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Comparative Studies on the Oral and Intraocular Routes of Administration of Newcastle Disease Vaccine, La Sota in Adult Chickens.

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Abstract: *Two hundred and fifty (250) white cockerels were used to compare the differences between the intraocular and oral routes of administration of ND vaccine La Sota in adult chickens. At 20 weeks of age, serum samples were collected from the chickens and used to estimate the antibody levels against Newcastle disease virus using heamagglutination inhibition (HI) test. The birds were randomly divided into two groups, G1 and G2. Birds in G1 were vaccinated with ND vaccine La Sota intraocularly while birds in G2 were vaccinated orally. Serum samples were collected from chickens in the two groups weekly and used to estimate the HI antibody. Result showed higher antibody level in the chickens vaccinated intraocularly when compared with those vaccinated orally. There was significant difference ($p < 0.05$) between the geometric HI titres of chickens in the two groups on days 7, 14 and 28 PV but it did not differ significantly on day 21 PV. Challenge studies showed 100% protection of chicken in the two groups on day 21 PV. We identified Harderian glands' possible roles in producing local and humoral immunity in intraocular vaccinations and the destruction of some of the vaccinal viruses by gastric secretions in oral vaccinations as the possible reasons for the production of higher antibodies in intraocular vaccination in adult chickens.*

Keywords: *Oral and intraocular routes, Newcastle disease, La Sota vaccine, chickens, immune responses*

I. Introduction

Newcastle disease (ND) is a disease of chickens, turkeys, pigeons, and other avian species caused by a filterable virus, Newcastle disease virus (NDV) [1]. 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 [2]. The disease is characterised by respiratory signs, nervous manifestations and diarrhoea [3]. Different isolates and strains of the virus may produce enormous variations in the severity of the disease in a given host [1]. In Nigeria, ND was first identified in Ibadan [4] and has since then be reported in other parts of the country being enzootic in both local and commercial poultry populations with occasional animal epizootics in highly susceptible flocks [5, 6, 7]. The disease in Nigeria is controlled by vaccination using ND vaccines, Hitchner B1, La Sota and Komarov [8]. 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 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 [9, 10], but these were mostly in young chickens.

There have been reports of frequent outbreaks of ND in vaccinated adult and laying chickens in Nigeria. An opinion among farmers and even professionals is that the oral route of administration on deep litter does not result in even distribution of the vaccine and that this may be one factor among other factors contributing to vaccine failure. It has been noted that drinking water vaccination can give varying results due to the variation in water intake between birds [11]. Because of this, it is becoming common to see farmers apply ND vaccine, La Sota through the intraocular route in adult birds and layers notwithstanding the labour involved. Since most of the comparative studies have been in young chicks, this study compared the two routes in grown or adult chickens with a view of noting whether there are differences in sero-conversion and protection.

II. Materials and Methods

Chickens: Two hundred and fifty (250) white cockerels obtained from a reputable hatchery in Nigeria, CHI Limited, Ibadan were used in the experiment. The birds were obtained at day old. The vaccination and medication history of the parent stock were not known. The birds were reared on deep litter system with food and water given *ad libitum*. Vaccination schedule as used locally was carried out on the chickens. At day old, ND vaccine, Hitchner B1 was given through intraocular route. At day 14, the chickens were vaccinated against Infectious bursal disease (IBD) using live attenuated IBD vaccine, which was repeated (booster dose) on day 28.

ND vaccine, La Sota was given on day 21 while the birds were vaccinated against fowl pox on day 35 using live attenuated fowl pox vaccine. Prophylactic medication against coccidiosis was done on day 24 – 27 and on day 37 – 40 using Embazin-forte,[R] a combination of sulphaquinoxaline, diaveridine and vitamin K.

ND vaccine, La Sota: The ND vaccine La Sota used in the experiment was sourced from the National Veterinary Research Institute Vom, Plateau State, Nigeria. It was a freeze dried live attenuated vaccine containing $10^{7.0}$ EID_{50} / ml.

Experimental design: At day 140 (20 weeks), serum samples were collected from 14 chickens and used to estimate the antibody levels against NDV using HI test as described by [12]. Thereafter the birds were divided into two groups, groups 1 and 2. The vaccines were reconstituted as recommended by the manufacturer. Birds in group1 were vaccinated, each with a single dose of vaccine through the intraocular route while birds in group 2 were vaccinated, each with a single dose of the vaccine through the oral route. The oral vaccination was done through drenching while the intraocular vaccination was by drop instillations into both eyes. On a weekly basis and up to 7 weeks post vaccination (PV), serum samples were collected from 13 – 14 chickens in each group and used to estimate the antibody response of the chickens in the groups using HI test.

Thirty chickens from each group and another 30 chickens from birds that were not vaccinated against ND were taken to a distant and isolated location on day 21 PV and challenged with velogenic NDV, Kudu 113. The birds were monitored for clinical signs against ND.

Haemagglutination inhibition (HI) test: This was performed according to the method described by [12]. Four (4) haemagglutinating units of the antigen were used. The serum samples were serially diluted with the antigen using two fold dilutions. 0.5% RBC was added to each well and this was incubated for 40 minutes. The reciprocal of the last dilutions that showed complete inhibition of agglutination of the RBCs were recorded as the HI titre.

NDV Inoculum/ challenge*:* The viral inoculum used was sourced from NVRI, Vom. It was a velogenic NDV isolated from duck, Kudu 113. Each chicken was inoculated with 0.2ml of the inoculum containing $10^{6.5}$ ELD_{50} /ml intramuscularly in the thigh muscle.

Statistical analysis: The geometric mean antibody titres (GMT) between the groups and within the groups were compared using student t-test.

III. Results

The antibody levels in the chickens at separation into groups (20 weeks of age) as estimated by HI test showed low titres with a GMT of 2.8 (Figure 1). The result of the antibody responses after vaccination showed a significant rise ($p < 0.05$) in both groups on a weekly basis with peak at 21 days PV after which it also declined significantly ($p < 0.05$) on a weekly basis. The result also showed that birds in group 1 had higher antibody titres, which shows better immune responses than birds in group 2(Figure 1). At 21 days PV which was the peak in both groups, birds in group 1 had a GMT of 256.0 while those in group 2 had a GMT of 207.9. However, there was no statistical difference ($p > 0.05$) between the mean HI titres of the two groups at peak levels (21 days PV) but it differed significantly ($p < 0.05$) at days 7, 14 and 28 PV.

The challenge studies showed 100% protection against morbidity and mortality in both vaccinated groups while the unvaccinated control showed 100% mortality.

IV. DISCUSSION

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 [13, 14]. This study compared the intraocular and oral routes of administration of ND vaccine La Sota in grown or adult chickens. The results of the above study favoured the intraocular route of La Sota administration in 20 weeks old white cockerels when compared with the oral route. However, it did not reveal any superiority of the intraocular route over the oral route in La Sota administration in these grown chickens as there was no statistical difference ($p > 0.05$) between the mean HI titres in the two groups on day 21 PV which happened to be period when peak antibody response was achieved. Moreover, both gave 100% protection from morbidity and mortality when challenged. Meanwhile, it is can be seen from the results that there were significant differences ($p < 0.05$) in the mean HI titres between the two groups on days 7, 14, and 28 PV. Figure 1 also showed that the intraocular route showed higher immune responses up to day 35 PV.

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 [15, 16]. 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 [9] compared immune responses of eye drop and drinking water method

and found that the eye drop method of vaccination gave a more uniform and better protection than drinking water method. According to, [17] that day old to 12 days old chicks showed better immune response than older birds when vaccinated by intranasal or intraocular route using Hitchner B1 strain [12] compared the intraocular and drinking water routes of administration of La Sota in broilers and found that the ocular vaccination resulted in significantly high level of circulating antibodies as compared to drinking water vaccination [18] 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. Studies have shown that the ocular route of administration of ND vaccines in chicks induced significantly higher levels of HI antibody titre with peak titre of log (2) 6.6 and 93% protection when challenged as compared to oral route that induced a peak HI antibody titre of log (2) 5.9 and 53% protection [9]. Field trials in Mozambique indicated that almost all farmers preferred the eye drop administration of ND vaccines as in their opinion, eye drop administration produced greater survival rate and had a lower frequency of administration [19]. Also, [20] using 6 day old chicks reported that administration of ND vaccine through drinking water gave more mortalities when compared with those that were vaccinated through beak dipping and intraocularly. However, [21] did not find any difference in the protection given by vaccination with either method as both gave 80 – 100% protection during their trials. The findings did not however agree with that of [22] who evaluated the two routes of administration in day old chicks using ND vaccine Hitchner B1 at a dose of 4 x 10^4 EID_{50} / chicken. They found that drinking water vaccination induced 90% – 100% protection against intraocular vaccination that induced 80% protection when chickens were challenged 3 weeks later.

Most widely used method of administration of ND vaccine La Sota worldwide is via drinking water. This has the advantage of ease of administration especially in adult chickens where it may be more difficult to catch the birds [1]. It also has advantage in intensive commercial farming where large numbers of birds are involved and this method reduces labour and stress on the birds due to catching. However, this method has the limitation that it may not ensure even distribution of the vaccine. Moreover, some of the vaccinal viruses given through the ocular route may have become destroyed by the gastric secretions. Gastric secretions can provide a non-specific barrier against invaders and destroy them [23]. This may have contributed in the lower immune response observed with the ocular route. This study did not in any way dispute the fact that the oral route is a good method of administration of ND vaccine La Sota in adult chickens as protective antibody levels were achieved and also, it gave the same level of protection (100%) as did the intraocular route 3 weeks PV. This is because of the lower antibody responses when compared with the intraocular method as observed in this study it may require more frequent vaccination.

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 [11]. The Harderian gland in chickens is a key organ in the development of immune responses [17]. Eye drop vaccinations result in significant increases in plasma cells in sections of the Harderian glands resulting in the production of necessary local and humoral antibodies [24, 12]. The direct movement of the vaccinal viruses into the Harderian and paranasal glands in intraocular vaccination makes large quantities of these viruses escape the humoral and phagocytic antiviral systems of the chickens leading to better immune responses when compared with ocular vaccination. This may have contributed significantly to the differences in antibody levels between the two routes of vaccination.

V. Conclusion

As reported for young birds, the intraocular route of vaccination in grown or older chickens provided better immune response when compared with the oral route of vaccination. However, the intraocular route of vaccination provided the same level of protection as did the intraocular route at 21 days PV as both achieved protective antibody levels, thus also making the oral route a good route of administration of ND vaccine La Sota in adult birds. This has an advantage when large numbers of birds are involved as it reduces the labour associated with catching of the birds. The intraocular route might be considered when the flock population is small. We identified the possible roles of the Harderian glands in producing local and humoral immune responses in intraocular vaccinations and the destruction of some of the vaccinal viruses by gastric secretions in oral vaccinations as the possible reasons for the production of higher antibodies in intraocular vaccination in adult chickens.

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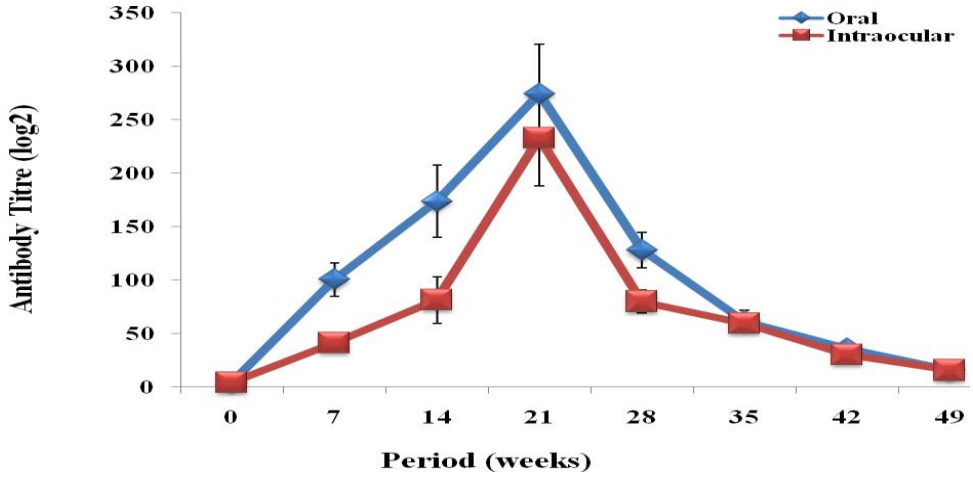

Figure 1: HI antibody titre in adult birds vaccinated with ND vaccine La Sota by oral and intraocular routes.