Archives of Veterinary Science, v.13, n.2, p.79-84, 2008 ISSN 1517 – 784X

ANTIGENIC RELATEDNESS AMONG NEWCASTLE DISEASE VIRUS ISOLATES FROM NIGERIAN FERAL BIRDS AND THE LA SOTA STRAIN

Relação antigênica entre o vírus da doença de Newcastle isolado de aves selvagens da Nigéria e das amostras La Sota

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ABSTRACT

The antigenic properties of thirteen isolates of Newcastle disease virus were assessed against La Sota strain. Using one of the previously recognized formulas significant antigenic differences were observed and marked inhibitory activities were noticed amongst the isolates and their hyper-immune sera as well as that of the La Sota strain. The implications of these differences for Newcastle disease epidemiology and control in Nigeria are discussed.

Key words: vaccine virus, wild virus, Newcastle disease, Nigeria.

RESUMO

As propriedades antigênicas de treze isolados do vírus da doença de Newcastle foram comparados com amostras La Sota. Usando uma das fórmulas previamente reconhecidas, significantes diferenças antigênicas foram observadas e marcantes atividades inibitórias foram percebidas entre os isolados e seus soros hiperimunes, bem como das amostras La Sota. As implicações destas diferenças para a epidemiologia e controle da Doença de Newcastle na Nigéria são discutidos.

Palavras-chave: vírus vacinal, vírus selvagem, doença de Newcastle, Nigéria.

INTRODUCTION

Newcastle disease virus (NDV) continues to pose a serious threat to the development of the poultry industry in Nigeria, other parts of Africa and Asia. The disease has economic and ecological impact on pet birds, free-living birds, as well as domestic birds.

Since the first detection and isolation of NDV in Nigeria in 1951 (Hill, et al., 1953), an average of 200 – 250 outbreaks of the disease are reported in the country annually (Okeke and Lamorde, 1988). Several of these outbreaks frequently occur in vaccinated flocks and are often attributed to the existence of antigenic differences between the vaccine virus and wild virus

Recebido para publicação em: 30/07/2008
Aprovado em: 21/11/2008

strains (Nawathe, 1975; Ugochukwu, 1982; Adu, 1985).

Although the various strains of the NDV so far identified worldwide are antigenically related, minor antigenic differences still exist among strains. The antigenic variations found within strains are similar to those found among mutants within the same serotype (Adu, 1987).

These differences are expressed by the two surface glycoprotein antigens on the ND Virus – Haemagglutinin (H) and Neuraminidase (N) (Gomez-Lillo et al., 1974).

La Sota strain of ND virus is one of the widely used live vaccines in Nigeria. This lentogenic vaccine is produced locally on commercial level at the National Veterinary Research Institute (NVRI, VOM) for routine immunization of commercial flocks.

In a recent survey, 13 NDV isolates were obtained from wild birds caught from Plateau, Benue and Kaduna States in central Nigeria. Using conventional and molecular techniques, there are differences in molecular aspects between the virus strains isolated (Ibu et al., 2008, in press). The present study was carried out to determine serologic antigenic relatedness between these isolates and the La Śota vaccine strain using the Cross Haemaglutination Inhibition test. The possibility of the isolates sharing a common origin with the vaccine strain and its implication in relation to vaccine failures is discussed.

MATERIAL AND METHODS

Production of Hyper-immune Serum

The ND virus hyper-immune serum (HIS) for all the 13 isolates and the La Sota vaccine strain was raised in six weeks old susceptible chickens. Seventy-five (75) specific antibody negative chicks were obtained from the Poultry Division of the National Veterinary Research Institute, (NVRI, VOM), Nigeria and raised under a conditioned environment at the experimental unit of the Viral Research Division of the Institute.

The birds were pre-screened for NDV antibodies and divided into 15 experimental groups with 5 birds per group. Following the final screening, groups 1 – 13 were inoculated with corresponding freshly harvested infective allantoic fluid of the virus isolate. Each bird received per-os (orally) 0.2 mL of 100 EID_{50} per mL of virus concentration containing a minimum of 6 log virus titre. Group 14 was given 0.2mL of NDV vaccine La Sota strain.

The virus inoculation procedure was repeated at weekly intervals for four weeks. Twenty one days after the last inoculation, blood were collected from the birds for serum and tested for the presence of ND virus antibodies. Chicks in group 15 were used as uninfected controls.

Cross Haemggulation Inhibition (HI) Test

A cross HI test (ß-procedure) (Oie, 2004), was conducted on the fourteen HIS raised against each of the thirteen field virus strains and the NDV La Sota.

Briefly described, 25µl of phosphate buffered saline (PBS) (P.H 7.2) was dispensed into each well of a 96-well, V-bottom plastic microtitre plate. Thereafter, 25µl of the test serum was placed in the first column of the microtitre plate. A two-fold serial dilution of the serum was made in PBS across the plate from 1:2 – 1:4096.

Serial dilutions of known positive and negative sera were used as controls. 25µl of four-haemagglutination unitage (4HA) of previously determined virus antigen was added to the HIS and the control wells and thoroughly mixed. A back titration of 4HA units of the antigen was made across the plate. The plate was then incubated at room temperature (≈25-30^{o}C) for 40 minutes. Afterward, 25µl of freshly prepared 1% v/v of chicken red blood cells (RBC) was added to each well and mixed using a micro-plate shaker. All wells of the RBC control (only

RBC and PBS) were allowed to settle to a distinct button.

Results were read for haemagglutination by tilting the plates and observation of a “tear-drop” streaming of the RBCs. A serum was considered positive if there was an inhibition (non-settling of RBC) at a serum dilution of $4Log_2$ or greater.

The extent of antigenic differences between two isolates was calculated from HI titre ratios using the formula of Archetic and Horsefall (1950) thus:

$R=\sqrt{r1xr2}$

Where R = ratio of extent of antigenic differences between two clones/strains when both clones/strains and their antisera are used in reciprocal cross serological reactions.

r1 = Heterologous titre (virus strain 2) / Homologous titre (virus strain 1)

r2 = Heterologous titre (virus strain 1) / Homologous titre (virus strain 2)

RESULTS

From this study, a reaction between viral isolates/antigens and their immune sera was observed as shown by the reciprocal of the mean HI titres in Table 1. The titres ranged between, 2 and 4096 (table 1). The highest geometric mean titre (GMT) was 955 and the lowest, 196 (table 1).

A titre ratio as high as 32 was observed in a reaction between isolate Pl-016 antigen and Pl-032 antiserum while a titre ratio as low as 0.016 occurred between isolate Bn 7 virus antigen and Pl-016 hyper-immune serum (table 2). The maximum R-value of 5.66 was observed in a cross reaction between isolate JZ 2 virus antigen and Pl-032 and Pl-038 hyper-immune sera (table 3). The lowest R-values of 0.13 was noticed between isolate Bn 2, and Jz2 and Pl-029 sera.

For the field isolates, 42 out of 78 (53.85%) heterologous reactions gave R-values above 1, while 28 (35.89%) had R-values below 1. Only 8 cross reactions (10.26%) had R-values equal to 1.

Of the 13 cross reactions between the field virus isolates and the La Śota strain, 4 (30.77%) had R-values above 1. Six cross reactions (46.15%) gave R-values below 1 while 3 (23.08%) cross reactions had R-values equal to 1 (table 3).

DISCUSSION

It is evident in this study that there is minor antigenic variability among the 13 field ND virus strains and the vaccine (La Śota) strain. A marked variation in the inhibitory capacity between the heterologous antigen and antisera is observed. For example, strains which show variability in terms of departure from the homologous HI titre (R> 1 or r < 1) accounted for 89.74% of the test result while strains which showed harmony (R=1) accounted for only about 10%. This high level of antigenic divergence could be of practical significance in relation to vaccination failures and the epidemiology of the disease in the localities. These findings are in agreement with those of other workers. Upton et al., (1953) and Bankosky et al., (1965) observed a significant diversity in antigenic components among the various strains of the ND Virus. Using different laboratory test, Gomez-Lillo et al., (1974); Schloer et al., (1975); and others identified antigenic differences between strains of ND viruses. Similarly, Alexander et al., (1999) using monoclonal antibodies, placed 102 ND virus isolates from 15 countries in 16 distinct groups. It is also suggested that antigenic divergence between clones of the same strain could be due to the variability in the function of external proteins (Adu, 1985). Similarly, the high level of reactivity between ND virus strains is also attributable to the presence of some mutants which react more broadly with heterologous virus (Bratt and Gallater, 1977). In the present study, all the strains had higher R-values with most of the

heterologous viruses than the homologous isolates. This observation is also true of their reaction with the La Sota vaccine strain.

Generally, a range of R-values ≤ 0.5 or ≥ 2 is an indication that there is a significant antigenic difference between two strains (Baba et al., 1998). In this report, 48.71% of heterologous reactions fall within this range. This level of antigenic diversity cuts across a significant range of the isolates. It is an indication that these ND virus strains may not have had a common origin. In conclusion, it is not likely that the 13 virus isolates used in this study may have evolved from the existing La Sota vaccine strain.

Table 1 - Reciprocals of cross–haemagglutination inhibition titres of homologous and heterologous ND virus antigens and antisera.

Antiserum	**Antigen**													
	Bn11	**Pl016**	**Pl032**	**Bn8**	**Pl038**	**Jz2**	**Jz6**	**Pl029**	**KD_4**	**Bn^2**	**Jz13**	**Bn^7**	**Jz4**	**NDV**
Bn11	512	512	64	128	256	256	128	128	512	256	1024	64	128	2856
Pl016	4096	2048	1024	512	4096	4096	2018	1024	4096	4096	4096	32	1124	1024
Pl032	2048	4096	128	128	256	2058	1024	128	512	1024	1024	64	256	256
Bn8	2048	1024	512	128	256	1024	512	156	4096	64	2048	128	256	512
Pl038	512	1024	256	64	128	2048	512	128	1024	2048	512	256	128	128
JZ2	1024	256	512	256	512	256	512	512	512	64	256	256	512	1024
Jz6	512	512	256	512	512	256	512	512	512	64	256	256	64*	256
Pl029	512	256	256	1024	4096	512	1024	512	1024	128	1024	256	64*	512
KD_4	128	256	128	64	256	128	256	128	512	256	256	512	128	256
Bn^2	256	512	256	256	1024	256	1024	256	256	4096	512	512	512	512
Jz13	512	2048	2048	512	2048	1024	1024	1024	512	256	1024	512	1024	2048
Jz4	256	1024	256	128	256	512	512	512	4096	512	512	256	128	1024
Bn^7	128	256	128	128	512	128	128	128	512	2048	512	128	128	128
Lasota	512	256	1024	512	512	1024	128	512	256	256	256	512	256	512
Range of HI titres[1]	128-4096	256-4096	64-2048	64-1024	128-4096	128-2048	128-2048	512-4096	64-2048	256-4096	32-512	64-1024	512-2048	128-2048
GMT	588	724	294	208	588	630	588	294	955	478	776	169	239	

[1]hyper-immune sera against field strains

Table 2 - Titre ratios derived from Cross HI titres.

Antiserum	**Antigen**													
	Bn 11	**Pl 016**	**Pl 032**	**Bn 8**	**Pl 038**	**Jz 2**	**Jz 6**	**Pl02 9**	**KD 4**	**Bn 2**	**Jz 13**	**Jz 4**	**Bn 7**	**NDV (L)**
Bn11	1	1	0.13	0.25	0.5	0.5	0.25	0.25	0.25	0.5	2	0.25	0.13	0.5
Pl016	2	1	0.5	0.25	2	2	1	0.5	2	2	2	0.5	0.016	0.5
Pl032	16	32	1	1	2	16	8	1	4	8	1	4	0.5	2
Bn8	16	8	4	1	2	8	4	2	32	0.5	16	2	1	4
Pl038	4	8	2	0.5	1	16	4	1	8	16	4	1	2	1
Jz2	4	1	2	1	2	1	2	2	2	0.25	1	2	1	4
Jz6	1	1	0.5	1	1	0.5	1	1	1	0.13	0.5	0.13	0.5	0.5
Pl029	1	0.5	0.5	2	8	1	6	1	2	0.25	2	0.13	0.5	1
KD4	0.25	0.5	0.25	0.13	0.5	0.25	0.5	0.25	1	0.5	0.5	0.25	1	0.5
Bn2	0.063	0.13	0.063	0.63	0.25	0.063	0.25	0.063	0.063	1	0.13	0.13	0.13	0.13
Jz13	0.5	2	2	0.5	2	2	1	1	0.5	0.25	1	1	0.5	2
Jz4	2	8	2	1	2	4	4	4	32	4	4	1	2	8
Bn7	1	2	1	1	4	1	1	1	4	16	4	1	1	1
NDV(L)	1	0.5	2	1	1	2	0.25	1	0.5	0.5	0.5	0.5	1	1

Table 3 - R-values calculated from titre ratios according to the formular of Archetti and Horsfall (1950).

Isolate / Antiserum	Bn 11	Pl 016	Pl 032	Bn 8	Pl 038	Jz 2	Jz 6	Pl0 29	KD 4	Bn 2	Jz 13	Bn 7	Jz 4	NDV (L)
Bn11	1	1.41	1.44	2	1.41	1.41	0.5	0.5	0.25	0.18	1	0.36	0.71	0.71
Pl016		1	4	1.41	4	1.41	1	0.5	1	0.51	2	0.18	2	0.5
Pl032			1	2	2	5.66	2	0.71	1	0.71	1.41	0.71	2.83	2
Bn8				1	1	2.83	2	2	2.04	0.18	2.83	1	1.41	2
Pl038					1	5.66	2	2.83	2	2	2.83	2.83	1.41	1
Jz2						1	1	1.41	0.71	0.13	1.41	1	2.83	2.83
Jz6							1	1.41	0.71	0.18	0.71	0.71	0.72	0.35
Pl029								1	0.71	0.13	1.41	0.71	0.72	1
KD4									1	0.18	0.5	2	3.83	0.5
Bn2										1	0.5	1.44	0.72	0.25
Jz13											1	1.41	2	0.32
Bn7												1	1.41	1
Jz4													1	2
NDV(L)														1

Acknowledgement

The authors thank Mrs. Josephine Kaigama for her laboratory assistance, Dr. H. M. Kazeem for his input and the Executive Director, National Veterinary Research Institute, Vom, Nigeria for permission to publish the manuscript.

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