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Effect of Intra-Ocular and Intra-Muscular Infectious Bursal Disease Vaccination on Immune Response against Newcastle Disease in Chicks

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Abstract
The aim of this study was to determine the best vaccine regimen for infectious bursal disease virus (IBDV) vaccination. It also determined the best regimen for IBDV Vaccine using intra-ocular and intra-muscular routes, which will minimize the effect of IBDV on Newcastle Disease virus(NDV) vaccine response. One hundred and twenty Isa-brown day old cockerels were kept in 10 groups with each group containing 12 birds. Each group was subdivided into two subgroups of six birds each based on the ages in days pre-inoculation. All the birds were administered with Newcastle disease Vaccine of Hitchner B1, Lasota and Komarov vaccines. Single inoculation of IBDV vaccine, were given to six groups while double IBDV vaccine were given to four groups. This was given by two different routes of intra-ocular and intra-muscular per each group. Serum samples were collected for six weeks for serological tests. Double intra-muscular IBDV inoculation on the (7+21) and (14+28) days against IBDV infections were the best vaccine regimen. Single intra-ocular IBDV inoculation was observed to be the best for IBDV vaccination which will minimize the effect of IBDV on NDV vaccine response. Histological, the bursa of fabricus of the birds with single and double intra-ocular IBDV inoculations showed no severe depletion of lymphoid follicle.Vaccination should be encouraged to prevent viral diseases such as NDV and IBDV in birds.

Keywords: Bursa Disease, Intraocular, Intramuscular, Inoculation, Infectious, Newcastle Disease, Vaccine

1. Introduction

Newcastle disease (ND) is a disease of chickens, turkeys, pigeons, and other avian species caused by a filterable virus, Newcastle disease virus (NDV) (Alexander, 1997). The disease

is a major problem in many countries of the world where poultry is kept and can cause mortality of 80 – 100% in unvaccinated chickens (Rahman *et al*., 2004). The disease is characterized by respiratory signs, nervous manifestations and diarrhoea (Alexander, 1990). Different isolates and strains of the virus may produce enormous variations in the severity of the disease in a given host (Alexander, 1997). In Nigeria, ND was first identified in Ibadan(Hill *et al*., 1953) and has since then been reported in other parts of the country being enzootic in both local and commercial poultry populations with occasional animal epizootics in highly susceptible flocks (Saidu and Abdu, 2008). The disease in Nigeria is controlled by vaccination using ND vaccines, Hitchner B1, La Sota and Komarov (Oladele *et al*., 2006). In Nigeria and more so in grown birds, ND vaccine La Sota seems to be the most popular because of its efficacy, availability, little or no adverse reaction and its easy method of administration in these adult birds. It has become traditional to administer ND vaccines; Hitchner B1 and La Sota through the intraocular routes in young chickens (from day old to 5 weeks) and this have been found to be very effective by farmers, if well applied. The most widely used method of administration of ND vaccine, La Sota in adult birds is orally through drinking water. Studies comparing the intraocular and oral routes of administration of these vaccines favoured the intraocular route (Degefa *et al*., 2004), but these were mostly in young chickens.

Infectious bursal disease (IBD), initially reported as Gumboro disease, is an acute, highly contagious virus infection of young chickens first described by Cosgrove 2 (1962), who found B lymphocytes to be the primary target cells (Kauffer and Weiss, 1980). IBDV is important because it causes clinical disease and mortality in chickens 3 weeks of age or older and prolonged immunosuppression of chickens infected early in life leading to other infections and vaccination failures (Lukert, 1997). Its immunosuppressive effects were reported by others (Allan *et al*., 1972). Infection with IBDV reduces antibody response to other vaccinations (Muller, 2003, Westbury 2008), but the response against IBDV itself is normal. The present study was consequently undertaken to evaluate the effects of experimental IBDV infection in chickens by assessing the humoral responses of chickens to influenza virus subtype H9N2 in addition of its effects on H9N2 AIV pathogenicity for broilers. The route of administration of each vaccine of NDV and IBDV is very important with a view to prevent their occurrences. This project work is based on studying the effects of intra-ocular administration of vaccines for NDV and IBDV.The aim of this study was to determine the best vaccine regime for IBDV and the best regime of IBDV vaccination which will minimize the effect of infectiousbursal disease virus on immune response against Newcastle disease virus in chicks

2. Materials and methods

2.1. Experimental birds

One hundred and twenty Isa-brown day old cockerels were bought for the research work.

They were fed with starter and growers mash respectively. The birds were given food supplements (vitamins). The birds were reared intensively in deep litter systems in an animal house.

2.2. Grouping

According to the method ascribed by Oguoma *et al* (2012), one hundred and twenty day old cockerels were grouped into ten groups consisting of 12 birds per group. Each group consists of two sub-groups with each of the sub-group consisting of six birds.

These groups included six groups for single vaccination which are groups (7), (14), (18), (21), (24), (28) and four groups for double vaccination which are groups (1+14), (7+21), (10+24) and (14+28). The groupings were based on the ages in days pre-inoculation.

2.3. Vaccines Source

Newcastle Disease Vaccines (NDV), Hitchmer BI, Lasota, Kamarov and Infectious Bursal Disease Vaccines (IBDV) were obtained and produced by the National Veterinary Research Institute, Vom in Plateau State, Nigeria were used for the research work. Each of the vaccines was reconstituted as recommended by the manufacturer in phosphate buffered solution using a sterile syringe and needle. These vaccines were reconstituted and given to the 120 birds.

2.4. Inoculation of Experimental Birds

2.4.1. Inoculation with Newcastle Disease Vaccines

According to the method described by Oguoma *et al* (2012), NDV Hitchmer BI was given intra-ocularly to all birds on the first day, NDV Lasota vaccine on the 14th day intra-ocularly and NDV Kamarov vaccine intra-muscularly on the 28th day of the bird's arrival.

2.4.2. Inoculation with Infectious Bursal Disease Vaccine (IBDV)

According to the method described by Oguoma *et al* (2012), reconstituted Gumboro vaccine was inoculated by intra-ocular and intra-muscular routes to two different routes for the two different sub-groups in each group (one route per sub-group). Reconstituted 0.2ml of Gumboro vaccine was inoculated into each of the birds according to ages in days. The groups inoculated were as follows: (7), (14), (18), (21), (24), (28) for single inoculation and groups (1+14), (7+21), (10+24), and (14+28) for double inoculation. The groupings are based on the ages in days pre-inoculation.

2.5. Collection of Blood Samples from Birds

According to the method described by Oguoma *et al* (2012), using 2mls syringes and 26G needles, blood samples was collected from all the birds through the jugular veins. The zone of insertion was properly swabbed with cotton wool, soaked in methylated spirit before collecting the blood. The syringe containing the blood sample was slanted and allowed to clot and retracted for a good yield of serum. The serum was then aspirated from the syringes into sterile labelled vials and stored in a freezer at -20^{o}c. The serum samples were used to test for antibodies against infectious bursa disease virus by Agar Gel Immuno-Diffusion Test (AGIDT). Blood collection was at weekly intervals for 5 weeks post IBD virus inoculation.

2.6. Harvesting of Bursa of Fabricus

According to the method described by Oguoma *et al* (2012), on the 5th and 10th days post infection respectively, one bird from each group was slaughtered and bursa of fabricus

surgically obtained for gross and histopathological examination. The bursa tissues were preserved in 10mls of 10% formalin (formal saline). Uninfected bursa of fabricus was also collected and preserved. The gross examination was made before fixing in 10% formal saline. The bursa samples were histologically processed, embedded in paraffin wax sectioned at 5µ thick using rotary (Glick, 1983).

2.7. Serology

Haemagglutination inhibition technique was used to detect Newcastle antibody and Agar-Gel Immuno-Diffusion Technique (AGIDT) was used to detect antibody production to IBD virus by the experimental birds (Culler and Wyeth, 1975; Marguardt *et al.,*1980; Thayer and Beard 1998).

3. Results

3.1. Effects of Single Intra-Ocular IBD Inoculation

Response to Newcastle Disease: Newcastle HI antibody was detectable throughout the five weeks post IBD inoculation assay at between $\log_2$ values of 1 and 10 with 2/3 between 4 and 10. Newcastle antibody titre increased from the 7th day post vaccination to the fourth week before declining (Fig.1).

Response to Infectious Bursa Disease: Single IBD inoculation at the 7,14 and 18 days failed to produce precipitating antibodies till the fifth week post-inoculation. Precipitating antibody was produced on day 21, 24 and 28 at the fifth week post inoculation (Table 1).

3.2. Effects of Double-Intra-Ocular IBD Inoculation

Response to Newcastle Disease: Newcastle disease antibody was detected throughout the five weeks though it was generally lower than those of single inoculation. Its $\log_2$ value of 2 to 7 with 2/3 between 3 and 7 (Fig.1).

Response to Infectious Bursa Disease: IBD inoculation produce detectable precipitating antibody on days (7+21), (10+24) and (14+28) only on the fifth week. No detectable antibody was detected in the first four weeks post-inoculation (Table 1).

Table1: Agar gel immuno-diffusion test (AGIDT) at weekly interval post inoculation for the various groups of birds inoculated with IBV vaccine by Intra-ocular route

Ages in days Pre-inoculation	1st week	2nd week	3rd week	4th week	5th week
GRP (7)	-	-	-	-	+
GRP (14)	-	-	-	+	-
GRP (1+14)	-	-	-	+	-
GRP (18)	-	-	-	+	-
GRP (21)		-		+	+
GRP (7+21)	-	-	+	+	+
GRP (24)	-	+	-	-	+
GRP (10+24)	-	+	-	-	+
GRP (28)	-	+	-	+	+
GRP (14+28)	-	+	-	-	+

Keys:
+ = Positive,
- = Negative

3.3. Effects of Single Intra-Muscular IBD Inoculation

Response to Newcastle Disease: Newcastle antibody was detectable throughout the five weeks post IBD inoculation assay at between $\log_2$ values of 1 and 9 with 2/3 between 4 and 9. Newcastle disease antibody increased from 2 at age of 7 days to 9 at fourth week and declined to 6 in the 5th week (Fig.2).

Response to Infectious Bursa Disease: No detectable antibody was produced in days 7 and 14 for the five weeks. Days 24 and 28 showed detectable antibody from the 2nd week to the 5th week post inoculation (Table 2).

3.4. Effects of Double Intra-Muscular IBD Inoculation

Response to Newcastle Disease: Newcastle disease antibody was detectable throughout the five week post inoculation. However Newcastle disease antibody titre increased from 4 to 9 within the first three weeks. Though 2/3 at between 3 and 9 (Fig.2)

Response to Bursa Disease: No detectable IBD antibody was detected for the five weeks of the assay in days (1+14). Only 14+28 days showed IBD antibody from 2 to 5th weeks. Days (7+21) and (10+24) showed antibody from the third week to the fifth week of assay (Table 2).

Table 2: Agar gel immuno-diffusion test (AGIDT) at weekly interval post inoculation for the various groups of birds inoculated with IBV vaccine by Intra-muscular route

Ages in days Pre-inoculation	1st week	2nd week	3rd week	4th week	5th week
GRP (7)	-	-	-	-	+
GRP (14)	-	-	-	-	-
GRP (1+14)	-	-	-	-	-
GRP (18)	-	-	-	-	+
GRP (21)		-	+	+	+
GRP (7+21)	-	-	+	+	+
GRP (24)	-	+	+	+	-
GRP (10+24)	-	-	+	+	-
GRP (28)	-	+	-	+	+
GRP (14+28)	-	+	+	+	+

Keys:
+ = Positive,
- = Negative

3.5. Gross Pathology of Bursa of Fabricus

3.5.1. Bursa of Uninfected and Experimental Birds

Appeared whitish, ovoid shaped and measuring up to 0.5cm in diameter. The cut surface displayed several lobulations. The colour of all the bursa had changed from creamy to white colour by the 5th day to grey colour by the 10th day. Reduction in size were noted from the 5th

to the 10th day post infection. Gelatinous surface was evident on the surface of all uninfected bursa. All displayed several lobulation on sectioned surfaces.

3.6. Histopathology Examination of Bursa of Fabricus

3.6.1. Bursa of Uninfected Birds (Normal Control)

Bursa of uninfected birds displayed large active follicles separated by clear stroma with little interfollicular tissue. Lymphoid cells were distinguished within the follicles (Fig.3).

3.6.2. Bursa of Infected Birds

Three types of histological lesions were distinguished among the bursa of infected birds. Patchy infiltration of the stroma by inflammatory cells (Fig.4 and 7). This was noted in groups 24,28,and (10+24),(14+28) that received infectious bursa disease virus intra-ocularly and in groups 21,24, (7+21), (14+28) that received infectious bursa disease virus intra-muscularly(Table 3)..

Generalized lesion with mild necrosis and minimal disintegration of follicle (Fig.6). This was noted in groups 7,14,18,21 and (1+14), (7+21) intra-ocularly and also in groups 7,18, (10+24) intra-muscularly Table 3).

Severe disintegration, necrosis and loss of follicles (Fig.5). These were noted in group 14 intra-muscularly and no group sustained this lesion intra-ocularly (Table 3).

Table 3: Histopathological examination of bursa for the various groups of birds inoculated intraocularly and intramuscularly with IBD virus

Description of bursal lesions	Groups of experimental birds			
	Single intraocular Dose IBDV	Double intraocular Dose IBDV	Single intramuscular Dose IBDV	Double intramuscular Dose IBDV
Patchy infiltration of the bursal stroma (mild inflammatory)	24,28	(10+24), (14+28)	22, 24, 28	(7+21), (14+28)
Generalized lessen with mild necrosis and minimal distintegration of follicle (moderate inflammatory)	7,14,18,21	(1+14), (7+21)	7,18	(10+24) (1+14)
Severe depletion of lymphoid follicle	None	None	14	(1+14)

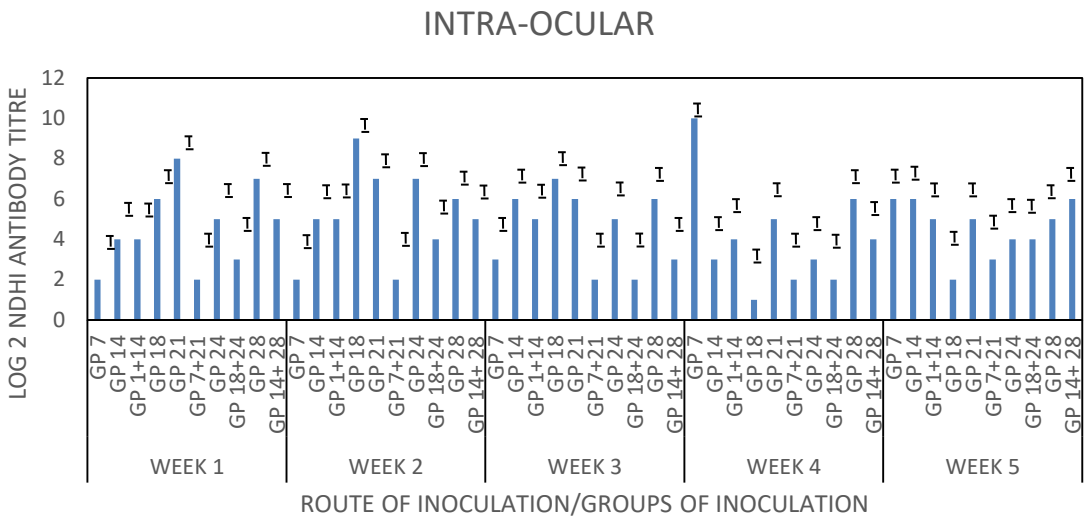

Fig. 1: Newcastle Disease Haemagglutination Inhibition (HI) Titre at Weekly Interval Post Inoculation for Various Groups of Birds Inoculated With IBV Vaccine By Intra-Ocular Route

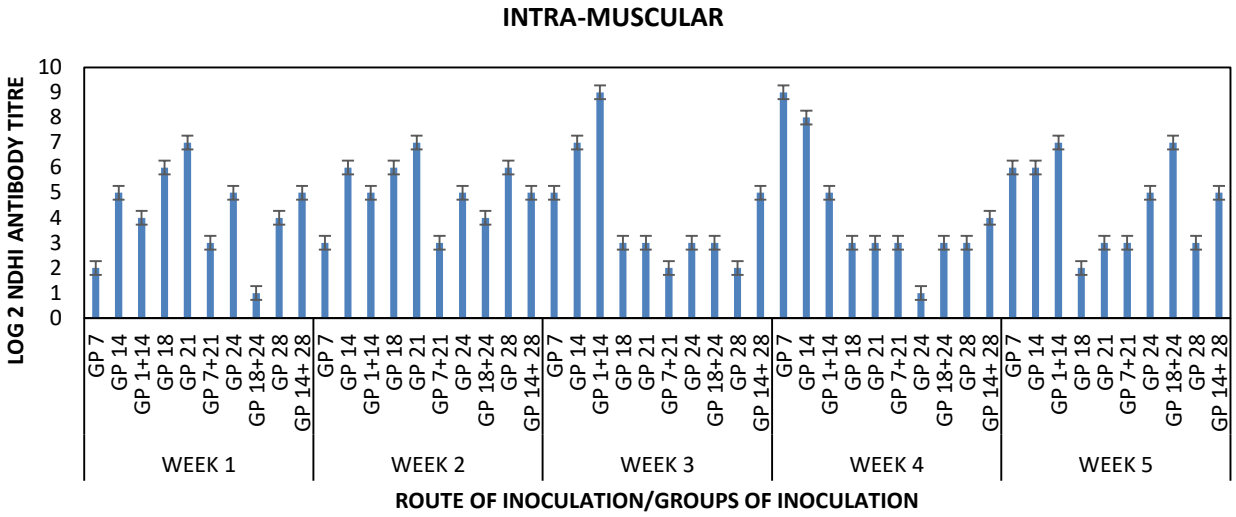

Fig. 2: Newcastle disease Haemagglutination inhibition (HI) titre at weekly interval post inoculation for various groups of birds inoculated with IBV vaccine by intra-ocular route

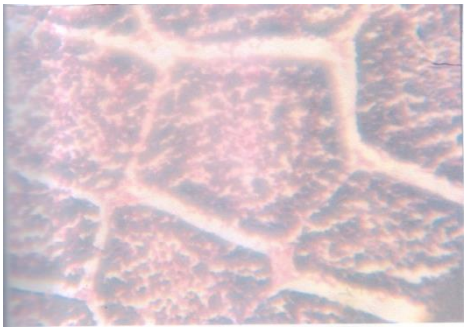

Fig. 3: Histopathological section of bursa of fabricus showing normal tissue with large active lymphoid follicles and clear stroma, H&E x 40

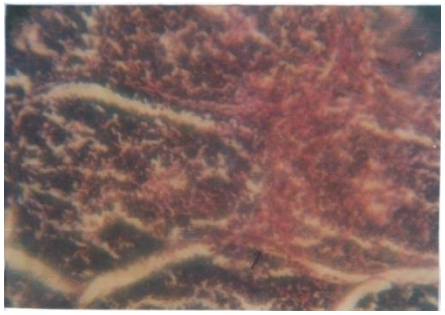

Fig 4: Histopathological section of bursa of fabricus of a bird inoculated with infectious bursal disease virus showing patchy lesions and infiltration of the stroma by inflammatory cells. H&E x 40

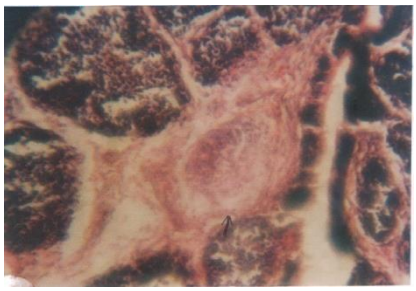

Fig. 5: Histopathological section of bursa of fabricus of a bird inoculated with infectious bursal disease virus showing severely depleted lymphoid follicle. H&E x 40

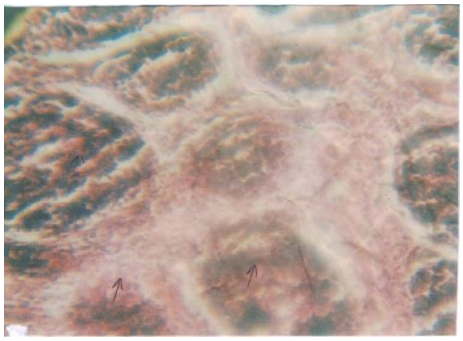

Fig. 6: Histopathological section of bursa of fabricus of a bird inoculated with infectious bursal disease virus showing general lesions, infiltration of the stroma by inflammatory cells. H&E x 40

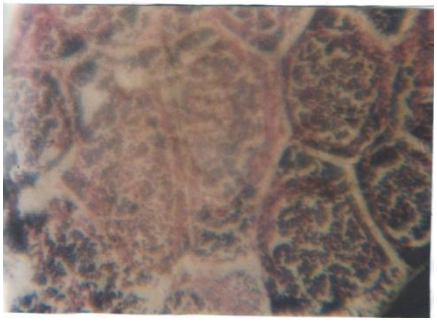

Fig. 7: Histopathological section of bursa of fabricus of a bird inoculated with infectious bursal disease virus showing patchy and mild infiltration of the stroma by inflammatory cells. H&E x 40

4. Discussion and Conclusion

Intraocular administration of ND vaccines is mostly restricted to Hitchner B1 in day old chicks and frequently to La Sota in chicks less than 5 weeks of age. Here vaccines reach the mucous membranes of the nose, the Harderian and paranasal glands at high concentrations and maternal antibodies do not interfere with immune response (Kouwenhoven, 1993). Alders and Spradbrow (2001) also reported that administration of ND vaccines via drinking water is easier, but provokes a lower level of immunity than eye drop administration and requires more frequent application. More so, the intraocular route of vaccination in grown or older chickens provides better immune response when compared with the oral route of vaccination (Okwor *et al*., 2013). Therefore, this research chose to administer the vaccines via intraocular and intramuscular routes.

In this research, it was observed that NDHI antibody was detectable throughout the five weeks in both single and double intraocular and intramuscular routes post IBD inoculation assay. However, ND antibody were generally lower in birds with double IBD inoculation than those with single IBD inoculation. Molecular interferences have been associated with this effect by previous researchers (Otim *et al*., 2005; Okwor *et al*., 2013).

Considering their responses to AGIDT, single intraocular IBD inoculation of the 21st and 28th day produced longer and higher NDV antibody in the experimental birds. Although the 24th Double intraocular IBD inoculation of the (1+14) and (14+28) also showed longer and higher antibody responses in birds. In both cases, the single inoculation of day 21 produced a better response than the other single and double inoculations. Various factors have been noted to affect the antibody production or responses in chickens, prominent among them are route of vaccine administration and the age of the chicken at vaccination (Okwor *et al*., 2013).

In this work, considering the single intraocular IBD inoculation, Newcastle HI antibody was detectable throughout the five weeks post IBD inoculation assay at between log2 values of 1 and 10. Newcastle antibody titre increased from the 7th day post vaccination to the fourth week before declining. The 21 and 8 days post inoculation show a greater antibody titre. Also, for the double intraocular IBD inoculation, Newcastle disease antibody was detected throughout the five weeks though it was generally lower than those of single inoculation. Its log2 value of 2 and 7 with 2/3 between 3 and 7. Otim *et al*., (2005) reported that Newcastle disease antibody levels after IBDV infection in chickens were lower than those of the control group, but they were still above log mean 25.2, the 100% protective titer. Ramirez *et al*. (2010) reported that previous history of IBDV infection in chickens may alter host range, tissue tropism or virulence.

Single intraocular IBD inoculation at 7, 14, and 18 days failed to produce precipitating antibodies till the 5th week post inoculation. These effects have been explained by previous researchers to be caused by maternal derived antibody which can stampede or impede the attenuated virus in the vaccine thereby producing limited response. However, considering

other results, Precipitating antibody was produced on day 21 at the 4th and 5th week post inoculation. Only day 28 showed on 2nd, 4th and 5th weeks. On day 24 detectable antibody was detected in the 2nd and 5th post inoculation. However, Double IBD inoculation produce detectable precipitating antibody on days (10+24) and (14+28) only on the 2nd and 5th week post inoculation.(7+21) showed IBD antibody from 3rd to 5th weeks for both intraocular and intramuscular and also in days(21). On the other hand (1+14) produced no detectable antibody for the 5 weeks post inoculation.

Double inoculation of IBDV reduced the production of antibody or response. However, Motamed *et al.,* (2013) reported that previous infection with infectious bursal disease virus promoted the propagation of avian influenza virus was reported by .Ramirez *et al*. (2010) reported that previous infection of IBDV in 25 chickens may render them more susceptible to avian influenza virus (AIV) infection, allowing for the potential introduction of AIVs in an otherwise resistant population. Inoculation of chicks with IBDV prolonged AI virus excretion from cloaca and trachea comparing with AIV group, suggesting that this immunosuppressive agent may have also interfered with immune mechanisms that could have prevented virus replication (Otim *et al*., 2005).

Though many authors have compared the routes of vaccination against ND, this has been mostly in young chickens less than 10 weeks of age and their results have either favoured one route or the other (with most favouring the intraocular route) or shown no difference (Orthel *et al*., 1981). The results of the above investigation are in agreement with that observed by many workers which showed higher antibody responses in chickens vaccinated through the intraocular route and found that the eye drop method of vaccination gave a more uniform protection. Therefore, intraocular vaccination is a better route of Newcastle disease vaccine in birds than oral route and hence should be encouraged for use by farmers (Nwiyi *et al*., 2013).

The histopathology of the bursa of fabricus of the birds in groups 24, 28 for single intraocular, groups (10+24), (14+28) for double intraocular, groups, 21, 24 and 28 for single intramuscular as well as in groups (7+21) and (14+28) for double intramuscular inoculation of infectious bursal disease vaccine showed patching agreement and mild inflammation of the stroma. This is in agreement with Hair-Bejo *et al.,* (2004) who reported in his study that broiler chickens vaccinated with intermediate strain of live attenuated IBD vaccine showed from day 21 to day 28, lesions ranging from mild to intermediate.

However, Igwe *et al.,* 2017 reported that between 21 days and 42 days, chickens are highly susceptible to severe IBD infection and also that the mortality rates is highest in weak chicken breeds. Perhaps the vaccination may have showed down the susceptibility of the chicken to the virus which resulted to patchy and mild inflammation of the stroma of bursa in those groups.

The study revealed that inspite of the intermediate vaccine fibrogumbovac which was used, generalized lesion with mild necrosis and minimal or moderate inflammation of the follicle were observed in groups 7,14, 18 and 21 for single intramuscular inoculation. This was also observed in groups (1+14), (7+21) and groups (10+24), (1+14) for double intraocular and intramuscular respectively.

Severe distintegration of the follicle necrosis of cell and loss of lymphoid cell and follicle were observed in bursal of fabricus of birds inoculated either by single or double intramuscular route. The gross pathology also showed that all these groups had atrophied bursa. However, severe disintegration of follicles, and loss of lymphoid cells was observed only in birds vaccinated in groups 14 for single and group (1+14) for double intramuscular routes respectively. This finding is in agreement with Al-sereah, (2007), that severe lesions like sblood vessel dilatation, inferior proliferation of the follicles in the bursa of fabricus and hyperemia after three days of vaccination was observed in 14th day administration of birds with intermediate vaccine (Cevac). The hiostopathological study showed that intraocular route of vaccination of the birds is the best.

5. Recommendation

Vaccination is a good means of preventing viral diseases particularly Newcastle disease in birds. Intraocular administration is a reliable method of delivering the vaccines and should be encouraged. From this research, Single inoculation on the on the 21st and 28th days as well as the double (7+21) inoculation were observed to be the best period for IBDV which will minimize the effects of IBDV on NDV vaccine response.

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