STUDIES ON THE PROFILE AND RELATIONSHIP OF MATERNAL AND VACCINAL ANTIBODIES IN THE PREVENTION OF INFECTIOUS BURSAL DISEASE IN CHICKENS

BY

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A Thesis Submitted to Ahmadu Bello University, Zaria in partial fulfillment for the degree of MASTER Of SCIENCE (Avian Medicine)

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NOVEMBER, 1985

DIGITIZED

DECLARATION

The work presented in this thesis is original and carried out under the supervision of Drs. S. U. Abdullahi, C. D. Ezeokoli and A. A. Adesiyun in the laboratory of the Department of Veterinary Surgery and Medicine.

The work of other investigators was referred to and acknowledged. No part of this thesis has previously been submitted for a degree or diploma.

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CERTIFICATION

This thesis entitled "Studies on the Profile and Relationship of Maternal and Vaccinal Antibodies in the Prevention of Infectious Bursal Disease in Chickens" by P. A. Abdu meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to
Ai'shatu, Shehu, Sa'adatu and
Abiodun.

ACKNOWLEDGEMENTS

I wish to thank Dr. S. U. Abdullahi for being brotherly and Dr. A. A. Adesiyun for encouragement. The contribution of Dr. C. D. Ezeokoli in the project is gratefully acknowledged.

I am also indebted to Drs. Wyeth and Wood of the Central Veterinary Laboratory Weybridge, Surrey for reagents and literatures.

The University Board of Research, Ahmadu Bello University fully funded the project.

ABSTRACT

The presence of infectious bursal disease virus maternal antibody in broiler chicks was checked every other day from day-old for 29 days and its decay pattern determined. Maternal antibody was found in the chicks sampled and its decay pattern linear. It was present in all the chicks sampled at day-old but absent in all the chicks sampled at 19 days old. These findings suggest that vaccinating these chicks against infectious bursal disease at day-old would be ineffective since maternal antibody has been shown to interfere with vaccination and that if these chicks were exposed to infectious bursal disease virus at day-old they would be protected. On the other hand, all the chicks would be expected to be susceptible at 19 days old.

Different sets of chicks similar to those used above were each vaccinated with infectious bursal disease vaccine at weekly intervals from day-old for 29 days. The presence of active infectious bursal disease virus antibody was determined at three, seven, 14 and 35 days post-vaccination. It was detected at 35 days post-vaccination in chicks

vaccinated at one, eight or 15 days old and at 14 and seven days post-vaccination in chicks vaccinated at 22 or 29 days old respectively. The prompt immune antibody response observed in chicks vaccinated at 22 or 29 days old than in. chicks vaccinated at one, eight or 15 days old was possibly due to the absence of maternal antibody in the chicks vaccinated at 22 or 29 days old since maternal antibody level decreases with age and is usually absent by the time a chick is three or four weeks old.

Chicks similar to those used in the vaccination trials above were challenged with a virulent field infectious bursal disease virus at weekly intervals from day-old for 29 days. Chicks challenged at eight, 15, 22 or 29 days old were susceptible based on bursal atrophy, bursal lesions and presence of infectious bursal disease virus precipitin antigen in the bursa of Fabricius. However, only 40 and 90% of the chicks challenged, at eight or 15 days old respectively were susceptible while all the chicks challenged at 22 or 29 days old were susceptible. Only the chicks challenged at 29 days old showed clinical signs. The results suggest that the initial level of maternal antibody at day-old in the chicks challenged

at 22 or 29 days old was not high enough to have lasted 22 or 29 days and that chicks as young as eight days old could be infected with infectious bursal disea virus without showing clinical signs. In the field this is possible because infectious bursal disease is enzootic in Nigeria. Chicks exposed this early may be permanently immune-suppressed.

In conclusion, it is recommended that maternally-immune chicks be vaccinated against infectious bursal disease at seven and 21 days of age and the chances of exposure to field infectious bursal disease virus be minimized by good management and hygienic practices.

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INFECTIOUS BURSAL DISEASE: A REVIEW OF THE LITERATURE

1.1. Introduction

1.1.1 Definition

Infectious bursal disease (IBD) is defined as a contagious, acute or, in many cases, subclinical viral disease which causes necrosis and atrophy of the bursa of Fabricius (BF), the organ responsible for immunocompetence in young chickens (Cosgrove, 1962; Cheville, 1967; Hopkins et al., 1979; Armstrong et al., 1981). The synonyms of the disease include the following: avian-nephrosis (Cosgrove, 1962); infectious bursal disease (Hitchner, 1970); Gumboro disease (Faragher, 1972); infectious bursitis (Ibragimov, 1976) and avian infectious bursitis (Roden, 1982).

1.1.2 Historical Perspective

Cosgrove (1962) was the first to describe an acute contagious disease in broilers in Gumboro county, Delaware, U.S.A. although the disease had occurred since 1957 and the local name was "Gumboro disease". He called the disease "avian-nephrosis" because of the prominent kidney lesions he observed

the BF. As bacteriological examinations were negative, he suggested a viral aetiology. Later in the same year, Winterfield and Hitchner (1962) described a "nephritis-nephrosis syndrome" in chickens with similar kidney lesions from the same area in the U.S.A. They isolated two strains of the infectious bronchitis virus (Holte and Gray strains) from the kidneys of affected chickens. Winterfield and Hitchner (1962) were able to isolate another agent from the BF of chickens affected by "avian-nephrosis" and designated it as infectious bursal agent (IBA). Edgar and Cho (1965) reported the isolation of a virus-like agent from affected birds and could transmit the agent to susceptible one to six-week-old chickens which caused a disease condition similar to that already described by Cosgrove (1962) and also by the IBA of Winterfield et al. (1962). The disease has since then been reported from various parts of the world. In Nigeria, Ojo et al. (1973) were the first to report a Gumboro-like disease which was later confirmed to exist by Onunkwo (1975).

1.2.1 Distribution

Infectious bursal disease has been reported in many parts of the world including Africa (Lüthgen, 1969; Faragher, 1972; EL. Sergany *et al*., 1974; Onunkwo and Momoh 1981). The disease is said to be more prevalent in commercial farms (Okeke, 1984).

1.2.2 Incidence

Infectious bursal disease appears with equal frequency throughout the year (Cosgrove, 1962). It recurs several times on many farms but with a decrease in the mortality rate during subsequent outbreaks (Cosgrove, 1962; Meroz, 1966). The prevalence of clinical IBD appears to be low, but serological evidence indicates that subclinical infection is widespread (Winterfield, 1969; Faragher, 1972). In Nigeria, there is serological evidence of high prevalence of IBD in local chickens in which clinical disease is rarely observed (Nawathe *et al*., 1978; Umoh *et al*., 1982; Abdu *et al*., 1984).

1.3.1 Classification

Infectious bursal disease virus (IBDV) has been classified as a reovirus by certain workers (Kosters et al., 1972) and an adenovirus by others (Almeida and Morris, 1973). The IBDV is however smaller than both adenoviruses and reoviruses and unlike adenoviruses, it replicates in the cytoplasm in association with vacuoles and inclusions of infected cells and four structural units are present along the edges of some viral particles (Kaufer and Weiss, 1976; Faragher, 1978; McFerran et al., 1980). It also differs from reoviruses in that its virion has a single instead of a double capsid and the viral RNA is single stranded (Kaufer and Weiss, 1976; Nick et al., 1976; McFerran et al., 1980). The capsomeres are also smaller than those of reoviruses and have been observed to be more than 32 in total number (Cho and McDonald, 1980).

The buoyant density of IBDV as determined in continuous sucrose gradient was reported to be 1.178 g/cm^3 (Cho and McDonald, 1980). Two populations of morphologically different but antigenically related virions with mean diameters of 60 nm and and 20 nm respectively have been detected in virus

preparations (Almeida and Morris, 1973; Pattison et al., 1975).

1.3.2 Resistance to Physical and Chemical Agents

Infectious bursal disease virus was found to be resistant to heat and ultraviolet irradiation (Benton et al., 1967b; Petek et al., 1973). The virus has also been found to be infective for chickens after exposure at 60°C for 90 minutes, at 25°C for 21 days and after storage for 3 years at -20°C (Yung Cho 1967). It was also found to be resistant to ether, chloroform, merthiolate, trypsin, phenol, staphene and hyamine (Benton et al., 1967a; Benton et al., 1967b). The virus has been reported to be acid and alkaline stable surviving at a pH range of 2 to 13 (Benton et al., 1967a). Two percent solution of chloramine, halamid, formaldehyde and glutaraldehyde were however, found to be effective against the IBDV (Meulemans and Halen, 1982).

The multiplication of the virus was not affected by 5-Iodo-2-deoxyuridine and cytosin arabinofuranoside but was inhibited by actinomycin D (Faragher, 1972; Okeke, 1984).

1.3.3 Antigenic Characteristics

Viral antigens are demonstrable in the cytoplasm of infected cells by immunofluorescence (Cheville, 1967). Infected birds develop antibodies detectable by virus neutralization (Winterfield, 1969) and by gel-diffusion precipitation (Hirai et al., 1972). A number of IBDV isolates from chickens, turkeys and ducks could be grouped into serotype I and serotype II based on the virus-neutralization test. It has been proposed (McFerran et al., 1980) that the cell culture adapted vaccine strain from chickens be designated the prototype virus for serotype I and the TY89 isolate from a turkey the prototype for serotype II. The isolates in serotype II consisted of an antigenically homogenous group of viruses from turkeys and chickens. However, within serotype I, which represented isolates from chickens and ducks, some isolates showed only a 30% cross reaction with the vaccine strain (McFerran et al., 1980).

Jackwood et al. (1982) found an IBDV isolate from chickens, two IBD vaccine viruses and an IBDV isolate from turkeys to be prototype viruses of serotype II. A common antigen shared by both serotype I and II viruses was detected using an

indirect immunofluorescent assay. The virions of both IBDV serotypes were determined to be non-enveloped icosahedrons with diameters ranging from 58 to 60 nm. In another survey, Jackwood and Saif (1983) found virus-neutralizing antibody to serotype I in 77% of the 75 chickens flocks they sampled and antibody to serotype II in 47% of the flocks. Of the eight flocks known to have been vaccinated with a serotype I IBDV vaccine, all had antibody to serotype I and four had antibody to serotype II. Eleven of the 17 known unvaccinated flocks had antibodies to serotype II. The study showed that antibodies to IBDV serotype I and II were present in both vaccinated and unvaccinated chickens. Since pathogenic and non-pathogenic strains of IBDV serotype I have been identified (Winterfield and Thacker, 1978), the possibility of the existance of a similar situation with serotype II was considered of practical importance (Jackwood and Saif, 1983).

1.3.4 Viral Propagation

Winterfield et al. (1972) were able to isolate IBDV from virtually all organs of susceptible chicken. At three days post-inoculation, they consistantly isolated the virus in the highest concentration from the BF and spleen while the brain

and blood gave low yields. Viremia was found to be transient and the virus was not isolated from tissues beyond 10 days post-inoculation. The spleen was preferred for virus isolation because it was found to be free of contaminating organisms.

The virus has been propagated in embryonated chicken eggs but because of the presence of interfering maternal antibody in the eggs of immune-hens, eggs from susceptible hens are required for successful propagation (Snedeker et al., 1967; Hitchner, 1970). The chlorioallantoic membrane was found to be the most sensitive for detecting small amounts of the virus (Hitchner, 1970).

Rinaldi et al. (1969) found that of the common laboratory animals, only white mice were sensitive to infection with IBDV. They reported that mice aged one to 11 days and inoculated intraperitoneally and older mice age 12 to 14 days and inoculated intracerebrally showed nervous signs and heavy mortality after five to 13 days.

The virus has also been grown on cell cultures of chicken macrophages and fibroblasts, bursal cells, kidney cells and epithelial cells of the chick embryo (Mora, 1966; Rinaldi et al., 1972a; Lukert

and Davis, 1974; Nick et al., 1976). Nick et al. (1976) found greater replicative ability of IBDV in chick embryo-fibroblast than in epithelial cell culture.

1.4 The Disease Entity

1.4.1 Susceptible Hosts

Natural and experimental infections with IBDV have been reported in chickens (Cosgrove, 1962; Helmboldt and Garner, 1964). To date, there is no firm evidence that IBDV occurs naturally in avian species other than the chicken. Edgar and Waggoner (1965) had however reported quails as being susceptible to IBD. Wild sparrows were also reported to have died from the disease (Edgar and Cho, 1965) and the village weavers have been found to be sero-positive for IBDV antibody (Nawathe et al., 1978). Coturnix quails and two breeds of turkey were experimentally infected with IBDV. Although no clinical signs were observed and no microscopic lesions were found in their bursae and the virus was not isolated from cloacal swabs, the turkeys developed both precipitin and virus neutralizing antibodies (Weisman and Hitchner, 1978). Similar results were obtained when susceptible ducks were challenged

(Yamada et al., 1982). In an experiment, Giron (1969) found 20 turkey poults completely refractory to infection with IBDV. In what seemed to be the first report of a natural outbreak of IBD in turkeys, McNulty et al. (1979) isolated a virus morphologically identical to some isolates of IBDV from the faeces of six-week-old turkeys showing diarrhoea.

1.4.2 Age and Breed Susceptibility

Infectious bursal disease has been reported in nine-day to 20-week-old chickens (Cosgrove, 1962; Hanson, 1967; Luthgen, 1969; Okoye and Uzoukwu, 1981). It is however most common in birds three to seven-week-old (Cosgrove, 1962; Hanson, 1967; Ojo et al., 1973). No clinical signs were observed in laying birds and chicks less than two weeks old but histophatological lesions were seen in the BF of the chicks (Hitchner, 1970; Hitchner, 1971). All breeds of chickens may be infected but it has been noted that light breeds show a more severe reaction to the virus than heavy breeds (Hitchner, 1978). However, no significant difference in mortality rate between the breeds was found (Meroz, 1966). In Nigeria, local breeds of chickens seem to be highly resistant to IBD (Onunkwo and

Momoh, 1981).

1.4.3 Transmission

Benton et al. (1967a) found IBDV highly contagious. They found houses that had housed infected birds infective for other birds 122 days after removal of the infected birds and that water, feed and droppings taken from infected pens remained infectious for 52 days. The lesser mealworm, Alphitobius disperinus, taken from a house eight weeks after an outbreak of IBD, was infectious for susceptible chickens when fed as a ground suspension (Snedeker et al., 1967). Experimental infections can be achieved by the oral, intramuscular, intrabursal and subconjunctival routes (Okeke, 1984).

1.4.4 Incubation Period

The first clinical signs and deaths were recorded 24 and 48 hours respectively after experimental oral infection (Kaufer and Weiss, 1976). Viral particles and histological lesions were first detected 24 hours after infection (Helmboldt and Garner, 1964; Kaufer and Weiss, 1976). The incubation period after natural infection is not known because the time of infection is usually unknown.

1.4.5 Clinical Signs

Depending on the age of the bird at the time of infection with IBDV, two forms (clinical and subclinical) of disease will occur. If they are affected prior to eight days of age, no apparent clinical disease will occur. Although these birds may appear normal, there may be extensive damage to the BF (Naqi, 1978; Giambrone, 1983). The onset of and recovery from the clinical disease are usually rapid (Rinaldi et al., 1965). The signs observed included diarrhoea, soiled vent feathers, severe depression, anorexia, ruffled feathers, droopy appearance, trembling, prostration and death (Cosgrove, 1962; Giambrone et al., 1977).

1.4.6 Morbidity and Mortality

In a fully susceptible flock, morbidity may be as high as 100%. Mortality usually begins on the third day post-infection and peaks half-way through the disease which usually lasts for five to seven days (Cosgrove, 1962; Parkhurst, 1964; Hitchner, 1978). The actual mortality may be nil but the rate can reach levels of 10 to 30% (Hitchner, 1978; Winterfield, 1969).

1.4.7 Pathology

Gross Lesions: Birds that died from IBD are usually in good bodily condition with dehydration of the subcutis and muscles (Cosgrove, 1962). The crops are usually empty and pectoral muscles discoloured (Luthgen, 1969; Hitchner, 1978). Frequently, haemorrhages may be present in the leg, thigh and pectoral muscles but may be absent as in mild outbreaks (Riggenbach, 1967; Hitchner, 1978). Haemorrhages could also be observed on the mucosa of the proventriculus and at the junction of the proventriculus and gizzard (Hanson, 1967; Hitchner, 1978). There is increased mucus in the intestine and renal changes are prominent, the latter occurs only in birds that die or are in advanced stages of the disease. In these birds, the kidneys are enlarged, the tubules distended with accumulated urates and the ureters are also distended (Cosgrove, 1962; Hitchner, 1978). The kidneys may vary in colour from white or pale gray to brown (Cosgrove, 1962; Barron et al., 1966). The spleen may be enlarged and very often has small gray foci uniformly dispersed on the surface (Rinaldi et al., 1965). Splenomegaly is always observed on the third day after infection and continues for several days (Okeke, 1984). Hyperemia due to injection of the

vessels of the thymus have been reported (DelBono et al., 1968). The size and colour of the BF vary depending on the number of days after infection. It is usually enlarged and white, yellowish or cream-coloured three to four days after infection and becomes atrophied and gray in appearance eight or more days after infection (Cheville, 1967). Infected bursae often show necrotic foci and at times petechial or ecchymotic haemorrhages on the mucosal surface. Occasionally, extensive haemorrhages throughout the bursa have been observed (Hitchner, 1978).

Histopathology: As IBDV has lymphocidal effect, histopathologic lesions are more commonly observed in lymphoid tissues including the BF, spleen, thymus and caecal tonsils with the BF being the primary site of damage (Helmboldt and Garner, 1964; Lukert and Davis, 1974). Hypertrophy, hyperplasia and mucus metaplasia of the epithelial lining of the BF have been observed (Cosgrove, 1962; DelBono et al., 1968; Cheville et al., 1978). Erosion and corrugation of the epithelium have also been reported (Winterfield et al., 1972; EL. Sergany et al., 1974; Thornton and Pattison, 1975; Naqi and Millar, 1979). The normal follicular structure have been reported to be altered by degeneration and necrosis of lymphocytes

(Cheville, 1967; Vindevogel et al., 1974). Lymphocytes have been shown to be replaced by heterophils, pyknotic debris, hyperplastic reticuloendothelial cells and oedema (Cheville, 1967; EL. Sergany et al., 1974; Thornton and Pattison, 1975; Okoye and Uzoukwu, 1981). Hemorrhages in the follicles of some bursae were noted in some outbreaks (Peters, 1967; Ibragimov, 1976). As inflammatory reaction declines, necrosis and phagocytosis of the heterophils and plasma cells occur (Cheville, 1967). After lysis of the cellular debris, the centres of bursal follicles appear as empty spaces lined by hypertrophied epithlial cells (Cheville, 1967; DelBono et al., 1968; Winterfield et al., 1972; EL. Sergany et al., 1974). Other features of IBD-infected BF are the hyperplasia and hypertrophy of the interfollicular connective tissue which is permeated with mononuclear leukocytes (Winterfield et al., 1972; Vindevogel et al., 1974).

In the early stages of IBD, there is hyperplasia of the reticuloendothelial cells around the adenoid sheath arteries of the spleen. By the third day, focal areas of necrosis of the lymphoid nodules and periarteriolar lymphoid sheath are usually present (Hitchner, 1978; Ley et al., 1983). Lymphocytes

and monocytes are found, even though rarely, on the periphery of blood vessels. Sometimes, haemorrhagic foci usually limited to the subcapsular area, may be present (DelBono et al., 1968). The thymus of some chickens may appear normal while others may show multifocal necrotic centres (Ley et al., 1983). Necrosis of lymphocytes in the thymus has been observed to be less pronounced than in the BF and spleen (DelBono et al., 1968).

Interstitial haemorrhages and perivascular accumulations of lymphoid cells, oedema, tubular necrosis and glomerular nephrosis in the kidneys of IBDV-infected birds have been reported (Mandelli et al., 1966; DelBono et al., 1968; Ley et al., 1983). Homogenous material (probably urate) sometimes infiltrated by heterophils and cellular debris have also been observed in the tubules and ureters of such birds (Helmboldt and Garner, 1964; DelBono et al., 1968; Ley et al., 1983).

Immunopathology: The non-lymphoid pathologic changes in IBD (haemorrhages in skeletal muscles and proventiculus, hepatic and renal changes) are still unexplained, although Ley et al. (1979) observed immunofluorescence in the renal glomeruli of infected chickens, indicating that gammaglobulins,

probably in the form of immune-complexes, had lodged in the glomeruli of IBDV-infected chickens. This, they believed, suggests that immune-complexes may play an important role in the pathogenesis of IBD in chickens. However, in a more recent study (Ley et al., 1983) glomerular fluorescence in the kidneys from normal and IBDV-infected chickens was observed.

Results obtained by Skeeles et al. (1979a) suggest that the clinical signs and mortality seen in IBD are associated with a depletion of circulating haemolytic complement from the formation of immune-complexes at sites of viral replication resulting in haemorrhage. In another study, however, Skeeles et al. (1980) reported that a clotting abnormality without changes in complement levels was associated with severity of clinical IBD.

In chickens, the development of an immune response is dependent upon two lymphoid organs, the BF and the thymus (Gatti et al., 1970). During incubation, the embryonating eggs haemopoietic cells migrate from the yolksac to the thymus and the BF. This process continues for three to five days after hatching until the yolk is fully absorbed. The thymus then develops the capacity to produce T-cells

responsible for cellular immunity. The BF on the other hand, produces stem cells, which migrate to the spleen, bone marrow, caecal tonsils and other lymphoid organs that produce B-cells which are responsible for humoral antibody production (Payne, 1971; Firth, 1977; Okeke, 1984).

Infection with IBDV suppresses both the humoral and cellular immune responses of chickens (Panigrahy et al., 1982). The severe depletion of B-lymphocytes observed in the BF and the decreased serum levels of IgG and IgA may be responsible for the increased susceptibility to pathogens and poor vaccination response observed in IBDV-infected chickens (Allan et al., 1972; Faragher et al., 1974; Wyeth, 1975; Ivanyi and Morris, 1976; Giambrone et al., 1977; Rosenberger and Gelb, 1978; Sivanandan and Maheswaran, 1980). This immunosuppression (IS) due to IBDV-infection depends on the age of birds at exposure to IBDV, the level of maternal antibody, the time interval between IBDV infection and exposure to a pathogen or vaccine, and the strain of IBDV involved (Meulemans et al., 1977; Wood et al., 1983). The older the bird, the higher the level of maternal antibody and the less the pathogenic the IBDV involved, the less the extent of immunosuppression

(Meulemans et al., 1977; Wood et al., 1983). For example, IBDV infection simultaneously or five days after Newcastle disease (ND) vaccination of day-old chicks did not decrease the resistance of chicks to ND challenge; a decrease in resistance was only observed when the chicks were infected at day-old with IBDV and later vaccinated against ND (Meulemans et al., 1977). Furthermore, Hirai et al. (1974) reported that IBDV suppressed the humoral antibody response of chickens to various vaccines. The virus had that effect whether injected at the same time with the vaccine or four or seven days before administration of the vaccine. The IS was slightly greater when injection was seven days before and was most pronounced in chickens inoculated with IBDV when six-weeks-old, slight in four-week-olds and absent in two-week-olds. However, the suppression of antibody response to Newcastle virus was found to be greatest in chicks exposed to IBDV at day-old. Chicks exposed to IBDV at seven days of age showed moderate suppression while negligible effect was observed in those exposed at 14 and 21 days of age (Faragher et al., 1974). Immunosuppression was also demonstrated in chickens which were infected with IBDV early in life and prior or shortly after vaccination with infectious bronchitis virus (IBV). Suppression was

evident by an increased susceptibility to respiratory tract infection with IB and reduced virus-serum neutralizing antibody levels (Winterfield et al., 1978). Suppression of response to Brucella abortus strain 19 caused by IBDV in day-old chicks was shown to persist for four weeks (Edwards et al., 1982). However, secondary immune response to Newcastle disease virus in IBD-infected chickens has been found to be unaffected (Giambrone et al., 1977; Fanigrahy et al., 1982).

1.4.8 Immunity

IBDV precipitin antibodies could initially be detected six to 28 days after infection or vaccination and were found to persist for between 42 and 238 weeks (Schneider and Haass, 1969; Faragher, 1971; Kosters, 1971; Ide et al., 1978; Cursiefen et al., 1979). Neutralizing antibodies were however detected as early as three days after vaccination (Curseifen et al., 1979). Neutralizing antibodies in naturally-infected or vaccinated chickens were able to neutralize homologous and heterlogous IBDV strains (Winterfield, 1969). Recently, however, some IBDV isolates (serotype I) from chickens and ducks have been shown to have only a 30% similar cross neutralization reaction with a vaccine strain of IBDV

(serotype I) (McFerran et al., 1980). It is possible therefore that some IBDV vaccines strains may not be able to fully protect birds from infections with some pathogenic strains of IBDV serotype I. Infectious bursal disease protection seven days after vaccination with a live IBDV vaccine has been observed and an immunity to challenge which lasted nine weeks has also been reported (Snedeker et al., 1967; Rosenberger, 1977).

The incidence of precipitins was found to be inversely related to the severity of clinical reaction in IBD and was also higher in birds that showed signs and lesions of IBD than in those vaccinated (Schneider and Haass, 1969; O'Brien, 1976). Precipitins were also found to be higher and persisted longer in chickens vaccinated orally than those vaccinated intramuscularly (Wyeth, 1980). Adult birds did not respond well to orally-administered IBDV but did produce antibody after intramuscular or subcutaneous inoculation of IBDV (Hitchner, 1976). Chickens exposed to IBDV at three days of age did not develop as significant neutralizing antibodies as those exposed at four weeks or later and the development of neutralizing antibodies was found to be dependent on the presence

of the BF (Winterfield, 1969).

The passive transfer of maternal antibody (MA) from IBD-immune hens to their progeny has been demonstrated. Such chicks were found to be resistant to experimental infection and eggs from the hens inhibited the growth of the IBDV (Faragher, 1971; Hitchner, 1971). It is worthy to note that only high MA level in chicks gives protection against early infection with virulent IBDV. The protection from IS lasts four weeks and protection from bursal atrophy (BA) for two weeks (Lucio and Hitchner, 1979). The level and persistance of MA in chicks depends on the age at vaccination or infection, the frequency of vaccination and the route and type of IBDV vaccine used in vaccinating the breeder hens (Wyeth and Cullen, 1978b; Wyeth and Chettle, 1982). At day-old, the percentage of chicks with MA within a flock is usually high (80 to 100%) but decrease with age (Ide et al., 1978; Wyeth and Cullen, 1978b). Maternal antibodies have been reported to last four to five weeks with a half-life of six to eight days and its rate of decay being roughly linear (Hitchner, 1971; Wyeth and Cullen, 1978b; Armstrong et al., 1981).

One important disadvantage of high MA level is the prevention of effective immunization with live

IBDV vaccines (Lukert et al., 1976; Wood et al., 1981). Only chicks with quantitative agar-gel precipitin titres below 32 U.K. units/ml and those with as low as 1:64 virus-neutralization (VN) antibody titres were successfully immunized against IBD (Lukert et al., 1976; Wood et al., 1981).

1.4.9 Prevention

The highly contagious nature of IBD and the extreme resistance of IBDV combine to pose a threat to the poultry industry in several countries. The virus is nearly impossible to keep away from non-infected poultry houses and once established on a farm, it tends to persist for a long time. Therefore, vaccination to prevent IBD has become a necessity in major poultry producing areas throughout the world (Giambrone, 1983).

There are at present two types of commercial IBDV vaccines available. These are the live and the inactivated vaccines. The live vaccines can be grouped by their pathogenicity as mildly or highly pathogenic (Haffer, 1982a). Mild IBDV vaccines are low in their invasiveness of the BF and may easily be neutralized by high levels of MA (Winterfield and Thacker, 1978) while highly pathogenic IBDV vaccines

are highly invasive and overcome the effect of MA (Fletcher et al., 1978; Haffer, 1982a). Chicks devoid of MA when given highly pathogenic vaccines will be immunosuppressed and are capable of spreading the vaccine virus to susceptible chickens (Muskett et al., 1979).

There are two methods of controlling the sub-clinical immunosuppressive form of IBD:

1. Vaccination of breeder hens in order to produce high levels of MA to be transferred to the offspring to prevent infection during the first two weeks of age, or
2. Vaccination of the broiler progeny at day-old to prevent early infection.

At present, broiler producers in the U.S.A and Britain are using the first method (Wyeth and Cullen, 1978a; Giambrone, 1983). This is because the essential element of controlling this form of IBD is the preservation of the BF during the first one or two weeks of life in the newly-hatched chick. This objective is achieved more effectively by MA than day-old vaccination of IBD susceptible chicks (O'Brien, 1976; Ide, 1979). Breeder flocks are being

vaccinat pregnant IBD in order to produce high levels of MA in the progeny chicks. The level of MA was found to be higher in chicks obtained from breeders vaccinated nearer to the point of lay than those whose hens were vaccinated early in life (Wyeth and Cullen, 1978a). Furthermore, higher, long-lasting and more uniform levels of MA in chicks comes from breeders receiving two live IBDV vaccine primers and one inactivated oil-emulsion IBDV vaccine during the growing period (Giambrone, 1983). Priming with live IBDV vaccine is important in order to provide the bird with adequate "memory" cells. These cells when introduced to a killed IBDV vaccine will respond by dividing and producing large number of antibody-producing cells. The large number of antibody-producing cells is needed for the production of antibody which will last for the breeder hen's 40-week period of lay (Giambrone, 1983).

Since MA declines steadily after hatch and is absent by the time a bird is three to four weeks of age, it cannot be dependent on to protect against clinical IBD (Naqi et al., 1983). The type of vaccine to use and the programme to follow for prevention of clinical IBD will depend upon the level and virulence of the field IBDV as well as the levels

of MA in the birds (Giambrone, 1983). If the field virus is very virulent and clinical IBD occurs as early as eight to 14 days of age and affects about 25 to 50% of the flock, vaccination with a highly pathogenic IBDV vaccine at five to eight days in drinking water is recommended. If on the other hand, clinical IBD is not occurring until at 18 days, then mild IBDV vaccines can be used at about 10 to 14 days of age in drinking water (Giambrone, 1983). Day-old vaccination with mild IBDV vaccines is usually confined to the progeny of parents that are not receiving the killed vaccine or parents that are older than 45 weeks of age and have become serologically negative to IBDV by the agar-gel precipitin test or below 1:100 on the VN test (Giambrone, 1983).

1.4.10 Diagnosis

A diagnosis of IBD is established from consideration of the histroy of the flock and of the onset and course of the disease, the clinical signs and the gross postmortem lesions of a sufficient number of chickens that have died (Faragher, 1972). Confirmation is based on the histopathology of infected BF, serology, inoculation of susceptible chicks and viral isolation (Cosgrove, 1962;

Helmboldt and Garner, 1964; Asdrubali and Gialletti, 1971; Weisman and Hitchner, 1978; Cho et al., 1979; Ide, 1979).

Serological methods used to detect flocks exposed to IBDV are: the agar-gel precipitin test; serum-neutralization test assayed in fully susceptible embryonated chicken eggs or susceptible chickens; plaque reduction test in chicken embryo kidney or fibroblast cell monolayers; immuno-fluorescent antibody test; and most recently, the indirect enzyme-linked immunosorbent assay (ELISA) (Cheville, 1967; Winterfield, 1969; Hirai et al., 1972; Lukert and Davis, 1974; Cho et al., 1979; Marquardt et al., 1980).

The quantitative gel diffusion test (QGDT) is widely used to measure IBD antibody levels (Cullen and Wyeth, 1975). However, in some laboratories, a tissue culture virus-neutralization (TCVN) test is employed (Ide, 1979, Eidson et al., 1980). While the QGDT is simple, rapid and inexpensive to perform, the TCVN test is more difficult, time-consuming and expensive; however, the latter test has been reported to be more sensitive and quantitative (Weisman and Hitchner, 1978; Phillips, Jr, 1981).

1.4.11 Differential Diagnosis

Infectious bursal disease may be confused with other poultry diseases. Lymphoid leukosis produces a swelling of the BF but it affects much older birds (16 weeks and older). In addition to the enlargement of the BF and spleen, the liver is very enlarged in this disease.

Marek's disease causes swelling of the spleen and atrophy of the bursal follicles but also causes other lesions not observed in IBD including enlargement of the sciatic nerves, ocular lesions and cellular infiltration of the visceral organs.

Inclusion body hepatitis causes atrophy of the bursa but is characterized by splash-type haemorrhage in thigh muscle and presence of inclusion bodies in the nucleus of hepatocytes (Okeke, 1984).

There is clinical resemblance between IBD and coccidiosis in the early stages of an outbreak (Barron et al., 1966); however, results of post-mortem examination can easily differentiate the two diseases.

Newcastle disease in which haemorrhages may occur in the muscles and on the mucosa of the proventriculus, is distinguished from IBD on the

basis of postmortem findings (Faragher, 1972). In that form of infectious bronchitis in which the kidneys are specifically affected, the gross changes in the kidney (enlargement and pinkish-grey in colour and with tubules and ureters distended with urates) are pathognomonic (Faragher, 1972).

Infectious bursal disease may also be confused with fatty liver and kidney syndrome, avitaminosis A and reticulo-endotheliosis (Faragher, 1972). Recovery from fatty liver and kidney syndrome is rare and the BF is not involved (Faragher, 1972). Avitaminosis A is characterized by the presence of caseous plugs in the BF and metaplasia of the epithelium of bursal plicae is the only histopathologic change observed (Faragher, 1972). In reticuloendotheliosis, the liver and spleen are enlarged while the thymus and BF and atrophied. Extensive reticuloendothelial proliferation and complete lymphoid depletion in the thymus and BF were the histopathologic changes observed (Olson, 1967).

Chapter 2

STUDIES ON THE DECAY PATTERN OF MATERNAL ANTIBODY AGAINST INFECTIOUS BURSAL DISEASE IN CHICKS

2.1 Introduction

Clinical and serological evidence indicate that infectious bursal disease (IBD) virus is widespread in Nigeria (Ojo et al., 1973; Onunkwo, 1975; Bishu et al., 1977; Nawathe et al., 1978, Umoh et al., 1984). Vaccination failures against IBD in Nigeria attributed to improper handling of vaccine have been suggested (Nawathe and Lamorde, 1982). Maternal antibody (MA) interference with vaccination has been reported in other countries (Winterfield et al., 1972; Wood et al., 1981) and has also been suspected to play a role in these vaccination failures (Nawathe and Lamorde, 1982). It is logical to expect young chicks in Nigeria to posses MA against IBD because of the following: IBD is enzootic; IBD live virus vaccines of various kind are frequently used; only birds three weeks or older are usually reported to succumb to clinical disease (Bishu et al., 1977) and fertilized eggs and day-old chicks are still imported from European countries in which breeder vaccination against IBD is practiced

(Wyeth and Cullen 1978b). Unfortunately, there is a dearth of information on the prevalence of MA against IBD and their possible role in interference with active immunization of chicks in Nigeria. Therefore, the objective of this study was to investigate the presence of MA and its decay pattern in chicks obtained from a commercial hatchery in Zaria.

2.2 Materials and Methods

2.2.1 Preparation of Specific IBD Virus Antigens and Antisera

Preparation of Field Virus Precipitin Antigen:

Typically affected bursae of chickens that died from natural IBD were removed, weighed, diluted 1:1(w/v) with PBS (pH 7.6) and ground with a pestle and motar with the aid of fine sterile sand. This mixture was then frozen, thawed and ground three times and clarified at 2,000 x g for 30 minutes. Two hundred and fifty I.U. of penicillin and 250 ug of streptomycin were added to the supernatant fluid (2 ml portions) and then stored in screw-cap vials at -20°C until used.

Three-week-old chicks were then inoculated intraocularly with 0.05 ml of the supernatent fluid

(bursal homogenate). Three days after inoculation the chicks were killed, the BF removed and treated as mentioned earlier. The homogenate was used as the antigen in quantitative agar gel precipitin test (QAGPT) (Cullen and Wyeth, 1975).

Preparation of Reference Precipitin Antigen: One ampoule of a freeze-dried IBDV Cheville strain (Kindly supplied by Dr. Wood, Central Veterinary Laboratory, Weybridge) was reconstituted in 1ml of PBS (pH7.6) and 0.05 ml of this was instilled into the open eyes of three-week-old chicks. Three days after inoculation, the chicks were killed and the bursae removed. A clarified homogenate was prepared and stored at -20°C until used as the reference antigen in the QAGPT.

Preparation of Field Virus Precipiting Antiserum: Some of the chicks infected with the field IBD above were used for the preparation of field virus anti-serum. Five weeks post-infection, two to five millilitres of blood were obtained from the wing vein or heart of the infected chicks. The blood was allowed to clot for two hours at room temperature and then at 37°C for 4 hours. The sera were separated, pooled and stored in vials at -20°C until used.

Reconstitution of British Standard Antiserum: British standard serum was kindly supplied by Dr. Wood of Weybridge. Each ampoule contained 1,000 U.K. units. The ampoule was reconstituted in 1 ml of PBS (pH. 7.6) to give a titre of 1,000 U.K. units/ml. The titre of an unknown serum was calculated by dividing the reciprocal of the endpoint dilution of the unknown serum by that of the standard serum and multiplying the number by 1,000. The titre was expressed in U.K. units/ml and the endpoint dilution was the last dilution in QAGPT at which a precipitin line could be seen.

2.2.2 Quantitative Agar Gel Precipitin Test

Gel diffusion agar was prepared by adding 8 g of sodium chloride, 0.02 g of sodium azide and 1.25 g of Difco agar to 100ml of distilled water and heating the mixture slowly to boiling. The agar plates were made by dispensing 25.7 ml agar into 9 x 9.5 cm perspex dishes. This gave an agar depth of three millimetres.

Wells of six millimetres with three millimetres seperation were cut into the already set agar. An offset linear pattern of 12 parallel rows was used with the middle row of every three having every

hole cut (Figure 2.1). The middle row was for antigen while the two complete rows to either side were for antiserum dilutions. Thus, one antigen well served the four adjacent antiserum wells. The agar plugs were removed from the wells with a hypodermic needle. A positive reaction system and a known negative antiserum or antigen was incorporated in each test run.

Standardization of Antigen: The field virus precipitin precipitin antigen was standardized by the following steps:

(1) 1:1.5, 1:2, 1:2.5 and 1:3 dilutions of antigen in sterile physiological saline were prepared. One millilitre of each dilution was required for the test.

(2) Serial two-fold dilutions of standard positive antiserum from 1:2 to 1:2048 using physiological saline as diluent were prepared. Approximately half of a millilitre of each dilution was sufficient for the test.

(3) One-twentieth millilitre (0.05 ml) of each of the dilutions of antigen was

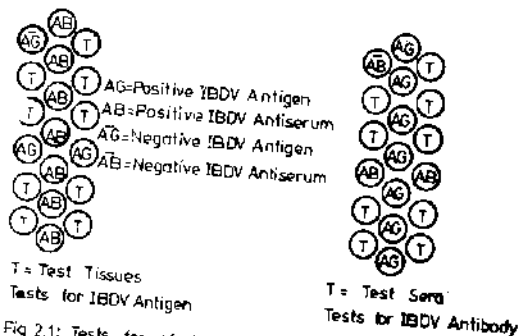


Fig 2.1: Tests for infectious bursal disease virus (IBDV) antigen and antibody.

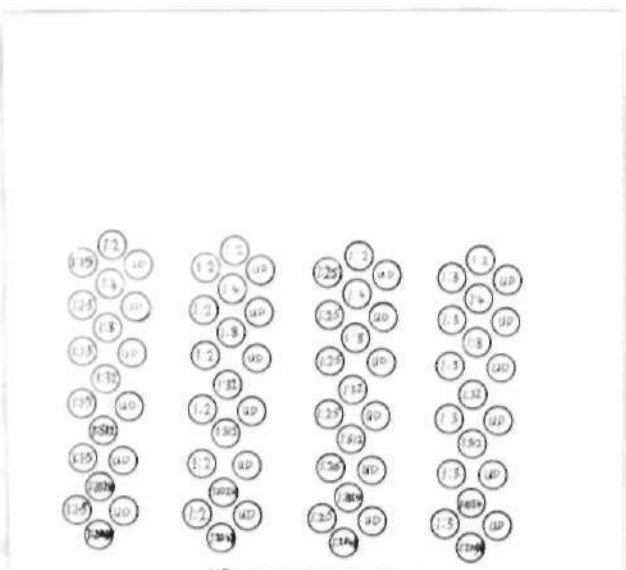
dispensed with a micropipette into each well of a vertical row of six wells. A row of wells containing undiluted antigen was included (*Figure* 2.2).

(4) One-twentieth millilitre of antiserum diluted 1:2 was dispensed into each of the first wells of the rows of seven wells.

(5) One-twentieth millilitre of antiserum diluted 1:4 was dispensed into each of the second wells. This process was repeated until the 1:2048 dilution was reached.

(6) The dishes were incubated at room temperature in a humid box and read after 48 hours. Strong reactions showed two or three lines between the antigen and antiserum. The number of lines eventually reduced to one. The end-point was taken as the dilution where this single line disappeared.

When reading the test, it was observed that the endpoints for all dilutions of antigen were



UD = Undiluted IBDV Antigen

Fig 2-2: Standardization of field infectious bursal disease virus (IBDV) antigen by the quantitative agar gel precipitin test.

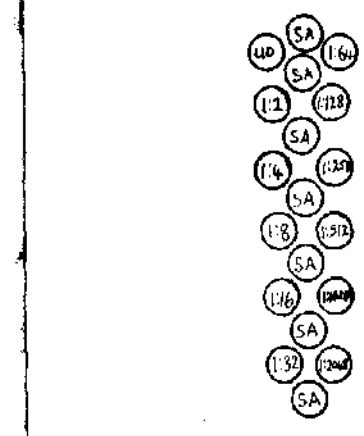
similar but the intensity and clarity of precipitin lines diminished as the antigen became more dilute. The highest dilution of antigen which produced a clear, sharp line was chosen for standard use.

Testing sera of Unknown Titre: The titres of unknown sera were determined by the following steps.

(1) Serial two fold dilutions of the test serum from 1:2 to 1:2048 using physiological saline were prepared.

(2) One-twentieth millilitre of undiluted test serum was placed in the first well of one of the rows of six wells (Figure 2.3). One-twentieth millilitre of the test serum diluted 1:2 was placed in the second well and the procedure repeated with each dilution of serum up to the 1:2048 (the second row of six wells was used as a continuation).

(3) One-twentieth millilitre of standardized antigen was placed in each of the wells in the centre row. Dishes were incubated at room temperature in a humid box for 48 hours



UD = Undiluted IBDV Antiserum
SA = Standardized IBDV Antigen

Fig 2-3: Testing infectious bursal disease virus (IBDV) antiserum of unknown titre

after which they were read. The endpoint of each serum was taken as the dilution which produced a precipitin line which could be easily seen without scrutinizing the agar gel.

2.2.3 Chicks

One hundred and fifty day-old starbro broiler chicks were purchased from a commercial hatchery in Zaria. The chicks were the offsprings of hens vaccinated with a live IBD vaccine just prior to point of lay. The parent stock were 32 weeks old at the time the eggs were collected and hatched. The chicks were reared in a room previously washed, disinfected (using a chlorine-containing disfectant) and sprayed with two percent formalin. Chemoprophylactic treatments were given against mycoplasma, coccidia and helminths. They were vaccinated against Newcastle disease at day-old. They were fed with a commercial broiler-starter ration ad libitum.

2.2.4 Experimental Design

From day-old for 29 days, a group of 10 chicks each were randomly picked and bled every other day. The antibody level of the sera obtained were measured by the QAGPT.

2.2.5 Statistical Analysis

A linear regression equation was calculated using the method of least squares from the mean titres of the chicks and the regression line plotted along with the actual mean values. Student t-test was performed on the correlation coefficient(r) (Colton, 1974).

2.3 Results

Although 10 chicks were bled on each occasion, only eight and five sera obtained from chicks at nine and 11 days old respectively were in enough quantities to be tested.

Infectious bursal disease virus maternal antibody (IBDV MA) was detected in the sera of the chicks tested. At day-old, 100% of the tested chicks had MA but by the nineth day of age, MA was present in only 50% of the tested chicks (Figure 2.4). It persisted for up to day 17 when only 10% of the chicks tested had MA, and absent from all the chicks tested at day 19. A sharp drop in the percentage and mean titre of seropositive chicks between day seven and nine was noted. The mean IBD MA level at day-old was 337.5 U.K. units/ml while the value at day nine was 78.1 U.K. units/ml. The highest

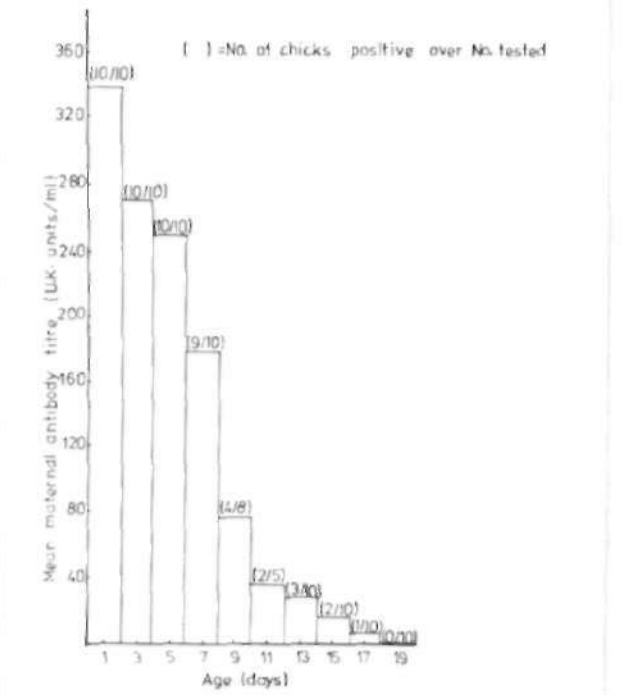


Fig 2-4 Pattern of infectious bursal disease virus maternal antibody decay in chicks

individual MA level recorded was 562.5 U.K. units/ml which was obtained at day-old. The standard deviations of the mean MA titres for each group of chicks were high. The correlation co-efficient(r) was calculated to be - 0.946 ($P<0.001$) and the standard error of r to be 0.115. The antibody half-life was found to be about 8.2 days (Figure 2.5).

2.4 Discussion

The presence of MA in the sera of the chicks studied confirmed earlier speculation of their presence in chicks in Zaria and agrees with earlier findings elsewhere (Hitchner, 1971; Ide et al., 1978; Wyeth and Cullen, 1978b). The natural linear decay of MA had already been shown (Wyeth and Cullen, 1978b). The absence of MA in the chicks at 19 days of age observed in this study is similar to the 17 and 18 days reported by Wyeth and Cullen (1978b) and Ide et al. (1978) respectively. The parents of the chicks they used were vaccinated with a live IBD vaccine as was the case in the parents of the chicks used in this experiment. It is possible that the MA would have persisted longer than 17 days if the initial antibody level in the chicks used was higher. There are currently two methods of obtaining a high, uniform and long-lasting MA in chicks: 1) to vaccinate

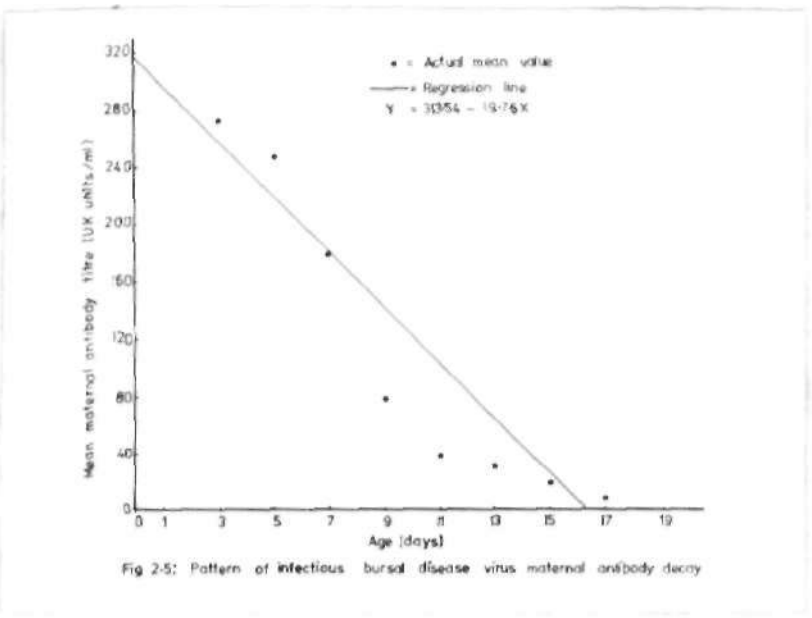


Fig 2-5: Pattern of infectious bursal disease virus maternal antibody decay

breeders at 12 weeks with a live IBD vaccine orally and at 20 weeks with inactivated IBD oil-emulsion vaccine (IBD OEV) intramuscularly or 2) vaccinate breeders at 18-22 weeks with an IBD OEV by subcutaneous or intramuscular routes and at 34-38 weeks with a live IBD vaccine at three times the normal dose orally (Eidson et al., 1980; Wyeth et al., 1981; Frazier, 1983). Maternal antibody of chicks from such breeders was reported to have lasted up to 27 or 28 days post-hatching while it lasted only 17 days in chicks whose parents received only the live or oil-emulsion vaccine (Wyeth and Cullen, 1978b; Wyeth and Chettle, 1982).

The MA half-life of 8 days obtained is longer than the 4 days reported by Wyeth and Cullen (1976) but agrees with the 6 to 8 days obtained by Wood et al. (1981). The test used by Wyeth and Cullen (1976) and Wood et al. (1981) and that used in this work measured essentially IgG whose half-life is about 4 days (Haffer, 1982b). A neutralizing MA half-life of 6½ days has been reported by Lukert (1977).

The presence of IBD precipitin antibodies in chicks is said to be synonymus with immunity to IBDV-induced BA and their absence was found to have correlated well with susceptibility to IBD challenge (Winterfield and Thacker, 1978, Wyeth and Cullen,

1978b). About 90 to 100% of the chicks studied therefore would be expected to be immune to BA within the first seven days of life and 50% and none at nine days and 19 days respectively. However due to the low sensitivity of the QAGDT as compared to virus neutralization (VN) test and its inability to detect precipitin antibody level of less than 26 U.K. units/ml, it was possible that a chick found to be negative by this test might have some neutralizing and precipitin antibodies and therefore resistant to IBD (Lukert et al., 1976; Ide et al., 1978; Wyeth and Cullen, 1978b; Wyeth, personal communication). However, VN antibody positive one-week-old chicks have been found to be susceptible to BA eventhough they did not show clinical disease (Weisman and Hitchner, 1978). It was earlier found that MA only delayed BA without reducing its extent (Muskett et al., 1979). Full protection against IBDV-challenge was only observed in chicks with antibody titres (Geometric mean virus neutralization titres) of 3.5 and above (Lucio and Hitchner, 1979). The rather sudden decrease in mean antibody titre on day seven from 181.25 U.K. units/ml with 90% of the tested chicks being sero-positive to 78.13 U.K. units/ml on day nine with only 50% of the tested chicks being sero-positive could not easily be

explained.

Since MA may interfere with active immune response of chicks to live IBD vaccine, it might be necessary to establish the immune status of the chicks before designing a vaccination programme. The MA level capable of interfering with immune response by live apathogenic IBD vaccine was found to be 34.0 U.K. units/ml (Wood et al., 1981). Based on the results obtained, it would appear that day-old and seven-day-old IBD vaccination of these chicks would be ineffective. By nine days and 19 days of age however, 50% and 100% of the chicks respectively would be expected to respond to vaccination. As there is usually wide variation in MA level between chicks, a uniform immune response may not be achieved. Resistance to clinical and subclinical IBD may also not be uniform. This lack of uniformity may be due to differences in the amount of antibody passed on to the chicks via the egg or differences in the sizes of the eggs as larger eggs usually contain more antibody (Kramer, 1973). Other chicks however may have lost their antibody at a faster rate than others. Even with the same hen, the transmission of IBDV precipitins to her chicks has been found to be irregular (Wyeth and Cullen, 1976).

Although, the antibody levels in the hens from which the chicks were derived were not determined, it would be safe to assume that they were high as IBD precipitins have been found to be transmitted to the chick at approximately one or two log lower quantity than in the parent bird (Wyeth and Cullen, 1978a).

To avoid MA interference with vaccination, it has been suggested that IBD vaccination be done with a pathogenic IBD vaccine or a cell - associated IBD vaccine at seven-day-old (Nawathe and Lamorde, 1982; Giambrone, 1983). Maternal antibody could also be avoided by direct injection of modified IBDV into the BF (Winterfield and Thacker, 1978). Since the sources of fertilized eggs and day-old chicks in Zaria are many and the IBDV exposure experiences (natural versus vaccination and live versus inactivated vaccine) of the hens from which the eggs or chicks are derived also varied and usually unknown, it will be difficult to formulate a uniform IBD vaccination programme for chicks in Zaria. Sero-monitoring chicks at different ages by the QAGDT would be useful. When about 70% of the chicks are negative to the test or their mean MA level falls below 34.0 U.K. units/ml, the chance of a response to vaccination is higher. From the results of this

study, these conditions were achieved when chicks were about two weeks old. It is possible, however, that delayed vaccination until two weeks of age would result in the establishment of the field IBDV. Unless there is lateral spread and persistence of the vaccine virus, effective protection is unlikely during the first nine days of life.

It appears from this work and the literature that a combination of the presence of high MA level and good hygiene or the use of wirefloors to reduce the chance and extent of exposure to field IBDV are required for effective control of IBD (Wood et al., 1981; Naqi et al., 1983).

Chapter 3

VACCINATION OF MATERNALLY-IMMUNE CHICKS WITH FIBROGUMBOVAC 10

3.1 Introduction

The choice of the type of vaccine, route of vaccination to use and the age at which to vaccinate chicks against infectious bursal disease (IBD) are dependent on the presence and level of maternal antibody (MA) in chicks and active antibody in their parents, the rate of MA decay in the chicks, the virulence of field virus strains and the length of protection desired (Ehizokhale, 1982). Winterfield et al. (1972) had observed IBD outbreaks in flocks vaccinated before ten days of age. Interference with vaccination with mild live IBD vaccine has been found in maternally-immune chicks as early as one day to as late as 21 days of age (Rinaldi et al., 1972b; Skeeles et al., 1979b; Wood et al., 1981).

In Nigeria, vaccination of chicks with live non-pathogenic IBD vaccines is widely practiced as a means of controlling IBD. The sources of these vaccines are varied and the recommendations for their use also differ. One of the vaccines, Fibrogumbovac 10 is locally produced (National Veterinary Research

Institute, Vom, Nigeria). It is more readily available and therefore most widely used. It is to be administered by the ocular route at day-old for both maternally-immune and susceptible chicks. The former are re-vaccinated at 30 days of age. Infectious bursal disease vaccination failures due to MA interference have been suspected (Nawathe and Lamorde, 1982). However, no studies have been conducted to confirm this. This study was therefore aimed at determining:

(1) whether MA would interfere with effective IBD vaccination of maternally-immune chicks from a commercial hatchery in Zaria, and
(2) the best age for vaccination when the MA have minimal interference effect.

3.2 Materials and Methods

3.2.1 Chicks

One hundred day-old chicks of the same breed, type, source and parents were bought on the same day as those used in earlier studies (Chapter 2). They were housed, fed and treated as earlier described (Chapter 2).

3.2.2 Vaccination

A freeze-dried live infectious bursal disease virus (IBDV) vaccine, Fibrogumbovac 10, was used. The vaccine contained a minimum of about $10^{4.5}$ tissue culture infective dose 50 per bird as stated by the manufacturer. A two millilitre vial contained 400 doses of the vaccine. One vial was reconstituted in 800 millilitres of distilled water. Each chick was given approximately two millilitres of the vaccine orally. Drinking water was witheld for three hours before the vaccine was administered.

3.2.3 Testing for Precipitin Antigen and Histopathology

The bursa of each vaccinated and control chick was sectioned into two halves. One-half of the bursae from the chicks in each group were pooled together, weighed and homogenized and tested for the presence of IBDV precipitin antigen (as in 2.2.3). The other half of the bursa was fixed in 10% neutral buffered formalin together with the spleen and thymus. Sections were made, stained with haematoxylin and eosin and examined microscopically.

3.2.4 Experimental Design

From day-old to 29 days old different sets of 20 chicks were vaccinated (as described in 3.2.2)

weekly. The same number of chicks were left unvaccinated to serve as controls. Different caretakers were delegated to manage the vaccinated and control chicks. At three and seven days post-vaccination, five chicks.from each group were euthenized, weighed and their bursae, spleens and thymi were examined grossly, removed and weighed. The bursal: body weight ratios were calculated as percentage for each chick and expressed as arithmetic means of groups of chicks. The tissues were fixed for histopathology (as described in 3.2.3). The bursae were homogenized for antigen detection. From each set of vaccinated chicks, the remaining 10 chicks were bled on three, seven, 14 and 35 days post-vaccination and their sera tested for the presence and level of active IBD antibody. After the last bleeding, vaccinated chicks were challenged ocularly with 0.05 ml of a field IBDV using bursal homogenate from IBD - infected chicks. All challenged chicks were closely observed for seven days for signs of IBD.

3.2.5 Statistical Analysis

The arithmetic mean of the body, bursal, splenic and thymic weights and bursal: body weight ratios of vaccinated and control chicks at three and seven days

post-vaccination were calculated and compared by the student t-test (Colton, 1974) to determine if there was a significant difference between the mean of the vaccinated and control chicks. Correlation coefficients were calculated to determine if any relationship existed between the age of control chicks and their mean body, bursal, splenic and thymic weights and bursal: body weight ratios (Colton, 1974).

3.3 Results

Chicks vaccinated at one, eight, 15, 22 or 29 days old became sero-positive at 36, 43, 50, 36 and 36 days of age respectively. The interval between vaccination and initial detection of active IBD precipitin antibody was 35 days for chicks vaccinated at one, eight or 15 days old, but 14 and seven days for chicks vaccinated at 22 or 29 days old respectively (Table 3.1). All the 10 chicks vaccinated at one, eight, 15, 22 or 29 days old were sero-positive when active IBD antibody in them was first detected. The IBD antibody titre at 35 days post-vaccination was highest in chicks vaccinated at 15 days old. Maternal antibody was detected on the third, seventh and fourteenth day post-vaccination in chicks vaccinated at day old. The MA

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Table 3.1: Mean infectious bursal disease virus antibody titres in maternally-immune chicks vaccinated with Fibrogumbovac 10

Age of chicks at vaccination* (days)	Days post-vaccination and titres (U.K units/ml)**			
	3	7	14	35
1	162.5 ± 42.7	46.9 ± 46.9	6.9 ± 6.9	320.3 ± 36.3
8	0	0	0	390.6 ± 32.9
15	0	0	0	562.5 ± 23.6
22	0	0	281.3 ± 28.4	381.3 ± 48.0
29	0	225.0 ± 31.8	444.4 ± 49.4	304.7 ± 39.9

* Ten chicks were tested at each age

** Values given as means and standard errors

in chicks vaccinated at day-old was lower than that of controls but was not statistically different ($P>0.05$) from that of controls (Table 3.2).

Infectious bursal disease virus precipitin antigen was not detected at three and seven days post-vaccination from the bursae of chicks vaccinated at one, eight or 15 days old. It was however detected at seven days post-vaccination from chicks vaccinated at 22 days old and at three days post-vaccination from those vaccinated at 29 days (Table 3.3).

There was highly significant correlation between the age and the mean body ($r = + 0.94$; $p<0.001$), bursal ($r = + 0.82$; $p<0.001$, splenic ($r = + 0.83$; $p<0.001$) and thymic weights ($r = + 0.84$; $p<0.001$) and bursal: body weight ratios ($r = + 0.58$; $p<0.001$) of control chicks. The mean body, bursal, thymic and splenic weights and bursal: body weight ratios and the significant level of the difference between the means of vaccinated and control chicks are shown in Table 3.4 and 3.5. As from 15 days of age, the mean bursal weight of control chicks was higher than that of vaccinated chicks. The mean bursal weight of control chicks increased from day-old to about 32 days of age when it remained constant and decreased

Table 3.2: Effect of vaccination on the level of maternal antibody against infectious bursal disease virus in chicks

Age** (days)	Group: vaccinated* Mean titre ± SE***	vaccinated* % Positive	control Mean titre ± SE	control % Positive
4	162.50 ± 42.69	80.00	268.75 ± 40.69	100.00
8	46.88 ± 46.88	20.00	56.25 ± 28.66	40.00
15	6.94 ± 6.94	10.00	18.75 ± 13.35	20.00

* Vaccinated at day-old

** Ten chicks were tested at each age for each group

*** Titre in U.K. units/ml

SE Standard errors

Table 3.3: Infectious bursal disease virus precipitin antigen in the bursae of maternally-immune chicks vaccinated with Fibrogumbovac 10*

Age of chicks at vaccination** (days)	Days post-vaccination	
	3	7
1	-	-
8	-	-
15	-	-
22	-	+
29	+	-

* Antigen detected by the QAGPT

** The bursae of five vaccinated chicks were pooled and tested

\- Antigen not detected

\+ Antigen detected

Table 3.4: Mean body, bursal, thymic and splenic weights and bursal: body weight ratios of maternally-immune chicks three days post-vaccination*

Group	Age** (days)	Body weight (g)	Bursal weight (g)	Bursal: body weight ratio (x 100)	Thymic weight (g)	Splenic weight (g)
Vaccinated	1	41.8 ± 3.2	0.055 ± 0.012	0.130 ± 0.027	0.145 ± 0.015[a]	0.025 ± 0.006[a]
	8	85.1 ± 6.9	0.106 ± 0.033	0.118 ± 0.028	0.172 ± 0.042	0.062 ± 0.027
	15	125.1 ± 0.1	0.263 ± 0.058	0.201 ± 0.028	0.454 ± 0.195	0.094 ± 0.016
	22	240.6 ± 10.8[a]	0.640 ± 0.053[a]	0.266 ± 0.019	1.101 ± 0.179[b]	0.260 ± 0.018
	29	465.9 ± 9.9	1.337 ± 0.207[b]	0.287 ± 0.446	2.219 ± 0.200[c]	0.755 ± 0.036
Control (Not vaccinated)	1	34.1 ± 2.6	0.035 ± 0.005	0.104 ± 0.018	0.081 ± 0.012[a]	0.088 ± 0.011[a]
	8	80.9 ± 15.4	0.144 ± 0.016	0.179 ± 0.015	0.242 ± 0.030	0.059 ± 0.011
	15	133.8 ± 10.8	0.259 ± 0.024	0.196 ± 0.020	0.527 ± 0.050	0.112 ± 0.014
	22	451.7 ± 48.8[a]	1.246 ± 0.055[a]	1.456 ± 0.130	1.714 ± 0.124[b]	0.326 ± 0,031
	29	498.8 ± 13.2	1.515 ± 0.228[b]	0.304 ± 0.045	3.257 ± 0.259[c]	0.835 ± 0.036
		a; P<0.005	a; P<0.001 b; P<0.005		a; P<0.025 b; P<0.050 c; P<0.005	a; P<0.025

* Values given as means and standard errors for five chicks

** Age at vaccination

Table 3.5: Mean body, bursal, thymic and splenic weights and bursal: body weight ratios of maternally-immune chicks seven days post-vaccination*

Group	Age** (days)	Body weight (g)	Bursal weight (g)	Bursal: body weight ratio (x 100)	Thymic weight (g)	Splenic weight
Vaccinated	1	63.9 ± 5.1	0.161 ± 0.022	0.249 ± 0.022	0.269 ± 0.040	0.041 ± 0.006
	8	144.8 ± 7.3[a]	0.271 ± 0.033	0.189 ± 0.024	0.708 ± 0.085[a]	0.128 ± 0.016[a]
	15	243.0 ± 11.6[b]	0.651 ± 0.149	0.264 ± 0.052	1.285 ± 0.061	0.208 ± 0.049
	22	376.9 ± 25.8[c]	0.958 ± 0.093[a]	0.259 ± 0.032	2.512 ± 1.393	0.599 ± 0.058
	29	512.4 ± 25.1	0.552 ± 0.051	0.108 ± 0.012	2.145 ± 0.212	0.723 ± 0.064
Control (Not vaccinated)	1	59.8 ± 2.1	0.113 ± 0.012	0.188 ± 0.018	0.204 ± 0.016	0.053 ± 0.006
	8	96.5 ± 4.5[a]	0.194 ± 0.021	0.194 ± 0.016	0.377 ± 0.037[a]	0.074 ± 0.006[a]
	15	314.4 ± 7.7[b]	0.906 ± 0.051	0.288 ± 0.014	1.525 ± 0.146	0.319 ± 0.023
	22	496.8 ± 9.9[c]	1.518 ± 0.128[a]	0.321 ± 0.026	2.752 ± 0.167	0.443 ± 0.041
	29	552.4 ± 39.0	0.848 ± 0.646	0.157 ± 0.013	1.954 ± 0.435	1.078 ± 0.129
		a; P<0.001	a; P<0.025		a; P<0.001	a; P<0.001

* Values given as means and standard errors for five chicks

a; P<0.001
b; P<0.005
c; P<0.001

**Age at vaccination

sharply by 36 days of age (Figure 3.1). The bursae of chicks seven days post-vaccination at 15 and 22 days old were grossly smaller than those of control chicks (Plate I).

None of the chicks vaccinated showed clinical signs when challenged five weeks after vaccination. Unfortunately, control chicks became naturally exposed to IBDV during the study. Clinical signs of IBD were first observed when the chicks were 32 days old.

Microscopic lesions were observed in the bursae of vaccinated and control chicks that were naturally exposed. They were present in the bursae of chicks vaccinated at 15, 22 or 29 days old and the bursae of control chicks that were naturally exposed at 32 and 36 days old. The lesions were observed seven days post-vaccination in chicks vaccinated at 15 or 22 days old and three and seven days post-vaccination in chicks vaccinated at 29 days old. The lesions included edema, necrosis of lymphocytes, atrophy of bursal follicles, interfollicular edema or fibroplasia, hypertrophy of the corticomedullary epitheloid layer and absence of epithelium (Plate II). There was an increase in the severity of these lesions and the number of bursae with lesions with

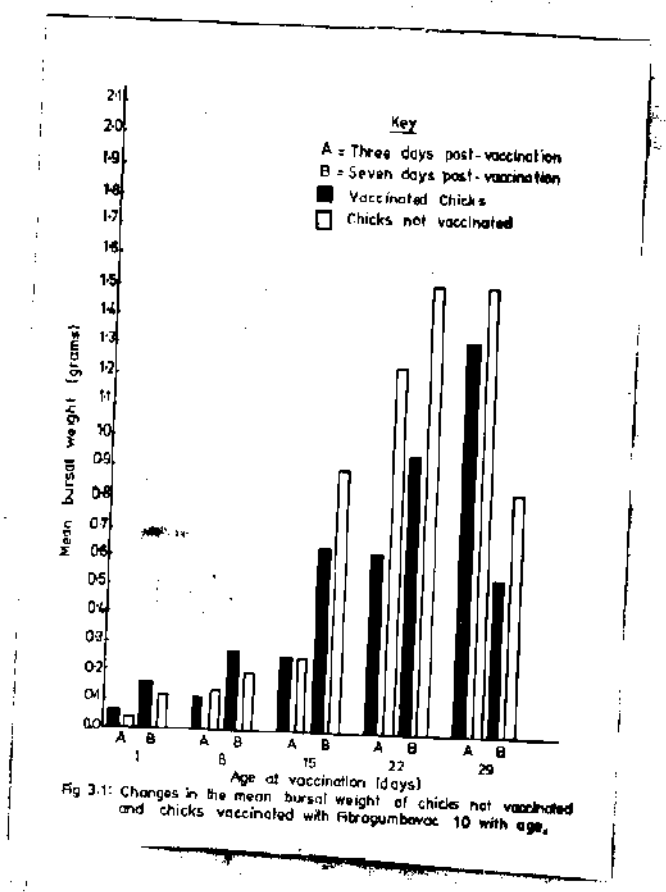


Fig 3.1: Changes in the mean bursal weight of chicks not vaccinated and chicks vaccinated with Fibrogumbavac 10 with age.

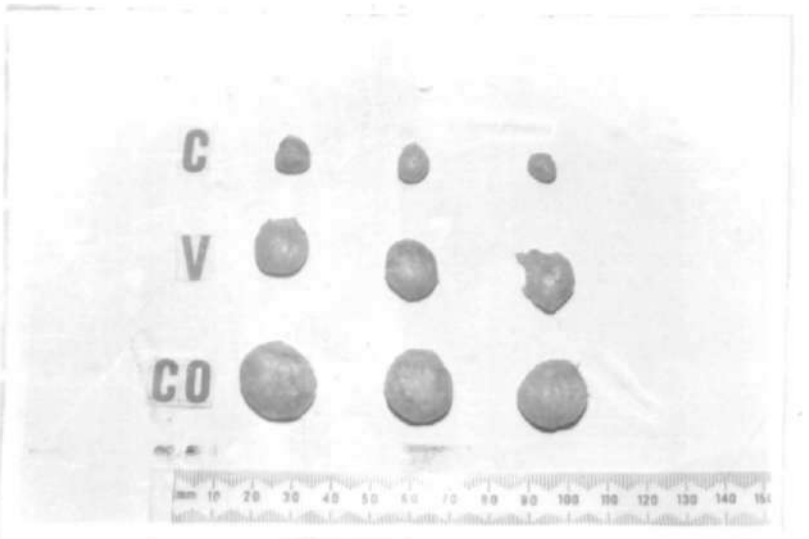
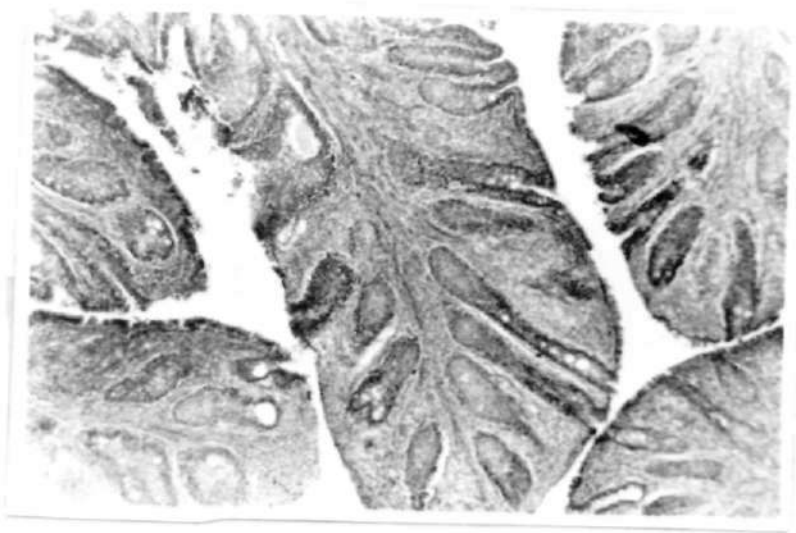


Plate I: The bursae of challenged (c) and vaccinated (v) chicks at seven days post-challenge or post-vaccination at 22 days of age and bursae of unchallenged, unvaccinated chicks (CO) at 29 days of age



Plate II: Photomicrographs of bursae from chicks vaccinated with infectious bursal disease vaccine (Fibrogumbovac 10) at 29 days of age. Note interfollicular oedema, follicular atrophy with lymphoid depletion and loss of surface epithelium on the third day post-vaccination (A) H & E stain; x 63



Evidence of regeneration by the seventh day post-vaccination (B) H & E stain; x 63



Normal bursa from a 25-day-old unvaccinated chick is shown in C; note the high number of lymphocytes in both the cortex and medulla, and scanty interfollicular connective tissue. H & E stain; x 63.

increase in age at vaccination. The thymi and spleens of a few chicks were congested. There was an increase in the number of germinal centres in the spleens of chicks seven days post-vaccination in chicks vaccinated at 15, 22 or 29 days old.

3.4 Discussion

The potent immunogenic nature of IBDV in susceptible chicks has been demonstrated (Naqi et al., 1980). Maternal antibody has however been shown to interfere with and delay IBD vaccination immune response (Naqi et al., 1983). In a study, immune response to IBDV (52:70 strain) in MA-free chicks inoculated at 14 or 17 days old was found to be rapid and stronger than in chicks possessing MA (Wyeth and Cullen, 1976). The presence of MA in vaccinated chicks in this study might have delayed the rate of sero-conversion observed in chicks vaccinated at one, eight or 15 days old. Naqi et al. (1983) also noted this lack of antibody response to live IBD vaccines (mildly invasive and invasive strains of IBDV) in chicks carrying high MA levels and vaccinated at two, three or 13 days of age. These chicks however showed antibody response to challenge with a field virus (FV) at

34 days of age. They also noted a response to the mildly invasive IBD vaccine and FV in two-day-old chicks possessing low levels of MA. The response to the FV was prompt but was delayed for about 14 days in the chicks vaccinated. The MA level in the chicks used in this study might have been higher than in the chicks used by Naqi et al. (1983) and/or Fibrogumbovac 10 may be less invasive than the vaccine they used resulting in the longer time (35 days) for immune response to develop in chicks vaccinated at up to 15 days old. The lower MA level in vaccinated chicks and the delay in immune response observed in this study could thus be explained. The MA might have neutralized some vaccine viral particles (Lucio and Hitcher, 1979) or stimulated T-suppressor cells to act on B-cell response or the interaction between MA and vaccine virus might have resulted in the depletion of complement required for the production of IgG from IgM (Subba Rao et al., 1978; Naqi et al., 1983).

In contrast to the result obtained in this study and that of Lucio and Hitchner (1979), little or no influence of vaccine virus on the depletion rate of MA was noted by Naqi et al. (1979). The use of live vaccines in

maternally-immune chicks may be associated with increased susceptibility for a certain period of time to FV (Henry and Williams, 1980). However, the mild, non-damaging IBD vaccine used by Wood et al. (1981) did not reduce the protective effect of MA. The level of MA necessary to neutralize IBDV may vary with the invasiveness and pathogenicity of the virus strain (Lucio and Hitchner, 1979). The higher the virulence of the IBDV vaccine the more the MA that can be overcome while the more attenuated strains overcome less MA antibody (Lukert, 1983). To overcome MA, Haffer (1982a) recommended the use of an intermediately virulent IBDV vaccine at $10^{2.7}$ egg infective dose 50 per bird dose orally in chicks eight days old and above. Highly virulent IBD vaccines such as Bursavac have been shown to overcome MA but caused bursal atrophy and immunosuppression in chicks (Muskett et al., 1979). In fact, based on these findings and the known resistance of IBDV to environmental conditions, Reece et al. (1982) suggested that the use of these vaccines be discontinued. To achieve adequate immune response using non-pathogenic IBDV in maternally-immune chicks, suspension of vaccination until the MA has waned, has been suggested (Baxendale, 1976). Double vaccination

at 15 and 36 days old (Cursiefen et al., 1979) or at day-old and 30 days old (Okeke and Tanimu, 1982) have also been recommended.

The positive relationship observed between the rate of immune response and the age of the chicks vaccinated with Fibrogumbovac 10 could be explained by the fact that MA wanes with the age of a chick (Wyeth and Cullen, 1976). Inhibition of active immune response by MA is particularly associated with avirulent IBDV strains and strains that must be administered by non-parenteral routes (Fletcher et al., 1978; Winterfield et al., 1980). The enzootic nature of IBD and the presence of MA in chicks may prevent successful vaccination with such vaccines in Nigeria (Nawathe and Lamorde, 1982). Fibrogumbovac 10 is said to be non-pathogenic. It did not produce gross and histopathological lesions in the BF of five-week-old chicks and was also found to be non-immunodepressive (Okeke and Tanimu, 1982). In this study however, lesions were observed in chicks vaccinated at 15, 22 or 29 days old.

The chicks vaccinated earlier than 22 days of age might have remained susceptible to FV for five weeks. However, precipitin-negative chicks have been shown to possess protective neutralizing

antibodies (Ide et al., 1978) especially as challenge test was not conducted in the chicks at this period. All the chicks vaccinated however, did not show clinical signs when challenged five weeks after vaccination. This may be due to the presence of active IBD viral antibody at the time of challenge.

The control chicks could not be prevented from getting exposed naturally for more than 32 days after hatching inspite of all the precautions taken to avoid this from happening. This further demonstrates the highly contagious nature of IBD and the necessity for vaccination. The outbreak must have resulted from lack of MA at this age. In contrast, chicks vaccinated at day-old did not succumb to challenge with virulent IBD virus at about the same age. The changes in the weights of the bursae and the lesions observed in the bursae of vaccinated chicks indicate that the virus might have grown in this organ. However, the absence of precipitin antigen at three and seven days post-vaccination in the BF of chicks vaccinated at one, eight or 15 days of age would indicate that the virus did not grow in the bursa. Based on the findings in chicks vaccinated at 22 or 29 days old, it is likely that precipitins would have been

detected if their bursae were checked at a time longer than seven days post-vaccination. On the other hand, the virus might have been present at a concentration undetectable by the test used. Another possibility is that the vaccine virus might have grown and persisted in other lymphoid organs such as the spleen to later infect the bursa when the MA has waned. Precipitin antigen was detected seven days post-vaccination rather than three days post-vaccination in the bursae of chicks vaccinated at 22 days old probably because of delay in the production of precipitin antibody that would neutralize the precipitin antigen. Contamination of the caretaker's footware with feces from IBD-infected chicks might have been the source of the virus for control chicks.

Vaccination appeared to have reduced the body, bursal and thymic weight gain. The bursal: body weight ratio was not, however, significantly affected by vaccination. In White-leghorns, bursal growth slows at six weeks and involution begins at 10 weeks of age (Glick, 1956). The growth of the bursa of control chicks in this study was arrested earlier than six weeks. This could be as a result of natural exposure to the IBDV.

Vaccination at one or eight days appeared safe based on the absence of bursal lesions. The immune response was, however, delayed for 35 days in chicks vaccinated at one or eight days of age but delayed for 14 and seven days in chicks vaccinated at 22 or 29 days of age respectively.

Based on the results of MA decay (Chapter 2) and those of vaccination trials in this study, vaccination of maternally-immune chicks at seven and 21 days of age with Fibrogumbovac 10 is recommended.

Chapter 4

CHALLENGE STUDY ON INFECTIOUS BURSAL DISEASE IN MATERNALLY-- IMMUNE CHICKS

4.1 Introduction

Infectious bursal disease (IBD) in chickens occurs as a clinical or subclinical disease depending on the age of the chicks at infection (Naqi, 1978). The prevalence of clinical IBD appears to be low but serological evidence indicates that subclinical infection is wide-spread (Winterfield 1969; Faragher, 1972). In Nigeria, there is serological evidence of high prevalence of IBD in local chickens in which clinical disease is rarely observed (Nawathe et al., 1979; Umoh et al., 1982; Abdu et al., 1984). Both clinical and subclinical IBD exist in Nigeria (Onunkwo, 1975; Okoye and Shoyinka, 1983).

In the subclinical form of IBD, no clinical sign or mortality is observed but histopathological lesions are seen in the bursa of Fabricius (BF) of chicks (Hitchner, 1971) after infection and the chicks become immunosuppressed (Naqi, 1978). Passive transfer of maternal antibody (MA) from IBD - immune hens to their progeny has been demonstrated (Hitchner, 1971). Such chicks were found to be resistant to experimental infection

(Hitchner, 1971). However, only high MA level in chicks gives protection against subclinical infection (Lucio and Hitchner, 1979). In the field, there is a chance that maternally-immune chicks might get infected at two to four weeks of age when their MA is waning (Giambrone, 1977). This study was aimed at determining the period in which maternally-immune chicks obtained from a commercial hatchery in Zaria were protected from challenge with a field IBD virus (FV).

4.2 Materials and Methods

4.2.1 Chicks

Two hundred and twenty day-old starbro broiler chicks from the same source and parents, and hatched on the same day as those used in Chapter 2 were used. They were also housed, fed and treated similarly.

4.2.2 Challenge

Each chick was inoculated intra-ocularly with 0.05 ml of bursal homogenate prepared from the bursae of chicks that suffered from natural IBD infection as described in Chapter 2 (section 2.2).

4.2.3 Testing for Precipitin Antigen and Antibody

The presence of precipitin antigen (PAG) in the bursae of control and challenged chicks was detected by the method used previously described in Chapter 2 (Section 2.3). The precipitin antibody (PAB) levels in the sera of control and challenged chicks was determined by the quantitative agar gel precipitin test (QAGPT) as described in Chapter 2 (Section 2.3).

4.2.4 Experimental Design

Ten chicks were challenged at day-old and another set of 10 chicks were left unchallenged to serve as controls. The ten chicks from each group were bled at four, eight and 15 days old. Sera obtained were tested for presence and level of PAB. Another set of 10 chicks were challenged at one, eight, 15, 22 or 29 days old. All challenged chicks were closely observed for seven days for deaths and clinical signs of IBD. At three and seven days post-challenge (PC) at the ages mentioned, a set of five chicks from each set were euthenized, the carcases weighed, the bursae, spleens and thymi examined grossly, removed and weighed. Sections of the bursae, spleens and thymi were fixed in 10% buffered formalin for histopathology as described in sub-section 3.2.3 of Chapter 3. Bursal

histopathological lesions were evaluated by a score criteria system (Table 4.1). The bursae for each group were pooled, weighed and a homogenate made from them for detection of precipitin antigen (PAG) as described in sub-section 2.2.3 of Chapter 2. Similar procedures were conducted on a set of five unchallenged chicks at four, eight, 11, 15, 18, 22, 25, 29, 32 and 36 days old (Table 4.2). Another set of 10 chicks were challenged at one, eight, 15, 22 or 29 days old and bled at three and seven days PC while a different sets of 10 chicks that were not challenged were bled at four and eight, 11 and 15, 18 and 22, 25 and 29 and 32 and 36 days old (Table 4.3). The PAB levels in challenged and unchallenged (controls) chicks were determined by the QAGPT.

4.2.5 Statistical Analysis

Data on mean body, bursal, thymic and splenic weights and bursal: body weight ratios were analysed by the Student's t-test (Colton, 1974) to detect significant differences between control and challenged chicks.

Table 4.1: Bursal histopathologic score criteria

Score	Description
0	Normal
1	No acute inflammation with some regeneration of lymphoid cells
2	Some oedema and depletion of lymphoid cells without frank necrosis; fibroplasia
3	Pyknosis and necrosis of lymphocytes, oedema, fibroplasia

Table 4.2: Experimental design for clinical and pathological studies in maternally-immune chicks challenged with a virulent infectious bursal disease virus

Age at challenge (days)	Age at euthanasia (days)	Group	
		Challenged	Not challenged (Control)
1	4	5*	5
	8	5	5
8	11	5	5
	15	5	5
15	18	5	5
	22	5	5
22	25	5	5
	29	5	5
29	32	5	5
	36	5	5

* Number of chicks euthanized

Table 4.3: Experimental design for serological studies in maternally-immune chicks challenged with a virulent infectious bursal disease virus

Age at challenge (days)	Number of chicks bled	Group	Age at bleeding (days)		
1	10	Challenged	4	and	8
	10	Not challenged	4	and	8
8	10	Challenged	11	and	15
	10	Not challenged	11	and	15
15	10	Challenged	18	and	22
	10	Not challenged	18	and	22
22	10	Challenged	25	and	29
	10	Not challenged	25	and	29
29	10	Challenged	32	and	36
	10	Not challenged	32	and	36

Table 4.4: Comparison of bursal histopathologic scores among maternally-immune chicks challenged with a virulent infectious bursal disease virus

Days post-challenge	Score*	Age at challenge (days)** and number of birds				
		1	7	15***	22	29
3	0	5	3	1	0	0
	1	0	0	0	0	0
	2	0	2	2	4	0
	3	0	0	2	1	5
7	0	5	3	0	0	0
	1	0	0	3	3	4
	2	0	2	1	2	1
	3	0	0	0	0	0

*Scores based on criteria in Table 4:1

**Ten birds were challenged at each age

***Only nine bursae were examined

4.3 Results

4.3.1 Clinical Findings

Clinical signs were not observed in chicks challenged at one, eight, 15 or 22 days old. They were however seen three days PC (ie at 32 days old) in chicks challenged at 29 days old. The signs observed included: diarrhoea (watery or in some cases white, yellow or greenish faeces were voided), soiled vent, decreased activity, anorexia, severe depression, dropped wings, ruffled feathers, prostration, (Plate III). Three of the 10 chicks challenged exhibited these signs and one of them died on the third day PC. At 32 days old, one of the unchallenged chicks became sick and died at 36 days old.

4.3.2 Gross Postmortem Findings

Haemorrhages in the gastrointestinal tract (proventriculus, duodenum, ileum and caecal tonsils), in some bursae and in the breast, leg and thigh muscles were noted in chicks challenged at 15, 22 or 29 days old. Three days PC in chicks challenged at 15, 22 or 29 days old, the bursae appeared oedematous, cream-coloured and were turgid. At seven days PC in the same set of chicks however,

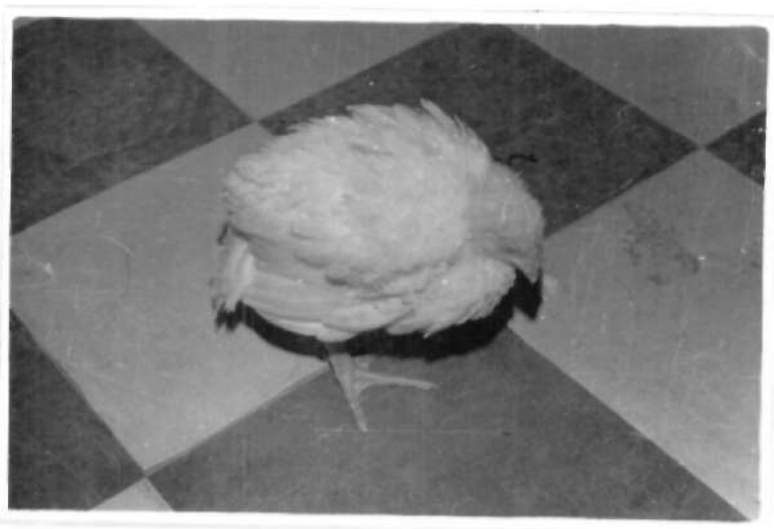


Plate III. Clinical signs of infectious bursal disease in a chick three days post-challenge at 29 days of age. The chick is depressed and has dropped wings and ruffled feathers.

they appeared grayish and were atrophied (Plate I). The spleens, thymic lobes, pancreas and bursae were congested at 15 days of aged or older. The spleens of dead chicks were enlarged and had diffused pin-point gray spots on their surfaces. The kidneys of the chicks that died were pale, enlarged, the tubules prominent and filled with urates (Plate IV). The livers were pale. Similar changes were observed in the organs of control chicks that were naturally exposed.

4.3.3 Histopathological Findings

Bursal lesions were not observed in the bursae of chicks challenged at day-old but were observed in chicks challenged at eight days or older and were observed in those of control chicks accidentally exposed at 32 and 36 days old only. The lesions were observed at both three and seven days PC in chicks challenged at eight days of age or older. Only 40% of the bursae examined at three and seven days PC in chicks challenged at eight days old had lesions while 80% and 100% of the bursae examined at three and seven days PC respectively in chicks challenged at 15 days old had lesions. All the bursae examined at three and seven days PC in chicks challenged at 22 or 29 days old had lesions.

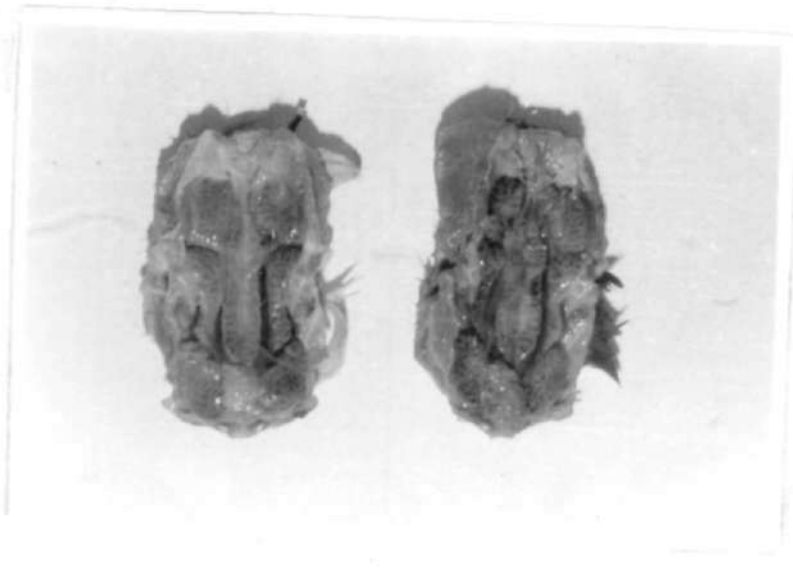


Plate IV: The gross pathological lesions in the kidneys of chicks that died of infectious bursal disease. The kidneys are pale and the tubules prominent.

The bursal lesions observed included: congestion of blood vessels haemorrhage, interfollicular oedema or fibroplasia, necrosis of lymphocytes, hypertrophy of follicular cortico-medullary epithelium, atrophy of lymphoid follicles, reticuloendothelial hyperplasia, cystic formations and epithelial erosion. The severity of lesions increased with age at challenge. There was regeneration seven days PC at 15, 22 or 29 days old (Table 4.4).

There was an increase in the number of germinal centres in the spleens of chicks seven days PC in chicks challenged at eight, 15, 22 or 29 days old.

4.3.4 Precipitin Antigen

Precipitin antigen was not detected three and seven days PC in chicks challenged at day-old but was detected three and seven days PC in chicks challenged at eight days old. It is consistently detected at three days PC in chicks challenged at 15, 22 or 29 days old (Table 4.5). At 32 days old, precipitin antigen was detected in the bursae of control chicks.

Table 4.5: Infectious bursal disease virus precipitin antigen in the bursae of maternally-immune chicks challenged with a field infectious bursal disease virus*

Age of chicks at challenge** (days)	Days post-challenge	
	3	7
1	-	-
8	+	+
15	+	-
22	+	-
29	+	-

* Antigen detected by the QAGPT

** The bursae of five challenged chicks were pooled and tested

\- Antigen not detected

\+ Antigen detected

4.3.5 Precipitin Antibody

The mean MA level in chicks challenged at day-old was lower than that of control chicks when it was determined at four, eight and 15 days old (Table 4.6). The mean MA level in control chicks was 337.5 U.K units/ml at day-old with 100% of them being seropositive. Precipitin antibody was detected at three days PC in chicks challenged at eight days old but was absent at seven days PC in the same set of chicks. Precipitin antibody was detected at seven days PC in chicks challenged at 15, 22 or 29 days (Table 4.7). Only 20% of the chicks challenged at 15 days of age and tested seven days PC had PAB while all the chicks challenged at 22 or 29 days of age and tested at seven days PC had PAB. Precipitin antibody was not detected at 22, 25, 29, 32 and 36 days of age in control chicks.

4.3.6 Gravimetric Findings

The mean body (carcass), bursal, thymic and splenic weights and bursal: body weight ratios and the significant level of the differences between the means of challenged and unchallenged chicks are shown in Tables 4.8 and 4.9. The mean bursal weight seven days PC was lower than the value three days PC at 15, 22 or 29 days old (Figure 4.1).

Table 4.6: Effect of challenge on the infectious bursal disease virus maternal antibody level in chicks

Age** (days)	Challenged*		Control (Not challenged)	
	Titre (U.K. units/ml)***	% Positive	Titre (U.K. units/ml)	% Positive
4	87.50 ± 46.77	60.00	268.75 ± 40.69	100.00
8	12.50 ± 12.50	20.00	56.25 ± 28.66	40.00
15	0.0	0.0	18.75 ± 13.35	20.00

* Challenged at day-old

** Ten chicks were tested at each age for each group

*** Titres given as means and standard errors

Table 4.7: Mean infectious bursal disease virus antibody titre in maternally-immune chicks challenged with a field infectious bursal disease virus

Age of chicks at challenge (days)*	Days post-challenge	Mean titre ± SE**	% Positive
1	3	87.5 ± 46.8	60.0
	7	12.5 ± 12.5	20.0
8	3	31.3 ± 16.7	40.0
	7	0	0
15	3	0	0
	7	12.5 ± 12.5	20.0
22	3	0	0
	7	162.5 ± 46.7	100.0
29	3	0	0
	7	237.5 ± 23.3	100.0

* Ten chicks were tested at each age

** Antibody titre in U.K. units/ml

SE Standard error

Table 4.8: Mean body, bursal, thymic and splenic weights and bursal: body weight ratios of maternally-immune chicks challenged with a virulent infectious bursal disease virus at three days post-challenge*

Group	Age (days)**	Body weight (g)	Bursal weight (g)	Bursal:body weight ratio (x 100)	Thymic weight (g)	Splenic weight (g)
Challenged	1	-	0.085 ± 0.007[a]	-	0.244 ± 0.037[a]	0.056 ± 0.085
	8	79.4 ± 3.8	0.155 ± 0.017	0.193 ± 0.043	0.181 ± 0.046	0.045 ± 0.085
	15	145.9 ± 7.7	0.463 ± 0.094	0.309 ± 0.045	0.735 ± 0.102	0.185 ± 0.007
	22	232.9 ± 10.10[a]	0.633 ± 0.058[b]	0.269 ± 0.016	1.210 ± 0.161	0.426 ± 0.043
	29	450.4 ± 19.1	0.891 ± 0.073[c]	0.197 ± 0.010[a]	2.145 ± 0.212[b]	0.723 ± 0.064
Control (Not challenged)	1	34.1 ± 2.6	0.035 ± 0.005[a]	0.104 ± 0.018	0.081 ± 0.012[a]	0.088 ± 0.011
	8	80.9 ± 15.4	0.144 ± 0.016	0.179 ± 0.015	0.242 ± 0.038	0.059 ± 0.011
	15	133.8 ± 10.8	0.259 ± 0.024	0.196 ± 0.020	0.527 ± 0.060	0.112 ± 0.014
	22	451.7 ± 48.8[a]	1.246 ± 0.055[b]	1.456 ± 0.130	1.714 ± 0.124	0.526 ± 0.031
	29	498.8 ± 13.2	1.515 ± 0.228[c]	0.304 ± 0.045[a]	3.257 ± 0.259[b]	0.935 ± 0.036
		a; P<0.005	a; P<0.005 b; P<0.001 c; P<0.005	a; P<0.05	a; P<0.01 a; P<0.005	

* Values given as means and standard errors for five chicks

**Age at challenge

\- Not weighed

Table 4.9: Mean body, bursal, thymic and splenic weight and bursal:body weight ratios of maternally-immune chicks challenged with a virulent infectious bursal disease virus at seven days post challenge*

Group	Age (days)**	Body weight (g)	Bursal weight (g)	Bursal: body weight ratio (x 100)	Thymic weight (g)	Splenic weight (g)
Challenged	1	63.0 ± 1.9	0.108 ± 0.014	0.169 ± 0.019	0.270 ± 0.002	0.060 ± 0.006
	8	131.0 ± 11.1[a]	0.251 ± 0.067	0.180 ± 0.025	0.627 ± 0.084[a]	0.128 ± 0.033[a]
	15	243.6 ± 10.5[b]	0.253 ± 0.023[a]	0.105 ± 0.009[a]	0.988 ± 0.203	0.282 ± 0.034[b]
	22	337.1 ± 20.3[c]	0.343 ± 0.056[b]	0.103 ± 0.018[b]	1.795 ± 0.121[b]	0.507 ± 0.054
	29	522.6 ± 26.1	0.647 ± 0.079	0.124 ± 0.147	3.079 ± 0.511	0.824 ± 0.128
Control (Not challenged)	1	59.8 ± 2.1	0.113 ± 0.012	0.188 ± 0.018	0.204 ± 0.016	0.053 ± 0.006
	8	96.5 ± 4.5[a]	0.194 ± 0.021	0.194 ± 0.016	0.377 ± 0.037[a]	0.074 ± 0.006[a]
	15	314.4 ± 7.7[b]	0.906 ± 0.051[a]	0.288 ± 0.014[a]	1.525 ± 0.146	0.319 ± 0.023[b]
	22	496.8 ± 9.9[c]	1.518 ± 0.128[b]	0.321 ± 0.026[b]	2.752 ± 0.167[b]	0.443 ± 0.041
	29	552.4 ± 39.0	0.848 ± 0.646	0.157 ± 0.013	1.954 ± 0.435	1.078 ± 0.129
		a; P<0.005	a; P<0.001	a; P<0.001	a; P<0.01	a; P<0.01
		b; P<0.001	b; P<0.001	b; P<0.001	b; P<0.01	b; P<0.05
		c; P<0.025				

* Values given as mean and standard errors for five chicks

** Age at challenge

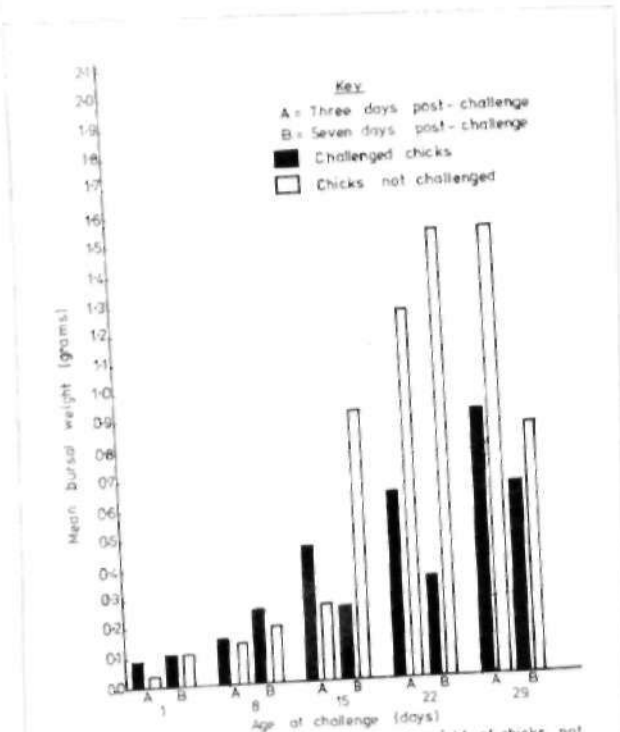


Fig 4-1: Changes in the mean bursal weight of chicks not challenged and chicks challenged with a virulent infectious bursal disease virus with age

4.4 Discussion

Similar to earlier observation, the BF was the organ most severely damaged by the FV used in this study (Ley et al., 1983). This is because the IBDV is most pathogenic for a B-cell subpopulation most abundant in the BF (Hirai and Calnek, 1979). These cells were postulated to be IgM - bearing (Hirai and Calnek, 1979). The lesions observed in the BF and spleens have also been reported earlier (Cheville, 1967; Winterfield et al., 1972; Ley et al., 1983). The changes in the bursal weight of challenged chicks correlated well with histopathologic changes. The increase in the mean bursal weight at three days and the decrease at seven days PC in chicks challenged at eight, 15, 22 or 29 days or age could be attributed to oedema and lymphoid depletion respectively (Cheville, 1967). Rapid lymphoid regeneration (Ley et al., 1983) or the presence of high MA level in the chicks prior to challenge might have been responsible for the absence of damage in some bursae examined seven days PC in chicks challenged at 15 days of age.

The presence of PAG correlated well with bursal lesions. For example, PAG and bursal lesions were absent at three and at seven days PC in chicks

challenged at day-old while present at three and at seven days PC in chicks challenged at eight days old. The MA present at day-old would have been able to completely neutralize the FV, the presence of which is necessary for production of bursal lesions. In this study, PAG was consistently present in the bursae of chicks three days PC in chicks challenged at 15, 22 or 29 days old. This falls within the period reported by Ulbrich and Zureck (1977) in which they demonstrated the presence of PAG in the BF of four-week-old chicks inoculated orally with 0.5 ml of IBDV infected bursal homogenate between 32 hours and five days. They regularly detected PAB in the BF from the eight day after infection. In another study, a cell-culture-adapted IBDV was detected in the bursae of three-week-old specific pathogen free chicks as early as one-day to as late as ten days after oral inoculation. The concentration of the virus was highest four days after inoculation (Takase et al., 1982).

The lower MA level in challenged than in control chicks and its earlier disappearance in challenged chicks could be due to MA neutralization by the FV used. Ley et al. (1983) were able to detect active PAB as early as three days PC in 35 days old IBD

susceptible chicks. This differs from the seven days obtained in this study. The presence of MA in the chicks used and the younger age at which they were challenged might have been responsible for this observed difference. It was noted from this study that the number of sero-positive chicks and the strength of immune response increased with age at challenge. The natural decay of MA (Wyeth and Cullen, 1978) and increase in immunocompetence with age (Vindevogel et al., 1975) might have been responsible. The MA level in chicks challenged at day-old was able to protect against the subclinical and clinical forms of IBD.

In Zaria, chicks were found to be most susceptible to clinical IBD as from the third to the eight week of age (Bishu et al., 1977). The age at which the chicks used in this study became susceptible to clinical IBD fell within this age range. However, the age range for susceptibility to subclinical IBD and the prevalence of subclinical IBD in Zaria are unknown. The wide variation in the levels of MA acquired and differences in their decay pattern in the chicks studied by Bishu et al. (1977) might have been responsible for the wide range they observed. Information on the subclinical form of IBD is needed

if the immunosuppressive effect of IBD is to be prevented or at least be controlled. In this study, the evidence of subclinical IBD (based on bursal atrophy, bursal lesions and presence of IBDV precipitin antigen) was first noticed at eight days old although not all the chicks at this age were susceptible. The variation in susceptibility indicate that IBD immunity in the flock was variable (immune and non-immune or high MA level and low MA level chicks were present). The evidence of infection at eight days old indicate that the initial MA level present at day-old in the chicks was not high enough to protect them for the first three weeks of life, the period in which exposure to IBDV would result in immunosuppression (Lukert, 1983). Since the parents of the chicks used were vaccinated with live IBD vaccine only, this finding further emphasizes the need for vaccinating breeding hens with inactivated oil emulsion IBD vaccine after priming them with a live IBD vaccine. This procedure has been found to result in higher, long-lasting and uniform MA level in the progeny chicks (Giambrone, 1983). The chance of chicks getting naturally exposed at eight days old is high because of the highly contagious nature of IBD (Benton et al., 1967a) and

the enzootic nature of IBD in Nigeria (Nawathe and Lamorde, 1982). It is therefore likely that the subclinical form of IBD is widespread as reported elsewhere (Winterfield, 1969; Faragher, 1971; Armstrong et al., 1981). This form of the disease has been reported to exist in Nigeria (Okoye and Shoyinka, 1983). The emphasis in controling IBD should be geared towards preventing this form of the disease. Although no mortality and clinical signs are observed in this form of the disease (Hitchner, 1971), the immunosuppression caused by IBDV in young chicks is usually permanent but transient in older chicks (Faragher et al., 1974; Meulemans et al., 1977; Edward et al., 1982). Moreover, it is only the primary immune response in older chicks that is depressed while the secondary immune response is unaffected (Giambrone, 1977). One of the methods used to control this form of IBD is by breeder vaccination (Giambrone, 1983). Day-old vaccination with mildly invasive IBD vaccines is also used if the MA level in the chicks is low but if the MA level is high, the highly invasive vaccines have been used (Giambrone, 1983). One of the things that would complicate the choice of an IBD vaccination programme is the wide age range between the

onset of subclinical and clinical disease which was found to be four weeks in this study.

The absence of clinical signs in the serologically negative chicks challenged at 22 days old could be explained by the possible presence of protective neutralizing antibodies not detected by the QAGPT.

The IBDV strains affecting chicks in Zaria may vary in their virulence. The FV used in this study is considered highly pathogenic because it caused severe damage to the bursae of challenged chicks and a cummulative mortality rate of up to 18% was recorded in the natural outbreak. However, the single passage in chicks and the handling it underwent could have had some effect on its pathogenicity.

From this study, maternally-immune chicks were protected from subclinical IBD for only one week and clinical IBD for four weeks. It is worthy to note that this study was conducted in a controlled environment and the time of infection was known while under field condition other factors may play a part in producing IBD and the time of infection is usually unknown.

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