EFFECT OF MULTIPLE INFECTIOUS BURSAL DISEASE VACCINATION ON ANTIBODY RESPONSE OF PULLETS TO NEWCASTLE DISEASE VACCINE LA SOTA AND PATHOLOGICAL CHANGES IN MAJOR IMMUNE ORGANS

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FEBUARY, 2018

DECLARATION

I declare that the work in this dissertation titled **" Effect of Multiple Infectious bursal disease vaccine on Antibody Response of Pullets to Newcastle disease vaccine La Sota and Pathological Changes in Major Immune Organs"** has been carried out by me in the Department of Veterinary Medicine, Faculty of Veterinary Medicine 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 dissertation was previously presented for another degree or diploma at this or any other institution.

Muhammad Babaji MUHAMMAD ________________ _____________

Signature Date

CERTIFICATION

This dissertation titled **"EFFECT OF MULTIPLE INFECTIOUS BURSAL DISEASE VACCINATION ON ANTIBODY RESPONSE OF PULLETS TO NEWCASTLE DISEASE VACCINE LAS SOTA AND PATHOLOGICAL CHANGES IN MAJOR IMMUNE ORGANS"** by Muhammad Babaji Muhammad, meets the regulations governing the award of the degree of Master of Avian Medicine of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This study is dedicated to my late Father, Muhammad Hassan Ahmad for his love, support and encouragement. And my late brother Yakubu Muhammad Hassan, My cousin brother late Ishaq Umar and his son late Abdurrahman Ishaq who lost their lives due to insurgent attack in our region. May Allah have mercy on their souls, amen.

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ABSTRACT

This study was conducted with the aim of determining the effect of multiple infectious bursal disease vaccination on antibody response of pullets to Newcastle disease (ND) vaccine La Sota and pathological changes on major immune organs. A total of 120 day-old pullets were used, the birds were randomly divided into 6 groups (A, B, C, D, E and F), consisting of 20 chicks each. A freeze-dried live attenuated infectious bursal (IBD) disease vaccines, M.B. strain (ABIC) and Newcastle disease vaccine La Sota strain live virus (ABIC) were administered orally. Group A pullets were vaccinated with IBD vaccine at 1st and 2nd weeks of age. Group B pullets received IBD vaccine at 2nd and 4th weeks. Group C pullets received IBD vaccine at 3rd weeks. Group D pullets received IBD vaccine at 2nd, 4th and 6th weeks. All the groups with the exception of group F received ND vaccine at 3rd and 6th weeks. Group F pullets received no vaccine. Ten serum samples from each group in this experiment were collected for 8 weeks and tested against ND for antibodies using ELISA test. Mean Elisa ND antibody titre had indicated statistical difference at week 5, where groups B and A ($P \leq 0.01$), C and B ($P \leq 0.01$) and D and C ($P \leq 0.05$). At week 7 groups D and C ($P \leq 0.01$). The multiple IBD vaccine had negative effect on ND vaccine La Sota, group C had highest ND mean Ab titre of 11480.6 ±827.45 at week 8. The gross lesions observed were more severe in groups B and D and milder in group E and F. Histopathologically lesions were more severe in groups that where vaccinated with multiple IBD vaccines. From this work it was recommended that it is safer to give two weeks interval before repeating infectious bursal disease vaccination for Newcastle disease vaccine La Sota vaccination to be effective.

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ABBREVIATION, DEFINATION, GLOSSARY AND SYMBOLS

^0C	Degree Centigrade
µ l	Micro Litre
Ab	Antibody
ABU	Ahmadu Bello University
AGPT	Agar Gel Precipitating Test
ANOVA	Analysis of Variance
APMV	Avian Paramyxovirus
BHI	Brain-Heart Infusion
CD	Cluster of Differentiation
DOP	Day Old Pullets
ELISA	Enzyme Linked Immunosorbent Assay
F	Fusion
FAO	Food and Agriculture Organization
HA	Heamagglutinin
HI	Haemagglutinin Inhibition
HN	Haemagglutinin-Neuraminidase
IBD	Infectious Bursal Disease
IBDV	Infectious Bursal Disease Virus
ICPI	Intercerebral Pathogenicity Index
ILT	Infectious Laryngotreichitis
L	Large polymerase protein
M	Matrix protein
MDA	Maternally Derived Antibody
Ml	Milliliter

Mm	Millimeter
N	Nuclocapsid protein
ND	Newcastle Disease
NDV	Newcastle Disease Virus
NDVS	Newcastle Disease Viruses
NP	Nucleoprotein
OIE	Office International des Epizootique
P	Phosphoprotein
PBS	Phosphate Buffered Saline
RT- PCR	Reverse Transcriptase Polymerase Chain Reaction
TBTB	Tris-Buffered Tryptose Broth
USA	United State of America
VVIBDV	Very Verulent Infectious Bursal Disease Virus
VNDV	Virulent Newcastle Disease Virus
VVNDV	Very Virulent Newcastle Disease Virus

CHAPTER ONE

1.0 Introduction

1.1 Historical Background

Poultry diseases are significant restrain to the efficiency and profitability of poultry production. From a global perspective basically the same range of poultry pathogens are responsible for losses in livability, egg production, growth rate and feed efficiency worldwide (Shane, 2004). In many developing countries, velogenic Newcastle disease (ND) is endemic, and represents an important limiting factor in the development of commercial poultry production and the establishment of trade links (Alexander *et al*., 1997). The constant loses from ND severely affect the quality and quantity of food for people on marginal diets (Saif *et al*., 2005). The endemicity of ND in Nigeria leads to high environmental dissemination of the virus and this is enhanced by poor husbandry practice (Okwor and Eze, 2010). The outbreaks of ND were reported to be more common in layers than in broilers (Abdu *et al*., 2005).

1.2 Statement of research problem.

In Nigeria, poultry contributes about 15% of the total annual protein intake with approximately 1.3 kg of poultry products consumed per head per annum (Ologbon and Ambali, 2012). However, major factors militating against the growth of the poultry industry include infectious diseases especially infectious bursal disease (IBD) and ND. Farmers administer ND and IBD vaccines simultaneously with the aim to reduce stress and minimize labour cost (Okwor *et al*., 2013). In Nigeria, farmers vaccinate birds against IBD and ND at regular intervals depending on the vaccination scheduled, some

vaccinate against IBD once, twice and thrice (Okwor *et al*., 2013). Vaccination using live IBD vaccine has been reported to affect response of birds to vaccination against other disease like ND (Ali *et al*., 2004). However, some viruses like IBD virus are immunosuppressive in nature and may interfere with chicken's immune responses to other viral vaccines which may be responsible for vaccination failure (Hair-Bejo *et al*., 2004). The use of live vaccines can result in negative vaccination reactions, especially if the birds are stressed (Alexander, 2004). Infectious bursal disease has been reported to be immunosuppressive and even the attenuated vaccines are reported to cause pathology in the bursa of Fabricius (Adamu *et al*., 2013). Several IBD vaccination failures have been reported in Nigeria (Abdu, 1986). The endemic nature of Newcastle disease in Nigeria ensures the ND virus is almost always present in poultry populations particularly village chickens and backyard flocks (Abdu, 1997). The village poultry flocks suffer from outbreaks of ND annually and these flocks are also in constant contact with backyard flocks. Vaccine failures in Nigeria were reported to occur primarily due to improper storage, transportation and administration of vaccine and secondarily due to interference with active immunization by Marternally derived antibody (Abdu, 1997). Research efforts on IBD control by conventional immunization or strict management practice is wroth with frustration.

1.3 Justification of the Study

Newcastle disease and infectious bursal disease have remained the two most important viral infectious diseases that are threatening commercial poultry production in most parts of the world (Agoha *et al.,* 1992; Sonaiya *et al*., 1999; Permin and Pederson, 2002). The epidemiology of these two diseases involves host's immune status, wide host range,

thermo-stability and antigenic variations in strains of the causative viruses (El-Yuguda *et al*., 2014). Unfortunately, severe outbreaks of ND still occur with high mortality rates in vaccinated and unvaccinated flocks (Musa *et al*., 2010). Economic losses of IBD are incurred as a result of the high mortality rate and the predisposition to secondary infection (Adewuyi *et al*., 1989). Field IBDV reports by different researchers indicated varying level of maternally derived antibody (MDA) in birds (Giambrone, 1983). Neutralization of IBDV by MDA at the time of vaccination was also reported (Okoye, 1984; Abdu, 1997). Immunosuppression caused by IBD has a significant economic impact due to widespread nature of the virus in chicken (Abdu, 1997). The infection at an early age compromise the humoral and the cellular immune response of chicken (Abdu, 1997). It has been observed, that ND outbreak occur more commonly in flocks recovered from IBD (Adewuyi *et al*., 1989). Therefore, this work was designed to find out whether IBD vaccinaton will have an effect on the antibody response to Newcastle disease vaccine (La Sota) strain.

1.4 Aim

The aim of the research was to study the effects of multiple IBD vaccinations on antibody response of pullets to ND vaccine La Sota and pathological changes on major immune organs.

1.5 Objectives of the Study were to;

1. Determine the antibody response of pullets to Newcastle disease La Sota vaccine following multiple IBD vaccine application in pullets.

2. Observe the gross and histopathological changes of some immune organs of pullets after IBD vaccination.

1.6 Research Questions

1. -Does IBD vaccination affect antibody response of pullets to ND La Sota vaccine?

2. -Does IBD vaccination cause pathological changes in major immune organs of pullets?

CHAPTER TWO

2.0 Literature Review

2.1 Introduction

2.2 Newcastle Disease

Newcastle disease (ND) is an acute and highly contagious viral infection that affect most of the species of birds. The disease is endemic in many parts of the world and causes big economic losses due to high mortality and reduced production. In rural areas, the disease can kill up to 80% of unprotected poultry, and is thereby, one of the biggest constraints to village poultry production and a considerable problem to rural development (Alexander, 2004). Newcastle disease is an economically important disease of poultry for which vaccination is applied as a preventive measure in many countries. Nevertheless, outbreaks have been reported in vaccinated flocks (Musa *et al*., 2010). The impact of ND is most notable in domestic poultry due to the high susceptibility of poultry and the severe consequences of outbreaks of the virulent strains on the poultry industries (Alexander *et al.,* 2003). In fact, it has been argued that ND may represent a bigger drain on the world economy than any other animal viral disease (Alexander *et al.*, 2003), although the current epizootics of H5N1 avian influenza in Southeast Asia and other part of the world are challenging this status (Alexander *et al*., 2003).

2.3 Historical background of Newcastle Disease

The first outbreaks to be recognized and termed Newcastle disease (ND) occurred in poultry in 1926, in Java, Indonesia, and in Newcastle-upon-Tyne, England. However, there are earlier reports of similar disease outbreaks in Central Europe before this date (Beach, 1942). Later it became clear that other less severe infections were caused by viruses almost identical to the original virus. In the United States of America, a relatively mild respiratory disease, often with nervous symptoms, was first reported in the 1930s and subsequently termed pneumoencephalitis (Beach, 1942). It was shown to be due to a virus indistinguishable from NDV in serological tests (Beach, 1944). Since then, numerous NDV isolates that produce an extremely mild disease or no evidence of disease in chickens, have been made around the world in waterfowl and other wild birds (Altizer, *et al*., 2011).

The pattern of outbreaks which are due to virulent NDV throughout the world suggest that several panzootics have occurred in poultry since 1926. The first appeared to have spread very slowly across the globe, apparently from the Far East. It probably took over 20 years to become a true panzootic and probably never reached poultry in the USA (Hanson, 1972). The beginning of the second ND panzootic was first recognized at the end of the 1960s and within four years had reached all corners of the earth (Hanson, 1972).

Antigenic and genetic evidences (Alexander *et al*., 1997; Lomniczi *et al*., 1998; Herczeg *et al*., 2001) indicated that there were probably worldwide spread of a third virulent virus

during the late 1970s, the start and spread of which is unclear, presumably due to the masking of disease by the almost universal use of vaccines since the mid-1970s.

Another ND panzootic occurred in the 1980s, but in racing and show pigeons (*Columba leavia*) rather than in poultry, although spread of the responsible virus did occur to poultry (Kaleta *et al*., 1985). The world population of racing or show pigeons is enormous and at the end of the 1970s these birds were still largely unvaccinated and fully susceptible to infection with NDV. Infections in pigeons with this variant NDV strain probably began in the Middle East in the late 1970s (Kaleta *et al*., 1985), and by 1984-5 had become a true panzootic. In many countries where outbreaks occurred there was also spread to feral pigeons and doves. The way pigeons are kept and raced has meant that this panzootic has proven difficult to control and in several countries it probably remains endemic in racing and possibly also in feral pigeons (Kaleta *et al*., 1985).

The effect of panzootics of ND that have had on the poultry populations of different countries has not always been well recorded. Alexander (2001) documented the history of ND in Great Britain in detail and considered it a good example of the effect ND may have on the poultry industry in a developed Western country where eradication policies have been employed.

In Africa, ND outbreaks are rampant and are the major constraint to poultry farming in villages (Spradbrow *et al*., 1988; Alders, 1998; Alders and Spradbrow 2001; Abolnik *et al*., 2004; Ortim *et al*., 2004). Phylogenetically distinguishable unique NDV strains have been reported from eastern and southern Africa, but reports of the molecular

epidemiology and genotype distribution of NDV strains from Western and Central Africa are scarce (Servan de Almeida *et al*., 2009 ; Snoeck *et al*., 2009).

2.4 Aetiology of ND

Newcastle disease is caused by a negative single stranded, non-segmented RNA virus belonging to the genus *Avulavirus* of the *Paramyxoviridae* family. So far, nine serotypes (APMV-1 to APMV-9) of avian paramyxoviruses have been found, of these APMV-1 is the most pathogenic serotype and is also referred to as Newcastle disease virus (NDV) (Caupa and Alexander, 2009). The three virus families Rhabdoviridae, Filoviridae and Paramyxoviridae form the order Mononegavirales (Lamb *et al*., 2000; Mayo 2002). Antigenic variation of ND viruses [APMV-1] detectable by conventional haemagglutination inhibition [HI] tests has been reported, although only rarely (Hannoun, 1977; Alexander *et al*., 1984). One of the most noted variations of this kind has been the virus responsible for the panzootic in racing pigeons. This ND virus, referred to as 'pigeon APMV-1 [PPMV-1]', was demonstrably different from standard strains in HI tests, but not sufficiently different antigenically that conventional ND vaccines were not protective (Alexander *et al.,* 1986). In recent years antigenic variations detected by monoclonal antibodies and genetic variations detected by nucleotide sequencing of the virus genome have proved invaluable in understanding the epidemiology of ND (Alexander *et al*., 1997; Herczeg *et al*., 1999).

The serotypes are usually classified into three groups depending on how virulent they are when inoculated into chicken embryo and chickens. The groups are velogenic (high virulent), mesogenic (moderate virulent) and lentogenic (low virulent) (Kahn, 2005).

Even if it is uncommon there have been reports that viruses of low virulence can mutate and become highly virulent (Capua and Alexander, 2009). Strains of NDV have been grouped into five pathotypes on the basis of the clinical signs seen in infected chickens (Alexander and Senne, 2008).

2.5. Structure and Composition of Newcastle Disease Virus

Newcastle disease virus is an enveloped virus, and its genome is a non segmented, single-stranded, negative sense RNA that contains six genes encoding the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion (F) protein, hemagglutinin-neuraminidase protein (HN), large polymerase protein (L), and an additional protein, V, that is expressed by RNA editing of P mRNA (Lamb and Parks, 2007). The two surface glycoproteins, HN and the F protein, are the viral neutralization antigens and the major protective antigens. Hemagglutinin-neuraminadase is responsible for attachment to the host cell, and the F protein mediates fusion of the viral envelope with the cell membrane (Scheid and Choppin, 1974). The F protein is synthesized as an inactive precursor (F0) that is cleaved by host cell protease into two biologically active F1 and F2 subunits that remain linked by a disulfide bond. Cleavage of the F protein is a prerequisite for virus entry and cell-to-cell fusion (Lamb and Parks, 2007; Samal, 2011). Like other paramyxoviruses, NDV encodes additional gene products, named V and W, which arise from the P gene translated from alternative mRNAs produced by RNA editing during P gene transcription (Steward *et al*., 1993). The two surface proteins of NDV are F and HN. HN is involved in virus attachment and release, and F mediates fusion of the viral envelope with cellular membranes. However, the HN protein is also required for fusion by interaction with F (Morrison, 1993; Lamb and Parks 2007). Newcastle disease virus

particles are pleomorphic as revealed by electron microscopy. Generally, they are rounded and vary in diameter from 100-500 nm, although filamentous forms of about 100 nm across and of variable length are often seen (Alexander, 1988). The virion consists of a core of genomic RNA encapsidated by nucleocapsid protein to which is bound an RNA polymerase complex composed of L protein and phosphoprotein. This core is surrounded by a lipid envelope coated with matrix protein on its inner surface (Nagai *et al*., 1989). The F and HN glycoproteins form spike-like protrusions on the outer surface of the virion. Both proteins play important roles in the initiation of infection (Nagai *et al*., 1989).

The nucleocapsid protein (NP) is the most abundant protein in NDV infected cells and in virus particles. The NP gene is 1.747 kb in size and is located at the 3' end of the genome adjacent to a 53-nucleotide ladder sequence in the genomic RNA of NDV (Ishida *et al*., 1986; Krishnamurthy and Samal, 1998; de Leeuw and Peeters, 1999). The NP protein is composed of 489 amino acids with a predicted molecular weight of 53 kDa (Ishida *et al*., 1986; Errington and Emmerson, 1997). The N-terminal region of the NP protein is involved with encapsidation of RNA and forms the helical nucleocapsid, whereas the C-terminal region binds P (Buchholz *et al*., 1994). The NP protein is essential for viral replication and serves several functions, including encapsidation of the genomic RNA into an RNase-resistant nucleocapsid which acts as the template for RNA synthesis, interaction with P and L proteins during transcription and replication, and association with M protein during virus assembly (Lamb and Kolakofsky, 2001).

The L protein is the least abundant protein in virus particles. The L gene is the last to be transcribed in the viral genome and encodes a protein consisting of 2200 amino acids

(Lamb and Kolakofsky, 2001). The L protein associates with P to form the active viral polymerase (Hamaguchi *et al*., 1983). The polymerase complex recognizes the helical nucleoprotein complex wherein the NP protein is tightly associated with the genomic RNA (Poch *et al*., 1990). L also has 5'capping and poly (A) polymerase activities on the nascent mRNA (Ishihama and Barbier, 1994).

The phosphoprotein of NDV is composed of 395 amino acids and has a predicted molecular weight of 42 kDa (Steward *et al.*, 1993). However, it has a relative mobility of 53 kDa bySDS-PAGE analysis (Collins *et al*., 1980; Chambers and Samson, 1980). P is an essential subunit of the viral RNA and dependent RNA polymerase (Lamb and Kolakofsky, 2001). In complex with the Lprotein, the P protein plays multiple roles during transcription and replication of the viral genome. The P protein acts as a chaperone to prevent uncontrolled encapsidation of non-viral RNA by NP (Errington and Emmerson, 1997).

The M protein is composed of 341 to 375 amino acids with a molecular weight of approximately 38.5 to 42.5 kDa. It is a basic protein (net charge at neutral pH of +14 to +17) and somewhat hydrophobic. M proteins are peripherally associated with membranes and are seen underlying the viral lipid bilayer (Lamb and Kolakofsky, 2001). The M protein is thought to orchestrate the assembly of virus particles at the plasma membrane of infected cells through interactions with the plasma membrane, the viral glycoproteins and the nucleocapsid (Peeples, 1991).

The HN glycoprotein is a type II integral membrane protein with a molecular weight of 74 kDa. The ectodomain consists of a stalk region that supports a terminal globular head.

Heamaglutinin neuraminidase exists on the surface of virions and infected cells as a tetramer consisting of pairs of homodimers (Thompson *et al.,* 1988). Heamaglutinin neuraminidase is a multifunctional protein. It is responsible for attachment of the virus to sialic acid-containing cell surface receptors. It also possesses neuraminidase (NA) activity that cleaves sialic acid from progeny virus particles to prevent viral self-aggregation. Heamaglutinin nueraminidase also promotes the fusion activity of the F protein (Scheid and Choppin, 1974). The attachment and neuraminidase activities of HN reside in the globular head. The crystal structure of the globular head region (residues 124-570) of HN from the Kansas strain of NDV was solved both ligand-free and in complex with either sialic acid or a NA inhibitor (Crennell *et al*., 2000). The ligand-free HN dimer was crystallized at pH4.6, whereas the liganded dimer was co-crystallized at pH 6.5.

However, the ligand-free structure poses a problem because the distance between amino acid residues at position 124 of each monomer is too large to allow disulfide bond formation between cysteine residues at position 123 in several NDV isolates (Sheehan *et al*., 1987). This led the authors to believe that the ligand-free structure may be an artifact of the low pH at which the crystals were grown. Subsequent studies by the same group showed evidence for a second sialic acid binding site, composed of residues from each monomer at the membrane-distal end of the dimer interface (Sheehan *et al.,* 1987).

The F protein is a class I transmembrane protein that is synthesized as a precursor protein F0. The cleavage of the precursor protein F0 into the disulfide-linked subunits F1 and F2 by cellular proteases is necessary for infectivity of progeny viruses (Nagai *et al*., 1976).

2.5.1 Pathogenicity of Newcastle disease virus

Although virulence of different APMV-1 strains appears to be modulated by both surface proteins. The F region is known as the main determinant of pathogenicity which was confirmed recently by a systematic study of chimeric NDVs with substitutions of genes between a mesogenic and a velogenic strain (Paldurai *et al*., 2014). Mesogenic and velogenic viruses exhibit a polybasic amino acid (aa) motif 112(K/R)-R-(Q/K)-(R/K)-R116 at the carboxy, terminus of F2 and a phenylalanine at the amino terminus of the F1 subunit (position aa 117) which are substrates for ubiquitously existing furin-like proteases detected in a wide range of cells and tissues, resulting in systemic infections (Sakaguchi *et al*., 1991). In contrast, the F protein of lentogenic viruses is characterized by a leucine at position 117 and a monobasic a a motif at the carboxy terminus of F2 112(G/E)-(K/R)-Q-(G/E)-R116, resulting in a virus that can be processed only by trypsin-like enzymes restricted to the respiratory and intestinal tract which limits virus replication (Glickman *et al*., 1988; Collin *et al*., 1993). Therefore, the F protein cleavage site is a major determinant of NDV virulence (Nagai *et al*., 1976).

2.5.2.1 Antigenic Variation of Newcastle Disease Virus

Newcastle disease Virus isolates vary widely in their pathogenicity for chickens. NDV strains are classified as highly virulent (velogenic), intermediate (mesogenic), or a virulent (lentogenic) on the basis of their pathogenicity for chickens (Alexander, 2000). All NDV strains are classified under a single serotype, but both antigenic and genetic diversities are recognized among NDV strains (Miller *et al*., 2010). NDV strains have

genome sizes of 15,186 nucleotides(nt), 15,192 nt, or 15,198 nt, for isolated isolates before 1960 and strains that have been isolated recently respectively (Czegledi *et al*., 2006). On the basis of genome length and the sequence of the F-protein gene (F gene), NDV strains have been classified into two major classes. The class I strains were mainly isolated from wild birds and are generally avirulent, whereas class II strains were being recovered from wild and domestic birds and include virulent and avirulent strains. Class I and II viruses are further divided into9 and 15 genotypes, respectively (Kim *et al*., 2008; Diel *et al*., 2012). Epidemiological studies have revealed that genotypes V, VI, and VII of class II NDV strains are the most prevalent genotypes currently circulating worldwide. Genotype VII has been reported to cause a number of outbreaks in East Asia and Western Europe (Munir *et al*., 2012). Early sublineages of Class II NDVs that occurred before the 1960s (genotypes I to IV) have a genomic size of 15,186 nucleotides, whereas late Class II NDV sublineages (genotypes VI to XI) have a genomic size of 15,192 nucleotides (Aldous *et al*., 2003).

2.5.2.2 Genetic Variation of Newcastle disease virus

The main reservoirs of the APMV-1 are waterfowl and migratory birds (Takakuwa *et al*., 1998; Ujvari *et al*., 2003). During infection with low pathogenic strains often they do not exhibit clinical signs, which allows for the “unnoticed” spread of the virus. The highest mortality (even higher than 90% in 2–6 dpi) among infected birds is observed in domestic fowl (*Gallus gallus domesticus*) (Alexander *et al*., 2003). Virulence of a strain depends mainly on the short amino acid sequence of the precursor protein responsible for fusion of membranes (Rott and Klenk 1988). Highly pathogenic NDV usually contains the following sequence: C-112R/K-R-Q/K/R-R/K-R-F117-N, while low pathogenic has C-

112G/E-K/R-Q-G/E-R-L117-N. These motifs are recognized by different cellular proteases which limit the ability for virus propagation only to specific host tissue (Nagai *et al*., 1976; Collins *et al.,* 1996). World Organisation for Animal Health (OIE, 2012) distinguishes five pathotypes of Newcastle disease virus: viscerotropic and neurotropic velogenic (highest mortality rate, Intracerebral Pathogenicity Index > 1.5), mesogenic (low mortality but moderate signs from respiratory system, ICPI 1.5–0.7), lentogenic (mild respiratory infection with no mortality, live-vaccine strains, ICPI < 0.7), and asymptomatic (no signs or subclinical enteric infections) (OIE, 2012).

2.5. 3 Molecular bases for virulence of Newcastle disease virus

During replication, NDV particles are produced with a precursor glycoprotein, F0, which has to be cleaved to F1 and F2 for the virus particles to be infectious (Rott and Klenk 1988). This post translation cleavage is mediated by host cell proteases. Trypsin is capable of cleaving F0 for all NDV strains and in vitro treatment of noninfectious virus will induce infectivity (Nagai *et al*., 1976).

The cleavability of the F0 molecule was shown to be related directly to the virulence of viruses invivo (Rott, 1985). It would appear that the F0 molecules of viruses virulent for chickens can be cleaved by a host protease or proteases found in a wide range of cells and tissues. This allows these viruses to spread throughout the host, damaging vital organs. Data from recent publications indicate that the effect of the HN protein on virulence was prominent and that both the stem region and the globular head of the HN protein seem to be involved in the determination of virulence (De Leeuw *et al*., 2005). The glycoprotein plays an important role in both recognization of sialic acid-containing receptors on cell

surfaces and promoting the fusion activity of the F protein (Scheid *et al*., 1972). This implies that the immune system of the hosts exerts pressure on the HN protein.

2.5.4 Classification of Newcastle Disease Virus

There are several classifications of NDVs based on antigenic and genotypic variations of their fusion protein and gene respectively (Collins *et al.*, 1998; Aldous *et al.,* 2003). Of special interest is the work by Czegledi *et al*., (2006), where the authors describe 2 classes of NDV (class I and class II) based on genomic data and sequencing of the fusion (F) and RNA-directed RNA polymerase (L) genes. These 2 classes contain the entirety of previously described genotypes and lineages. The work of Aldous *et al*., (2003) grouped all avirulent APMV-1 in lineage 1 and lineage 6, which contain among other groups the H group NDVs as defined by monoclonal antibodies. This particular group H contains mainly isolates from wild birds such as MC110/77 (isolated from a shelduck in France), NZ/1/97 and Fin/97 (isolated from mallard ducks in New Zealand and Finland respectively) (Aldous *et al*., 2003). Although isolates belonging to this group are usually of low pathogenicity (Alexander, 2001), this group also contains two chicken isolates (APMV-1/chicken/Ireland/34/90 and APMV-1/chicken/Ireland/48/90) that were shown to be highly virulent for poultry and that caused outbreaks in Ireland in 1990 (Alexander *et al.,* 1992).

Wild birds are reservoirs of NDV (Takakuwa *et al*., 1998; Alexander, 2001). Alexander *et al*. (1986) isolated 14 isolates of NDV in Western Australia from wild birds belonging to the orders *Charadriiformes, Passeriformes* and *Anseriformes* and showed that the isolates could be divided in two groups based on their reactivity to monoclonal

antibodies: V4-like (class II NDV), similar to APMV-1/chicken/Qld/V4/66 (Simmons, 1967) and MC110-like (class I NDV), similar to APMV-1/shelduck/France/MC110/77. The V4-like isolates were found mainly in *Charadriiformes* and *Passeriformes* and the MC110–like isolates were found in *Charadriiformes* and *Anseriformes* (Simmons, 1967). The authors concluded that the isolation of V4-like NDV in passerine birds was significant as they are more likely to come in contact with poultry (Simmons, 1967). Similarly, the testing of 257 samples from Anseriform birds (Magpie geese) from the Kakadu National Park (Diallo *et al.* 2007) failed to detect any APMV-1. However, in 2006 the testing of an ibis that died of unknown causes resulted in the isolation and identification of an NDV belonging to class I (Gordon and Field, 2006). The virus isolated from the ibis (Qld/116603/06) is a class I NDV and is different from the more widespread and more described Queensland V4 isolated by Simmons in 1966 (Simmons, 1967), which belongs to class II NDVs. Its fusion and matrix genes were not amplified by any of the then published NDV PCRs, which were developed using sequences from poultry isolates and therefore detect only V4-like NDV.

2.6. The role of ND Vaccine on the evolution of Virulent Newcastle Disease

Inactivated vaccines were first made commercially available to the poultry industry in 1946, but because they provided incomplete protection against ND, they were replaced with live lentogenic NDV vaccines (Hitchner, 1964). Although these vaccines reduce disease, they do not always prevent infection and birds can shed both vaccine and challenge strains of the virus (Kapczynki and King 2005; Miller *et al*., 2007; Van Boven *et al*., 2008). Viruses belonging to genotypes I-IV have circulated since the 1930's. Genotype I and II consist of both lentogenic and velogenic viruses and have been

associated with ND outbreaks in Australia and North America, respectively. These viruses have been attenuated in culture and are used as modified live vaccines. Ribonucleic acid viruses typically have a high mutation rate due to low fidelity and processivity of their polymerase (Moya *et al.,* 2000), which coupled with a high replication rate and short generation time (Elena and Sanjauan, 2005) lead to high evolutionary rates. In addition, evidence is accumulating that recombination is an important process driving genotype diversity for many RNA viruses (Bruen and Poss, 2007; Herrewegh *et al.,* 1998; Palmenberg *et al*., 2009). Although recombination was not thought to contribute to APMV-1 evolution (Sakaguchi *et al*., 1989; Toyoda *et al*., 1989), evidence of recombination in NDV has recently been reported (Han *et al*., 2008; Qin *et al*., 2008; Miller *et al*., 2010).

2.7. Response of Newcastle Disease Virus to Chemical, Natural and Physical condition

Several studies have shown that environmental temperature may influence the immune response of poultry (Henken *et al*., 1982; Beard and Mitchell, 1987; Donker *et al.,* 1990). Thermostable ND vaccines exhibit a relative resistance to inactivation on exposure to elevated temperatures (Spradbrow, 1992). They are prepared from a strain of NDV that retains its ability to infect cells after storage outside a cold chain for a short period of time and involves isolation of naturally occurring thermostable variants of the virus and increasing the thermostability of this variant by artificial selection in the laboratory (Spradbrow, 1992). The heat resistant V4 (NDV4-HR) vaccine against ND has yielded encouraging results in many countries in Africa and Southeast Asia (Alders and Spradbrow, 2001). NDV4-HR vaccine is a living vaccine which is thermostable, retaining

its activity for 12 weeks at a temperature of 28°C in freeze-dried form (Spradbrow, 1992).

It is, however, expected that physical factors such as temperature are responsible for decreasing the polymerase activity of the virus which ultimately affects its replication activity (Stenwich and Hallum, 1976). NDV proved to have high resistance to ambient temperature. However, it was inactivated at 56° C after 45 minutes exposure (Qayyum *et al.,* 1999).

At low temperature, low concentration of formalin will destroy the infectivity without markedly affecting the haemagglutination ability of the virus and without effect of immugenicity. All known virucidal chemicals will destroy NDV (Alexander, 1997). Formalin, beta propiolactone and phenol have been used to destroy the infectivity of NDV without severely damaging the immunogenicity of the virus (Alexander, 1997).

2.8 Epidemiology of Newcastle Disease Virus

Newcastle disease in poultry continues to occur in almost all continents of the world, except in Europe, where sporadic epizootics of the disease usually occur (Munir *et al*., 2012; Naveen *et al*., 2013). Infection of domestic poultry with low virulence NDV contributes to lower productivity (Garba *et al*., 2012).

Even in countries that have long been recognised as free of ND, monitoring surveys often reveal symptomless infections with avirulent viruses which have presumably spread from waterfowl or other wild birds (Alders and Spradbrow, 2001). However, there can be little doubt that the highly pathogenic form of ND is a serious problem, either as an enzootic disease or as a cause of regular, frequent epizootics throughout Africa, Asia, Central

America and parts of South America (Spradbrow, 1988; Rweyemamu *et al*., 1991; Alders and Spradbrow, 2001). In other areas such as Europe, the situation appears to be one of sporadic epizootics occurring despite vaccination programmes (Kaleta and Heffels-Redmann, 1992). Different parts of the world recorded major panzootics of Newcastle disease at different periods of time. The first panzootic was reported in 1926 in Southeast Asia from Java, Indonesia and in Europe from Newcastle-Upon-Tyne, England (Seal *et al*., 1995; Arifin *et al*., 2011), and it remained till late 1950s (Qiu *et al*., 2011). This was followed by another panzootic that started in Middle East in late 1960s and spread to other countries of the world up to 1973 (Ashraf and Shah, 2014). The most devastating was the third panzootic that occurred in the Middle East in late 1970s which was caused by pigeon paramyxovirus type 1 (Ashraf and Shah, 2014). In 1981, Newcastle disease reached Europe and then spread rapidly throughout the globe (Mase *et al.,* 2002). The fourth pandemic occurred in late 1980s in which many continents were affected (Qiu *et al*., 2011). The first documented outbreak of Newcastle disease in Nigeria occurred between December 1952 and February 1953 in and around Ibadan (Hill *et al.,* 1953). Since then, Newcastle disease was reported from all parts of the country (Hill *et al.*, 1953; Fatumbi and Adene, 1979; Ezeokoli *et al*., 1984; Gomwalk *et al*., 1985; Baba *et al*., 1995). The disease had also been reported to be endemic in both Local and Commercial poultry with annual epidemics being recorded in highly susceptible poultry flocks (Adu *et al*., 1986; Sa'idu *et al.,* 1994; Halle *et al*., 1999; Orajaka *et al*., 1999).

2.8.1 Spread of Newcastle Disease Virus

Transmission is primarily through direct contact between healthy birds and bodily discharges of infected birds (Solomon, 2010). Infection of domestic poultry with low

virulence NDV contributes to lower productivity (Garba *et al*., 2012). Movement of infected birds and transfer of virus, especially in infected faeces, by the movement of people and contaminated equipment or litter are the main methods of viral spread between poultry flocks (Altomonte *et al*., 2010). Transmission occurs by exposure to feacal and other excretions from infected birds, and through contact with contaminated food, water, equipment and clothing (Tu *et al*., 1998). The disease is also transmitted mainly through newly introduced birds, selling or given away sick birds and carrier birds (Tu *et al*., 1998). However, suitable climatic factors and high poultry farm concentrations favour air transmission. The windy harmattan also encourages the spread of the virus (Abdu *et al.,* 1992; Musa *et al*., 2010). Outbreaks of ND were reported to be more likely in farms that kept exotic birds together with local chickens and other poultry species like ducks and turkeys (Abdu *et al*., 2005).

2.8.2 Newcastle disease virus Reservoir

The virus can survive in poultry premises for 120 days (Jordan, 1990), and remain a source of infection for susceptible chickens in the vicinity. Newcastle disease was reported in both local and exotic chickens (Hill *et al*., 1953; Fatumbi and Adene, 1979; Abdu *et al*., 1985; Echeonwu *et al*., 1993; Sa'idu *et al*., 1994a; Baba *et al*., 1995; Oyeduntan and Durojaiye, 1999). The diseases was also reported more common during the dry harmattan period (Sa'idu *et al*., 1994; Halle *et al*., 1999) and the stress of harmattan period is known to worsen the outcome of the disease (Abdu *et al*., 1992). However, the disease continues to occur in both vaccinated and unvaccinated flocks (Halle *et al*., 1999; Sa'idu *et al*., 2006)

2.8.2.1 Other domestic birds

Infection of domestic poultry with low virulence NDV contributes to lower productivity (Garba *et al*., 2012). The disease was reported in Guinea fowls, and Turkeys (Okaeme, 1983; Ezeifeka *et al*., 1992; Echeonwu *et al.,* 1993; Haruna *et al*., 1993; Sa'idu *et al.,* 1994; Mohammed *et al*., 1996). Serological evidence of ND in pigeons, Laughing dove, Muscovy duck, Khaki Campbell ducks, geese, Mallard duck, peacock, ostrich, quil, village weavers, gray-headed sparrow, red bishop, grey canary, scaly fronted weaver and bearded berbet had been reported (Ezeifeka *et al*., 1992; Oladele *et al*., 1996; Sa'idu *et al*., 2004; Bisalla *et al*., 2005, Ibrahim *et al*., 2005).

2.8.2.2 Carrier chickens

Partially immune birds can develop infection with virulent NDV and display mild or no clinical signs. Such infected birds can then shed the virus for up to 5 weeks (Lancaster, 1996). It is unclear whether chickens can become long-term carriers of NDV. Although, ND is mostly pathogenic for the domestic chickens, turkeys and guinea fowls; other poultry and wild birds may not show clinical disease but do develop antibodies to the virus (Orajaka *et al.,* 1999; Mohammed *et al*., 1998; Sa'idu *et al*., 2004). These birds act to maintain ND viruses which serve as sources of frequent outbreaks in rural and backyard poultry (Alexander, 2001; Sa'idu *et al*., 2004).

2.8.2.3 Wild birds

Newcastle Disease Virus strains of low virulence are prevalent in poultry and wild birds, especially waterfowl. Reports on the status of ND in wild birds in Nigeria are scanty as a result of limited surveillance (Assam, 2014). Captive reared gamebirds, such as pheasants

and Hungarian partridge, have died of ND. However, large-scale illness and death from NDV in free-ranging wild birds has only occurred in double crested cormorants in Canada and the United States (Meteyer, 1997). White pelicans, ring-billed gulls, and California gulls were also reported to have died from NDV in association with cormorant mortalities in Canada (Meteyer, 1997). In most parts of Africa and Nigeria, ND is endemic often causing outbreak in backyard and commercial poultry (Ezeokoli *et al*., 1984; Adene, 1996). The impact of ND is more in Nigeria where over 90% of the poultry are rural poultry that are left to roam freely to scavenge for food and water bringing them into close activity space with wild birds (Adene and Oguntade, 2006). Wild birds and semi-domestic birds are known to be susceptible and develop antibodies to ND hence, play major roles in the spread of ND viruses especially in Nigeria as well as other African Countries with poor poultry husbandry practice (Ezeokoli *et al*., 1984; Oladele *et al.,* 1996; Sa'idu *et al.,* 2004).

2.8.3 Host range of Newcastle disease virus

It has been shown that many different species can be infected with NDV. It is believed that all bird species are most likely at risk to be infected, but the effects of the disease varies very much with different species, but chickens are most sensitive whereas ducks and geese are least sensitive (Okwor *et al*., 2013). Newcastle Disease Virus is capable of infecting a wide variety of avian species. In addition to poultry, more than 230 species from more than half of the 50 orders of birds have been found to be susceptible to natural or experimental infections with avian paramyxoviruses (Alexander, 1997).

2.8.4 Species Susceptibility

Over 200 species of birds have been reported to be susceptible to natural and/or experimental infection with ND virus and it seems probable that more are fully susceptible (Alexander, 1997). Chickens are highly susceptible to velogenic strains and usually become severely ill if they are infected, turkeys develop less severe sings than chickens, and the susceptibility of other gallinaceous game birds is variable (Houston, 2002). Infections are usually inapparent in ducks and geese; however, some isolates have caused outbreaks among geese in China since the 1990s (Wan *et al*., 2004). Young domestic fowls and guinea fowls are more commonly affected than adults (Nwanta *et al.,* 2006). The virus of ND has been demonstrated in domestic pigeons (Oladele *et al*., 1996; Bisalla *et al*., 2005).

2.8.5 Breeds Susceptibility

Newcastle disease is one of the major problems in village chickens in most parts of the world (Mazengia, 2012). In rural areas, the disease can kill up to 80% of unprotected poultry, and is thereby, one of the biggest constraints to village poultry production and rural development (Alexander, 2004). The susceptibility of both local and exotic breeds to ND has been documented in the field (Uzoukwu, 1967). The rural dwellers believe that there is difference in susceptibility to ND between the different breeds of indigenous chickens (Ibrahim and Abdu, 1992). However, village chickens appear to be more resistant to ND infection than the exotic chickens (Oluyemi *et al*., 1979). Exotic breeds of chickens are about twice more likely to be infected with ND than the local chickens

(Halle *et al.,* 1999). But Sa'idu *et al*., (2005) reported that all breeds of local chickens are equally susceptible to ND.

2.8.6 Age Susceptibility

All ages of chickens are susceptible to ND however, Spradbrow (1987) reported that maternally derived antibodies (MDAs) in newly hatched village chickens may be present for up to 5 weeks and chicks become fully capable of mounting immunity at about the same time. It was reported by Abdu, *et al*. (2005) that ND affects both young and old. Chicks 3-4 weeks old are at high risk of suffering from ND which may be as a result of decline in MDAs level (Halle *et al*., 1999). Chickens within 9-10 weeks of age are more resistance to ND which was attributed to the presence of substantial antibody tire to ND vaccination at 6 weeks of age (Halle *et al*., 1999).

2.9 Clinical manifestation of Newcastle Disease

The clinical signs in infected bird vary greatly from very high morbidity and mortality (up to 100 %) to asymptomatic carriers. Some of the clinical sings are gasping, stretching of the neck, sneezing, coughing, tracheal rales, dyspnoea and opisthotonus (Fatumbi and Adene, 1979; Okoye *et al*., 2000). There are also ruffled feathers, oedema of the head, cloudy eyes, conjunctivitis, severe depression, yellow and whitish diarroea, dehydration, emaciation and sudden death (Abdu *et al*., 2004). Oladele *et al*. (2005) reported a rise in rectal temperature following infection by NDV. Other general signs that can be seen are greenish diarrhoea, depression and inappetence, partial or complete drop in egg production and an increase production of deformed eggs (Kahn, 2005). In ND morbidity rate of 80% has been recorded in chickens (Fatumbi and Adene, 1979), in another report,

an average mortality rate of 28% was recorded and the disease has been found to be responsible for 56% of all mortalities in the birds (Abdu *et al*., 1992). Mortality due to ND may range from 24-100% although in most outbreaks 45-100% of the birds in a flock have been reported to have died of the diseases (Abdu and Sa'idu 1990). Beard and Hanson (1984), summarized ND into pathotypes, based on clinical signs in chicken as:

2.9.1 Viscerotropic velogenic

Viscerotropic velogenic ND, also known as Doyle's form in which, clinical signs often begin with listlessness, increased respiration and weakness, prostration and death. Oedema around the eyes and head may occur. Greenish diarrhoea, muscular tremors, torticolis, paralysis of legs and wings and opisthotonus may occur and mortality may reach 90- 100% in fully susceptible flock (Cynthia *et al.,* 2005).

2.9.2 Neurotropic velogenic

The neurotropic velogenic form (Beach's form) of ND presents with sudden onset of severe respiratory distress, followed by neurologic signs. Egg production falls dramatically but diarrhoea is usually absent. Morbidity may reach 100% and mortality may be up to 50 to 90% (Saif *et al*., 2005).

2.9.3 Mesogenic strain

Mesogenic strains of ND virus causes respiratory disease with marked drop in egg production and the mortality rate is usually low (Kahn, 2005).

2.9.4 Lentogenic

Lentogenic or respiratory form: This is a form of ND that presents with mild or subclinical respiratory infection. The greatest loss among laying birds frequently results from reduced egg production and impaired eggshell and albumen quality. (Quinn *et al*., 1953). In young birds, respiratory disease may occur and death may result from secondary bacterial infection (Quinn *et al*., 1953).

2.10 Incubation Period

The incubation period varies from 2-15 days, the severity of an infection is dependent on factors like the virulence and tropism of the virus, host species, age of the host, immune status, other diseases and environmental conditions (Kahn, 2005). Newcastle disease virus has an incubation period of 3-6 days or, on rare occasions, 2-15 days (Beard and Hanson, 1984).

2.11 Gross Pathology of Newcastle disease

Petechiae in the proventriculus and on the submucosae of the gizzard are typical; there is also severe enteritis of the duodenum. The lesions are few in hyperacute cases (Garba *et al*., 2012). Pronounced gross lesions such as swelling of the head or periorbital region and oedema of the interstitial tissue of the neck especially near the thoracic inlet are usually found in birds infected with velogenic strains (Alexander, 1998; Brown *et al*., 2008). Predominant postmortem lesions reported include matting of vent feathers, petechial haemorrhage on the tip of the proventricular papilla and caecal tonsils, congestion of duodenal and ileal mucosa and degeneration of ovarian follicles (Mozaffor *et al*., 2010). Other lesions reported by Abdu and Sa'idu (1990) includes haemorrhages in

the legs, thigh and breast muscles, trachea, abdominal and coronary fat, air sacculities, pale spleen and kidneys. There were necrosis in the gastrointestinal tract, caecal tonsils and bursa of fabricious and enlargement of spleen, liver and kidney (Sa'idu and Abdu 2008).

2.12 Microscopic Lesions

In most tissues and organs where changes occur, they consist of hyperaemia, necrosis, cellular infiltration and oedema. Changes in the central nervous system are those of nonpurulent encephalomyelitis (McFerran and McCracken, 1988). Necrosis of the cerebral parenchyma and markedly thickened arteries were also observed (Okoye *et al*., 2000; Oladele *et al*., 2005). Histopathological lesions are as varied as clinical signs and gross lesions and can be affected by virus pathotype, species of bird, concurrent disease and preexisting immunity (Kumbish, 2013). Histologic changes following virulent NDV infection include, depletion of lymphoid tissues and necrosis in various visceral organs (Alexander, 1997).

2.13 Differential Diagnosis

Outbreaks of ND may resemble many other poultry diseases such as infectious bronchitis, avian influenza, Marek's disease, fowl cholera and infectious laryngotracheitis (Aliyu, 2013).

2.13.1 Infectious laryngotreichitis

Avian infectious laryngotracheitis (ILT) is a respiratory disease of chickens caused by an alpha herpesvirus, gallid herpesvirus 1 (Williams *et al*., 1994). It can also affect

pheasants, partridges and pea fowl. In the virulent form, the history, clinical signs and very severe tracheal lesions are highly characteristic of the disease, but the mild form may be indistinguishable from other mild respiratory diseases. Laboratory diagnosis depends on the demonstration of the presence of the virus or viral components (Scholz *et al*., 1994; Williams *et al*., 1994; Guy and Bagust, 2003) or specific antibodies in the serum (Meulemans and Halen, 1978; Adair *et al*., 1985). Clinically, the morbidity is high and mortality may exceed 50%. Some birds may die in good body condition before the appearance of signs, which are characteristic and comprise difficulty in breathing with extension of the neck and gasping in an attempt to inhale. There is also gurgling, rattling and coughing when birds try to expel obstructions in the trachea. Conjunctivitis may also be observed. Clots of blood may be coughed up and can be found on the floor and walls of the house (OIE, 2014).

2.13.2 Avian Influenza (AI)

Avian influenza is a highly contagious viral disease affecting several species of birds. It is caused by various serotypes of influenza A virus of the Orthomyxoviridae family (Capua and Alexander, 2009). Type A influenza can be divided into subtypes based on the relationship between the glycoproteins on the surface, haemagglutinin (H1-H16) and neuraminidase (N1-N9). Each virus has one haemagglutinin and one neuraminidase antigen in any combination (Capua and Alexander, 2009). In most cases the infection is fulminant, sudden deaths, anorexia, drop in egg production, mild to severe respiratory distress and/or nervous signs like tremors and incoordination are often seen. There might also be respiratory discharge and swelling of periorbital tissues and sinuses, Mortality

may be as high as 100 %. ND cannot be distinguished from AI without laboratory testing (Siri, 2009).

2.13.3 Marek's Disease

Marek's Disease Virus (MDV) is a highly infectious lymphotropic alphaherpesvirus of poultry that induces Marek's disease (MD), characterised by immunosuppression, paralysis and rapid-onset of visceral lymphomas of the CD4+ T cells. Marek's disease remains a major concern for the poultry industry due to the unpredictability of outbreaks and the evolution of more virulent strains of MDV 1(Read *et al*., 2015). In some of these birds, lymphoid infiltration of peripheral nerves leads to paralytic symptoms. Marek's disease-associated mortality, due to neoplastic lesions and paralysis in birds from 3-4 week onwards, can cause serious economic losses (Osterrieder *et al*., 2006).

2.13.4 Infectious bronchitis

Avian infectious bronchitis (IB) is primarily a respiratory infection of chickens. The nephretic form of IB is characterised by mild and transient respiratory signs followed by depression, ruffled feathers, hunched stance, reluctance to move, excessive water intake, rapid weight loss and diarrhea. Death occurs four to five days after infection and ceases by day twelve after infection (Cumming, 1969). In adult chickens clinical signs may not be present or may take the form of a mild respiratory disease with coughing, sneezing and rales which can go unnoticed unless the flock is examined carefully. A decline in egg production usually follows within seven to twelve days (Mc Martin, 1968).

2.13.5 Fowl cholera

Fowl cholera (FC), caused by *Pasteurella multocida* can result in either an acute septicemia or chronic localized infections in domestic and wild birds (Sander *et al.,* 1998). It affects birds of all ages, however, chickens less than 16 weeks of age are generally resistant. Death from FC in chickens usually occur in laying flocks (Rhoades and Rimler, 1991).

2.14 Diagnosis of Newcastle disease.

The diagnosis of ND in Nigeria is based mainly on history of the flock, clinical sings and post mortem examination (Dipeolu *et al*., 1998), histopathology (Okoye *et al*., 2000) and serological test haemaglutination (HA), ELISA (Ezeokoli *et al*., 1984; Abdu and George, 1986; Oyewola *et al*., 1996 ; Orajaka *et al*., 1999). Viral isolation and identification using RT-PCR and other techniques are use in diagnosis of ND (OIE, 2012). The polymerase chain reaction (PCR) is frequently used to detect NDV (Fuller *et al*., 2009; Jang *et al*., 2011) in conjunction with virus isolation and biological characterization for index cases. However, some have shown lack of sensitivity of RT-PCR in detecting virus in some organs and particularly in feaces (Creeland *et al*., 2002). Multiplex PCR test have been developed to allow simultaneous detection and differentiation of several viruses, such as NDV, avian pneumovirus and avian influenza (Malik *et al*., 2004). Multiplex PCR techniques have also been used experimentally to differentiate between velogenic, lentogenic and mesogenic strains from chickens (Shane *et al*., 2003).

2.15 Laboratory Specimens

Samples from live birds should include both tracheal or oropharyngeal and cloacal swabs, the latter should be visibly coated with faecal material (OIE, 20l2). Swabbing may harm small, delicate birds, but the collection of fresh faeces may serve as an adequate alternative. Samples from dead birds should consist of oro-nasal swabs, as well as samples collected from lung, kidneys, intestine, caecal tonsils, spleen, brain, liver and heart tissues (OIE, 2012). These may be collected separately or as a pool, although brain and intestinal samples are usually processed separately from other samples (OIE, 2012).The samples should be placed in isotonic phosphate buffered saline (PBS), pH 7.0–7.4, containing antibiotics. Protein-based media, example brain–heart infusion (BHI) or tris-buffered tryptose broth (TBTB), have also been used and may give added stability to the virus, especially during shipping (OIE, 2012). The antibiotics can be varied according to local conditions, but could be, for example, penicillin (2000 units/ml); streptomycin (2 mg/ml); gentamycin (50 µg/ml); and mycostatin (1000 units/ml) for tissues and tracheal swabs, but at five-fold higher concentrations for faeces and cloacal swabs (OIE, 2012).

2.16 Diagnostic Techniques of Newcastle Disease

2.16.1 Virus Culture

According to OIE (2012), supernatant fluids of faeces or tissue suspensions and swabs, obtained through clarification by centrifugation at 1000 g for about 10 minutes at a temperature not exceeding 25°C, are inoculated in 0.2 ml volumes into the allantoic cavity of each of at least five embryonated SPF chicken eggs of 9–11 days incubation. If SPF eggs are not available, at least NDV antibody negative eggs are required. After

inoculation, these are incubated at 35–37°C for 4–7 days. Eggs containing dead or dying embryos as they arise, and all eggs remaining at the end of the incubation period, should first be chilled to 4°C for 4 hours or overnight and the allantoic fluids tested for haemagglutination (HA) activity. To accelerate the final isolation, it is possible to carry out two passages at 3-day intervals, obtaining results comparable to two passages at 4–7-day intervals (Alexander and Senne, 2008).

2.16.2 Virus Identification

Newcastle disease virus can be confirmed by the use of specific antiserum in a haemagglutination inhibition (HI) test. Usually chicken antiserum that has been prepared against one of the strains of NDV is used. In the HI test, some level of cross-reactivity may be observed among the various avian paramyxovirus serotypes (OIE, 2012). Cross-reactivity can be observed between APMV-1 and APMV-3 viruses (particularly with the psittacine variant of APMV-3, commonly isolated from pet or exotic birds) or APMV-7. The risk of mistyping an isolate can be greatly reduced by using a panel of reference sera or monoclonal antibodies (MAbs) specific for APMV-1, APMV-3 and APMV-7 (OIE, 2012).

At present, RT-PCR-based techniques for the detection and typing (pathotyping and genotyping) of APMV-1 RNA in allantoic fluid of inoculated fowl eggs is becoming increasingly common in diagnostic laboratories (OIE, 2012). However, the genetic variability of APMV-1 isolates should be considered carefully as potential cause for false negative results of genetic-based laboratory tests (Thayer and Beard, 2008).

2.16.3 Monoclonal Antibody

Mouse monoclonal antibodies (MAbs) directed against strains of NDV have been used in HI tests to allow rapid identification of NDV without the possible cross-reactions with other APMV serotypes that may occur with polyclonal sera (Alexander *et al*.,1997). Many MAbs have been produced that give reactions in HI tests that are specific for particular strains or variant NDV isolates (Alexander *et al*., 1997).

2.16.4 Serological tests

Virus neutralization, HI and ELISA tests are available, and new ELISAs have been described for use with feature marker and subunit vaccines (Mackay *et al*., 1999). At present, the HI test is most widely used for detecting antibodies to APMV-1 in birds while the use of commercial ELISA kits to assess post-vaccination antibody levels is common (Mackay *et al*., 1999).The HI test and ELISA may measure antibodies to different antigens; depending on the system used ELISAs may detect antibodies to more than one antigen while the HI test is probably restricted to those directed against the HN protein (Brown *et al*., 1999). However, comparative studies have demonstrated that the ELISAs are reproducible and have high sensitivity and specificity; they have been found to correlate well with the HI test (Brown *et al*., 1990).

2.17 Treatment of Newcastle Disease

There are no reports of successful treatment of ND and no antiviral drugs for ND are commercially available. Antibiotics are given to reduce or prevent secondary bacterial infections and prevention through vaccinations is widely practice. Electrolytes and vitamins are use as supportive therapies (Aliyu, 2013).

2.18 Prevention and Control of Newcastle Disease

Good management, biosecurity and hygiene remain the basis for prevention of ND, but in areas with an intensive poultry industry control of ND without vaccination is uncommon (Altimonte *et al*., 2010). Only in geographically isolated areas, with a very low risk of introduction of NDV and a relatively small economic impact of an outbreak, vaccination may be reserved for emergency only (Altimonte *et al*., 2010). Control of ND by vaccination is a routine in commercial chicken flocks in many countries. Inactivated vaccines have been used for inducing mainly systemic immunity (Rauwa *et al*., 2009). Live attenuated vaccines prepared from lentogenic strains such as Hitchner B1, LaSota, Clone 30 and VG/GA are widely used because they provide high efficacy of protection through the induction of both systemic and local immunity (Sellers, 2000; Rauwa *et al*., 2009). Many of the vaccination programs have been used in commercial chicken flocks to achieve reasonable protection against NDV (Khalifeh *et al*., 2009). The vaccination programs and procedures differ between countries and even between farms in the same country, depending on local circumstances (Rehmani, 1996). Newcastle disease is an economically important disease of poultry for which vaccination is carried out as a preventive measure in many countries. Orakaja *et al.,* (1999) reported vaccination as the only safe option in control strategies of infection. Mariana *et al.,* (2016) also reported that vaccination of chickens is able to prevent internal egg contamination, reducing egg shell surface contamination and reducing shedding from digestive and respiratory tracts in virulent NDV challenged hens. However, live vaccines have traditionally been heat-sensitive and require storage at or below 4°C (Spradbrow, 1992). Complete and effective

cold chains are expensive and difficult to maintain and extensively raised birds are difficult to catch, hence making vaccination labour intensive (Spradbrow, 1992).

2.19 Immunity to Newcastle Disease Virus

Because parasites and pathogens can have a critical effect on individual fitness, protection against disease is of major importance. However, pathogen avoidance, and investment in immune function often interact with other vital parameters, and even nonpathogenic immune challenge scan induce relevant physiologic changes in the host (Norris and Evans, 2000).

Immune system of poultry is a complex network of different cell types and soluble factors that give rise to an effective response to pathogenic challenges (Sarker *et al*., 2000) both cellular and humeral response have been suggested to play important roles in the hosts defense against NDV infection (Reynolds and Maraqa, 2000; Al-Shahery *et al.,* 2008). A research conducted on immune response of day old broiler chicks support the concept that shows humeral immunity to NDV is a key component in the protection against ND (Al-Zubeedy, 2009). Chicks from vaccinated parent stock contained high level of maternally derived antibody at day old which then declined gradually below protective level within 15-20 days after hatching. Saeed *et al.* (1988) reported that maternally derived antibody level declined to zero at day 25. High level of maternal antibody in day-old-chicks was also reported by Balla (1986). The rate of decline of maternally derived antibody was about half by every 5 days. But Mahmud *et al*., (2007) reported the persistence of MDA upto 27 days of age which may be due to the high MDA titres.

2.20 INFECTIOUS BURSAL DISEASE

Infectious bursal disease (IBD) is a highly contagious acute viral disease of young chickens of 3-6 weeks old that causes a fatality or immunosuppression by damaging bursa of Fabricius and impaired growth of young chickens which results in significant economic losses in the poultry industry (Lukert and Saif, 1997; Islam and Samad., 2004).

2.21 History of Infectious Bursal Disease

The first report of a specific disease affecting the bursa of Fabricius in chickens was made by Cosgrove (1962). The causative virus of this disease has affinity for bursa of Fabricius, hence the name infectious bursal disease (Hitchner, 1970). Infectious bursal disease has been of great concern for the poultry industry for a long time, particularly for more than the past two decades. In the late 1980s classical serotype 1 IBDV evolved into variant strains, in the USA or very virulent forms in Europe, Asia and other major parts of the world (Synder, 1990; Van den Berg, 2000). Some of the IBD outbreaks occurred in broilers at the end of fattening period, at farms where all hygienic and prophylactic measure have been taken care of (Van den Berg, 2000), in vaccinated flocks and, maternally immuned chicks (Wood *et al*., 1984), as well as adult birds.

Infectious bursal disease is included in the OIE (Office International des Epizooties) list of notifiable diseases as an avian disease (OIE, 2004). Infectious bursal disease has been diagnosed and identified in Nigeria (Ojo *et al*., 1973; Onunkwo, 1975; Oluwayelu *et al*., 2007). It is of high economic importance in Nigeria as it results in tremendous loss to poultry farmers interms of mortality and immunosuppression (Abdu *et al*., 2001). It is generally believed that the indigenous chicken is less susceptible to infections than the

exotic breeds (Okpe, 2001; Mdegela *et al*., 2002). Studies by other scientist in Nigeria showed that IBD has acquired an endemic status among the Nigerian poultry population (Abdu *et al*., 2001; Okoye and Uzoukwu, 2001; Oluwayelu *et al*., 2007). Oni *et al*. (2008) in a study on the serological status of unvaccinated indigenous chickens using ELISA in Abeokuta, Nigeria found a seroprevalence rate of 89.7%. Several studies have shown that the disease is of major concern to the poultry industry in the country (Tong *et al*., 1993; Mbuko *et al*., 2010). Infectious bursal disease virus (IBDV) has tropism to actively dividing precursor B lymphocytes, primarily in the bursa of Fabricius, but other immune organs are also involved (Wang *et al*., 2011).

2.22 Aetiology of Infectious Bursal Disease

Infectious bursal disease is caused by Infectious bursal disease virus (IBDV), which is a member of the genus *Avibirnavirus* of the family *Birnaviridae* (Delmas *et al*., 2004). IBDV is a double-stranded (dsRNA) virus (Muller *et al*., 1979), which targets immature B lymphocytes of bursa of fabricius, which is a primary B lymphoid organ in avian species, and subsequently causes B-cell depletion in bursal follicles (Muller *et al*., 1979). They cause severe, long-lasting immunosuppression due to destruction of immature lymphocytes in the bursa of Fabricius, thymus and spleen (Jean, 2010). There are two known serotypes: serotype I viruses cause disease with a range of severity in chickens and are further classified into classic, variant, and highly virulent strains, and serotype II viruses are nonpathogenic (Jean, 2010).

2.22.1 Antigenic characteristics of Infectious Bursal Disease Virus

Infectious bursal disease virus is endemic throughout the world but several different antigenic and pathogenic types exist in specific geographic locations (Mcferren *et al*., 1980). Two serotypes of IBDV occur in Europe and USA as recognized by the virus neutralization test. These two serotypes are antigenically distinct (Mcferren *et al.,* 1980). Serotype 1 viruses are pathogenic to chickens and differ in their virulence (Winterfield and Thacker, 1978). They cause lesions in the bursa of fabricius by lymphocytic depletion (Schroder *et al*., 2001). Whereas serotype 2 viruses are avirulent to chickens and isolated mainly from turkeys (Kibange *et al*., 1991) and chickens. Serotype 1 viruses can be broadly divided into classic, variant and very virulent IBDVs. Indeed, Adamu *et al.,* (2013) reported a high level of genetic heterogeneity and two distinct genetic clusters, specifically VV1 and VV2. In Nigeria the high degree of sequence diversity among Nigerian (VV) IBDV isolates suggests that West-Africa may be the origin of the newly emerging (VV) IBDV variant found across the Old World (Owoade *et al*., 2004).

2.22.2 Biochemical Properties of Infectious Bursal Disease Virus

The genome consists of two pieces of high Mr ds RNA that sediments at 14S components in sucrose gradients (Kibange *et al*., 1988; Todd and McNulty, 1979). The molecular weights of the two segments are 2.2 x 10^6 and 2.5 x 10^6 (Muller and Nitschke, 1987). It was reported that the two segments migrated similarly when co-electrophoresed. The RNA segments from serotype 2 viruses migrated similarly, but differed from serotype 1 viruses when co-electrophoresed (Jackwood *et al.*, 1984).

Bouyant density of 1.62 g/ml in cesium sulphate, melting point of 95.5 C in the presence of RNase, pairing of Adenine and Uracil and Thymine and cytosine, precipitation from 4M but not from 2M LiCl, green staining with acridine orange, all point towards a dsRNA genome (Kibenge *et al*., 1988). Both genome segments contain 94 x 10 3 5′ genome-linked protein (VPg). There are no poly (A) tracts at the 3′ ends of the RNA segments. The defective particles binding at 1.30 g/cm3 appear to have a truncated A segment (Kibenge *et al*., 1988).

2.22.3 Classification and Morphology of Infectious Bursal Disease

Infectious bursal disease virus is a nonenveloped, icosahedral capsid with diameter of about 60 nm. Formerly the virus of infectious bursal disease virus (IBDV) was improperly classified as Picorna or Reovirus. However, the known biological and structural properties of IBDV didn't allow its classification in one of the established taxonomic groups (Van den Berg, 2000; Ven den Berg, *et al*., 2002). Characterization of viral genome as bi-segmented double strand RNA. Muller *et al.*, (2003) allowed placing IBDV into a new family of viruses the Birnaviridae. There are two distinct serotypes (1 and 2) of infectious bursal disease virus (IBDV) that have been reported (Lukert and Saif, 1997). However, only serotypes 1 has been associated with clinical disease, thereby all commercial vaccines available have been prepared against serotype 1 (OIE, 2008). Out of the four existing pathotypes of serotype 1, very virulent IBD (vvIBDV) have been incriminated for most vaccination failures (Lukert and Saif, 1997). Strains of serotype 2 are naturally avirulent for chickens (Cummings *et al*., 1986; Ismail *et al*., 1998). Infectious bursal disease Virus can also be grouped into pathotypes based on their pathogenicity in chickens: They are referred to as classical virulent (CV),

antigenicvariant, very virulent (VV) and attenuated (Műller *et al*., 2003). As with all viruses, IBDV requires a receptor to penetrate target cells to cause infection. The distribution of this virus receptor mainly determines the target cells and the tissue specificity (Bass and Greenberg, 1992) and thereby the site of pathological changes associated with infection (Haywood, 1994). Infectious bursal disease virus (IBDV) has tropism to actively dividing precursor B lymphocytes, primarily in the bursa of Fabricius, but other immune organs are also involved (Wang *et al*., 2011).

2.22.4 Resistance to Chemical and Physical agents

The hardiness of the virus makes it difficult to eradicate from poultry houses after outbreaks of IBD (Alexander and Chattle, 1998). The virus is non-enveloped and resistant to physical and chemical agents. Certainly, the hardy nature of this virus is one reason for its persistent survival in poultry houses even when thorough cleaning and disinfection procedures are followed (Benton *et al*., 1967; Eterradossi and Saif, 2008). The virus is inactivated at a pH of 12.0 but not at pH 2.0 it can also survive a temperature of 37 ^{0}C for 90 min and 56 ^{0}C for 5 hours (Benton *et al*., 1967). A marked reduction in infectivity of the virus was observed after treatment with 0.5% Formalin for 6 hours. Cho and Edgar (1969) reported that the virus was inactivated by exposure for 1 hour to 1% formalin, 1% cresol and 1% phenol. It remained stable at 60 ^{0}C for 90 min and was still infectious at room temperature for 21 days. Petek *et al.,* (1973) observed that IBDV was more resistant than Reovirus to heat, ultraviolet irradiation and photodynamic inactivation. Landgraf *et al*. (1967) found that the virus survived 60 ^{0}C but not 70^{0}C for 30 minutes, and exposure to 0.5% chloramine after 10 minutes and 0.5% formalin for 6 hours destroyed the virus. In addition, iodine complex had deleterious effects on the virus

at 23^0C for 2 minutes (Eterradossi and Saif 2008).Heat resistance of IBDV is an important factor to be considered in trade of poultry due to extensive international trade of processed and partially processed poultry meat. Alexander and Chettle, (1998) constructed the heat inactivation curves of classical IBDV at 70^0C, 75^0C and 80^0C. These biphasic multiple kinetic curves showed an initial rapid drop in infectivity followed by a more gradual decline. In the second phase it took 18.8 min at 70^0C, 11.4 min at 75^0C and 3.0 min at 80^0C for reducing the infectivity by 1 log 10.

2.22.5 Pathogenicity and Pathotype of Infectious Bursal Disease Virus

Variation in IBDV antigenicity depends on amino acid changes in peak A and B. Serotype two strain 23/82, North American antigenic variants A, E, GLS-5 and DS 326 neutralization resistance escape mutants all exhibit amino acid changes in the hydrophilic peaks. Only serotype 1 strains show changes in hydrophobic domain (Eterradossi *et al*., 1998). Two smaller hydrophilic areas of VP2 variable domain also influence IBDV antigenicity (Etarradossi *et al*., 1998).

Deletion mutagenesis studies with Australian strain 002/73 showed that virus neutralizing monoclonal antibody recognized a discontinuous epitope on VP2 (Azzad *et al*.,1987). The antigenic region responsible for the production of neutralizing antibody is highly conformation dependent (Becht *et al*., 1988; Fahey *et al*., 1989) since the antibodies immune precipitate VP2 but did not react with the denatured protein in the immune blot (Oppling *et al*., 1991). The VP2 carries the epitopes which elicit neutralizing antibodies and distinguish the two strains as well as those which elicit non-neutralizing antibodies and are common to both strains (Cruz-coy *et al*., 1993). The antigenic region responsible

for the production of neutralizing antibody is highly conformation dependent. Monoclonal antibodies have also defined a common sequence dependent antigenic site located at the VP2 region (Becht *et al*., 1988).

2.23 Transmission of Infectious Bursal Disease Virus

Chickens infected with the IBD virus shed the virus in their feces (Gary and Richards, 2008). Feed, water, and poultry house litter become contaminated (Van den Berg, 2000). Other chickens in the house become infected by ingesting the virus in the contaminated litter, feed, water or dead birds. Because of the resistant nature of the IBD virus, it is easily transmitted mechanically among the farms by people, equipment and vehicles Some of the IBD outbreaks occurred in broilers at the end of fattening period, at farms where all hygienic and prophylactic measures have been taken care of (Van den Berg, 2000), in vaccinated flocks and, maternally immuned chicks (Wood *et al*., 1984), as well as adult birds. (Gary and Richard, 2008).

2.24 Epidemiology of Infectious Bursal Disease

Infectious bursal disease has been of great concern for the poultry industry for a long time, particularly for the past two decades. In the late 1980s classical serotype 1 IBDV evolved into variant strains, in the USA or very virulent forms in Europe, Asia and other major parts of the world (Synder, 1990; Van den Berg, 2000). In Nigeria IBD was first reported in 1973 (Ojo *et al*., 1973) with the highest incidence between the months of April-July and November-December (Abdu *et al.,* 2001). The outcome of IBD is largely depended on the strain and the amount of the virus, age and breed of birds, the route of inoculation, the presence or absence of neutralizing antibodies, intercurrent primary and

secondary pathogens and environmental and managemental factors (Muller *et al.,* 2012). Subsequent studies shows that the disease has acquired an endemic status in Nigeria (Abdu *et al*., 2001; El-Yuguda and Baba, 2004; Mai *et al*., 2004). Infectious bursal disease has been reported in vaccinated flocks and, maternally immuned chicks as well as adult birds (Durojaiye *et al*., 1984; Abdu, 1986).

2.25 Species of Birds Affected by Infectious Bursal Disease

Infectious bursal disease affect mainly chickens but the virus has been reported in Ostriches (Gough *et al*., 1998), in Baltic ducks and herring Gulls (Hollmen *et al*., 2000), various raptors and passerine species in Japan (Ogawa *et al.,* 1989). Anti-IBDV antibodies have been reported from Antarctic penguins (Gardner *et al.,* 1997). Oluwayelu *et al.* (2007) demonstrated the presence of IBDV antibodies in the sera of ducks. 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 Sa'idu, 1990). 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 and cockerels (Okoye and Uzuokwo, 1982), breeders (Abdu, 1986) and different strains of exotic chicken, white Leghorn (Ojo *et al*., 1973) and Star cross (Okoye and Uzuokwo, 1981). Experimental inoculation of IBDV to pheasants, partridges, guinea fowls and quails showed no signs of disease (Van den Berg *et al*., 2001). The IBDV has been isolated in domesticated pigeons (Bisalla *et al.,* 2005) but the Japanese quails are reported to be refractory to IBDV infection (Greenfield *et al*., 1986).

2.26 Pathogenesis of Infectious Bursal Disease

In studies conducted on tissues from chickens orally infected with IBDV. The viral antigens were detected in macrophages and lymphoid cells in the cecum at 4 hr PI and in the lymphoid cells of duodenum and jejunum at 5 hr PI using Immunoflourence test (Muller *et al*., 1979). The virus reaches the liver at 5 hrs PI and enters the bloodstream from where it is distributed to other organs; the bursal infection is followed by second viremia (Lukert and Saif, 2003). The virus persists in the bursa of experimentally inoculated SPF chickens for up to 3 weeks of age but the presence of maternal antibodies in the commercial chicken decreases the duration of its existence in bursa (Abdel-Alim and Saif, 2001a).

Various studies have shown that the variants and classic viruses exhibit similar pathology but differ from each other with respect to their pathogenicity and immunogenicity (Hassan *et al*., 1996). Reduction in the number of B cells in the BF due to viral infection is the major cause of immunosuppression. Besides lymphocyte lysis, apoptosis also plays a role in immunosuppression (Vasconcelos and Lam 1994; Ojeda *et al*., 1997; Tanimura and Sharma, 1998). Apoptosis could occur in a variety of organs (Allan *et al.,* 1972) like thymus (Inoue *et al*., 1994), BF and spleen (Vasconcelos and Lam, 1994; Lam, 1997). Ma *et al*. (2013) reported that vv, IBDV proliferated in 99% of the kupffer cells, the the largest reservoirs of resident tisse macrophages, rather than hepatocytes or endothelial cells of liver.

2.27 Clinical Signs of Infectious Bursal Disease

Infectious bursal disease is extremely contagious, in infected flocks, morbidity is high with up to 100% sero conversion after infection, whilst mortality is variable (Van den Berg *et al*., 2000). Early subclinical infections are the most important forms of the disease because of economic losses (Jean, 2010). They cause severe, long-lasting immunosuppression due to destruction of immature lymphocytes in the bursa of Fabricius, thymus and spleen (Jean, 2010). The disease manifests as acute and subclinical form(s) in chicks of age 0-3 weeks as immunosuppression or also in clinical form depending on the age of the bird (Okoye and Ozoukwo, 2001; Singh *et al*., 2015). The chicks become anorectic, reluctant to move, and show ruffled feathers with whitish yellowish diarrhea, trembling and severe prostration (Okoye and Ozoukwo, 2001; Singh *et al*., 2015). The disease is manifested by debilitation, dehydration and the development of depression with watery diarrhea, swollen and blood stained vent (Islam and Samad, 2004). Severity of the signs depends on the virus strain and the age and breed of the chickens (Van den Berg *et al*., 1991). Classical strains cause bursal inflammation and severe lymphoid necrosis in infected chicken, resulting in immunodeficiency and moderate mortality from 20 –30% in specific pathogen free (SPF) chicken (Lim *et al*., 1999). Hypervirulent strains of IBDV, 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. 28 Gross Pathological lesions of Infectious Bursal Disease

Birds infected with IBDV show enlargement, congestion, necrosis, haemorhages and oedema of bursa of Fabricius (Ojo *et al*., 1973; Bishu *et al*., 1977; Abdu, 1986). Kidneys of the infected birds become congested and enlarged with prominent tubules (Okoye and Uzuokwo, 1982). The spleen becomes enlarged, haemorhagic and necrotic (Onunkwo, 1975). Haemorhages are also observed at the junction between the proventriculus and gizzard, caecal tonsil and duodenum (Onunkwo, 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, 1986). The heart in affected birds show hydropericardium (Abdu*.,* 1986). There is increased mucus in the intestine and renal changes that are due to dehydration are observed in diseased birds (Lukert and Saif, 2003). The kidneys, tubules and ureters are also distended and filled with urates that they appear white (Cosgrove, 1962). Characteristic pattern of bursal changes observed during the course of infection differ for the classic and variant viruses (Rosenberger and Cloud, 1986). During the infection with classic viruses, the bursa increases transiently in size accompanied with inflammation. After the inflammation subsides, rapid bursal atrophy occurs. Extensive hemorrhages could be seen on the entire bursa. Pathologic changes in the spleen and thymus were less prominent than those of the bursa (Inoue *et al*., 1994). The spleen might be slightly enlarged and usually had small gray foci uniformly dispersed on the surface (Lukert and Saif, 2003). Lesions in these organs were noticed at the same time as the changes occurred in the bursa and resolved within 1 or 2 days of appearance (Helmboldt and Garner, 1964).

2.29 Microscopic lesions of Infectious Bursal Disease

The first signs of infection occur in the bursa and it is the most severely affected organ. Degeneration and necrosis of individual lymphocytes in the medullary region of the bursa occur as early as 1 day post infection. Lymphocyte degeneration is accompanied by nuclear pyknosis and formation of lipid droplets in the cytoplasm (Cheville, 1967). The microscopic lesions reported were intra and inter folicular oedema, necrosis of lymphocytes, interfollicular fibroplasias, heterophilic infiltration and reticular hyperplasia of the bursa of Fabricius (Okoye, 1984; Okoye and Ozoukwu, 2001). Kidney shows necrosis of tubules, congestion, mononuclear cell infiltration and vascular degeneration (Okoye, 1984; Okoye and Uzuokwu, 2001). The spleen, thymus and, caecal tonsil show necrosis and depletion of lymphocytes (Okoye, 1984; Okoye and Uzuokwu, 2001). The proventriculus shows congestion, desquamation and necrosis of hepatocytes and the muscle of the thigh and breast shows congestion, oedema, haemorhages and necrosis (Okoye, 1984; Okoye and Uzuokwu, 2001). Infected thymus, spleen, and caecal tonsils become normal a week after infection, while the bursa of Fabricius, kidney and skeletal muscles take 2-6 weeks to become normal (Abdu, 1988; Okoye and Uzuokwu, 2001). Follicular regeneration and repopulation of follicles with the lymphocytes occur but healthy follicles are not formed during the observed time span of 18 days (Helmboldt and Garner, 1964).

2.30 Diagnosis of Infectious Bursal Disease

Clinical disease due to infection with the IBDV can usually be diagnosed by combination of characteristic signs and postmortem lesions (OIE, 2008). Diagnosis can be obtained by

detection of antigen or antibody to IBD using the following technique: (1) macerated bursa used as antigen in ELISA or agar gel diffusion tests against a known positive antiserum, (2) microscopic examination of tissues for lesions or antigen following immunoperioxidase staining; or antigen detection by immunofluorescence of frozen bursal section, (3) virus isolation following inoculation of 9-11 day old embryonated eggs or cell cultures and, (4) antibody detection by ELISA, neutralization, or precipitating tests (Jordan and Pattison, 1996). Viral antigen can be demonstrated by agar gel precipitin assay or by antigen capture enzyme linked immunosorbent assay (AC-ELISA) (Dwight *et al*., 2004). Various molecular technique for the detection of IBDV particularly the real time RT-PCR, have also been developed (Van den Berg, *et al*., 1991; Kong *et al*., 2009). Real time RT-PCR and conventional PCR were usually followed by genetic characterization techniques such as heteroduplex mobility assay, RFLP and Neuclotide sequence comparisons (Bauda *et al*., 2004; Gomes *et al*., 2007; Sareyyuoglu and Akan, 2006). Rapid antigen/antibody detection of IBDV using monoclonal antibody/antigen by immunochromatography (Zhang *et al*., 2004).

2.30.1 Differential Diagnosis of Infectious Bursal Disease

The gross lesions and symptoms of coccidiosis are very similar to IBD (Lukert and Saif, 2003). However, muscular hemorrhages and edema differentiate IBD from coccodiosis. Other diseases that resemble IBD are infectious bronchitis virus, hemorrhagic syndrome and Marek’s disease (Lukert and Saif, 2003).

2.30.2 Detection of infectious bursal disease virus

Cell cultures containing 50% bursal lymphocytes and 50% CEF have been used to isolate and serotype IBD virus successfully (Lukert and Saif, 1997). The fibroblasts served as a matrix for lymphocytes and infected lymphocytes are detected by immunoflorescence (Lukert and Saif, 2003).

Immunoflorescence (MacDonald, 1980) and electron microscopy (McNulty *et al.,* 1970) of the infected cell culture or embryonated eggs are valuable tools for monitoring the growth of IBDV particularly those strains lacking pronounced CPE (Kibenge *et al*., 1988; Kibenge *et al*., 1991). . It is possible to grow the virus in transformed cell lines (Kibenge *et al.,*1988; Kibenge *et al*., 1991).The isolation, antigenic analysis and pathogenicity studies of the viruses isolated from field cases are done to detect the changes in the wild virus population (Lukert and Saif, 2003).

2.30.3 Serology

Serological tests generally used for the detection of IBDV are ELISA, VN and AGP.The ELISA allows the quantification of antibodies to IBDV and is therefore used for monitoring the immune status of the chicken flocks (Marquardt, 1980), to check response to vaccination, natural field exposure and decay of maternal antibody titre (Lasher and Shane, 1994; Lukert and Saif 2003). However, ELISA cannot differentiate between the antibodies specific to the two serotypes (Ismail and Saif 1990; Lukert and Saif 2003). Therefore, while using ELISA for monitoring the chicken flocks for antibodies to IBDV, careful consideration should be given to the fact that the serotype 2 viruses are widespread in commercial chickens and could result in erroneous impression of antibody

levels of the flock (Abdel-Alim and Saif, 2001b). Another method used to detect antibodies to IBDV is the AGP test. This test has been adapted to the quantitative format (Cullen and Wyeth, 1975). Virus Neutralization (VN) test differentiates between antibodies elicited by two serotypes as well as subtypes of serotype 1 viruses (Ashraf, 2005).

2.31 Prevention and Control of Infectious Bursal Disease

Disinfection, cleaning, good biosecurity and good chicken source are important in ensuring that the chicken would be free from any diseases. There is evidence, however, that thorough cleaning and disinfection of houses between flocks and the practice of all-in all-out management reduces the challenge thus allowing more time for vaccine to induce immunity (Ingrao *et al*., 2013). Formaldehyde and iodophors have been shown to be effective disinfectant (Jordan *et al*., 2002). Rigorous biosecurity measures have to be implemented in order to stop the spread of the virus from one flock to the next. No therapeutic treatment has been found to have an effect on the course of the viral infection (Cosgrove, 1962; Lukert and Saif, 2003). There are no reports of the use of the antiviral compounds and interferon inducers for the treatment of IBD (Lukert and Saif, 2003).

2.32.1 Vaccination against infectious bursal disease

Infectious bursal disease virus is highly infectious and resistant to inactivation therefore, despite strict hygienic measures; vaccination is inevitable under high infection pressure. It is recommended to protect chickens against infection during the first week after hatch (Kaufer and Weiss, 2005). Most commercially available conventional live IBDV vaccines are based on classical virulent strains (Jackwood, 1990). Those classified as

mild vaccines exhibit only poor efficacy in the presence of certain levels of maternally derived antibodies against vvIBDV (Van den Berg and Meulemans, 1991). Intermediate and intermediate plus or hot vaccines have better efficacy by breaking through higher levels of maternally derived antibodies but they can induce moderate to severe bursal lesions and may not fully protect chicken against infection by vvIBDV or antigenic variant strains (Muller *et al*., 2012). Despite acceptable reduction of mortality from the use of hot IBDV vaccine, their safety still remained a major concern (Vander Berg, 2000). Infectious bursal disease killed vaccine have been experimentally shown to confer protection against challenge with vvIBDV (Chen *et al*., 2016; Martinez-Torrecuadrada *et al*., 2003). The use of killed IBD vaccine candidates would eliminate the risk of circulation of vaccine IBDV that may undergo antigenic variation or increase in virulence (Sreedevi and Jackwood, 2007). Live classical IBD vaccines show a degree of attenuation; many of them may cause bursal atrophy and thus immunosuppression with poor immune response to vaccination against other pathogens and an increase in vulnerability to various types of infections as possible consequences (Muller *et al*., 2012). However, the time of vaccination is crucial as persisting mertanally derived antibody might neutralised live IBDV vaccine and revaccination may be necessary (Muller *et al*., 2003). The use of live IBD vaccines contributed to the high sequence diversity of emerging field strains and spread of IBDV strains from one region to another (Jibrin, 2014). There is no IBD vaccination schedule that can be routinely recommended (Segal, 2009). Factors influencing a vaccination scheduled include the type of chicken to be vaccinated (broilers or commercial layers) and level of MDA (Segal, 2009). The higher the start level of MDA the later the age at which vaccination is possible. If the variation

in MDA levels is too high (Cv > 30%) a second IBD live vaccination is required to effectively immunize the flock (de Wit and Baxandale, 2013). The MDA break through titre of the vaccine being used (intermediate or intermediate plus is 200 and 500 MDA titre (de Wit and Baxandale, 2013). The choice of live vaccine to be used depends on the virulence of field infection (Segal, 2009). Mild and intermediate vaccine strains have poor efficacy against virulent IBDV as these vaccines cannot be administered at an early age due to MDA interference (de Wit and Baxandale, 2013). The earlier the vaccination age, the higher the level of MDA, requiring a stronger vaccine. High quality chicks (high and uniform MDA) are to be vaccinated with intermediate vaccine at 26 to 30 days and with intermediate plus vaccine at 8 to 10 weeks to boost the immunity, low quality chicks (low and uneven MDA) are to be vaccinated with intermediate vaccine at 5 to 10 days or with intermediate plus vaccine at 16 and 22 days and with intermediate plus at 8 and 10 weeks to boost the immunity (Segal, 2009). Some commercial layers companies prefer to use only inactivated vaccines combined with intermediate vaccines to protect their birds thereby, eliminating the use of intermediate plus vaccines from their programme, due to some negative effects on the bird (Segal, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Birds

A total of one hundred and twenty day-old chicks were purchased from a reputable hatchery located in Ibadan, Nigeria. Prior to the arrival of the pullets, the pens were thoroughly cleaned and disinfected using 0.5% formalin.

3.2 Housing

The birds were raised in deep litter (with floor space of 0.10 m^2per bird) in the Poultry Research Pen of the Veterinary Teaching Hospital, Ahmadu Bello University Zaria. They were fed commercial chick mash throughout the experiments and water was provided *ad libitum*. Access to the pen by people or other animals as well as rodents were restricted as much as possible. At day one, the birds were randomly divided into six groups (A, B, C, D, E and F), consisting of 20 chicks each. The groups were kept in separate cages.

3.3 Vaccines

A freeze-dried live attenuated IBD (ABIC) Vaccine (M.B. Strain, Biological Laboratories Ltd a division of Phibro Animal Health) and Newcastle disease Vaccine La Sota Strain live vaccine (Biological Laboratories Ltd a division of Phibro Animal Health). The vaccine were reconstituted with clean and dechlorinated water and administered via drinking water in accordance with manufacturer's instruction.

3.4 Experimental design

Group A pullets were vaccinated with infectious bursal disease vaccine at 1st and 2nd weeks while Newcastle disease La Sota vaccine at 3rd and 6th weeks.

Group B pullets were vaccinated with infectious bursal disease vaccine at 2nd and 4th weeks while Newcastle disease La Sota vaccine at 3rd and 6th weeks.

Group C pullets were vaccinated with infectious bursal disease vaccine at 3rd week while Newcastle disease La Sota vaccine at 3rd and 6th weeks.

Group D pullets were vaccinated with infectious bursal disease vaccine at 2nd, 4th and 6th weeks while Newcastle disease La sota vaccine at 3rd and 6th weeks.

Group E pullets were vaccinated with Newcastle disease La Sota vaccine at 3rd and 6th weeks.

Group F received non of infectious bursal disease and Newcastle disease La Sota vaccines.

Table 3.1 Experimental design

Groups	Age (Weeks)							
	1	2	3	4	5	6	7	8
A	IBV	IBV	NDLV	-	-	NDLV	-	-
B	-	IBV	NDLV	IBV	-	NDLV	-	-
C	-	-	IBV+NDLV	-	-	NDLV	-	-
D	-	IBV	NDLV	IBV	-	IBV+NDLV	-	-
E	-	-	NDLV	-	-	NDLV	-	-
F	-	-	-	-	-	-	-	-

Key :

IBV = Infectious bursal disease vaccine (ABIC)

NDLV = La Sota vaccine (Newcaste disease)

- = No vaccine

3.5 Blood Sample Collection

A total of 10 birds from each group were randomly bled with sterile 2ml disposable syringes and needles through cardiac puncture. The blood samples were collected on weekly basis for 8 weeks and were transferred to labeled 5ml plain plastic test tube and allowed to clot at room temperature. The blood samples were centrifuged at 2,054 g for 15 minutes using a Hermler centrifuge. The serum was extracted using a plastic micropipette and transferred into labeled sample bottles, which were stored at -20 ^{0}C until used.

3.6 Serology

The antibody titre of Newcastle disease was determined by using enzyme linked immunosorbent assay (ELISA).

3.7 Determination of Newcastle disease antibody titre

Enzyme linked immunsorbent assay kit (ELISA) for Newcastle disease antibody was obtained from IDvet Laboratories (310, rue Louis Pasteur – Grabels – France). The reagents preparation and the test procedures were strictly carried out according to the manufacturer's instruction. In the ELISA micropalte, add:

- 100 µl of the Negative Control were added to wells A1 and B1, 100 µl of the Positive Control were added to wells C1 and D1. 90 µl of Dilution Buffer 14 to as many wells as there are samples to be tested. 10 µl of the pre-dilution samples prepared were added.

- The plates were covered and incubated for 30 minutes ± 3 minutes at 21 ^{0}C (±5 ^{0}C). – The conjugate 1X were prepared by diluting the Concentrated conjugate 10X to 1:10 in dilution Buffer 3.

-The wells were empted and washed each well 3 times with approximately 300 µl of the wash solution 1X. Drying of the wells between washings were avoided. -100 µl of the Subtrate Solution were added to each well. –Incubated for 15 minutes ± 2minutes at 21 ^{0}C (± 5 ^{0}C) in the dark. –100 µl of the Stop Solutions were added to each well in order to stop the reaction as in step N ^{0}9. – Ordinary Dilution (OD) were read and recorded at 450 nm.

3.8 Gross and Histopathological Examinations

Two birds were randomly and humanely sacrificed (slaughtered) from each group to observe for gross lesions in the bursa of Fabricius, thymus and spleen at the end of the experiment. Tissues for histopathology were harvested and fixed in 10% phosphate-buffered neutral formalin. The fixed tissues were embedded in paraffin and sectioned at 5 microns and then stained with Haematoxalin and Eosin (H&E) (Luna, 1968). The slides were observed under a light microscope using various magnifications and snapped using digital camera.

3.9 Data Analysis.

Two way analysis of variance (ANOVA), followed by Tukey's multiple comparism post-hoc test using GraphPad prism 5.0 (2007) for windows were used to compare the differences between the Mean titers obtain from control and treated groups, values of $P<0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Antibodies to Newcastle disease virus

The antibodies to NDV were expressed as mean ± SEM as presented in Table 4.1. The mean antibodies titre to NDV for all the experimental groups at the first week were high in group A with 3876.5 ± 185.0, while the lowest mean antibody titre of 3671.4 ± 25.07 was recorded in group E chickens (Table 4.1). In the second week of the experiment, the highest ND mean antibody titre of 45664.4 ± 58.06 was recorded in group A while the lowest ND mean antibodies titre of 3648.3 ± 90.18 was observed in group E (Table 4.1). For the 3rd week of the experiment, group D had the highest mean ND antibody titre of 4452.6 ± 158.67 while the lowest mean ND antibody titre of 3804.5 ± 430.14 was observed in group F (Table 4.1). At the 4th week group B had the highest ND mean antibody titre of of 5626.2 ± 193.4 and group F had the lowest ND mean antibody titre of 3379.2 ± 442.91 (Table 4.1). At the 5th week group A had the highest antibody titre of 9339.8 ± 776.42, then followed by group D that had ND mean antibodies titre of 8034.7 ± 679.85 and group C which had the lowest mean antibody titre of 4913.9 ± 946.67. The ND mean antibody titre of group A was significantly different lower ($P \leq 0.01$) with that of group B and there was also significant difference ($P \leq 0.001$) with mean antibody titre of group C. In the 5th week also the mean antibody titre of group C demonstrated significant difference ($P \leq 0.05$) with mean antibody titre of group D (Table 4.1). At the six week group D with the highest mean ND antibody titre was significant difference ($P \leq 0.01$) from that of mean ND antibody mean titre of group B and ND mean antibodies titre of

Table 4.1: Mean antibody titre to Newcastle disease of pullets vaccinated with IBD and ND vaccines using different vaccination schedules

WEEKS	GROUPS					
	A MEAN±SEM(n=10)	B MEAN±SEM(n=10)	C MEAN±SEM(n=10)	D MEAN±SEM(n=10)	E MEAN±SEM(n=10)	F MEAN±SEM(n=10)
1.	3876.5 ± 18.50^{a}	3874.7 ± 3.747^{a}	3716.1 ± 37.14^{a}	3757.7 ± 32.31^{a}	3671.4 ± 25.07^{a}	3874.5 ± 19.26^{a}
2.	45664.4 ± 58.06^{a}	4348.3 ± 111.31^{a}	3753.2 ± 18.80^{a}	3678.6 ± 28.71^{a}	3648.3 ± 90.18^{a}	4269.8 ± 119.0^{a}
3.	3980.7 ± 226.93^{a}	4176.0 ± 250.98^{a}	4143.1 ± 286.7^{a}	4452.6 ± 158.67^{a}	4328.4 ± 302.64^{a}	3804.5 ± 430.14^{a}
4.	3722.7 ± 579.28^{a}	5626.2 ± 193.48^{a}	4296.7 ± 542.01^{a}	4560.9 ± 333.86^{a}	4017.2 ± 397.92^{a}	3379.8 ± 442.94^{a}
5.	5334.5 ± 1004.07^{a}	9339.8 ± 776.42^{ba}	4913.9 ± 946.67^{cb}	8034.7 ± 697.85^{dc}	7507.3 ± 1196.17^{a}	7084.2 ± 664.21^{a}
6.	4979.5 ± 1496.02^{a}	2861.9 ± 1284.72^{a}	3985.5 ± 1161.56^{a}	7187.2 ± 986.04^{db}	5815.8 ± 1114.49^{a}	3104.6 ± 611.84^{fd}
7.	9059.0 ± 1805.96^{a}	10916.8 ± 957.49^{a}	12134.3 ± 647.0^{a}	10224.3 ± 446.87^{dc}	1006.8 ± 603.69^{a}	$6288.0\pm1091.^{fbcde}$
8.	7886.63 ± 1792.67^{a}	9044.8 ± 1153.75^{a}	11480.6 ± 827.45^{ca}	7812.63 ± 1977.87^{a}	7497.22 ± 1754.93^{ec}	7281.29 ± 1698.38^{fc}

Data with the same superscript indicate that there is no significant difference. Data with different superscript indicate significant difference.

group F (Table 4.1). At the 7th week of the experiment, the highest ND mean antibody titer was observed in group C. Group F ND mean antibody titre was significant difference ($P \leq 0.001$) with ND mean antibody titre of group B. The mean ND titre of group C ($P \leq 0.001$) was significant difference with ND mean titre of group D (Table 4.1). At week 8, ND mean antibody titre of group C was significant difference ($P \leq 0.05$) with ND mean antibodies titres of groups A, F and C respectively (Table 4.1).

4.2 Gross Pathological Changes observed in Major Immune Organs

Postmortem examination were conducted on the sacrificed pullets at the end of the experiment of some immune organs and the lesions observed were recorded (Table 4.2).

4.2.1 Gross lesions observed in group A

At postmortem, there were severe enlargement and congestion of the bursa of Fabricius, enlargement of the spleen and enlargement of the thymus compared to group F which showed mild enlargement of the bursa of Fabricius, mild congestion of spleen and no lesions in the thymus (Table 4.2).

4.2.2 Gross lesions observed in group B

At postmortem, there were enlargement and congestion of bursa of Fabricius, congestion of the spleen and congestion of the thymus compared to group F which showed mild enlargement of the bursa of Fabricius, mild congestion of spleen and no lesions in the thymus (Table 4.2).

4.2.3 Gross lesions observed in group C

At postmortem, there were mild enlargement of the bursa of Fabricius, enlargement and congestion of the spleen and congestion of the thymus compared to group F which showed mild enlargement of the bursa of Fabricius, mild congestion of spleen and no lesions in the thymus (Table 4.2).

Table 4.2 Gross lesions observed in major immune organs of pullets of different groups at the end of the experiment

Organs evaluated	Gross Lesions observed	A	B	C	D	E	F	+ve	(%)
		Groups							
Bursa of Fabricius	Enlargement	+	+	+	+	+	+	6/6	(100)
	Congestion	+	+	-	-	-	-	2/6	(33)
	Haemorrhage	-	-	-	+	-	-	1/6	(16)
Spleen	Enlargement	+	-	-	+	+	-	3/6	(50)
	Congestion	-	+	+	+	-	+	4/6	(66)
Thymus	Enlargement	+	-	-	+	+	-	3/6	(50)
	Congestion	-	+	+	+	-	-	3/6	(50)
Total +ve per group		4	4	3	6	3	2		

Key

+ve = Positive

+ = Presence of lesion

- = Absence of lesion

4.2.4 Gross lesions observed in group D

At postmortem, there were severe enlargement, haemorrhages and congestion of the bursa of Fabricius, enlargement and congestion of the spleen and enlargement and congestion of the thymus compared to group F which showed mild enlargement of the bursa of Fabricius, mild congestion of spleen and no lesions in the thymus (Table 4.2).

4.2.5 Gross lesions observed in group E

At postmortem, there were mild enlargement of the bursa of Fabricius, enlargement of the spleen and slight enlargement of the thymus compared to group F which showed mild enlargement of the bursa of Fabricius, mild congestion of spleen and no lesions in the thymus (Table 4.2).

4.2.6 Gross lesions observed in group F

At postmortem, there were milder enlargement of the bursa of Fabricius, congestion of the spleen and no gross lesion in the thymus (Table 4.2).

4.3 Histopathological Changes in Tissues of Major immune Organs of Different Groups

The histopathology observed in all the experimental groups are shown in Table 4.3. In group A include lymphocytic depletion, focal necrosis, thickened interfollicular space and formation of cyst in the bursa of Fabricius (Plate 4.1). In group B, lymphocytic depletion, necrosis of the follicles and oedema in the lumen in the bursa of Fabricius (Plate 4.2). In group C, lymphocytic depletion, thickened interfollicular space and formation of cyst in the bursa of Fabricius (Plate 4.3). In group D, lymphocytic depletion and necrosis of follicles, thickened intefollicular space and formation of cyst in the bursa of Fabricius (Plate 4.4). For group E, the bursa of Fabricius was oedematous, lymphocytic depletion and atrophy of the follicles (Plate 4.5). In group F, the bursa of Fabricius showed mild lymphocytic depletion (Plate 4.6). In group A the spleen was congested and had depletion of lymphocytes (Plate 4.7). In group B there was congested with depletion of lymphocytes in the spleen (Plate 4.8). In group C the spleen had congestion and depletion of lymphocytes (Plate 4.9). In group D the spleen had oedema and depletion of lymphocytes (Plate 4.10). In group E the spleen had depletion of lymphocytes (Plate 4.11). In group F the spleen showed mild depletion of lymphocytes (Plate 4.12). In group A the thymus had depletion of lymphocytes in the medullary portion (Plate 4.13). In group B had haemorrhages, necrosis and depletion of lymphocytes of the thymus (Plate 4.14). In group C the thymus had depletion of lymphocytes and haemorrhages (Plate 4.15). In group D the thymus had interlobular congestion, haemorrhages and depletion of the lymphocyte in medullary portion (Plate 4.16). In group E the thymus had depletion of lymphocytes in the medullary (Plate 4.17). In group F the thymus had mild depletion of lymphocytes (Plate 4.18)

Table 4.3 Histopathological changes in major immune organs of all the groups

Organs Evaluated	Histopathology Observed	Groups							
		A	B	C	D	E	F	+ve	%
Bursa of Fabricius	Depletion of lymphocytes	+	+	+	+	+	+	6	100
	Focal necrosis	+	+	-	+	-	-	3	50
	Thickened interfollicular space	+	-	+	+	-	-	3	50
	Cyst formation	+	-	+	+	-	-	3	50
	Oedema	-	+	-	-	+	-	2	33.3
	Atrophy of follicules	-	-	-	-	+	-	1	16.6
Thymus	Depletion of lymphocytes	+	+	+	+	+	+	6	100
	Necrosis	-	+	-	-	-	-	1	16.6
	Haemorrhages	-	+	+	+	-	-	3	50
	Congestion	-	-	-	+	-	-	1	16.6
Spleen	Depletion of lymphocytes	-	+	+	+	+	+	5	83.3
	Congestion	+	+	+	-	-	-	3	50
	Oedema	-	-	-	+	-	-	1	16.6
Total +ve from groups		6	8	7	9	5	3		

Key: - = Negative , + = Positive, % = Percentage Positive and +ve = Total number Positive

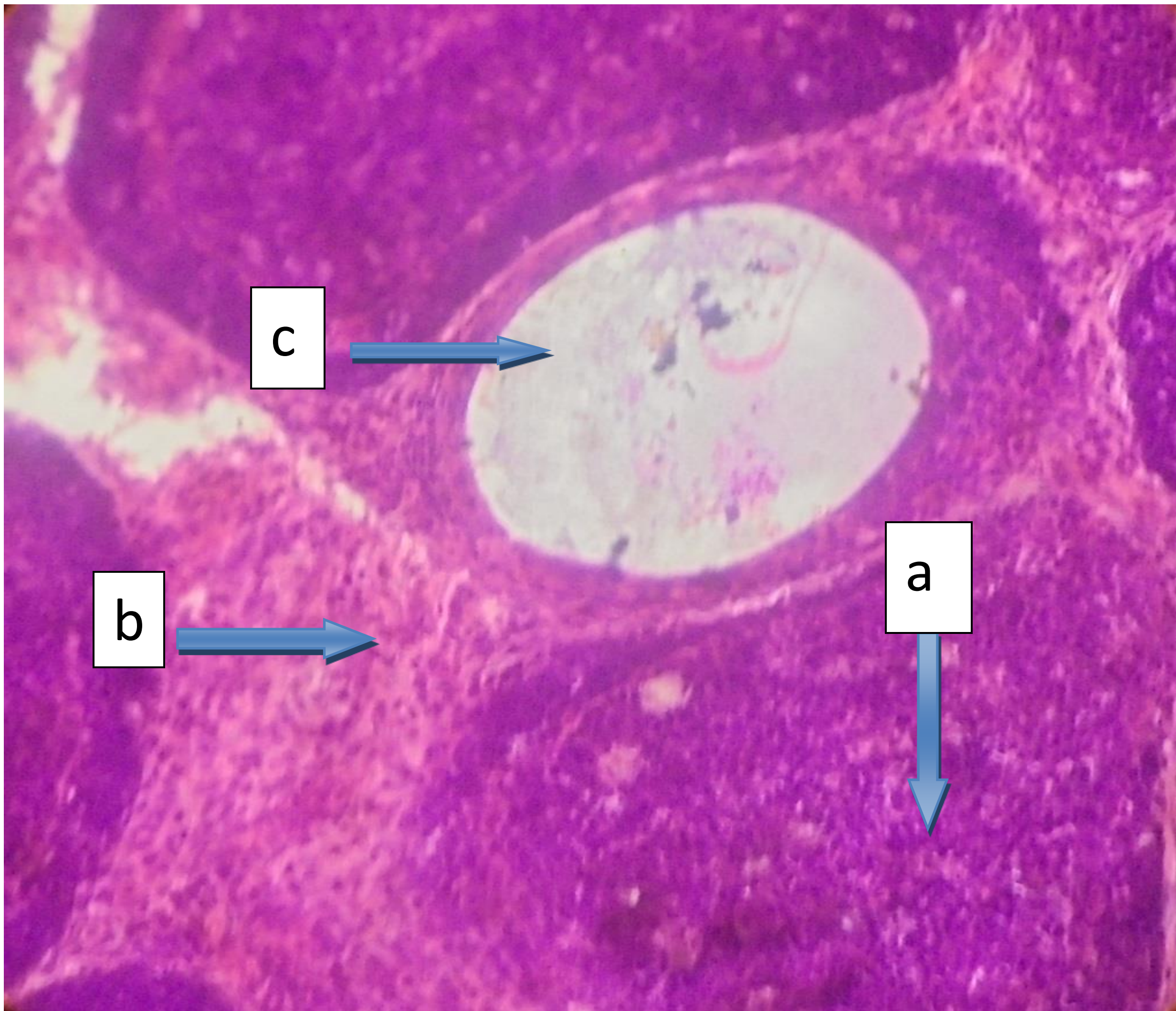

Plate 4. 1: Photomicrograph of section of the bursa of Fabricius of pullets in group A showing depletion and necrosis of lymphocytes (arrow a), thickened interfollicular space (arrow b) and cyst formation (arrow c) . H&E x400**.**

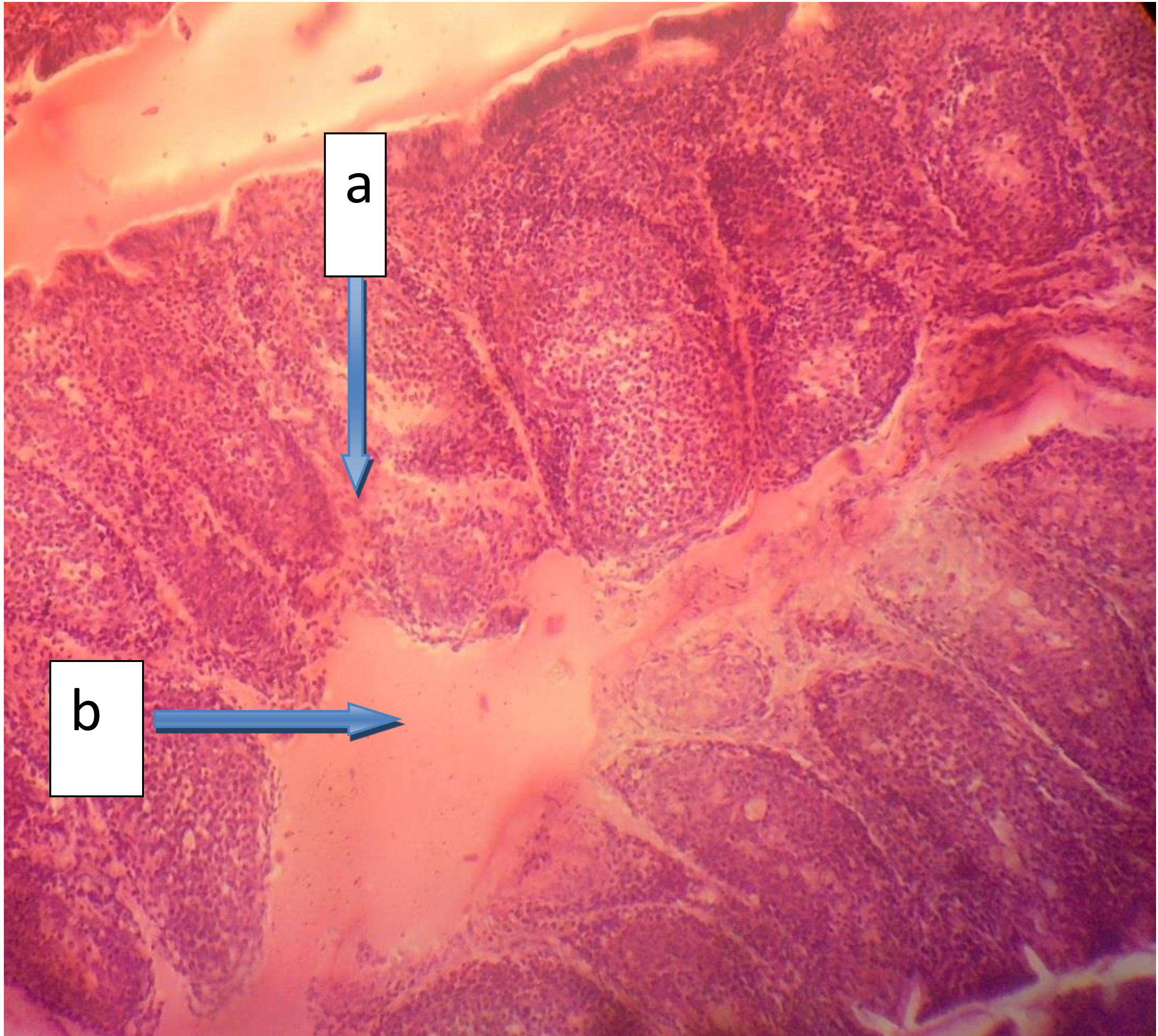

Plate 4.2: Photomicrograph of section of the bursa of Fabricius of pullets in group B showing depletion and necrosis of lymphocytes (arrow a) and oedema of the lumen of the follicles (arrow d). H&E x400.

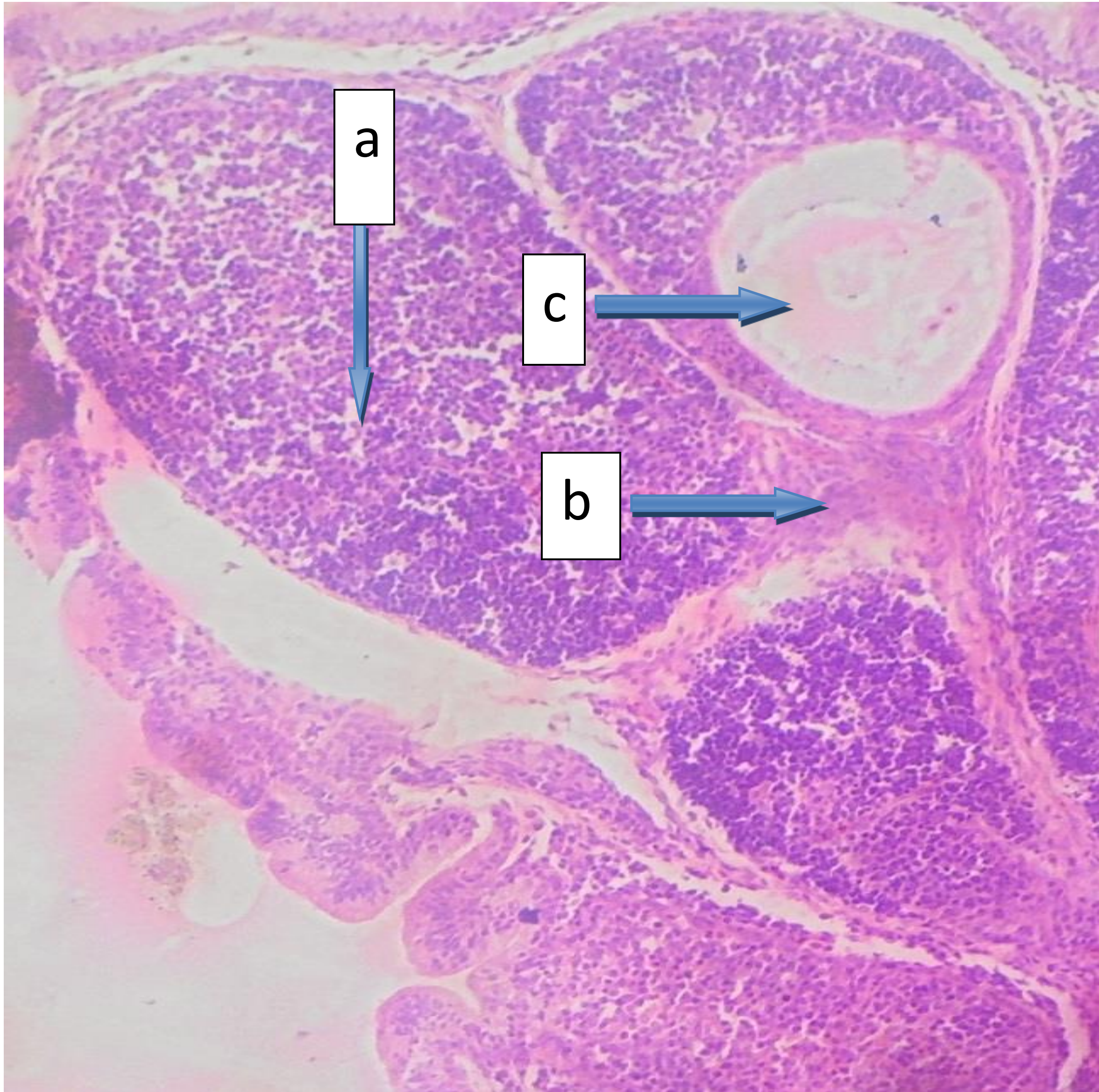

Plate 4.3: Photomicrograph of section of the bursa of Fabricius of pullets in group C showing depletion and necrosis of lymphocytes (arrow a), thickened interfollicular space (arrow b) and cyst formation (arrow c). H&E x200

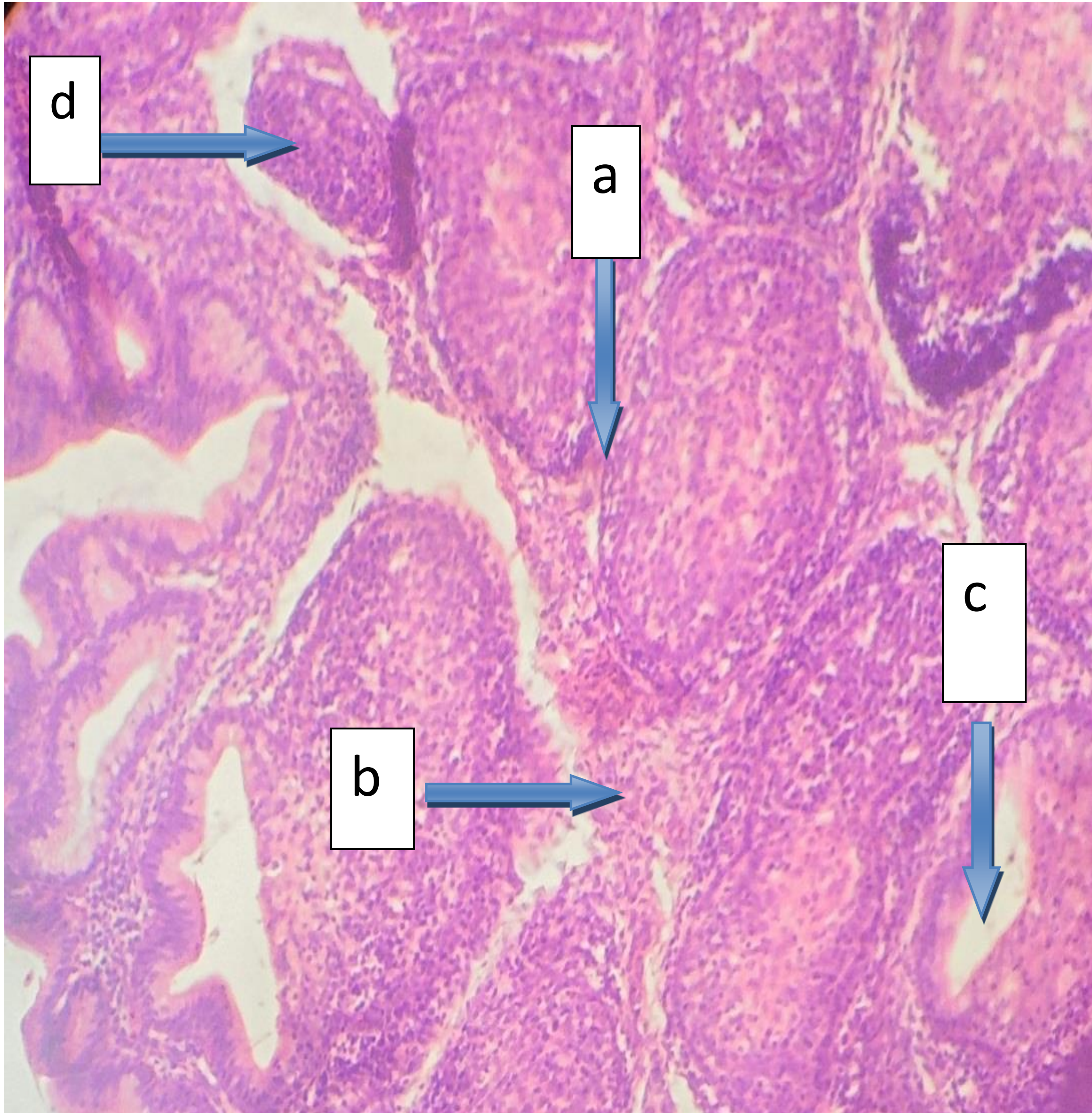

Plate 4. 4: Photomicrograph of section of the bursa of Fabricius of pullets in group D showing depletion and necrosis of lymphocytes (arrow a), thickened interfollicular space (arrow b), cyst formation (arrow c) and atropy of follicles (arrow d). H&E x200

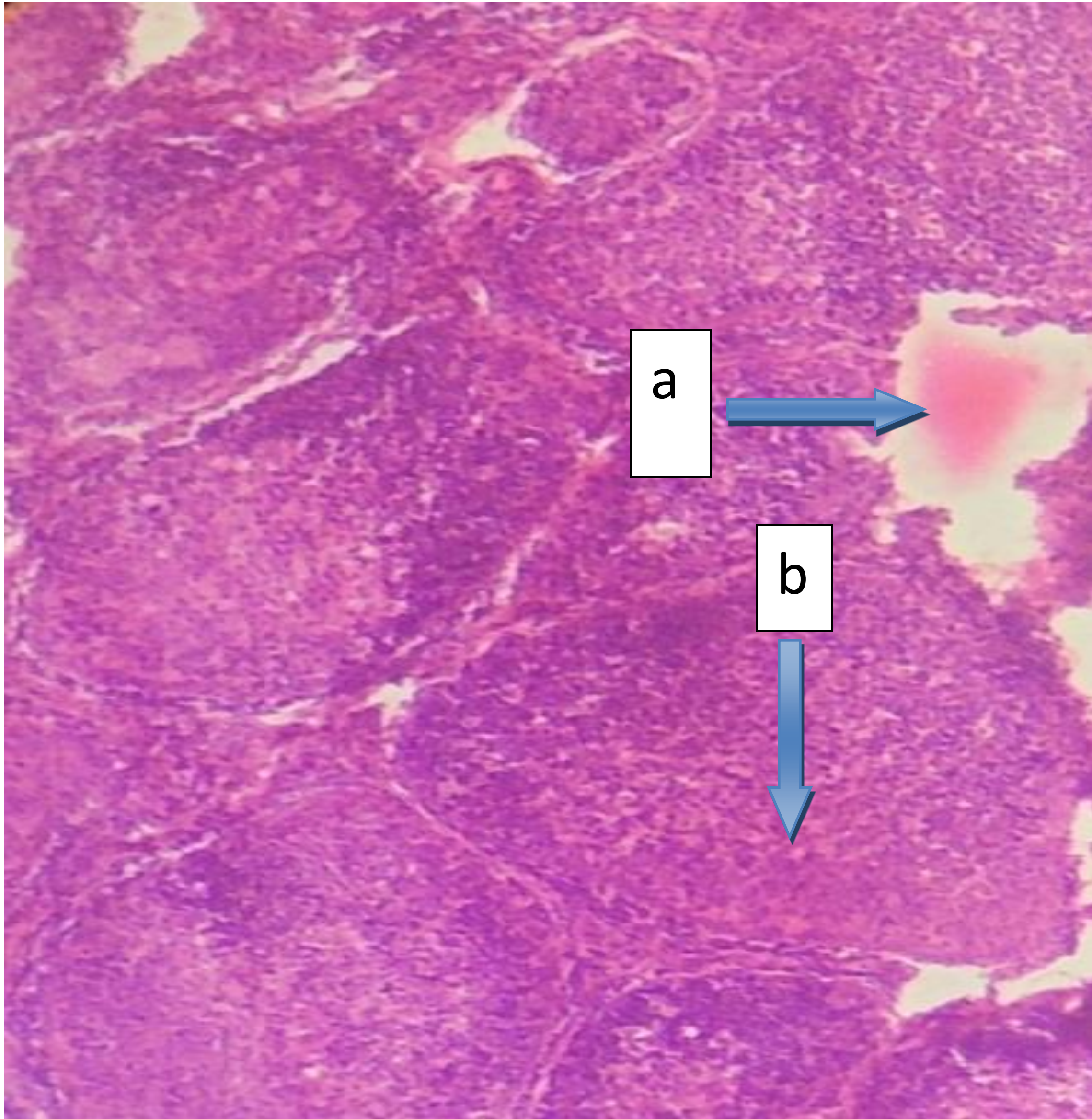

Plate 4.5: Photomicrograph of section of bursa of Fabricius of pullets in group E showing oedema (arrow a), and depletion of lymphocytes (arrow b) . H&E x100.

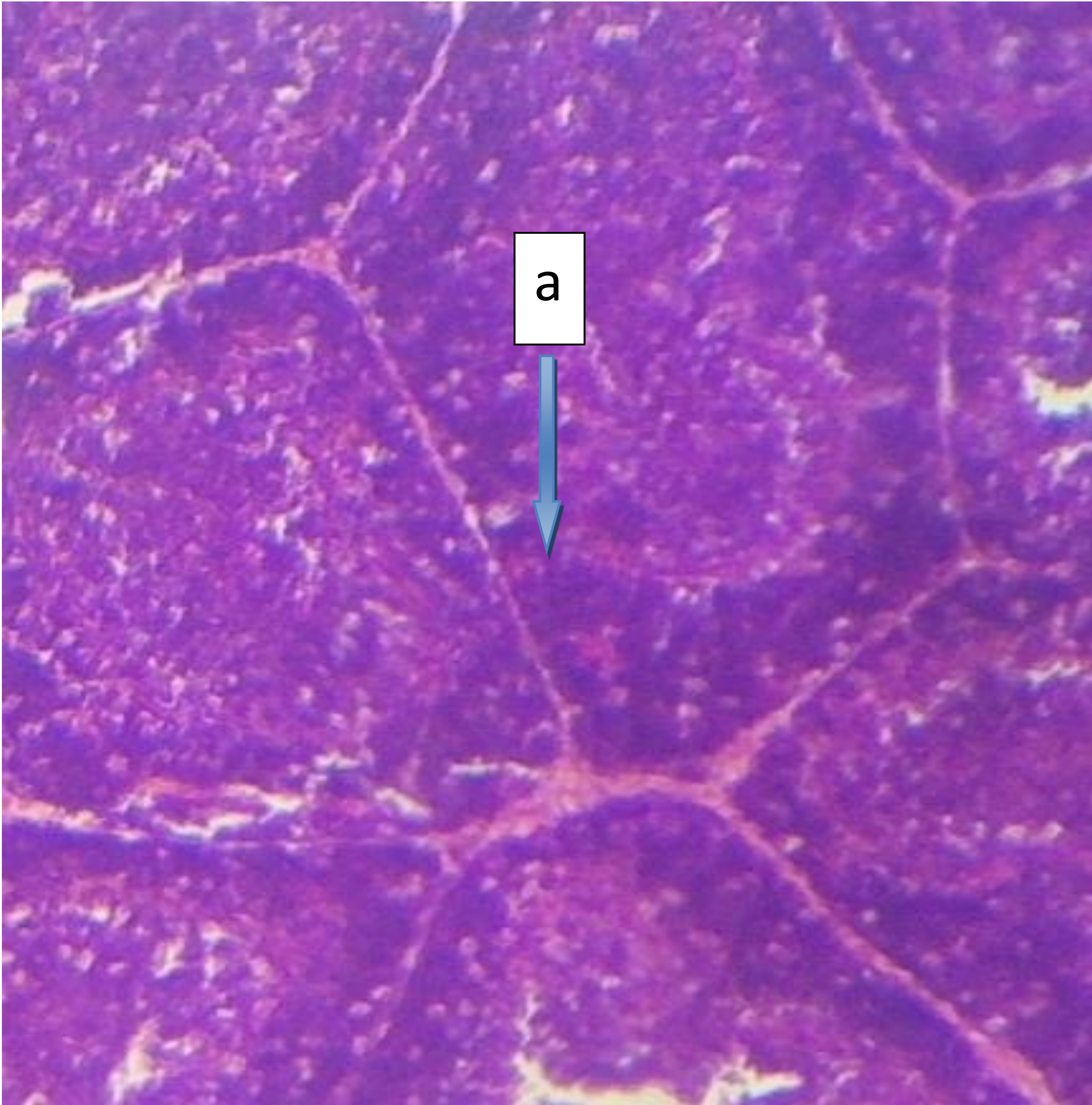

Plate 4. 6: Photomicrograph of section of bursa of Fabricius of pullets in group F showing depletion of lymphocytes (arrow a). H&E x100

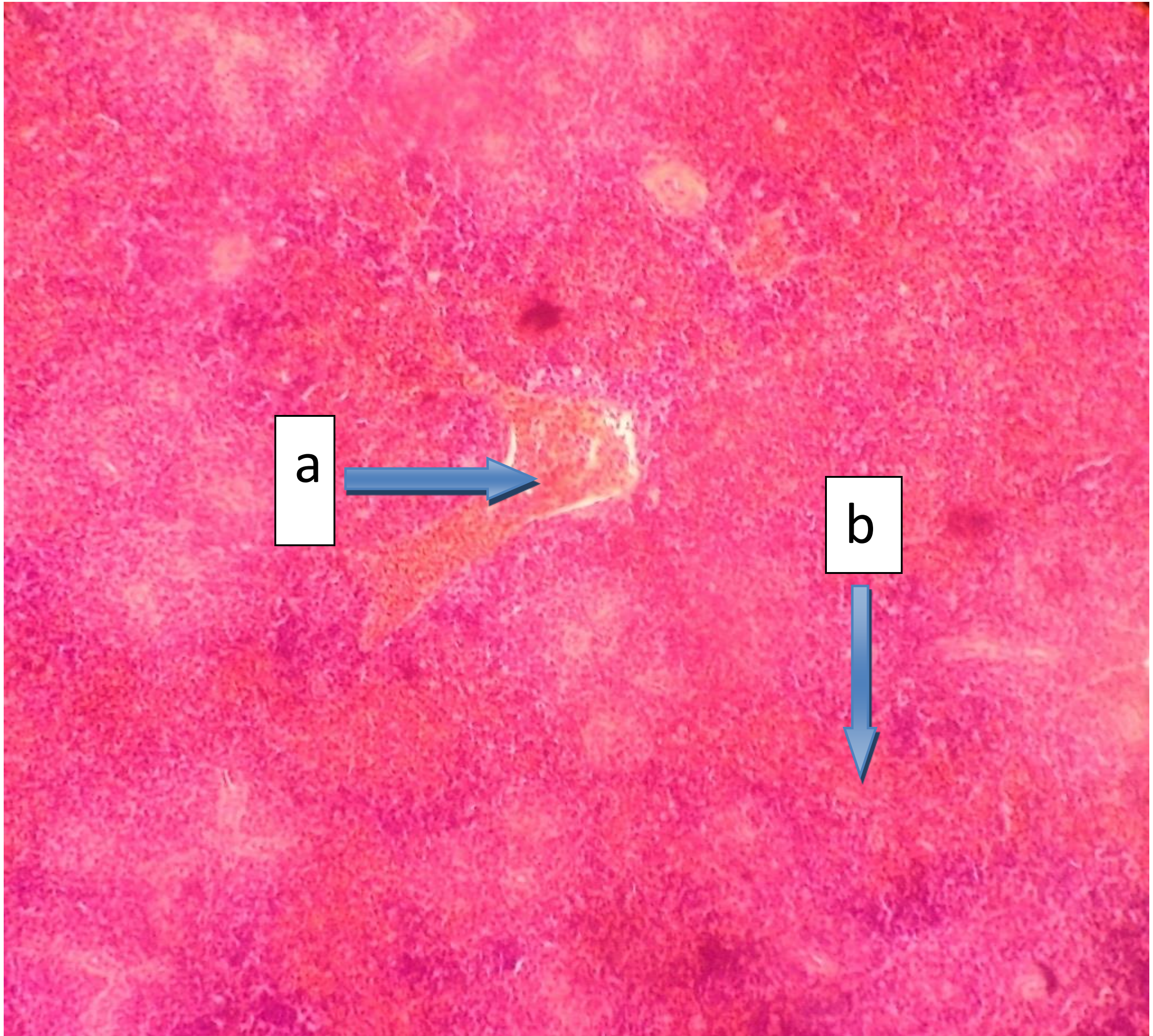

Plate 4.7: Photomicrograph of section of the spleen of pullets in group A showing congestion (arrow a) and depletion of lymphocytes (arrow b). H&E x200

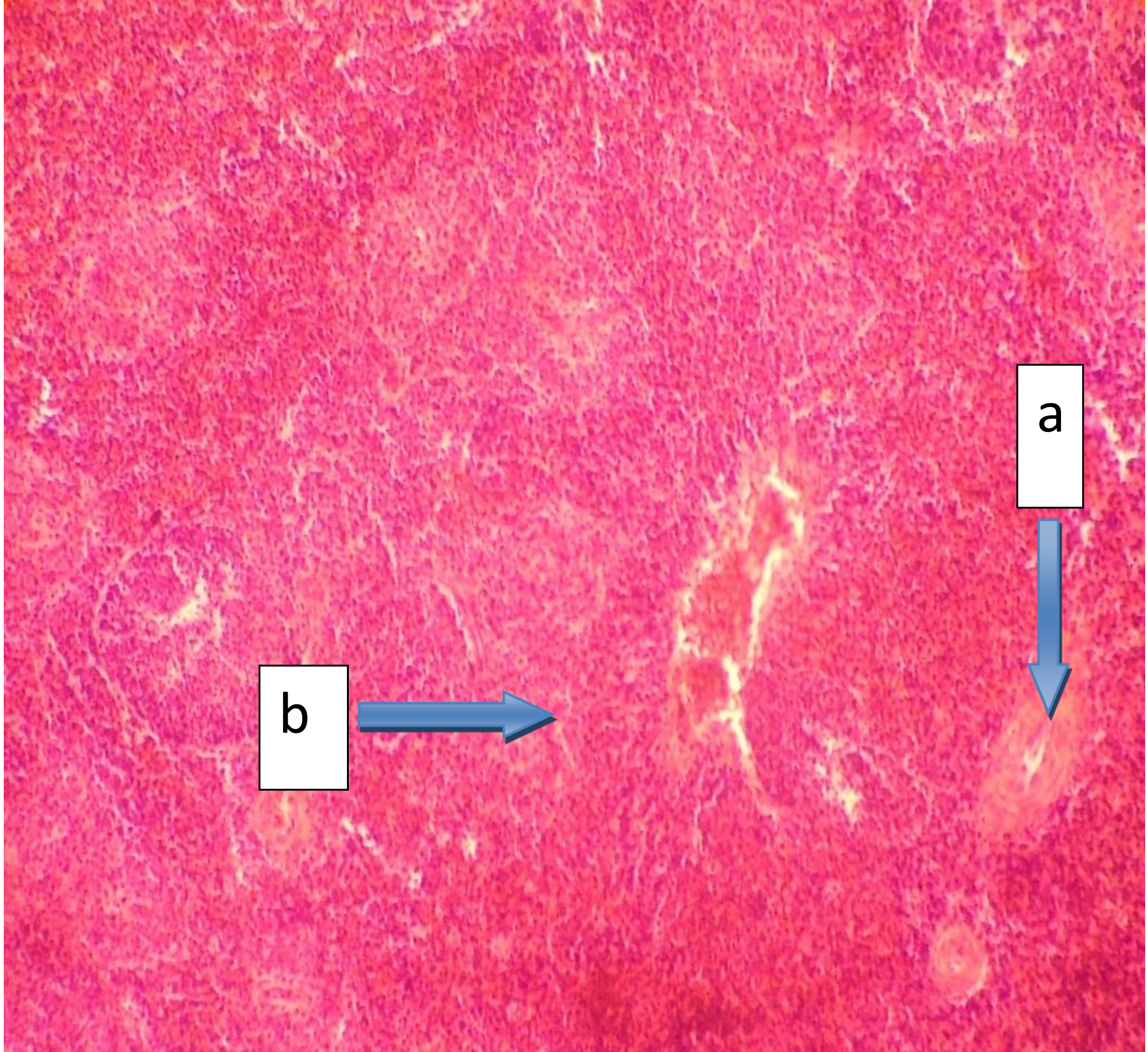

Plate 4.8: Photomicrograph of section of the spleen of pullets in group B showing congestion (arrow a) and depletion of lymphocytes (arrow b). H&E x200

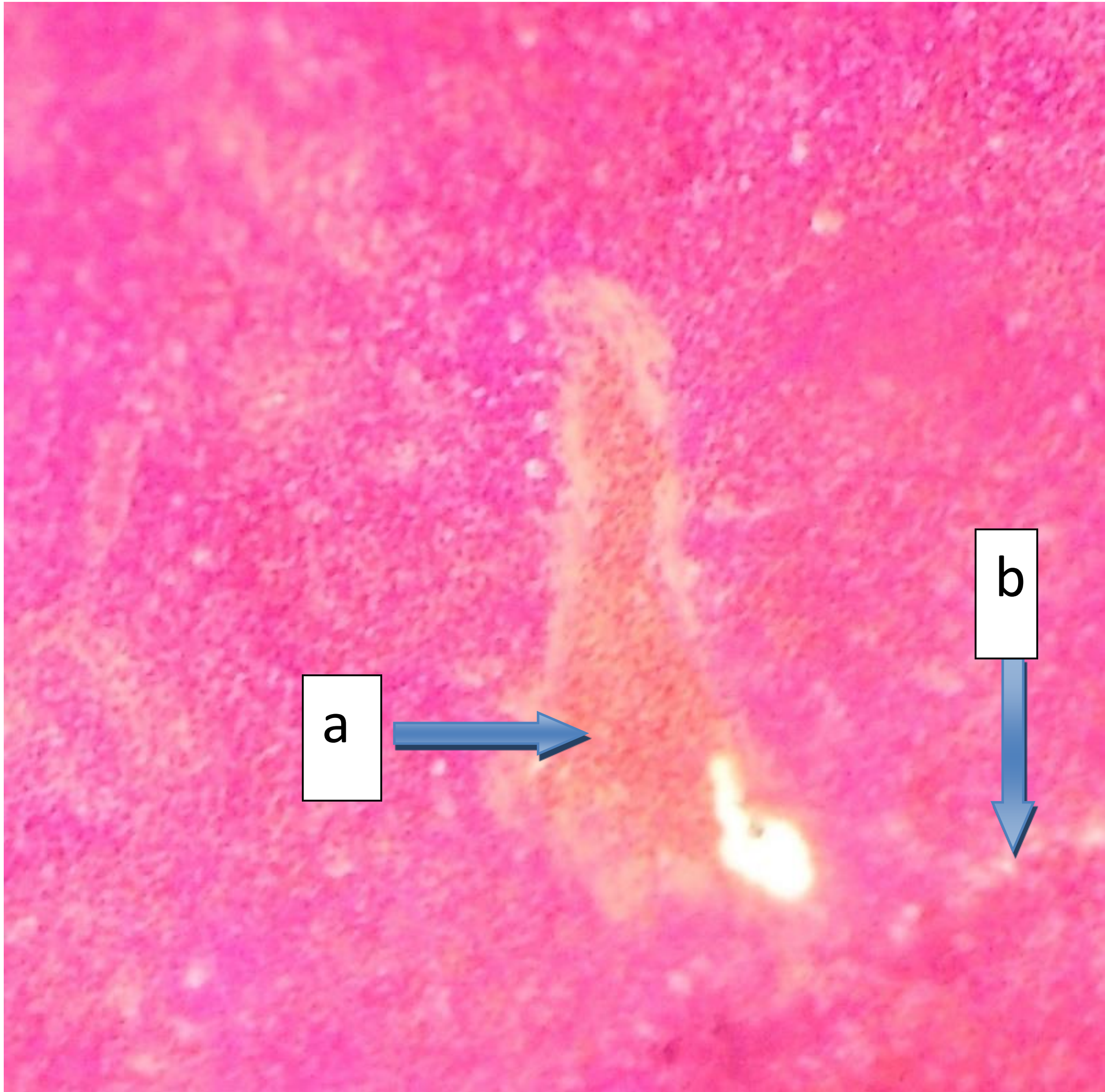

Plate 4.9: Photomicrograph of section of spleen of pullets in group C showing congestion (arrow a) and depletion of lymphocytes (arrow b). H&E x200

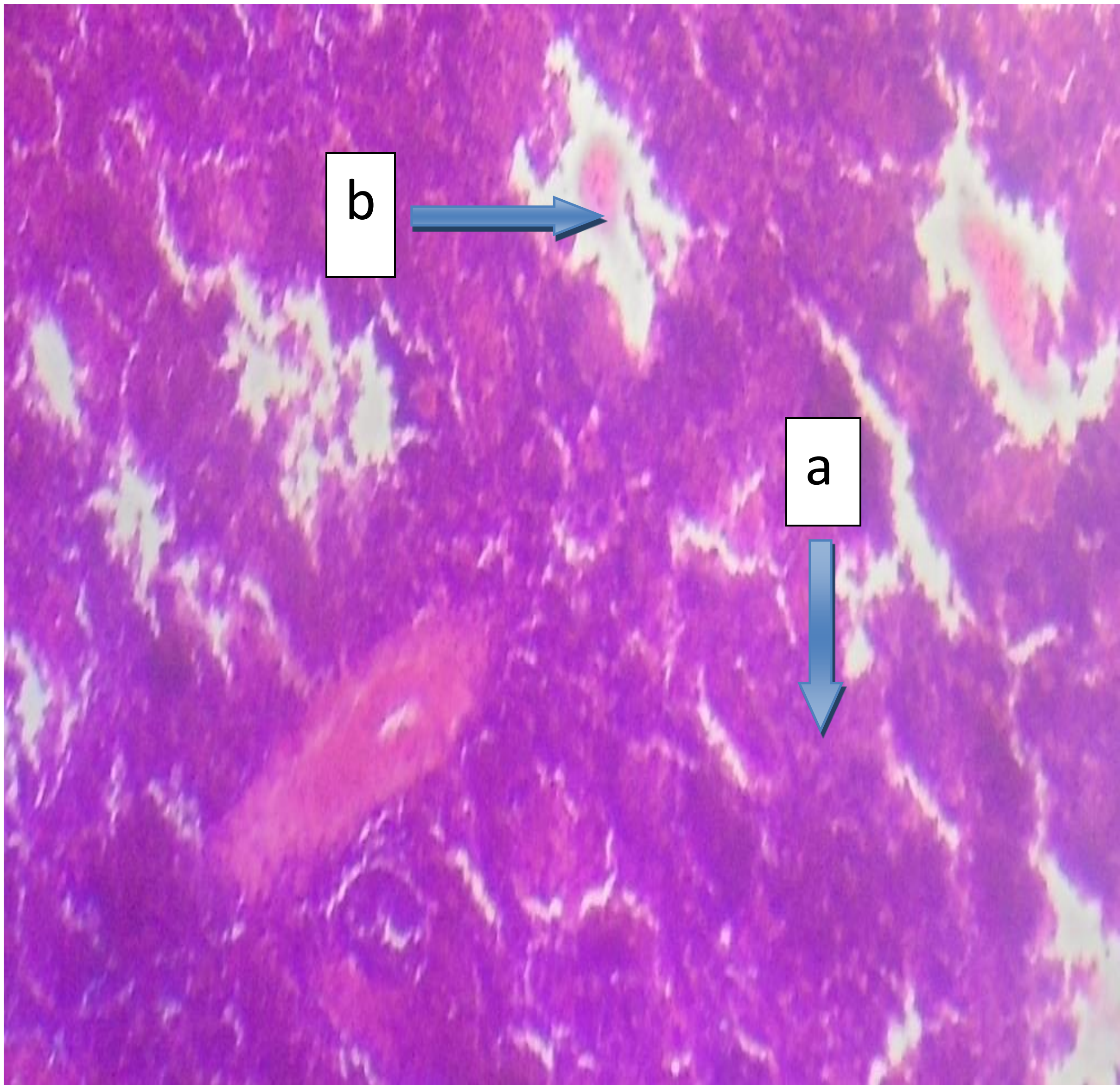

Plate 4.10: Photomicrograph of section of spleen of pullets in group D showing depletion of lymphocytes (arrow a) and oedema (arrow b). H&E x200.

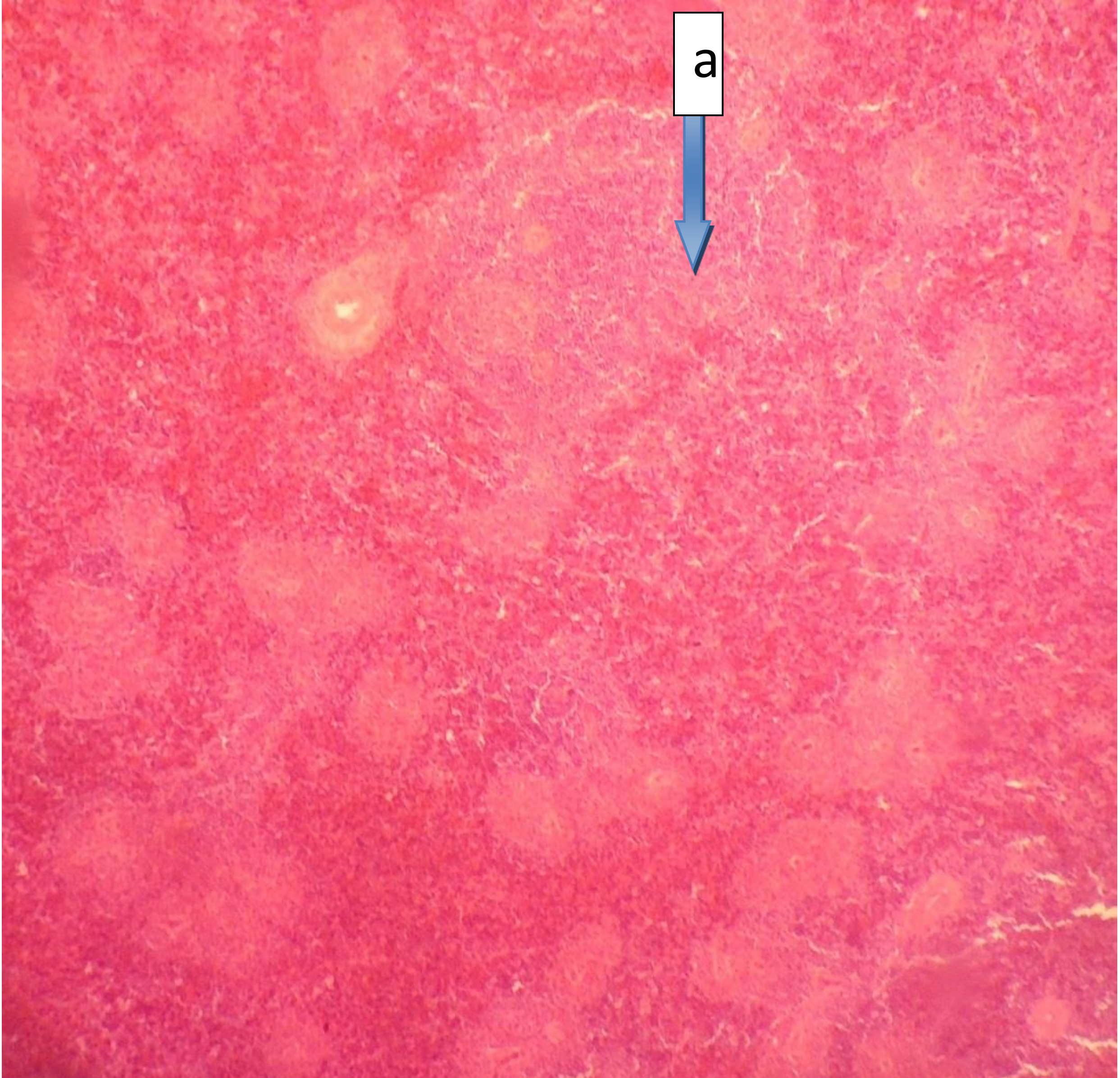

Plate 4. 11: Photomicrograph of section of spleen of pullets in group E showing mild depletion of lymphocytes (arrow a). H&E x100

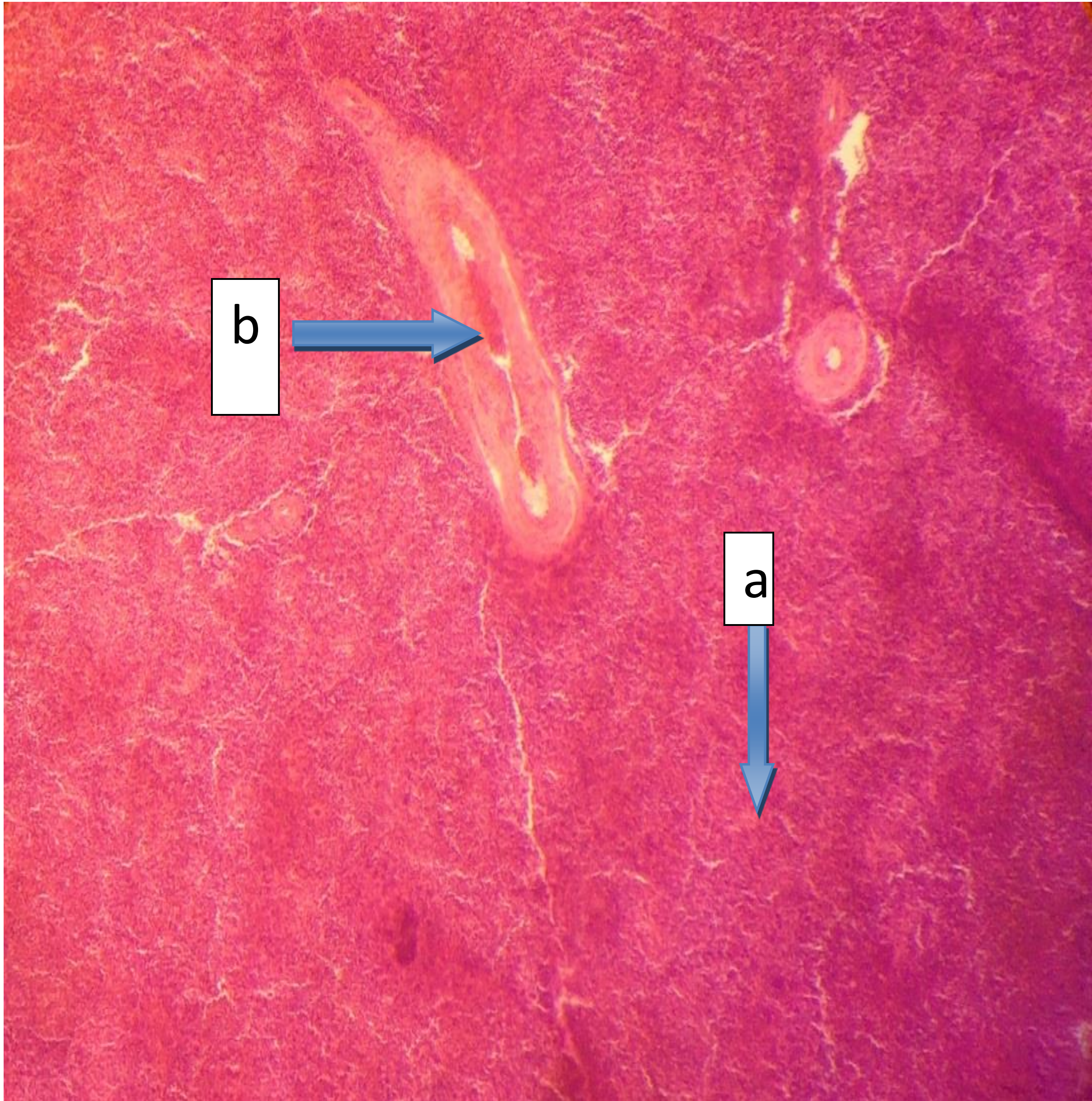

Plate 4. 12: Photomicrograph of section of spleen of pullets in group F showing mild depletion of lymphocytes (arrow a) and congestion (arrow b). H&E x100

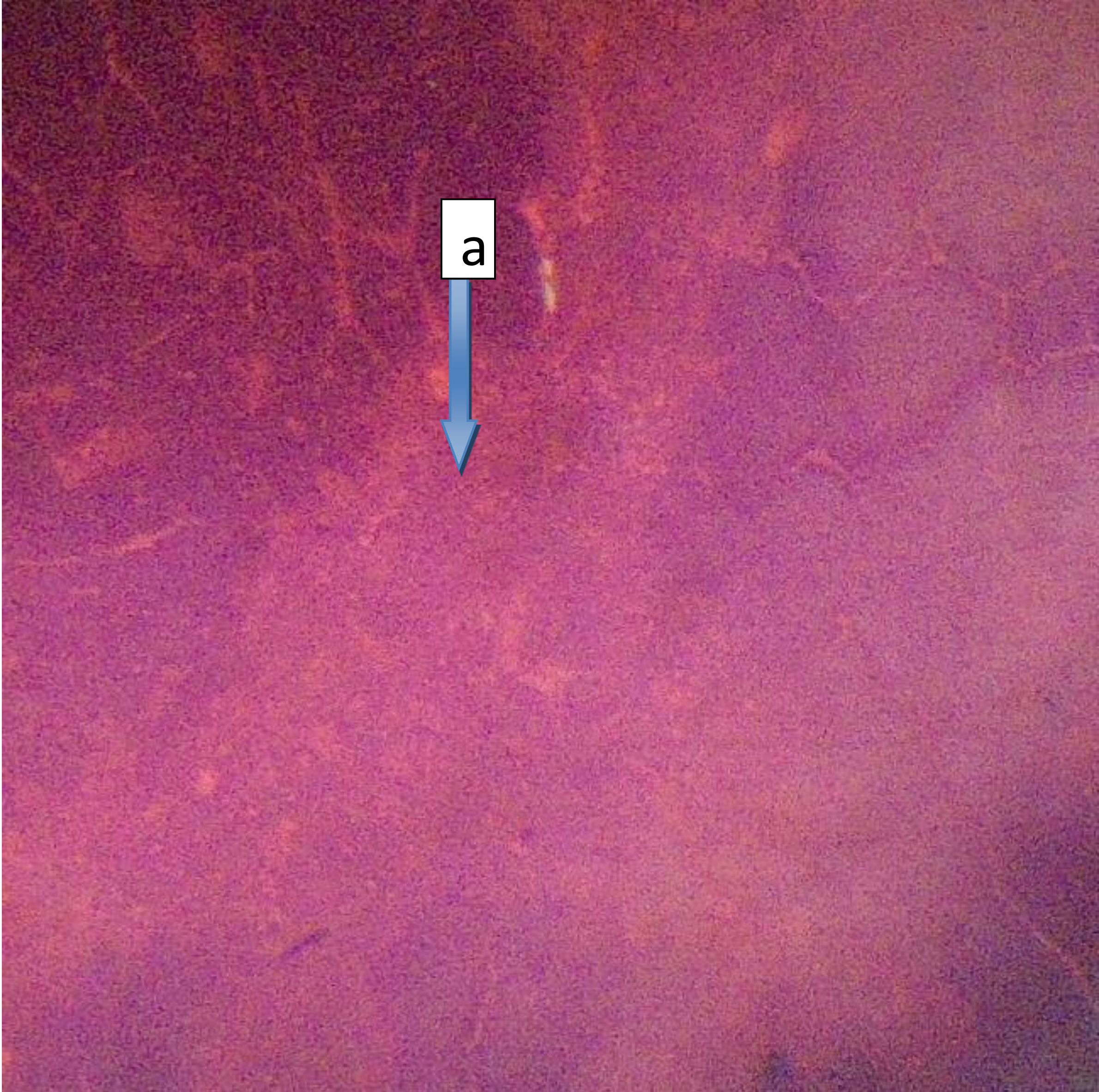

Plate 4.13: Photomicrograph of section of the thymus of pullets in groups A showing depletion of lymphocytes (arrow a). H&E x200

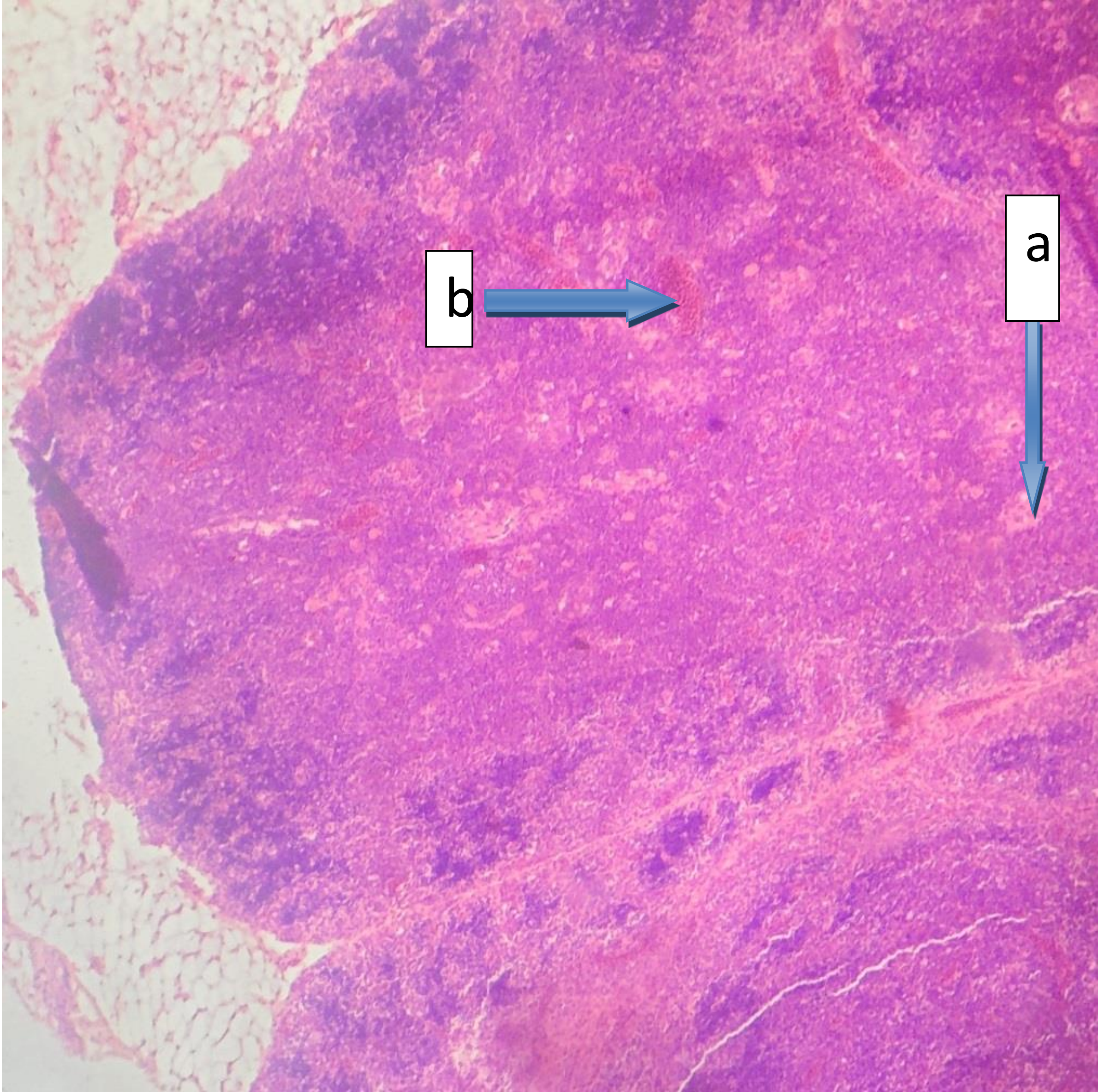

Plate 4.14: Photomicrograph of section of the thymus of pullets in group B showing depletion of lymphocytes (arrow a) and haemorrhages (arrow b). H&E x200

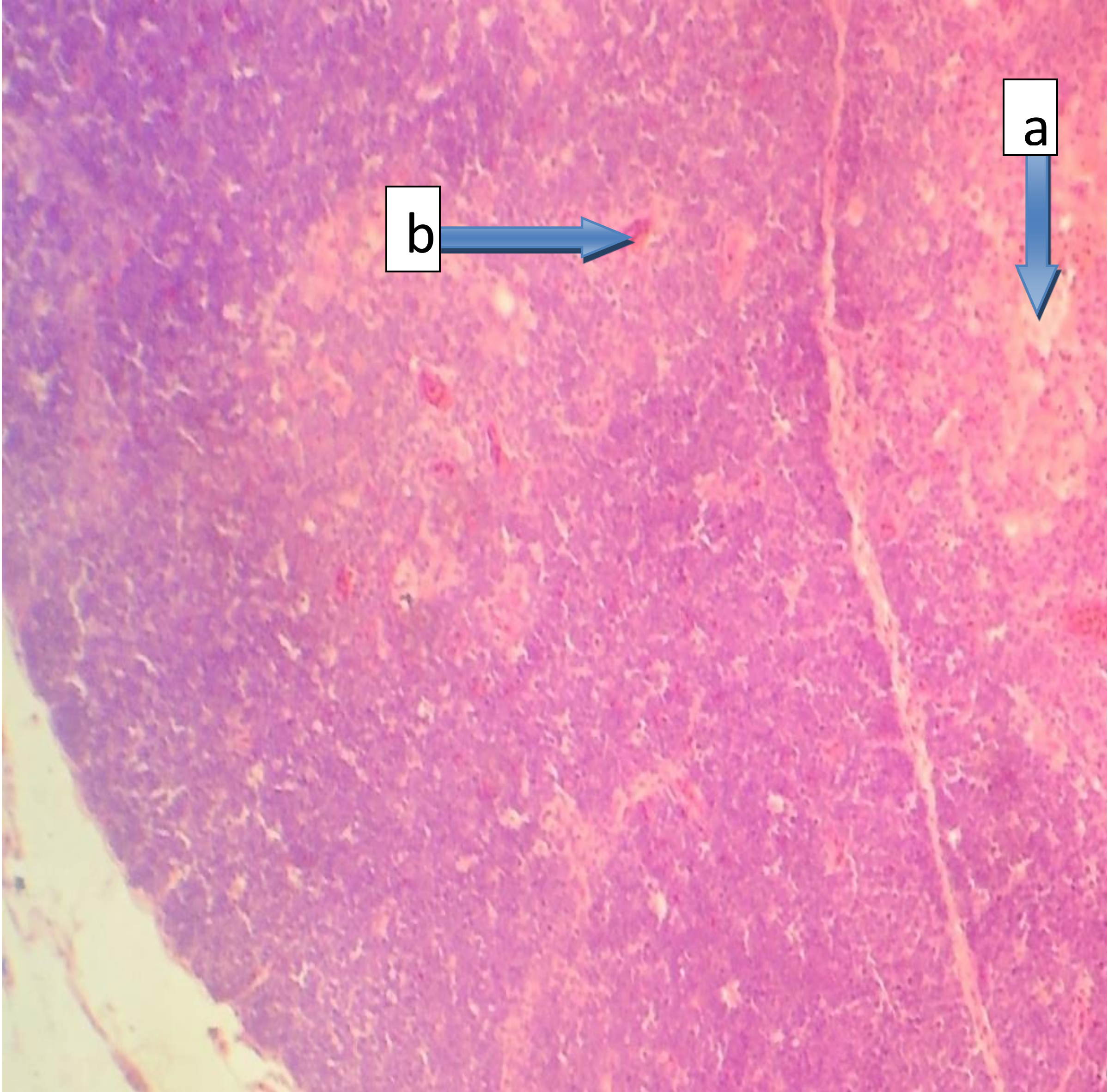

Plate 4.15: Photomicrograph of section of thymus of pullets in group C showing depletion and necrosis of lymphocytes (arrow a) and haemorrhages (arrow b). H&E x200.

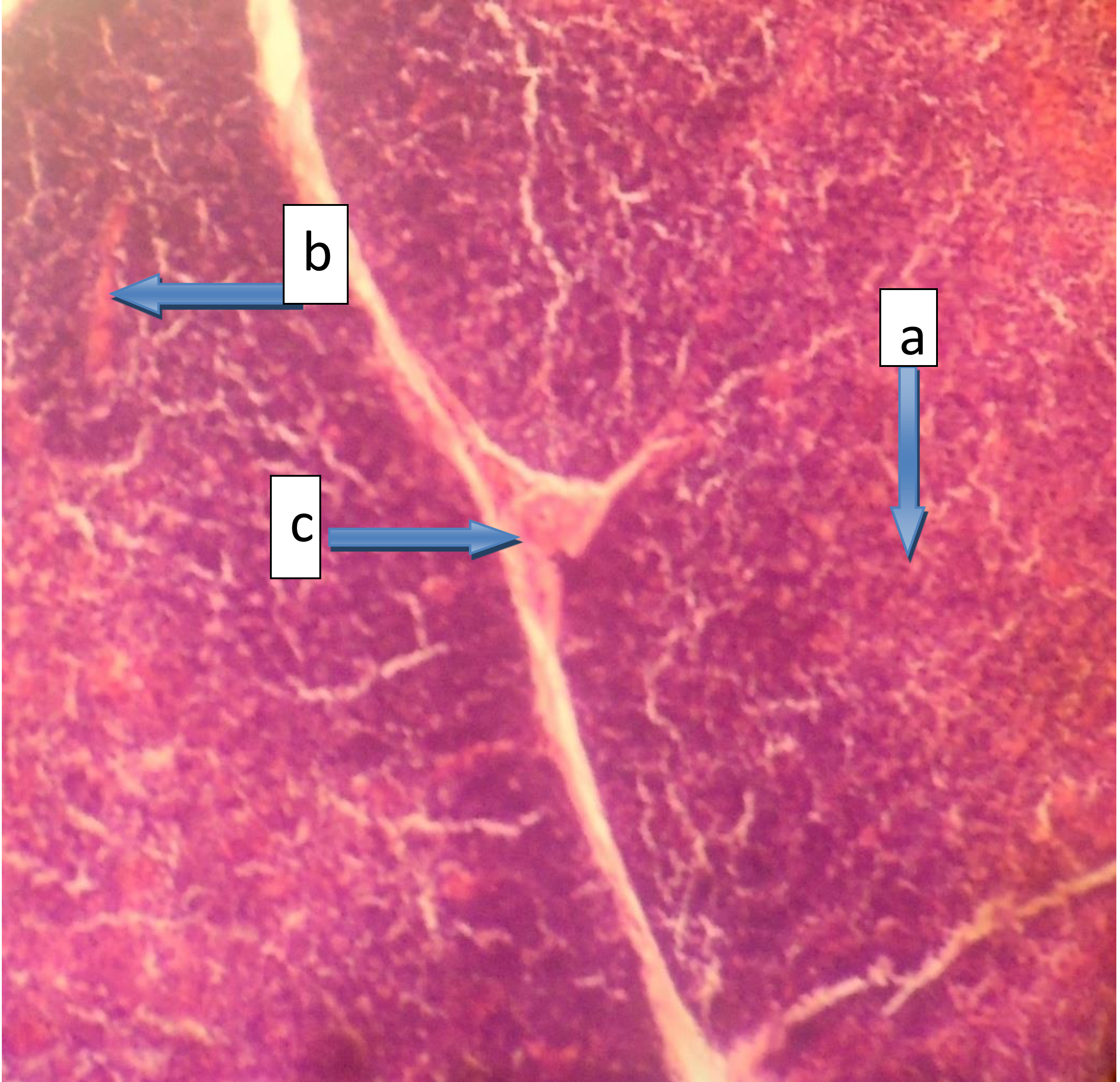

Plate 4.16: Photomicrograph of section of thymus of pullets in D showing depletion and necrosis of lymphocytes (arrow a),haemorrhages (arrow b) and congestion (arrow c). H&E x200**.**

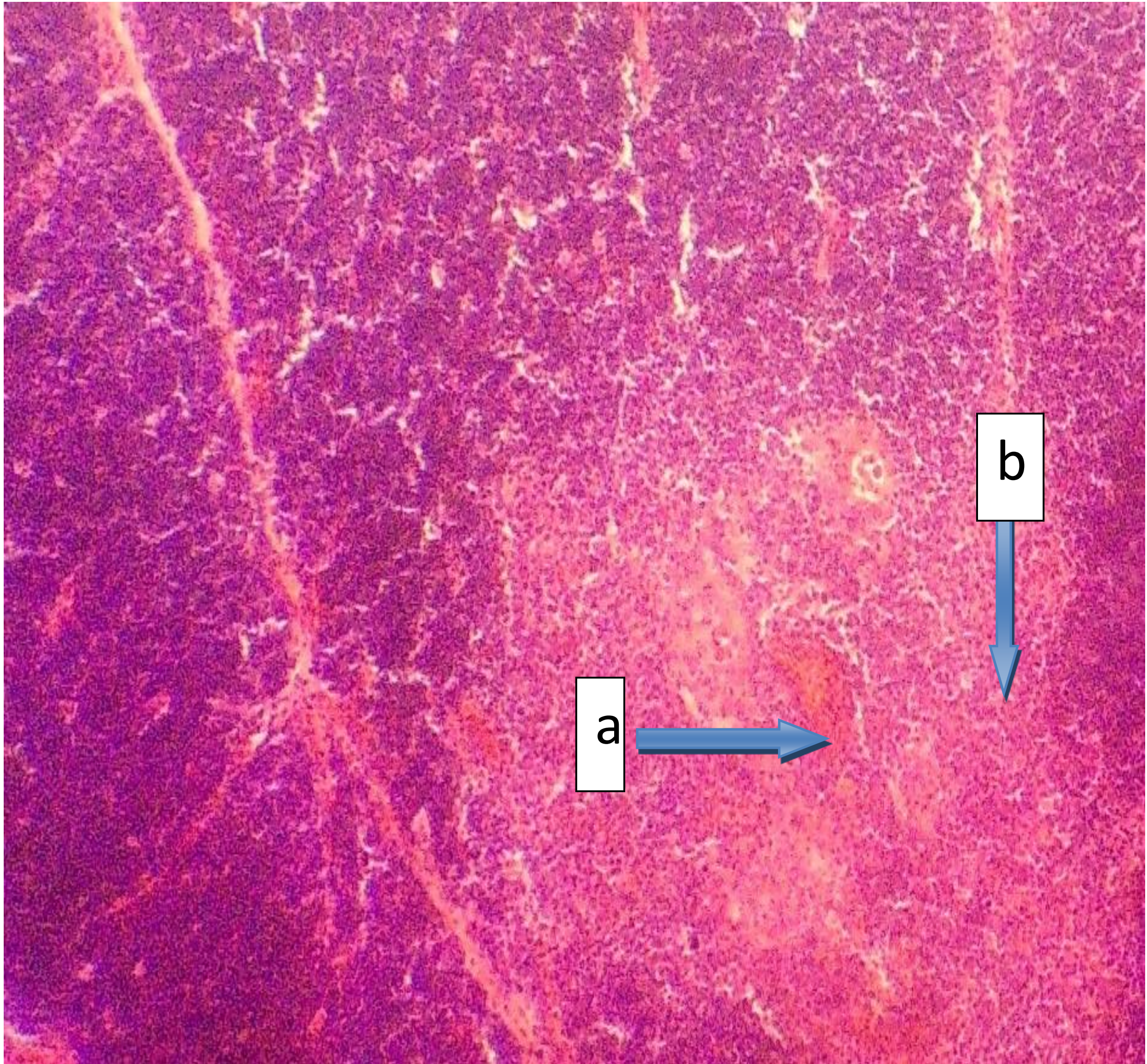

Plate 4.17: Photomicrograph of section of thymus of pullets in group E showing haemorrhages (arrow a) and depletion of lymphocytes in the medullary portion (arrow b). H&E x200.

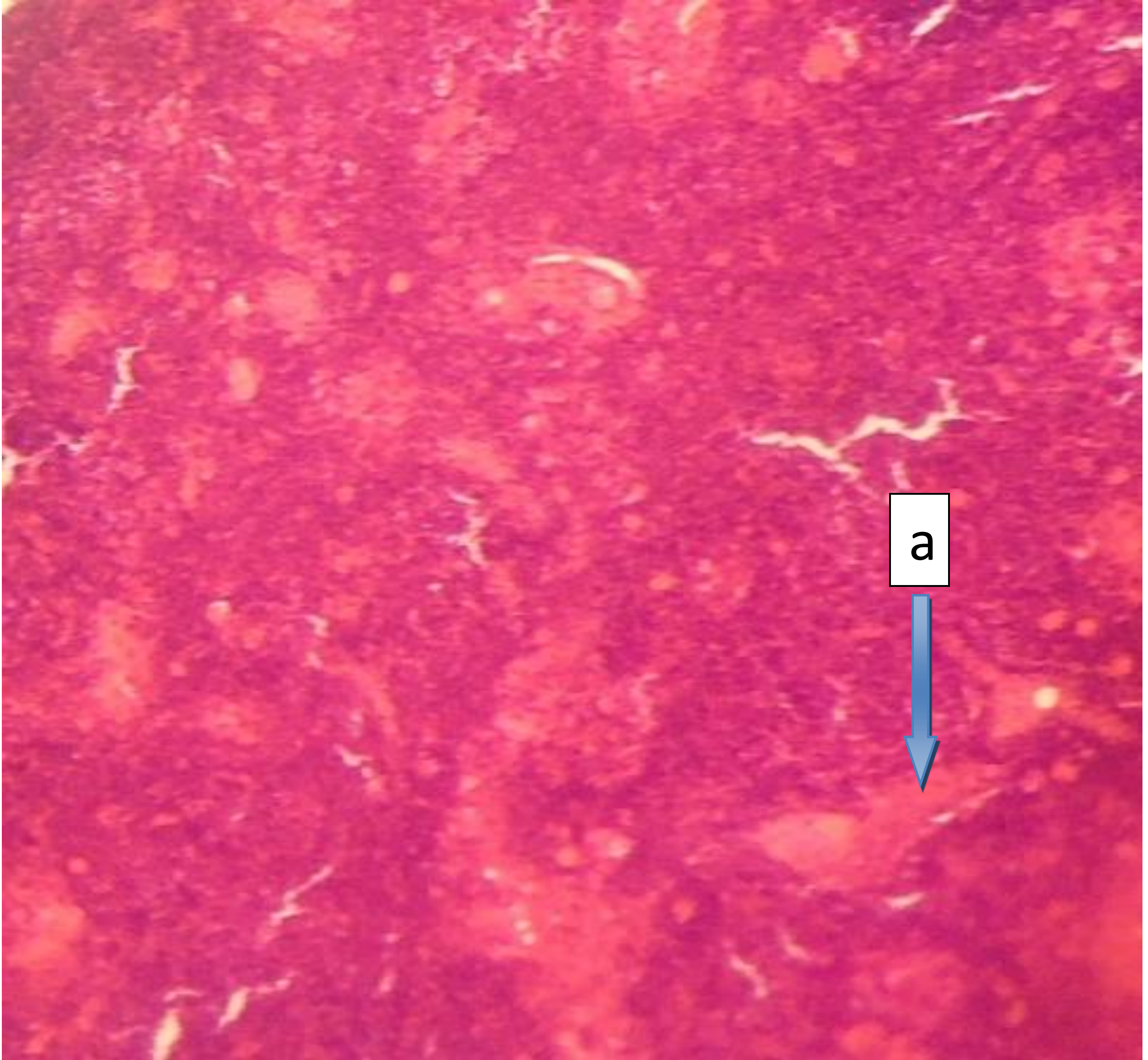

Plate 4.18: Photomicrograph of section of thymus of pullets in group F showing depletion of lymphocytes (arrow a). H&E x200.

CHAPTER FIVE

DISCUSSION

There is no statistical significant difference between the mean ND antibody titre of the chickens in all the groups at the first two weeks of the experiment. This is may be due to maternally drived antibodies (MDA) received by the chicks from parent stock and or the chicks might have been vaccinated with ND vaccine I/O. This is in agreement with the report of Zalelam *et al*. (2014) that Newcastle disease antibody titre of the chicks were protective for eighteen days, due to the fact that MDA is protective until 18 days.

Similarly, the reduced mean ND antibody titre of the chicks in groups A and B at the third week may be due to the 1st and 2nd Gumboro vaccine consecutivelly in an interval of one week.The Gumboro vaccine is known to cause pathology in the bursae which will affect the response to vaccination with ND vaccine La Sota. This is consistence with the report of El-Yuguda *et al.* (2014) who reported that is better to give IBD and ND vaccines within the time frame of more than one week interval.

The higher mean ND antibody titre found in chickens of groups B, C and D when compare to that of group E at week 4 can be attributed to personnel error. This was because the chicks in group E were expectedly to have higher mean ND antibody titre as no IBD vaccines administered to have affected the bursa of Fabricius for adequate response. The trend of ND humoral response at 5 weeks of age was higher in group B and indicated a significant difference with group A ($P \leq 0.01$) and group C ($P \leq 0.01$) as compared with group F. This can be attributed to the time interval of IBD and ND vaccines which was in consistence with the report of El-Yuguda *et al*. (2014), recommended that IBD live vaccine should be given at two weeks interval.

The highest mean ND antibody titre observed in chicks from group D at week 6 may be related to the fact that the previous vaccines (IBD) were administered at week 2 and 4 respectively. This was similar to what was reported by El-Yuguda *et al*. (2014), who reported that it is better to give the vaccines within the time frame of more than one week interval or they should be given simultaneously. The above observations were similar to what was reported by Cardoso *et al*. (2006) who reported that the utilization of polyvalent vaccinal programs has a different efficacy compare to monovalent vaccinations when ND, avian infectious bronchitis, and infectious bursal disease vaccinations are applied.

The trends of the mean ND antibody titre observed at 7th and 8th weeks of the experiment, group C had the highest response to ND compare to other groups, this may be due to single dose of IBD that cause less lesions on the immune organs making it efficient to response to booster dose of ND vaccine received at 6 week and indicated that IBD vaccine had effect on immune response of pullets to ND vaccine La Sota . This was in agreement with the work of El-yuguda *et al*. (2014) and Ali *et al*. (2004) who worked on interaction between ND and IBD vaccine commonly used in Sudan, found that vaccination of chicks with ND vaccine containing La Sota strain will be affected by live IBD vaccine when the IBD was administred first. However, this is in disagreement with work of Okwor *et al*. (2013) who have shown that mixed vaccination against ND and IBD using live vaccine did not affect immune response, feed intake and weight gain in healthy broilers. The difference may be due to breeds used, method and age of the birds at the time of the experiment.

The more severe gross pathological changes observed in major immune organs in the groups vaccinated with IBD live vaccine compared to unvaccinated groups may be due to the effect of IBD vaccine on the organs. These results are in line with findings of Tartar *et al*. (1995) and Boudaoud *et al*. (2008) who showed that severe lesions were observed on bursa of Fabricius of vaccinated chickens with IBD live vaccines which also revealed the immunosuppressive tendencies of the IBD live intermediate plus vaccine. But these results are not in agreement with those of Bolis *et al*. (2003) who found that birds vaccinated at 14 days of age, with 228E (intermediate-plus type of IBD live vaccine) did not induce IBD-typical microscopic lesions in the bursa of Fabricius and also agrees with that of Moraes *et al.* (2004) who recorded that gross lesions caused by the intermediate IBD live vaccines did not differ from that of the control. However, the microscopic results of the present study are in agreement with those of Abdul Ahad (2002) who evaluated the degree of bursal damage produced by the local Bangladeshi isolate in the local cross breed Sonali chickens which was characterized by severe depletion of lymphocytes, many vacuoles, pyknotic nuclei, severe edema were also demonstrated in the interfollicular septa, cystic cavities developed in the medullary areas of the follicles and loss of demarcation between the follicular septa. Ezeokoli *et al*. (1990) evaluated the histopathological modifications of bursa of Fabricius associated with poultry vaccination against IBDV, describing severe lesions in the bursa between three and seven days after vaccination. On the other hand, many authors supported the findings of this study, Alloui (2005) found that broiler chicks vaccinated against IBD with an Intermediate-plus vaccine had lesions score more than the half (53%) of the examined organs which were characterized by atrophy, lymphocyte depletion. In the

more serious cases, a follicular lymphoid necrosis and interfollicular interstitial fibrosis had been noticed (Alloui, 2005). These pathological implications on the major immune organs causes insufficient response to other vaccination or immunity against other pathogens.

CHAPTER SIX

6.1 CONCLUSION

From this study it was concluded that:

1- Multiple infectious bursal disease vaccination had negative effect on antibodies response to Newcastle disease vaccination La Sota because the group that received single IBD vaccine had the highest mean Elisa ND ntibody titres (11480.6 ± 827.45) at eight week.

2- Multiple infectious bursal disease vaccination cause severe pathological lesions on immune organs (Bursa of Fabricius, spleen and thymus).

3- Histopathological changes (such as depletion of lymphocytes, focal necrosis, thickened interfollicular space and cyst formation of the bursae, depletion of lymphocytes and congestion of the thymus and the spleen) were more severe in groups that received multiple IBD vaccinations (twice or thrice).

6.2 RECOMMENDATIONS

From this work it was recommended that:

1. It is safer to give two weeks interval before repeating infectious bursal disease vaccination for Newcastle disease vaccine La Sota vaccination to be effective.

2. Further study should be carried out to determine the effect of multiple infectious bursal disease vaccinations on the antibodies response of pullets to Newcastle disease vaccines other than La Sota.

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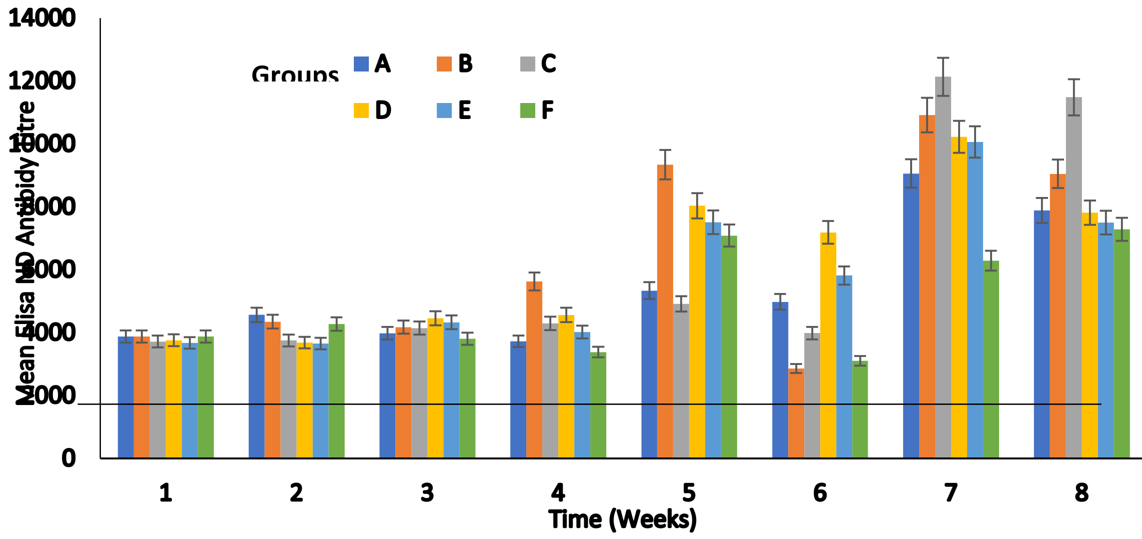

Figure showing mean ND antibodies titers of various groups

__________ Antibody protective level