SEROLOGICAL STUDIES ON NEWCASTLE DISEASE IN SERA OF LOCAL CHICKENS IN PLATEAU STATE

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ABSTRACT

Prevalence of Newcastle Disease (ND) in local chicken in Plateau State was serologically determined in one thousand seven hundred and fifty (1750) sera and forty (40) samples of cloaca swabs from sick birds. The samples were heat inactivated at 56°C for 30 minutes and subjected to hemagglutination inhibition test (HI) controlled with standard antisera. 1210 (69.14%) sera were positive for ND with titres ranging from 2 logs to 128 logs. 4 isolates were obtained using 10 days old embryonated chicken eggs. The isolates were compared with antisera from vaccinated birds and were found different from the vaccine strains. This may suggest the emergence of new ND strains in the field. The positive sera from the local unvaccinated birds may also suggest the circulation of ND in local chicken and the birds may serve as reservoir and source of new infection to vaccinated and unvaccinated flocks. This however, suggested that control of ND in local chicken may be an additional step in the need to eradicate Newcastle disease in Nigeria.

Key words: Antibody, Newcastle Disease, and Vaccine

INTRODUCTION

Newcastle disease (ND) is a contagious fatal vital disease, which affects all ages and species of birds (Alexander, (1991). It is one of the major causes of economic loss and is the most important viral infection of poultry worldwide (Fenner *et al* 1987, Spradbrow, 1993-94, Alexander, 1995 and Olabode *et al* 1992). An enveloped single stranded ribonucleic acid virus of the paramyxoviridae causes it. The symptoms include dullness, cough, sneezes, gasping, depression, prostration, and profuse greenish diarrhoea. There is paralysis of the winds and legs with complete torticollis twisting of the neck as well as backward somersaults (Allan, 1982; Topley and Wilson (1990).

Exotic and rural scavenger chickens are kept in both the rural and urban areas of Nigeria, unlike the exotic birds which number about 30 million, the local scavenger chicken numbering about 120 million are not routinely vaccinated against prevailing disease, prominent among these is Newcastle Disease (ND) (Yahaya Personal Communication, Olabode *et al* 1992; Nawathe and Lamorde 1987). Currently, ND is

viewed as one of the most serious fatal poultry disease of economic importance in Nigeria among the exotic and local chickens (Fatunbi and Adene (1979).

Although little or no veterinary care is given to the rural poultry, they are present in greater numbers than the exotic breeds. They are found in village and cities and are kept by both the low and high income earning classes of people. The local chickens provide income and a cheap source of animal protein to the rural population (Egege, (1990); Fatunbi and Adene (1979)).

In Nigeria as well as many African countries the population of free roaming local chickens that wonder like scavengers is very high and the menace of the poultry is real and emphasis appear to be laid more on disease of cattle such as rinderpest and contagious boving pleuro-pneumonia than on any poultry disease (Olabode *et al* (1992); Okeke and Lamorde (1988)). High mortality of the local chicken due to ND may be militating against realizing the much-needed national livestock protein, on one hand and poverty alleviation of the rural populace on the other hand.

This study is therefore designed to investigate the possible roles that may be played by the local chickens in the spread of annual outbreak of ND in both vaccinated exotic poultry birds and unvaccinated local chickens.

MATERIALS AND METHODS

Standard virus

The standard Newcastle disease viruses were obtained from the Virology Department of the National Veterinary Research Institute Vom, Plateau State of Nigeria. The viruses were tested and confirmed by hemogglutination inhibition test using specific type antibodies to ND viruses.

Blood Sample Collection

Sera samples were obtained from one thousand seven hundred and fifty (1750) blood samples collected through vene-puncture with sterile syringes and needles from local chickens in five Local Government Areas of Plateau State. 5ml of blood were collected into sterile evacutainer tubes (venoject, Terumo Europe N. V. – Beigium) and al'owed to clot and serum separated. The locations and the number of samples collected in each location are shown in table 1.

TABLE I: Samples Distribution of Newcastle Disease Virus HI Antibody in The Different Locations.

Location (L.G.A.)	Total Samples	No. of Samples Positive (%)	No. of Samples Negative (%)
Jos North	650	428 (24.46)	222 (12.69)
Jos South	450	271 (15.49)	179 (10.23)
Barkin Ladi	260	208 (11.89)	52 (2.97)
Riyom	200	155 (8.86)	45 (2.57)
Mangu	190	148 (8.46)	42 (2.40)
Total	1750	1210 (69.14)	540 (30.86)

NB: Over 50% of the sample tested in each location had positive haemagglutinations inhibition antibodies to Newcastle disease virus. The higher percentage values of HI

antibodies in samples from Jos North and South may be a reflection of the number of samples collected from the area since most of the poultry farms in the state are located in the area, the local birds may have been infected due to in contact vaccination with the vaccines strains of the Newcastle disease virus

Swab Sample Collection

Inserting the swab into the cloaca of the sick birds until the cotton wool is not seen collected the cloaca swab samples. The swab was gently swirled in the cloaca. It was removed and placed in a sterile universal bottle containing transport medium (E99 medium with penicillin 1miu/1 and 2g 1 of streptomycin plus fungizone antibiotics). The swabs were allowed to elute for 3 hours at 4°C with gentle agitations at intervals. The swabs were removed and the medium centrifuged at 300rpm for 20 minutes in a cold refrigerated centrifuge (M.S.W. Ltd. London). The supernatant was decanted and stored at – 20°C until used.

Serum Treatment

One milliliter (1ml) of the serum was added to 0.9ml of acid washed kaolin plus 0.2mls of 10% chicken red blood cells washed in phosphate buffered saline, pH 7.2. The mixture was gently shaked and centrifuged at 2500rpm for 15 minutes in M.S.E. Refrigerated centrifuge. The supernatant was decanted in sterile bijou bottle. The sera were finally heated at 56°C for 30 minutes in a water bath (B&T, A Searle Co. U.K) and stored at –20°C until used.

Virus Isolation

The ND virus isolation was done by inoculating samples into 10 days old embryonated chick eggs. The eggs were candled to select fertile eggs. The fertile eggs were swabbed with 70% alcohol and route of inoculation-drilled 3mm from the air space mark. O.2mls of the sample dilutions of 10^{-1} to 10^{-2} were inoculated into the eggs. The holes were sealed and the eggs incubated at 37°C in a humidified incubator (B&T, A Searle Co. U.K). The eggs were candled each day for three days. The life eggs, post inoculation were chilled at 4°C, swabbed with 70% alcohol and cut open with sterile scissors. The allantoic fluids were harvested into sterile universal bottle ready for virus identification by micro HI test. (Spradbrow, *et al.* (1995).

ND Virus Identification

The ND virus in allantoic suspension was identified with type specific antisera to Newcastle Disease Virus using hemagglutination inhibition test (HI) (Burnet, 1942). 25 microlitre of the different samples were diluted in U-shaped 96 wells plastic micro titre plates. 25 microlitre of 1:10 dilution of specific ND antisera was added to dilute antigens. The mixture was allowed to react at 4°C for 15 minute after which 25 microlitres of 1% chick red blood cells was added and the final mixture was gently rocked and incubated at 4°C for 45 minute in the fridge. The confirmation of the virus was done with positive HI test result.

Serum Assay

The titre of the standard virus was determined by hemagglutination test (HA) with 1% wash chick red blood cells in U-shaped 96 wells microlitre plastic plates using 25-microlitre volumes in 2-fold serial dilutions. The 4 HA units of the virus titre were prepared with phosphate buffered saline, pH 7.2 in sterile universal bottle, kept at 4°C until used.

The titration of the sera samples was done in 2-fold serial dilution using hemagglutination inhibition (HI) test (Burnet, 1942) in U-shaped 96-wells plastic Microtitre plates. 25 micro litre of the serum was diluted in phosphate buffered saline (PBS), pH 7.2. 25 microlitre of the 4 HA units of the antigen was added and the mixture was allowed to react for 15 minutes at 4°C after which 25 microlitre of 1% chick red blood cells was added to each test well and the controls. The serum titre was determined after 45 minutes incubation at 4°C fridge temperature and expressed as HI unit.

RESULTS

Sample analysis gave 69.14% positive results that is 1210 out of 1750 samples contained antibody to Newcastle Disease while 30.86% (540) of the samples did not show any detectable Newcastle disease antibody (Table 1).

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Over 50% of the samples collected from the different locations contained no antibody.

The hemagglutination inhibition (HI) antibody titre obtained ranges from 2 to 512, while the overage HI antibody titre ranges from 13.01 to 33.00. Barkin Ladi samples had the highest range as well as highest average HI antibody to Newcastle disease (Table II).

TABLE II: Samples Range and Average Hemaglutination Inhibition (HI) Antibody Titres to Newcastle Disease

Location (L.G.A.)	Total Samples	No. of Samples Positive (%)	No. of Samples Negative (%)	Average HI Antibody Titre
Jos North	650	428	2 - 128	25.52
Jos South	450	271	2 - 128	24.80
Barkin Ladi	260	208	2 - 512	33.00
Riyom	200	155	2 - 64	14.15
Mangu	190	148	2 - 128	13.01

NB: . Haemagglutination inhibition antibody above 64 HI unit conferred protections against challenges with field strains ND virus. The samples from Barkin Ladi had the highest average titres of 41 antibodies of 33.00 although the sample size was less than that collected from Jos North with 650 samples.

Swab sample for ND virus isolation were obtained only in four locations. No sick birds for sample was found in Riyom Local Government Area at the time of sampling. Five isolates of ND were obtained from only two locations 3 isolates from; Barkin Ladi Local Government Area gave no isolates (Table III).

TABLE III: Samples Range and Average Hemaglutination Inhibition (HI) Antibody Titres to Newcastle Disease

Location (L.G.A.)	Total Samples	No. of Samples Positive (%)	No. of Samples Negative (%)	Average HI Antibody Titre	Average log E.I.D. 50
Jos North	14	2	5.00	30.00	$10^{10.90}$
Jos South	5	0	0.00	12.50	-
Barkin Ladi	9	3	7.50	15.00	$10^{10.30}$
Riyom	0	0	0.00	0.00	-
Mangu	12	0	0.00	30.00	-
Total	40	5	12.50	87.50	

NB: Isolates were obtained only from clinically confirmed sick birds with ND characteristics symptoms using 9 – 10 days old chick embryonated eggs.

The results of haemagglutination test of the test samples are as shown in table IV.

TABLE IV: HI antibody and Antigen to influenza A and B with Newcastle disease antibody and antigen.

Virus Serotypes	Influenza A HINI Antibody	Influenza H3N2 Antibody	Influenza Antibody	NDV Positive Antibody	Influenza Negative Control Antibody
Inf A HINI	+	-	-	-	-
Inf A H3N2	-	+	-	-	-
Inf B	-	-	+	-	-
NDV – L	-	-	-	+	-
NDV – K	-	-	-	+	-
NDV – 1/0	-	-	-	+	-
NDV Is 1	-	-	-	+	-
Is 2	-	-	-	+	-
Is 3	-	-	-	+	-
Is 4	-	-	-	+	-
Is 5	-	-	-	+	-

NB: + = Positive Inhibition reaction. Is = Isolates (1, 2, 3, 4, 5)
- = Negative Inhibition reaction. NDV = Newscastle disease Virus
L = Lasota. K = Komatov. I/o = IntraOcula

The cross reaction of influenza positive antibodies with ND virus antigen and ND virus positive to Influenza virus antigen did not show any evidence of cross reaction or identify any relatedness or relationship between the isolates.

DISCUSSION

Local chickens contribute immensely and account greatly and also remain one of the cheapest sources of animal proteins to the local and the urban populations in Nigeria and most African countries. Newcastle disease is a dreaded disease to both exotic and local village birds (Adu, 1987; Onunkwo and Momoh, 1981).

As village chickens continues to die due to Newcastle disease unnecessarily an important source of protein from village chickens will be lost (Alders and Spradbrow, (1999) Village chicken require access to Newcastle disease vaccines now and flock owners deserve this services (Fatunbi and Adene, 1979).

Strictly speaking local scavenger birds in Nigeria are not routinely vaccinated like the exotic poultry birds hence they often maintain pockets of disease outbreaks like Newcastle disease or they receive very little or none of veterinary services (Olabode *et al*; 1992).

The problem of controlling ND in local chickens is many because the flocks are small, scattered and multi-aged. The owners of the chicken (often women) lack economic or political influence and veterinary and extension services are seldom responsive to their needs (Spradbrow, 1993 – 94).

Serological studies carried out on 1750 local birds sera in Plateau State showed that 1210 sera were positive for Newcastle disease antibodies. The fact that these birds were not vaccinated may suggest that we have some strains of Newcastle disease virus

circulating in the field. Also five isolates of ND viruses were obtained from swabs collected from ND suspected sick birds. This finding agreed with the studies of Echeonwu et al (1993) in which velogenic Newcastle virus was recovered from dead and healthy free roaming birds in Nigeria.

This result obtained from this study with the local chicken sera may imply that the current NDV strains causing Newcastle disease among local birds may be antigenically related to the vaccine strain used for the exotic birds in the field or an indication of the vaccine strain mutant circulating in the field. The antibody identified by the vaccine virus strains may be due to incontact vaccination or spread from the vaccinated exotic poultry birds to the local birds in he areas and not infection by a new ND virus strain. It is very possible for incontact vaccination since NDV Lasota and Komarov have been in use in the field for the past three decades (30 yrs) in Nigeria

Olabode *et al*, (1992) in a separate study on the use of thermostable NDV_4 vaccine in the control of ND in local chicken discovered that the villages are loosing their birds in there hundreds to Newcastle disease. Some of these occurred as small pocket outbreaks, which are not reported. This study therefore has shown that the success in the control and eradication of ND in Nigeria should start first with the use of potent ND vaccine prepared for routine vaccination of the local chickens.

However, the risks of having village flocks with locally produced vaccines must be balanced against the outcomes of contact with circulating strain of Newcastle disease virus in the field (Spradbrow, 1993-94). In line with the outbreaks of avian flu virus in Combodia, China, Hong Kong, Indonesia, Japan, Korea Laos, Thailand and Vietnam in the recent past, efforts were made to identify any presence of influenza virus antigens in local birds in Plateau State of Nigeria using the available earlier circulated human influenza virus strains antibody and antigen to H1N1 and H3N2. Although it was not possible to get the recent influenza A (H5 N1) virus currently in circulation in Asia, the Haemagglutination inhibition tests conducted with field isolates of Newcastle disease virus which were cross matched with influenza antibodies to Sero type A and B influenza virus and vise-visal with NDV antibody does not show any evidence of cross reaction and or implication of influenza virus antigen among local chickens in the area of study (Table iv). This study meanwhile wish to suggest that urgent attention be given to local village birds in the control of known poultry disease like Newcastle disease in Nigeria.

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