THE SEROLOGICAL STATUS FOR NEWCASTLE DISEASE IN LOCAL CHICKENS OF LIVE BIRD MARKETS AND HOUSE HOLDS IN NSUKKA, ENUGU STATE, NIGERIA

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Abstract: Newcastle disease (ND) is the most important viral disease of poultry in the world and a major constraint against both Industrial and village poultry production in Africa and Asia. A serological study was conducted to estimate the level of circulating antibodies against ND in unvaccinated local chickens, raised under traditional management system in Nsukka, Enugu State, Nigeria. Haemagglutination inhibition (HI) test was used to analyze 400 chicken sera (252 live bird market and 88 household chickens) for NDV antibodies from four Local Government Areas (LGAs) -Udenu, Nsukka, Igbo-Eze South and Igbo-Eze North- all within Nsukka Zone. The overall seroprevalence rate was 60.3% and only 47.1% of chicken had HI antibody titre of > 41og2 which was considered protective. A seroprevalence rate of 65.1% and 46.6% and a Geometric Mean titer (GMT) of 207.9 and 11.3 were obtained from live bird markets and households chickens respectively. About 52.9% of chickens sampled were at risk of suffering clinical ND. There was a significant difference ($P < 0.05$) in the overall antibody prevalence rate between live bird market chickens and household chickens and between the age groups. A seroprevalence rate of 60.3% NDV antibodies in apparently healthy chickens observed in this study is suggestive of the presence and continuous circulation of NDV in the study area. Hence, improvement of extension services, biosecurity measures, and routine vaccination with thermostable NDV vaccines are highly recommended.

Keywords: Haemagglutination inhibition (HI), Household chickens, Live bird market, Newcastle disease (ND), Newcastle disease virus (NDV), Seroprevalence.

INTRODUCTION

Newcastle Disease (ND) is the most important viral disease of poultry in the world including developing countries (Adene, 1999; Spradbrow, 1997). ND is a highly contagious and commonly fatal viral infection of birds which can cause up to 100 percent mortality in susceptible chickens. Many avian species may become infected but dramatic losses are seen most often in domestic fowl and to a lesser extent in turkeys and pheasants (Rosenbeger *et al.*, 1975). In Nigeria, ND has been reported as one of the greatest constraints to the development of rural poultry production (Shamaki *et al.*, 1989; Oladele *et al.*, 2003).

ND is caused by avian paramyxovirus type-1 (APMV-1) which is classified with other paramyxoviruses in the genus Avulavirus, subfamily paramyxovirinae, family paramyxoviridae, and order mononegavirales. It is an enveloped virus and has a negative sense single strand RNA genome (Lamb *et al.*, 2005). The genome contains six genes 3′-MP-P-

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M-F-HN-L-5' that encode six major proteins: nucleoprotein, phosphoprotein, matrix protein, fusion protein, haemmaglutinin-neuraminidase, and RNA- dependent RNA polymerase respectively (Chamber *et al.*, 1986; Alexander and Senne, 2008). Beard and Hanson (1984) classified Newcastle disease virus (NDV) strains into highly virulent (Velogenic), intermediate virulent (Mesogenic) or avirulent (Lentogenic) based on their pathogenicity in chickens. Lentogenic strains produce mild or inapparent respiratory infections, the mesogenic strains produce low mortalities, acute respiratory disease and neurological signs in some birds while the velogenic strains which can be either neurotropic velogenic NDV (NVNDV) or viscerotropic velogenic NDV (VVNDV), cause disease with high mortality (Huang *et al.*, 2004; Piacenta *et al.*, 2006). The known surface antigens are the haemagglutinin and neuraminidase (Nagai *et al.*, 1976) and the fusion (F) protein. The first, haemagglutinin-neuraminidase (HN), is important in the attachment and release of the virus from host cells, in addition to its role in serologic identification (Beard and Hanson, 1984; OIE, 2008). The other, the fusion (F) protein, has a critical role in the pathogenesis of the disease (Beard and Hanson, 1984; OIE, 2008).

According to FARD (2006), the poultry population of Nigeria is estimated to be 137.6 million, with backyard poultry population constituting 84% (115.8 million) and exotic poultry 16% (21.7 million), with a higher percentage of these poultry raised for subsistence production. Ogundipe (1998) reported that close to 75% of the Nigerian population live in rural areas where most households keep small number of local and village chickens, which scavenge on free range and usually taken care of by women and children. Establishment of the disease status in Nsukka is of great importance to avoid economic losses caused by the disease. Therefore, the aim of this study was to determine the seroprevalence of NDV within Nsukka Area and its environs.

MATERIALS AND METHODS

Experimental Design

Four selected Local Government Areas (LGA) in Nsukka were covered in the study. The LGA include: Nsukka, Igbo-Eze North, Igbo-Eze South, and Udenu. All the Local Government Areas are in Nsukka, Enugu State which is located in the South East Savannah derived zone of Nigeria. The study was carried out for a period of seven months between August 2013 and March 2014.

Selection of Birds

A total of four hundred (340) apparently healthy chickens, including cocks, cockerels, hens and pullets were used in this study. Out of 340 chickens, two hundred and fifty two (252) were purchased from live bird markets while eighty eight (88) chickens were bought from households. Five live bird markets and 12 villages were selected for the study. The markets include Obollo-Afor (in Udenu LGA), Ikpa (in Nsukka LGA), Nkwo Ibagwa-Aka and Orie Igbo-Eze (both in Igbo-Eze South LGA), and Eke Ozzi (in Igbo-Eze North LGA). The villages include Orba, Obollo-Eke, Obollo-Orie, all in Udenu LGA; Edem Ani, Opi and Obukpa, all in Nsukka LGA; Ugoo, Ovoko and Iheakpu-Awka, all in Igbo-Eze South LGA; Umuopu, Umuonu and Uda, all in Igbo-Eze North LGA.

In live bird markets, forty-seven (47) chickens were randomly purchased from Obollo-Afor; sixty-one (61) chickens from Ikpa, sixty (60) from Nkwo Ibagwa-Aka and Orie Igbo-Eze, and eighty-four (84) chickens from Eke Ozzi. For the households, twenty-two (22) chickens each were randomly purchased from three different villages in each of the four LGAs as stated above. Age was estimated in chickens purchased from households by the farmers and were then grouped into two: those whose age ranged from four to twenty-four (4-24) weeks (growers) comprised forty-four (44) chickens and the remaining forty-four (44) chickens were those whose age fell above twenty-four weeks (adults).

Blood Sample Collection and Storage

The skin of the wing vein was swabbed with seventy percent (70%) ethanol and allowed to dry. Blood sample was collected from each bird through the wing vein using sterile 2 ml hypodermic syringe and 21G needle into sterile bijou bottles. This was slanted for the blood to clot, and then placed in ice packs during transportation to the laboratory. Bijou bottles containing clotted blood were then left on the bench for 1-2 h at room temperature for sera to separate from the clotted blood. Using Micropipettes, the separated serum was collected into a new clean plain bottle and refrigerated at about 4°C until tested.

Serology

Washing of Erythrocyte

Five milliliters (5 ml) of blood sample was collected aseptically from young chicken and transferred to a sample bottle containing 1 ml of sodium citrate (4% solution) as anticoagulant. The blood was centrifuged in a desk centrifuge (HME Global, England), at 1200 g for 15 min and the supernatant (plasma) and buffy coat pipetted off. The packed red blood cells (RBCs) were resuspended in 10 ml phosphate buffer saline (PBS). The process of RBCs suspension in PBS and centrifugation was repeated until a clear supernatant was obtained (3 times on the average). Then 10% and 1% RBCs suspensions in PBS were made to be used in spot test and HI test respectively.

Reconstitution of Antigen

A 200 dose La Sota virus strain of the antigen obtained from Veterinary Teaching Hospital, UNN was reconstituted in 10 ml of fresh PBS as diluents in a sterile bijou bottle. The bottle was recapped and gently shaken to homogenize before using it in spot and haemagglutination tests.

Spot test

Spot test was done using a clean white tile. A drop of viral antigen was placed at the centre of the tile. A drop of 10% chicken RBC was added to the antigen, both were mixed together and rocked gently. The mixture was observed for haemagglutination. This test was used to check the potency of the antigen.

Haemagglutination (HA) test

The antigen titer was determined by haemagglutination (HA) test as described by OIE (2008). The titer was taken as the reciprocal of the highest dilution giving a 100% agglutination of 10% chicken RBC. This amount represents 1 haemagglutination unit (HAU). Four HAU of the virus antigen

was calculated and diluted accordingly for use in HI test.

Haemagglutination Inhibition (HI) test

HI test was performed against 4 HAU of the virus antigen following standard procedure described by OIE (2008). The titers were expressed as log2 of the highest dilution of serum giving 100% inhibition of the 4 HAU. Titers equal or higher than 4log2 were considered protective.

Data Analysis

Geometric mean of HI antibody titer (GMT) and percentages of detectable NDV HI antibody titer were calculated. The GMTs of all the sera were determined according to the method described by Brugh (1978). Data analysis was performed using chi-square test for the two variables of location and age. Differences in prevalence rates between variables and disease were considered statistically significant at $P < 0.05$.

RESULTS

The overall seroprevalence of Newcastle disease (ND) from the four selected LGAs was 60.3% as shown in table 1. There was a significant difference ($P < 0.05$) in the overall seroprevalence of ND between live bird market and household (table 1). On individual LGA, Udenu had the lowest seroprevalence of 46.4%, followed by Igbo-Eze South with 54.9%, then Igbo-Eze North with 67.0% and Nsukka with the highest seroprevalence of 68.7% (tables 2 – 5). While the seroprevalence of live bird market followed the same pattern, the seroprevalence of the household showed that Igbo-Eze North and South had the highest seroprevalence of 54.5%, followed by Nsukka with 45.5%, and Udenu with 31.8% (tables 2 – 5). There was no significant difference ($P > 0.05$) in ND seroprevalence between live bird markets and household in each of the LGA except in Nsukka LGA ($P < 0.05$). The seroprevalence of ND was significantly higher ($P < 0.05$) in the adult birds than in growers (table 6). Table 7 shows the overall HI titers against NDV in both live bird markets and households. More birds had titer of 2^{10} than lower dilutions.

DISCUSSION

HI test is still the most widely used assay that requires cheap reagents and easy interpretation. It is a conventional serological method for measuring anti-NDV antibody levels in poultry sera and considered the standard laboratory method for diagnosis of NDV (Jestin *et al.*, 1989). The present serological study showed that out of 340 chickens sampled, 205 of them had detectable antibodies to NDV which indicated evidence of virus infection and revealed the presence of widespread circulating antibodies of Newcastle disease virus in the area. Antibodies detected may be a result of natural infection since these birds are hardly ever vaccinated and roam about acting as reservoir and carriers of the disease to commercial farms and to themselves (Olabode *et al.*, 1992). Based on the result of this work, 60.3% of samples tested positive to NDV antibodies. Similar studies in other parts of Nigeria have reported variable HI antibody seroprevalence rates. For instance, 60%, 72%, and 74.3% seroprevalence were reported in Southeastern Nigeria (Orajaka *et al.*, 1999), in Zaria (Ezeokoli *et al.*, 1984) and in Maiduguri (Nwanta *et al.*, 2008)

respectively. These are relatively higher than the seroprevalence rate observed in this study. However, lower seroprevalence rates of 38%, and 54.0%, have been reported in Southwestern Nigeria (Oyewole *et al.*, 1996) and FCT Abuja (Olabode *et al.*, 2006) respectively. These observed regional differences in ND seroprevalence showed ecological area variation in NDV activity and may perhaps be a reflection of the impact of environment on the viability and spread of NDV and its epidemiology (Orajaka *et al.*, 1999). Only Nsukka LGA, out of the four LGAs studied, showed a significant difference ($P < 0.05$) in seroprevalence rate between live bird markets and households chickens. This may be as a result of high concentration of commercial poultry in Nsukka LGA. Commercial chickens are routinely vaccinated against NDV and rural chickens may come in contact directly or indirectly with them in live bird markets, which could lead to transmission and spread of vaccine virus among local birds. Live bird markets are also a common source of ND as a result of bringing sick, infected and uninfected birds together for sales (Nwanta *et al.*, 2008). This is reflected in the differences seen in the overall antibody prevalence rate between samples from live bird markets (65.1%) and those from households (46.6%). Other studies conducted on local chickens at live bird markets in Nigeria by Ameji *et al.* (2011), Chollom *et al.* (2013), and Jibril *et al.* (2014) showed 96%, 35.8% and 25.5% seroprevalence rates respectively. There was a significant difference ($P < 0.05$) between the two age groups which is in line with similar studies carried out in Vietnam (Vui *et al.*, 2002), and Thailand (Thitisak *et al.*,1988).

A ND-HI titer of 4log2 or above is generally accepted as indicative of specific immunity based on OIE recommendation of 2008. Using these criteria in our work, 41.7%, of live bird market and 85.2% of household chickens showed no serological signs of specific immunity. According to Awan *et al.* (1994), low HI antibody prevalence is suggestive of an interepidermic phase or early phase of infection. Problems with ND outbreaks in the near future may have to be expected unless the vaccination practice is improved substantially. In this study, the log2 titer ranged from 1 to 10 which is comparable to a study among unvaccinated chickens in Vietnam in which the log2 titer ranged from 0 to 11. The wider range of anti-NDV titers in unvaccinated chickens may be due to natural infection which is known to produce higher antibody titer than vaccination (Luc *et al*,. 1992; Ricardo *et al.*, 2000). Okwor and Eze (2011) reported that most village chickens in the Eastern part of Nigeria are not vaccinated and acquire antibodies to NDV after survival from an active infection. Therefore, the seroprevalence of NDV in these apparently healthy chickens shows that they may have either survived clinical disease or subclinical infections and could thus act as reservoirs (Bell and Mouloudi, 1988; Olabode *et al.*, 1992 and Orajaka *et al.*, 1999). A reinfection or immunization some weeks after antibody begins to decline produces a secondary response (Allan and Gough, 1976). Thus, the high GMT observed in this study may be due to constant reinfection with NDV.

In conclusion, this study has demonstrated the presence and continuous circulation of NDV in apparently healthy chickens in the study area. Lack of biosecurity measures and poor veterinary awareness among rural poultry farmers were observed. It is recommended that extension services to the farmers through extension workers should be improved and the farmers should be educated on the need to quarantine any bird purchased before mixing with household chickens.

Table 1: The overall seroprevalence of ND in Local Chickens of Live Bird Markets and Households

S/N	Location	No of sample	No of positive sample	% prevalence	HI titre ≥ 4log2	
					Frequency (f)	Percentage (%)
	Live bird Market	252	164	65.1	147	58.3
	Household	88	41	46.6	13	14.8
	Total	**340**	**205**	**60.3**	**160**	**47.1**

X2 = 9.313a, df=1, P-value = 0.002 (significant)

Table 2: Seroprevalence of ND in Udenu Local Government Area

S/N	Location	No. of Sample	No of positive sample	% prevalence
1	**Live Bird Market**	47	25	53.2
2	**Household**	22	7	31.8
	Total	**69**	**32**	**46.4**

X2 = 2.753a, df=1, P-value = 0.97 (Not significant)

Table 3: Seroprevalence of ND in Nsukka Local Government Area
X2 = 7.503a, df=1, P-value = 0.006 (Significant)

S/N	Location	No. of Sample	No of positive sample	% prevalence
1	**Live Bird Market**	61	47	77.0
2	**Household**	22	10	45.5
	Total	**83**	**57**	**68.7**

Table 4: Seroprevalence of ND in Igbo-Eze South Local Government Area
X2 = 0.001a, df = 1, P-value = 0.971 (Not significant)

S/N	Location	No. of Sample	No of positive sample	% prevalence
1	**Live Bird Market**	60	33	55.0
2	**Household**	22	12	54.5
	Total	**82**	**45**	**54.9**

Table 5: Seroprevalence of ND Igbo-Eze North Local Government Area

S/N	Location	No. of Sample	No of positive sample	% prevalence

1	Live Bird Market	84	59	70.2
2	Household	22	12	54.5
	Total	106	71	67.0

X2 = 1.941a, df = 1, P-value = 0.164 (Not significant)

Table 6: Seroprevalence of ND in chickens According to Age

Age	4-24 weeks	Above 24 weeks
No. of sample	44	44
No of positive sample	13 (29.5%)	28 (63.6%)
HI titre ≥ 4log2	2 (4.5%)	11 (25.0%)

X2 = 10.275a, df = 1, P-value = 0.001 (Significant)

Table 7: Overall HI Titres against NDV in Local Chickens of both Live Bird Markets and Households

No of positive sample	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	GMT
206	9	13	24	16	11	10	11	24	22	66	119.4

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