STUDIES OF THE PROBLEMS ASSOCIATED WITH VACCINATION AGAINST INFECTIOUS BURSAL DISEASE IN NIGERIA

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DECLARATION

The work presented in this thesis is original and conducted under the supervision of Professors J.U. Umoh and S.U. Abdullahi 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 of the problems associated with vaccination against infectious bursal disease in Nigeria" by Paul Ayuba Abdu meets the regulations governing the award of the degree Doctor of Philosophy of Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

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The thesis is dedicated to the memory of late Mallam Stephen Audu, Mallam Nuhu Bayero, Mallam Joseph Adamu Maiwada and Mallam Musa Zakari.

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ABSTRACT

Chicks vaccinated with a local infectious bursal disease (IBD) vaccine (batch 01) at day-old and 4 weeks, 2 and 4, 2 and 5, and 1, 2 or 3 weeks of age and then challenged with a virulent infectious bursal disease virus (IBDV) 2 weeks later became sick and or died although the disease was more severe in unvaccinated chicks. None of the chicks vaccinated at 3 weeks of age became sick or died. About 33% to 75% of the challenged birds tested at 1 week post-challenge (PC) had detectable precipitin antibodies (PAb) in their sera and 100% had PAb at 2 and 3 weeks PC. The mean Ab titre ranged from 1.3 to 5.8 Log2 with 57% of the chicks having a titre of 4 or 5 Log2. Except chicks vaccinated at 3 weeks of age, the IBDV-infected birds responded poorly to inoculation with sheep blood cells.

Four and 5-week-old chicks with no PAb to IBDV vaccinated orally (PO), intranasally (IN), intraocularly (IO), subcutaneously (SC), intramuscularly (IN), or by vent drop (VD) became sick and died 3 days post-vaccination (PV) with a batch (03) of a local IBD vaccine. Fewer birds became sick or died when the vaccine was administered PO or IN. The signs and lesions were reminiscent of those caused by very virulent IBDV strains. The vaccine was also found to spread to in-contact unvaccinated chicks.

The vaccine (batch 03) also caused disease 3 days PV in passively-immunized 3-week-old chicks when administered by SC

or VD routes. Only chicks vaccinated IN or IM did not die when challenged with a virulent IBDV.

Three-week-old chicks vaccinated PO with 4 different batches of a local IBD vaccine purchased from 4 veterinary shops in Zaria became sick following challenge with a field IBDV when it was inoculated at 2 weeks PV. Only chicks vaccinated with 2 of the 4 batches did not die.

None of the chicks vaccinated with two imported IBD vaccines (B and C) at day-old, 1 or 2 weeks of age sero-converted 2 weeks PV. Only those vaccinated at 2 and 4 weeks and 3 weeks old with vaccine C sero-converted. Both vaccines produced PAb 2 weeks PV in chicks vaccinated at 2 and 4 weeks. None of the chicks vaccinated with vaccine B at 2 and 4 week of age became sick when challenged. When 3 imported IBD vaccines (A,B, and C) were administered once to 3-week-old chicks, they could not be protected from sickness and death when challenged with a virullent field IBDV at 2 weeks PV. The disease was more severe in chicks vaccinated with vaccine C than in unvaccinated chicks.

When PAb-positive antisera from IBD-vaccinated, naturally infected, or local chickens were incubated with a virulent field IBDV, virus neutralization did not occur with some sera. While some sera showed an appreciable level of neutralization, no sera was able to completely neutralize the field IBDV because the virus multiplied in the bursa of Fabricius (BF) of susceptible chicks inoculated with the serum-virus mixture.

Chicks should be vaccinated against IBD with the local vaccine (01) at 2 and 4 weeks or 3 weeks of age and be prevented from exposure to a virulent IBDV by maintaining high standard of hygiene and biosecurity to avoid vaccination failure and immunodepression.

The local IBD vaccine (batch 03) should be used only via IN, PO or IM route and unvaccinated chicks should be prevented from contact with vaccinated chicks. This vaccine needs to be attenuated further.

IBD-susceptible chicks should be vaccinated with imported vaccines (B and C) at 2 and 4 weeks of age and prevented from exposure to a virulent IBDV for up to 4 weeks PV in the case of vaccine C. The cause of the increase in severity of IBD in chicks vaccinated with vaccine A need to be investigated.

The field IBDV is highly immunogenic but virulent and highly invasive for the BF. Vaccination and passive immunization may not protect a certain proportion of chicks against IBD in case of exposure to a field virus. There is a need to type IBDV present in Nigeria.

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Chapter 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 desease(IBD) are dependent on the active antibody in hens, presence and level of maternal antibody (MA) in their chicks and the rate of MA decay in chicks, the virulence of the field virus strains and the length of protection desired (Ehizokhale, 1982).

Winterfield et al. (1972) had observed IBD outbreaks in flocks vaccinated before 10 days of age due to the presence of MA. 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., 1972a; Skeeles et al., 1979a; Wood et al., 1981).

The ability of MA necessary to neutralize IBD vaccines depends on the invasiveness and pathogenicity of the virus strain used in the vaccine (Lucio and Hitchner, 1979a). A MA level of 34 U.K. units per ml of serum has been reported to have interfered with vaccination (Wood et al., 1981).

In Nigeria, vaccination of chicks with live IBD vaccines is widely practiced as a means of controlling IBD. The sources of these vaccines are varied and recommendations for their use also differ (Abdu, 1985).

The incidence of IBD outbreaks in vaccinated flocks

appears to be common (Abdu et al., 1986a; Tong et al., 1993) and widespread (Okoye, 1984b; Abdu, 1986a; Nwosuh et al., 1989; Awolaja and Adene, 1995). These IBD outbreaks in vaccinated flocks were either a result of vaccine breaks (Okoye, 1985d; Abdu et al., 1986a; Tong et al., 1993) or vaccination failures (Okoye, 1984b; Abdu, 1986b; Nwosuh et al., 1989; Awolaja and Adene, 1995). Vaccination failures appeared to be more common than vaccine breaks (Abdu et al., 1986a).

Vaccination failures were reported to occur primarily due to improper storage, transportation and administration of vaccine and secondarily due to interference of active immunisation by MA (Nawathe and Lamorde, 1982). Avirulent (highly attenuated) vaccines do not elicit a satisfactory immune response in the presence of MA (Haffer, 1982; Mousa et al., 1988). Mildly attenuated vaccines are highly invasive for the bursa of Fabricius (BF) and could overcome the negative effect of MA on the bird's immune response (Fletcher et al., 1978; Winterfield and Thacker, 1978; Sharma, 1985). Some of these vaccines are capable of producing clinical disease, immunosuppression and spreading to susceptible chickens (Muskett et al., 1979).

Other factors that make the control of IBD in Nigeria difficult are the presence of highly pathogenic field strains of the infectious bursal disease virus (IBDV) all over the country (Onunkwo and Momoh, 1981; Okoye and Uzoukwu, 1982 a

and b), poor biosecurity measures and inconsistency in the manner IBD vaccines are being administered by some poultry farmers (Abdu, 1995; Anonymous, 1995). Antigenic variation, absence of 'hot intermediate' vaccines and the highly variable nature in which MA decays in chicks are other causes of IBD outbreaks in vaccinated flocks (Okoye, 1996).

Clinical IBD has been reported in previously vaccinated flocks (Okoye, 1984b; Ezeokoli et al., 1985; Abdu et al., 1986a). Between 21.1% to 61.5% of IBD outbreaks in Zaria area had occurred in vaccinated flocks (Abdu et al., 1986a; Abdu, 1986a). An appreciable percentage (40%) of the IBD cases in vaccinated birds had received IBD vaccine within the first 2 weeks of life (Anonymous, 1977; Abdu et al., 1986a). Most of the outbreaks occurred in chicks more than 3 weeks of age (93%) and in chicks 2 weeks or more after vaccination (71%) (Abdu et al ., 1986a).

Mortality in IBD outbreaks in vaccinated flocks ranged from 4.4% to 46% (Ezeokoli et al., 1985) and 5.7% to 37.5% (Abdu, 1986a). Mortality rate appears not to be related to flock size, type of vaccine (local or imported) or interval between vaccination and outbreak. However, chickens with one vaccination history against IBD were 4.9 times more likely to have IBD compared to non-vaccinated chickens and those with two vaccinations were 2.4 times more likely to have the disease (Tong et al., 1993).

Neutralization of vaccine virus by MA present at the time

of vaccination (Okoye, 1984b; Abdu, 1986b; Abdu, 1991) the inability of vaccinal antibodies to neutralize highly pathogenic IBDVs (Okoye, 1984b) and the use of mildly attenuated vaccines in areas where the very virulent IBDV exist (Abdu, 1986a) have been suggested as the possible causes of IBD outbreaks in vaccinated flocks.

MA is the most probable cause of the vaccination failures (Abdu, 1986b). Vaccination has been shown to increase the rate of decay of MA (Abdu et al ., 1987b). If the vaccine administered fails to elicit immune response it renders vaccinated chickens vulnerable to IBD earlier than expected. Therefore, the more the frequency of vaccination the less the likelihood of an outbreak of IBD (Tong et al., 1993).

Some vaccines increase the severity of IBD in vaccinated chicks (Ezeokoli et al., 1985). Vaccines in the Nigerian market differ in their immunogenicity (Okeke et al., 1982; Adene et al., 1985b; Okoye, 1985c; Aba-Adulugba et al., 1992).

Following vaccination, precipitin antibody (PAb) negative chicks might still be resistant to IBD (Ezeokoli et al., 1985; Okoye, 1985c) and therefore the agar gel precipitin test (AGPT) is not a good indicator of protection in recently-vaccinated birds. While some IBD vaccines are lymphocidal (Okoye, 1985c) others could not prevent bursal lesions in some birds following infection with a virulent virus (Aba-Adulugba et al., 1992). Precipitin antibodies appear 1 week post-vaccination (PV) but they are found in 100% of the birds 2 or

3 weeks PV (Okeke et al., 1982; Aba - Adulugba et al., 1992). There is an increase in the level and rate of antibody production with increase in age at vaccination and vaccination results in a drop in MA level which delays the rate of active antibody production and the level of antibody produced (Abdu et al., 1987b).

It can be clearly seen from the forgoing that the nature, safety and potency of vaccines in use differ. Manufacturer's recommendations as to the vaccination schedule and route of administration also differ. These are certainly some of the factors that contribute in our failure to control IBD in Nigeria. Other factors are poor administration (Nawathe and Lamorde, 1982).

The main objectives of the study were to determine: (i) the antibody response and pathogenicity of IBD vaccines in chicks with or without passive antibodies to IBDV vaccinated with some batches of locally-produced vaccine (0,01, 03, 05 and 06) and imported vaccines (A,B and C); (ii) the ability of the vaccines to prevent morbidity, mortality and immunodepression in vaccinated chicks following challenge with virulent viruses obtained from field outbreaks and (iii) the ability of sera obtained from IBD vaccinated, naturally infected or local chickens to neutralize in vitro virulent IBDVs from field outbreaks.

Chapter 2

LITERATURE REVIEW

2.0 Definition

Infectious bursal disease is an acute or in many cases subclinical and highly contagious viral disease of young chickens which initially causes gross enlargement of the BF and later atrophy of the organ (Cosgrove, 1962; Cheville, 1967; Hopkins et al, 1979; Armstrong et al., 1981; Gonzales, 1982; Savova, 1984). The synonyms of the disease include avian-nephrosis (Cosgrove, 1962), infectious bursal disease (Hitchner, 1970), Gumboro disease (Faragher, 1972), infectious bursitis (Ibragimov, 1976) and avian infectious bursitis (Rodon, 1982).

2.1 History

Cosgrove (1962) was the first to report an acute infectious disease in broilers in Gumboro district, Delaware, United States of America (USA). Ojo et al. (1973) were the first to report the disease in the South Western States of Nigeria. It is now nation-wide (Agbede, 1975; Onunkwo, 1975; Okoye and Uzoukwu, 1981; Onunkwo and Momoh, 1981; Nawathe and Lamorde, 1982).

2.2 Distribution and Incidence

The IBD has a world-wide distribution (Luthgen, 1969; Faragher, 1972; Okoye, 1984c; Abdu et al., 1986b). It is more prevalent in commercial farms (Okeke, 1984) and occurs

throughout the year (Cosgrove, 1962). The prevalence of clinical IBD is low (Faragher, 1972) while subclinical disease is wide-spread (Winterfield, 1969).

2.3 Aetiology

2.3.1 Classification and Morphology

Infectious bursal disease virus is classified as a birnavirus (Dobos et al., 1979; Sharma and Adlakha, 1984). It is non-enveloped with a single layered capsid consisting of 32 capsomeres arranged in a 5:3:2 symmetry. It is icosohedral in shape and has a diameter of 55 to 60 nm with a genome consisting of two segments of double-stranded ribonucleic acid (RNA). A cylindrical structure of 570 nm long has also been observed (Hirai and Shimakura, 1974; Nick et al., 1976; Dobos et al., 1979; Bakulin et al.,1991). The RNA has a molecular weight (MW) of about 2×10^6 (Nick et al., 1976). The buoyant density of IBDV as determined in continuous sucrose gradient was found to be 1.78 g/cm^3 and in caesium chloride between 1.29 and 1.34 g/ml by rate zonal and density-equilibrium method (Hirai and Shimakura, 1974;Nick et al., 1976; Cho and McDonald, 1980; Fahey et al., 1985; Lee and Lukert, 1986a).

2.3.2 Resistance to Physical and Chemical Agents

The IBDV was found to be resistant to heat and ultraviolet irradiation (Benton et al., 1967b; Petek et al., 1973; Cernik, 1983). The virus could withstand heating at 37°C for 90 min and 56°C for 5 h (Petek et al., 1973; Lukert and Davis, 1974; Okoye, 1984c). It was found to be infective for chickens after exposure at 60°C for 90 min at 25°C for 21 days and after storage for 3 years at -20°C (Yung Cho, 1967). The virus is also resistant to either, chloroform, hyamine, merthiolate, phenol, staphene and trypsin (Benton et al., 1967a; Benton et al., 1967b). The virus has been reported to be acid and alkaline stable surviving at pH range of 2 to 13 (Benton et al., 1967a). Two percent solutions of chloramine, formaldehyde, glutaraldehyde or halamid were found to be effective against the virus (Meulemans and Halen, 1982). Dimethyleneimine and sodium dichloroisocyanurate were also effective against IBDV (Aliev et al., 1986; Hirata and Goto, 1986).

2.3.3 Antigenic Characteristics

Two antigenically distinct IBDV serotypes (1, 2) using neutralization test have been identified in the USA and the UK (McFerran et al., 1980; ; Lukert, 1983; Giambrone, 1985). Serotype 1 and 2 IBDV from the USA and UK are antigenically similar. Within the main serotypes further antigenic differences can be demonstrated with monoclonal antibodies and

cross-neutralization test (Jackwood and Saif, 1987; Snyder et al., 1988b; Jordan, 1990). For example six substypes were distringuished among some serotype 1 strains using cross-neutralization test (Jackwood and Saif, 1987).

Using an antigen capture enzyme-immunosurbent assay (ELISA) and monoclonal antibodies, two variants of the classic serotype 1 IBDV were discovered. One group of the variant viruses (Delaware strains-DEL) lacked a critical neutralization site on the IBDV while the other group of viruses (Grayson Laboratory strains-GLS) lacked a common critical neutralization site on the IBDV (Snyder et al., 1988a; Synder, 1989). Antisera against the classic vaccine strains were not able to neutralize the GLS strains to the same extent as the homologous virus (Snyder, 1989). In another study a variant strain of IBDV was not neutralized by MA and hyperimmune sera produced by vaccination with two commercial vaccines (Hellwig. 1986). Data of a survey for variant IBDV in the USA suggests that classic viruses are replaced first by DEL type viruses, and subsequently DEL by GLS type viruses (Snyder, 1989).

Serotype 1 and 2 IBDVs have been isolated from chickens (McFerran et al., 1980) turkeys (McNulty et al., 1979; Chettle et al., 1985) and ducks (McFerran et al ., 1980; Jordan, 1990). The prevalence of antibodies to both serotypes is high in chickens but low to medium in turkeys (Barnes et al., 1982; Eddy et al., 1985). The geometric mean antibody titres to

serotype 1 IBDV were low while the titres for serotype 2 IBDV were high in turkeys (Chin et al., 1984). Most chicken IBDV isolates tend to be classified as serotype 1 while turkey isolates are usually serotype 2. Although, serotype 1 isolates can infect turkeys, serotype 2 infections in turkeys is widespread (Cummings et al., 1986).

Pathogenic and non-pathogenic strains of IBDV serotype 1 have been identified in chickens (Winterfield and Thacker, 1978). They differ in virulence from mild to highly pathogenic (Jordan, 1990). Although, most serotype 2 viruses are infectious and not pathogenic for chickens, a strain was reported to have caused severe lesions in lymhoid organs (Sivananda et al., 1986; Ismail et al., 1988). Chickens with antibodies to serotype 2 viruses could not be protected against challenge with serotype 1 IBDV (Jackwood et al., 1985). Maternally derived antibodies to serotype 1 viruses were unable to protect against serotype 2 viruses and vice visa (Chettle and Wyeth, 1989).

2.3.4 Viral Propagation

IBDV has been propagated in embryonated chicken eggs but because of the presence of interfering MAs in the eggs of immune hens, eggs from susceptible or specific- pathogen-free (SPF) hens are required for successful propagation (Snedeker et al., 1967; Hitchner, 1970). The chorioallantoic membrane is the most sensitive for detecting small amount of virus

(Hitchner, 1970). The virus can be adapted to grow in cell culture by serial passages. It has been grown in chick embryos (Rinaldi et al., 1969; Aliev et al., 1986) lymphocytes of the BF, spleen and thymus of chicken embryos (Schunemann, 1986), epithelial (Nick et al., 1976) and kidney cells (Lukert and Davis, 1974), fibroblasts (Rinaldi et al., 1972b; Lee and Lukert, 1986a) and macrophages of chick embryos (Mora, 1966).

2.4 Susceptibility

Natural infections with IBDV have been reported in chickens and ducks (McFerran et al., 1980; Jordan, 1990) and turkeys (McNulty et al., 1979; Perelman and Heller, 1981; Barnes et al., 1982; Sivanandan et al., 1984; Chettle et al., 1985).

Village weavers (Ploceus cucullatus) (Nawathe et al., 1978), guinea fowls (Adewuyi et al., 1989) and wild rats (Okoye and Uche, 1984), exotic (Okoye and Uzoukwu, 1982b) and local chickens (Agbede, 1975; Audi, 1977; Nawathe et al., 1978; Umoh et al., 1982; Abdu, 1985; Sokale and Oyejide, 1986; Okoye et al ., 1992) have been found to be positive for IBDV antibodies. Following experimental infection with IBDV, turkeys, quail-chicken hybrids and ducks developed PAbs to IBDV without showing any clinical sign and gross lesions (Weisman and Hitchner, 1978; Yamada et al., 1982; Greenfield et al., 1986). Quails (Coturnix coturnix) (Greenfield et al., 1986) and pigeons (Vindevogel, 1979) appeared to be completely

refractory to the infection by IBDV. Following natural exposure to the IBDV clinical disease is seen mainly in chickens (Cosgrove, 1962; Faragher, 1972; Hitchner, 1978) and rarely in turkeys (Owoade and Durojaiye, 1995). Clinical signs, gross lesions and PAbs were observed in ducklings, keets and local chickens experimentally inoculated with the IBDV (Christopher, 1982; Adewuyi et al., 1989; Okoye and Aba-Adulugba, 1996).

IBD has been reported in 9-day to 26-week-old chickens (Onunkwo, 1975; Ley et al., 1979b; Okoye and Uzoukwu, 1981; Durojaiye et al., 1984; Kumar et al., 1984; Abdu, 1985; Salami et al., 1989;. It is, however, most common in birds 3- to 7-week-old (Cosgrove, 1962; Hanson, 1967; Ojo et al., 1973; Okoye and Uzoukwu, 1982b; Abdu, 1986a; Abdu, 1987a; Tong et al., 1993). Clinical signs are not observed in laying birds and chicks less than 2 weeks old but histopathological lesions are seen in the BF of the chicks (Hitchner, 1970; Hitchner, 1971; Koster, 1971). The age resistance to clinical IBD is believed to be independent of the ability of the virus to replicate and induce lesions (Fadly and Nazerian, 1983) but dependent on the absence of a functional BF (Hitcher, 1978). Improved and indigenous breeds of chickens are susceptible to IBD (Hitchner, 1978; Mousa et al., 1984; Abdu and George, 1986; Okoye, 1987; Tong et al., 1993). Of the improved breeds, pullets show a more severe reaction to the virus than broilers (Hitchner, 1978) because their bursal index is higher (Okoye

and Aba-Adulugba, 1996). However, no significant difference in mortality rate was found between improved breeds (Meroz, 1966). IBD has been reported in layers, broilers, cockerels (Okoye and Uzoukwu, 1982b; Abdu et al., 1986a) and different strains of exotic chickens (Ojo et al., 1973; Okoye and Uzoukwu, 1981; Okoye and Uzoukwu, 1982b; Durojaiye et al., 1984).

2.5 Transmission

The IBDV is highly contagious and spreads readily by direct contact (Benton et al., 1967a). The disease spreads rapidly in a flock and recur in successive flocks (Hungerford, 1969). Litter, drinking water, and feed from IBD flocks were found to be infectious when added to rations of susceptible chickens but were not when added to drinking water (Edgar and Cho, 1976). The IBDV has been found to remain viable for 52 days in contaminated water, feed and faeces; for 180 days in dry litter and for 122 days or 360 days in dry chicken houses that had infected birds (Benton et al., 1967a). A ground suspension of lesser meal worms (Alphitobius disperinus) or litter mites taken from a house 56 days after an outbreak of IBD, were successfully used to infect susceptible chickens (Snedeker et al., 1967; Brady, 1970; Jordan, 1990).

2.6 Pathogenesis

Clinical signs and deaths were initially recorded 24 h and 48 h respectively after oral infection (Kaufer and Weiss,

1976). Viral particles and histopathological lesions in the BF and thymus were detected first 24 h after infection and most pronounced 72 h after (Helmboldt and Garner, 1964; Kaufer and Weiss, 1976; Savova and Bazhkov, 1985). IBDV replicates initially and mainly in the cytoplasm of lymphocytes and at a later stage in the macrophages of the medullar of the bursal follicle (Mandelli and Valeri, 1972; Savova and Bazhkov, 1985). It also grows in heterophils, reticulocytes and epithelial cells of the BF (Mandelli and Valeri, 1972; Kaufer and Weiss, 1976; Bakulin et al., 1985; Savova and Bazhkov, 1985). The development of clinical signs of IBD seems to depend on the rate and amount of virus production within B-lymphocytes (Becht, 1980).

The non-lymphoid pathologic changes in IBD (haemorrhges in skeletal muscles, proventriculus, and hepatic and renal changes) are still unexplained although Ley et al. (1979a) observed immunofluorescence in the renal glomeruli of infected chickens, indicating that gammaglobulins in the form of immune-complexes had lodged in the glomeruli of IBDV-infected chickens. This suggests that immune-complexes may play a role in the pathogenesis of IBD in chickens. However, the presence of glomerular fluorescence in the kidneys of normal and IBDV-infested chickens questioned the validity of the immune-complex theory (Ley et al., 1983).

The clinical signs and mortality associated with IBD may be due to depletion of circulating haemolytic complement from

the formation of immune-complexes at sites of viral replication resulting in haemorrhages (Skeeles et al., 1979a). A clotting abnormality without changes in complement levels was found to be associated with the severity of clinical IBD (Skeeles et al., 1980). The decrease in the serum calcium level in IBDV-infected chickens may be responsible for prolongation of blood clotting time seen in IBD (Chineme and Cho, 1984; Ray et al.,1985). IBD infection also causes a decrease in erythrocyte count, packed cell volume, haemoglobulin, albumin, albumin:globulin ratio, and glucose and an increase in the level of serum globulins (Mazurkiewicz and Wachnik, 1972; Panigraphy et al., 1986). The presence of urates in kidney and increase in serum uric acid could be due to renal damage, decreased fluid intake, diarrhoea and polyuria observed in IBD (Siller, 1959; Ley et al., 1983). An increase in serum lactate dehydrogenase and serum glutamic oxalic transaminase and decrease in cholesterol and potassium 3 to 5 days PI with IBDV was believed to be due to liver damage (Ley et al., 1983; Panigraphy et al., 1986). The severity of IBD has been found to be more intense in chicks fed a high protein (24%) diet (Proudfoot, 1975) because they grow faster and therefore have lower bursal index.

2.7 Clinical Findings

The onset of and recovery from clinical IBD are usually rapid (Rinaldi et al., 1965). The signs observed include

diarrhoea, soiled vent feathers, severe depression, anorexia, ruffled feathers, droopy appearance, trembling, prostration and death (Cosgrove, 1962; Giambrone et al., 1977b). After exposure to the standard IBDV, mortality may vary from 0%-30% (Winterfield, 1969; Hitchner, 1978). Morbidity of as high as 100% has been reported (Hitchner, 1978). IBD usually begins with high mortality on the third day PI and peaks half-way through the disease which usually last for 5 to 7 days (Cosgrove, 1962; Parkhurst, 1964; Hitchner, 1978). In birds exposed to the very virulent strain of IBDV (VVIBDV) mortality rate of 30% to 70% occurs coupled with early onset of clinical signs (Guan and Chua, 1993).

2.8 Pathology

2.8.1 Gross Findings

Birds that died from IBD are usually in good body condition with dehydration of the subcutis and muscles (Cosgrove, 1962). The crops are usually empty and the pectoral muscles discoloured (Luthgen, 1969; Hitchner, 1978). Frequently, paint-brush haemorrhages may be present in the leg, thigh and breast 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. Renal changes are prominent in birds that die or

are in advanced stages of the disease. In these birds, the kidneys are enlarged, lobules distended with accumulated urates and ureters are also distended (Cosgrove, 1962; Hitchner, 1978). The kidneys may vary in colour from white or pale grey to brown (Cosgrove, 1962; Barron et al., 1966). The spleen may be enlarged and very often has small grey foci uniformly dispersed on the surface (Rinaldi et al., 1965). Hyperemia due to injection of the blood vessels of the thymus have been reported (DelBono et al., 1968). In the acute phase of IBD, the BF is enlarged (there might be upto 100% to 200% increase in size) white, yellowish or cream-coloured 3 or 4 days PI and becomes atrophied and grey in the convalescent phase as from the fourth or sixth to eight or more days PI (Cheville, 1967; Hungerford, 1969; Ibragimov, 1976). Bursal atrophy may last for 3 weeks (Vindevogal et al., 1974). Infected bursae often show necrotic foci and at times petechial or ecchymotic haemorrhages on the mucosal surface. Occasionally, extensive haemorrhages throughout the BF may be observed (Hitchner, 1978).

2.8.2 Histopathology

Histopathological lesions are more commonly observed in the lymphoid organs; the BF, spleen, thymus and caecal tonsils, in order of decreasing level of destruction, with the BF being the primary site of damage followed by the spleen (Helmboldt and Garner, 1964; Lukert and Davis, 1974; Savova,

1984; Savova and Lyupke, 1984; Lam, 1988). Hypertrophy, hyperplasia, heterophil infiltration and mucous metaplasia of the epithelium of the BF has been observed (Cosgrove, 1962; DelBono et al., 1968; Cheville et al., 1978; Henry et al., 1980). 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 Miller, 1979). The follicular structure may be altered by degeneration and necrosis of lymphocytes (Cheville, 1967; Vindevogal et al ., 1974; Rampin et al., 1975). Intracellular oedema, condensation and margination of nuclear chromatin, increased number of lysosomes were observed in macrophages, lymphocytes and reticuloendothelial cells of the BF (Panisup et al., 1988). Lymphocytes are replaced by heterophils, pyknotic debris, hyperplastic reticuloendothelial cells and oedema (Cheville, 1967; El.Sergany et al.,1974; Vindevogel et al., 1974; Rampin et al., 1975; Thornton and Pattison, 1975; Ibragimov, 1976; Henry et al., 1980; Okoye and Uzoukwu, 1981; Okoye and Uzoukwu, 1984). Haemorrhages 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 cavities lined by hypertrophied epithelial cells (Cheville, 1967; DelBono et al., 1968; Winterfield et al ., 1972; El.Sergany et al ., 1974). Other

features of IBDV-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). Follicular regeneration and repair occurred at about 21 or 30 days PI (Asdrubali et al., 1974; Vindevogal et al., 1974; Rampin et al., 1975; Nandapalan et al., 1981).

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. Some times, haemorrhagic foci usually limited to the subcapsular area, may be present (DelBono et al., 1968). There is also fatty degeneration of macrophages of the spleen (Vindevogal et al., 1974). The thymus of some chickens may appear normal while others may show multifocal necrotic centres (Confer and MacWilliams, 1982; Ley et al., 1983). Necrosis of lymphocytes in the thymus has been observed to be less pronounced than in the BF and spleen in infections by IBDV serotypes 1 standard strains (DelBono et al., 1968). In the caecal tonsils, there is lymphoid necrosis and depletion (Okoye, 1984a). In the muscles of the thigh and breast, congestion, oedema, haemorrhages and necrosis are seen (Okoye and Uzoukwu, 1982a; Okoye, 1985b).

There is proventricular congestion, desquamation and necrosis (Okoye and Uzoukwu, 1982a).

Interstitial haemorrhages and perivascular accumulation 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 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).

2.9 Immunosuppression

Infection with IBDV suppresses both the humoral and cellular immune response of chickens (Giambrone et al., 1977c; Nandapalan et al., 1981; Confer and MacWilliams, 1982; Panigraphy et al., 1982; Perelman and Heller, 1983; Chui and Thorsen, 1984; Montgomery et al., 1986). Supression appear to be independent of the infective dose of the IBDV inoculated but suppressive effects varied with the strain of IBDV (Faragher et al., 1974; Chui and Thorsen, 1984). The virus causes atrophy of the BF, depletion of bursal lymphoid cells, decreased humoral responsiveness of blood lymphocytes and monocytes (Hudson et al., 1975; Ivanyi and Morris, 1976; Sivanandan and Maheswaran, 1981; Confer and MacWilliams, 1982). These changes caused by IBDV on the immune organs might be responsible for the decrease in the levels of complement,

IgA, IgG and IgM in serum and a decrease in serum albumin and the existence of IgM exclusively in monomeric form (Ivanyi and Morris, 1976; Giambrone et al., 1977a; Hirai et al., 1979; Fadly and Nazerian, 1983; Perelman and Heller, 1983). As a result of these, previously infected birds are highly susceptible to diseases such as haemorrhagic aplastic anaemia, gangrenous dermatitis, Clostridium perfringens, C. septicum and aflatoxicosis (Rosenberger et al., 1975; Giambrone et al., 1978; Hafacre et al., 1986).

Also chickens exposed to IBDV had low antibody titres, high mortality, extensive lesions, prolonged excretion of pathogens, and do not respond to treatment when challenged with coccidia (Anderson et al., 1977; Mcdougald et al., 1979), Crytosporidium baileyi (Levy et al., 1988), spirochaetosis (Rao and Rao, 1981), Salmonella pullorum (Lucio and Hitchner, 1979a), S. typhimurium (Wyeth, 1975), Haemohilus gallinarum (Hirai et al., 1974b), E. coli (Rosenberger et al., 1975), Alcaligenes faecalis (Jackwood et al., 1982), Mycoplasma gallisepticum (Uchida, 1984). M. synoviae (Giambrone et al., 1977b), infectious bronchitis, infectious laryngotracheitis (Giambrone et al., 1977a) and adenoviruses (Menendez et al., 1983; Jones and Georgiou, 1984). IBDV-infected chickens also respond poorly to vaccinations and are poorly protected following vaccination with Marek's disease (herpes virus of turkeys) (Jen and Cho, 1980; Liu et al., 1982; Sharma, 1984), infectious bronchitis (Winterfield et al., 1978) and Newcastle

disease (Snyder et al., 1986) vaccines and Pasteurella multocida bacterin (Khan et al., 1988) and inoculation with Brucella abortus (BA) (Hopkins et al., 1979; Edwards et al., 1982); sheep red blood cells (SRBCs) (Giambrone et al., 1977c), rabbit red blood cells (Giambrone et al., 1977a), bovine serum albumin (Lucio and Hitchner, 1979a) and human serum albumin (Perelman and Heller, 1983). Immunosuppression by IBDV depends on the age of the bird at the time of exposure, the absence or presence (Winterfield et al., 1978) and MA (Wood et al., 1983), the virulence of the IBDV strain used (Minta et al., 1985) and time of inoculation of the antigen used for determining immunosuppression (Meulemans et al., 1977; Sharma, 1984).

Generally the younger the bird, the more severe the suppression (Faragher et al., 1974; Wood et al., 1983). Suppression is absent in chicks with high level of MA, partial when MA is low and complete when MA is absent (Wood et al., 1983). Chicks infected with IBDV within the first week of age show no clinical signs of the disease but the virus multiplies and destroy the BF and limits the participation of the BF in the maturation and "seeding" of B-lymphocytes in the spleen, caecal tonsils, harderian glands and lymphoid foci in the intestines (Naqi, 1978). Affected birds are highly susceptible to infectious agents, and respond poorly to vaccinations (Naqi, 1978). This immunosuppression has been found to be permanent (Naqi, 1978; Giambrone et al., 1982). Chickens

exposed at day-old to a strain of IBDV that cause extensive bursal lesions 5 days before Newcastle (ND) vaccination were immunosuppressed while others exposed simultaneously or 5 days after ND vaccination were not suppressed (Meulemans et al., 1977). Strains of the IBDV that do not damage the BF are non-immunosupressive (Zanella et al., 1977). Secondary immune response to ND virus and SRBCs has been found to be unaffected by IBDV (Giambrone et al., 1977a; Giambrone et al., 1977b; Panigraphy et al., 1982).

2.10 Immunity

2.10.1 Active Antibodies

The IBDV is highly immunogenic (Naqi et al., 1980). IBDV PAbs appear from the 5th to the 28th day PI or PV and may persist for between 42 and 238 weeks PI (Schneider and Haass, 1969; Faragher, 1971; Koster, 1971; Vindevogel et al., 1975; Cursiefen et el., 1979; Ulbrich and Zureck, 1977; Ide et al., 1978). Neutralizing antibodies (NAb) are detected as early as 3 days PV(Curseifen et al., 1979).

Neutralizing Ab in naturally-infected or vaccinated chickens are able to neutralize homologous IBDV strains (Winterfield, 1969). Chickens exposed to IBDV at 3 days of age did not develop as significant NAb as those exposed at 4 weeks or later and the development of NAb was found to be dependent on the presence of the BF (Winterfield, 1969).

The incidence of PAb was found to be inversely related to

the severity of clinical reaction in IBD. Precipitin Ab levels were higher in birds that suffered clinical IBD than those that were vaccinated (Schneider and Haass, 1969; O'Brien, 1976). PAb levels were higher and persisted longer in chickens vaccinated orally than those vaccinated intramuscularly (Wyeth, 1980). Adult birds did not respond adequately to orally administered IBDV but produced Ab after intramuscular or subcutaneous inoculation of IBDV (Hitcher, 1976; Hitchner, 1978). PAb levels in IBD-infected chickens may be as high as 9 Log2 (Cullen and Wyeth, 1975).

IBD protection 7 days PV with a live IBDV vaccine has been observed and an immunity to challenge which lasted 9 weeks has been reported (Snedeker et al., 1967; Rosenberger, 1977).

PAb is regularly detected in the BF from the eighth day PI and occasionally in the heart, spleen and kidneys (Ulbrich and Zureck, 1977). Pathogenic and attenuated strains of IBDV induce the production of interferon. The level of the interferon in the serum peaks two to three-and-a-half days PI and is followed by production of NAb (Gelb et al., 1979).

2.10.2 Maternal Antibodies

Passive transfer of MA from IBD-immune hens to their chicks through eggs has been demonstrated (Hitchner, 1971; Abdu, 1985; Angelov and Lyupke; 1985; Abdu, 1986b; Abdu and Audu, 1987). Such chicks were found to be resistant to IBDV

infection and clinical IBD and eggs from the hens inhibited the growth of the IBDV (Faragher, 1971; Hitchner, 1971; Lucio and Hitchner, 1979b; Giambrone et al., 1982; Naqi et al., 1983; Panisup et al., 1984). MAs interfere with vaccination and prevent active antibody production (Abdu et al., 1987b). The level of MA vary with the source and age of birds. The higher the level of MA at day old the longer they last in circulation (Abdu and Audu, 1987). The MA levels and percentage of birds with MA are low or absent in chicks 3 weeks of age and absent in all birds 4 weeks old (Wyeth and Cullen, 1978; Abdu and Audu, 1987).

2.11 Diagnosis

2.11.1 Tentative Diagnosis

A tentative diagnosis of IBD is established from consideration of the history of the flock and the onset and course of the disease, the clinical signs and gross lesions of a sufficient number of chickens that have died (Faragher, 1972).

2.11.2 Confirmatory Diagnosis

Confirmatory diagnosis of IBD is based on the histopathology of infected BF(Cosgrove, 1962; Helmboldt and Garner, 1964), electron microscopy of the BF (Bakulin et al., 1985), serology (Asdrubali and Gialletti, 1971; Ide, 1975; Weisman and Hitchner, 1978; Cho et al., 1979), inoculation of

susceptible chicks (Cosgrove, 1962; Helmboldt and Garner, 1964) viral isolation (Ide, 1975) and detection of IBDV antigens in the BF by AGDT (Hirai et al, 1972).

2.11.3 Differential Diagnosis

IBD may be confused with lymphoid leukosis, Marek's disease, inclusion body hepatitis, coccidiosis, Newcastle disease, infectious bronchitis, fatty liver and kidney syndrome, avitaminosis A and reticuloendotheliosis (Barron et al., 1966; Faragher, 1972; Okeke, 1984). These diseases are distinguished from IBD on the bases of clinical, post-mortem and histopathological findings, serology, isolation and identification of the aetiologic agent.

2.12 Control and Prevention

The highly infectious nature of IBD coupled with the resistance of IBDV has made vaccination a necessity in major poultry producing areas throughout the world. There are two methods of controlling subclinical IBD, namely, vaccination of breeders to produce high MA levels in their off-springs which should prevent infection during the first few weeks of life, or vaccination of the progeny at day-old to prevent early infection (Giambrone, 1983). Producers in the USA and Britain have been using the first method (Wyeth and Cullen, 1978a; Giambrone, 1983). This is because the essential element of controlling subclinical IBD and immunosuppression is

preventing the infection of BF during the first few weeks of life. This is achieved more effectively by MA than day-old vaccination of IBD-susceptible chicks (O'Brien, 1976; Ide, 1979).

Since MA wanes steadily after hatch and is absent by 3 or 4 weeks of age, it cannot be depended upon to protect against clinical IBD (Naqi et al., 1983). The type of vaccine to use and the programme to follow will depend on the level and virulence of the field virus and level of MA in birds (Giambrone, 1983). If the field virus is very virulent and clinical IBD occur as early as 8 to 14 days of age and affects about 25% to 50% of the flock, vaccination with a highly pathogenic IBDV vaccine at 5 to 8 days of age in drinking water has been recommended (Giambrone, 1983). If clinical IBD occur at 18 days, mild vaccines could be used at about 10 to 14 days of age in drinking water (Giambrone, 1983). Day-old vaccination with mild vaccines is usually confined to progeny of parents that are not receiving killed vaccine or those that are older than 45 weeks and have become sero-negative to IBDV by AGPT or below 1:100 on the VNT (Giambrone, 1983).

Chapter 3

THE ANTIBODY RESPONSE, SURVIVAL AND IMMUNE RESPONSE TO SHEEP RED BLOOD CELLS BY CHICKS VACCINATED WITH A LOCAL VACCINE AND CHALLENGED WITH A PATHOGENIC INFECTIOUS BURSAL DISEASE VIRUS

3.1.0 Introduction

For the effective control of clinical IBD in chicks, two vaccinations are necessary (Giambrone, 1985). Chickens are most susceptible to IBD at 6 weeks of age (Cosgrove, 1962). The age at which to vaccinate for IBD before 6 weeks of life is controversial. For initial vaccination, some scientists(Winterfield and Thacker, 1978; Winterfield et al ., 1980) recommend early vaccination (at day-old), others (Cullen and Wyeth, 1979; Hitcher, 1971) late vaccination (at 14 to 21 days old) or an intermediate period for vaccination (at 4 to 8 days) (Ehizokhale, 1982). Chicks 1 to 8 days old may possess high levels of MA capable of neutralizing some vaccine virus while those 14 days or older would have low MA levels or are devoid of MA and therefore susceptible to field IBDV (Ehizokhale, 1982).

Vaccine 01, National Veterinary Research Institute (NVRI), Vom, Nigeria contain an attenuated and supposedly apathogenic strain of IBDV. Flocks without MA are to be vaccinated at day old only while those with MA at day-old and repeated at 30 days of age (Okeke and Tanimu, 1982). Vaccination failures following the use of local vaccine and the recommended vaccination schedule have been reported

(Ezeokoli et al., 1985). This study was, therefore, conducted to determine: (i) the immune response of chicks following vaccination with vaccine 01 at various ages and frequencies; (ii) the morbidity and mortality rates and antibody response of vaccinated chicks following challenge with a pathogenic IBDV from a field outbreak; (iii) the antibody response of chicks to sheep red blood cells after vaccination with vaccine 01 and challenge with a pathogenic IBDV.

3.2.0 Materials and Methods

3.2.1 Serum

Whole blood was obtained via cardiac puncture. It was allowed to clot at room temperature and then centrifuged at 5,000 rpm (6.175 Xg)for 15 min (Alton et al., 1990). The serum at the top was aspirated and stored at -20^{0}C until tested for PAb to IBDV.

3.2.2. Vaccine and Vaccination

Vaccine 01, a live virus vaccine was obtained from NVRI, Vom Nigeria . A 0.5 ml of the vaccine in phosphate-buffered saline (PBS) (pH 7.6) containing one field dose ($10^{4.5}$ tissue culture infective dose, TCID, 50 per bird) was administered orally to each bird.

3.2.3 Challenge and Challenge IBD Virus

A pooled clarified bursal homogenate (50% w/v) in PBS (pH

7.6) of bursae from birds that died of IBD was used as the challenge virus. A 50 ul of the homogenate containing an unknown quantity of virus was dropped onto the open eyes (25 ul per eye) of each bird. The chicks were observed for sickness and death daily for 1 week. The number sick and number dead were recorded.

The bursal infective dose (BID) 50 of the inoculum was determined in 3-week-old IBD-susceptible chicks. The titre of the virus which was $10^{4.4}$ bursal infective dose-BID-50 per ml of undiluted viral suspension was calculated according to the method of Reed and Muench (1938) (Appendix 1).

3.2.4 Quantitative Agar Gel Precipitin Test

Preparation of IBDV precipitin antigen: Bursae of chickens that died from natural IBD were removed, weighed, and ground in PBS (pH 7.6) (1:1 w/v) using a pestle and mortar with the aid of sterile fine sand. This mixture was then frozen, thawed and ground three times. The mixture was centrifuged at 5,000 rpm(6.175 Xg) for 15 minutes. The supernatant obtained was clarified by centrifuging at 15,000 rpm (18.525 Xg) for 15 minutes in a refrigerated centrifuge(model J - 21B Beckman^{R}). Two hundred and fifty IU of penicillin and 25 ug of streptomycin were added to the supernatant fluid (2 ml portions) and then stored in screw-cap vials at -20^{o}C until used.

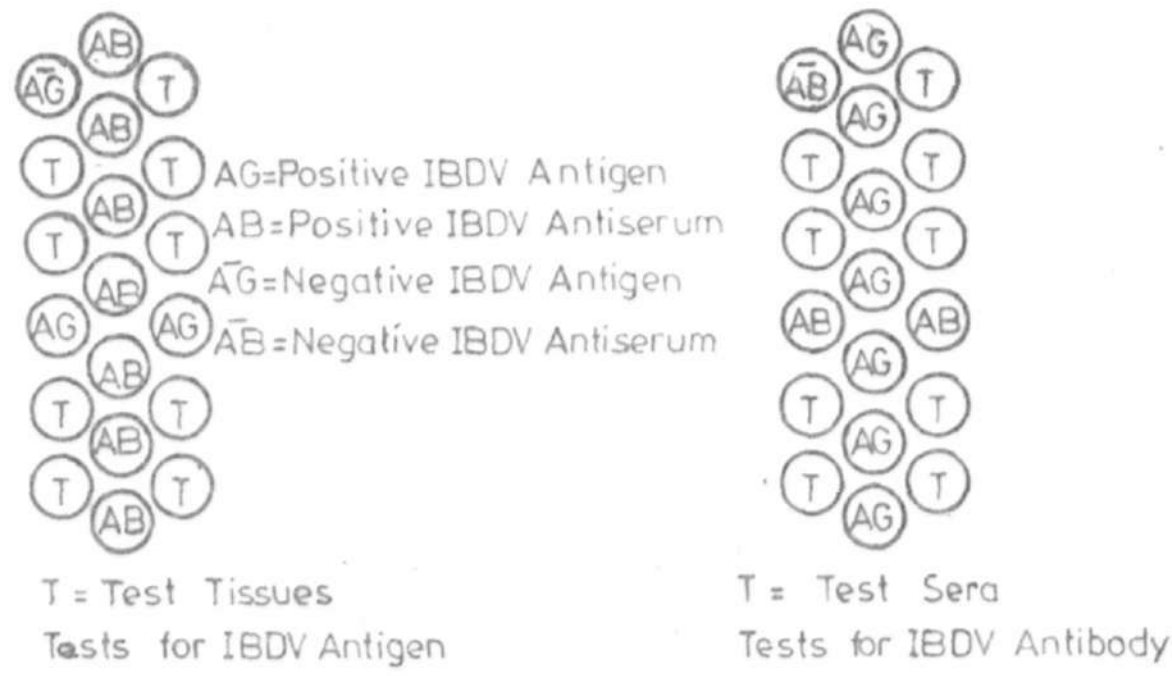
Three-week-old chicks were then inoculated intraocularly

with 50 ul of the inoculum. Three days after inoculation the chicks were killed, the BF removed and treated as mentioned earlier(3.2.2). The homogenate was used as antigen in a quantitative agar gel precipitin test (QAGPT) (Cullen and Wyeth, 1975).

Preparation of IBDV precipitin antiserum: Some of the chicks infected with the field IBD above were used for the preparation of IBDV antiserum. Two weeks PI, 2-5 ml of blood were obtained from the wing vein or heart of infected chicks. The blood was allowed to clot for 2 h at room temperature and then at 37^{o}C for 4 h . The sera were separated, pooled and stored in vials at -20^{o}C until used.

Preparation of agar gel plates: 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 100 ml of distilled water and heating the mixture slowly to boiling. The agar plates were made by dispensing 25.7 ml of agar into 9 x 9.5 cm perspex dishes. This gave an agar depth of 3 mm.

Wells of 6 mm width with 3 mm. separation were cut into the already set agar. An offset linear pattern of 12 parallel rows was used with the middle row of every 3 having every hole cut (Figure 3.1). The middle row was for antigen while the 2 complete rows to either side were for antiserum dilutions. The agar plugs were removed from the wells with a hypodermic needle.

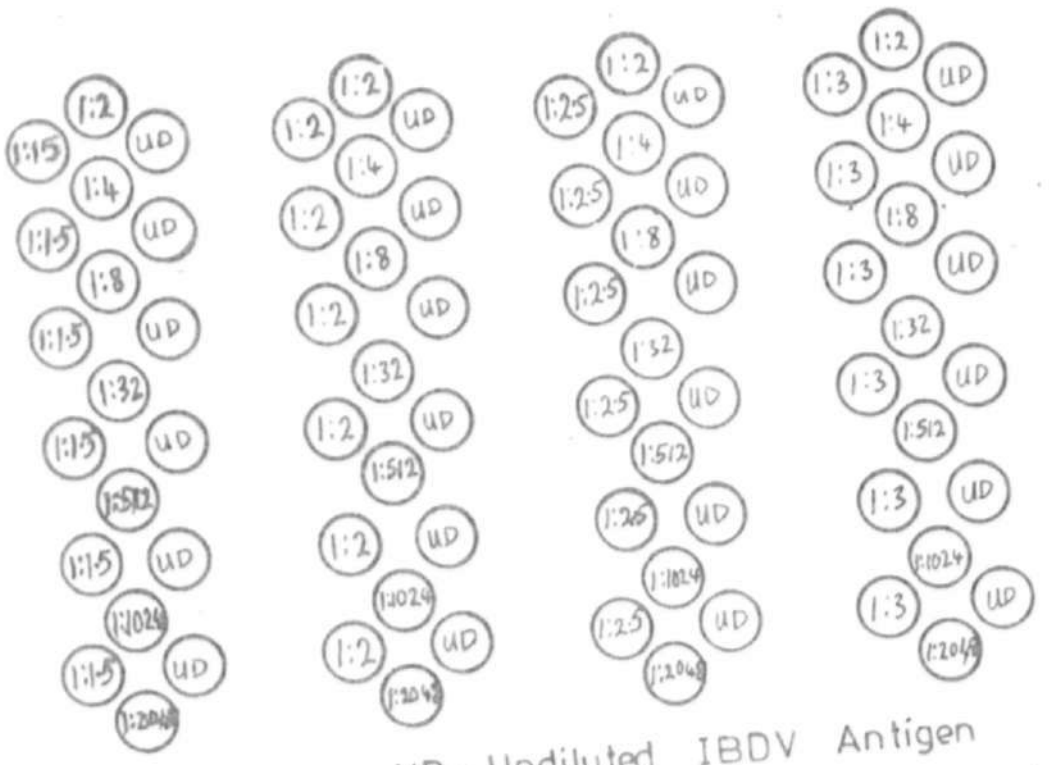


Fig·3·1: Tests for infectious bursal disease virus (IBDV) antigen and antibody.

Standardization of precipitin antigen: The field virus precipitin antigen (PAg) was standardized by the following steps. (1) 1:1.5, 1:2, 1:2.5 and 1:3 dilutions of antigen in PBS (pH 7.6) were prepared. 1 ml of each dilution was required for the test. (2) Serial two fold dilutions of standard positive antiserum from 1:2 to 1:2048 using PBS as the diluent were prepared. Approximately 0.5 ml of each dilution was sufficient for the test. (3) 25 ul of each of the dilutions of antigen was dispensed with a micro pipette into each well of a vertical row of 6 wells. A row of wells containing undiluted antigen was included (Figure 3.2). (4) 25 ul of antiserum diluted 1:2 was dispensed into each of the first wells of the rows of 7 wells. (5) 25 ul of antiserum diluted 1:4 was dispensed into each of the serum 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 h. Strong reactions showed 2 or 3 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. The highest dilution of antigen which produced a clear, sharp line was chosen for standard use.

Testing serum of unknown titre: The titre of test serum was determined by the following steps. (1) Serial two fold dilutions of the test serum from 1:2 to 1:2048 in PBS (pH 7.6) were prepared. (2) 25 ul of undiluted test serum was placed



UD = Undiluted IBDV Antigen

Fig.3.2: Standardization of field infectious bursal disease virus (IBDV) antigen by the quantitative agar gel precipitin test.

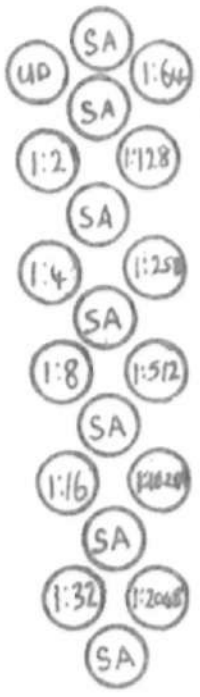
in the first well of one of the rows of 6 wells (Figure 3.3). 25 ul of the test serum diluted 1:2 was placed in the second well and the procedure repeated with each dilution of serum up to the 1:2048.

(3) 25 ul 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 h after which they were read. The end point of each serum was taken as the dilution which produced a precipitin line. The Ab titre was considered as the reciprocal of the end point. A geometric mean was used to express the average titre in each group.

3.2.5 Test for Immunodepression

Sheep red blood cells (SRBCs): SRBC were collected in Alsever's solution and washed three times in PBS (pH 7.6) by centrifugation at 1,500 rpm(1.853 Xg) for 20 min. After the final washing, the cells were suspended in PBS(pH 7.6) in a concentration of 10% (v/v).

Inoculation of chickens with SRBCs: After 1 week of challenge with a pathogenic IBDV, birds that survived were inoculated intramuscularly with 1 ml of a 10% SRBC and with 2 ml of 10% SRBCs 3 days and with 3 ml of 10% SRBCs 6 days later. The birds were bled at 7 and 14 days after the initial inoculation of SRBC for sera.



UD = Undiluted IBDV Antiserum
SA = Standardized IBDV Antigen

Fig.3·3: Testing infectious bursal disease virus (IBDV) antiserum of unknown titre

Haemagglutination (HA) assay for SRBCs antibodies: Two fold dilutions of sera in PBS (pH 7.6) were made in microtitre plates. An equal amount of 50 ul of 0.5% dilution of SRBC was added to each well. The plates were sealed and floated in a water bath at 32°C for 1 h. They were refrigerated overnight and read the following day. The Ab titre recorded for each sample was the highest dilution in which complete HA occurred. A geometric mean was used to express the average HA titre in each group (Sharma et al., 1989).

3.2.6. Experimental Design

One hundred and thirty 6-day-old Isa-brown pullets chicks from 75-week-old hens vaccinated with a live IBD vaccine at 10 days old were purchased from SIMTU, Zaria and 136 from the National Animal Production Research Institute (NAPRI), Shika-Zaria. The chicks from each source were divided into six groups , 1,2,3,4,5, and 6, containing 28, 17, 17, 30, 26 and 18 each respectively. Chicks in group 1 were vaccinated orally at day-old and 4 weeks, those in group 2 at 1 week of age, those in group 3 at 2 weeks of age, those in group 4 at 2 and 4 weeks of age, those in group 5 at 2 and 5 weeks and those in group 6 at 3 weeks of age. Six 4-week-old IBD PAb-negative chickens were also vaccinated with the vaccine and their bursae tested for IBD viral antigens 3 days PV.

Chicks in group 1 were challenged with a virulent virus at 6 weeks of age and chicks in groups 2,3,4,5,and 6 at

3,4,6,7 and 5 weeks of age respectively. One week after challenge surviving chicks were inoculated with SRBCs and bled at 1 and 2 weeks PIc and the level of their response to SRBCs determined by the HA test.

3.2.7 Statistical Analysis

One way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used to compare the levels of antibody titre between the different experimental groups (Kleinbaum and Kupper, 1978). Statement of statistical significance were based on $P<0.05$.

3.3.0 Results

All the chicks from SIMTU and NAPRI in all groups had no detectable PAbs to IBDV from day-old and 1, 2 and 3 weeks of age, 1 and 2 weeks PV. Vaccinal antigens were not detected in the bursae of chickens without IBDV Ab vaccinated at 4 weeks of age.

About 81% of the chicks in group 1 from NAPRI, 71% in group 2, 33% in group 3, 22% in group 4, 31% in group 5 and 33% in group 6 became sick after virus challenge. Only 9% of the chicks in group 1, 11% in group 3 and 8% in group 6 died as a result of challenge. No bird from groups 2,3, and 5 died of IBD. Only chicks vaccinated at 3 weeks from SIMTU did not become sick after challenge and only those vaccinated at 2,3 or 2 and 4 weeks of age did not die (Table 3.1).

Generally, the older the chicks at the time of vaccination the lower the mortality and morbidity. Morbidity and mortality in vaccinated chicks was lower than those of unvaccinated chicks. The differences between the mean morbidity and mortality between the different age groups was statistically significant ($P<0.05$). The difference was found between chicks vaccinated at 2 weeks and those at 1 week, at 2 weeks and those at 3 weeks, at 3 weeks and those at 1 week, at 3 weeks and those at 2 weeks and, at 3 weeks and those at 1-day old and 4 weeks.

Challenged chicks had detectable PAb in their sera 1 week PC when between 33 to 75% of the birds, depending on the group, had sero-converted. By 2 and 3 weeks PC 100% of the chicks had PAbs. The mean PAb titres of chicks three weeks PC was higher than at 2 weeks PC. Individual PAb titre ranged from 0 to 8 Log2 with 57% of birds possessing a titre of 4 or 5 Log2. The mean PAb titre for each group ranged from 1.3 to 5.8 Log2. NAPRI chicks produced significantly higher ($p<0.05$) amount of PAb than SIMTU birds following challenge with IBDV. The mean PAb level was significantly ($p<0.05$) lower in chicks vaccinated at 3 weeks old than in chicks vaccinated at 1 week, 2 weeks or 1 day old for NAPRI birds sampled at 2 weeks PC. For chicks from SIMTU sampled at 3 weeks PC, there were significant differences in the PAb level between chicks vaccinated at 1 day old and 4 weeks, 1 week, and 2 weeks, and

Table 3.1: The morbidity mortality of chicks challenged after vaccination with a batch (01) of local IBD vaccine

Source of chicks	Age at (weeks)		Morbidity (%)	Mortality (%)	Protection rate
	Vaccination	Challenge			
NAPRI	ID[a] and 4	6	9/11 (82)	1/11 (9)	72
	1	3	(5/7) (71)	(0/7) (0)	100
	2	4	(2/6) (33)	(0/6) (0)	100
	3	5	2/9 (22)	1/9 (11)	67
	2 and 4	6	(5/16) (31)	(0/16) (0)	100
	2 and 5	7	4/12 (33)	1/12 (8)	76
SIMTU	1D and 4	6	3/14 (21)	1/14 (7)	78[A]
	1	3	3/7 (43)	2/7 (29)	12[B]
	2	4	5/7 (71)	0/7 (0)	100[AB]
	3	5	0/6 (0)	0/6 (0)	100[AB]
	2 and 4	6	3/6 (50)	0/6 (0)	100[AB]
	2 and 5	7	2/6 (33)	1/6 (17)	48[C]
Control	-[b]	7	6/6 (100)	2/6 (33)	0

a, day-old
b, not vaccinated
means with different superscripts on the same column are statistically different

1 week and 3 weeks (Table 3.2).

The mean HA titre of each group of SRBC-inoculated chicks at 1 week PIc was lower than the mean titre at 2 weeks PIc irrespective of the source of the birds. The mean HA titres of chicks from SIMTU at 1 (3.4 Log2) and 2 (6.3 Log2) weeks PIc with SRBc were higher than those of uninoculated controls at 1 (2.0 Log2) and 2 (2.5 Log2) weeks PI. The same was the case with chicks from NAPRI. The mean HA titre between groups was more variable at 1 week PIc and at 2 weeks PIc for chicks from SIMTU. At 1 week PIc the mean HA titre of chicks from NAPRI (2.0 Log2) and SIMTU (3.4 Log2) that were vaccinated, challenged and inoculated was lower than the value (4.8 Log2) from unvaccinated unchallenged and inoculated chicks. At 2 weeks PIc, only the main HA titre (6.3 Log2) of chicks from SIMTU that were vaccinated at or 2 weeks old, challenged and inoculated was higher that the value (5.8 Log2) for unvaccinated, unchallenged and inoculated chicks. While the mean HA titre of chicks vaccinated at 3 weeks old from NAPRI and SIMTU was higher than that of any other group at 1 week PI, it was highest in chicks vaccinated at 2 and 5 weeks old at 2 weeks PIc. Chicks from SIMTU had higher HA titre than those from NAPRI and not all chicks from NAPRI developed Ab to SRBCs at 1 week PIc. Only chicks vaccinated at day-old and 4 weeks of age, 1 week of age and those vaccinated at 2 and 4 weeks from NAPRI had HA titre lower than that of uninoclated control birds at 1 week PIc. The same was true for chicks

Table 3.2: The Ab response of chicks challenged after vaccination with a batch of a local IBD vaccine

Source of chicks	Age at (weeks)		Weeks post-challenge/% with Ab/Ab titre Log2±SD			
	Vaccination	Challenge	0	1	2	3
NAPRI	ID[a] and 4	6	-[b]	NT[c]	4.6[A] ±2.0	3.8 ±2.0
	1	3	-	33	6.8[A] ±2.9	5.7 ±2.0
	2	4	-	40	4.3[A] ±0.6	4.6 ±1.9
	3	5	-	NT	1.8[B] ±2.4	4.8 ±2.4
	2 and 4	6	-	NT	3.7[AB] ±0.6	3.8 ±1.7
	2 and 5	7	-	NT	3.4[AB] ±1.1	4.8 ±1.7
SIMTU	ID and 4	6	-	NT	3.3 ±1.4	3.2[AB] ±1.8
	1	3	-	75	4.0 ±1.0	5.5 ±1.3
	2	4	-	67	2.4 ±0.9	4.8[BD] ±0.8
	3	5	-	50	1.5 ±0.7	1.3[C] ±0.6
	2 and 4	6	-	NT	1.7 ±1.5	4.7[D] ±0.6
	2 and 5	7	-	NT	±4.3	±4.8
Control	-[d]	7	-	NT	0.6 ±2.0	1.7 ±3.3
					0.5	1.0

a, day-old b, no Ab to IBDV c, not taken d, not vaccinated
means with different superscripts on the same column are statistically different

obtained from SIMTU that were vaccinated at 2 weeks of age (Table 3.3). For chicks from NAPRI, the older the age of bird at vaccination the higher the HA Ab produced when sampled at 1 week PIc with SRBC. Significant differences ($p<0.05$) were found in the HA Ab levels between chickens vaccinated at 1 day and 4 weeks and 3 weeks, 1 week and 2 weeks, 1 week and 3 weeks and 2 weeks and 3 weeks. For SIMTU chicks, there was no significant differences ($p>0.05$) in the HA Ab levels at 1 week PIc; significant differences ($p<0.05$) were observed between chicks vaccinated at 1 week and 3 weeks at 2 weeks PIc.

3.4.0 Discussion

It was not possible to compare the antibody responses of birds to vaccine OI when administered at different age or when administered once or twice because the QAGPT used could not detect PAb 2 weeks PV. Using the same vaccine but a different batch, Sa'idu (1995) obtained the same result. However, over 50% of the chicks vaccinated twice had PAb in their sera 2 weeks after a second vaccination even in birds vaccinated with 50% or 75% of the recommended dose. It is likely that the batch of vaccine used in this study contained little amount or no viable virus or the vaccine was highly attenuated and was neutralized by low undetectable antibodies present in the serum of vaccinated chicks. This is supported by the fact that viral antigens could not be detected in the bursae of IBDV-PAb-negative birds. Some of the reasons for the frequent and

Table 3.3: Effect of vaccination at different ages with a batch (01) of a local IBD vaccine and challenged with a virulent IBDV on Ab response to sheep red blood cells

Group	Source of birds	Age at vaccination in weeks[a]	Age at challenge in weeks	Mean HA titre (Log2) ± SD(%+) 1 week after SRBC inoculation	2 weeks after SRBC inoculation[b]
Vaccinated challenged SRBC inoculated	NAPRI	10[c] and 4	6	1.7± 1.0 (70)^{AB}	5.1± 0.4 (100)
		1	3	1.0± 0.0 (67)^{AC}	5.7± 2.7 (100)
		2	4	2.0± 1.0 (75)^{AD}	4.3± 3.0 (100)
		3	5	3.8± 1.8 (100)^{AE}	5.3± 2.1 (100)
		2 and 4	6	1.7± 0.7 (100)^{A}	5.6± 1.1 (100)
		2 and 5	7	2.3± 1.2 (85)^{A}	5.8± 1.5 (100)
Vaccinated challenged SRBC inoculated	SIMTU	10 and 4	6	3.0± 2.0 (100)	6.7± 1.5 (100)^{A}
		1	3	3.0± 1.0 (100)	4.7± 0.6 (100)^{AB}
		2	4	1.7± 0.5 (100)	4.7± 1.4 (100)^{A}
		3	5	6.5± 1.0 (100)	7.7± 1.5 (100)^{AC}
		2 and 4	6	4.5± 2.1 (100)	6.3± 0.5 (100)^{A}
		2 and 5	7	2.0± 1.0 (100)	7.8± 2.2 (100)^{A}
Unvaccinated Unchallenged SRBC inoculated				4.8± 1.5 (100)	5.8± 1.5 (100)
Unvaccinated Unchallenged SRBC Un-inoculated				2.0± 0.0 (40)	2.5± 1.6 (45)

a, chicks challenge 1 week post-vaccination and inoculated with SRBC 1 week later
b, sheep red blood cells; c, one day old;
SD, standard deviation
means with different superscripts on the same column are statistically different

widespread vaccination failures observed in the field in Nigeria could be the administration of non-viable or highly attenuated vaccines or those containing little amounts of virus.

The vaccine used could not have lost its viability and potency as a result of poor storage, transportation or a fault during its manufacture. The vaccine was obtained directly from Dr. Aba-Adulubgba of NVRI, Vom, Nigeria. A cold chain was maintained during storage at the NVRI, during transportation and storage in our laboratory.

Winterfield et al. (1981) had identified that changes may occur in the identity of an IBDV vaccine from manipulation in the laboratory, mutation, storage and errors in labelling. In the event of shortage of IBD vaccine and high demand, batches of vaccines to be discarded might be smuggled out and sold or the label on vaccine vials other than those for IBD produced in excess might be replaced with those of IBD and sold to unsuspecting buyers.

The challenge virus was found to be immunogenic. It resulted in the production of PAb in 33% to 75% of the chicks at 1 week PC and in 100% of challenged chicks at 2 and 3 weeks PC. Chicks vaccinated with vaccine 01 at 1 week of age and challenged at 3 weeks produced higher PAb than those vaccinated at other ages.

The Abs produced due to primary immune response to SRBC are said to be 2-Mercaptoethanol(2-ME) sensitive and therefore

of the IgM class (Vanderzijpp et al., 1982; Sharma et al., 1989). The HA antibodies to SRBCs measured in the present experiment are the results of primary and secondary immune responses because three injections of SRBCs were given and therefore could have contained both 2-ME sensitive and resistant (IgG) immunoglobulins. The multiple injections of SRBCs might also explain why the HA titres in chicks were as high as those of chicks inoculated intravenously (IV)(5-7 Log2) (Sharma et al., 1989). The IM route is safer as birds might react very strongly to the SRBCs when inoculated IV possibly resulting in death. Allergic reactions were observed in rabbits injected IV with SRBCs (Rockborn et al., 1990).

Whether chicks are infected at day-old or at 4 weeks of age with IBDV there is a reduction in the primary Ab response to SRBCs (Ivanyi and Morris, 1976; Giambrone et al ., 1977a). In this study, the challenge IBDV used also delayed the development of Ab by 1 week and reduced the level of HA Ab produced to SRBCs.

IBDV causes necrosis of the lymphocytes in the BF and thymus (Sharma et al., 1989). Severe necrosis in the thymus was seen by 4 or 5 days but absent by 10 or 12 days PI (Okoye, 1984a; Sharma et al., 1989). These changes in the BF and thymus result in a decrease in the levels of complement, IgA, IgG and IgM in serum (Ivanyi and Morris, 1976) and IBDV-infected birds are highly susceptible to other infectious pathogens and respond poorly to vaccinations (Wyeth, 1975;

Naqi, 1978). In Nigeria, chicks previously exposed to a pathogenic IBDV were found to respond poorly to vaccination with the Hitchner B1 vaccine for Newcastle disease (Okoye and Shoyinka, 1983; Okoye, 1985a) and to have suffered more severely when exposed to Aspergillus flavus and coccidia infections (Okoye, 1983; Okoye et al., 1991).

Chicks from SIMTU reacted better to SRBCs inoculation than those from NAPRI. There is the possibility that the destruction of the immune organs by challenge IBDV in SIMTU birds was less severe and widespread than those of chicks from NAPRI. These birds are of different breed and from different parent stocks with different exposure experience to vaccinal or field IBDV and of different age and differences in susceptibility among genetic lines (Okoye and Aba-Adulugba, 1996).

The rate of immune response to SRBCs differed between chicks. Some reacted promptly while others reacted slowly. Differences in the rate of repopulation of the thymus with T-lymphocytes and BF with B-lymphocytes following exposure to pathogenic IBDV 2 weeks earlier might explain the differences. In a previous study, IBDV infection at day-old reduced HA titres to SRBCs. Repopulation of the BF by lymphocytes occurred by 4 weeks and the serum Ab levels to SRBCs returned to near normal by 5 weeks following infection (Nandapalan et al., 1981).

Vaccine 01 could not elicit detectable antibody response in chicks devoid of PAb vaccinated at 1 day old and 4 weeks, 1, 2, 3, 2 and 4 or 2 and 5 weeks of age. Some vaccinated birds could not be prevented from becoming sick or dying when challenged with a pathogenic field virus. Immunodepression occurred in vaccinated birds that were subsequently challenged. There is the need to routinely determine the potency of each batch of local vaccine produced in order to reduce the incidence of IBD vaccination failures and subsequent immunodepressions being experienced in the field. Low undetectable levels of MA in chicks might be capable of neutralizing vaccine 01, a vaccine which contains apathogenic IBDV strain.

Farmers should vaccinate their chicks against IBD at 2 and 4 weeks of age and prevent vaccinated chicks from a virulent IBDV before they are 3 weeks old to avoid immunodepression.

Chapter 4

DETERMINATION OF THE PATHOGENICITY AND ANTIBODY RESPONSE OF VACCINE 03 ADMINISTERED VIA DIFFERENT ROUTES TO CHICKS WITHOUT MATERNAL ANTIBODIES TO INFECTIOUS BURSAL DISEASE VIRUS

4.1.0 Introduction

Attenuated IBDV vaccines are administered via various routes (Ehizohale, 1982). However, the most common route employed is the oral in drinking water. This is because it is the most convenient, not labour intensive and can be used for vaccinating large number of chickens at once. Despite these advantages, the method has some shortcomings. Firstly, the birds have to be water-starved. Secondly, the quantity (invariably the dose) of the vaccine-treated water that a bird will consume cannot be accurately controlled as what is consumed will depend on the size of the bird, the weather condition, the position of the bird in the peck order and the watering space provided. Furthermore, the water used in reconstituting the vaccine must be free from antiviral agents. Therefore, chickens vaccinated orally may have varying levels of immune responses and this may contribute to the high incidence of the immunization failures reported in the field in Nigeria (Okoye, 1985d; Abdu, 1986b).

Earlier studies indicated that some batches of local IBD vaccine may be pathogenic (Abdu, 1985; Adene et al., 1985a; Okoye, 1985d). Vacine 03 was manufactured by the NVRI Vom, and is widely used by farmers. It is recommended for oral, intraocular or intramuscular administration (Okeke and Tanimu,

1982). The objectives of the study were to determine:
(i) The pathogenicity of vaccine 03 in chicks without MA to IBDV when administered by different routes (ii) The presence of vaccinal antigens in the BF of vaccinated chicks (iii) The amount and rate of antibody production in vaccinated chicks.

4.2.0 Materials and Methods

4.2.1 Experimental Birds

One hundred day-old chicks were purchased from two hatcheries, fifty from SIMTU, Zaria and fifty from Kaduna Hatchery, Kaduna. They were fed grower's mash. The feed and water were provided ad libitum. The chicks were housed in a room previously cleaned, washed, disinfected and fumigated.

4.2.2 Vaccination

Vaccine 03 was purchased from a veterinary shop (Aldisa Ventures) in Zaria. For oral route, (PO) 0.5 ml of the vaccine reconstituted in PBS (pH 7.4) and containing one field dose was administered to each bird. Exactly 0.2 ml of reconstituted vaccine was used for subcutaneous (SC) and intramuscular (IM) administration and 50 ul for intranasal (IN), eye (IO) and vent drop (VD) methods. Each of these volumes also contained one field dose(10^{4-5}TCID 50 per bird) of the vaccine.

4.2.3 Experimental Design

The chicks were reared for 4 weeks (those from Kaduna) and 5 weeks (those from Zaria) of age and then bled by cardiac puncture for sera. Groups of birds from each source were then vaccinated IO, IN, SC, IM, VD or PO and housed in separate wire-floored cages. Five of the birds were left unvaccinated but housed together with those vaccinated PO (referred to in the text as in-contact). The chicks were observed for clinical signs and deaths for 1 week after which they were bled for sera. Another bleeding was conducted 2 weeks PV. The sera were tested for the presence and concentration of PAbs to IBDV by the QAGPT (Cullen and Wyeth, 1975) before vaccination, and at 1 week and 2 weeks PV. The bursae of chicks that died 3-7 days PV were removed and processed as stated earlier (3.2.4) and the presence of viral antigens determined by the AGPT.

4.2.4 Statistical Analysis

With the aid of a computer (EVEREX - 286PC) and using a soft-ware, statistical package for social sciences, a one way analysis of variance (ANOVA) was used to determine if the means of the PAb levels due to vaccine 03 between groups were different. In cases where the means differ, the Duncan's multiple range test was used to identify the individual groups that were different. The Student's t-test was also used to compare the means of PAb levels of 2 groups of chicks (Kleibaum and Kupper, 1978) at a significance level of $P<0.05$.

4.3.0 Results

In the chicks from Zaria, 20% of the chicks vaccinated IM or VD, 40% of chicks vaccinated IO, SC or PO and 100% of in-contact birds became sick 3 days PV. The mortality rates for chicks vaccinated via VD, SC or for in-contact chicks were 20%, 40% and 40% respectively (Table 4.1). In the chicks from Kaduna, morbidity were 20%, 33%, 40%, 40%, 60%, 40% and 60% for PO vaccinated, in-contact chicks, IN, SC IM, VD and IO vaccinated birds respectively. The mortality rates for these groups were 20%, 0%, 40%, 20% 20%, 40% and 40% (Table 4.2). Fewer chicks became sick or died when vaccine 03 was administered IN, IM or PO.

The clinical signs of sick chicks were: ruffled feathers, yellowish diarrhoea, anorexia, severe depression, weakness and abdominal respiration. At post-mortem, the muscles, lungs, pancreas, thymus and the BF were congested. Haemorrhages were seen in the leg, thigh, breast and wing muscles, proventriculus, duodenum, ileum, caecum and caecal tonsils. The liver was enlarged and streaked and the kidneys enlarged with prominent lobules; the ureters were

Table 4.1: Morbidity, mortality and Ab response of IBDVAb-negative 5-week-old chicks vaccinated with a batch (03) of local IBD vaccine via different routes

Route of vaccination	Morbidity (%)	Mortality (%)	Viral antigens	Mean antibody (Log2) level in sera (%) ± SD		
				Days post-vaccination 0	7	14
Intraocular	2/5 (40)	0/5 (0)	-[a]	-[c]	2.0 (100)[A] ±2.4	3.0 (100)[A] ±0.7
Intranasal	0/5 (0)	0/5 (0)	-	-	2.2 (100)[A] ±1.3	3.4 (100)[A] ±1.7
Subcutaneous	2/5 (40)	2/5 (40)	+[b]	-	0.3 (100)[B] ±0.6	2.7 (100)[B] ±0.6
Intramuscular	1/5 (20)	0/5 (0)	-	-	2.2 (100[A] ±2.2	3.8 (100)[AC] ±1.3
Vent drop	1/5 (20)	1/5 (20)	+	-	3.5 (100)[A] ±1.9	4.0 (100)[A] ±2.9
Oral	2/5 (40)	0/5 (0)	-	-	3.3 (100)[A] ±2.9	3.2 (100)[A] ±0.8
In-contact	5/5 (100)	2/5 (40)	+	-	1.7 (100)[A] ±0.6	4.0 (100)[A] ±0.0
Overall	13/35 (37)	5/35 (14)			2.2 (100)[A] ±1.9	3.4 (100)[B] ±1.4

a, IBD viral antigens absent from BF; b, IBD viral antigens present in BF
c, IBD viral antibodies absent in sera; SD, standard deviation
means with different superscripts on the same row or column are statistically different

Table 4.2: Morbidity, mortality and Ab response of IBDVAb-negative 4-week-old chicks vaccinated with a batch (03) of local IBD vaccine via different routes

Route of vaccination	Morbidity (%)	Mortality (%)	Viral antigens	Mean antibody (Log2) level in sera (%) ± SD		
				Days post-vaccination 0	7	14
Intraocular	3/5 (60)	2/5 (40)	+[a]	-[c]	1.0 (20)^{A} ±0.0	2.5 (100)^{A} ±2.1
Intranasal	2/5 (40)	2/5 (40)	+	-	0 (40)^{A} ±0.0	3.0 (100)^{A} ±1.0
Subcutaneous	2/5 (40)	1/5 (20)	+	-	1.0 (20)^{A} ±0.0	2.5 (100)^{AB} ±0.6
Intramuscular	2/5 (40)	1/5 (20)	+	-	2.0 (60)^{A} ±2.0	2.8 (100)^{A} ±1.5
Vent drop	2/5 (40)	2/5 (40)	+	-	0.5 (40)^{A} ±0.7	4.3 (100)^{AC} ±0.6
Oral	1/5 (20)	1/5 (20)	+	-	-	3.5 (100)^{A} ±1.0
In-contact	1/3 (33)	0/3 (0)	-[b]	-	0 (33)^{A} ±0.0	2.3 (100)^{A} ±1.5
Overall	13/33 (38)	9/33 (27)			0.9 (30)^{A} ±1.3	3.0 (100)^{B} ±1.2

a, IBD viral antigens present in the BF; b, IBD viral antigens absent in BF
c, IBD viral antibodies absent in sera; SD, standard deviation
means with different superscripts on the same row or column are statistically different

distended with urates. The chicks became sick and died irrespective of the route of vaccination. While the overall morbidity for 5-week-old chicks and 4-week-old chicks were comparable, the mortality rate was higher in the younger birds (almost double) although the difference was not statiscally significant ($P>0.05$). All the chicks that died had viral antigens in their BF irrespective of the route and age of vaccination. The batch of vaccine administered caused sickness and death and gross lesions that were similar to those caused by virulent IBDV isolated from the field (Okoye and Uzoukwu, 1982b).

None of the chicks had detectable PAb to IBDV at the time of vaccination. At 1 week PV, all the chicks vaccinated at 5 weeks were positive for PAb while only 20% to 60% of the birds vaccinated at 4 weeks were positive depending on the route of vaccination. By 2 weeks PV, however, all the chicks vaccinated in the two age groups were positive for IBDV Ab. The amount of PAb produced by 5-week-old chicks vaccinated IN, IO or in-contact was significantly higher ($P<0.05$) than the amount produced by 4-week-old chicks. For chicks vaccinated at 4 weeks or 5 weeks old, the mean Ab titres for each route of inoculation was significantly ($P<0.05$) higher 2 weeks PV than at 1 week PV. Based on the speed of eliciting an immune response and the amount of Ab produced, the VD, IM and PO in decreasing order of efficiency (Table 4.1 and 4.2) were the best.

One week PV at 5 weeks old the PAb levels of chicks vaccinated SC was significantly lower ($P<0.05$) than the level of chicks vaccinated via IN, IM, VD, PO or in-contact route. At 2 weeks PV the PAb level of chicks vaccinated SC was also significantly lower ($P<0.05$) than that of chicks vaccinated via IO or VD route.

4.4.0 Discussion

The morbidity and mortality observed following vaccination with vaccine 03 were unexpected. The experiment confirmed the pathogenicity of this batch of the vaccine for 4- and 5-week-old MA-free chicks. These findings are contrary to the manufacturers observations (Okeke and Tanimu, 1982; Aba-Adulugba et al., 1992) that the local IBD vaccine was safe for use in MA-free 5-week-old chicks at ten times the normal field dose ($10^{4.5}$ TCID 50 per bird) (Aba-Adulugba et al., 1992). In the present experiment, even at the recommended field dose, this vaccine produced clinical signs and gross lesions similar to those of birds exposed to the very virulent strain of the IBDV (VVIBDV) (Guan and Chua, 1993; Younus, 1994).

A batch (02) of the local IBD vaccine earlier administered to MA-positive chicks did not produce clinical signs but produced bursal atrophy and lymphoid depletion in chicks 2 weeks of age and older (Abdu, 1985; Abdu et al., 1987a). Okoye (1985d) stated that immunoprophylaxis against

IBD in Nigeria was unreliable. He reported two cases of IBD in birds vaccinated 2 to 4 days earlier and attributed these outbreaks to improperly attenuated vaccines. Adene et al. (1985a) also found a batch of the local IBD vaccine pathogenic and immunosuppressive. Based on the observations of Adene et al. (1985a), Okoye (1985d) and the findings of the present study, it is apparent that some batches of the local IBD vaccine being marketed may be pathogenic. The ability of some vaccines to cause infection and mortality have also been reported in the USA (Winterfield and Thacker, 1978; Lucio and Hitchner, 1979a).

It is possible that the vaccine strain virus has reverted back to virulence after several passages in susceptible chickens (Muskett et al., 1985) or the CEF cells in which it was produced (Okeke and Tanimu, 1982). The vaccine virus has earlier been reported to be highly pathogenic for CEF cells (Okeke and Tanimu, 1982).

If this batch of vaccine has been tested for pathogenicity in the laboratory, it would have been withdrawn from the market. However, it would have been the assumption of the producers of the vaccine that since IBD is endemic and wide-spread in Nigeria (Onunkwo and Momoh, 1981) chicks hatched from the country's hatcheries would invariably possess some level of IBDV MA in their sera and therefore be capable of resisting the pathogenic effects of the vaccine virus.

In UK and USA, several vaccines showed significant

variation in their safety, efficacy and immunosuppressive effect (Thornton and Pattison, 1975; Winterfield and Thacker, 1978). Commercial vaccines can be grouped, based on their effect on the BF essentially into non-pathogenic, mildly, intermediately or highly pathogenic vaccines. The first two are only effective in birds devoid of MA or those with low MA titres, while the highly pathogenic vaccines are needed for use in birds with high MA titres (Haffer, 1982). Intermediate vaccines are reported to be safe and able to elicit an immune response in birds with moderate MA titres. The vaccine examined in this study belong to the highly pathogenic group and therefore should be used only in chicks with high MA levels.

In a survey for MA in chicks only 30% to 32% and 8% to 11% of the chicks 1-day and 1-week-old respectively were found to possess high MA titres (Abdu and Audu 1987). The vaccines that would be most suitable for use in Nigeria should be mildly or intermediately pathogenic. Pathogenic vaccines could lead to IBD and the presence of incompletely attenuated vaccine 03 in the market might explain the vaccination breaks often observed in the field (Okoye, 1985d; Abdu, 1986a; Abdu et al., 1986a; Tong et al., 1993).

The local vaccine has been recommended for use in drinking water but could be administered by the IO or IM routes (Okeke and Tanimu, 1982). Of the six routes used in this study, the SC route produced the poorest results in terms

of safety, amount of PAb produced and the rate of PAb production. Although the VD route resulted in the production of the highest amount of PAb in vaccinated chicks, it was associated with more death than other routes. When all criteria were considered, the IM route appeared to be the best. Okeke and Tanimu (1982) found that the IM route resulted in the production of higher amounts of Ab to local IBD vaccine than the PO route even though the two routes were able to prevent sickness, death and bursal lesions in 100% of 5-week-old chicks when challenged with a VVIBDV. The most effective route for conferring immunity to MA-free chicks for most live IBD vaccines is the vent drop although the oral, eyedrop and subcutaneous routes could be used (Ehizokhale, 1982). The IM route is preferred for killed emulsion vaccines. Adult birds can be immunized against IBDV by IM or SC inoculation and not the oral route because the immune response was absent or minimal, however, the IM route was superior than the SC route (Hitchner, 1976).

Vaccine 03 spread from vaccinated to unvaccinated chicks; it is therefore highly transmissible. Having been widely distributed in the country it might be responsible for some of the outbreaks of IBD in vaccinated flocks in this country thereby complicating the efforts being made in controlling the disease. The amount of virus in the faeces or contaminated water or feed of vaccinated chicks ingested by in-contacts could not be controlled. When all the chicks consume high

amounts it is likely that they will all become sick.

An ideal IBD vaccine should elicit prompt, high level, uniform, and long lasting immunity in chicks and breeding stock without causing morbidity, mortality, reduced weight gain and damaging the immune organs (Ehizokhale, 1982).

Farmers are encouraged to report outbreaks of IBD in chicks recently vaccinated with vaccine 03 or any other vaccine for proper documentation and reporting to the manufacturers for the withdrawal of the product from the market for further attenuation.

It could be concluded from this study that vaccine 03 is capable of producing clinical disease in MA-negative chicks and therefore safer for use in the MA-positive chicks. Vaccine 03 is highly transmissible and therefore only a few birds in a flock need be vaccinated and the vaccine can easily be spread from vaccinated to unvaccinated flocks. Vaccinal antigens could easily be demonstrated in the bursae of vaccinated birds; making it possible for checking the viability of the vaccine within 3 days. In order to reduce the pathogenic effects of vaccine 03 and still produce high amount of PAb in vaccinates the IN and PO routes are recommended. Unvaccinated chicks should be prevented from contact with vaccinated chicks. Vaccine 03 should be attenuated further to reduce its pathogenicity.

Chapter 5

DETERMINATION OF THE SAFETY, IMMUNOGENICITY AND POTENCY OF VACCINE 03 IN CHICKENS WITH PASSIVE ANTIBODIES TO INFECTIOUS BURSAL DISEASE VIRUS

5.1.0 *Introduction*

An ideal IBD vaccine should elicit a prompt and uniform lasting immunity without causing damage to immune organs, and should not cause morbidity, mortality or reduced growth (Naqi et al., 1979). Each batch of vaccine produced is subjected to various quality tests; for sterility, viability, safety, potency and immunogenicity (Cullen, 1990).

Vaccines are to be free from bacteria, fungi, mycoplasmas and pathogenic viruses. A dilution of the vaccine is made in duplicate and subcultured in McConkey agar, nutrient broth, blood agar and mycoplasma media (PPLO base + 10% fresh yeast extract + 12 % horse serum). One set is incubated at room temperature and the other at $37^{0}C$ for 48 h and observed for growth of contaminants (Aba-Adulugba et al., 1992).

The viability of IBD vaccine is determined by injecting one field dose of the reconstituted vaccine into 10 nine-day old chick embryos onto the chorioallantoic membrane (CAM) (Thorton and Pattison, 1975). The extra embryonic fluids, CAM, and embryos are harvested 96 h later and ground and titrated for virus quantity. Titres below 3.0 Log 10 egg infective dose (EID) 50 per ml is considered as non-viable. Serial dilutions

of the vaccine from 10^{-3} to 10^{-8} could also be inoculated into chick-embryo-fibroblast-cell cultures. Vaccines with less than 6.5 Log 10 TCID 50 per ml are considered as non-viable (Aba-Adulugba et al., 1992).

In order to test for safety (vaccine reaction, pathogenicity), 1-day-old and 7-day-old chicks are vaccinated with one or 10 field doses by eyedrop or 100 doses IM of vaccine or inoculated with a virulent virus. Sickness and death are recorded over a period of 24 days. Depression of weight gain is assessed 4 days PV or PC. The bursal index is determined at 4,14 and 24 days after PV or PC. Gross and histological examination of the BF is also made (Cullen, 1990). Immunosuppression is determined by measuring the level of haemagglutination inhibition antibodies to ND vaccine (Thorton and Pattison, 1975; Sharma et al., 1989). The vaccine fails the test if it causes sickness, death, depression of weight gain, decrease in bursal index, any lesions in the BF or immunosuppression (Okeke et al., 1982; Cullen, 1990).

To test for potency (challenge reaction), 7-day-old chicks were vaccinated with one field dose of the vaccine and challenged with a virulent virus present in a clarified bursal homogenate, 10-14 days later together with unvaccinated chicks. The chicks are observed for 10-14 days for sickness and death. The bursal index is determined and histological examination made 14 days PC (Thorton and Pattison, 1975; Cullen, 1990). Controls should die (50%) or become sick and

survivors have lesions in the BF. Not more than 12% of the vaccinated birds should die, show signs or have bursal lesions (Cullen, 1990).

For immunogenicity (antibody response) test, serum samples are obtained from 14 and 21 days PV and tested for the presence of antibodies by the AGPT or VNT. The rate of immune response, the amount of antibodies produced, the percentage of birds with antibodies and how long the antibody lasts are determined. The vaccine fails if none of the chicks vaccinated have detectable antibodies by the AGPT (Okeke and Tanimu, 1982) or a titre of less than 1:640 or 5 Log2 by the VNT (Okeke and Tanimu, 1982; Aba-Adulugba et al., 1992).

In Nigeria, IBD outbreaks have been reported soon after vaccination (Okoye, 1985d; Abdu, 1986a; Abdu et al., 1986a). It has also been observed that vaccinated flocks were about five times more likely to suffer from IBD than unvaccinated flocks (Tong et al., 1993). The administration of poorly attenuated and pathogenic IBD vaccines has been advanced as a probable cause of these outbreaks (Okoye, 1985d; Abdu et al., 1986a). A batch (03) of local IBD vaccine has been found to be pathogenic for MA-free chickens (Chapter 4).

This study was, therefore, conducted to determine the safety, transmissibility, immunogenicity and potency of vaccine 03 in chickens passively immunized with antibodies to IBD virus (IBDV) when vaccinated via different routes.

5.2.0 Materials and Methods

5.2.1 Chickens

Isa-brown pullet chicks were purchased from a commercial hatchery in Zaria. They came from 20-week-old hens vaccinated with a live IBDV vaccine at 12 days of age. They were housed from day-old in a room previously washed, disinfected and fumigated. They were fed with grower's mash and given tap water ad libitum. At 3 weeks of age, they were moved into wire floored cages until the end of the experiment.

5.2.2 Vaccine and Vaccinations

Vaccine 03 (NVRI, Vom), a live virus vaccine for control of IBD was used. Each chicken was given orally (PO) 0.5 ml of reconstituted vaccine in PBS(pH 7.6) containing one field dose. A 0.2 ml of vaccine containing one field dose was given subcutaneously (SC) or intramuscularly (IM) per bird. For ocular (IO), nasal (IN), and vent drop (VD) routes, 50 ul of reconstituted vaccine was used. The chickens were observed for clinical signs and mortality for 1 week PV.

5.2.3 Challenge Virus and Challenge Studies

Bursae of birds that died as a result of an outbreak of IBD were pooled and a clarified homogenate (50% w/v) prepared in PBS (pH 7.6) containing penicillin, streptomycin, and amphotericin B at a concentration of 10,000 IU, 100 ug and 1 mg/ml respectively was used as a challenge virus. A 50 ul of

the homogenate was dropped onto the open eyes (25 ul per eye) of each bird. The homogenate contained $10^{6.7}$ BID 50 of the virus per ml (Appendix 2). Challenged chicks were observed for clinical signs and mortality for 1 week.

5.2.4 Infectious Bursal Disease Virus Antiserum

The antiserum was a pooled sera obtained from 8-week-old chickens challenged with a virulent IBDV at 5 weeks of age. The PAb titre of the antiserum was 4 Log2 by the QAGPT (Cullen and Wyeth, 1975).

5.2.5 Experimental Design

Thirty two chicks at 3 weeks of age were divided into 8 groups of 3, 4 or 5 chickens each and housed separately. The chicks were bled at 3 weeks of age via cardiac puncture and then inoculated intramuscularly in the leg with 0.5 ml of the IBDV antiserum. Three days after the inoculation, all the chickens were vaccinated with vaccine 03. Four chicks were not passively immunized and unvaccinated (controls) while three immunized birds were not vaccinated but housed together with chicks vaccinated orally(in-contacts). On the day of vaccination, the chicks vaccinated SC were bled for sera. At 7 and 14 days PV the chicks in all the groups were challenged with a virulent IBDV. At 7, 14 and 21 days PC, the chicks from all groups were bled for sera. The number of chicken that became sick or died within 1 week PV and PC were noted. The

sera collected were tested for the presence and or level of PAb to IBDV by the QAGPT.

5.3.0 Results

At 3 weeks of the age, none of the chicks had PAb to IBDV. On the day of vaccination (i.e 3 days after injection with antiserum) the sera of all the chicks in one of the groups passively-immunized had PAb to IBDV. The mean PAb titre was 0.5 Log2. At 7 days PV, none of the chicks had PAb in their sera but by 14 days PV, all or some of the chicks vaccinated via IM (33%),SC(100%) or VD (67%) route had PAb in their sera. Only chicks vaccinated SC (50%) or VD(40%) became sick and died within 7 days PV.

Chicks vaccinated via IO (60%),IM,(33%), VD(33%) or PO(40%) and non-passively-immunized unvaccinated (25%) became sick after IBDV challenge. Of these groups only chicks vaccinated via VD(33%) or PO(20%) and non-passively-immunised unvaccinated (25%) chicks died after IBDV challenge. Irrespective of the route of vaccination, at 7, 14 and 21 days PC all the chicks had PAb in their sera. The PAb titres at 14 days PC were higher than at 7 days PC for all the groups. The PAb titres 14 days and 21 days PC were comparable (Table 5.1).

Table 5.1: Challenge results in 3-week-old chicks passively immunised with IBDVAb and vaccinated with a batch (03) local IBD vaccine

Route of vaccination	One week post-vaccination Morbidity (%)	Mortality (%)	One week post-challenge Morbidty (%)	Mortality (%)	Mean antibody in Log2 (%) ± SD Days post-vaccination 0	7	14	Days post-challenge 7	14	21
Intraocular	0/5	0/5	3/5 (60)	0/5	ND[a]	-[b]	-	1.8 ±1.0 (100)	2.4 ±1.5 (100)	2.0 ±1.9 (100)
Intranasal	0/3	0/3	0/3	0/3	ND	-	-	1.5 ±0.0 (100)	2.7 ±0.6 (100)	3.0 ±1.7 (100)
Subcutaneous	2/4 (50)	2/4 (50)	0/2	0/2	0.5 ±0.5 (100)	-	+[c] (100)	2.0 ±0.0 (100)	3.0 ±0.0 (100)	2.3 ±1.2 (100)
Intramuscular	0/3	0/3	1/3 (33)	0/3	ND	-	+ (33.3)[d]	1.5 ±0.0 (100)	2.7 ±2.1 (100)	2.0 ±1.7 (100)
Vent drop	2/5 (40)	2/5 (40)	1/3 (33)	1/3 (33)	ND	-	+ (66.7)	2.0 ±1.0 (100)	3.0 ±0.0 (100)	3.7 ±1.0 (100)
Per os	0/5	0/5	2/5 (40)	1/5 (20)	ND	-	-	1.8 ±0.5 (100)	3.5 ±0.6 (100)	3.3 ±0.5 (100)
In-contact	0/3	0/3	0/3	0/3	ND	-	-	ND	2.0 ±1.0 (100)	1.7 ±0.6 (100)
Control	0/4	0/4	1/4 (25)[e]	1/4 (25)[f]	-	-	-	ND	3.0 ±0.0 (100)	2.5 ±0.7 (100)
Overall (%)	4/32 (13)	4/32 (13)	8/28 (29)	3/28 (11)				1.7 ±0.7 (100)	2.7 ±1.1 (100)	2.5 ±1.3 (100)

a, not done; b, negative for IBDV antibodies; c, positive for IBDV antibodies
d, percentage of birds with IBDV antibodies; e, percentage of birds sick; f, percentage of birds dead

5.4.0 Discussion

The results of this study shows that vaccine 03 would cause sickness and death when administered via the SC or VD routes. The study also show that only chicks (100%) vaccinated by the SC route were immunized 2 weeks PV and birds vaccinated via IO, IN, VD or PO route became sick, and or died following challenge with a virulent IBDV. The only group of chicks that did not become sick or died after vaccination and challenge were those vaccinated via the IN route. The safest route for administration of vaccine 03 in passively-immunized chicks is, therefore, IN.

Since vaccine 03 is pathogenic for MA-free chicks (Chapter 4) and passively-immunized chicks, all chicken flocks are, therefore, at risk of developing IBD after vaccination. The product is most probably one of the causes of many of the outbreaks of IBD in vaccinated birds observed in the field.

In a sequential study conducted by Lucio and Hitchner (1979a) on the interaction of passive Ab and age with IBDV infections, chickens with high levels of passive Ab were protected from BF atrophy for 2 weeks and immunosuppression for 4 weeks and their active immune response to IBDV was inhibited. As their passive Ab waned, their active immune response increased. The effect of the vaccine on the BF and immunosuppression in the passively-immunized chicks in this study were not examined; however, passive Ab prevented active immune response in chicks vaccinated IO, IN and PO.

Antibodies were expected to have appeared by the 7th or 14th day viously observed in MA-free chicks (Okeke et at., , 1988). The passive Ab might have been responsible for this delay observed in the antibody response in vaccinated chicks.

The results suggest that vaccine 03 will not cause sickness or death when administered to passively-immunized chicks via the IO,IN and PO routes. Furthermore, only chicks vaccinated IN with vaccine 03 will be protected from clinical disease when exposed to a virulent IBD virus 14 days PV. In addition vaccine 03 is capable of inducing the production of antibodies in passively-immunized chicks vaccinated via the SC,IM or VD route 14 days PV.

Chapter 6

DETERMINATION OF THE ANTIBODY RESPONSE AND PROTECTIVE ABILITY OF FOUR BATCHES OF A LOCAL IBD VACCINE IN CHICKS WITHOUT ANTIBODY TO INFECTIOUS BURSAL DISEASE VIRUS

6.1.0 Introduction

A live IBD vaccine was manufactured in Nigeria for the control of infectious bursal disease (IBD) (Okeke and Tanimu, 1982). Since its initial production in 1982, it has been widely used by poultry farmers. However, outbreaks of IBD in chicks that had been vaccinated with this vaccine appeared to be wide-spread (Okoye, 1984b; Ezeokoli et al., 1985; Okoye, 1985d; Abdu, 1986a). Several factors, amongst which is the use of inpotent vaccines, have been advanced as the causes of these outbreaks (Abdu, 1985; Okoye, 1985d; Abdu, 1986a). This study was therefore aimed at determining Ab response and presence of vaccinal Ag in chicks vaccinated with four batches of local IBD vaccine and the morbidity, mortality, presence of viral Ab and Ag of vaccinated chicks following challenge with a pathogenic IBDV.

6.2.0 Materials and Methods

6.2.1 Chicks

Sixty day-old cockerel chicks were purchased from SIMTU, Zaria. The chicks were housed in a room previously cleaned, washed, disinfected and fumigated. They were fed grower's mash and given tap water ad libitum.

6.2.2 Vaccines and Vaccination

Four batches of a live chicken-embryo-fibroblast cell culture-adapted IBDV vaccine manufactured by the NVRI, Vom were used for the vaccinations. They were batches 0 (Expiry Date - ED - 12/95), 01(ED1/96) 05(ED9/96) and 06 (ED7/95) and were all purchased from four different veterinary shops (Aldisa Ventures, GATS Group of Company, Mercy Holdings and IS-Agric) in Zaria. They were used before the expiry dates. Each individual bird was vaccinated orally with 1.0 ml of reconstituted vaccine in PBS (pH 7.6) containing one field dose per bird at 3 weeks of age.

6.2.3 Challenge Virus and Challenge Studies

A pooled clarified bursal homogenate (50% w/v) in PBS (pH 7.6) of bursae from birds that died of an outbreak of IBD was used as the challenge virus. The titre of the virus in susceptible chicks was $10^{4.4}$ BID 50 per ml of the bursal suspension. Penicillin, streptomycin and amphotericin B were added to the suspension at the rate of 10^5 IU/l, 100 mg/l and 2.5 mg/l respectively. Exactly 50 ul of the virus suspension was dropped onto the open eyes (25 ul per eye) of each bird. The chicks were observed for clinical signs and mortality daily for 1 week.

6.2.4 Experimental Design

Sixty 5-day-old chicks were divided into 5 groups of 13 birds each. The groups were identified as 0, 01, 05 and 06 representing the batches of the local IBD vaccine used, the 5th group was used as unvaccinated control. Six chicks from group 05 were bled at day-old and 3 weeks of age and their sera tested for the presence of IBDV PAb by the QAGPT (Cullen and Wyeth, 1975). Five chicks were bled from each group on the day of vaccination and 3 days PV. Two birds each from each group were sacrificed on days 3 and 7 PV and their bursae examined for IBD viral antigens. At 14 days PV, 5 birds from each group were bled for the detection of antibodies in their sera. The remaining 9 birds in each group were challenged at 5 weeks of age with a virulent IBDV as stated in 6.2.3 and observed for clinical signs and deaths. An additional 2 birds each from all the groups were euthanized and their bursae examined for viral antigens at 3 and 7 days PC. At 7 and 14 days PC, the sera of surviving chicks were tested for viral antibodies.

6.3.0 Results

At day-old, 33.3% (2/6) of the chicks sampled in group 05 had MA in their sera but by 3 weeks old (the day of vaccination) none (0/6) of the chicks tested carried detectable PAb to IBDV. At 2 weeks PV, none of the chicks had PAb detectable by AGPT. Antibodies to IBDV were detected in

[illegible] only after challenge. It was found in 40% of them [illegible] PC and in all (100%) chicks 2 weeks PC. Fifty percent of unvaccinated chicks had PAb in their sera at 1 week and all at 2 weeks PC. Viral antigens were not detected at 3 [illegible] days PV in the 5 groups but at 3 day PC they were detected in bursae of unvaccinated chicks and at 7 days PC in group 05 (Table 6.1).

All vaccinated and unvaccinated groups challenged with the IBDV became sick; 40% of birds in group 05, 40% of group 06, 60% of group 0, 20% of group 01 and 40% of control group [illegible]ome vaccinated chicks died; 20% of groups 05 and 0 and none from groups 06, 01 and control. The clinical signs and [illegible] lesions of challenged birds were similar to those [illegible]d for IBD. Signs seen were greenish yellow diarrhoea, dehydration, anorexia, weakness, ruffled feathers, dropped wings, depression and somnolence. Gross lesions were congestion of pancreas, spleen, thymus, BF and muscles. Others were pale kidneys with u[illegible] in the ureters, haemorrhages in the caecal tonsils, duodenal loop, thigh and leg muscles.

Table 6.1: Morbidity, mortality and Ab response due to IBDV in MAb-negative chicks after vaccination with four batches of a local IBD vaccine at 3 weeks of age

	Group of birds																			
	05				06				0				01				Control			
Age in days	Ab[a]	Ag[b]	Morb. (%)	Mort. (%)	Ab	Ag	Morb. (%)	Mort. (%)	Ab	Ag	Morb. (%)	Mort. (%)	Ab	Ag	Morb. (%)	Mort. (%)	Ab	Ag	Morb. (%)	Mort (%)
24 (3DPV)	0/3 (0) -	-			0/3 (0) -	-			0/3 (0) -	-			0/2 (0) -	-			0/2 (0) -	-		
28 (7DPV)	0/5 (0) -	-			0/4 (0) -	-			0/4 (0) -	-			0/4 (0) -	-			0/5 (0) -	-		
35 (14DPV)	0/5 (0) -	-			0/4 (0) -	-			0/5 (0) -	-			0/5 (0) -	-			0/5 (0) -	-		
38 (3DPC)		-[g]				-				-				+				+		
42 (7DPC)	1/4[c] (25)[d] 0Log2[e] ±0.0[i]	-[h]	2/5 (40)	1/5 (20)	3/4 (75) 1Log2 ±1.0	-	2/5 (40)	0/5 (0)	3/3 (100) 1.7Log2 ±1.2	-	3/5 (60)	1/5 (20)	2/4 (50) 1Log2 ±0.0	-	1/5 (20)	0/5 (0)	2/4 (50) 1Log2 ±1.4	-	2/5 (40)	0/5 (0)
49 (14DPC)	3/3 (100) 3.3Log2 ±1.2				3/3 (100) 5.3Log2 ±2.3				3.3 (100) 4.7Log2 ±0.6				3/3 (100) 7.7Log2 ±2.3				4/4 (100) 4.8Log2 ±1.0			

a, precipitin antibodies to IBDV in sera
b, IBD viral antigens in bursae
c, no of birds with antibodies over those tested
d, % positive for antibodies
e, mean antibody titre in Log2
f, negative for viral antibodies
g, negative for viral antigens
h, positive for viral antigens
i, standard deviation

6.4.0 Discussion

The 4 batches of local IBD vaccine administered in this experiment did not multiply in the bursae to amounts detectable by AGPT and could not elicit enough Ab response in all vaccinated chicks which subsequently succumbed to IBD following challenge with a virulent virus.

Outbreaks of IBD in birds that had 2 vaccinations with local IBD vaccine have been reported earlier (Okoye, 1984b; Ezeokoli et al., 1985; Kazeem, 1986). The failure of 4 batches of the local IBD vaccine to protect challenged birds and the occurrence of field outbreaks of IBD in vaccinated birds show that several batches of this vaccine being released into the Nigerian market may contain live virus incapable of multiplying in the bursal cells of the birds to induce an immune response. Birds vaccinated with such vaccines remain susceptible to IBD and outbreaks occur following natural exposure to virulent field viruses. These vaccination failures have continued to cause severe psychological stress and huge financial losses to poultry farmers in this country.

It might be argued that the vaccines used had lost their viability through poor handling, transportation, storage or administration. However, the local IBD vaccine is in a freezed-dried state, a condition that to some extent improves the thermostability of live vaccines (Spradbrow, 1991). In addition, the manufacturer of the vaccine had stated that the field dose is calculated so that at least one guaranteed dose

is still available to each bird even after incubation at 37°C for 7 days (Okeke and Tanimu, 1982). Moreover, IBDV is fairly resistant to heat (Benton et al., 1967b).

Batches of local IBD vaccine used by various workers in the 1980s were found to be immunogenic (Okeke et al., 1982; Abdu, 1985; Okoye, 1985c; Abdu et al., 1988). What had happened between the 1980s and now that affected the quality of the vaccine is difficult to explain. Could it be that the long continuous passage of the stock IBDV in chicken-embryo-fibroblast cells had adversely affected the quality of the batches of the vaccine produced now. High number of passages might have rendered the vaccinal virus less infective and less immunogenic. The methods of packaging, labelling and distribution of the vaccine may be open to abuse. For example, the original label on the vial can easily be removed and replaced with a new one and the identification number and expiry date can be altered since it is hand written. The label can also easily be produced by unscrupulous persons. It is pertinent to note that other workers had also observed the poor labelling system on vials of the local IBD vaccine (Aba-Adulugba et al., 1992).

The level of and percentage of birds with antibody to IBDV at day-old were low. This indicates that the parents of the chicks were either old or had not received IBD vaccine as a booster at point of lay. At 3 weeks of age (the time of vaccination) none of the chicks had detectable antibodies.

This is in agreement with earlier observations that MA is invariably absent in most or all chicks at 21 days of age or older irrespective of the source of birds (Abdu, 1985; Abdu and Audu 1987).

Viral antigens were detected at 3 or 7 days PC. However, not all infected and tested birds had viral antigens in their BF. Therefore, the absence of viral antigens in the BF by AGPT is not synonymous with the absence of IBDV infection. Previous exposure to the IBDV could best be confirmed by the presence of PAb in the sera either at 7 or 14 days PI. There is an urgent need to continuously test the viability, potency and safety of all vaccines imported or produced within the country. The testing should be conducted by an independent body other than the producers of the vaccines. It is also the responsibility of poultry farmers to organize into a strong body to ensure the marketing and sale of potent vaccines in the country and ensure that their birds are sero-monitored to determine sero-conversion after vaccination.

As the administration of 4 different batches of a local IBD vaccine could not result in Ab production and could not confer protection against challenge inoculation, the use of such vaccines in the field could explain the widespread vaccination failures commonly encountered in Nigeria. There is the need to ensure that potency tests are conducted on each batch of local IBD vaccine produced. Vaccinated chicks should be prevented from exposure to a virulent IBDV.

Chapter 7

STUDIES ON THE ANTIBODY RESPONSE AND PROTECTIVE ABILITY OF THREE IMPORTED INFECTIOUS BURSAL DISEASE VACCINES

7.1.0 Introduction

Locally-produced and imported live vaccines for the control of IBD are available in Nigeria. The imported vaccines are obtained from countries such as France, America, Italy, India, Holland, Germany, Israel, and the Cameroon. They are widely used because they are relatively cheaper and more easily available. However, because of delays on transit, the viability and potency of imported vaccines cannot always be guaranteed. Some of the vaccines are produced specifically for the control of IBD in their countries of origin. The climatic conditions, poultry management practices and epidemiology of IBD in these countries differ from those in Nigeria. It is therefore, not surprising that vaccination failures after the use of these vaccines in the field are being encountered (Okoye, 1984b; Ezeokoli et al., 1985; Abdu, 1986a). The strains of the IBDV used in producing the vaccines differ from one source to another and the vaccination schedules differ between vaccines. While some of the imported vaccines contain apathogenic virus, others contain attenuated virus. One of the vaccines, is recommended for use at 10 to 18 days of age while another, for vaccination at 1 and 4 weeks of age. Some of the vaccines are produced in bursal cells while others are derived from fibroblast cells or chick embryos (Giambrone, 1985).

This study was conducted to investigate the antibody response and protective ability of three imported IBD vaccines (A,B and C) when administered using different schedules or when given once to MA-free chicks.

7.2.0 Materials and Methods

7.2.1 Chicks

One hundred day-old Isa-brown pullet chicks were purchased from a commercial hatchery in Zaria. The chicks were housed in a room cleaned, washed, disinfected and fumigated. They were fed with grower's mash and given tap water ad libitum.

7.2.2 Vaccines

Live virus bursal disease vaccines A, B, and C were used. Four 3-day-old chicks were inoculated orally with one field dose of each vaccine. Two chicks were killed at 3 and 7 days PV and their bursae tested for viral antigens.

7.2.3 Challenge Virus and Challenge Studies

A pooled clarified bursal homogenate (50% w/v) in PBS (pH 7.6) of bursae from birds that died of IBD was used as the challenge virus. The titre of the virus in susceptible chicks was $10^{6.7}$ BID 50 per ml (Chapter 7) . Penicillin, streptomycin and amphotericin B were added to the suspension at the rate of 105 IU/l, 100 mg/l and 2.5 mg/l respectively. Exactly 50 ul of

the virus suspension was dropped on the open eyes (25 ul per eye) of each bird. The chicks were then examined daily for clinical signs and deaths for 2 weeks.

7.2.4 Vaccination

Each chick was vaccinated orally with 0.1 ml of reconstituted vaccine in PBS (pH7.6) containing one field dose per bird.

7.2.5 Experimental Design

Thirty-five of the day-old pullet chicks purchased were divided into 5 groups of 7 chicks each. One group was vaccinated with vaccine B vaccine at day-old and the other groups at 1, 2, 3 or 2 and 4 weeks of age. Another group of 35 day-old chicks were grouped as mentioned above and vaccinated with vaccine C using the vaccination schedules as for birds vaccinated with vaccine B. Two weeks PV each group was challenged with a virulent IBDV. Six and 4 unvaccinated chicks were also challenged at 2 and 3 weeks of age respectively. The birds were observed for sickness and death and the BF of dead birds examined for viral antigens by the AGPT (Cullen and Wyeth, 1975). Twenty 3-week-old chicks were divided into 4 groups of 5 chicks each. One group was vaccinated with vaccine B, another with vaccine A and two groups (A and B) with vaccine C. At 2 weeks PV, at 5 weeks of age, they were challenged with a virulent IBDV and observed

daily for 2 weeks for clinical signs and deaths. Dead birds were examined at post-mortem and the BF tested for viral antigens by the AGPT. In all the experimental groups mentioned above, each chick was bled and its serum tested for presence of Ab on the day of vaccination, at 1 and 2 weeks PV.

7.2.6 Statistical Analysis

The chi-square test was used to determine statistical differences in the number of chicks with PAb after vaccination at 3 or 2 and 4 weeks of age and the number of chicks that became sick or died in vaccinated and unvaccinated groups (Colton, 1974).

7.3.0 Results

None of the chicks vaccinated with vaccine B at 2 and 4 weeks of age became sick or died as a result of challenge. Two of the 5 chicks vaccinated with vaccine B at 2 weeks became sick after challenge but none of them died. About 20% to 100% and 17% to 33% of the birds vaccinated with vaccine C became sick and died respectively after challenge. Mortality was lowest (17%) in birds vaccinated with vaccine C at 2 and 4 weeks of age. Generally, morbidity and mortality rates were significantly ($P<0.05$) lower in vaccine B vaccinated chickens and higher in unvaccinated chickens (Table 7.1). Antigens were not detected in the bursae of vaccinated chicks at 3 and 7 days PV. Antigens were present in the BF of chicks vaccinated with vaccine C at 3 weeks and challenged at 5 weeks and in

Table 7.1: Challenge results in chicks vaccinated with iported IBD vaccine B

Age at vaccination (weeks)	Age at challange (weeks)	Unvaccinated			Vaccine B		
		No. of sick (%)	No. of dead (%)	Viral antigens in BF	No. of sick (%)	No. dead (%)	Viral antigens in BF[e]
1D[a]	2	4/6 (67)	4/6 (67)	-[b]	2/6 (33)	2/6 (33)	-
1	3	3/4 (75)	2/4 (50)	-	3/6 (50)	3/6 (50)	+[d]
2	4	ND[c]	ND		2/7 (29)	0/7 (0)	
3	5	ND	ND		2/5 (40)	1/5 (20)	+
2/4	6	ND	ND		0/7 (0)	0/7 (0)	
Over all		7/10 (70)[1]	6/10 (60)[2]		10/31 (32.3)[3]	6/31 (19.4)[4]	

a, day old
b, negative for viral antigens
c, not done
d, positive for viral antigens
e, bursa of Fabricius
1,3 x^2 = 5.07(P<0.05)
2,4 x^2 = 6.03(P<0.05)

Table 7.2: Challenge results in chicks vaccinated with imported IBD vaccine C

Age at vaccination (weeks)	Age at challange (weeks)	Unvaccinated			Vaccine C		
		No. of sick (%)	No. of dead (%)	Viral antigens in BF	No of sick (%)	No. dead (%)	Viral antigens in BF[e]
1D[a]	2	4/6 (67)	4/6 (67)	-[b]	ND[c]	ND	ND
1	3	3/4 (75)	2/4 (50)	-	1/5 (20)	1/5 (20)	-
2	4	ND	ND[c]		3/6 (50)	2/6 (33)	-
3	5	ND	ND		5/5 (100)	1/5 (20)	+[d]
2/4	6	ND	ND		5/6 (83)	1/6 (17)	+
Over all		7/10 (70)[1]	6/10 (60)[2]		14/22 (63.6)[3]	5/22 (22.7)[4]	

a, day old
b, negative for viral antigens
c, not done
d, positive for viral antigens
e, bursa of Fabricius
1,3 χ^2 = 0.17 (P>0.05)
2,4 χ^2 = 4.23 (P<0.05)

Table 7.3: Antibody response of chicks vaccinated with IBD vaccine D

Group	1	2	3	4	5
Age at vaccination (weeks)	1D[a]	1	2	3	2 and 4
Weeks after vaccination	Antibody response				
0	-[b] (0/5)	- (0/7)	- (0/7)	- (0/5)	- (0/7)
1	+[c] (1/6)	- (0/6)	- (0/7)	- (0/5)	- (0/7)
2	- (0/6)[d]	- (0/6)	- (0/5)	+ (4/5)	+ (5/5)
3					+ (5/5)
4					(5/5)
Over all	(4/15)[1]				(15/29)[2]

a, day old
b, absence of precipitin antibodies
c, presence of precipitin antibodies
d, no.positive for precipitin antibodies over the no. tested
1,2 $x^2 = 2.5$ (P<0.05)

Table 7.4: Antibody response of chicks vaccinated with IBD vaccine C

Group	1	2	3	4	5
Age at vaccination (weeks)	1D[a]	1	2	3	2 and 4
Weeks after vaccination	Antibody response				
0	ND[b]	+[c] (4/8)	-[d] (0/7)[e]	- (0/6)	- (0/7)
1	ND	- (0/7)	- (0/6)	- (0/5)	- (0/6)
2	ND	- (0/6)	- (0/5)	- (0/5)	- (0/5)
3					- (0/5)
4					+ (2/5)
Over all		(0/16)[1]			(2/28)[2]

a, day old
b, not done
c, presence of precipitin antibodies
d, absence of precipitin antibodies
e, no. positive for precipitin antibodies over no. tested
1,2 $x^2 = 0.04$ (P>0.05)

Table 7.5: Challenge results in 3-week-old chicks vaccinated with three imported IBD vaccines

Vaccine used	Day post-vaccination/ presence of vaccinal antibodies		Presence of viral antigens at 5, 6 or 8 days post challenge	Morbidity at three days post-challenge	Mortality after 14 days post-challenge
	7	14			
Vaccine D	-[a] (0/5)[b]	- (0/5)	+[e] (2/5)[f]	5/5[c]	2/5[d]
Vaccine C	- (0/5)	- (0/5)	+ (3/5)	5/5	3/5
Vaccine C	- (0/5)	- (0/5)	+ (1/5)	5/5	1/5
Vaccine A	- (0/5)	- (0/5)	+ (3/5)	5/5	3/5
Unvaccinated	- (0/5)	- (0/5)	+ (2/5)	5/5	2/5

a. absence of precipitin antibodies in sera
b. no. with precipitin antibodies over no. tested
c. no. of chicks sick over no. of chicks challenged
d. no. of chicks dead over no. of chicks challenged
e. presence of precipitin antigens in the BF
f. no. with precipitin antigens over no. tested

Antibodies were detected 1 week after vaccination with vaccine B in 17% of chicks and on the day of vaccination at 1 week of age with vaccine C in 50% of chicks. Birds vaccinated at 3 weeks and at 2 and 4 weeks of age with vaccine B had detectable antibodies 2 weeks PV. Only chicks vaccinated at 2 and 4 weeks of age with vaccine C had detectable antibodies 4 weeks PV (Table 7.3).

7.4.0 Discussion

Of the two vaccines used initially, vaccine B appeared to be the most immunogenic and protective. Vaccinating earlier than 3 weeks of age or using a single vaccination schedule gave poor results with both vaccines. Vaccinating chicks with vaccine B at 2 and 4 weeks of age gave the best results. Therefore, a single vaccination schedule with any of the vaccines will not confer immunity against exposure to a virulent IBDV in the field.

Vaccine C was developed to control the very virulent form of IBD (VVIBD)(Guan and Chua, 1993). It is to be administered to broilers at day-old, 11 and 21 days of age and to layers at day-old, 11 and 24 to 28 days of age. The rationale for day-old vaccination is to occupy the receptor sites of the BF and other primary lymphoid organs by a vaccinal IBDV strain as early as possible thereby preventing the entry and multiplication of the VVIBDV (Guan and Chua, 1993) in birds with high MA levels and antibody production in those with low

MA levels (Anonymous, 1995). This programme has been found to be effective in preventing VVIBD in Indonesia and China (Guan and Chua, 1993). As this vaccination schedule was not employed in the present study and no field tests have been conducted, it is difficult to confirm whether this vaccination programme will work for the control of IBD in this country.

Generally, day-old vaccination against IBD in MA-positive and negative chicks will not protect for more than 4 weeks. A booster dose is needed about 7 days prior to when clinical IBD occurs (Giambrone, 1985).

Vaccine B is also an "intermediate" vaccine developed by Dr. Lukert (Giambrone, 1983). The virus strain is not very invasive and is recommended for use in drinking water at 21 to 28 days of age when MA is absent (Giambrone, 1983; Mousa et al ., 1988). To leave chicks unvaccinated until this age in Nigeria is dangerous as the highest incidence of clinical IBD commences at 3 weeks of age (Okoye and Uzoukwu, 1982b; Tong et al., 1993). In the present study, vaccine B was found to be most effective when administered at 2 and 4 weeks of age.

"Intermediate" vaccines are said to be safe, invasive and immunogenic and also capable of spreading to susceptible chickens (Giambrone, 1985).

The comparative study on vaccine A,B and C showed that none of them was able to prevent sickness and death when exposed to a virulent IBDV 2 weeks after a single vaccination at 3 weeks of age. Of the three vaccines, vaccine A was the

worst. Chicks vaccinated with this vaccine died more acutely and gross lesions were more severe and widespread. It might be that the administration of vaccine A vaccine had increased the susceptibility of the birds to IBDV. This type of phenomenon has been observed by Ezeokoli et al. (1985).

The apparent immunization failures by the three vaccines when administered once might be due to loss of viability or potency, antigenic differences between the vaccine virus and the challenge virus, or the presence of high concentration of the VVIBDV in the challenge inoculum used. These factors highlight the need to isolate, purify and attenuate the IBDV strains in Nigeria for production of prototype vaccine.

The results of this study differ from those of Okoye (1985c) and Aba-Aduluyba et al. (1992). They found imported vaccines protective after exposure with a virulent IBDV. The sources of the vaccines used were different from those used in the present study, thus the strain of vaccine virus they used also differ from the ones used in this study. In addition, while the chicks in this study were 3 weeks old, the previous studies utilized 5-week-old birds, an age when chickens are most susceptible to IBD in this country (Bishu et al., 1977; Nawathe and Lamorde, 1982; Tong et al., 1993). The vaccines they used also caused histological changes in the BF of the vaccinates and the chickens sero-converted 2 weeks after vaccination. The vaccines used in these studies could be easily grouped as "hot" mildly-attenuated invasive vaccines

(Winterfield and Thacker, 1978; Giambrone, 1985). These "hot" vaccines were found to be non-immunosuppressive but were highly invassive and immunogenic in MA-positive and MA-negative chicks (Mousa et al., 1988). These vaccines will most likely be more appropriate for use in Nigeria.

Vaccination with three imported vaccines at 1 day old, 1, 2, or 3 weeks old could not completely prevent sickness or death due to challenge with a field virus 2 weeks PV. IBD-vaccinated chicks should be protected from virulent IBDV.

Generally vaccination with vaccine B and vaccine C reduced the percentage of chicks that became sick(3%-40%) or died(37%-40%) after challenge with a virulent field virus.

Vaccination of MA-free chicks with vaccine B at 2 and 4 weeks of age resulted in a prompt sero-conversion in all vaccinates and all birds subsequently challenged were protected from clinical disease. Vaccination of chicks free of MA with an "intermediate" IBD vaccine at 2 and 4 weeks of age is recommended.

Chapter 8

NEUTRALIZATION OF VIRULENT FIELD INFECTIOUS BURSAL DISEASE VIRUSES BY SERA OF CONVALESCENT, VACCINATED AND LOCAL CHICKENS

8.1.0 Introduction

Infectious bursal disease outbreaks in previously vaccinated flocks have been reported (Nawathe and Lamorde, 1982; Okoye, 1984b; Abdu, 1986a; Onunkwo and Okoye, 1991). Poor transportation, storage and administration have been suggested as some of the causes of the vaccination failures (Nawathe and Lamorde, 1982). Other causes suspected were the administration of pathogenic or non-potent vaccines (Okoye, 1985d), the presence of MA (Abdu, 1986b), the presence of highly invasive IBDV in the field (Okoye and Uzoukwu, 1982b) and the presence of IBDV serotypes, and subtypes (variant strains) (Okoye, 1996). In the western world serotype 1 and 2 IBDV exist (Abdu et al., 1986). Serotype 1 and 2 viruses are only 30% related; therefore, cross-protection is minimal (Giambrone, 1985). The viruses are highly invasive and pathogenic and able to overcome high levels of MA long before vaccine viruses can be effective (Giambrone, 1985).

This study was undertaken to determine whether antisera of birds (i) that had suffered from clinical IBD or (ii) have been vaccinated against the disease or (iii) local chickens whose experience with field or vaccine virus is unknown could neutralize two pathogenic IBDV obtained from natural outbreaks.

8.2.0 Materials and Methods

8.2.1 Chickens and Antisera

Six out of 8 6-week-old local chicks (T42) vaccinated with a local IBD vaccine at 3 weeks of age were bled via cardiac puncture for sera. Five apparently healthy adult local chickens (L) without any history of clinical IBD or vaccination against IBD were bled via the wing veins. The sources of these birds were unknown and varied as they were brought to Samaru market for sale.

Five out of 50 20-week-old layer cocks (C-20) without a history of vaccination against IBD and clinical IBD were bled via the wing vein for sera.

Five out of 100 13-week-old Harco black pullets (BLP 13) that had clinical IBD at 4-week-old after vaccination with a local IBD vaccine at 2 weeks old were bled for sera.

Five out of 100 16-week-old Harco black pullets (BLP 16) that had clinical IBD at 6 weeks of age after vaccination with a local IBD vaccine at 2 weeks old were bled for sera.

Five out of a total of 198, 13-week-old brown pullets (BrLP13) that had been vaccinated at 2 and 4 weeks of age with a local IBD vaccine were bled for sera.

Five out of a total of 131 16-week-old black pullets (BKLP 16) that had been vaccinated with a local IBD vaccine were bled via the wing vein for sera.

Five out of 5 5-week-old Isa brown pullet chicks (VB3WO) that were vaccinated at 3 weeks old with an imported IBD

vaccine were bled via cardiac puncture for sera.

Five out of 5 5-week-old Isa brown pullets (05) chicks vaccinated at 3 weeks old with a local IBD vaccine were bled via cardiac puncture for sera.

The blood samples were allowed to clot at room temperature and then centrifuged at 6.175 Xg for 15 min, the sera at the top were aspirated.

The sera from each flock were tested individually for the presence of PAb by the AGPT. Positive sera from each flock were pooled together and the amount of PAb present were determined by the QAGPT. The pooled sera were then stored at -20^{0}C until used in vitro neutralization testes.

8.2.2 Viruses

Inoculum 7327: This was the clarified bursal homogenate that contained viral antigens to IBDV obtained from Harco black pullets chicks that were vaccinated with a local IBD vaccine at 2 weeks and had IBD at 4 weeks old. Thirty eight of the 198 birds in the flock (19%) died. The presence of viral antigens in the bursa was confirmed by the AGPT. The virus titre was $10^{4.4}$ BID 50 per ml of the homogenate.

Inoculum 7558: This was obtained from Harco black pullets that were vaccinated with a foreign IBD vaccine at 6 weeks and had IBD at 14 weeks old. Eighty nine out of the 250 birds in the flock (36%) died. The virus titre was $10^{6.7}$ BID 50 per ml of the homogenate.

8.2.3 Quantitative Agar Gel Precipitin Test (QAGPT)

The QAGPT was conducted as described previously (Chapter 3) to determine the Ab levels in the pooled sera. The Ab titre was considered as the reciprocal of the highest dilution of serum in which precipitin was observed.

8.2.4 Virus-Neutralization Test (decreasing virus-constant serum method) (VNT) in Susceptible Chicks

The VNT as described by Cursiefen et al (1979) and Cullen (1990) with slight modifications was used.

The test was conducted as follows:

The pooled serum from each flock was inactivated at 56^{0}C in a water bath for 30 min. Ten fold serial dilutions of the bursal homogenate was made from 10^{-1} to 10^{-5} in PBS (pH 7.6) was made. 1 ml of the serum and 1 ml of diluted virus were mixed. The serum-virus mixture was shaken and incubated at 37^{0}C for 30 min. The steps in 3 and 4 were repeated with virus diluted 10^{-2} to 10^{-5}. 50 ul of virus diluted 10^{-1} to 10^{-5} was dropped each onto the eyes of 5 susceptible chicks to serve as positive controls. The chicks were sacrificed at 3 days PI and their bursae tested for IBD viral antigens by the AGPT.

8.3.0 Results

Of the 6 vaccinated flocks sampled, 5 had detectable PAbs that ranged from 0 to 3 Log2. The two flocks that had suffered from IBD and the local chickens also had detectable PAb(2-4

Log2) in their sera.

No end point for the viruses using the VNT could be obtained as viral antigens could be detected in the bursae of infected chicks even at the highest virus dilutions (10^{-5}). However, 4 antisera appeared to have better neutralizing ability than the other 6 tested (Table 8.1 and 8.2). The neutralizing ability was independent of the level of PAb in the antisera and the previous exposure experience with the IBDV either in the form of a vaccine or virulent virus.

Table 8.1: IBD virus neutralization tests in 3-week-old susceptible chicks

Identification of flocks	History of birds	Titre of undiluted serum Log2	Virus (7327) dilutions					
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Overall(%)
			No.with viral antigen/No. infected					
BLP 13	Vaccinated	1	1/3	0/3	1/3	1/3	0/3	3/15 (20)
BLP 16	-do-	0	1/3	1/3	1/3	1/3	1/3	5/15 (33)
T42	-do-	2	3/3	1/3	3/3	2/3	3/3	12/15 (80)
BrLP 13	-do-	2	3/3	3/3	2/3	0/3	3/3	11/15 (73)
C-20	Natural exposure	4	3/3	2/3	3/3	3/3	2/3	13/15 (87)
BkP 16	-do-	2	3/3	3/3	1/3	3/3	3/3	13/15 (87)
LC5	-do-	0	1/3	1/3	2/3	1/3	0/3	5/15 (33)
LC3	-do-	2	1/3	1/3	0/3	3/3	1/3	6/15 (40)
Control		-[a]	4/5	3/5	2/5		2/5	11/18 (61)

a, absence of precipitin antibodies

Table 9.2: IBD virus neutralization tests in 3 week old susceptible chicks

			Virus (7558) dilutions					
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Overall (%)
VB390	Vaccinated	3	5/5	1/5	4/5	2/5	3/5	18/25/(72)
05	-do-	a	5/5	2/5	4/5	3/5	3/5	17/25/(68)
Control				5/5	4/5	3/5	1/5	13/20/(65)

a, absence of precipitin antibodies

8.4.0 Discussion

The study showed that IBDV PAb-positive antisera could only partially neutralize IBDV even at a very high viral dilution. This disagrees with the findings of Winterfield and Thacker (1978) that detection of PAb always meant that a bird is immune to IBD.

The antisera used in the VNT might have contained some varying amounts of NAb as some of them were able to prevent the virus from infecting and multiplying in the bursae of infected chickens. It was observed earlier that NAb-positive chicks were susceptible to IBDV challenge and developed bursal atrophy in the absence of clinical disease (Weisman and Hitchner, 1978). It has also been observed (Lucio and Hitchner, 1979b) that the levels of NAb necessary to the IBDV vary with the invasiveness and pathogenicity of the virus strain. It is, therefore, possible that the virus used in this study is a highly invasive and pathogenic strain of the IBDV. This is supported by the fact that the virus was obtained from chicks that had died of IBD. The concentration of the virus used might have been very high. The control of IBD due to pathogenic viruses might be difficult as a vaccine capable of eliciting a strong immune response and producing high NAbs in vaccinated chickens is needed.

There is also the problem of the existence of antigenic diversity among IBDV. For example, using a cross-

neutralization test, six subtypes (variant strains) of serotype 1 IBDV were detected (Jackwood and Saif, 1987). Again chickens with Ab to serotype 2 IBDV cannot be protected against challenge with serotype 1 virus (Jackwood et al., 1985). Hyperimmune serum prepared by vaccinating chickens with two commercial vaccines could not prevent bursal lesions when the serum and a variant virus were incubated at room temperature for 2 h and inoculated into susceptible chicks (Hellwig, 1986). If the viruses used in this study were different subtypes, of the IBDV that produced the antibodies in the sera tested, complete neutralization would not occur, because some sites on the viruses critical for pathogenicity might not be destroyed by the Ab in the sera.

This study showed that vaccination or passive immunization may not protect a certain proportion of birds against IBD in case of exposure to the field virus. There is a need for antigenic characterization of IBDVs strains present in Nigeria.

Chapter 9

GENERAL CONCLUSIONS AND RECOMMENDATIONS

9.1.0 General Conclusions

Some batches of a local IBD vaccine are apparently highly attenuated, avirulent and could not confer complete resistance to young chicks with or without passive Ab to IBDV against a pathogenic field virus although fewer birds became sick or died when the local vaccine was administered at 2 and 4 weeks of age.

A batch of the local IBD vaccine is mildly attenuated, highly transmissible and pathogenic to chicks without passive Ab to IBDV. However, using the IN, IM or PO routes may reduce the virulence of the vaccine in chicks with passive Ab to IBDV.

Live imported IBD vaccines also failed to confer immunity to chicks without passive Ab to IBDV when administered PO once at day-old, 1,2 or 3 weeks of age. One of the vaccines was, however, able to confer solid immunity to chicks without passive Ab to IBDV when administered orally at 2 and 4 weeks of age.

Field viruses will cause immunodepression in birds previously vaccinated with a local IBD vaccine. Sera from IBD-vaccinated and naturally infectd chickens could not completely neutralize IBDV strains obtained from field outbreaks; therefore, some vaccinated chicks still stand the risk of having IBD.

9.2.6 Recommendations

There is the need to (i) further attenuate some batches of local IBD vaccine (ii) clearly label and indicate the strain and level of attenuation of each IBD vaccine being marketed (iii) isolate and characterize the field IBDVs in Nigeria, (iv) produce vaccines from local isolates of the IBDV and, (v) increase the frequency of vaccinations to three when highly attenuated batches of the local IBD vaccine are used; at 2, 3 and 4 weeks of age.

A uniform chick and breeder vaccination schedule for IBD control needs to be formulated for nation-wide adoption. IBD vaccines should be readily available at subsidized prices and in convenient or economic doses. IBD vaccines should be properly stored, transported, handled and administered. Other species of domestic birds, wild birds and vermin should be kept out of all chicken houses and farms. All forms of stress should be prevented and a high standard of hygiene and biosecurity be maintained.

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Appendix I: Calculation of virus titre

Log of virus dilution	Presence of viral antigen (Ag) in bursa (BF). Ratio	Individual No. without viral Ag in BF	No. with viral Ag in BF	Accumulated No. without viral Ag in BF	No. with viral Ag in BF	Total	Percentage with viral Ag in BF
0	3/4	1	3	1	14	15	93
-1	4/5	1	4	2	11	13	85
-2	3/3	0	3	2	7	9	78
-3	2/5	3	2	5	4	9	44
-5	2/5	3	2	8	2	10	20
-7	0/5	5	0	13	0	13	0

Proportionate distance = $\frac{\text{infected at dilution next above} - 50}{\text{infected at next above} - \text{infected at next below}}$

$$= \frac{78 - 50}{78 - 44} = \frac{28}{34} = 0.82$$

Titre = $10^{-2.82}$ bursal infective dose (BID)50 per 0.05 ml of undiluted viral suspension

Titre = $10^{4.41}$ BID50 per ml of undiluted viral suspension

Appendix 2: Calculation of virus titre

Log of dilution	Presence of antigen (Ag) in the Pf ratio	Individual No. without viral Ag	Individual No. with viral Ag	Accumulated No. without viral Ag	Accumulated No.with viral Ag	Total	Percent with viral Ag
-2	5/5	0	5	0	16	16	100
-3	4/5	1	4	1	12	13	92
-4	3/5	2	3	3	8	11	73
-5	4/5	1	4	4	5	9	56
-6	1/5	4	1	8	1	9	11

Proportionate distance = $\frac{56 - 50}{56 - 11} = \frac{6}{45} = 0.13$

Titre = $10^{-5.13}$ bursal infective dose (BID50) per 0.05ml of undiluted viral suspension

Titre = $10^{6.74}$ BID50 per ml of undiluted viral suspension.