*, 1992). After oral infection or inhalation, the virus replicates primarily in the lymphocytes and macrophages of the gut-associated tissues. Then virus travels to the bursa via the blood stream, where replication will occur. By 13 h post inoculation (pi), most follicles are positive for virus and by 16 h pi, a second and pronounced viraemia occurs with secondary replication in other organs leading to disease and death (Müller *et al.*, 1979). Similar kinetics is observed for vvIBDVs but replication at each step is amplified. Actively dividing, surface immunoglobulin M-bearing B cells are lysed by the virus (Hirai and Calnek, 1979; Hirai *et al.*, 1981; Rodenberg *et al.*, 1994), but cells of the monocyte–macrophage lineage can be infected in a persistent and productive manner, and play a crucial role in dissemination of the virus

(Burkhardt and Müller, 1987; Inoue *et al.*, 1992; van den Berg *et al.*, 1994) and in the onset of the disease (Sharma and Lee, 1983; Kim *et al.*, 1998; Lam, 1998). The exact cause of clinical disease and death is still unclear but does not seem to be related only to the severity of the lesions and the bursal damage. Indeed, after infection, some birds with few bursal lesions can be found dead, while others can survive despite extensive bursal damage. Moreover, mortality rates are often variable and the establishment of median lethal dose for standardization has always been hazardous. In addition, the narrow age range for susceptibility to clinical disease has not yet been clearly explained. Depletion of lymphoid cells in the bursa of Fabricius after IBDV infection is due to both necrosis and apoptosis. Apoptosis, or programmed cell death, is a process where, in response to specific stimuli, cells die in a controlled, programmed manner. Many different cell species can undergo apoptosis but immature B and T cells are particularly susceptible to apoptotic cell death (Sharma and Lee, 1983; Kim *et al.*, 1998; Lam, 1998).

2.5 Epidemiology of Infectious Bursal Disease

Infectious bursal disease occurs throughout the year (Anon, 1977) with a high incidence from April to July and November to December (Abdu, 1987; Tong *et el.,* 1993). However, it is most common in April and July in Zaria, Nigeria (Abdu *et al.,* 1985). Mortality due to IBD was observed to be highest during the dry season (Abdu *et al.,* 1992). The two periods coincide with the times of peak production due to anticipated increased demand for poultry meat during the religious festival of Eid-El-Fitr, Eid- El-Kabir, Easter and Christmas celebrations (Abdu *et al.,* 1985).

The age of maximum susceptibility is between three and six weeks, corresponding to the period of maximum bursal 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). Light strains of laying stock are more susceptible to disease than the heavy broiler strains (Bumstead *et al*., 1993). The virus is sensitive to sodium hydroxide (it is totally inactivated when pH exceeds 12), but it is not affected at pH 2 (Benton *et al*., 1967). The iodinated and chlorinated derivatives, as well as the aldehydes (formaldehyde, glutaraldehyde) are also active (Ley *et al*., 1979). 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 animals and poultry meat. Infectious bursal disease is an OIE List B disease (OIE, 1999)

2.6 Mortality and Morbidity 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. Until 1987, the field strains isolated were of low virulence and caused only 1 to 2% specific mortality. However, since 1987 an increase in specific mortality has been described in different parts of the world. In the USA, new strains responsible for up to 5% of specific mortality were described (Rosenberger and Cloud, 1986). At the same time, in Europe and subsequently in Japan, high mortality rates of 0 to 60% in laying hens and 25 to 30% in broilers were observed. These hypervirulent field

strains caused up to 100% mortality in specific-pathogen-free (SPF) chickens (van den Berg *et al*., 1991; Nunoya *et al*., 1992).

The mortality and morbidity rates due to IBD could be up to 100 % (average 20 %) and 75% (average 15%), respectively (Abdu *et al*., 2001). In Zaria, an average of morbidity rate due to IBD was 19.9 % (Abdu, 1986). Infectious bursal disease mortality range between 5% and 57% (Anon, 1977). Infectious bursal disease average mortality rates reported were 7.3% (Abdu, 1986), 12.5% (Ojo *et al*., 1973) and 14.7% (Bishu *et al*., 1977). It has been observed that difference in age, breed, type, management practice, climatic condition, concurrent infection and degree of virulence of the infecting IBDV may be responsible for the variability observed in the morbidity and mortality rates (Abdu, 1986).

2.7 Breed Susceptibility of Infectious Bursal Disease

Infectious bursal disease has been reported in chickens as young as 9 days to 2 weeks and as old as 20 to 24 weeks of age (Durojaiye *et al.,* 1984; Abdu and saidu, 1995). The most susceptible age range was found to be 3-11 weeks. Incidence of IBD begins to rise when birds are 3 or 4 weeks and peaks when they are about 5 to 8 weeks. Infectious bursal disease has been reported in all types of chicken, layers, broilers, cockerels (Okoye and Uzuokwo, 1982), breeders (Abdu *et al*., 1986) and different strains of exotic chicken, white Leghorn (Ojo *et al*., 1973) and Star cross (Okoye and Uzuokwo, 1981). Cockerels, broilers and layers are found to be more susceptible compared to breeders and local chicken. Other susceptibility factors include growth rate, level of veterinary care and type of management (Abdul *et al*., 2001).

2.8 Clinical Signs and forms of Infectious Bursal Disease

Clinical signs of IBD in chicken are sudden onset of sickness including trembling, weakness, depression, somnolence, ruffled feathers, drooped wings, anorexia, prostration and yellow or whitish diarrhoea (Bishu *et al*., 1977; Okoye and Uzoukwu, 2001) and reluctance to move (Onukwo, 1975; Okoye and Uzoukwu, 2001). The clinical signs of IBD vary considerably from one farm, region, country or even continent to another. Schematically, the global situation can be divided into three principal clinical forms, as follows:

2.8.1 The classical form of infectious bursal disease

As described since the early 1960s, is caused by the classical virulent strains of IBDV. Specific mortality is relatively low (20%), and the disease is most often subclinical, occurring after a decline in the level of passive antibodies (Faragher, 1972)

2.8.2 The immunosuppressive form of infectious bursal disease

Principally described in the USA, is caused by low-pathogenic strains of IBDV, as well as by variant strains, such as the Delaware variant E which partially resist neutralisation by antibodies against the so-called 'classical' viruses (Jackwood and Saif, 1987; Snyder, 1990)

2.8.3 The acute form of infectious bursal disease

This form of disease, first described in Europe and then in Asia, is caused by 'hypervirulent' strains of IBDV, and is characterised by an acute progressive clinical disease, leading to high mortality rates (50-70%) on affected farms (Chettle *et al.,* 1989; Stuart, 1989; van den Berg *et al.,* 1991).

2.9 Gross Lesions of Infectious Bursal Disease

Birds infected with IBDV show enlargement, congestion, necrosis (Abdu, 1986), haemorhages and oedema (Ojo *et al.,* 1973, Bishuet *et al*., 1977) of bursa of Fabricius. Kidneys of the infected birds become congested and enlarged with prominent tubules (Okayed and Uzuokwo, 1982). The spleen becomes enlarged, haemorhagic and necrotic (Onukwo, 1975). Haemorhages are also observed at the junction between the proventriculus and gizzard, caecal tonsil and duodenum (Onukwo, 1975). The liver of the infected chicken is usually enlarged (Ojo *et al.*, 1973). There is congestion of the thigh and breast muscles (Bishu *et al*., 1977), and haemorhages in the muscles of the leg, thigh, breast, wing, neck and back (Abdu *et al.,* 1986). The heart in affected birds show hydropericardium (Abdu *et al.,* 1986).

2.10 Microscopic Lesions of Infectious Bursal Disease

The microscopic lesions reported were intra and inter folicular oedema, necrosis of lymphocytes, interfollicular fibroplasias, heterophilic infiltration (Okoye and Uzuokwu, 1981; Okoye and Uzuokwu, 2001) and reticular hyperplasia (Okoye, 1982; Okoye and Uzuokwu, 2001) of the bursa of Fabricius. Kidney shows necrosis of tubules, congestion, mononuclear cell infiltration and vascular degeneration (Okoye, 1982; Okoye and Uzuokwu, 2001). The spleen, thymus and, caecal tonsil show necrosis and depletion of lymphocytes (Okoye, 1982; Okoye and Uzuokwu, 2001). The proventriculus shows congestion, desquamation and necrosis (Okoye 1982; Okoye and Uzuokwu, 2001). There is necrosis of hepatocytes (Okoye, 1982; Okoye and Uzuokwu, 2001). The muscle of the thigh and breast shows congestion oedema, haemorhages and necrosis (Okoye, 1982; Okoye and Uzuokwu, 2001). Infected thymus, spleen, and caecal tonsils become normal one week after infection (Okoye, 1982; Okoye and Uzuokwu, 2001) and takes two

(Abdu, 1988) to six (Okoye, 1982; Okoye and Uzuokwu, 2001) weeks for bursa of Fabricius, kidney, spleen and skeletal muscles (Abdu *et al*., 1986; Okoye and Uzuokwu, 2001).

2.11 Infectious Bursal Disease Antibodies

Infectious bursal disease antibodies (IBDAbs) in breeders and maternal antibodies (MA) in chicks are prevalent and their concentration depends on the source and age of birds and the type of vaccine (Live or Attenuated) used (Okoye and Uzuokwu, 2001). It has been reported that chicks that are 3 or 4 weeks old are invariably devoid of MA. The presence of active immunity prevents antibody production or delays its rate of production and consequently the level produced (Okoye and Uzuokwu, 2001). Chicks should be vaccinated before the age of 6 weeks, preferably 3 and 4 weeks of age (Abdu *et al*., 2001).

Maternal antibody does interfare with vaccination preventing active antibody production (Abdu *et al.,* 2001). All vaccinated birds usually sero-convert at one (Okeke *et al*., 1982) two (Abdu, 1988) or three (Aba- Adulugba *et al*., 1992) weeks after vaccination. The level of antibodies produced depend on type of IBD vaccine administered (Okeke *et al*., 1982; Aba- Adulugba *et al*., 1992; Okoye and Uzuokwu, 2001) and ranged between 5 and 6 $\log_2$ and the highest antibody level recorded for vaccinated birds was 10.0 $\log_2$ (Aba- Adulugba *et al*., 1992; Abdu *et al.,* 2001). A survey of antibody in vaccinated birds showed that 75 to 100% of chicks that are one, two, three or four weeks old remained sero negative despite vaccination but by the third week of age or older 53 to 100% of vaccinated bird had sero converted (Abdu, 1990). Result so far suggested that the outcome of vaccination depend on the type of vaccine administered, how the

vaccine was handled and administered and the presence or absence of MA at time of vaccination. Antibodies following IBDV challenge were detected 7 days post infection in 100% of susceptible chicks aged 22-29 days and the level of antibodies was highest in older birds (Abdu, 1987).

2.12 Diagnosis of Acute Infectious Bursal Disease

2.12.1 Base on clinical signs and lesions of the disease

Hypervirulent IBDV infections are characterised by severe clinical signs and high mortality. The vvIBDVs produce disease signs similar to conventional type 1 infection, with the same incubation period (4 days), but the acute phase is exacerbated and more generalized in the affected flock. Severe outbreaks are characterized by sudden onset of depression in susceptible flocks. Birds in the acute phase of the disease are prostrate and reluctant to move, with ruffled feathers and frequently watery or white diarrhoea. The age susceptibility is extended, covering the entire growing period in broilers, and the peaks of mortality show a sharp death curve followed by rapid recovery (Chettle *et al.*, 1989; van den Berg *et al.*, 1991; Nunoya *et al.*, 1992; Tsukamoto *et al.*, 1992). On post mortem examination of birds that died during the acute phase of vvIBD, the bursa of Fabricius is the principal diagnostic organ: it is turgid, oedematous, sometimes haemorrhagic and turns atrophic within 7 to 10 days. This atrophy might be more rapid, even 3 to 4 days after inoculation (Tsukamoto *et al.*, 1992). In addition, dehydration and nephrosis with swollen kidneys are common, and ecchymotic haemorrhages in the muscle and the mucosa of the proventriculus are observed in the majority of the affected birds. Severe depletion of lymphoid cells is observed not only in the bursa of Fabricius, but also in the non-bursal lymphoid tissues. Pathogenicity of IBDV has been associated with virus distribution in

non-bursal lymphopoietic and haematopoietic organs. Using various immunostaining methods, a higher frequency of antigen-positive cells could be demonstrated after infection of birds with vvIBDV compared with other strains, in the thymus (Nunoya *et al.*, 1992; Sharma *et al.*, 1993; Inoue *et al.*, 1999), the spleen and the bone marrow (Tanimura *et al.*, 1995; Tsukamoto *et al.*, 1995; Inoue *et al*, 1999). In particular, atrophy of the thymus has been associated with the acute phase of the disease and might be indicative of the virulence of the isolate, although it is not associated with extensive viral replication in thymic cells (Sharma *et al.*, 1993). An increased number of macrophages are found in various organs (Tanimura *et al.*, 1995). Thrombocytes also represent a target for IBDV, and acute disease is characterized by disseminated haemorrhages probably related to an impairment of the clotting mechanism (Skeeles *et al.*, 1980).

2.12.2 Isolation of infectious bursal disease virus

A filtered homogenate of the bursa of Fabricius is inoculated in nine to eleven days old embryonated eggs originating from hens free of anti-IBDV antibodies. The most sensitive route of inoculation is the chorio-allantoic; the yolk sac route is also practicable, and the intra-allantoic route is the least sensitive. The specificity of the lesions observed must be demonstrated by neutralising the effect of the virus with a monospecific anti-IBDV serum. Isolation in embryonated eggs does not require adaptation of the virus by serial passages, and is suitable for vvIBDVs. In the absence of lesions, the embryo from the first passage should be homogenised in sterile conditions and clarified, and two additional serial passages should be performed (Hitchner, 1970).

2.12.3 Detection of viral antigens

2.12.3.1 In thin sections of the bursa of Fabricious

The viral antigens specific to IBDV may be detected by direct and indirect immunofluorescence (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 from the tenth day. However, the virus can be isolated from bursae sampled from the second to the tenth day, with a maximum infectious titre after four days (Vindevogel *et al.,* 1976). The use of monoclonal antibodies for detection of the virus enhances the specificity of the test (Cho *et al.,* 1987).

2.12.3.2 In suspensions of the bursa of Fabricius

The agar gel immunodiffusion (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.,* 1976). 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 *et al.,* 1995). 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 (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 characterisation of the captured viruses.

Different batteries of monoclonal antibodies enable a tentative identification of the variants from the USA (Snyder *et al*., 1988) vvIBDV (Eterradossi *et al.,* 1997).

2.13 Serology of Infectious Bursal Disease Virus

In areas contaminated by IBDV, most broiler flocks have anti-IBDV antibodies when leaving the farm. 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 interest in endemic zones. Nonetheless, the quantification of IBDV induced antibodies is important for the medical prophylaxis of the disease in young birds, in order to measure the titre of passive antibodies and determine the appropriate date for vaccination (Muskett, 1979) or in laying hens to verify success of vaccination (Lucio, 1987). Serology is likewise essential to confirm the disease-free status of specific pathogen free (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 agar gel immunodiffusion (AGID) (Hirai *et al*., 1997), enzyme-linked immunosorbent assay (ELISA) (Marquardt *et al*., 1980), and serum neutralization (SN) in cell culture (Weisman and Hitchner, 1978). Agar gel immunodiffusion is the simplest, but least sensitive technique. Results are obtained after an incubation period of 48 hours. Variability in results may be due to the investigator, as well as the nature of the viral strain used as an antigen (Becht *et al.*, 1988). Serum neutralization presents the disadvantages that specialised 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 (Rooney and Freund, 1988).

The ELISA is the most rapid and sensitive method, and presents the fewest variations due to the viral strain used as an antigen (Rooney and Freund, 1988). Considerable inter and intra-laboratory variability can occur with certain commercial kits. Although the correlation between results obtained using SN and ELISA is high, ELISA remains less sensitive, and does not detect low neutralising 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).

2.14 Differential Diagnoses of Infectious Bursal Disease

The clinical diagnosis of the acute forms of IBD is based on disease evolution (a mortality peak followed by recovery in five to seven days), and relies on the observation of the symptoms and post-mortem examination of the pathognomonic lesions, in particular of the bursa of Fabricius. The conditions most liable to be clinically mistaken for IBD are Newcastle disease in some visceral forms, stunting syndrome, chicken infectious anaemia, mycotoxicoses and nephropathogenic forms of infectious bronchitis. In all acute cases, the presence of bursal 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).

2.15 Prevention and Control of Infectious Bursal Disease

Due to the high resistance of IBDV to environmental exposure and its wide distribution, hygienic measures alone, while essential, are often insufficient. Vaccination is thus essential (Lütticken, 1997). The economic impact of both clinical and sub-clinical diseases warrants the search for

and the use of efficient vaccines. While the protective role of cellular immunity cannot be ruled out, good protection is achieved by the induction of neutralizing antibodies, as proven by the excellent passive protection of young chicks against infection. This satisfactory protection can be achieved by immunization with live or inactivated vaccines (Lütticken, 1997). Classical live vaccines achieve lifelong and broad protection, but possess residual pathogenicity and a proportional risk of reversion to virulence. Inactivated vaccines, although costly, were used successfully until the emergence of the hypervirulent strains (Box, 1989). It was a normal practice in broiler production to vaccinate hens with an oil-emulsion vaccine just before laying in order to induce a high level of passive immunity in the offspring, which could protect them until an age where infection is less detrimental with regards to immunosuppression (Box, 1989). This procedure was satisfactory until the emergence of vvIBDV when all classical prophylactic measures were called into question. The first cases occurred in flocks where all hygienic measures and vaccinations had been properly applied (Lambrecht *et al.*, 2000). It was no longer possible to protect broilers passively during the whole growing period and a live vaccination became necessary. But the interference of maternally derived antibody (MDA) became the crucial problem in establishment of the vaccination schedule (Box, 1989).

Serological monitoring is usually necessary to determine the optimal timing for vaccination (van den Berg and Meulemans, 1991; Kouwenhoven and van den Bos, 1994). In this context, the development of tests allowing the differentiation between passive and active immunity could be of considerable help (Lambrecht *et al.*, 2000). Inactivated vaccines might prove helpful if they can induce higher antibody levels in breeders, which will then be passively transmitted to the offspring and protect them during their entire growing period. Subunit IBDV proteins expressed

in yeast (Macreadie *et al.*, 1990) or via the baculovirus system (Vakharia *et al.*, 1994; van den Berg *et al.*, 1994; Pitcovski *et al.*, 1998; Dybing and Jackwood, 1998; Yehuda *et al.*, 2000) might help to reach this goal. Another advantage of these technologies is that a vaccine based on VP2 alone should allow monitoring of the field situation by the discrimination between vaccinal (anti-VP2 only) and infectious antibody (anti-VP2 and VP3). There is no evidence of antigenic variation in the very virulent strains as described in the US: they belong to classical serotype 1 (van der Marel *et al.*, 1990; van den Berg *et al.*, 1991; Eterradossi *et al.*, 1992) and, therefore, they can be controlled adequately under experimental conditions by vaccination with commercial vaccines prepared from classical attenuated strains (Oppling *et al.*, 1991; van den Berg and Meulemans, 1991; Eterradossi *et al.*, 1992). Live vaccines have another advantage in that they are excreted in the environment, where they can compete with field viruses. Unfortunately, most intermediate vaccines are inadequate for interfering with vvIBDVs that could break through higher MDA levels. However, the use of less attenuated ('hot') vaccines, even with an acceptable reduction of mortality, is dangerous as these vaccines induce immunosuppression and carry the risk of reversion to virulence (Guittet *et al.*, 1992).

2.16 Haematological Parameters

It has been established that in the avian species, knowledge of blood parameters is used in the diagnoses of certain disease, especially those involved in the destruction of blood cells (Onyeyili *et al.,* 1991; Julian, 1999). Haematological parameters could be of value in developing efficient comprehensive flock health programme for prophylaxis and therapy of diseases, especially those involving cardiovascular, nutritional, parasitic, haemolytic and haemorhagic disorders (Oladele and Samuel, 2014)

2.16.1 Erythropoiesis

In the birds, erythropoiesis occurs in the yolk sac, and to lesser extent, in the bone marrow. After hatching in healthy birds, only the bone marrow is erythropoietic (Hawkey and Dennett, 1989). In adult healthy birds, erythropoiesis goes on continuously in the bone marrow, and corpuscles are released into the blood stream to replace the erythrocytes that are being destroyed (Reece and Swenson, 2005). Erythropoiesis in birds is stimulated by bleeding and environmental hypoxia and is suppressed by induce polycythaemia indicating that the physiologic response is similar to that in mammals (Prehale and Pastore, 2004). The importance of the erythropoietin in stimulating erythropoiesis in mammals is well established. In domestic fowls and Japanese quails, for example, the role of erythropoietin in the stimulation of erythropoiesis is inactive in mammals and vice versa. Since erythropoiesis in ducks is stimulated by cobalt, it is assumed that the kidney is also the source of erythropoietin in domestic fowls as in the mammal (Campbell and Coles, 1986). Haematological values are partly hereditary, and therefore, vary between and within species and breeds of birds, and mammals. They also depend on the nutritional status and prevailing environmental condition (Oladele and Ayo 1999). The influence of climatic environment on haematological parameters of poultry in most countries of the world has not been given much attention (Oladele *et al.,* 2005c).

2.16.2 Erythrocytes

Avian erythrocytes unlike those of non-mammalian animals are nucleated and contain mitochondria. The erythrocyte of birds appears elliptical in outline and has oval nucleus. The dimension of mature erythrocyte does not show any significant change during the life of the bird. The erythrocyte of mature male is larger than that of the female (Hayhoe and Flemans, 1990).

Erythrocyte life span varies among species of birds just as it does among mammals. The erythrocyte life span for chicken is 28-35 days, 42 days for ducks, 35-45 days for pigeon and 33-35 days for quails (Sturkie, 1976). The short life span in comparison to mammals is due partly to high body temperature and metabolic rate of the bird. It is not uncommon to find immature erythrocytes in the circulating blood and, therefore, the recognition of normal and abnormal erythrocyte precursors is of diagnostic importance (Campbell and Coles, 1986; Hawkey and Dennett, 1989). Many factors influence the erythrocyte count in birds. The count may vary among individual birds of the same species as well as among birds of different species. The number of erythrocyte varies with age, sex, hormonal influence, hypoxia and prevailing environmental conditions (Campbell and Coles, 1986).

2.16.3 Packed Cell Volume (PCV) and Haemoglobin Concentration (Hb)

The value of PCV is a good indicator of an animal's haemogramme. This is because it is a good index of the number of circulating erythrocyte (Benjamin, 1985). The PCV determination has been regarded as the quickest and most practical method establishing the status of erythrocytes (Oladele *et al*., 2000). Most caged birds have PCV of 35-55% (Campbell and Coles, 1986). In Chicken PCV, Hb concentration and erythrocyte count less than 30%, 8g/dl and 2x 10^9, respectively indicate anaemia, while PCV greater than 55% suggest dehydration. Haemoconcentration is also associated with hypothermia. Birds respond to hypoxia by increasing PCV and total erythrocyte count. In acute response to hypoxia, PCV of 60 to 80 % can be observed (Campbell and Coles, 1986) as a result of increased erythropoietic response to hypoxaemic condition (Scheele *et al*., 2003). Result of investigation demonstrated that the male had higher values of PCV and Hb than the female. The differences were attributed to the level of

sex hormonrs. In many avian species, androgen was found to increase the level of PCV and Hb in males (Scheele *et al*., 2003).

2.16.4 Morphology and function of avian white blood cell

The morphologic features of leucocyte of Red jungle are similar to those of other species of chicken (Aengwanich and Tanomtong, 2007). The white blood cell of poultry is influenced by age, sex, environmental temperature, nutritional status and degree of stress (Scheele *et al*., 2003).

2.16.5 Basophils (BAS)

Basophils tend to be round cells, the cytoplasmic granules often hide nucleus, the cytoplasmic granule has a slightly basophilic stain using Giemsa - Wright's stain method and they are about the same size as the heterophils (Aengwanich and Tanomtong, 2007). In some species of birds, basophils are more numerous than the eosinophils (Lucas and Jamroz, 1961). The number of basophils present in blood is small. A slightly greater number may be found in the blood of young chicken than in the blood of adult chicken (Scheele *et al*., 2003). The function of basophils and their counterparts, the mast cell are not well known (Campbell and Coles, I986).

2.16.6 Thrombocytes

Thrombocytes are oval with more nearly round nucleus in the center of nearly clear cytoplasm and they are the smallest cell seen in blood of fowl (Gentry, 2005). It is believed that the thrombocytes play important part in blood coagulation. They are slightly more numerous in the female than male and more numerous in young than adult chicken (Campbell and Coles, 1986; Aengwanich and Tanomtong, 2007).

2.16.8 Eosinophils (EOS)

Eosinophils are round in shape, with abundant amount of blue gray cytoplasm containing numerous large rounds to spherical granules. The functions of eosinophils are not well understood. It is suspected that they act as detoxifying agents. In some animals and birds they are increased in verminous infestation and are found in the tissue in certain allergic states (Hayhoe and Flemans, 1990; Aengwanich and Tanomtong, 2007).

2.16.9 Monocytes (MON)

Monocytes are the largest leukocytes found in the peripheral blood. The nuclear lobes vary from round to bilobed and cytoplasm colored blue gray which is larger than the lymphocytes and heterophils (Aengwanich and Tanomtong, 2007). Monocyte developmental sequence includes the monoblast, early promonocyte, late promonocyte, and mature monocyte (Hawkey and Dennett, 1989). They may then engulf and digest not only bacteria, but also particulate matters, such as cellular debris. It is believed that monocyte is numerous in the blood of adult male chicken than in adult female chicken (Woerpel and Rosskopg, 1984). They are more in chicken kept in outdoor environment than those confined indoors (Campbell and Coles, 1986).

2.16.10 Heterophils (HET)

Heterophil is the avian equivalent of mammalian neutrophil, is a single round to irregular cell with spindle shape cytoplasmic granules. Mature heterophils have a lobed nucleus (Aengwanich and Tanomtong, 2007). Their characteristic feature is the presence of many acidophilic crystalline granules in a clear colorless cytoplasm. They are known to be highly phagocytic and respond well to various chemotactic stimuli (Olson, 1997).

Immature heterophils are rarely seen in the blood of birds and if seen represent a poor prognosis (Woerpel and Rosskopg, 1984). Heterophil can exhibit toxic granulation with partial or total degranulation (Scheele *et al*., 2003).

2.16.11 Lymphocytes (LYMP)

Lymphocytes are typically round cells seen in a blood film. The nucleus is usually rounded and centrally located (Aengwanich and Tanomtong, 2007). They are believed to be most frequently occurring leucocyte in some birds. The lymphocyte series begins with the lymphoblast followed by the prolymphocyte. The prolymphocyte transform to mature lymphocyte in peripheral blood, is small to medium size, while the lymphoblasts are more likely immature and are infrequent in healthy birds (Gentry, 2005).

2.17 Serum Biochemical Parameters

2.17.1 Cholesterol (CHOL) and triglyceride (TRG)

Cholesterol levels in birds may accompany various physiological or pathological conditions, but there is inconsistency and a lack of specificity associated with abnormal findings (Lumeij, 1990). Generally, elevations are associated with liver disease, hypothyroidism, high fat diets and starvation, especially in obese birds. Subnormal levels may be observed with endotoxaemia, aflatoxicosis, spirochaetosis and low dietary fat. Again, normal values do not rule out the aforementioned conditions (Lewandowski *et al*., 1986; Harris, 2009).

Cholesterol, triglycerides, and high-density lipoproteins are important constituents of the lipid fraction of the body. Cholesterol is an unsaturated alcohol of the steroid family of compounds; it is essential for the normal function of all animal cells and is a fundamental element of their cell membranes (Lumeij, 1990). It is also a precursor of various critical substances such as adrenal and gonadal steroid hormones and bile acids (Lewandowski *et al*., 1986).

Triglycerides are fatty acid esters of glycerol and represent the main lipid component of dietary fat and fat depots of animals (Walker *et al*., 1991). Cholesterol and triglycerides being nonpolar lipid substances (insoluble in water), need to be transported in the plasma associated with various lipoprotein particles. Plasma lipoproteins are separated by hydrated density; electrophoretic mobility; size; and their relative content of cholesterol, triglycerides, and protein into five major classes: chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) (Lumeij, 1990; Walker *et al*., 1991). The fact that all the cholesterol required by the body can be produced by biosynthesis points to the essential nature of this substance. As an estimated loss of cholesterol occurs daily through desquamation and fecal loss, this amount must be replaced. Usually this replacement is obtained from dietary sources, but another portion is synthesized in multiple cells of the body. Triglycerides are also obtained from the diet as well as synthesized by the liver (Lumeij, 1990; Walker *et al*., 1991). The origin of circulating lipoproteins is less understood than is their uptake, transport, and degradation. The lipid transport system in plasma has been described as involving two pathways: an exogenous route for the transport of cholesterol and triglycerides absorbed from dietary fat in the intestine, and an endogenous system through which cholesterol and triglycerides reach the plasma from the liver and other nonintestinal tissues

(Walker *et al*., 1991).Cholesterol and triglycerides, like many other essential components of the body, attract clinical attention when present in abnormal concentrations. Increased or decreased levels usually occur because of abnormalities in the synthesis, degradation, and transport of their associated lipoprotein particles. When hyperlipidaemia or hypolipidaemia are defined in terms of the class or classes of increased or decreased plasma lipoproteins, the names hyperlipoproteinaemia or hypolipoproteinaemia are preferentially employed (Lewandowski *et al*., 1986; Walker *et al*., 1991).

Multiple epidemiologic studies have demonstrated that increased levels of plasma total cholesterol and low-density lipoproteins are strongly and directly related to a greater incidence of coronary heart disease. Elevated plasma triglycerides and very-low-density lipoproteins are directly associated with the risk of heart disease (Lewandowski *et al*., 1986; Walker *et al*., 1991). Clinical manifestations of hyperlipoproteinaemia include a greater incidence of ischemic vascular disease, acute pancreatitis, and visible accumulations of lipid deposits (xanthomas and xanthelasmas). The localization of these lesions is of great help in many instances to categorize the lipoprotein dysfunction present (Lewandowski *et al*., 1986; Walker *et al*., 1991).

2.17.2 High-density lipoproteins (HDL) and low-density lipoproteins (LDL)

High-density lipoproteins are a heterogeneous group of macromolecules with different physical properties and chemical components; two subclasses of HDL have been identified (HDL_2 and HDL_3) within which several subspecies have also been demonstrated (Lewandowski *et al*., 1986). The predomination function of HDL seems to be the reverse transport of cholesterol from different tissues into the liver, where it is eventually removed. Subclass HDL_2 has been reported

to have a better correlation with coronary artery disease protection than total HDL cholesterol (Walker *et al*., 1991).

The serum concentration of HDL and its components derives from various complex intravascular and cellular metabolic events (Lewandowski *et al*., 1986). These events include secretion of precursor HDL particles from the liver and small intestine, interaction of these particles with lipids and proteins released during the catabolism of triglyceride-rich lipoproteins, and production of cholesteryl esters (the core substance in HDL) from the action of lecithin–cholesterol acyltransferase (LCAT), an enzyme that originates in the liver (Walker *et al*., 1991). This enzyme acts on unesterified cholesterol released into plasma from cellular turnover (Lewandowski *et al*., 1986). The cholesterol esters formed in this reaction are in turn transferred to VLDL and subsequently appear in LDL. The end result is a system that allows the transfer of cholesterol through LDL to peripheral cells and its return to the liver through HDL, and that prevents excessive accumulation of cholesterol in the body (Walker *et al*., 1991).

2.17.2 Creatinine

Elevations have been associated with kidney disease, but creatinine is not considered to be a reliable indicator of renal function (Harris, 2009). Serum creatinine (a blood measurement) is an important indicator of renal health because it is an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced via a biological system involving creatine, phosphocreatine (also known as creatine phosphate), and adenosine triphosphate (ATP) (Taylor, 1989; Lumeij, 1990). Creatine is synthesized primarily in

the liver from the methylation of glycocyamine (guanidino acetate, synthesized in the kidney from the amino acids arginine and glycine) by S-adenosyl methionine. It is then transported through blood to the other organs, muscle, and brain, where, through phosphorylation, it becomes the high-energy compound phosphocreatine (Taylor, 1989; Lumeij, 1990). During the reaction, creatine and phosphocreatine are catalyzed by creatine kinase, and a spontaneous conversion to creatinine may occur (Lumeij, 1990). Creatinine is removed from the blood chiefly by the kidneys, primarily by glomerular filtration, but also by proximal tubular secretion. Little or no tubular reabsorption of creatinine occurs. If the filtration in the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which correlates with the glomerular filtration rate (GFR). Blood creatinine levels may also be used alone to calculate the estimated GFR (eGFR) (Shemesh *et al.,* 1985).

2.17.3 Blood urea nitrogen (BUN)

The usefulness of serum blood urea nitrogen (BUN) is limited because of the low measurable levels of urea in avian blood. Also, the avian kidney appears able to excrete most urea as long as the patient's hydration is adequate. Therefore, blood urea may be a better indicator of hydration than renal function, and an elevated urea would imply dehydration (Lewandowski *et al*., 1986; Harris, 2009). Serum blood urea nitrogen (BUN) determinations are considerably less sensitive than BUN clearance (and creatinine clearance) tests, and levels may not be abnormal until the BUN clearance has diminished to <50%. Clinicians frequently calculate a convenient relationship, the urea nitrogen/creatinine ratio: serum BUN (Burtis *et al*., 2006). Significantly lower ratios denote acute tubular necrosis, low protein intake, starvation or severe liver disease.

High ratios with normal creatinine levels may be noted with catabolic states of tissue breakdown, prerenal azotaemia, high protein intake, etc. High ratios associated with high creatinine concentrations may denote either postrenal obstruction or prerenal azotaemia superimposed on renal disease (Lewandowski *et al*., 1986; Burtis *et al*., 2006). Because of the variability of both the BUN and creatinine assays, the ratio is only a rough guide to the nature of the underlying abnormality. Its magnitude is not tightly regulated in health or disease and should not be considered an exact quantity (Burtis *et al*., 2006).The liver produces urea in the urea cycle as a waste product of the digestion of protein. Individual laboratories may have different reference ranges, and this is because the procedure may vary (Deepak *et al*., 2007). Serum blood urea nitrogen is an indication of renal health. If glomeruler filtration rate and blood volume decrease (hypovolemia) then BUN will increase. Other factors responsible for its increments are fever, increased catabolism, high-protein diet and gastrointestinal bleeding (Deepak *et al*., 2007).

2.17.4 Total Protein (TP)

A total serum protein level must be evaluated in light of its components, albumin and globulin. The total value is influenced by various factors, but a normal value does not rule out abnormalities of the individual protein components (Lumeij, 1990). Overall dehydration and immune stimulation may cause a hyperproteinaemia. Hypoproteinaemia may be caused by overhydration, protein loss associated with renal disease, starvation, hepatic disease or intestinal disease (Oladele *et al.,* 2005c; Harris, 2009).

2.17.5 Glucose

Elevations in blood glucose levels are the principal pathological change noted in avian species. Hypoglycaemia is extremely rare in birds and, when present, is almost never associated with starvation. The primary cause of hypoglycaemia in birds is septicaemia ((Lewandowski *et al*., 1986); Harris, 2009).

2.17.6 Potassium

Potassium is the major positive ion (cation) found inside of cells. The proper level of potassium is essential for normal cell function. Among the many functions of potassium in the body are regulation of the heartbeat and the function of the muscles (Lewandowski *et al*., 1986; Lumeij, 1990). A seriously abnormal increase in potassium (hyperkalemia) or decrease in potassium (hypokalemia) can profoundly affect the nervous system and increases the chance of irregular heartbeats (arrhythmias), which, when extreme, can be fatal. Potassium is normally excreted by the kidneys, so disorders that decrease the function of the kidneys can result in hyperkalemia (Lewandowski *et al*., 1986). Certain medications may also predispose an individual to hyperkalemia. Hypokalemia, or decreased potassium, can arise due to kidney diseases; excessive loss due to heavy sweating, emesis, or diarrhoea, eating disorders, certain medications, or other causes (Lumeij, 1990; Harris, 2009). As with sodium, pathological changes in potassium levels indicate a serious and usually life-threatening clinical situation. Hyperkalaemia develops with advanced kidney disease, adrenal disease, and during episodes of acidosis. Hypokalaemia may result from potassium loss through diarrhoea, and during states of alkalosis (Lewandowski *et al*., 1986; Harris, 2009).

2.17.7 Sodium

Changes in sodium values usually reflect serious conditions. Elevated levels occur with salt poisoning, water deprivation and dehydration. Decreased levels occur due to sodium loss through renal disease or diarrhoea (Harris, 2009). Three elements that are predominant in satisfying the electrolyte balance within the body are sodium (Na+), potassium (K+) and chloride (Cl-). Sodium and Cl are minerals with important physiological functions in avian and may reduce leg abnormalities. These electrolytes are essential in osmotic pressure regulation and acid base balance. Sodium is also obligatory for absorption of amino acid and sugars in the small intestine; therefore utilization of digested protein and carbohydrates is diminished with an insufficiency of this cation (Kiddi and Kerr, 1998). Likewise, both sodium and chloride aid in nutrient passage and waste removal in cell nourishment and maintenance. While sodium is seldom deficient in the diet, a deficiency of this element will cause growth reduction, increased feed conversion, gonadal dormancy, bone softening, corneal keratinization, decreased cellular volume and changes in cellular function (Lewandowski *et al*., 1986). In contrast to potassium, sodium is the major cation in extracellular fluid and is closely associated with chloride and bicarbonate in the management of acid-base balance. Sodium functions in the maintenance of osmotic pressure of body fluid and the protection against excessive body fluid loss (Barros *et al.,* 1998). The permeability of cells and the sustenance of normal muscle irritability are additional functions of sodium (Kiddi and Kerr, 1998).

2.17.8 Bicarbonate

Measurement of bicarbonate levels provides an indication of the patient's acid–base status. Increases in bicarbonate imply alkalosis, while decreases imply acidosis (Lumeij, 1990; Harris, 2009). The bicarbonate ion acts as a buffer to maintain the normal levels of acidity (pH) in blood and other fluids in the body. Bicarbonate levels are measured to monitor the acidity of the blood and body fluids. The acidity is affected by foods or medications and the function of the kidneys and lungs. The bicarbonate test is usually performed along with tests for other blood electrolytes. Disruptions in the normal bicarbonate level may be due to diseases that interfere with respiratory function, kidney diseases, metabolic conditions, or other causes (Lumeij, 1990; Harris, 2009).

CHAPTER 3

MATERIALS AND METHODS

3.1 Sources of Birds, Feed, Challenged virus and Vaccines

Two hundred and fifty (250) day- old dominant black marshal cockerel (DBMC) chicks were purchased from a hatchery in Ibadan. The birds were fed on Vital feed® (Grand Cereals) chick mash which were purchased from commercial sales outlet in Zaria.

Very virulent strain of infectious bursal disease virus (vvIBDV) field strain (uncharacterized) isolated from chicken in Zaria, was obtained from Prof. Paul Abdu of Department of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. Vials of live freeze dried intermediate strain of infectious bursal disease vaccines (Indovax Private Limited Siswala P.O. Khurd Hismar Harna India) with manufacturing license No 353-B(H) were purchased from a commercial sales outlet in Zaria.

3.2 Source of Enzyme-linked Immunosorbent Assay (ELISA) Kit

Enzyme-linked immunosorbent assay (ELISA) kit was purchased from Biogal Galed Laboratories in Israel, to assay for IBDV antibodies.

3.3 Experimental Design

The chicks on arrival were randomly divided into five groups (A-E) with 50 chicks per group. The chicks were housed in separate rooms and maintained at recommended ambient temperature and environmental conditions. Feed and water were provided *ad libitum*.

Twenty five chicks, five from each group were randomly selected from the 250 DBMC chicks at day one and sacrificed. Two milliliters (2ml) of blood was collected from each chick into two sets of Bijou bottles, one containing ethylene diamine tetra acetic acid (EDTA) for the determination of haematological values; and the other without EDTA. Blood in the tubes without EDTA was allowed to clot and sera extracted into sterile sample Bijou bottles and kept at -20^{0}C until used. The sera were used for determination of antibody titers to IBDV.

To each group of birds, the following treatments were administered:

Table 3.1. Experimental design of the study

Group of birds	Weeks when birds were challenged with vvIBDV	Weeks when birds were vaccinated against IBD	Sampling weeks
A(n = 50)	NC	2 and 4	3 and 5
B(n = 50)	4	NV	5 and 6
C(n = 50)	6	NV	7 and 8
D(n = 50)	8	NV	9 and 10
E(n = 50) Control	NC	NV	1-10

Key: NV = Not vaccinated; NC = Not challenged

The experimental design gave room for sequential studies of haematological and biochemical changes from week 1 to 10 weeks of age in the cockerels challenged with vvIBDV or those vaccinated against IBD.

From each group above, blood samples were aseptically collected from 10 randomly selected birds during each experiment using 23G sterile hypodermic needles and syringes into two sets of Bijou bottles, one containing EDTA for the determination of haematological parameters and the other without EDTA for serum biochemical parameters and IBDV antibodies.

3.4 Enzyme Linked Immunosorbent Test for Antibodies to Infectious Bursal Disease Virus

The ELISA kit was designed to determine IgG antibody titers to IBD, ND and infectious bronchitis (IB) in chickens (Appendix 7). The immunocomb is a self contained portable kit based on solid phase immunoassay principle. The immunocomb provide result within 38 minutes. The immunocomb is a plastic card shaped like a comb, on which purified IBD, ND and IB antigen were attached. Serum samples from birds were deposited into separate wells in Row A. Positive control and negative control samples, supplied in the kit were applied in separate wells in Row A. Comb was inserted into separate wells so that antibodies on sample bound with antigen on the comb's teeth. Non-bound antibodies were washed off in Row B. Row C contained an anti-chicken/turkey IgG antibody labeled with an enzyme. While comb is immersed in this "conjugate" the bound antibody was labeled. After two washing steps the Comb was inserted into a row where the enzyme reaction took place. Then there was a color change, its intensity indicated the amount of antibodies present in each sample. Purple-grey color intensity obtained at each spot was converted to the antibody level by using the Comb scale.

3.5 Challenge of Birds with Infectious Bursal Disease Virus

Each chick in groups B, C and D was inoculated with 0.4 ml of vvIBDV inoculums equivalent to 16 x10 $^{4.6}$ ELD_{50} of undiluted viral suspension via conjuctival instillation at 4, 6 and 8 weeks of

age respectively. Birds in group A were vaccinated against IBD at a dose of 10 ml per chick which contained equivalent of 10^3 plague forming unit (PFU) intermediate strain (Georgia) IBD virus at 2 and 4 weeks of age.

3.6 Determination of Biochemical Parameters:

Total protein (TP) was determined by Biuret method (Pilar *et al.,* 2005). The express plus clinical chemistry analyzer (Bayer Germany) was used to analyze serum electrolyte, which included potassium, K (mmol/L), sodium, Na (mmol/L), biocarbonate, HCO_3 (mmol/L) and chloride, Cl^- (mmol/L). Other parameters included low density lipoprotein, LDL (mmol/L)**,** high density lipoprotein, HDL (mmol/L)**,** triglyceride, TRG (mmol/L)**,** creatinine, CR (µmol/L), total glucose, TGL (mmol/L), urea (mmol/l) and albumin ALB(g/L), which were determined using the various Bayer diagnostic reagents as approved by International Federation of Clinical Chemistry (IFCC), Bayer Express plus Clinical Chemistry Autoanalyzer (Bayer® Germany).

3.7 Determination of Hematological Parameters

Differential white blood cell counts was determined by use of monolayer blood films, fixed with 95% ethyl alcohol for 5 minutes and stained with Giemsa-Wright's stain.

Total white blood cell count (WBC) was determined by the following formula:

Total WBC= Number of cells counted×10×32×100/(%H+E)×18 (Campbell, 1995; Sinkalu *et al*., 2010), where H is heterophils and E eosinophils.

Red blood cell count (RBC) was determined using Mindray auto analyser, model BC-3000 PLUS, manufactured by Shenzhen Mindray Biomedical Electronic Limited, China (Freitas-Neto *et al*., 2007)

Packed cell volume (PCV) was determined by a manual technique using microhaematocrit capillary tubes and centrifuged at 327 x g for 8 min.

Haemoglobin concentration (Hb) was measured by haemoglobinometer.

Erythrocyte indices, such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were calculated using the RBC, PCV and Hb value obtained (Ritchie *et al*., 1994).

3.8 Clinical Signs, Gross and Histopathological Examinations

Birds in each group were observed for clinical signs. Signs observed were recorded and photographed. Gross lesions observed in the lymphoid and non lymphoid organs were recorded. Tissue sections were collected and fixed in 10 % neutral buffered formalin. They were processed for histopathological assessment using the method outlined by Baker *et al.* (2000). The processing of tissues consists of series of dehydration, clearing, impregnation and embedding. It was performed manually and appropriate timing in series of chemicals was adhered to. Sections were cut with microtome and floated in water in tissue floating bath; the tissues were then mounted on microslide with the aid of egg albumin solution, stained with Hematoxylene and

Eosin stains and were viewed under light microscope. Pictures of lesions were taken using digital camera.

3.9 Data Analysis

GraphPad Prism Version 5 was used for the analysis. Data were expressed as mean ± standard error of the mean. Differences in the means between groups were examined with Student's t-test. Values of $P < 0.05$ were statistically significant.

CHAPTER FOUR

RESULTS

4.1 Clinical Signs Observed in the vvIBDV Infected Groups

In general, the clinical signs recorded after challenge of groups B, C, and D with vvIBDV were whitish diarrhoea, depression, morbidity, recumbency, muscle trembling, somnolence, ruffled feathers, postration, drooped wings, reluctance to move, head hidden beneath wings and mortality as shown in Plates 1-6.

4.1.1 Clinical signs observed in group A

In group A (vaccinated against IBD and not challenged), no clinical signs were observed in this group.

4.1.2 Duration of clinical signs in group B

In group B (challenged at 4 weeks), whitish diarrhoea lasted up to day 7 post inoculation (pi), depression and morbidity lasted up to day 6 pi. Recumbency, somnolence lasted up to day 5 pi. The morbidity rate was 100%, while the mortality rate was 54% (Table 4.1).

4.1.3 Duration of clinical signs in group C

In group C (challenged at 6 weeks), whitish diarrhea, depression, hurdling and morbidity lasted up to day 7 pi. Recumbency, somnolence lasted up to day 6 pi. The morbidity rate was 100%, while the mortality rate 72% (Table 4.2).

4.1.4 Duration of clinical signs in group D

In group D (challenged at 8 weeks), whitish diarrhoea, depression, hurdling and recumbency lasted up to day 5 pi; morbidity lasted up to day 7 pi; somnolence lasted up to day 5 pi. The morbidity rate was 100%, while the mortality rate was 50% (Table 4.3).

4.1.5 Clinical signs observed in group E

In group E (not vaccinated nor challenged), no clinical signs were observed in this group.

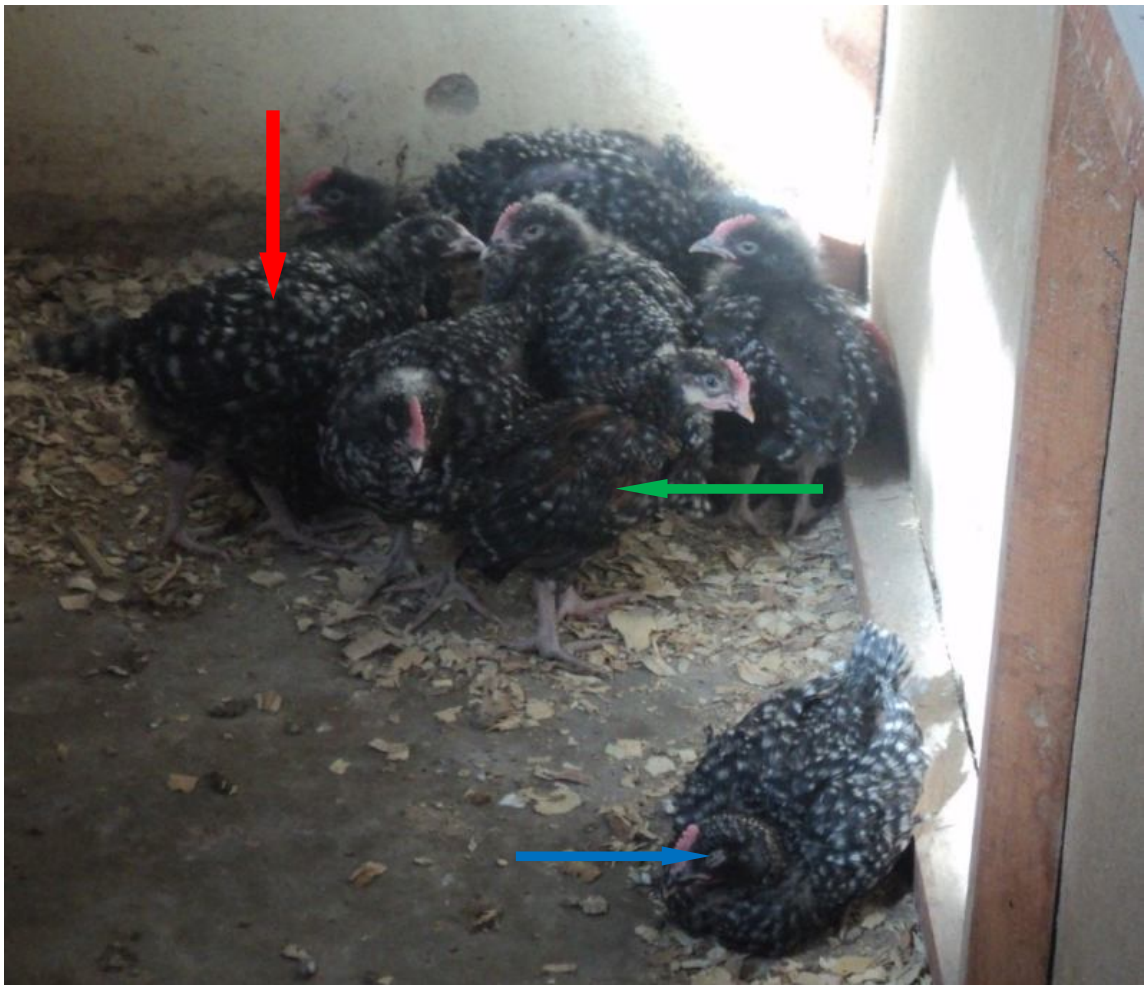


Plate 1. Cockerels in group B inoculated with vvIBDV at 4 weeks showing the signs of hurdling, ruffled feathers (red arrow), drooped wings (green arrow), and somnolence (blue arrow) at day three post inoculation.

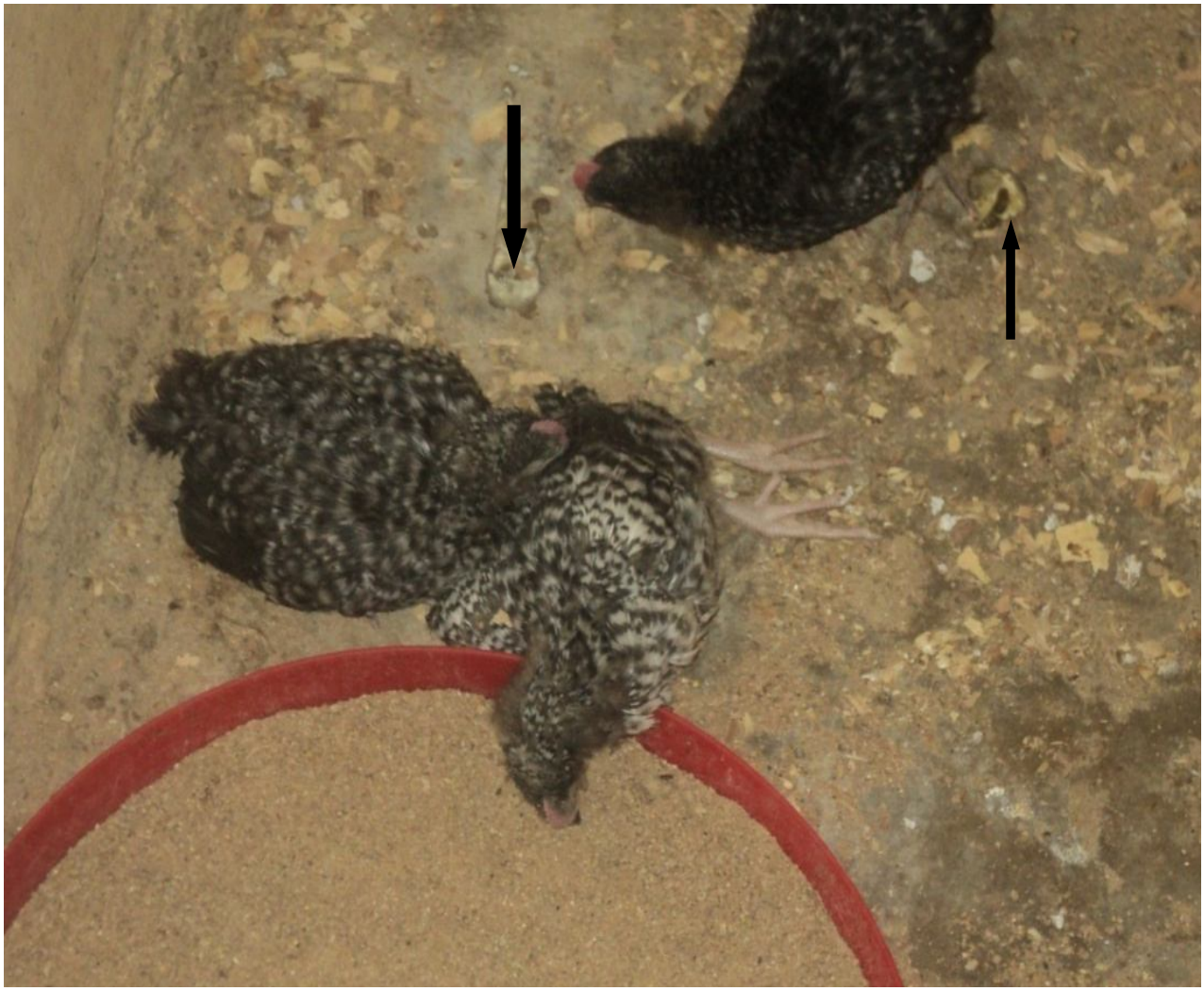


Plate 2. Cockerels in group B inoculated with vvIBDV at 4 weeks showing signs of recumbency and the floor stained with whitish diarrhoea (arrows) at day four post inoculation.



Plate 3. Cockerels in group C inoculated with vvIBDV at 6 weeks showing signs of slight weakness and depression day one post inoculation.



Plate 4. Cockerels in group C inoculated with vvIBDV at 6 weeks showing hurdling together with 100% morbidity at day two post inoculation.



Plate 5. Cockerels in group C inoculated with vvIBDV at 6 weeks showing mortality in flock at day four post inoculation.

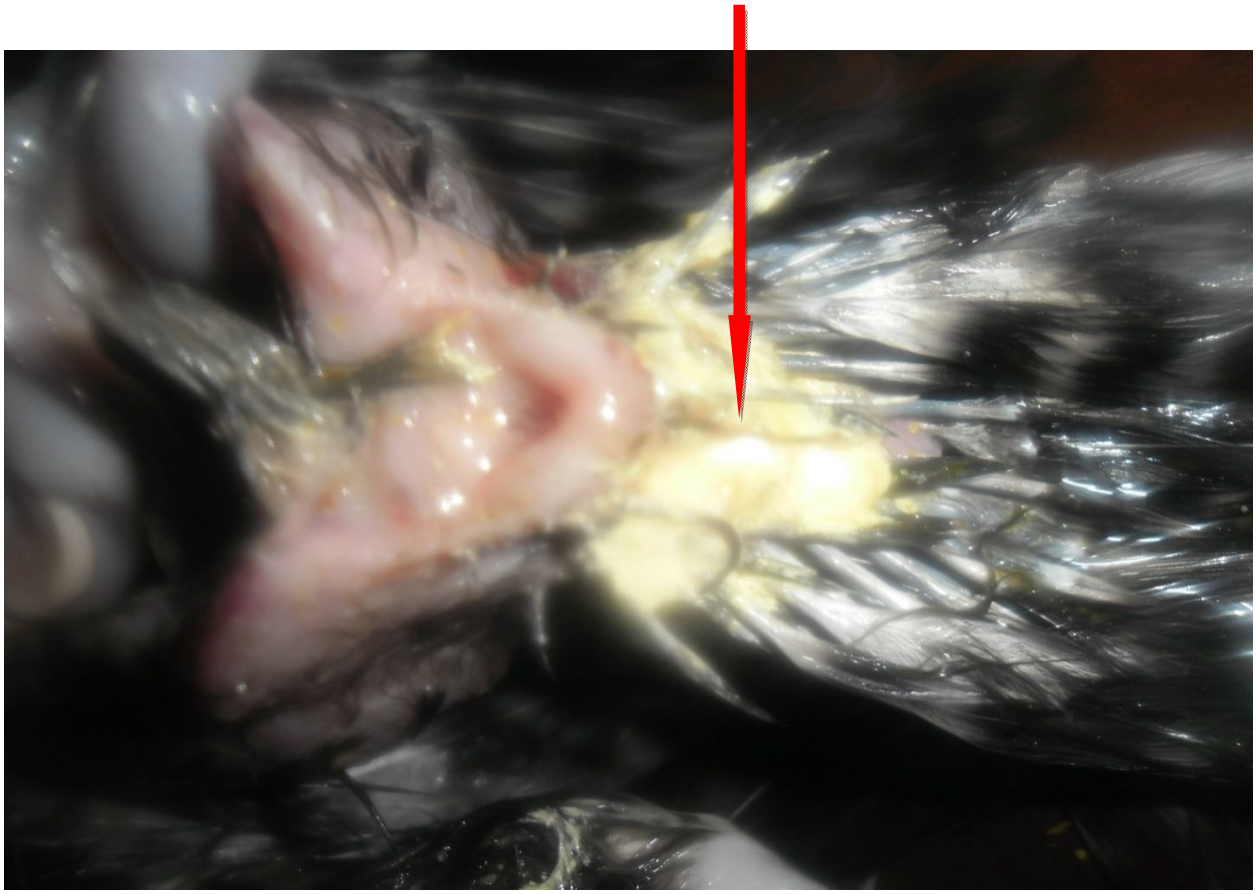


Plate 6. Cockerels in group D inoculated with vvIBDV at 8 weeks showing whitish- yellowish mucoid diarrhoea on the perianal area of live bird(arrow).

Table 4.1. Clinical signs recorded in group B (birds challenged with very virulent infectious bursal disease virus at 4 weeks of age).

	Clinical signs						
Day (pc)	WD n(%)	DEP n(%)	MORB n(%)	REC n(%)	HURD n(%)	SOMN n(%)	MORT n(%)
1	0	10(20)	15(30)	0	0	0	0
2	8(16)	35(70)	36(72)	0	0	23(46)	0
3	30(65.2)	40(87)	50(100)	2(4.3)	40(86.9)	17(40)	4(8)
4	14(46.7)	45(80)	40(87)	5(8.7)	18(60)	13(43)	20(48)
5	12(44.4)	12(40)	18(78.3)	1(3.3)	5(18.5)	2(8.7)	3(54)
6	10(37)	8(29)	10(43.5)	0	0	0	0
7	6(22)	0	3(13)	0	0	0	0

Key:

n: Number of birds

pc: Post challenged

WD: Whitish diarrhea

DEP: Depression

REC: Recumbency

HURD: Hurdling

SOMN: Somnolence

MORB: Morbidity

MORT: Mortality

Table 4.2. Clinical signs recorded in group C (birds challenged with very virulent infectious bursal disease virus at 6 weeks of age).

	Clinical signs						
Days (pc)	WD n(%)	DEP n(%)	MORB n(%)	REC n(%)	HURD n(%)	SOMN n(%)	MORT n(%)
1	0	7(14)	7(14)	0	0	0	0
2	8(16)	35(70)	35(70)	8(16)	30(60)	26(52)	0
3	21(52.5)	22(55)	40(100)	7(17.5)	31(77.5)	20(50)	10(20)
4	15(60)	20(80)	25(100)	3(12)	21(84)	10(40)	15(50)
5	8(32)	5(33.3)	10(66.7)	3(20)	11(73.3)	5(33.3)	10(70)
6	3(21)	2(14.3)	5(35.7)	1(7.14)	7(46.7)	0	1(72)
7	1(7)	1(7.14)	3(21.4)	0	5(35.7)	0	0

Key:

n: Number of birds

pc: Post challenged

WD: Whitish diarrhea

DEP: Depression

REC: Recumbency

HURD: Hurdling

SOMN: Somnolence

MORB: Morbidity

MORT: Mortality

Table 4.3. Clinical signs recorded in group D (birds challenged with very virulent infectious bursal disease virus at 8 weeks of age).

	Clinical signs						
Day (pc)	WD n(%)	DEP n(%)	MORB n(%)	REC n(%)	HURD n(%)	SOMN n(%)	MORT n(%)
1	0	9(18)	9(18)	0	0	0	0
2	0	15(60)	35(70)	0	20(40)	20(40)	0
3	10(20)	35(72.9)	50(100)	3(6.25)	30(62.5)	15(31.3)	2(4)
4	30(77)	30(81)	33(100)	5(13.5)	36(97.3)	11(29.7)	11(26)
5	21(70)	18(60)	26(86.7)	1(33.3)	16(80)	6(20)	7(40)
6	13(52)	9(36)	16(64)	1(4)	8(32)	0	5(50)
7	3(12)	0	8(32)	0	2(8)	0	0

Key:

n : Number of birds

pc: Post challenged

WD: Whitish diarrhea

DEP: Depression

REC: Recumbency

HURD: Hurdling

SOMN: Somnolence

MORB: Morbidity

MORT: Mortality

4.2 Result of Haematological Parameters

4.2.1 Result of haematological parameters of vvIBDV infected groups

The haematological parameters of group B showed that at 5 weeks TRBC, PCV, Hb, MCV, MCH, MCHC, HET, and EOS were lower when compared with the control group, while LYMP, MON, and TWBC were higher, but not statistically significant ($p > 0.05$). At 6 weeks, the values of TRBC, PCV, Hb, MCV, MCH, MCHC, HET, and EOS were higher, however, only PCVand Hb had statistically significant increase in their values ($p < 0.05$). The values of TWBC, LYMP and MON were lower, but only TWBC and LYMP had statistically significant ($p < 0.05$) decrease in their values (Table 4.4).

The haematological parameters of group C showed that at 7 weeks, the values of TRBC, PCV, Hb, MCH, MCHC, LYMP, and MON were lower compared with control group, while the values of EOS, MCV, TWBC, and HET were higher, but the decrease was not statistically significant ($p > 0.05$). At 8 weeks, the values of TRBC, PCV, Hb, MCH, MCHC, LYMP and TWBC were higher, while the values of HET, MCV, MON , and EOS were lower, but not statistically significant ($p > 0.05$) (Table 4.5).

The haematological parameters of group D showed that at 9 weeks, the values of TRBC, PCV, Hb, MCV, MCHC, TWBC and LYMP were lower when compared with control group, but only the values of LYMP and TWBC were statistically decreased ($p < 0.05$), while the values of MCH, EOS, MON and HET were higher, but only the value of HET was significantly increased ($p < 0.05$). At 10 weeks, the values of TRBC, PCV, Hb, MCV, MCH, LYMP and TWBC were lower, but only the values of TRBC, PCV and Hb were statistically significant ($p < 0.05$). On the

other hand the values of MCHC, HET and EOS were increased, but the values were not statistically significant at ($p < 0.05$) (Table 4.6).

4.2.2 Result of haematological parameters of vaccinated group

The haematological parameters of group A showed that at 3 weeks, the values of TRBC, MCV, Hb, PCV, MCHC, HET, LYMP and TWBC were lower when compared with the control group, but only the value of LYMP were statistically significant ($p < 0.05$) while MCH and EOS values were higher, they were not statistically significant at $p > 0.05$. At 5 weeks, the values of MCH, MCHC, LYMP and TWBC were higher while the values of TRBC, PCV, Hb, MCV HET, EOS, and MON were lower, but not statistically significant ($p > 0.05$) (Table 4.7).

Table 4.4. Effect of infectious bursal disease virus on haematological parameters of cockerels challenged at 4 week of age and assayed for hematological parameters at 5 and 6 weeks

	Age of cockerels			
	5th week		6th week	
Parameters	E(Control)	B(Challenged)	E(Control)	B(Challenged)
TRBC(10^{12} /L)	3.838±0.056	3.597±0.147	4.616±.0.080	4.835±0.140
PCV(%)	23.2±0.490	21.7±0.883	27.9±0.482□	29.9±0.809□
Hb(g%)	7.68±0.167	7.260±0.261	8.95±0.182□	9.94±0.275□
MCV(fl)	60.13±0.170	60.07±0.159	59.76±0.273	60.057±0.097
MCH(g/dL)	19.77±0.095	19.49±0.324	1911.±0.365□	19.96±0.025□
MCHC(pg)	32.89±0.205	31.89±0.629	31.87±0.601	32.88±0.311
LYMP(%)	84.80±1.665	87.00±1.033	84.67±1.344□	72.90±3.358□
HET(%)	9.8±0.998	9.40±1.077	9.556±1.425□	23.20±2.054□
EOS(%)	3.7±0.517	2.2±0.742	3.22±.9829	4.100±0.527
MON(%)	1.7±0.4955	1.9±0.433	2.56±0.556	1.00±0.527
TWBC(10^9/L)	14.40±1.662	16.1±2.211	15.77±2.31□	7.8±1.144□

Values are expressed as mean±SEM, □ Significant difference at $p<0.05$.

Table 4.5. Effect of infectious bursal disease virus on haematological parameters of cockerels challenged at 6 week of age and assayed for hematological parameters at 7 and 8 weeks

	Age of cockerels			
	7th week		8th week	
Parameters	E(Control)	C(Challenged)	E(Control)	C(Challenged)
TRBC(10^{12} /L)	4.382±0.121	4.180±0.225	4.157±0.192	4.329±0.226
PCV(%)	26.7±0.746	25.30±1.342	25.00±1.145	26.10±1.378
Hb(g%)	8.70±0.224	8.42±0.454	8.29±0.383	8.66±0.458
MCV(fl)	59.99±0.112	60.09±0.138	60.13±0.142	60.01±0.155
MCH(g/dL)	19.98±0.398	19.84±0.090	19.84±0.090	20.494±0.613
MCHC(pg)	33.23±0.035	33.01±0.171	33.01±0.172	33.18±0.029
LYMP(%)	84.5±1.857	82.7±2.638	79.89±2.188	83.3±2.226
HET(%)	11.8±1.451	16.3±2.697	13.6±1.213	13.4±2.001
EOS(%)	2.5±0.833	3.1±0.795	4.2±1.114	2.5±0.946
BAND(%)	0.2±0.2	0	0.4±0.221	0.1±0.1
MON(%)	1.0±2.012	0.8±0.4163	1.3±0.597	0.6±0.340
TWBC(10^9/L)	8.78±1.067	11.26±1.278	11.06±1.278	13.91±2.382

Values are expressed as mean±SEM

Table 4.6 Effect of infectious bursal disease virus on haematological parameters of cockerels challenged at 8 week of age and assayed for hematological parameters at 9 and 10 weeks

	Age of cockerels			
	9th week		10th week	
Parameters	E(Control)	D(Challenged)	E(Control)	D(Challenged)
TRBC(10^{12} /L)	3.67±0.198	3.339±0.169	4.204±0.129□	3.660±0.1419□
PCV(%)	22.1±1.169	20.125±1.043	25.4±0.812□	22.1±0.849□
Hb(g%)	7.33±0.397	6.588±0.382	8.480±0.3247□	7.360±0.29□
MCV(fl)	60.272±0.126	60.239±0.114	60.193±0.323	59.768±0.102
MCH(g/dL)	19.862±0.324	20.026±0.388	19.885±0.052	19.85±0.035
MCHC(pg)	32.94±0.536	32.48±0.485	33.058±0.115	33.213±0.023
LYMP(%)	85.5±1.241□	78.00±0.309□	88.25±1.652	84.3±1.862
HET(%)	10.6±2.675□	15.625±1.426□	8.25±0.4787	10.0±0.989
EOS(%)	3.4±1.222	5.625±1.179	1.0±1.0	5.3±1.342
BAS(%)	0.1±0.1	0	0.125±0.125	0.1±0.1
MON(%)	0.4±0.4	1.00.±0.564	0.667±0.494	1.100±0.9
TWBC(10^9/L)	14.65±1.075□	8.8±0.835□	18.5±1.996	13.35±1.641

Values are expressed as mean±SEM, , □ Significant difference at $p<0.05$.

Table 4.7. Effect of infectious bursal disease vaccines on haematological parameters of cockerels vaccinated at 2 and 4 weeks of age and assayed for hematological parameters at 3 and 5 week.

	Age of cockerels			
	3rd week		5th week	
Parameters	E (Control)	A(Vaccinated)	E (Control)	A(Vaccinated)
TRBC(10^{12} /L)	4.712±0.096	4.674±0.0984	3.838±0.086	3.748±0.084
PCV(%)	28.40±0.56	28.21±0.098	23.2±0.489	22.62±0.4875
Hb(g%)	9.44±0.187	9.379±0.193	7.680±0.167	7.492±0.488
MCV(fl)	60.659±0.248	59.86±0.0866	60.127±0.170	59.963±.116
MCH(g/dL)	19.746±0.263	19.90±0.028	19.769±0.095	19.78±0.091
MCHC(pg)	32.673±0.468	33.259±0.028	32.89±0.206	33.01±0.193
LYMP(%)	88.8±1.397□	83.50±1.405□	84.80±1.665	85.23±1.199
HET(%)	9.5±1.47□	13.79±1.09□	9.8±0.998	8.769±1.199
EOS(%)	0.9±0.379	1.786±0.494	3.7±0.518	3.615±0.5722
MON(%)	0.8±0.359	0.7143±0.266	1.70±0.4955	1.615±0.513
TWBC(10^9/L)	19.510±2.367	13.22±1.928	14.40±1.239	14.08±0.887

Values are expressed as mean±SEM, □ Significant difference $p<0.05$.

4.3 Biochemical Parameters

4.3.1Result of biochemical parameters of vvIBDV infected groups

The results of biochemical parameters of group B showed that at 5 weeks the concentrations of TC, LDL, HDL, TG, UREA, Na, Cl, HCO_3, ALB and TP were lower when compared with the control group, but only the concentrations of UREA and TP were statistically significant ($p < 0.05$), while TRY, Cr, K concentrations were higher, but not statistically significant ($p > 0.05$). At 6 weeks of age the concentrations of TC, LDL, Cr, TGL, UREA, ALB and TP were higher, while those of TRY, HCO_3, Na, K and CL were decreased, but not statistically significant ($p > 0.05$) (Figures 4.1a and 4.1b).

The results of biochemical parameters of group C showed that at 7 weeks the concentrations of TC, HDL, LDL, Cr, TP, Cl and HCO_3, were lower when compared with the control group, but only TC was statistically significant ($p < 0.05$), while the concentrations of TRY, K, Na, and ALB were higher, but not statistically significant ($p > 0.05$). At 8 weeks of age, the concentrations of TC, LDL, TRY Cr, K and HCO_3 were lower, while those of HDL, UREA, Cl, Na, ALB and TP were higher, but only UREA was statistically significant ($p < 0.05$) (Figures 4.2a and 4.2b).

The results of biochemical parameters of group D showed that at 9 weeks, the concentrations of Na, TGL, TRY, and HCO_3, were higher, but not statistically significant ($p > 0.05$), while the concentrations of TC, LDL, K, Cl, HDL, UREA, ALB and TP were lower, but not statistically significant ($p > 0.05$). At 10 weeks of age, the concentrations of TC, LDL, TRY, Na, and HCO_3

were increased, while those of HDL, TG, UREA, TP, ALB, K, and CL were lower, but only UREA was statistically significant ($p < 0.05$) (Figures 4.3a and 4.3b).

4.3.2 Result of biochemical parameters of vaccinated group

The results of biochemical parameters of group A showed that at 3 weeks the concentrations of TC, LDL, HDL, TRY, Na and K were lower, but were not statistically significant ($p > 0.05$), while the concentrations of TGL, Cl, ALB and TP were lower, but only K was statistically significant ($p < 0.05$). At 5 weeks of age the concentrations of K and TRY were lower, while those of TC, LDL, HDL, UREA, Na, Cl, TGL, HCO_3, ALB and TP were higher, but only TP was statistically significant ($p < 0.05$) (Figures 4.4a and 4.4b).

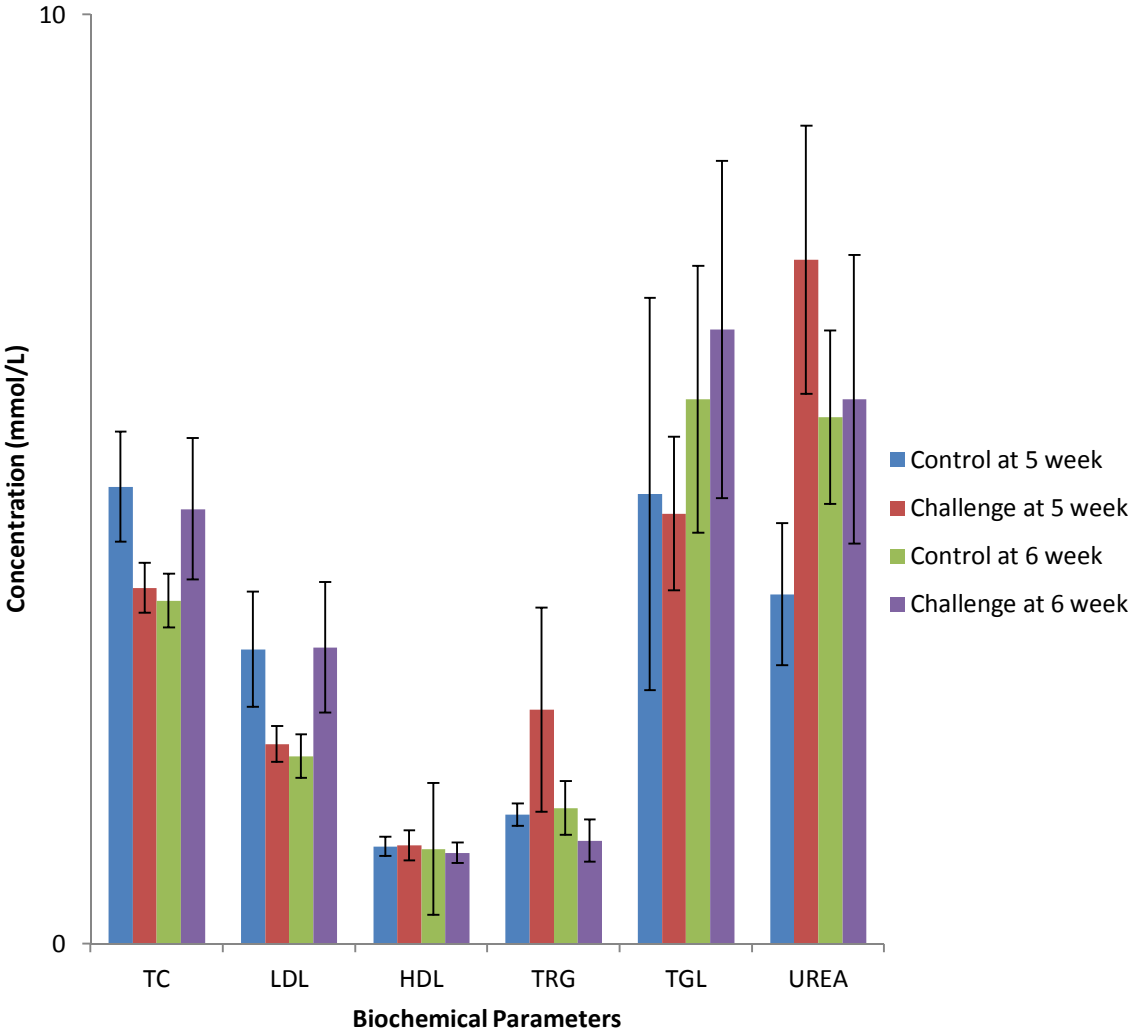


Fig.4.1a: Effect of infectious bursal disease virus on some serum biochemical parameters of cockerels challenged at 4 weeks of age and assayed for biochemical parameters at 5 and 6 weeks

Key:

HDL (High-density lipoprotein); TRG (Triglyceride); TGL (total glucose); TC (total cholesterol) LDL (low-density lipoprotein)

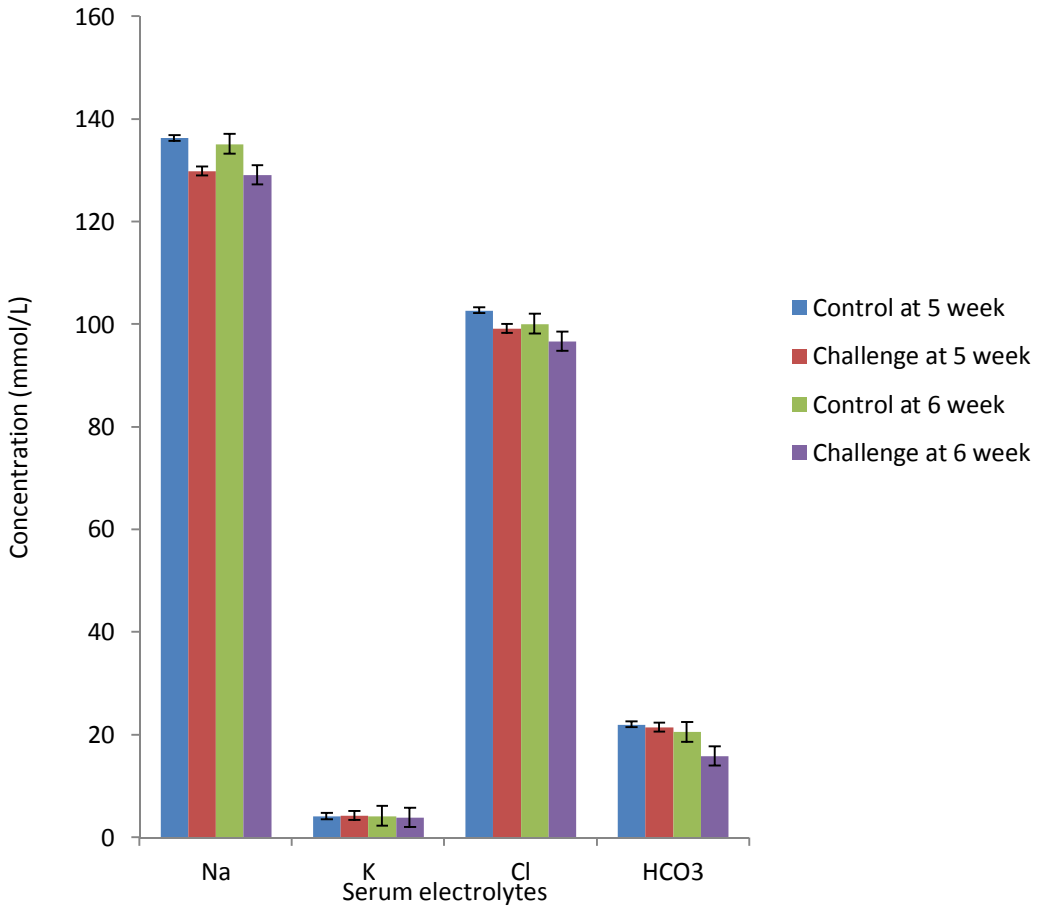


Fig.4.1b: Effect of infectious bursal disease virus on some serum biochemical parameters of cockerels challenged at 4 weeks of age and assayed for serum electrolytes at 5 and 6 weeks

Key:

Na (sodium); k (potassium) CL (chlorine); HCO_3 (bicarbonate)

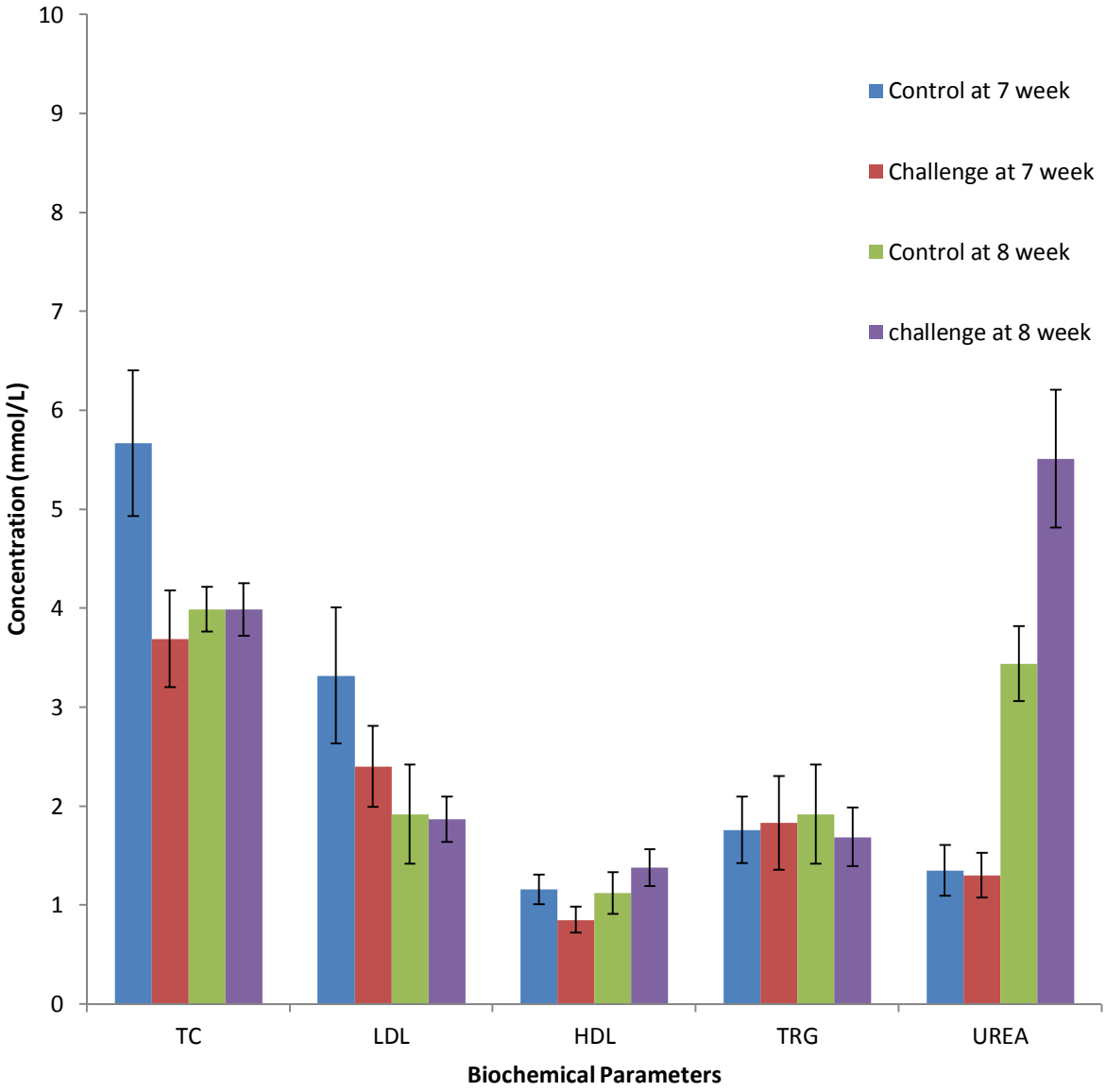


Fig.4.2a: Effect of infectious bursal disease virus on some serum biochemical parameters of cockerels challenged at 6 weeks of age and assayed for biochemical parameters at 7 and 8 weeks

Key:

HDL (High-density lipoprotein); TRG (Triglyceride); TGL (total glucose); TC (total cholesterol)
LDL (low-density lipoprotein)

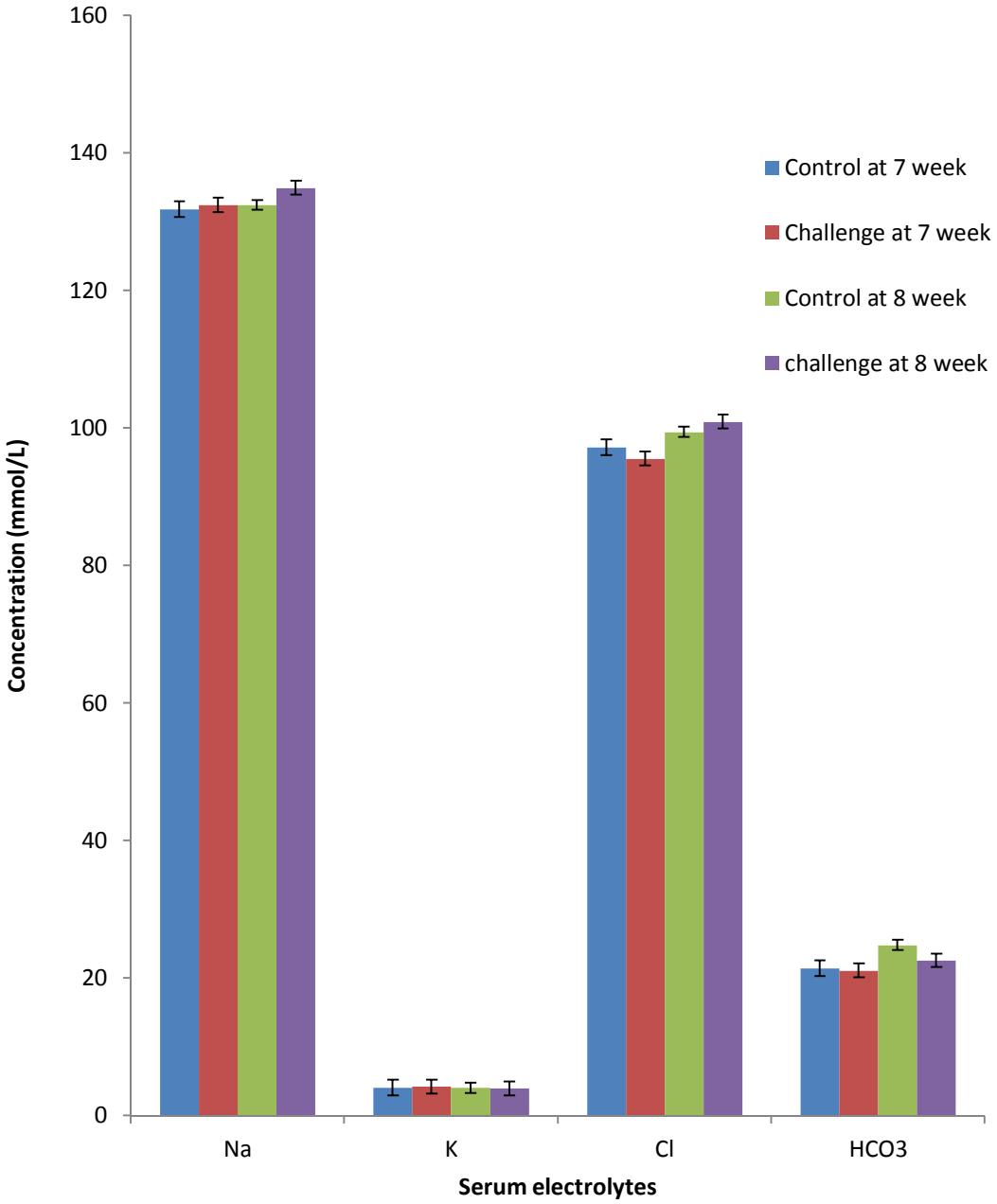


Fig.4.2b: Effect of infectious bursal disease virus on some serum biochemical parameters of cockerels challenged at 6 weeks of age and assayed for serum electrolytes at 7 and 8 weeks

Key:

Na (sodium); k (potassium) CL (chlorine); HCO_3 (bicarbonate)

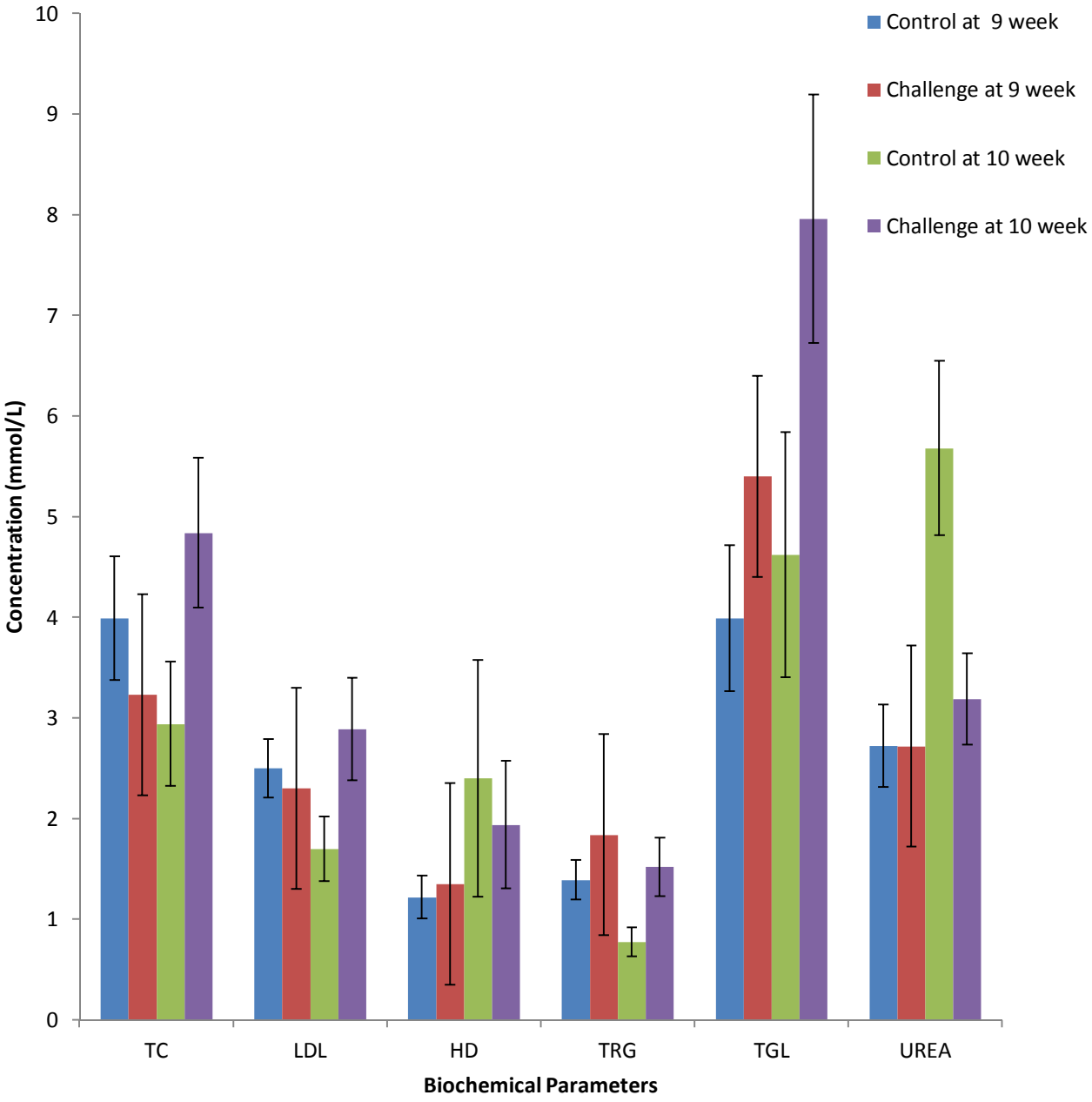


Fig.4.3a: Effect of infectious bursal disease virus on some biochemical parameters of cockerels challenged at 8 weeks of age and assayed for biochemical parameters at 9 and 10 weeks

HDL (High-density lipoprotein); TRG (Triglyceride); TGL (total glucose); TC (total cholesterol) LDL (low-density lipoprotein)

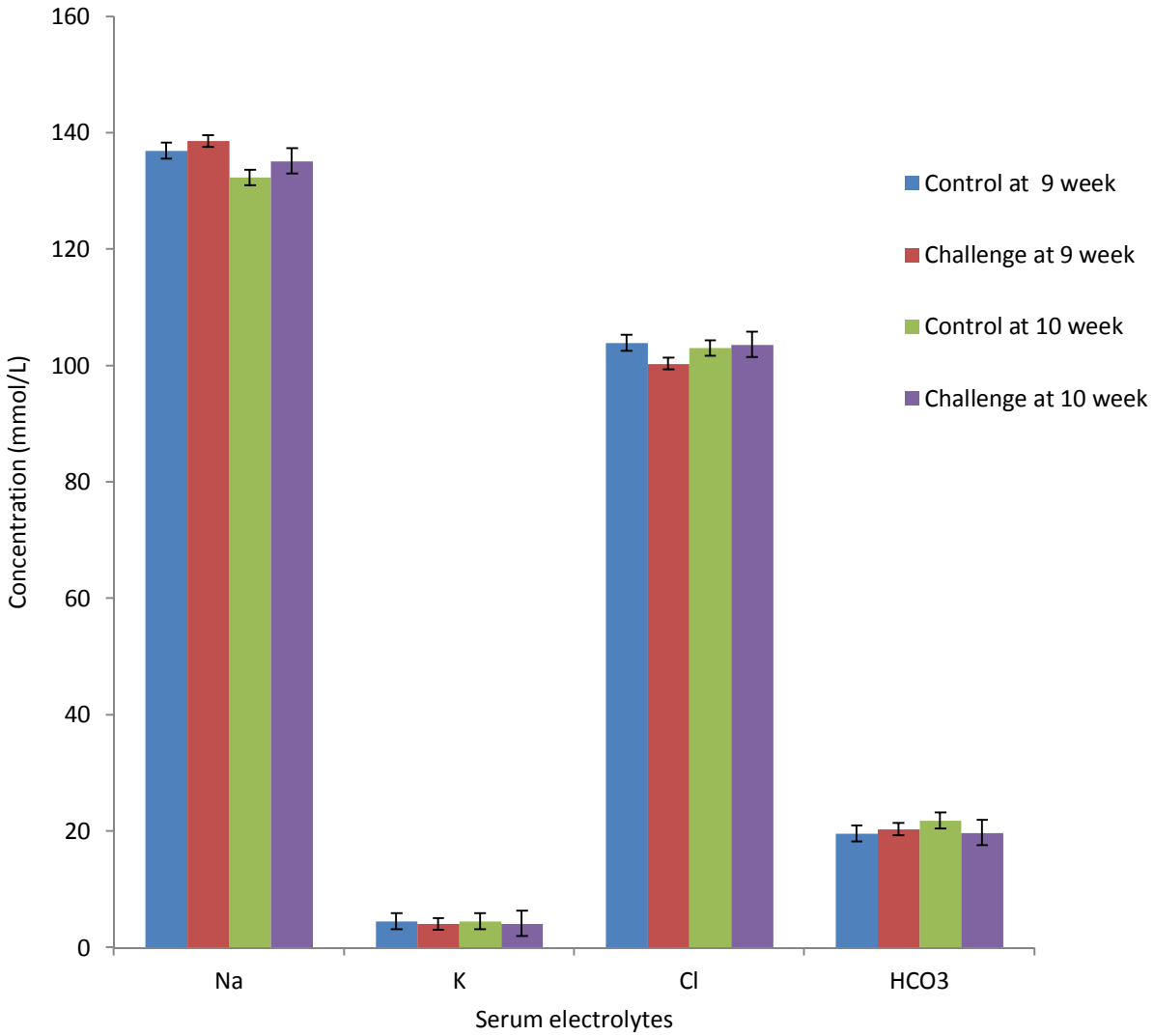


Fig.4.3b: Effect of infectious bursal disease virus on some serum biochemical parameters of cockerels challenged at 8 weeks of age and assayed for serum electrolytes at 9 and 10 weeks

Key:

Na (sodium); k (potassium) CL (chlorine); HCO_3 (bicarbonate)

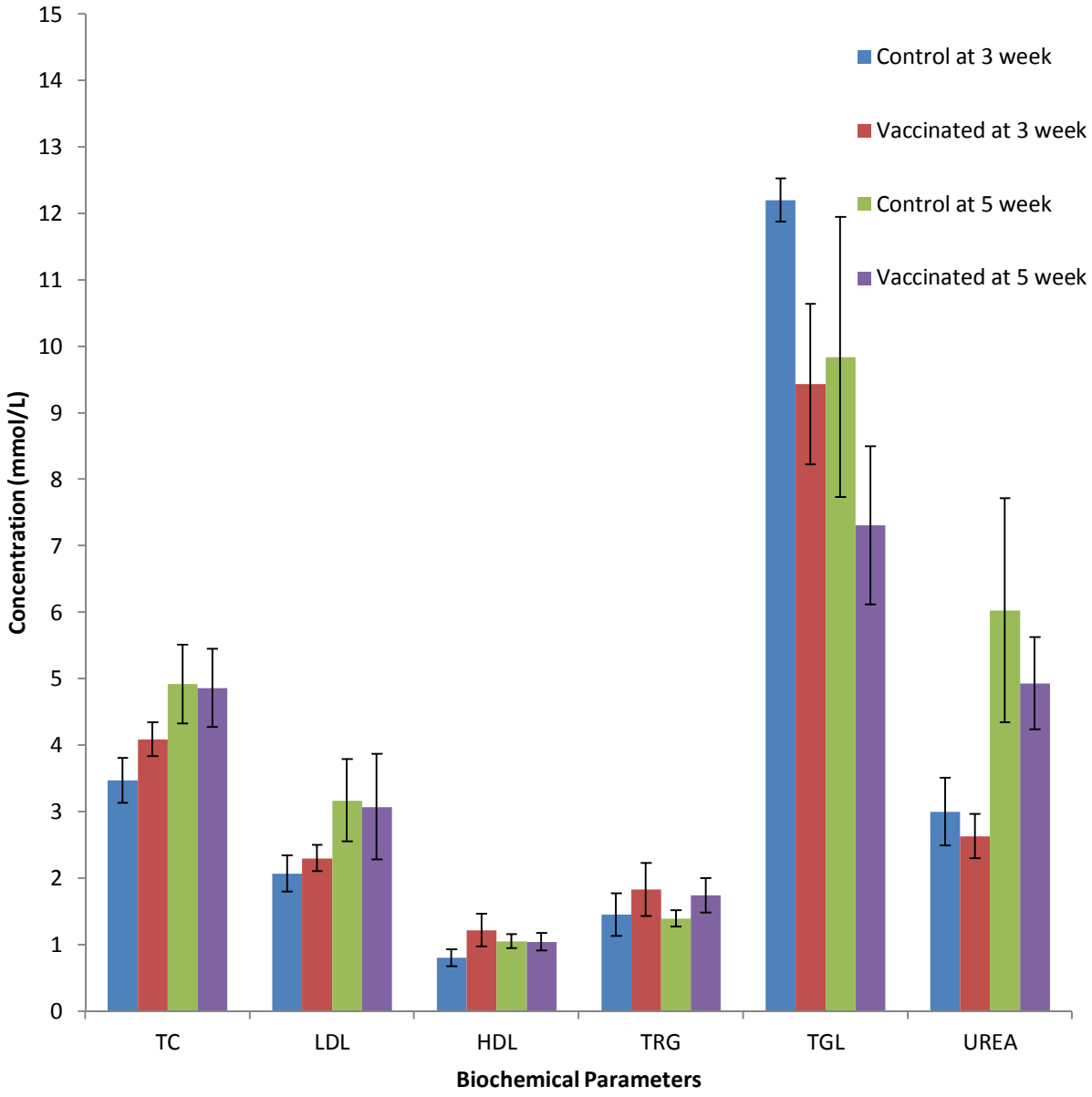


Fig.4.4a: Effect of infectious bursal disease vaccine on some serum biochemical parameters of cockerels vaccinated at 2 and 4 weeks of age and assayed for biochemical parameters at 3 and 5 weeks

HDL (High-density lipoprotein); TRG (Triglyceride); TGL (total glucose) TC (total cholesterol) LDL (low-density lipoprotein)

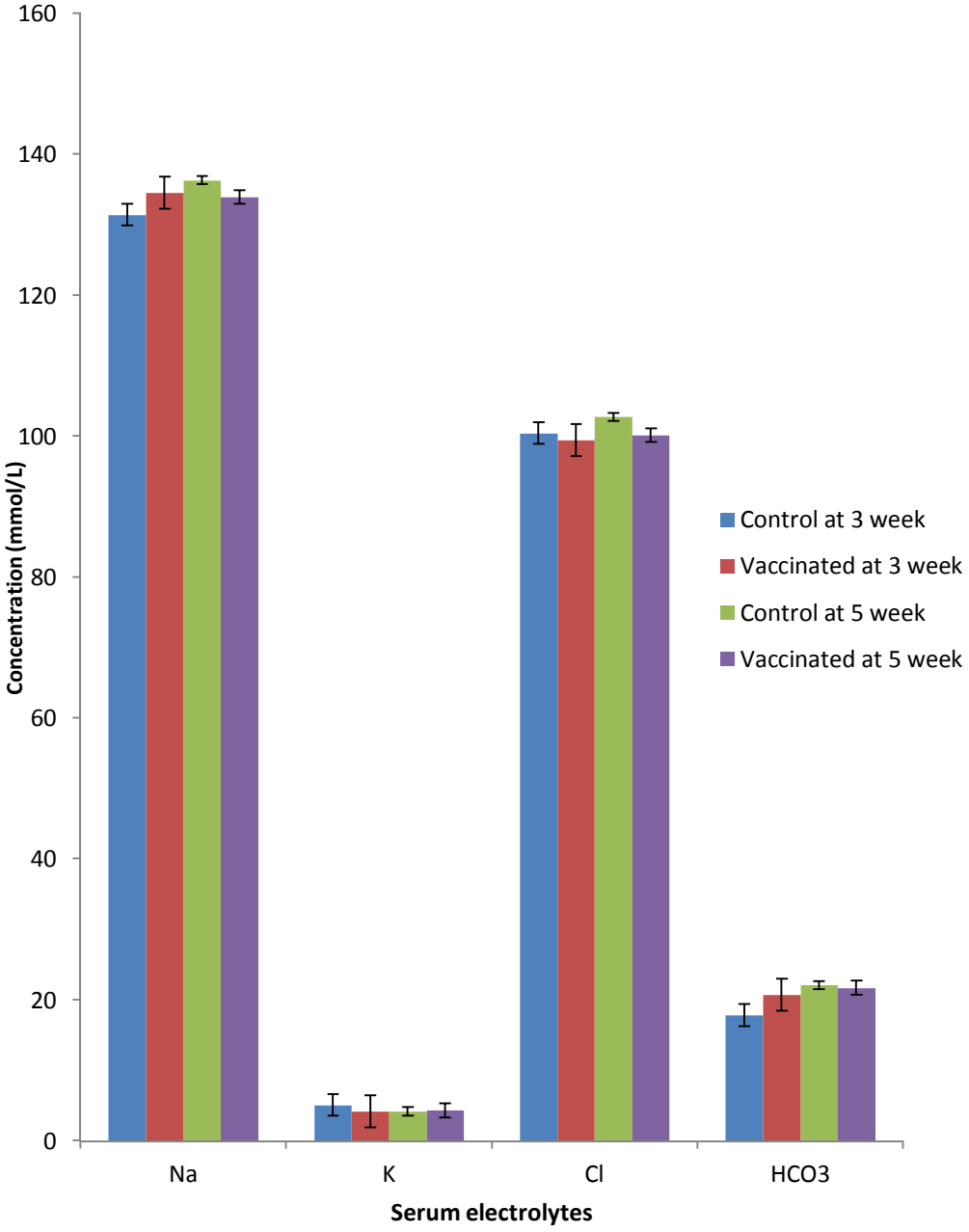


Fig.4.4b: Effect of infectious bursal disease vaccine on some serum biochemical parameters of cockerels vaccinated at 2 and 4 weeks of age and assayed for serum electrolytes at 3 and 5 weeks

Na (sodium); k (potassium) CL (chlorine); HCO_3 (bicarbonate)

4.4 Antibody Titre

4.4.1 Maternal antibody (MA).

Group E (control) was screened for MA against IBDV from day old to 10 weeks of age. It was observed that MA persisted up to 4 weeks of age and it dropped to zero by 5 weeks (Figure 4.9).

4.4.2 Vaccinal antibody.

Group A, was screened for vaccinal antibody against IBDV at 3 and 5 weeks after vaccination at 2 and 4 weeks. At 3 weeks after vaccination the antibodies were present, but at 5 weeks after vaccination the Ab titre dropped (Figure 4.9).

4.4.3 Convalescence antibody.

In Group B there was increase in Ab after the challenge at 4 weeks, but it dropped to zero 14 days pi. While in group C the Ab increased at 7 weeks and slightly decreased at 8 weeks. Group D Ab titre showed slight increase at 7 days pi and it rose to 3.09 at 14 days pi (Figure 4.9).

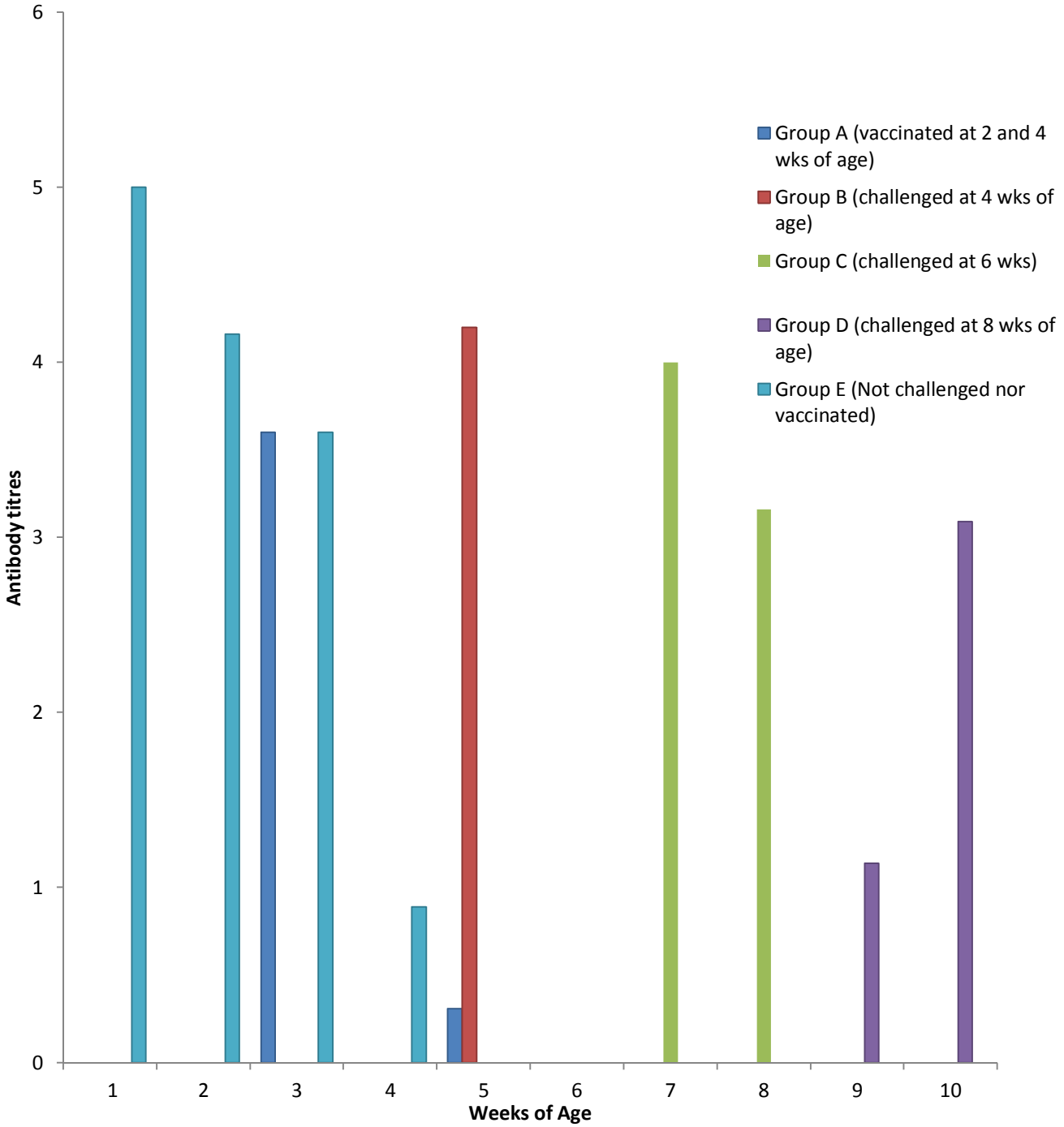


Fig. 4.9:` Antibody titres to infectious bursal disease virus in different experimental groups

4.5 Gross Pathology

4.5.1 Gross pathology in vvIBDV infected groups

The post mortem lesions observed in challenged groups (B, C and D) were as follows: Bursa of Fabricius were congested, enlarged and turgid (Plate 7). Caecal tonsils were congested and haemorhagic (Plate 8). Deposits of urates were seen in the ureter (Plate 9). Enlarged and congested spleen (Plate10). Kidneys were congested, haemorhagic and enlarged (Plate 11). In some cases the kidneys bulged out from the pelvic cavity. Thigh and breast muscles showed congestion and haemorhages (Plates 12 and 13). Enlargement and congestion of organ were observed in the liver (Plate 14), and thymus (Plate15). The proventriculus showed mucosal haemorhages (Plate 16). The lesions were all seen in all the groups (B, C, and D). Table 4.8 shows the summary of each lesions observed.

4.5.2 Gross pathology of vaccinated group

The post mortem lesions observed in vaccinated group (A) were as follows: Pinpoint haemorrhages on thigh, leg and breast muscles (Plate 17); congested and oedematous bursa of Fabricius (Plate 18); kidneys were enlarged and congested (Plate 19).

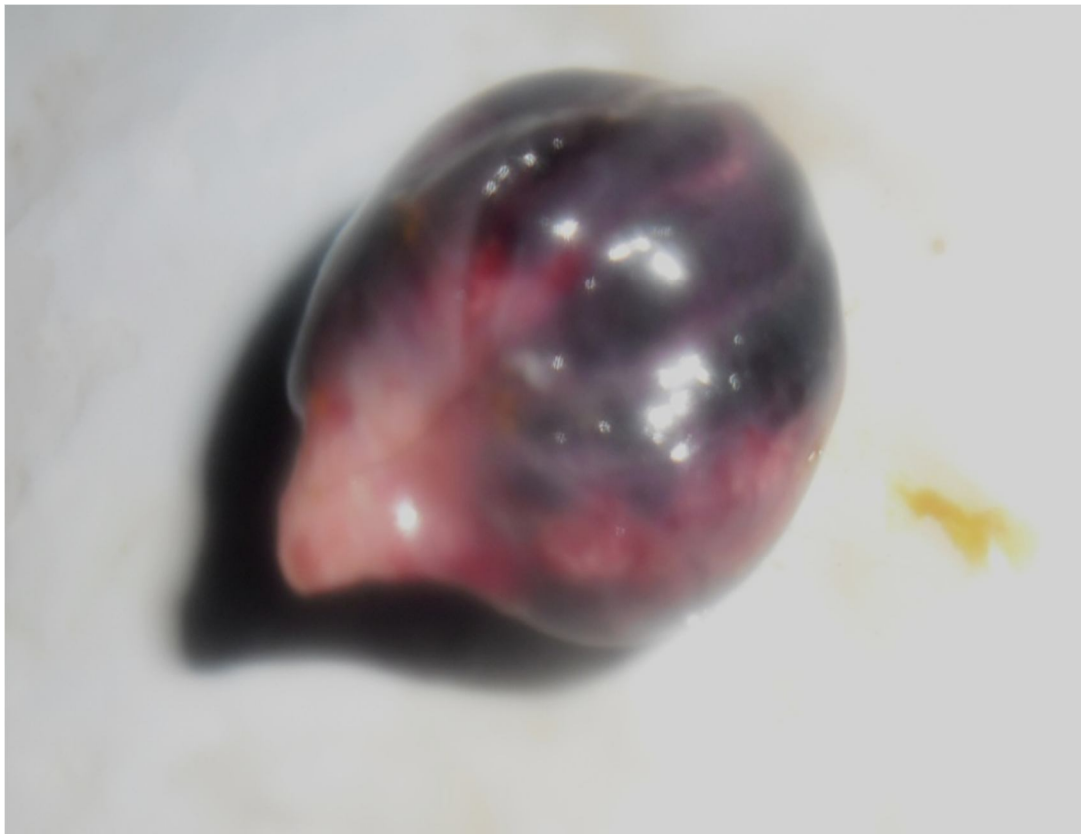


Plate 7. Enlarged, congested, haemorhagic and turgid bursa of Fabricius of a cockerel in group B inoculated with vvIBDV at 4 weeks

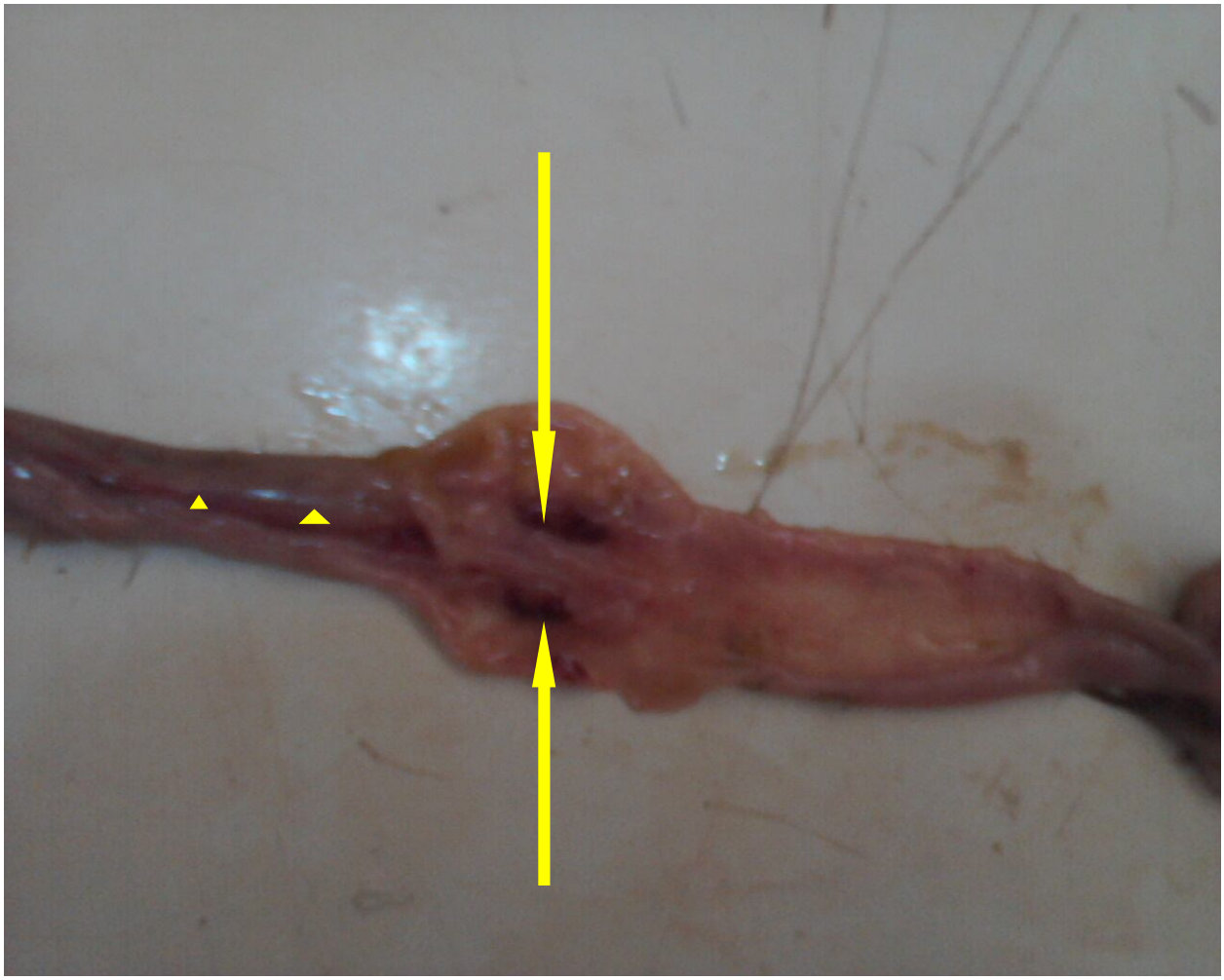


Plate 8.Haemorhages in the caecal tonsil (arrows) and intestine (arrow heads) of a cockerel in group B inoculated with vvIBDV at 4 weeks

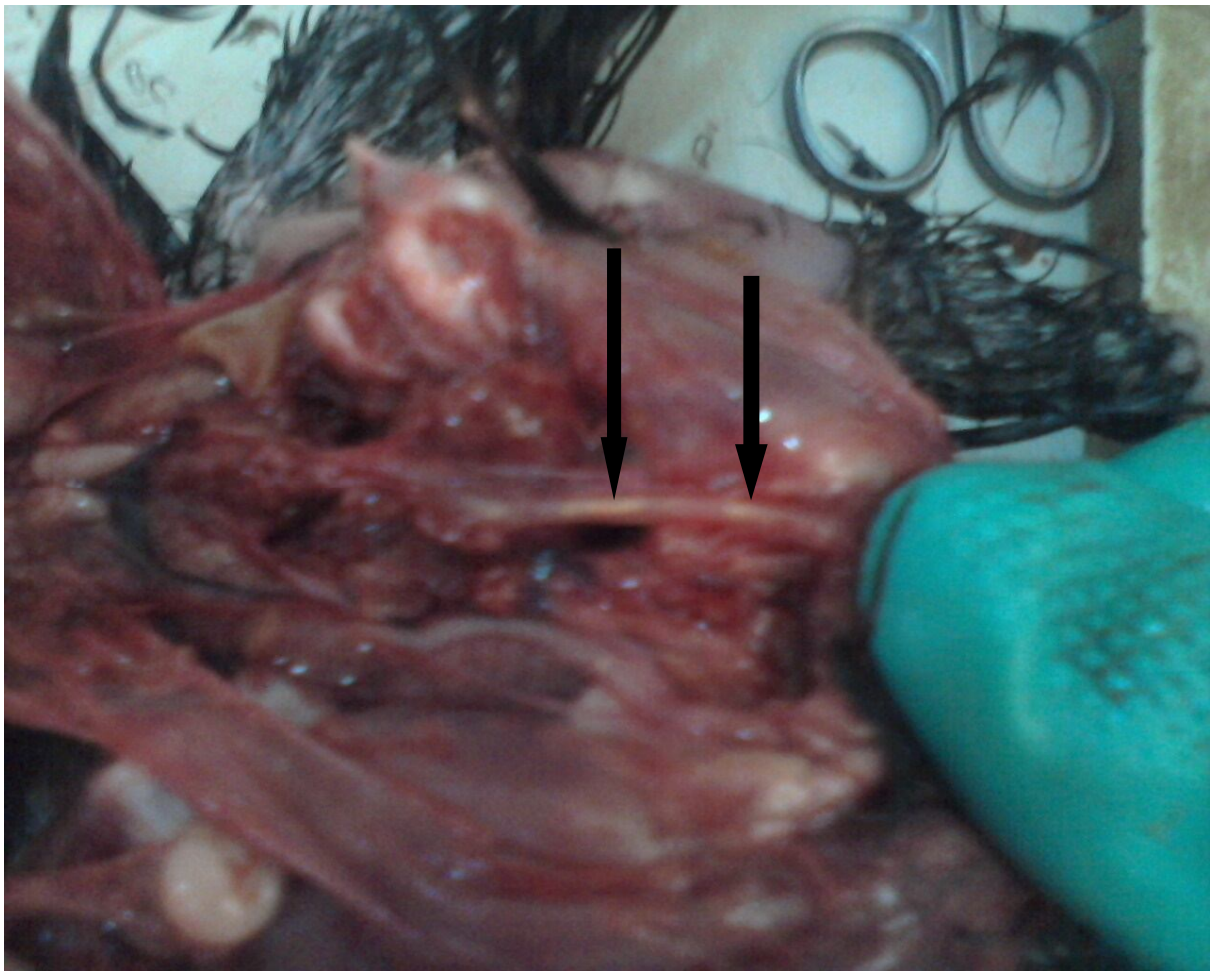


Plate 9. Urate (arrows) deposition in ureter of a cockerel in group B inoculated with vvIBDV at 4 weeks

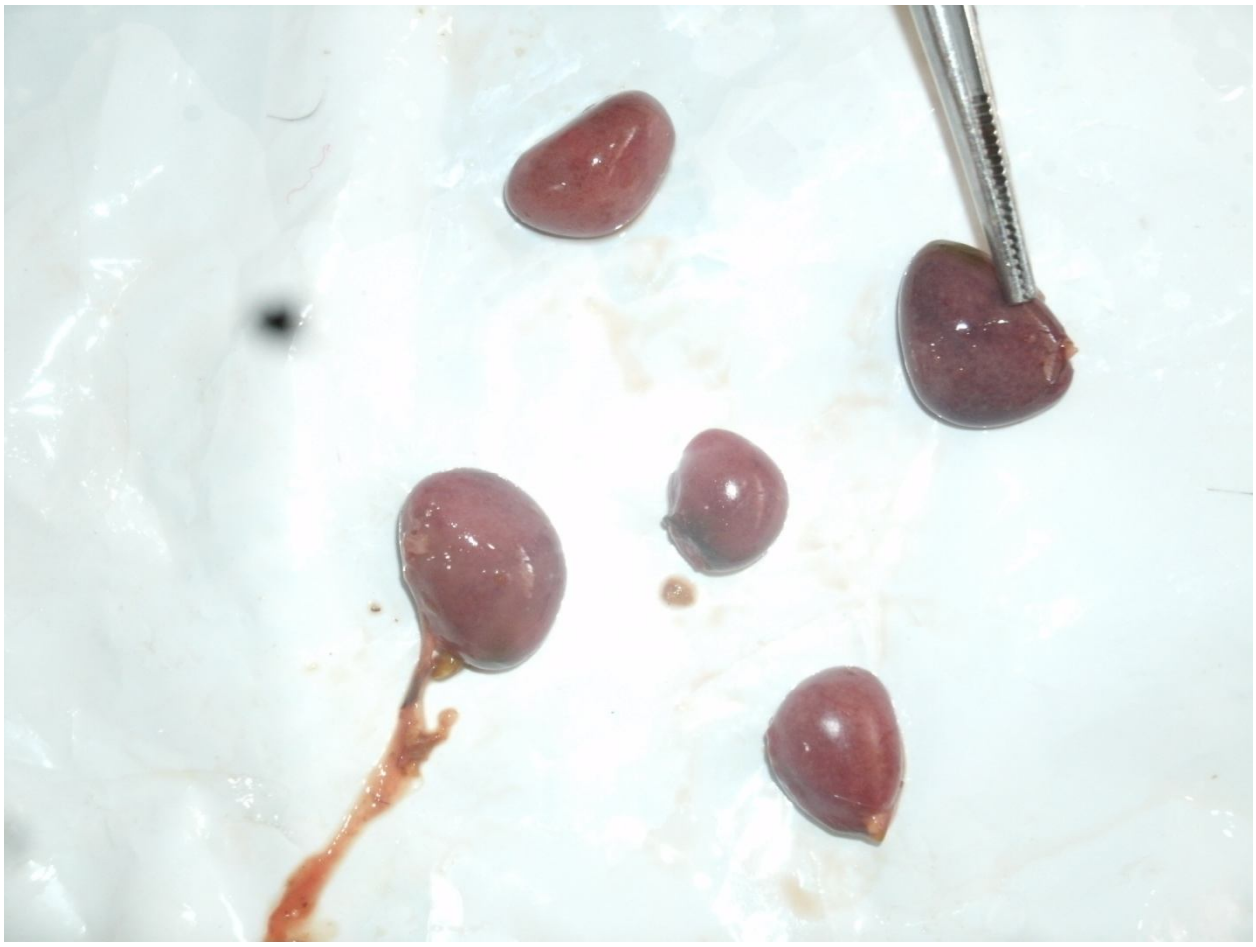


Plate 10. Shiny enlarged and congested spleen of cockerels in group B inoculated with vvIBDV at 4 weeks

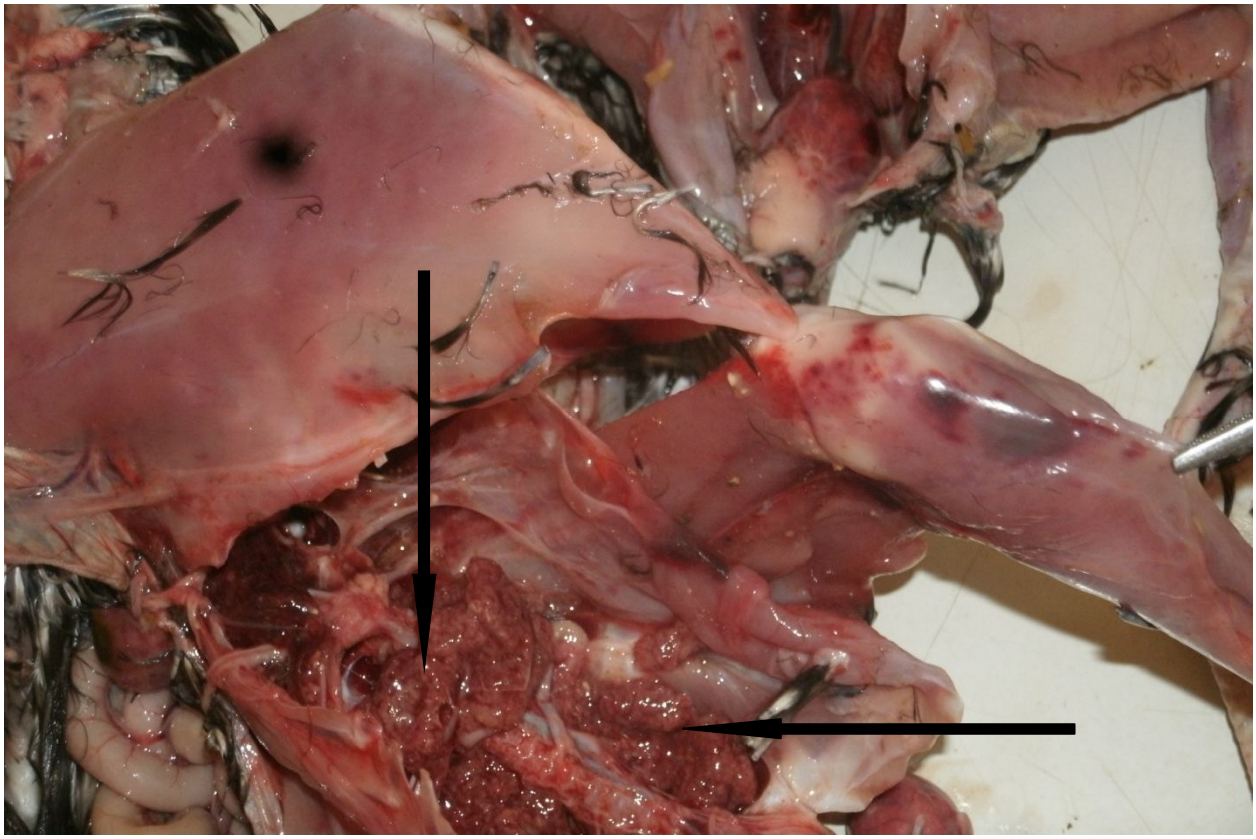


Plate 11. Enlarged and congested kidneys (arrows) of a cockerel in group C inoculated with vvIBDV at 6 weeks

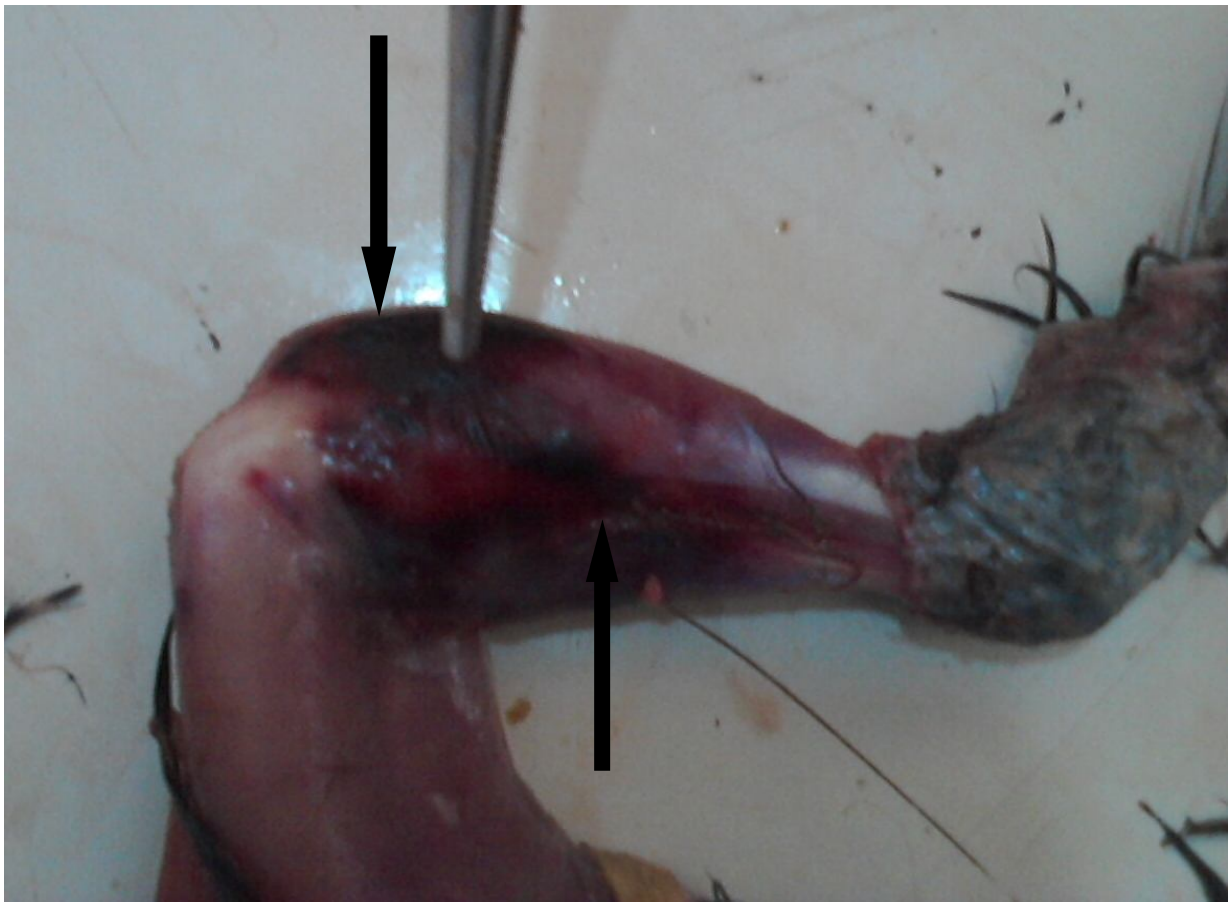


Plate 12. Severe heamorhages on leg and thigh muscles (arrows) of a cockerel in group C inoculated with vvIBDV at 6 weeks



Plate 13. Paint brush haemorhages (arrows) on breast muscle of a cockerel in group C inoculated with vvIBDV at 6 weeks



Plate 14. Enlarged and congested liver of a cockerel in group C inoculated with vvIBDV at 6 weeks

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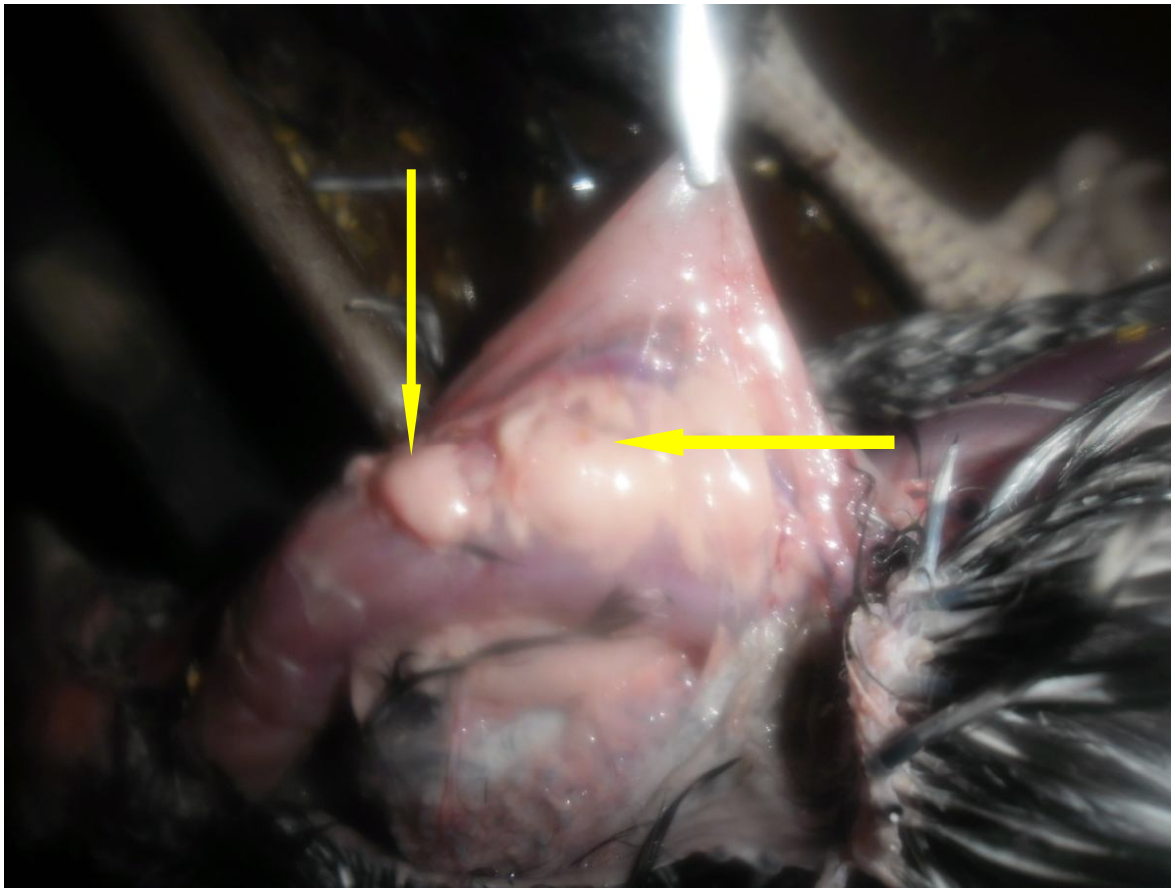


Plate 15. Enlarged thymus (arrows) of a cockerel in group C inoculated with vvIBDV at 6 weeks

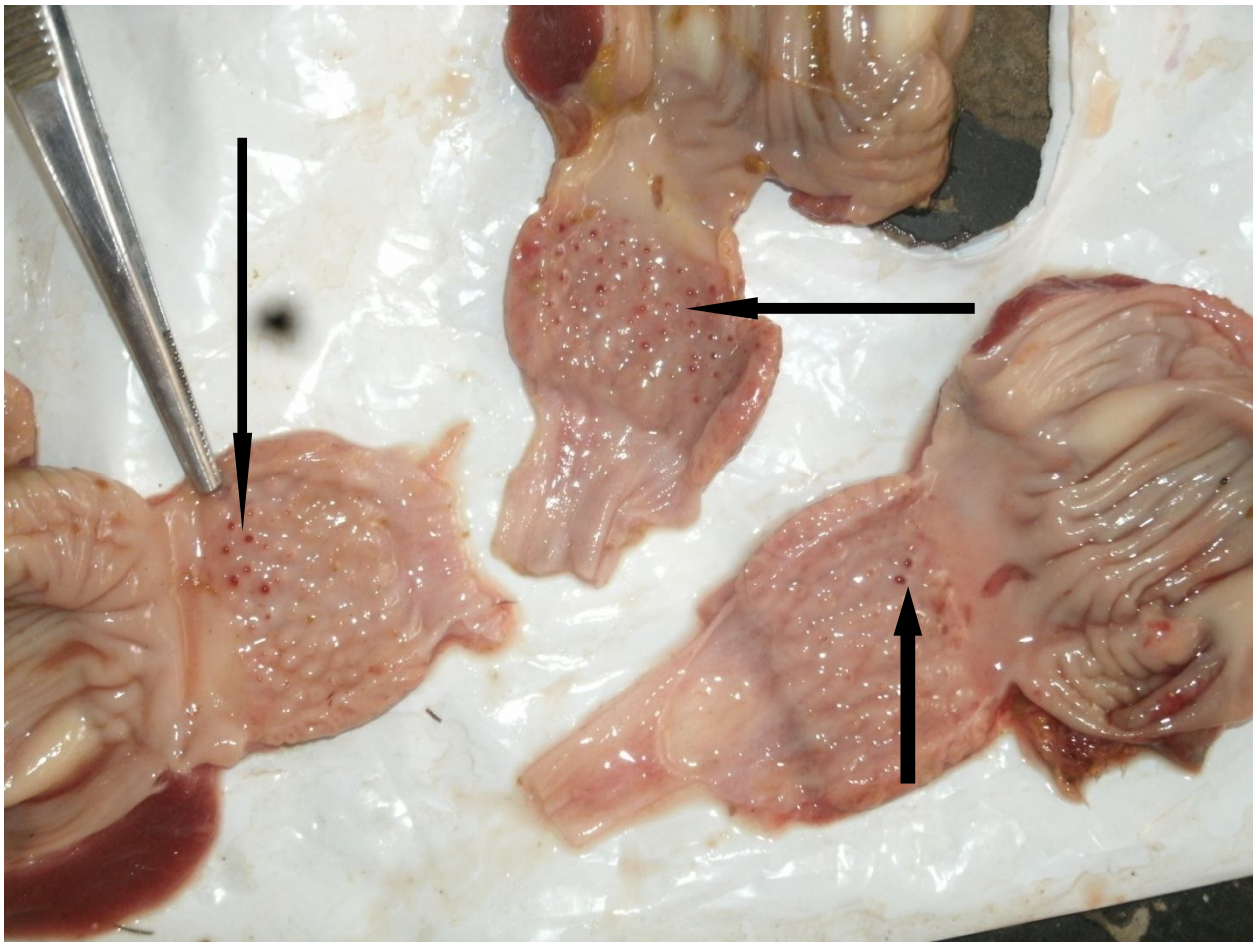


Plate 16. Pinpoint haemorhages in proventriculus (arrows) of cockerels in group C inoculated with vvIBDV at 6 weeks

Table 4.8 Summary of the gross lesions recorded at necropsy in all the groups (B, C and D) challenged with very virulent infectious bursal disease virus

Organ	Gross lesion
Bursa of Fabricius	Congested, haemorrhagic, enlarged and turgid
Kidneys	Congested and enlarged with haemorrhages
Thymus	Enlarged and slightly congested
Spleen	Congested and enlarged
Liver	Congested, enlarged with discoloration
Thigh and breast muscles	Haemorrhages
Breast muscle	Haemorrhages and congestion
Caecal tonsil	Haemorrhages and congestion
Proventriculus	Haemorrhages
Ureter	Deposits of urate crystal

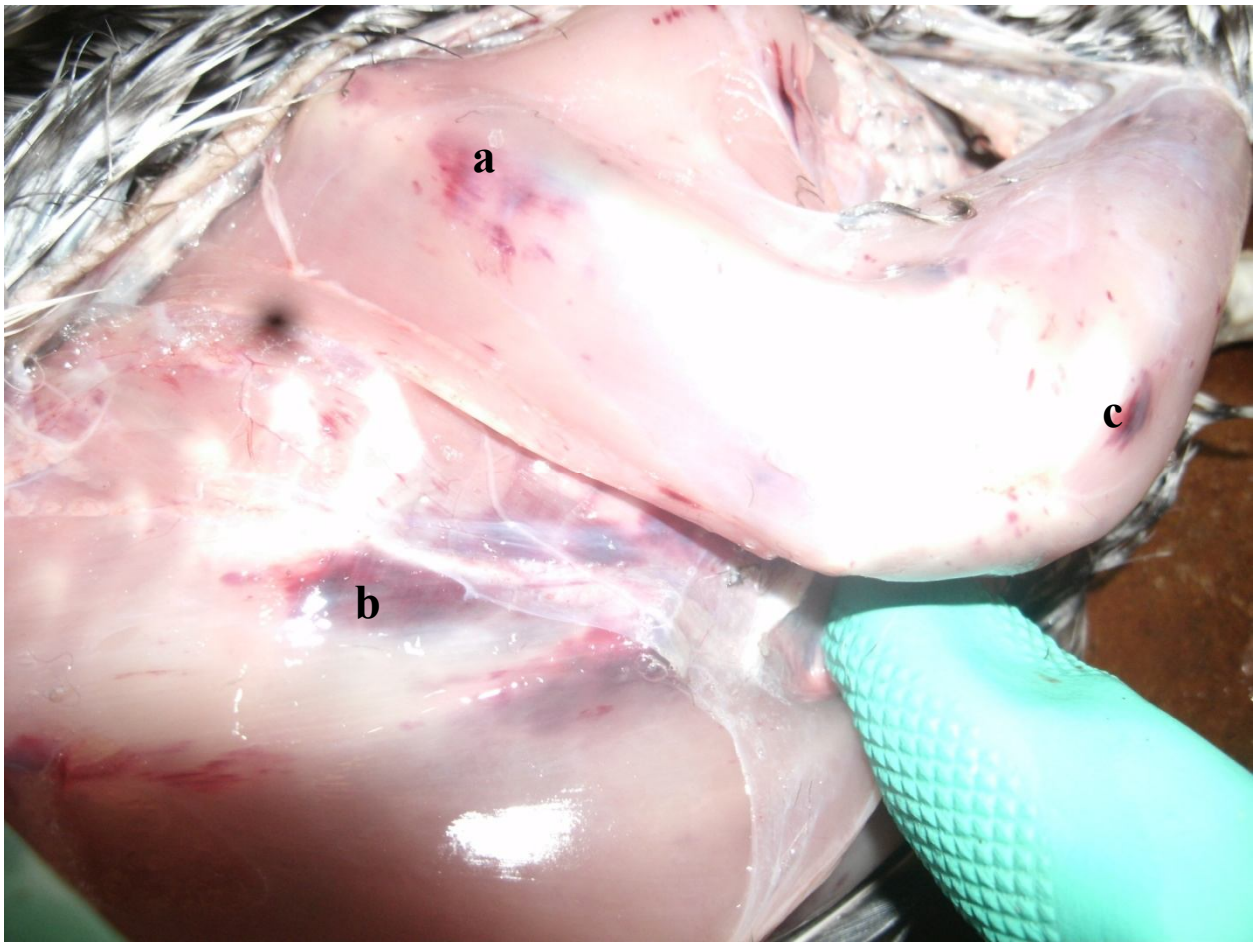


Plate 17. Haemorrhages on thigh (a) breast (b) and leg (c) muscles from cockerel in group A vaccinated with IBDV vaccine at 2 and 4 weeks

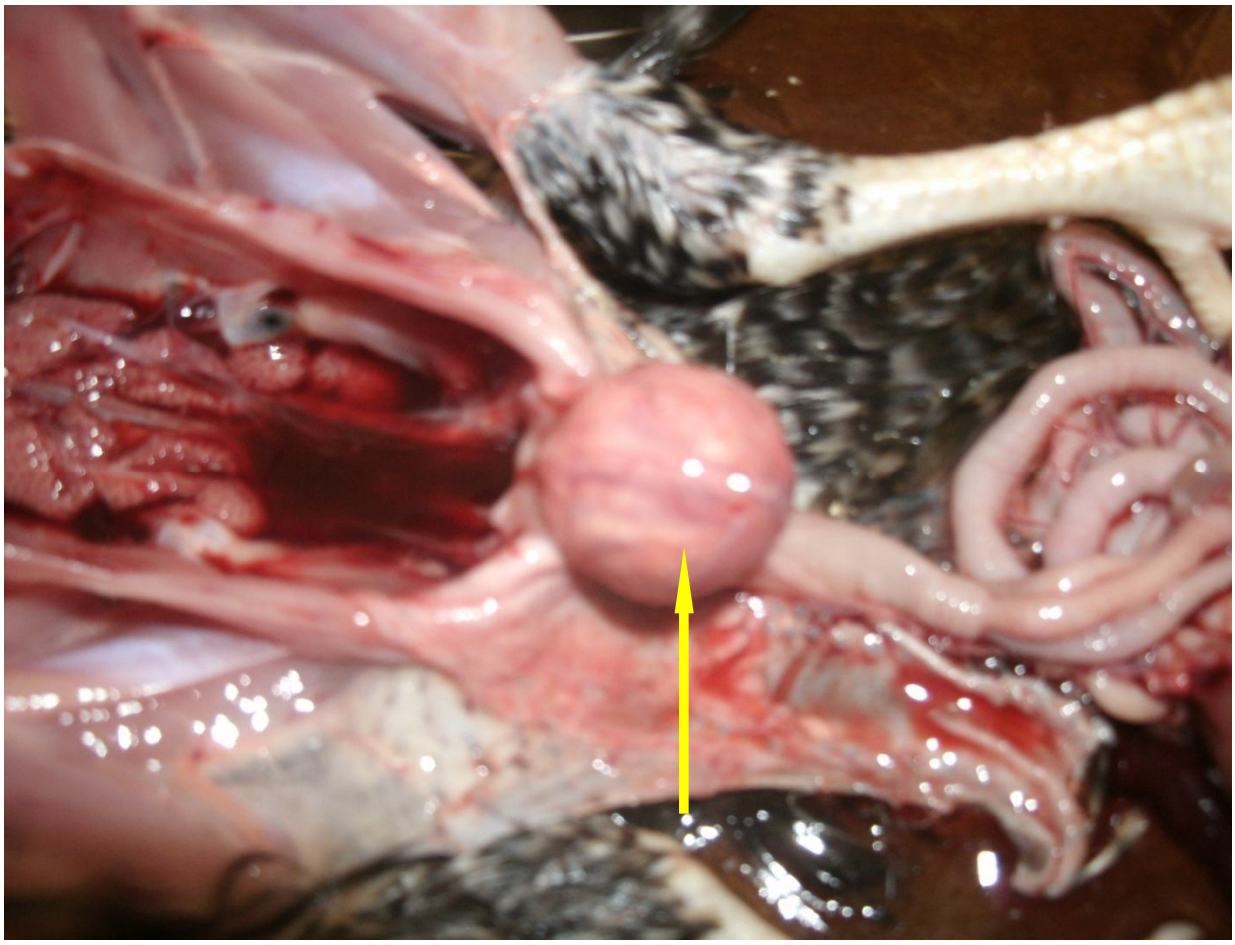


Plate 18. Congested and oedematous bursa of Fabricius (arrow) of a cockerel in group A vaccinated with IBDV vaccine at 2 and 4 weeks

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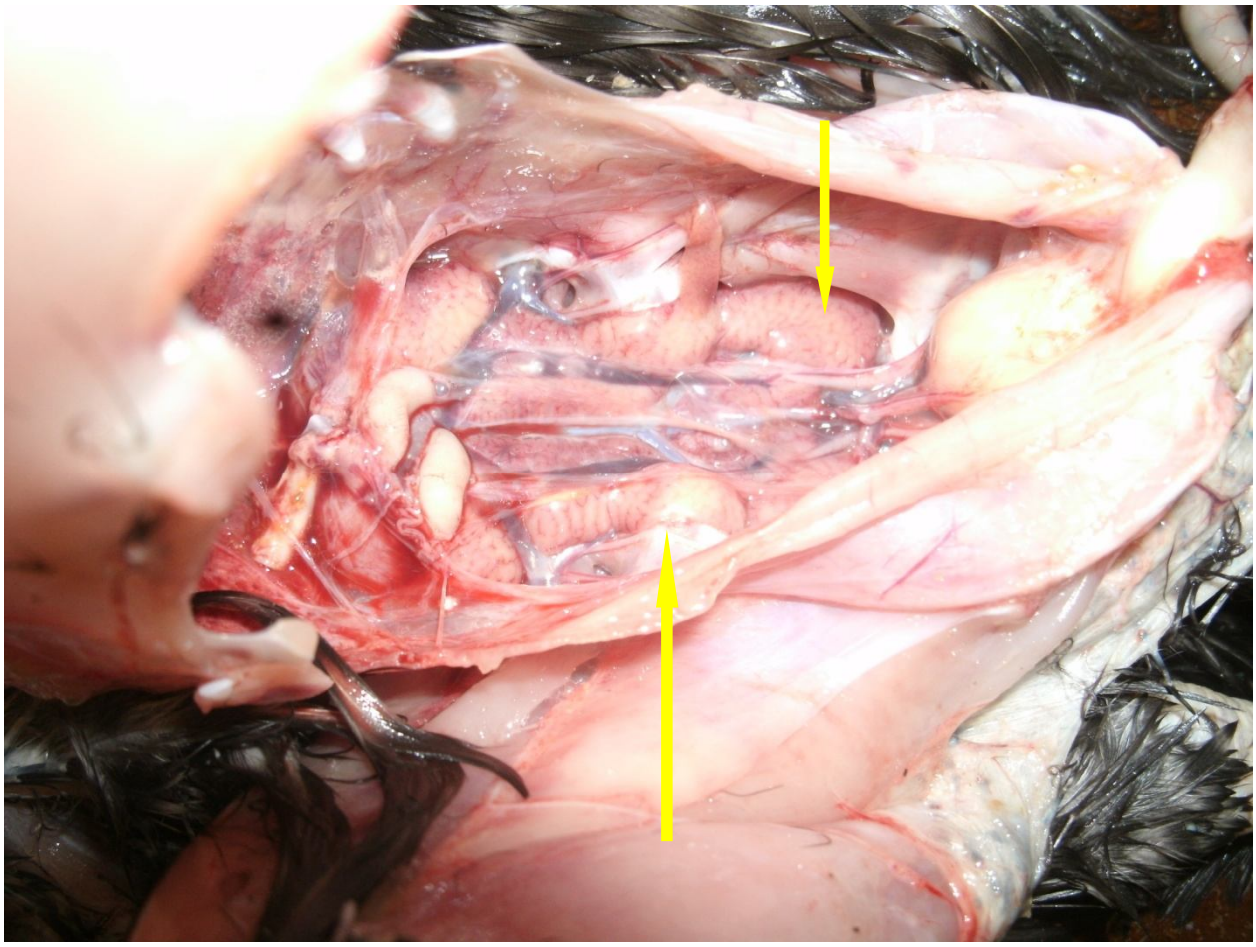


Plate 19. Enlarged and congested kidneys (arrows) of a cockerel in group A vaccinated with IBDV vaccine at 2 and 4 weeks

4.6 Microscopic Lesions

4.6.1 Microscopic lesion in the vvIBDV infected groups

The microscopic lesions observed in the challenged groups were as follows: There were areas of necrosis haemorhages in the thigh muscle (Plate 20). Proventriculus showed areas of necrosis and desquamation of the gland (Plate 21). Bursa of Fabricius showed areas of follicular atrophy, vacuolation, and interfolicular necrosis (Plate 22). Caecal tonsil showed area of necrosis, desquamation and haemorrhages (Plate 23).

4.6.2 Microscopic lesions in the vaccinated groups

The microscopic lesions observed in vaccinated groups were as follows: Proventriculus showed intact gland (Plate 24). Bursa of Fabricius had some hypertrophied follicles (Plate 25). Caecal tonsil showed mild necrosis of villi (Plate 26).

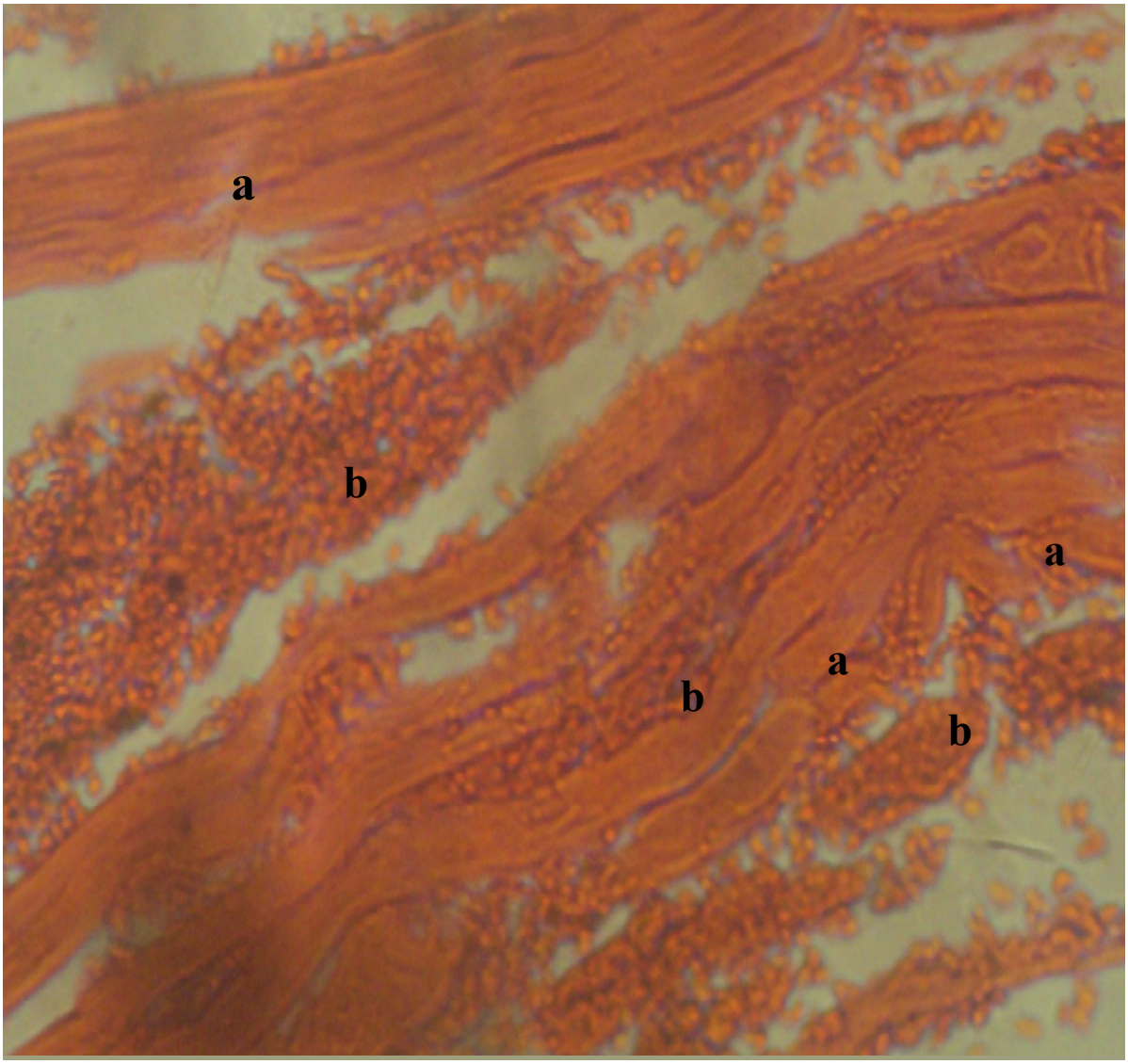


Plate 20.Thigh muscle from cockerel in group B inoculated with vvIBDV at 4 weeks showing areas of necrosis (a) and haemorrhages (b) of the muscle H and E stain ×250

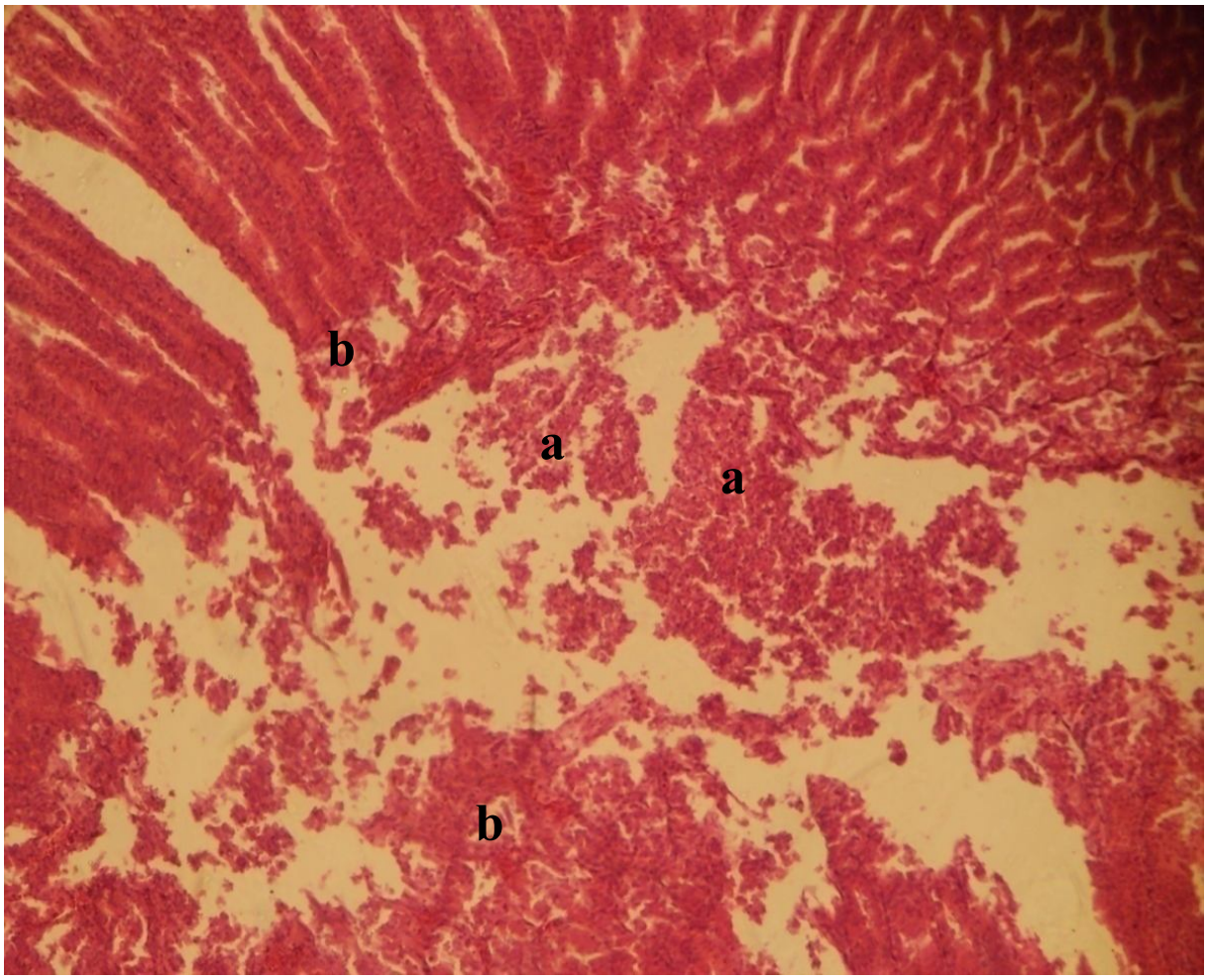


Plate 21. Proventriculus from cockerel in group C inoculated with vvIBDV at 6 weeks showing areas of necrosis (b) and desquamation (a) of the glands. H and E stain ×200.

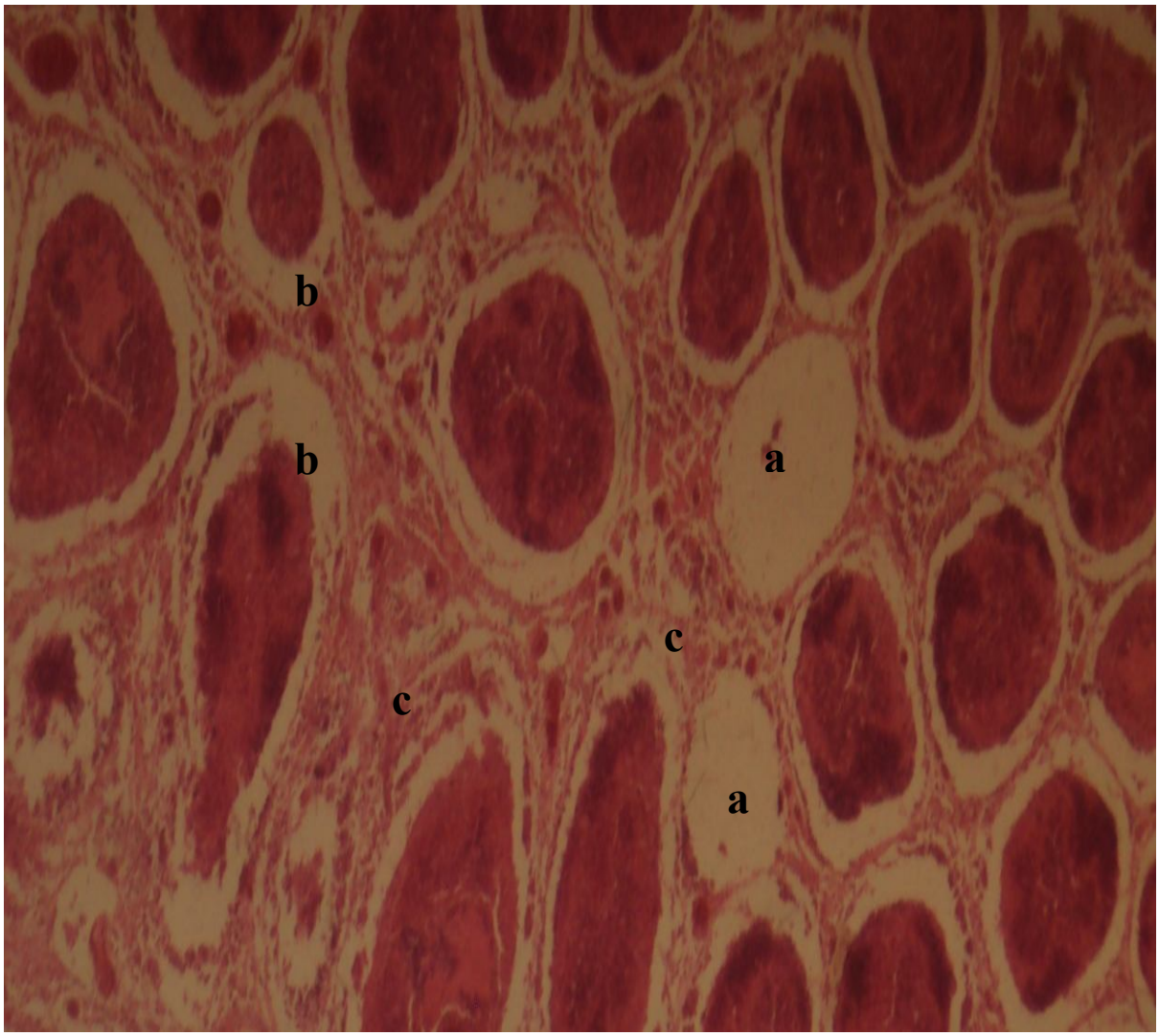


Plate 22. Bursa of Fabricius of a cockerel in group C inoculated with of vvIBDV at 6 weeks showing areas of folicular vacuolation (a), folicular atrophy (b) and interfolicular necrosis (c) H and E stain ×160.

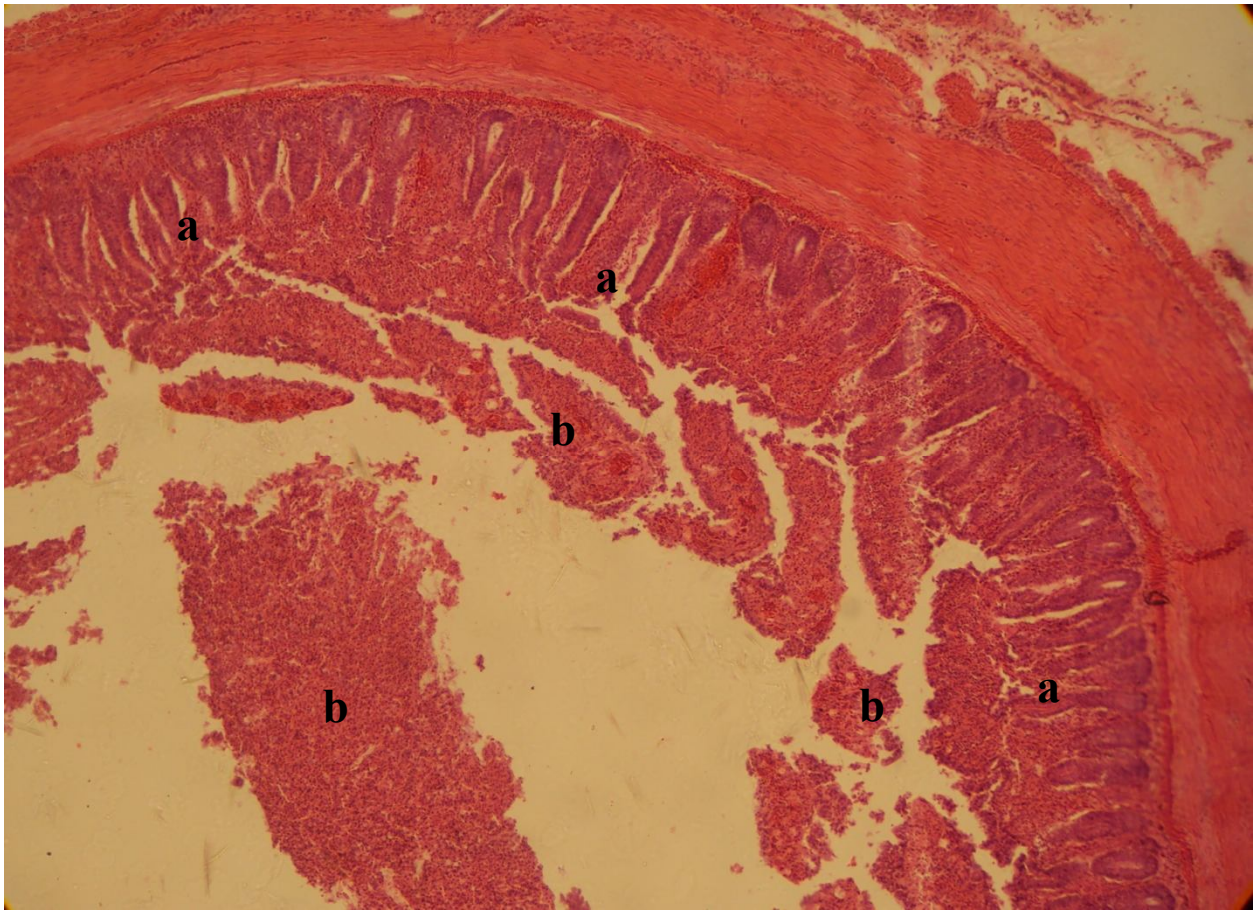


Plate 23. Caecal tonsil from cockerel in group C inoculated with vvIBDV at 6 weeks showing areas of necrosis of the villi (a) and desquamation of mucosae into the lumen (b) (enteritis). H and E stain ×150.

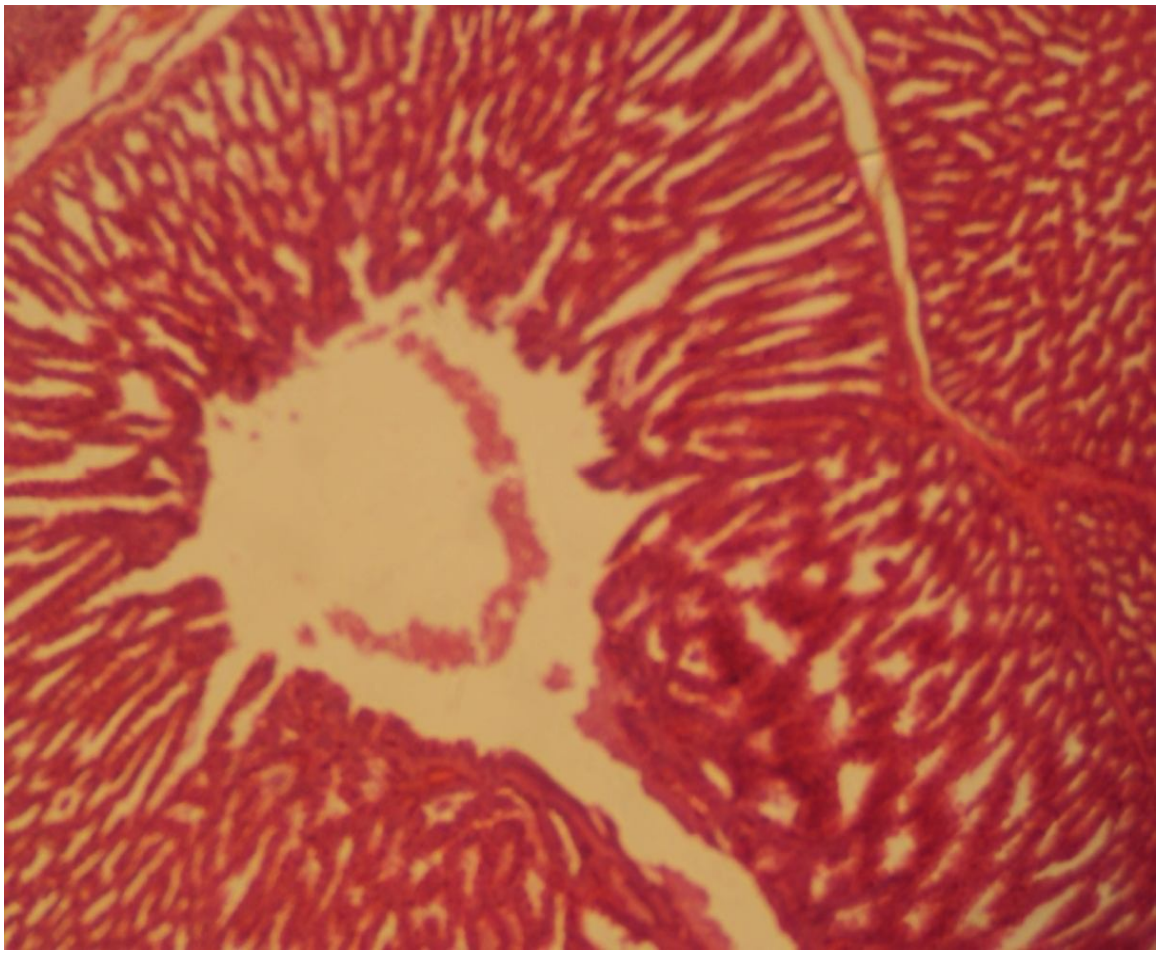


Plate 24. Proventriculus of a cockerel in group A vaccinated with IBDV vaccine at 2 and 4 weeks showing intact proventricular glands. H and E stain ×160.

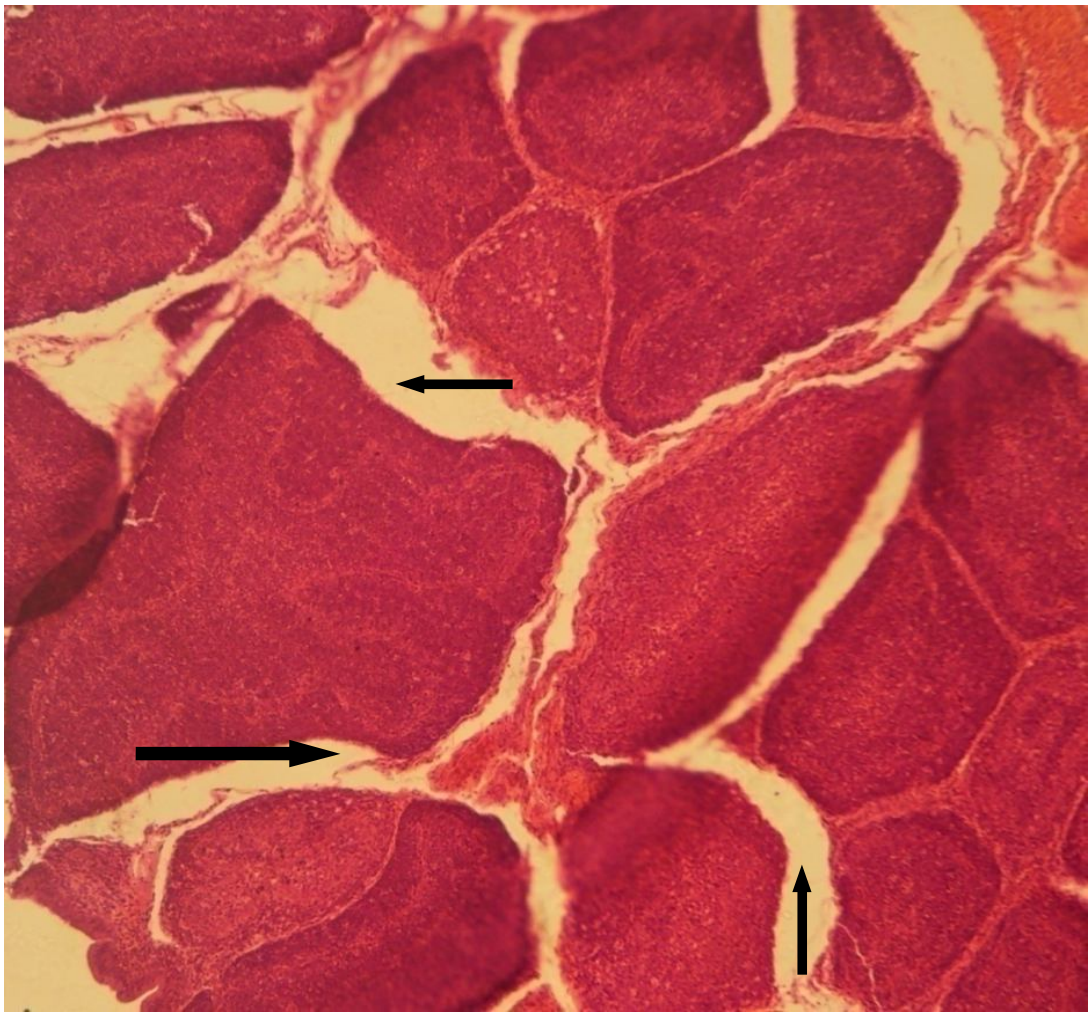


Plate 25. Bursa of Fabricius of a cockerel in group A vaccinated with IBDV vaccine at 2 and 4 weeks had hypertrophied follicles and interfolicular edema (arrows) H and E stain ×160.

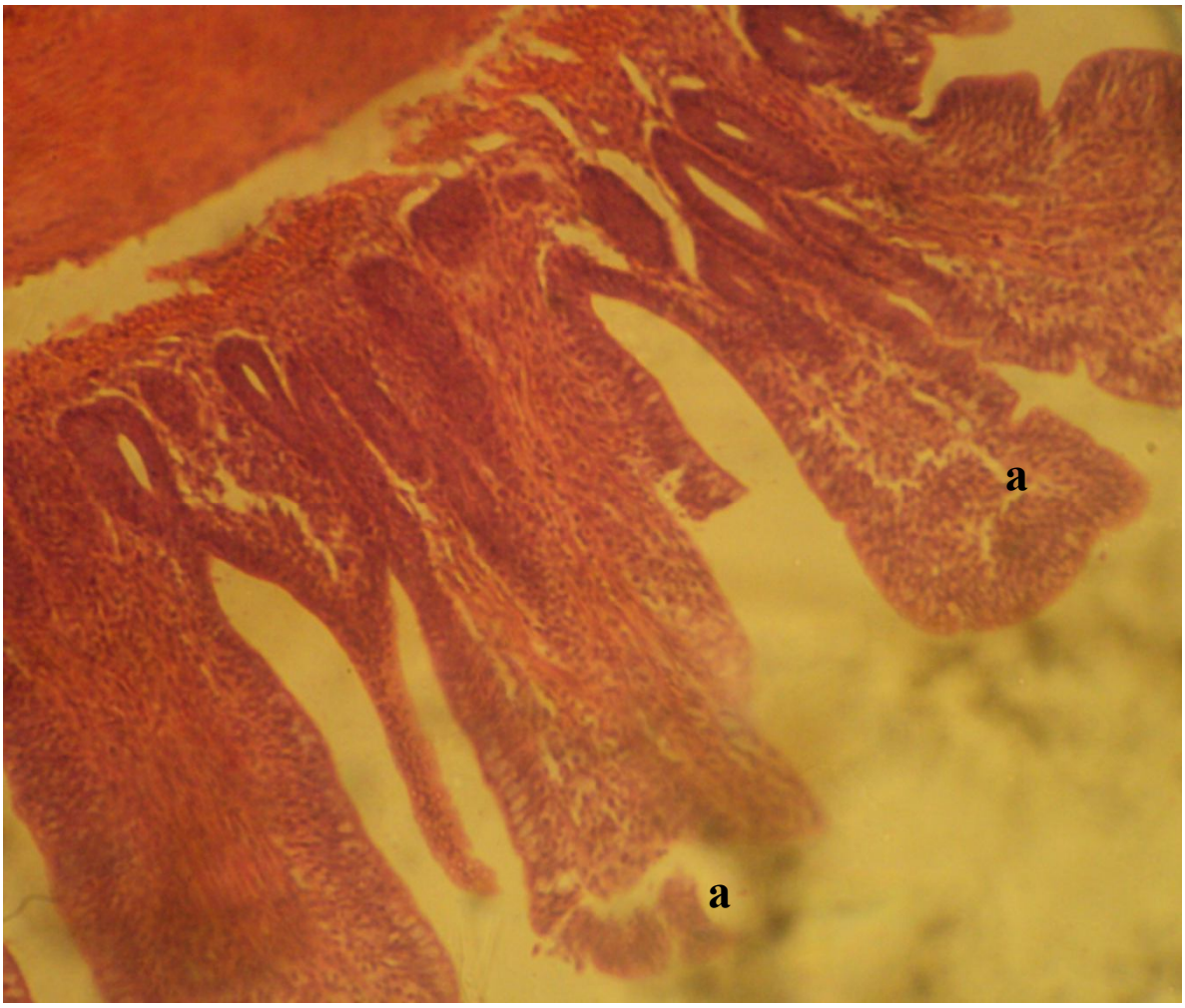


Plate 26. Caecal tonsil of a cockerel in group A vaccinated with IBDV vaccine at 2 and 4 weeks showing areas of necrosis of villi (a) H and E stain ×160.

CHAPTER FIVE

DISCUSSION

The findings in this study that the challenged cockerels showed typical clinical signs of IBD, characterized by ruffled feathers, drooped wings, head hidden beneath wing, reluctant to move, somnolence, whitish diarrhoea, depression, prostration, muscle trembling and recumbency are consistent with the findings in previous reports (Onukwo, 1975; Bishu *et al*., 1977; Oluwayelu *et al*., 2002; Santos *et al.,* 2011).

The Morbidity rate of up to 100% recorded in groups B, C and D, and mortality rates of 54%, 72% and 50% recorded in groups B, C and D, respectively were consistent with findings in works of other scientists who reported that mortality of IBD ranges between 5% - 57% (Anon, 1977; Abdu, 1986; Salami *et al*., 1989; Oluwayelu *et al*., 2002; Santos *et al.,* 2011).Variation of mortality rates among the groups could be due to so many factors. For example, it has been observed that differences in age, breed, type of bird, size of bursae, management practices, climatic conditions, concurrent infection, and degree of virulence of infecting IBDV may be responsible for the variability in the mortality rate (Abdu, 1986; Tsukomota *et al.,* 1995; Oluwayelu *et al*., 2002; Santos *et al.,* 2011).

The haematological values of the vaccinated group (A) in this study when compared with the control group revealed significant reduction ($p < 0.05$) in LYMP (lymphopaenia); and significant increase ($p < 0.05$) in HET (heterophilia). The lymphopaenia was propably due to viraemia caused by the IBD vaccine virus. The gross and microscopic lesions showed slight pathologic lesions in the bursa of Fabricius probably caused by the vaccines virus which could be the cause

of the lymphopaenia. The heterophilia observed could be associated with tissue destruction seen in the organs grossly and microscopically. This tissue destruction might have been caused by IBD vaccine virus.

The Changes in haematological values of challenged group B were not statistically significant ($p > 0.05$) at 7 days pi, but at 14 days pi there were significant increase ($p < 0.05$) in PCV, Hb, and HET (heterophilia); and significant decrease in lymphocytes (lymphoapenia) and TWBC (panleucopaenia).Group C that were challenged at 6 weeks showed no significant differences ($p > 0.05$) in erythrocyte indices as well as the differential leucocytes count at 7 and 14 days post inoculation. At 7 days pi, the following were reduced when compared to the control group: TRBC, PCV, Hb, and LYMP. Total white blood cells (TWBC), EOS and HET were all increased. At 14 days pi the haematological values of the challenged group were close to the control group, signifying some level of recovery. Group D result showed significant increase ($p < 0.05$) in LYMP (lymphopaenia), TWBC (panleucopaenia) and HET (heterophilia) at 7 days pi, while TRBC, PCV and Hb were significantly reduced ($p < 0.05$) at 14 days pi.

In all the challenged groups as well as the vaccinated group there were reduced values of the TRBC, PCV and Hb, indicating some levels of anaemia, which is in consistent with the work of Panigrahy *et al*. (1986) who reported a significant reduction in the values of total erythrocyte count, packed cell volume and haemoglobin concentration 5 days pi. In group D, anaemia persisted up to 14 days pi with the value of TRBC, PCV and Hb remaining significantly reduced. Also, the values of TRBC, PCV and Hb were found to be increased at 14 days pi which indicated polycythaemia that could be due to dehydration, a characteristic finding in IBD infection. These

results have added further insights to the pathenogenesis of IBD. The significant reduction in lymphocytes in this study as evidenced from the microscopic lesions observed in the bursa of Fabricius and thymus could be due to increase in multiplication of IBDV, and consequent, destruction of lymphocyte forming tissues. This is in conformity with work of Oladele *et al.* (2005). According to Campbell and Coles (1986), lymphopaenia occur in acute viral diseases due to glucocorticoide excesses. The significant decrease in TWBC (panleucopenia) which coincides with significant increase in heterophils (heterophilia) in this study is consistent with the work of Oladele *et al.* (2005) that such signify an acute viral infection and cellular necrosis in the tissues. Although, the heterophilia could also be due to secondary bacterial infection.

In this study, it was found that vaccination had no effect on the TCL, LDL, HDL, TRY, TGL and UREA, which is consistent with previous works (Kudair and Alhussairy, 2010) that revealed that vaccination had no effect on levels of glucose, total protein, total lipids cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) and the activity of alanine aminotransferase (ALT). However, the significant reduction ($p < 0.05$) in albumin reported by Kudair and Alhussairy (2010) is contrary to the findings of this study. Total protein in this study was higher in the vaccinated birds and statistically significant ($P < 0.05$) when compared with the control group. This result is contrary to the findings of Kudair and Alhussairy (2010), but consistent with the result of Talebi (2006) who revealed that only values of triglyceride, total protein and albumin of vaccinated chickens were significantly ($P < 0.05$) different from those of corresponding biochemical parameters of control chickens. The concentrations of some serum electrolytes assayed for in this study such as Na, HCO_3, Cl, were not affected by vaccination. This is consistent with previous work of Telabi (2006) where the

values of magnesium, phosphorus, calcium and chloride were not significantly higher ($p > 0.05$) when compared with the control. However, the concentrations of potassium were significantly lower ($p < 0.05$) in the vaccinated group than the control group in this study.

In group B, the concentrations of TCL, LDL, HDL, TRG, CR, Na, K, CL, HCO_3 and ALB in the challenged cockerels were similar to the control cockerels. Similarly in groups C and D the concentrations of biochemical parameters were not significantly different ($p > 0.05$) in both the cockerels challenged and the control, except the concentrations of urea in group C that were statistically increased ($p > 0.05$) than the control.

Changes in urea concentration observed in this study could be due to pathologic lesions, such enlarged and haemorrhagic kidneys; and glomerular necroses seen grossly and microscopically in the kidneys. Whitish diarrhoae and urate crystal deposit in the ureter seen in this study might also be contributory factors that significantly altered urea concentration. Significantly lower ($p<0.05$) cholesterol concentrations in this study might have been due to lesions, such as congestion and haemorrhages seen in the liver.

From this study maternally derived antibodies (MA) in chicks were present from day old which correspond with the previous report that MA are present in chicks (Abdu, 1986; 1988; 1990). The MA from day old lasted up to 4 weeks in this study which is contrary to the results of Abdu (1990) and Kumar *et al*. (2000) who reported that by 4 weeks all birds were devoid of MA. The MA varies with source of birds, and the higher the MA from day old, the longer it last in circulation (Abdu, 1990).

The group of cockerels vaccinated at 2 weeks, when screened for vaccinal antibody at 3 weeks showed almost equal antibody titres when compared with the control group. This might be due to the presence of MA which interferes with the seroconversion as reported by (Abdu *et al.,* 2001). At 5 weeks following vaccination at 4 weeks the antibody titre was present but low. It was reported that all vaccinated birds sero- converted at one week (Okeke *et al*., 1982), two weeks (Abdu*.,* 1988) three weeks (Abu- adulugba *et al*., 1992) after vaccination. The chicks were believed not to have completely sero- converted in this study. This could be due to the type of vaccine administered or presence of MA at the time of vaccination (Okeke *et al*., 1982 and Aba-Adulugba *et al.,* 1992).

Abdu (1987) reported that antibodies following IBDV challenge were detected 7 days post infection, which is consistent with the results of the present work, where all the 3 groups challenged with vvIBDV had antibodies 7 days pi, with highest titre in the group that was challenged at 4 weeks, followed by those challenged at 6 weeks, then 8 weeks. Contrary to the report of Abdu (1987) that antibodies are highest in older birds, in this study highest antibody were observed in younger birds 7 days pi and in the older birds at 14 days pi. This variability might be due to age differences.

Frequency of birds encountered with haemorrhages and congestion was higher in bursa of Fabricius, the thigh, leg and breast muscles; while the mucosae of proventriculus in the challenged chicks also showed variable haemorhages . These results are also consistent with those that had been reported in other studies (Onukwo, 1975; Bishu *et al*., 1977; Okoye, 1982 Oluwayelu *et al*., 2002; Santos *et al.,* 2011). The finding that the spleen and thymus were

enlarged and congested and there were haemorhages on caecal tonsils, and the Harderian gland are consistent with those findings of previous authors (Okoye, 1982; Henry *et al.,* 1988; Tsukomota *et al.,* 1995; Inoue *et al.,* 1999; Oluwayelu *et al*., 2002). Enlarged, haemorrhagic and congested kidney, with deposits of urate crystals in the ureter were also reported by Ojo *et al*. (1973); and Santos *et al.* (2011).

The gross lesions observed in the vaccinated cockerels (group A) were congested and oedematous bursa of Fabricius, congested kidneys and liver, pinpoint haemorrhages on thigh, leg and breast muscles; and the microscopic lesions observed were congested liver, hypertrophied and edematous bursa of Fabricius. Caecal tonsil and proventriculus were apparently intact. These lesions are comparable with the challenged groups, but they were milder. These findings are in consistent with works of other authors (Giambrone *et al*., 2001; Luengo *et al*., 2001; Hussain *et al*., 2001; Block *et al.,* 2007; Moura *et al*., 2007). They reported bursal lesions in flocks vaccinated against IBDV 7 days post vaccination.

The microscopic lesions observed in the challenged groups in this study such as folicular vacuolation, folicular atrophy, interfolicular necrosis, congestion; and haemorrhages of bursa of Fabricius; necrosis, congestion and haemorrhages of breast, leg and thigh muscles; necrosis, congestion, haemorrhages and desquamation of proventriculus and caecal tonsil; congestion and haemorrhages of the liver; Necrosis, congestion, haemorrhages and depletion of thymus are similar to the findings of other authors (Okoye and Uzoukwu 1981; Okoye, 1982; Oluwayelu *et al*., 2002).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

From this study it was concluded that:

1. Very virulent IBDV has effect on some haematological parameters, resulting in decrease in PCV (13%), TRBC (13%) and Hb (13%). Panleucopaenia (51%), lymphopaenia(10%), heterophilia (59%) and eosininophilia (81%).

2. Infectious bursal disease vaccine also causes some changes on the haematological parameters such as heterophilia (31.1%) and lymphopaenia (5.9%).

3. Very virulent IBDV has effect on some biochemical parameters, resulting in decrease in TP (20%) and TC (34.9%); and an increase in urea (49%).

4. Infectious bursal disease vaccine used in this study caused a decrease in potassium (17.6%) and total protein concentration (11.55%).

6.2 Recommendations

1. Further studies should be carried out at the molecular level on the effect of field IBDV infection on serum total protein, urea and cholesterol levels.

2. Some of the parameters can support and aid the diagnosis of IBD.

REFERENCES

Aba- Adulugba, E.P., Ibu, J.O. Ohenekwu, H. and Majiyagbe, K. A. (1992). A comparative evaluation of a Nigerian and three strains of live infectious bursal disease (Gumboro) vacccines. *Bulletin of Animal Health and Production in African,* 41: 8-9.

Abdu, P. A. (1986). Infectious bursal disease vaccination failure in chicken in Nigeria. *Tropical Animal Health and Production,* 18 (2): 123-125.

Abdu, P. A. (1987). A retrospective study of infectious bursal disease. *In*: Proceeding of the 11th *Annual Conference of the Nigerian Society for Animal Production, Zaria.* Pp 65-69.

Abdu, P. A. (1988). A case report: Infectious bursal disease in a flock of broilers and local Nigeria chickens. *Bulletin of Animal Health and Production in African,* 36 (3): 269-271.

Abdu, P. A. (1990). Precipitin antibodies to infectious bursal disease of chicken in Zaria, Nigeria, Unpublished Report.

Abdu, P.A. and George, J.B. (1986). Outbreak of infectious bursal disease among local chicken in Zaria Nigeria. *Tropical Veterinarian,* 4: 143-144.

Abdu, P.A. and Saidu, L. (1995). Disease of local chicken and possible method of their control. Workshop on small animal and poultry production. NVRI Vom, May 30th- June 3rd, 1995.

Abdu, P. A., Abdullahi, S.U. and Adesiyun, A. (1985). Infectious bursal disease virus antibody in chickens from Fulani nomads around Zaria. *Nigerian Veterinary Jour*nal, 14 (1): 61-62.

Abdu, P.A., Salami, J.O. and Rahamatullah. P.M. (1986). Infectious bursal disease of chicken in Zaria and Kaduna, Nigeria. 23rd *Annual Conference of the Nigeria Veterinary Medical Association,* Jos. Nov 17th-20th, 1986.

Abdu, P.A., Mera, U.M. and Saidu, L. (1992). A study on acute chicken mortality in Zaria, Nigeria. National Workshop on Livestock and Veterinary Service, NVRI, Vom. August 11th- 14th, 1992.

Abdu, P.A., Umoh, J.U., Abdullahi, S.U. and Saidu, L. (2001). Infectious bursal disease (Gumboru) of chicken in Nigeria. *Tropical Veterinarian,* 19(4): 216-236.

Aengwanich, W. and Tanomtong, A. (2007). Blood cell characteristics and hematological valuess of free ranging- Red jungle fowl*(Gallus gallus*) in Northeastern Thailand. *Journal of Biological Science,* 7(4): 689-692.

Agbede, R.I.S. (1975). Gumboro disease. *Student Veterinarian*, 2: 11-15.

Al-Homidan, A., Al-Qarawi, A.A., Al-Waily, S.A., and Adam, S.E. (2002). Response of broiler chicks to dietary *Rhazya stricta* and *Nigella sativa. Brazilian Poultry Science,* 43: 291-299.

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 Pathology,* 13: 419-427.

Almeida, J. D., Berry. D. M., Cunningham, C. H., Hamre, D., Hofstad, M. S., Mallucci, L., McIntosh, K.. and Tyrrell. D. A. J. (1968). Coronaviruses. *Nature,* 220:650.

Anonymous. (1977), Gumboru disease in the Federal Republic of Nigeria. *Bullatin de office Internationale des Epizootic*, 88: 391-392.

Aqawane, S.B. and Lonkar, P.S. (2004). Effect of probiotic containing *Saccharomyces boulardii* on experimental ochratoxicosis in broilers. *Journal of Veterinary Science,* 5: 359-367.

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

Bacon, L. D., Witter, R. L., Silva, R. F. (2001). "Characterization and experimental reproduction of peripheral neuropathy in White Leghorn chickens". *Avian Pathology,* 30 (5): 487–499. doi:10.1080/03079450120078680. PMID 19184938.

Baker, F.J., Silverton, R.E. and Pillister, C.J. (2000). Dehydration, impregnation, embedding technique and section preparation. *Introduction to Medical Labouratory Technology,* 7*th* edition. 199 – 242.

Barger, A.M. (2004). The blood cell count: A powerfull diagnostic tools in veterinary clinic: *Small Animal Practice*, 33:1207-1222.

Barros, J.M.S., Gomes, P.C., Albino, L.F.T., and Nascimento, A.H. (1998).Sodium levels over performance parameters of broilers chicken from 1 to 21 days of age. Avian *Conference of Science and Technology*, Brazil.

Bayliss, C.D., Spies, U., Shaw, K., Peters. R.W., Papageorgiou. A., Muller, H. and Boursnell, M.E.G. (1990). A comparison of the sequences of segment A of four infectious bursal disease virus strains and identification of a variable region in VP2. *Journal of General Virology,* 71: 1303-1312.

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

Benjamin, M.M. (1985). Outline of veterinary clinical pathology, 3rd Edition, The Iowa state university press, Ames, USA pp 361.

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.

Bishu, G., Alhaji, I. and Akerejola, O.O. (1977). Effect of Infectious bursal disease on poultry production in Nigeria. *World's Poultry Science Association (Nigeria branch) Ile-ife Nigeria.* March 25th-26th 1977.

Block, H., Meyer-Block, K., Rebeski, D. E., Scharr, H., deWit, H., 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 antibodies. *Avian Pathology,* 36:5, 401-409.

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

Brandt, M., Yao, K., Liu, M., Heckert, R.A. and Vakharia, V.N. (2001). Molecular determinants of virulence, cell tropism, and pathogenic phenotype of infectious bursal disease virus. *Journal of Virology*, 75.

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

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

Burtis, C.A., Ashwood, E.R., Bruns D.E., Saunders, W.B. Tietz Textbook of Clinical Chemistry. Fourth edition . Company, Philadelphia, 2006;24:801-803

Campbell, T.W. (1995). Avian Haematology. *In:* Avian Haematology and Cytology, 2nd Edition T.W. Campbell (Ed). Iowa State University Press, Ames, Iowa. Pp 3-25.

Campbell, T.W. and Coles, E.H. 1986. Avian clinical pathology. *In*: Coles, E.H (ed). *Veterinary Clinical Pathology*, W.B. Saunders company, Philadephia; 279-301.

Cavanagh, D., and S. A. Naqi. (2003) Infectious bronchitis. *In: Diseases of poultry,* 11th ed. Y. M. Saif, ed. Iowa State University Press, Ames, IA. pp. 101–120.

Cetin, M., Deniz, G., Polat, U., Yalcin, A. and Tarihi, G. (2002). The effects of inorganic and organic selenium supplementation on biochemical blood parameters in broiler. Uludag University, *Journal of Veterinary Medicine,* 21: 59-63.

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

Chineme, C.N. and Cho, Y. (1984). Clinicopathological and morphological changes in chickens experimentally infected with infectious bursal disease virus. *Tropical Veterinarian,* 2:218-224.

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

Cho. Y. and Edgar, S.A. 91972). Characterization of infectious bursal agent. *Poultry Science* 51: 60-69.Onukwo.O. (1975). An outbreak of infectious bursal (IBD) of chicken in Nigeria. *Veterinary Research,* 97:433.

Christie, G. and Halliday, W.G. (1979). Haematology and biochemical aspects of an *Escherichia coli* in Brown leghorn. *Avian Pathology,* 8: 45-55..

Coles H. E. (1986). Veterinary Clinical Payhology, 4th Edition Philaldephia, PA, UAS, Wb Saunders, Pp 17-23.

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

Csatary, L.K., Moss, R.W. Beuth, J., Töröcsik, B., Szeberenyi, J., Bakacs, T. (1999). "Beneficial treatment of patients with advanced cancer using a Newcastle disease virus vaccine (MTH-68/H)". *Anticancer Research,* 19 (1B): 635–8. PMID 10216468.

Deepak A. R., Le, T., Bhushan, V. (2007). *First Aid for the USMLE Step 1 2008 (First Aid for the Usmle Step 1)*. McGraw-Hill Medical. ISBN 0-07-149868-0.

Durairaj, V., Sellers, H.S., Linnemann, E.G., Icard, A.H. and Mundt, E. (2011). Investigation of the antigenic evolution of field isolates using the reverse genetics system of infectious bursal disease virus (IBDV). *Archives of Virology*, 156: 1717-1728.

Durojaiye, O.A., Ajibode, H.A., Olafimihan, G.O. (1985). An outbreak of an infectious bursal disease virus in 20 weeks old birds.*Tropical Veterinarian,* 2(3):175-176.

Dybing, J.K. and Jackwood, D.J. (1998). Antigenic and immunogenic properties of baculovirus-expressed infectious bursal disease viral proteins. *Avian Diseases, 42*, 80– 91.

Eeckeren, N., Mass, A., Saatkanpp, H. and Verschuur, M. (1995) Small scale poultry production in the tropics. *Wageninger Agromisa*, Agrodok No4.

Eroksuz, Y., Eroksuz, H., Ozer, H., Canatan, H., Yaman, I. and Cevik, A. (2001). Toxicity of dietary *Heliotropium dolosum* seed to broiler chickens. *Veterinary and Human Toxicology*, 43: 334-338.

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., Picault, J.P., Drouin, P., Guittet, M., L'Hospitalier, R., Bennejean, G. (1992). Pathogenicity and preliminary antigenic characterization of six infectious bursal disease virus strains isolated in France from acute outbreaks. *Zentralbl Veterinary Medicine,* B 39: 683–691.

Fabricant, C.G and Fabricant, J. (1999). "Atherosclerosis induced by infection with Marek's disease herpesvirus in chickens". *American Heart Journal,* 138:5-2: S465–8. doi:10.1016/S0002-8703(99)70276-0. PMID 10539849.

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

Fenner, F. J., Gibbs, E., Paul, J., Murphy, F. A., Rott, R., Studdert, M. J., White, D. O. (1993). *Veterinary Virology (2nd ed.)*. Academic Press, Inc. ISBN 0-12-253056-X.

Gary, D.B. and Richard, D.M. (2003). Infectious bursa disease in commercial broiler, *In*:VM84n Veterinary Medicine-Larege Clinic Sciences Department, Florida cooperative Extension Extension Service, Institute of Food and Agricultural Science, University of Florida. Retrieved October 16,2008 from: *http://idis.ifas.ufl.edu*.

Giambrone, J. J., Dormitorio, T., and Brown, T. (2001). Safety and efficacy of in ovo administration of infectious bursal disease viral vaccines. *Avian Diseases*, 45: 144-148.

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

Gunes, N., Polat, U., Petek, M. and Tarihi, G. (2002). Investigation of changes in biochemical parameters of hens raised in alternative housing systems. Haematobiochemical studies. *Journal of Veterinary Medicine,* 21: 39-42.

Harris, D. J. (2009). Clinical Tests: *In Hand book of Avian Medicine* Chapter 4, Edition: Thomas Tully, Elsevier Limited Publcation.

Hawkey, C., Samour, J.H., Ashton, D.G., Hart, M.G., Cindey, R.N., Finch, J.M. and Jones, D.M. (1983). Normal and clinical haematology of captive cranes (Griform) *Avian Pathology,* 12: 73-74.

Hawkey, C.M., and Dennatt, P. (1989): Acolour atlas of comparative veterinary haematology. Wolfe Medical Publication, England; 192.

Hayhoe, G.F. and Flemans, R.J. (1990) A colour atlas of haematology and cytology. ELBS. The Neitherlands; 240.

Henry C.W., Brewer R.N., Edgar S.A. and Gray B.W. (1980). Studies on infectious bursal disease of chickens: 2 – scoring microscopic lesions in the bursa of Fabricius, thymus, spleen and kidney in gnotobiotic and battery reared white Leghorns experimentally infected with infectious bursal disease vims. *Poulry Science***,** 59: 106-101.

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., Kawamoto E. and Shimakura S. (1976). Some properties of precipitating antigens associated with infectious bursal disease virus. *Infectious Immunology***,** 10: 1235-1240.

Hirai K., Shimakura S. and Hirose M. (1997). Immunodiffusion reaction to avian infectious bursal virus. *Avian Diseases*, 16: 961-964.

Hirai, K . (2001). *Current Topics in Microbiology and Immunology: Marek's Disease (Current Topics in Microbiology and Immunology)*. Springer: Berlin. ISBN 3-540-67798-4.

Hirai, K. and Calnek, B.W. (1979). *In vitro* replication of infectious bursal disease virus in established lymphoid cell lines and chicken B lymphocytes. *Infection and Immunity, 25*: 964–970

Hirai, K., Funakoshi, T., Nakai, T. and Shimakura, S. (1981). Sequential changes in the number of surface immunoglobulin-bearing B lymphocytes in infectious bursal disease virus-infected chickens. *Avian Diseases, 25*: 484– 496.

Hitchner S.B. (1970). Infectivity of infectious bursal disease virus for embryonating eggs. *Poultry Sci*ence, 49: 511-516.

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

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

Hussain, I., Ahmad, A., Ashfaque, M., Mahmood, M. S., Akthar, M. (2001). Pathenogenic properties of infectious bursal disease virus. *Pakistani Veterinary Journal*, 21 (4).

Ifon, E.T. and Basir, O. (1980) The nutritive value of some Nigeria leafy vegetable, particularly vitamin and mineral contents. *Food Chemistry,* 4: 263-267.

Iheukwumere, F.C. and Herbert, U. (2000). Physiological responses of broiler chickens to quantitative water restrictions: Haematology and serum biochemistry. *International Journal of Poultry Science,* 2: 117-119.

Ikuta, N., El-Attrache, J., Villegas, P., Garcia, E.M., Lunge, V.R., Fonseca, A.S., Oliveira, C. and Marques, E.K. (2001). Molecular characterization of Brazilian infectious bursal disease viruses. *Avian Diseases,* 45: 297–306.

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

Inoue, M., Yamamoto, H., Matuo, K. and Hihara, H. (1992). Susceptibility of chicken monocytic cell lines to infectious bursal disease virus. *Journal of Veterinary and Medical Science, 54*: 575–577.

Islam, M.R., Zierenberg, K., Eterradossi, N., Toquin, D., Rivallan, G. and Müller, H. (2001). Molecular and antigenic characterization of Bangladeshi isolates of infectious bursal disease virus demonstrate their similarities with recent European, Asian and African very virulent strains. *Journal of Veterinary Medicine,* B 48: 211–221.

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.

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

Jain, N. C. (1986). Shalm's Veterinary heamatology 4th edition Philaphdelphia, PA , USA, Lea and Febiger publisher, pp 747-748.

Julian., R.J. (1999): Cardiovascular diseases. In: Poultry Disease. F.T.W. Jordan and M. Pattison, Eds. W.B. Saunders company, Limited, London; 279-301.

Kibenge, F.S.B., Dhillon, A.S. and Russel, R.G. (1988). Biochemistry and immunology of infectious bursal disease virus. *Journal of General Virology*, 69: 1757–1775.

Kiddi M.T. and Kerr B.J. (1998). Dietary Arginine and lysine in large White Toms. 2. Lack of interaction between argnine:lysine ratios and electrolyte balance. *Poultry Science*, 77: 864-869.

Kim, H.R., Kwon, Y.K., Bae, Y.C., Oem, J.K. and Lee, O.S. (2010). Genetic characteristics of virion protein 2 genes of infectious bursal disease viruses isolated from commercial chickens with clinical disease in South Korea. *Poultry Science,* 89: 1642-1646.

Kim, I.J., Karaca, K., Pertile, T.L., Erickson, S.A. and Sharma, J.M. (1998). Enhanced expression of cytokine genes in spleen macrophages during acute infection with

infectious bursal disease virus in chickens. *Veterinary Immunology and Immunopathology,* 61: 331– 341.

Koinarski, V., Angelov, G. and Lalev, M. (2001). Haematologic and blood electrolyte changes in broiler chickens experimentally infected with *Eimeria tenella*. *Bulgarian Journal of Veterinary Medicine,* 4: 237-24.

Kouwenhoven, B. and Van den Bos, J. (1994). Control of very virulent infectious bursal disease (Gumboro disease) in the Netherlands with more virulent vaccines. *Proceedings of the International symposium on infectious bursal disease and chicken infectious anaemia* (pp. 262–271). Rauischholzhausen, Germany.

Kral, I. and Suchy, P. (2000). Haematological studies in adolescent breeding cocks. *Actatica Veterinary Journal,* 69: 189-194.

Kudair, I.M. and Al-hussary, N.A.J. (2010). Effect of vaccination on some biochemical parameters in broilers chicken. *Iraqi Journal of Veterinary Science*, 24(2): 159-164.

Kumar, A., Jindal, N., Shukla, C.L., Pal, Y., Ledoux, D.R., and Rottinghaus, H. (2003). Effect of Ochratoxin A on *Escherichia coli*-challenged broiler chicks. *Avian Diseases,* 47: 415-424.

Kumar, K., Singh, K. C. P., Prasad, C. B. (2000). Immune responses to intermediate strain IBD vaccine at different levels of maternal antibody in broiler chickens. *Tropical Animal Health and Production*, 32(6):357-360.

Kurtoglu, F., Kurtoglu, V., Celike, I., Kececi, T. and Nizamlioglu, M. (2005). Effects of dietary boron supplementation on some biochemical parameters, peripheral blood lymphocytes, splenic plasma cells and bone characteristics of broiler chicks given diets with adequate or inadequate cholecalciferol (vitamin D3) content. *Brazil Poultry Science,* 46: 87-96.

Lütticken, D. (1997). Viral diseases of the immune system and strategies to control infectious bursal disease by vaccination. *Acta Veterinaria Hungarica, 45*: 239–249.

Lam, K.M. (1998). Alteration of chicken heterophil and macrophage functions by the infectious bursal disease virus. *Microbiologica Pathogenesis, 25*: 147–155.

Lambrecht, B., Gonze, M., Meulemans, G., Van den Berg, T.P. (2000). Production of antibodies against chicken interferon-g : demonstration of neutralizing activity and development of a quantitative ELISA. *Veterinary Immunology and Immunopathology*, 74: 137– 144.

Letzel, T., Coulibaly, F., Rey, F.A., Delmas, B., Jagt, E., Van Loon, A.A.M.W. and Mundt, E. (2007). Molecular and structural bases for the antigenicity of VP2 of infectious bursal disease virus. *Journal of Virology,* 8: 12827-12835.

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

Lucas, A.J. and Jamroz, C. (1996). Oil pollution and petroleum toxicity to wild life. In: Non infectious diseases of wild life. A. Fairbrother, L. N. Locke and G.L. Hoff. Eds published by Manson Publishing Limited, London NW117DL; pp141-156

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

Luengo, A., Butcher, G., Kozuka, Y., Miles, R. (2001). Histopathology and transmission electron microscopy of the bursa of Fabricius following IBD vaccination and IBD virus challenge in chickens. *Revista Científica*, 11(6):533-544.

Lukert, P.D. and Saif, Y.M. (1991). Infectious bursal disease. *Diseases of Poultry* In *B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid and H. W. Yoder (Eds.). 9th Edition. Iowa* State University Press. *Iowa State University Press, Americans,USA*. Pp: 648-663.

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

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

M¨uller, R., Kaufer, I., Reinacher, M. and Weiss, E. (1979). Immunofluorescent studies of early virus propagation after oral infection with infectious bursal disease virus (IBDV). *Zentralblad Veterinarmedicine [B],* 26: 345–352.

Macreadie, I.G., Vaughan, P.R., Chapman, A.J., McKern, N.M., Jagadish, M.N., Heine, H.G., Ward, C.W., Fahey, K.J. and Azad, A.A. (1990). Passive protection against infectious bursal disease virus by viral VP2 expressed in yeast. *Vaccine,* 8: 549–552.

Marquardt W.W., Johnson R.B., Odenwald W.F. and Schlotthober B.A. (1980). - An indirect enzyme-linked immunosorbent assay (ELISA) for measuring antibodies in chickens infected with infectious bursal disease virus. *Avian Diseases*, 24: 375-385.

Mbuko, I.J., Musa, W.I., Ibrahim, S., Saidu, 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*, 7(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 J.B. McFerran and M.S. McNulty (Eds.), *Virus Infections of Birds* (pp. 213–228). Amsterdam: Elsevier Science Publishers B.V.

Moura, L. Vakharia, V. Liu, M. and Song, H. (2007). *In ovo* Vaccine against infectious bursal disease. *International Journal of Poultry Science*, 6 (11): 770-775.

Mundt, E., Beyer, J. & Muller, H. (1995). Identification of a novel viral protein in infectious bursal disease virus-infected cells. *Journal of General Virology*, 76: 437-443.

Muskett J. C., Hopkins I.G., Edwards K.R. and Thornton, D.H. (1979). Comparison of two infectious bursal disease vaccine strains. Efficacy and potential hazards in susceptible and maternally immune birds. *Veterinary Research*, 104: 332-334.

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,* 43: 123-130.

Nawathe, D.R., and Lamorde, A. (1982). Proublem of disease Control in Nigeria. *Bulletin* de *l Office Internationale des epizooties revue Scietifique ET Technique,* 1 (4): 1163-1168.

Nelson, C.B., Pomeroy, B.S., Schrall, K. Park, W.E., Lindeman, R.J. (1952). An outbreak of conjunctivitis due to Newcastle disease virus (NDV) occurring in poultry workers.". *American Journal of Public Health and the Nation's Health,* 42 (6): 672–8. PMC 1526237. PMID 14924001.

Nielsen, O.L., Sorensen, P., Hedemand, J.E., Laursen, S.B. and Jorgensen, P.H. (1998). Inflammatory response of different chicken lines and B haplotypes to infection with infectious bursal disease virus. *Avian Pathology, 27*: 181–189.

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

Odunsi, A.A., Onifade, A.A. and Babatunde, G.M. (1999). Response of broiler chicks to virginiamycin and dietary protein concentrations in the humid tropics *Archive de Zootechnologe*, 48: 317-325.

Office International of Epizooties (OIE) (1999).Infectious bursal disease (Gumboro disease). In International animal health code: mammals, birds and bees, 8th Ed, OIE, Paris, 247-248.

Oguz, H., Kececi, T., Birdane, Y.O., Onder, F. and Kurtoglu, V. (2000). Effect of clinoptilolite on serum biochemical and haematological characteristics of broiler chickens during aflatoxicosis. *Research of Veterinary Science*, 69: 89-93.

Ojo, M.A. and Oduye, O.O.B., Naoibi, L.M. and Idowu, L.A. (1973). Gumboru-like disease in Nigeria. *Tropical Animal Health and Production,* 5:52-57.

Okeke,E.N., Choji, C., Njike. M.C. (1982). The effect of IBD Fibrogumbovac and a commercial vaccine on the body weight, bursal weight and development of antibody in broilers. *Nigeria Journal of Animal Production,* 9(2): 102-105.

Okoye, J.O.A. (1982). Characterization of Nigerian strain of infectious bursal disease virus of chicken: *Bulletin Animal Health Production in Africa,* 30(3): 185-191.

Okoye, J. A., and Uzuokwu, M. (2001). Histopathogenesis of local Nigeria isolates of infectious bursal disease among chickens between 16 and 20 weeks old chicken. *Avian Diseases*, 25:1034-1038.

Okoye, J.O.A., and Uzoukwu, M. (1982). Characterization of Nigerian strain of infectious bursal disease virus of chickens: clinicopathalogical manifestations of naturally occurring field outbreaks. *Bulettin Animal Health and Production in Africa,* 20:193-197.

Okoye, J.O. A.and Uzoukwu, M. (1981). An outbreak of infectious bursal disease among chicken between 16-20th weeks old. *Avian Diseases, 25* (1):1034.

Oladele, O.A., Adene, D.F., Obi, T.U., Nottidge, H.O. and Ayedun, A.I. (2005). Sequencial hematological study of experimental infectious bursal disease virus infection in chickens, turkeys and duck. *Revue des Elev Mediciene Vetenaria Pay Tropicale.* 58 (4): 211-215.

Oladele S. B. (2000). Hematological parameters of some apparently healthy and some clinically sick indigenious poultry species in Zaria. Msc Thesis, Ahmadu Bello University, zaria; 116.

Oladele, S.B. and Samuel, J. (2014). Whole blood coagulation time, haematocrit, haemoglobin and total protein of turkeys reared in Zaria. *Sokoto Journal of Veterinary Sciences,* 12(1): 13-17.

Oladele, S.B and Ayo J.O. (1999). Comparative studies on haematocrit, haemoglobin and total protein values of apparently healthy and clinically sick indigenous chickens in Zaria, Nigeria. *Bulletin of Animal Health and Production in Africa,* 47: 1673-165.

Oladele, S.B., Ayo, J.O. Esievo, K. A. N. and Ogundipe, S. O. (2000). Effect of season and sex on packed cell volume, haemoglobin and total protein of indigineous chicken in Zaria, Nigeria *Journal of Medical Sciences*, 3: 137-177.

Oladele, S.B., Ayo J.O., Ogundipe, S.O. and Esievo K.A.N. (2005a). Seasonal and sex variations in packed cell volume, haemoglobin and total protein of the guinea fowl*(Numida maleagris*) in Zaria, Northern Guinea Savannah Zone of Nigeria. *Journal of Tropical Biosciences, 5(2*):67-71.

Oladele, S.B., Ayo J.O., Ogundipe, S.O. and Esievo K.A.N. (2005b). Seasonal and species variations in erythrocyte osmotic fragility of indeginious poultry in Zaria, Northern Guinea Savannah Zone of Nigeria. *Bullettin of Animal Health And Production in Africa*, 51:204-214.

Oladele, S.B., Nok, A.J., Esievo, K.A.N., Abdu, P.A., Useh, N.M. (2005c). Haemaglutination inhibition antibodies, rectal temperature and total protein of chicken infected with a local Nigerian isolate of velogenic Newcastle disease virus. *Veterinary Research Communication,* 29:171-179.

Oladele, S.B. (2009). The significance of haematology in the diagnosis and therapy of avian diseases: A review. *Nigeria Veterinary Journal,* 30(2):34-39.

Oladele, S.B. and Audu S.B. (2010). Packed cell volume, haemoglobin, total protein and whole blood coagulation time of the geese (*Anser anser*) reared in Zaria, Northern Nigeria. *Zariya Veterinarian*, 7(1): 1-5.

Olson, C. (1997) Avian Haematology. *In*: Disease of poultry. H. E. Biester and L. H. Schwarle Eds The Iowa state university press, Ames, USA.pp 232.

Oluwayelu, D. O., Emikpe, B. O., Ikheloa, J.O., Fagbihun,O. A. and Adeniran, G. A. (2002). The pathology of infectious bursal disease in cross breed of Harco cocks and indigenous hens. *Journal of Clinical and Experimental Microbiology,* 3: (2).

Onukwo, O. (1975). An outbreak of infectious bursal disease virus of Nigeria. *Veterinary Research,* 97: 433.

Onunkwo, O and Momoh, M.A. (1981). Laboratory diagnosis of infectious bursal disease (IBD) in Northern Nigeria (1975-1999). *Bulletin of Animal Health and Production in Africa*, 29: 243-249.

Onyeyili, P.A., Egwu, G.O., Jibike, G.I., Pepple, D.J. and Ohaebuler, J.O. (1991): Seasonal variation in the haematological indices in the grey breasted guinea fowls. *Nigerian Journal of Animal Production*, 18:1108-1110.

Oyewale, J. O. and Fajimi, J. L. (1988). The effect of egg laying on haematological and plasma biochemistry of guinea hen. *Bulletin of Animal Health and Production in Africa*, 36:229:232.

Öppling, V., Müller, H. and Becht, H. (1991). Heterogeneity of the antigenic site responsible for the induction of neutralizing antibodies in infectious bursal disease virus. *Archives of Virology, 119*: 211– 223.

Organisation of International Epizootic, (2010). Marek's Disease.in *Terrestrial Manual*, "Chapter 2.3.13.

Panigrahy, B., Rowe, L.D. and Corrier, D.E. (1986) . Haematological values and changes in blood chemistry in chickens with infectious bursal disease. *Research in Veterinary Science,* 40: 86-88.

Parkhurst, R.T. (1964). Pattern of mortality in avian nephrosis. *Poultry Science,* 43: 788-789.

Pilar, L. M., Victoria, B. M., Manuel I. S., Manuel, F. and Cassidy, R. (2005). Haematologic, protein electrophoresis, biochemistry and cholinesterase values of free-living black storknestlings (*Ciconia nigra*). *Journal of Wildlife Diseases,* 41(2): 379-386.

Pitcovski, J., Goldberg, D., Levi, B.Z., Di-Castro, D., Azriel, A., Krispel, S., Maray, T. and Shaaltiel, Y. (1998). Coding region of segment A sequence of a very virulent isolate of IBDV-comparison with isolates from different countries and virulence. *Avian Diseases, 42*: 497– 506.

Polat, U., Cetin, M., Turkyilmaz, O. and Ak, I. (2003). Effects of different dietary protein levels on the biochemical and production parameters of ostriches (*Struthio camelus*). *Veterinary Archive,* 73:73–80.

Prehale, J.T. and Pastore., Y.D. (2004) Erythropoietin and erythropoiesis: Polycythaemia due to distribution of oxygen homeostasis. *Haematology Journal,* 3: 110-113.

Quintavalla, F., Bigliardi, E. and Bertoni, P. (2001). Blood biochemical baseline values in the Ostrich (*Struthio camelus*). Universita degli studi di Parma, *Annali della Facolta di Medicine Veterinary*, XXI: 61-71.

Rebar, A. H., Mac Williams, P. S., Feldam B. F. Metzgar, F. L. Pollock, R. V. H., and Roche J. (2005). A Guide to Haematology in Dogs and Cat. (Eds). Publisher: Teton NewMedia, Jackson, W. Y. (www. Veterinarywire.com) Internet Publisher: International Veterinary Information Service, Ithaca New York (www. Ivis.org): A3307.0505.

Reece, W.O. and Swenson, M. (2005) Composition and functions. In; Dukes Physiology of Domestic Animals. W.O. Reece, (Eds) Published by Pamina Publishing corporation, New Delhi; Pp 26-52.

Ritchie, B. W., Harrison, J. G. and Harrison, R. L. (1994). *Avian Medicine-Principle and Application*. Winger's Publishing, Inc, Florida. 2nd Edition, Pp 176-198.

Rodenberg, J., Sharma, J., Belzer, S.W., Nordgren, R.M. and Nagi, S. (1994). Flow cytometric analysis of B cell and T cell subpopulations in virus. *Avian Diseases, 38*, 16– 21.

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. 37th Western Poultry Disease Conference, 29th February – 2nd March, Davis, California.University of California, Davis, Pp 17-20.

Rosenberger J.K. and Cloud S.S. (1986). Isolation and characterization of variant infectious bursal disease viruses. In Abstracts 123rd American Veterinary Medical Association. (AVMA) Meeting, 20-24 July, Atlanta, Georgia. AVMA, Schaumburg, Illinois, Abstract No. 181, 104.

Santos, B. M., Realpe, M. I., Periara, C. G. (2011). Occurrence of infectious Bursal Disease After vaccination with a low- passage attenuated virus. Event xxii Latin American Poultry Congress.

Scheeles, C. W. van der klis, J.D. Kwarkernaak, C., Buys, N. and Decuypere, E. (2003). Hematological characteristics predicting susceptibility for ascitis 2. High haematocrit values in juvenile chicken *Britain Poultry*, 44: 484-489.

Schnitzler, D., Bernstein, F., M¨uller H. and Becht, H. (1993). The genetic basis for the antigenicity of the VP2 protein of the infectious bursal disease virus. *Journal of General Virology, 74*, 1563–1571.

Sharma, J.M. and Lee, L. (1983). Effects of infectious bursal disease virus on natural killer cell activity and mitogenic response of chicken lymphoid cells. *Infection and Immunity, 42*, 747–754.

Sharma, J.M., Dohms, J., Walser, M. and Snyder, D.B. (1993). Presence of lesions without virus replication in the thymus of chickens exposed to infectious bursal disease virus. *Avian Diseases,* 37: 741-748.

Sharma, J. M., Rautenschlein, S. and Yen, H. Y. (2000). Infectious bursal virus of chickens. Pathenogenesis and immune-suppression. *Comparative Immunology*, 24: 223-235.

Shemesh, O., Golbetz, H., Kriss J. P., Myers B.D. (1985). "Limitations of creatinine as a filtration marker in glomerulopathic patients". *Kidney International Journal* 28 (5): 830–8. PMID 2418254.

Sinkalu, V. O., Ayo, J. O., Kan Esievo and Adamu, S. (2010). Is it possible to count differential leucocyte of avian in Nigeria using the conventional staining technique. *47th Book of Proceeding Nigeria Veterinary Medical Association*, 2010.

Skeeles, J.K., Slavik, M., Beasley, J.N., Brown, A.H., Meinecke, C.F., Maruca, S. and Welch, S. (1980). An age-related coagulation disorder associated with experimental infection with infectious bursal disease virus. *American Journal of Veterinary Research, 41*, 1458–1461.

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.

Sommer, F. and Cardona, C. (2003). Chicken anemia virus in broilers: dynamics of the infection in two commercial broiler flocks. *Avian Diseases,* 47 (4): 1466–73. doi:10.1637/7048. PMID 14708998.

Spies, U., Muller, H. and Becht, H. (1987). Polymerase activity associated with infectious bursal disease virus and characterization of its reaction products. *Virus Research*, 8: 127-140.

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

Stuckie, P.D. (1976): Physical characteristics, formed elements, haemoglobin, and coagulation. In: Avian Physiology. P.D. Sturkie, Ed. Springer-Verlag, New York.

Talebi, A. (2006). Biochemical parameters in broiler chickens vaccinated against Newcastle disease, infectious bronchitis and infectious bursal disease. *International Journal of Poultry Science*, 5(12):1151-1155.

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.

Taylor, E. H (1989). *Clinical Chemistry*. New York: John Wiley and Sons. pp. 4, 58–62. Allen, P. J (2012). Creatine metabolism and psychiatric disorders: Does creatine supplementation have therapeutic value. *Neuroscience Biobehaviour,* 36 (5): 1442–62. doi:10.1016/j.neubiorev.2012.03.005. PMC 3340488. PMID 22465051.

Tong, J.C., Umoh, J.U., Abdu, P.A. and Sa'idu, L. (1993). Retrospective studies of Gumboro disease seen in Ahmadu Bello University Veterinary Teaching Hospital Zaria, Nigeria (1985-1990). *Bulletin of Animal Health and Production in Africa,* 41, 173-179.

Tripathy, D.N. (2004). The impact of vaccine and the future of the genetically modified pox virus vaccines for poultry *Animal Health Research Revolution,* 5: 263-266.

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 Veterinary Medicine 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.

Vakharia, V.N., Snyder, D.B., Lutticken, D., Mengel-Whereat, S.A., Savage, P.K., Edwards, G.H. and Goodwin, M.A. (1994). Active and passive protection against variant and classic infectious bursal disease virus strains induced by baculovirus-expressed structural proteins. *Vaccine, 12*, 452– 456

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

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 Proceeding Xlth International Congress of the World Veterinary Poultry Association, 18-22 August, Budapest. World Veterinary Poultry Association, Budapest, pp 57.

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*: 133–143.

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*: 409– 421.

van den Berg, T.P., Godfroid, J., Morales, D., and Meulemans, G. (1994). The use of HD11 macrophage cell line in the study of IBDV. *Proceedings of the International Symposium on Infectious Bursal Disease and Chicken Infectious Anaemia* (pp. 133–142). Rauischholzhausen, Germany.

van der Marel, P., Snyder, D. and Lutticken, D. (1990). Antigenic characterization of IBDV field isolates by their reactivity with a panel of monoclonal antibodies. *Deutsch Tierarztl Wochenschrift, 97*: 81–83.

vecerek, V., Strakova, E., Suchy, P. and Voslarova, E. (2002). Influence of high environmental temperature on production and haematological and biochemical indexes in broiler chickens. *Czechlovakia Journal of Animal Science,* 47: 176-182.

Vindevogel H, Gouffaux M., Meulemans G., Halen P. and Schyns P. (1974). Maladie de Gumboro. II : Inoculation expérimentale : étude clinique et anatomo-pathologique. *Médicine Vétenaria,* 118: 375-386.

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

Walker, H. K. Hall, N. D., Haurst, J.W. (1991). In: Clinical method. Raphael, A. C. Manog, R. G. 3rd Edition butterworth publisher PMID 21250192 pp 153-160.

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

Witter, R. L., Gimeno, I. M., Pandiri, A. R., and Fadly, A. M. (2010). Tumor diagnosis manual: the differential diagnosis of lymphoid and myeloid tumors in the chicken. *American Association of Avian Pathologists,* 1: 1-84.

Woerpeel, R. W. and Rosskopg, W. J. (1984). Clinical experience with avian, Labouratory diagnostic veterinary clinic North America *Small Animal Practice*,14: 249.

Yehuda, H., Goldway, M., Gutter, B., Michael, A., Godfried, Y., Shaaltiel, Y., Levi, B.Z. and Pitcovski, J. (2000). Transfer of antibodies elicited by baculovirus-derived VP2 of very virulent infectious bursal disease virus strains to progeny of commercial breeder chickens. *Avian Pathology, 29*: 13–19.

Zaman, Q., Khan, M.Z., Islam, N. and Muhammad, G. (1995). Experimental furazolidone toxicosis in broiler chicks: effect of dosage, duration and age upon clinical signs and some blood parameters. *Actatica Veterinary Hungaria,* 43: 359-367.

APPENDICES

Appendix 1. Antibody titre for infectious bursal disease virus in different groups

AGE(wks)	Day old	1	2	3	4	5	6	7	8	9	10
GROUP E	5	4.16	4	3.6	0.89	0	0	0	0	0	0
GROUP A			□ □	3.5	□ □	0.31					
GROUP B					□	4.2	0				
GROUP C							□	4	3.6		
GROUP D									□	1.14	3.09

□ □ Vaccination □ Challenge

Appendix 2. Effect of infectious bursal disease virus on some serum biochemical parameters of cockerels challenged at 4 weeks of age and assayed for biochemical parameters at 5 and 6 weeks.

	Age of cockerels			
	5th week		6th week	
Parameters	E(control)	B(challenge)	E(control)	B(challenge)
TC(mmol/l)	4.92±0.591	3.83±0.267	3.690±0.289	4.68±0.76
LDL(mmol/l)	3.17±0.618	2.150±0.190	2.02±0.232	3.190±0.705
HDL(mmol/l)	1.050±0.104	1.060±0.16	1.020±0.71	0.980±0.112
TRG(mmol/l)	1.39±0.121	2.520±1.098	1.46±0.288	1.110±0.225
CR(µmol/l)	76.50±10.48	84.6±12.77	91.78±9.989	93.33±9.671
TGL(mmol/l)	4.840±2.108	4.63±0.829	5.860±1.434	6.610±1.816
UREA(mmol/l)	3.763±0766□	7.363±1.44 □	5.667±0.935	5.860±1.552
Na(mmol/l)	136.3±1.23	129.8±1.37	135.111±2.874	129.1±3.853
K(mmol/l)	4.13±0.268	4.29±0.157	4.156±0.303	3.878±±0.279
Cl(mmol/l)	102.7±1.506	99.2±1.569	100.1±3.478	96.7±5.203
HCO3(mmol/l)	22.0±0.577	21.50±0.86	20.56±1.944	15.89±1.837
ALB(g/l)	38.70±3.910	32.0±3.707	30.56±3.888	36.56±4.503
TP(g/l)	69.30±4.356□	55.80±3.747□	56.89±6.317	68.30±4.072

Values are expressed as mean ± SEM, □ Significant differences from control group at $p<0.05$.

Appendix 3. Effect of infectious bursal disease virus on some serum biochemical parameters of cockerels challenged at 6 week of age and assayed for biochemical parameters at 7 and 8 weeks.

	Age of cockerels			
	7th week		8th week	
Parameters	A(control)	C(challenge)	A(control)	C(challenge)
TC(mmol/l)	5.667±0.738□	3.689±0.487□	3.989±0.225	3.989±0.266
LDL(mmol/l)	3.32±0.689	2.4±0.411	1.92±0.501	1.867±0.229
HDL(mmol/l)	1.16±0.150	0.850±0.131	1.123±0.211	1.378±0.185
TRG(mmol/l)	1.76±0.337	1.83±0.472	1.92±0.501	1.689±0.298
CR(µmol/l)	39.20±5.199	38.8±4.139	63.6±7.389	58.9±4.398
UREA(mmol/l)	1.350±0.259	1.3±0.226	3.44±0.377□	5.511±0.699□
Na(mmol/l)	131.8±2.230	132.4±1.8333	132.4±1.833	134.9±1.882
K(mmol/l)	4.04±0.301	4.23±0.263	4.03±0.146	3.956±0.305
Cl(mmol/l)	97.2±1.982	95.5±2.018	99.4±1.522	100.9±1.602
HCO3(mmol/l)	21.4±1.157	21.1±1.016	24.8±0.727	22.556±1.002
ALB(g/l)	28.8±3.47	37.4±2.948	33.25±2.153	33.556±1.796
TP(g/l)	69.56±2.678	65.1±5.174	71.5±2.099	73.222±2.493

Values are expressed as mean ± SEM, □ Significant differences from control group at $p<0.05$.

Appendix 4. Effect of infectious bursal disease virus on some biochemical parameters of cockerels challenged at 8 weeks of age and assayed for biochemical parameters at 9 and 10 weeks.

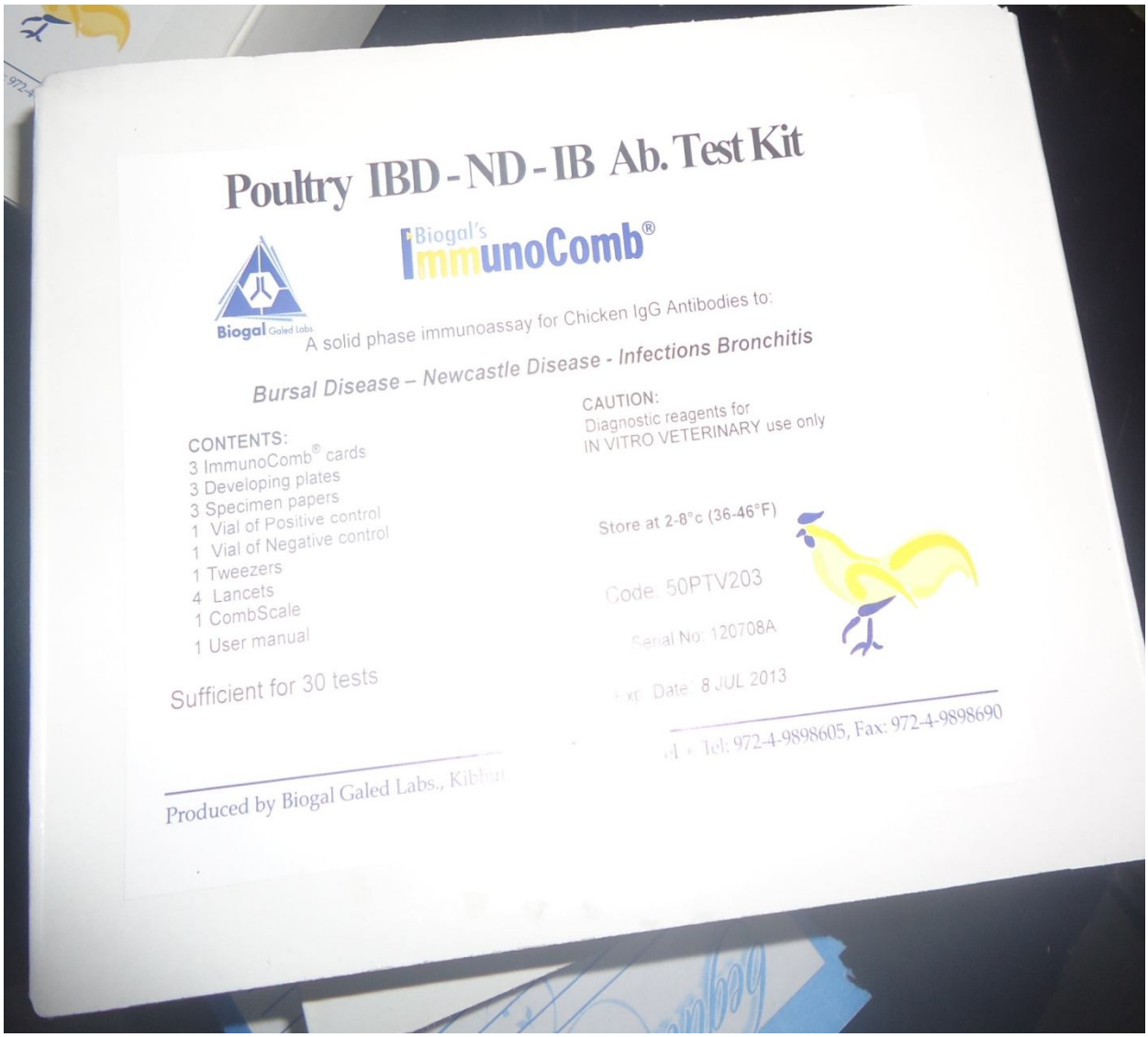
	Age of cockerels			
	9th week		10th week	
Parameters	E(Control)	D(Challenge)	E(Control)	D(Challenge)
TC(mmol/l)	3.99±0.613	3.23±0.474	2.94±0.618	4.840±0.7442
LDL(mmol/l)	2.50±0.291	2.30±0.3266	1.7±0.3194	2.89±0.508
HDL(mmol/l)	1.22±0.211	1.35±0.306	2.4±1.178	1.94±0.635
TRG(mmol/l)	1.39±0.196	1.84±0.335	0.775±0.144	1.52±0.292
TGL(mmol/l)	3.99±0.725	5.4±0.6502	4.620±1.217	7.96±1.233
UREA(mmol/l)	2.722±0.409	2.72±0418	5.680±0.867□	3.189±0.454□
Na(mmol/l)	136.9±2.030	138.6±1.166	132.3±3.376	135.15±1.048
K(mmol/l)	4.53±0.186	4.04±0.178	4.48±0.471	4.090±0.212
Cl(mmol/l)	103.9±1.853	100.3±3.353	103.0±1.807	103.6±1.204
HCO3(mmol/l)	19.6±1.384	20.3±1.023	21.8±1.356	19.7±2.196
ALB(g/l)	33.8±2.954	27.6±5.743	41.6±3.669	38.2±2.004
TP(g/l)	63.9±3.987	52.0±7.135	68.8±5.083	74.2 ±2.740

Values are expressed as mean±SEM ,□ Significant differences from control group at $p<0.05$.

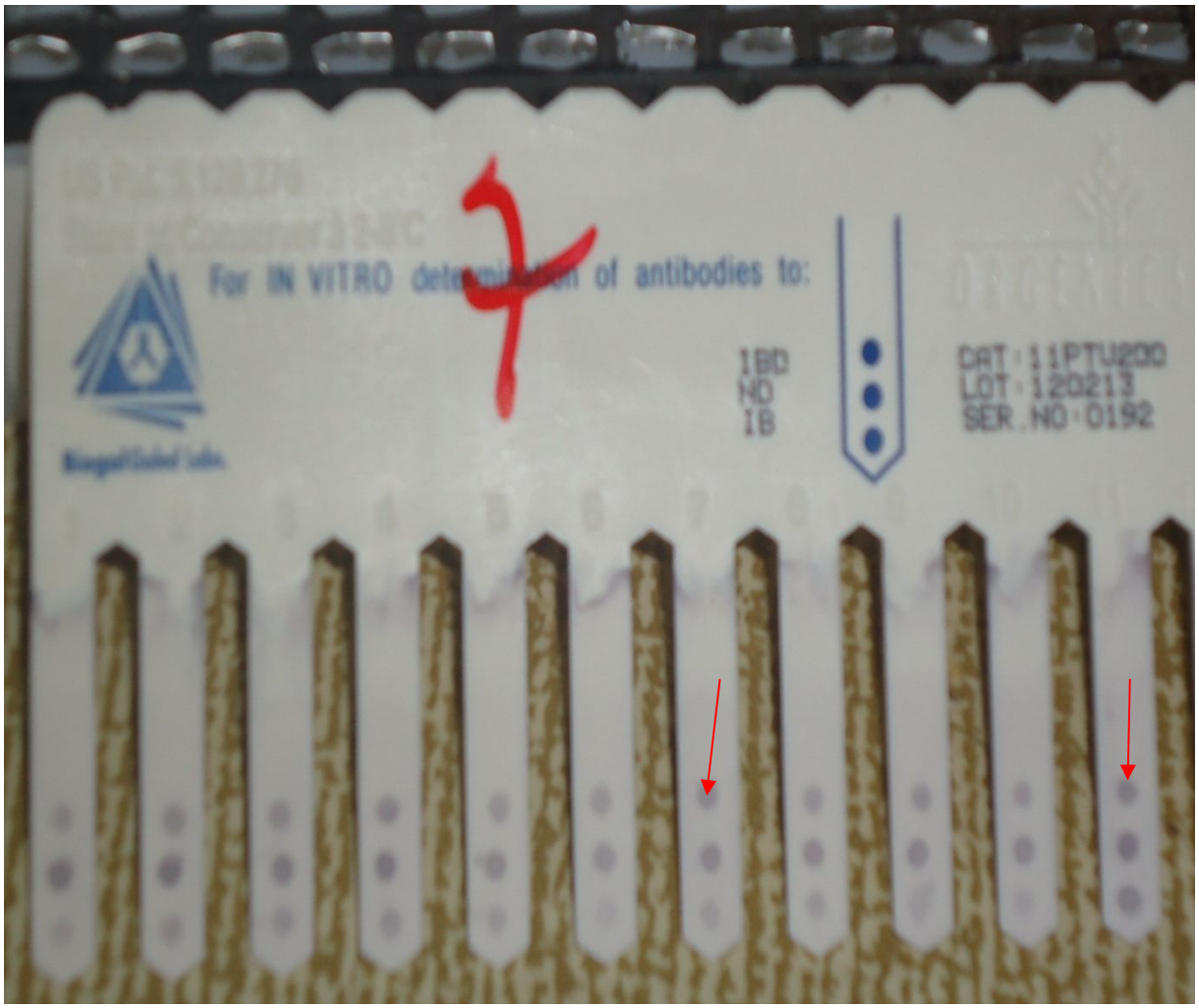
Appendix 5. Effect of infectious bursal disease vaccine on some serum biochemical parameters of cockerels vaccinated at 2 and 4 weeks of age and assayed for serum biochemical parameters at 3 and 5 weeks of age.

	Age of cockerels			
	3rd week		5th week	
Parameters	E(Control)	A(Challenge)	E(Control)	A(Challenge)
TC(mmol/l)	3.47±0.337	4.0870.257	4.920±0.591	4.86±0.590
LDL(mmol/l)	2.07±0.272	2.30±0.197	3.17±0.617	3.073±0.797
HDL(mmol/l)	0.80±0.125	1.213±0.245	1.050±0.1035	1.04±0.130
TRG(mmol/l)	1.450±0.322	1.827±0.401	1.39±0.121	1.74±0.259
CR(µmol/l)	37.67±3.296	36.53±3.617	76.5±10.48	75.27±8.901
TGL(mmol/l)	12.2±0.322	9.433±1.207	9.84±2.108	7.307±1.189
UREA(mmol/l)	3.0±0.506	2.63±0.333	6.030±1.688	4.933±0.6935
Na(mmol/l)	131.4±2.334	134.5±1.759	136.3±1.23	133.9±1.452
K(mmol/l)	5.03±0.358□	4.147±0.223□	4.13±0.2679	4.273±0.161
Cl(mmol/l)	100.4±1.00	99.4±173	102.7±1.506	100.1±1.087
HCO3(mmol/l)	17.8±1.548	20.670±2.25	22.0.8±0.577	21.67±0.988
ALB(g/l)	38.5±4.023	40.29±2.165	38.70±2.360	38.20±2.962
TP(g/l)	65.5±4.643	64.4±2.047	73.11±2.36□ □	64.47±1.8952□ □

Values are expressed as mean ± SEM, □Significant differences from control group at $p<0.05$



Appendix 6. ELISA kit purchased from Biogal-galed laboratory Isreal, used for antibody detection in this study.



Appendix 7. Immunocomb showing presence of infectious bursal disease maternal antibodies (arrows) in cockerel at week 2 of control group.



Appendix 8. Group E (control group) at 3 weeks of age.