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COMPARATIVE TISSUE REACTIVITY OF THREE LENTOGENIC STRAINS OF NEWCASTLE DISEASE VACCINES IN NIGERIA

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SUMMARY

The comparative safety of a viscerotropic and lentogenic Newcastle disease vaccine strain, NDvac-1 (*VG/GA* strain) and two other proprietary lentogenic Newcastle disease vaccine strains in Nigeria, NDvac-2 (*R_2B*) and NDvac-3 (*LaSota*) were studied. Safety parameters adopted in the investigation included: post vaccination clinical reactions; gross and histopathological changes in various tissues. The eyelids, sinus, trachea, lung and ceacal tonsil were examined for post vaccination reactions and observed lesions were scored on a scale of 1 - 4.Although clinical reactions and gross pathological lesions were absent following vaccination in each of the four experimental vaccination groups, there were histological reactions with total lesion scores attaining 3,5,14, 12, and 1 in the NDvac-1, double dose NDvac-1, NDvac-2, NDvac-3 and the unvaccinated control group respectively at day 3 post vaccination. The least scores of 3 and 5 in the NDvac-1 vaccinated groups implied superior safety in association with the conventional and double dosage of the vaccine. The ND-vac-2 vaccinated group with the maximum score of 14 was found to be the histopathologically most reactive of the three vaccines.

KEYWORDS: Comparative, Tissue reaction, Newcastle disease vaccines

INTRODUCTION

Newcastle disease (ND) is a major viral disease of economic importance in poultry. It occurs in enzootic proportions in most countries except perhaps Australia and New Zealand (Alexander, 1986). ND has been observed to be widespread throughout Nigeria after its fist recognition by Hill *et al* (1953). ND is currently one of Nigeria's most problematic diseases in the poultry industry (Adu *et al.,* 1986).

In Nigeria, the main control measure for ND is by vaccination. The National Veterinary Research Institute (NVRI) Vom, Nigeria produces 3 different strains of live ND vaccines namely HB-1, *LaSota* and *Komarov* strains. Compelling supply and demand factors have continued to favour the introduction of imported supplementary ND vaccines into Nigeria. Some of these newer vaccines make certain claims which apart from higher immunogenicity include freedom from respiratory side effects such as sneezing, coughing, and nasal discharge which could result to stress, morbidity and in some cases mortality. In view of the fact that live ND virus vaccines, unlike the inactivated ND vaccines, frequently produce adverse reactions such as mild respiratory disease and drop in egg production (Van Roekel *et al.,* 1948), It was thought necessary to experimentally investigate the comparative safety of this recently introduced viscerotropic lentogenic ND vaccine and two other available live lentogenic strains of ND vaccines under prevailing humid tropical environment in Nigeria.

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 chicks were fed ad-libitum on a proprietary broiler ration containing supplements. Prophylactic medication including anti-coccidials, and anti-myocoplasmals were administered. The chicks were also vaccinated against infectious bursal disease at days 9 and 18.

Experimental design

Two hundred and thirty day-old chicks (doc) were randomly divided into five 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 from the other group of chicks (Table I).

Vaccination

At day 1, 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 a dose of NDvac-1 (Table I).

TABLE 1: Grouping, vaccines and vaccination schedules

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

KEY: NVL = New Visceroptropic Lentogenic , 2NVL = Double dose NVL, PPL = Proprietary Pneumotropic Lentogenic, PPLS = Proprietary Pneumotropic Lentogenic (*LaSota*)

Sampling

At day 1 before sorting of the chicks into groups, 20 doc were euthanized (in chloroform buckets) and necropsied for detailed pathology. Subsequently at days 2 and 3 four chicks per group (i.e. A to E) were also euthanized (in chloroform buckets) and necropsied for detailed pathology. For histopathology, samples of the eyelids, sinus, trachea, lung, and ceacal tonsil were fixed in 10% Formal saline for 24 hours, processed embedded in paraffin wax, and examined under a light microscope. Histopathological examination of selected target tissues i.e. eyelids, sinus trachea, lung and ceacal tonsil was adopted as a means of microscopic screening for possible and otherwise grossly undetectable tissue reactions. The histopathological lesions observed were assigned scores ranging from 1 to 4 depending on the severity of the lesions observed.

RESULTS

Generally, no clinical reactions were detectable during days 1-3 post-vaccination (P.V) in any of the 5 groups (A to E). Also, no gross lesions were also detectable during days 1-3 P.V in any of the five groups (A to E).

The histopathological lesions observed at 2-3 days P.V including hyperaemia and congestion, epithelial proliferation, mononuclear cell infiltration was in most cases mild to severe attaining scores of 2,3 and up to 4. Thus in the eyelids it consisted of vascular changes and proliferation of the epithelium. In the sinus, it consisted of vascular changes including congestion and haemorrhage, mononuclear cell infiltration and hyperplasia of the mucous glands.

The lesions in the trachea, lung and ceacal tonsil were similar but included hyperaemia and congestion especially in between the cartilage and mucous membrane in the trachea, mild to serve congestion in the lung and mild

cellular infiltration of the ceacal tonsil were also observed. The group lesion scores are depicted in table 2. From chronological analysis there were no histopathological lesions observed in the samples of the eyelid, sinus, trachea, lung and ceacal tonsil in any of the five groups (A to E), at day 1 pre-vaccination. At day 2 P.V the eyelid tissue specimens revealed an absence of lesions in any of the samples examined.

The lesion scores for the sinus tissues were zero for A, B and E and 2 each for groups C and D. The lesion scores for the tracheal tissues were 2, 2, 1 and 2 for groups A, B, C and D respectively but zero for the control group E. There were no detectable post- vaccination lesions in the lung tissues in all groups except in group D where the lesions were scanty and attained a score of 1. The lesions scores for the ceacal tonsil tissue was 1 for groups A, B, D and E. At day 3 P.V. increased intensity of lesions were observed in most tissues with scores attaining a maximum of 3 or 4 in some cases (Table 2).

The scores for eyelid tissues were 2 in groups B and 3 for group C. The sinus tissues presented high scores of 3, 3 and 4 in group B, C and D respectively. The lesion scores for the tracheal, lung and ceacal tonsil tissues ranges from 0-4 with group C and D consistently recording higher lesion scores than both the group A and B tissues.

The experiment was terminated on day 4 P.V to avoid complications by secondary infections.

TABLE II: Histopathological lesions scores

Pre-vac		EL	SN	TR	LG	CT	THS
		0	0	0	0	0	0
Days 2		EL	SN	TR	LG	CT	THS
Groups	A	0	0	2	0	$1_{(MI)}$	3
	B	0	0	2	0	1	3
	C	0	$2_{(MP)}$	$1_{(VC)}$	0	0	3
	D	0	$2_{(MP)}$	$2_{(VC)}$	$1_{(VC)}$	$1_{(MI)}$	6
	E	0	0	0	0	$1_{(MI)}$	1
Days 3		EL	SN	TR	LG	CT	THS
Group	A	0	$1_{(VC,MI)}$	$2_{(VC)}$	0	0	3
	B	$2_{(VC,EP)}$	$3_{(VC,MP)}$	0	0	0	5
	C	$3_{(VC,EP)}$	$3_{(VC,MP)}$	$2_{(VC)}$	$4_{(VC)}$	$2_{(MI)}$	14
	D	0	$4_{(VC,MP)}$	$2_{(VC)}$	$4_{(VC)}$	$2_{(MI)}$	12
	E	0	$1_{(VC,MI)}$	0	0	0	1

***Pre-vac = pre vaccination**

Key

Tissues EL = Eyelid, SN = Sinus, TR = Trachea
LG = Lung, CT = Ceacal tonsil
THS = Total histopathological lesions score

Lesions VC = Vascular changes (hyperemia and congestion)
EP = Epithelial proliferation
MP= Mucous gland proliferation
MI = Mononuclear cell infiltration

Rating 0 = Nil
1 = Mild
2 = Moderate
3 = Severe
4 = Very severe

TABLE III: Chronological total histopathological lesion scores in groups

TIME	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
Day 1 -(Pre-vac)	0	0	0	0	0
Day 2	3	3	3	6	1
Day 3	3	5	14	12	1

***Pre vac = Pre vaccination**

TABLE IV: Chronological organ distribution of histopathological lesion scores

TIME	Eyelid	Sinus	Trachea	Lung	Ceacal tonsil
Day 1-(Prevac)	0	0	0	0	0
Day 2	0	4	4	1	3
Day 3	5	12	6	8	4

*** Pre vac = Pre vaccination**

DISCUSSION

Local velogenic NDV was used in this experiment. The clinical signs and lesions observed in this study were typical of the clinical signs of velogenic ND previously described by various authors (Copland, 1987; Kouwenhoven, 1993 and Alexander, 1997). The low antibody titres of 1:4 detected in the serum before infection were probably due to maternal antibody. Allan *et al* (1978) estimated that each two-fold decay in maternal derived HI antibody titre takes about 4-5 days. The high antibody titres of 1:789 detected in the surviving birds were probably due to the good immune response to the velogenic NDV.

There were more viral concentrations in the organs examined at 7-10 days PI. However, the concentration declined with time. Lancaster (1966) noted that virus builds up in the infected organs and later declined appreciable as a result of the development of circulating antibodies. It is therefore, probable that the rise in antibody level towards day 21 PI may have rapidly cleared the viruses, resulting in the low concentrations.

There were no significant differences in the means between of the viral concentrations of the organs examined in this study. This may be due to the strain of the virus and the method used for the analysis. The strain of NDV used in this study is a virulent strain which attacks and destroys many organs of the body (Al-Sheikly and Carlson, 1975). The widespread of the virus in various organs examined is in line with the result of Alexander (1997) who observed wide dissemination of lesions in the organs following velogenic ND. The method of virus isolation in embryonated, chicken's eggs is believed to affect the virus multiplication in the embryos. Therefore, variations in results have be observed from different authors. Pomeroy (1951) and Sinha (1958) for example, in their work found some organs more useful than others in the isolation of NDV. This may be due to the method used for analysis of the organs.

CONCLUSION

The results of this study have shown that internal organs can be used for the isolation of local strains of velogenic NDV in embryonated chicken's eggs. Samples should be collected early in infection, especially on or before the 10th day post infection, as the development of circulating antibodies may rapidly clears the virus from the tissues as infection progresses. Selection of organs should be based on the organs that showed conspicuous or marked lesions of ND. In the field, it is suggested that

samples be collected immediately clinical signs are noticed. Alexander (1997) noted the incubation period of ND after natural infection to be 2-15 days with average of 5-6 days.

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