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THE COMPARATIVE IMMUNOGENICITY OF THREE LENTOGENIC BRANDS OF NEWCASTLE DISEASE VACCINES IN NIGERIA

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SUMMARY

The comparative immunogenicity of a new lentogenic viscerotropic Newcastle disease vaccine, NDvac-1 (*VG/GA* strain) and two other existing proprietary pneumotropic lentogenic Newcastle disease vaccines in Nigeria, NDvac-2 (*R_2B*) and NDvac-3 (*LaSota*) were studied. Immunogenicity was assessed on the basis of antibody production and protectivity ratio indices following experimental challenge. Results showed that the relatively high pre-vaccination maternal haemagglutination-inhibition antibody titres were counter-productive to effective vaccination at day old. The mean geometric titres were 2.4, 3.5, 2.1 and 4.4 for NDvac-1, double dose NDvac-1, NDvac-2 and NDvac-3 vaccinated groups respectively at day 20 post vaccination. The protection indices were 2.5, 2.5, 1.5, 3.0 and 1.0 for NDvac-1; double dose NDvac-1, NDvac-2, NDvac-3 and the unvaccinated control group respectively following experimental challenge. These results therefore presented superior immunogenicity shared by NDvac-3 and NDvac-1 while NDvac-2 was found to be the least immunogenic of the three vaccines.

KEYWORDS: Comparative immunogenicity, Newcastle disease vaccines

INTRODUCTION

Newcastle disease (ND) has been observed to be enzootic in Nigeria (Orajaka *et al.*, 1999; Oyewola *et al.*, 1996 and Ezeokoli *et al.*, 1984). There are two types of ND vaccines generally in use, namely: the inactivated and the live varieties. Live ND virus vaccines consist mainly of lentogenic strains like *H-B1* but also of mesogenic strains like the *Komarov*. Several criteria of quality are taken into consideration in the evaluation of live lentogenic ND vaccines and they include not only freedom from post-vaccination respiratory reactions (Borland and Allan, 1980 and Meulemans, 1988), but also the immunogenicity of such vaccines.

In this paper, a new and recently introduced viscerotropic and lentogenic brand of ND vaccine was evaluated and compared with other existing or conventional brands/strains in terms of immunogenicity.

MATERIALS AND METHODS

Chicks

Two hundred and fifty day-old broiler chicks unvaccinated for ND, were employed in this study. They were kept in conventional open sided houses on deep litter floor. Brooding was based on charcoal fire pots for the first 2 weeks. The chickens were fed ad-libitum on a proprietary broiler ration containing supplements. Prophylactic medication including anti-coccidials, and anti-mycoplasmals were administered. The chicks were also vaccinated against infections bursal disease at 9 and 18 days of age.

Experimental design

Two hundred and thirty day old chicks (doc) were randomly divided into five different groups. Four groups of 46 doc each for the vaccination experiment, while one group of 46 doc served as the unvaccinated control. The experimental groups were designated A, B, C and D for

NDvac-1, double dose NDvac-1, NDvac-2 and NDvac-3 respectively, while the control Group was designated group E. The group E chicks were reared in isolation at a remote location.

Vaccination

At day 1 of age or post hatch each one of the chicks in groups A, C, and D was vaccinated with a single dose of the respective reconstituted vaccine according to manufacturer's direction by eye drop application, except group B which received a double dose of NDvac-1. At day 16 of age, each one of the chicks in groups A, C and D was re-vaccinated with a single dose of the respective reconstituted vaccine according to manufacturer's direction by drinking water application, except group B which again received a double dose of NDvac-1.

TABLE I: Experimental design

GROUP	A	B	C	D	E
Day 1 ND vaccination	NDvac-1	Double dose NDvac-1	NDvac-2	NDvac-3	Nil
Day 16 ND vaccination	NDvac-1	Double dose NDvac-1	NDvac-2	NDvac-3	Nil
	NVL	2NVL	PPL	PPLS	

KEY: NVL=New viscerotropic lentogenic, 2NVL =Double dose NVL, PPL=Proprietary Pneumotropic lentogenic, PPLS=Proprietary Pneumotropic lentogenic (LaSota)

Serum collection

At day 1 of age (Pre vaccination) serum from blood was collected terminally by cardiac puncture of 20 randomly selected chicks representing the whole flock. Subsequently serum was obtained from blood collected by jugular veni puncture at days 10 and 20 of age respectively. The sera were centrifuged at 3000 revolutions per minute (RPM) before testing.

Antigen

Newcastle disease vaccine virus *LaSota* strain obtained from National Veterinary Research Institute (NVRI) Vom was used as antigen after reconstitution of 200 dose vial in 8ml of distilled water. The HA titre was determined as baseline.

Indicator

0.5% washed chicken red blood cells (RBC) suspension was prepared essentially as described by Adene and Ogunji (1980).

Haemagglutination *(HA) test*

The HA test was done by the microtest method as described by Adene and Njoku (1980). The titre was taken as the reciprocal of the highest dilution giving 100% agglutination of the 0.5% chicken RBC. This amount of virus also represents one haemagglutination (HA) unit.

Haemagglutination inhibition (HI) test

The HI test was performed using the beta procedure (constant virus and varying serum) against 4 HA units of the ND-Lasota virus. The titres were taken as the reciprocal of serum dilutions giving 100% inhibition of the chicken RBC agglutination.

Experimental challenge

At day 33 of age fifteen randomly selected chickens from each of the five groups were subjected to experimental challenge with 0.2ml of a Nigerian velogenic strain of ND virus (i.e. Kudu strain) with stock titre of 2×10^6 ELD_{50} per ml.

Statistical Analysis

Data on antibody (HT) titre was statistically analyzed using 1- way ANOVA to determine group significant differences In addition Pearsons "r" correlation method was used to determine the relationship between the mean geometric titres (MGT's) at day 20 post vaccination and the protection indices (PI) following experimental challenge.

RESULTS

Immunology

The Newcastle disease haemagglutination inhibition (ND-HI) titres obtained from the samples collected were used as a measure of the immune response elicited by the individual vaccines.

The ND-HI titres of sample collected at day 1 (pre vaccination) are as presented in table 2. The ND antibody seroprevelence was 100% with titres ranging from 3 log_2 to 7log_2 and a mean geometric titre (MGT) of 5.25. Subsequently the ND antibody titres persisted with 100% prevalence but with moderate decline in titres at day 10 of age. Thus the MGT's were 3.8, 3.7, 3.6, 3.9 and 4.0 in groups A, B, C, D and E respectively at day 10 of age (Table III). The decline in titres continued especially in groups A, C, and E at day 20 of age despite re- vaccination at 16weeks (Table III). Thus the MGT's were 2.4, 3.5, 2.1,4.4 and 1.2 in groups A, B, C, D and E respectively at day 20 of age (Table IV).

Experimental challenge

Following experimental challenge at day 33 of age there were 6 dead chickens each from groups A and B, 10 dead chickens from group C, 5 dead chickens from group D and 15 dead chickens from group E (as shown in table 5). All the mortalities occurred between 2 and 6 days post-challenge after displaying typical clinical signs of ND including torticollis, nasal discharge, ruffled feathers, depression anorexia and diarrhoea. The protection index (PI) was 2.5, 2.5, 1.5, 3.0, and 1.0 for groups A, B, C, D and E respectively.

Statistical analysis

At day 10 post vaccination there was no statistical difference ($P < 0.01$) in MGT in groups A, B, C, D and E. (Table 3). At day 20 post-vaccination the MGT of group E chicks was significantly lower ($P < 0.01$) than that of group A, B, C and D chicks (Table 4). The MGT of group D chicks was significantly higher ($P < 0.01$) than that of groups A and C chicks at day 20 post-vaccination. A high positive correlation ($P < 0.05$) existed between the MGT's at day 20 post-vaccination and the PI following experimental challenge (i.e. the higher the MGT's the higher the PI).

TABLE II: Pre-vaccination Newcastle disease haemaggulitination inhibition antibody titres (Log2)

S/No*	Titre	S/No*	Titre
1	6	11	5
2	6	12	5
3	6	13	7
4	6	14	6
5	3	15	5
6	3	16	5
7	5	17	5
8	3	18	6
9	6	19	6
10	6	20	5

***S/No: Sample number**
Total tested = 20
Total seropositive = 20 (100%)
Mean geometric titre = 5.25

TABLE III: Day 10 post-vaccination Newcastle disease haemagglutination inhibition antibody titres (Log_2)

Group	A	B	C	D	E
No. tested	10	10	10	10	10
%seropositive	100	100	100	100	100
Titres (range)	3-4	3-4	3-4	3-5	3-6
MGT*	3.8^a	3.7^a	3.6^a	3.9^a	4^a
	(0.42)	(0.48)	(0.52)	(0.57)	(0.94)

***MGT: Mean geometric titre**
a = same superscripts in a row indicate no significant difference between the means, a: P<0.01
Standard deviation in brackets

TABLE IV: Day 20 post-vaccination Newcastle disease haemagglutination inhibition titres (Log2)

Group	A	B	C	D	E
No. tested	10	10	10	10	10
%seropositive	100	100	100	100	100
Titres (range)	1-3	3-4	1-4	3-6	1-2
MGT*	2.4^a	3.5^b	2.1^a	4.4^c	1.2^d
	(0.70)	(0.53)	(0.88)	(0.97)	(0.42)

***MGT: Mean geometric titre a b c d Different superscripts in a row indicate significant difference between the means, a b c d: P<0.01**
Standard deviation in brackets

TABLE V: Post challenge mortalities

Group	A	B	C	D	E
Total	15	15	15	15	15
Dead	6	6	10	5	15
%Mortality	40	40	66.7	33.3	100
PI*	2.5	2.5	1.5	3	1.0

*PI: Protection index = $\frac{\text{Total number of birds}}{\text{Number of dead birds}}$

DISCUSSION

The ND-HI titres of serum samples collected at day old showed high prevalence of maternal antibody to ND in the stock of chicks employed for this study. Levels of such antibodies will be directly related to titres in the parents (Heller *et al.*, 1977). Following vaccination at day 1 and day 16 of age the titres declined appreciably to a much lower level. This drop in the titre could be attributed to antibody decay and neutralization between the vaccine virus and the maternally derived ND antibody in all the experimental chicks. This is in consonance with an earlier report that the immune response of chicks appears to be inversely related to the level of maternal antibody present at the time of vaccination (Box, 1965).

The practical implication of the presence of such levels of maternal antibodies in chicks is that the timing of vaccinations should be delayed to ensure non-interference by maternal antibodies. In general, there were noticeable group differences in the ND antibody titres especially at day 20 of age. Thus with an MGT of 1.2 in the unvaccinated group E as the baseline, groups, A and C (2.4; 2.1) were intermediate while groups B and D were better with MGT's of 3.5 and 4.4 respectively. In effect therefore, the double dose NDvac-1 and the single dose LaSota (NDvac-3) were superior to the others.

Indeed this finding is consistent with the reputation of LaSota as one of the most antigenic within the lentogenic range of ND vaccines. In consonance with its reputation of high antigenicity, Borland and Allan (1980) found LaSota to be one of the most pathogenic among the lentogenic range of vaccines.

Following experimental challenge, the post-challenge mortalities were used to measure the degree of protection afforded by the different strains employed in vaccination of the chicks. The PI of 2.5, 2.5, 1.5, 3.0 and 1.0 in groups A, B, C, D, and E respectively showed the superiority of NDvac-3 (LaSota) vaccine in group D while group A (NDvac-1) and group B (double dose NDvac-1) were intermediate in this regard. Thus, NDvac-2 (group C) offered the least protection. The equal protections offered to groups A and B chicks suggest that an increase in dosage of vaccinations with the attendant increase in antibody response does not necessarily translate to higher protectivity, directly. This is a lesson, which needs to be further studied.

CONCLUSION

In conclusion, in Nigeria where ND is enzootic and constitutes a serious disease problem in the poultry industry with ND being reported in vaccinated flocks (Ugochukwu, 1982), the more immunogenic and potent strains of ND vaccines like *LaSota* as well as the new viscerotropic and lentogenic ND vaccine (NDvac-1) are recommended for disease control, as primary vaccines, to cater for risks of neonatal Newcastle disease virus infection.

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