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The Carrier Rate of Newcastle Disease Virus in Pigeons in Owerri Area of Imo State, Nigeria

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Abstract

The role of domestic pigeons (*Colombia livia domestica*) in the transmission of Newcastle disease was investigated in 3 Local Government Area (LGAs) of Owerri Zone of Imo State – Owerri Municipality, Owerri North, and Owerri West LGAs of Imo state, Nigeria between February and May, 2004. Isolation of Newcastle disease virus (NDV) was attempted from 20 apparently healthy pigeon collected per LGA. Cloacal swabs from pigeons coloured mainly black and white were inoculated into 9-11 day old embryonated hens' eggs via the allantoic route and incubated for 96 hours at 37^0C in a humidified incubator. Three (5%) of the 60 pigeons tested positive for NDV. Two (10%) were positive for Owerri Municipal Area, 1 (5%) for Owerri North LGA and none for Owerri West LGA. Two (16.6%) of 12 white pigeons and one (14.3%) of 7 black pigeons showed evidence on NDV. The carrier rate of NDV in pigeons in Owerri Zone of Imo State was 5%. Because of the seriousness of this disease in birds, including chickens, more studies of their nature needs to be conducted in Imo State.

Keywords: Newcastle Disease Virus, Pigeon, Owerri

1.0 Introduction

Newcastle disease is an acute, rapidly spreading, nervous and respiratory disease of birds of all ages. The disease has been referred to variously as avian-pneumoencephalitis, pseudo fowl pest, pseudo poultry plaque, and Ranikhet disease in various areas of the world (Doyle, 1992; Adu, 1987; AL-Jumaily *et al.*, 1980). Newcastle disease was first identified in 1953 (Echeonwu, *et al.*, 1997) and with Newcastle disease virus (NDV) being the causative agent. Newcastle Disease Virus (NDV) is the etiologic agent. It is a serious and fatal disease of chickens caused primarily by a paramyxo-virus (Alexander and Alan 1993). The first description of the disease was from Java (Indonesia) in 1926, in India the disease was first described by Ranikhet hence the name Ranikhet disease in that part of the world (Al-Jumaily, *et al.* 1989). In most developing countries, Newcastle disease is the most important infection disease affecting village chicken (Park and Shaw, 2000).

Newcastle Disease Virus (NDV) is parmyxo-virus and is made up of various strains. Among the various strains of NDV there are various levels of lethality. The most virulent (velogenic) strains can cause rapid onset of disease and kill almost 100% of the infected birds (Echeonwu *et al.*, 1997). There are naturally milder forms that are not deadly (Lentogenic). The virus can infect all species of birds-both domesticated and wild bird populations. The impact of the disease even in mild forms is a drastic reduction in the commercial product of eggs and broilers.

In a study of Newcastle disease outbreak, Echeonwu *et al.* (1997) reported a carrier rate of the disease in 5.56% of pigeons in Kaduna state, Nigeria. In Nigeria the factors tending to produce high incidence of Newcastle disease include the large population of chickens, ducks and feral birds whose movement are completely unrestricted (Okeke and Lamorde, 1988). The free flying birds have been regarded as reservoirs of NDV for the free roaming domestic chickens. These carrier birds maintain the virulent strain of the virus in circulation and constitute effective foci of challenge to susceptible intensively reared large poultry flocks (Echeonwu *et al.*, 1997) and subsequently to humans, who work around them (Dawson 1993). Infection of chicken with NDV through pigeon feces has been documented (Alexander *et al.*, 1984). Recovery of NDV from

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pigeons has also been reported (Hanson and Sinha 1952; Vitiak, 1958; Echeonwu *et al.*. 1997). In a study of Newcastle disease outbreak, Echeonwu *et al.* (1997) isolate NDV from 1 out of 19 pigeons in Kaduna state, Nigeria.

In Owerri area of Imo State, pigeons are domesticated and maintained by families for money making and by traditional worshippers for healing and for other fetish purpose. Due to poor financing of back yard poultry enterprise, make shift structures are mostly relied upon for housing of the birds. These result in the exposure of poultry to competition for space, feed and water with free flying pigeons. These pigeons shed the virus in their faces, which easily contaminate feeds for poultry. Also, erosolized fecal dust remains a potent source of infection to poultry workers. The purpose of this study is to find out the prevalence of carrier rate of Newcastle Disease virus (NDV) in pigeons in the Owerri (Zone) of Imo State.

2.0 Materials and Methods

2.1 Egg Inoculation

Viable hens' eggs used for virus inoculation were selected through candling to determine their fitness. After selection, site of inoculation was identified, marked and disinfected using ethyl alcohol soaked in cotton wool to swab the surface of the eggs. The marked area was punched using an egg puncher. The cloacal samples were diluted 1:100ml with phosphate-buffered saline (PBS). The embryonated hens' eggs were inoculated aseptically through the egg allantoic cavity using 0.5ml of cloacal sample per egg. This was done by, first, drawing the cloacal sample into a 5 ml of sterile syringe and needle. The needle was then introduced into the allantoic cavity to a depth of 16mm via the egg membrane and 0.5 ml of cloacal sample released into the allantoic cavity from the syringe.

After inoculation, eggs were sealed with colodium and incubated at 37ºC in a humidified incubator. Eggs that died after 48 hours were chilled and the allantoic fluid harvested, pooled, labeled clearly and used for test. Death of eggs within the first 24 hours was regarded as non-specific and was ignored.

2.2 Spot Agglutination Test

The pooled allantoic fluids were tested for the presence of virus using spot agglutination test. The procedure involves placing 3 separate drops (approximately 0.02 ml) of 10% saline suspension of washed chicken red blood cells (RBC) on clean glass slide. That first drop (auto-agglutination control) was mixed with a drop of phosphate buffered saline (PBS). To the second drop was added a loopful of negative control allantoic fluid. The last drop was mixed with loopful of the unknown sample to be tested. Positive sample showed agglutination within a minute. No agglutination was observed in negative samples. The fluid that showed no agglutination (Negative) for the spot test was subjected to hemagglutination inhibition (HI) to confirm the type of virus present.

2.3 Chicken Erythrocytes (RBC)

This was obtained by bleeding health chicken (via the wing vein) and mixing the blood obtained with citrate anticoagulant (ACD) to prevent clotting. The freshly obtained blood sample was washed three times in phosphate buffered saline (PBS) using an MSE centrifuge and then preparing a 10% stock of the chicken erythrocytes. Furthermore, a 0.5% suspension was prepared by mixing 1ml of the 10% stock with 19ml of PBS.

2.4 Hemagglutination Titration (HA)

The hemagglutination (HA) test was done in U-bottom microliter plates. Using single channel pipette, 50 microliters of the PBS was dispensed into V-bottom microplates beginning from the first to the last. A 2-fold serial dilution of the virus was carried out. This involved placing an equal volume (50 microliters) of the virus in the first well and mixing thoroughly 5 times with the pipette. About 50 microliters of the mixed content of the first well was transferred to the second well. The process was repeated up to the 11th well and the last 50 microliters discarded. A freshly prepared 50 microliters of 0.5% chicken red blood cell was added to each well including the last well (12th), which stood as the control well. The plate was allowed to incubate for 30-60 minutes at room temperature before reading. The HA titer was recorded. This was used to cal-

calculate the 4 HA unitage used in the hemagglutination inhibition (HA) test.

2.5 Haemaflutination Inhibition Test

2.5.1 Beta Procedure (Constant Virus Plus Diluted Serm)

This procedure was given in methods for examining poultry biologics and quantifying avian pathogens of the national academy of science, Washington D.C. 1971. The 4HA unitage of stock virus by HA test was used.

Into each well of a U-bottom microliter plate was dispensed 25 miccroliters of phosphate buffered saline (PBS). About 25ml of serum was dispensed into the first well and the last (control) well of a row of microwells. A two-fold serial dilution was done along the row until the 2nd last well from the end. The last well being the serum control was not diluted. About 25ml of the 4HA dilution of antigen was added to each well excluding the control wells in the last column. The plates were gently tapped by the sides to mix the reagents. About 25ml of p0.5% washed red blood cells was added to each well including the control wells. The reagents were mixed and incubated at room temperature for 45 minutes. The setting pattern was observed and the end point noted.

3.0 Results

Of the cloacal swabs collected from 60 pigeons and inoculated into embryonated eggs (F1-F60), only 3 isolates (F3, F7, and F26) derived from 3 pigeons gave positive results for Newcastle disease virus

Figure 1: Map of Nigeria Showing the Location of Imo State.

(NDV). This result represents a carrier rate of 5% of NDV in pigeons examined in the 3 Local Government Areas (Owerri municipality, Owerri north, Owerri west) of Imo State, Nigeria. Two (10%) of the positive samples (F3 and F7) were isolated from white pigeons and recorded for Owerri municipal. Only 1(5%) positive result (F26) was recorded for Owerri North LGA and was recovered from a black pigeon (Table 1).

4.0 Discussion and Conclusion

Domestic pigeons have been identified as reservoirs of Newcastle Disease Virus (NDV) in many parts of the world. Pigeons while appearing normal, shed the virus in their feces, which can initiate outbreaks of fatal disease in chicken and amongst poultry workers (Rithie, 1985; Echeonwu *et al.* 1997; Alexander *et al.* 1984). In the present study, a 5% (3 out of 60) carrier rate of NDV was detected in the pigeons from Imo state. Echonwu *et al.* (1994)

Table 1: Spot Test Results of Cloacal Swabs of Pigeons Sampled From Three Local Government Areas

Pigeon Type	Owerri Municipality		Owerri North		Owerri West	
	Number Examined	Number Positive & (Percent)	Number Examined	Number Positive & (Percent)	Number Examined	Number Positive & (Percent)
White	12	2 (16.6)	13	0 (0)	7	0 (0)
Black	8	0 (0)	7	1 (14.3)	13	0 (0)
Total	20	2 (10)	20	1 (5)	20	0 (0)

Total Number of Pigeons examined in Imo State = 60
Total Number Positive (in %) = 3 (5%)

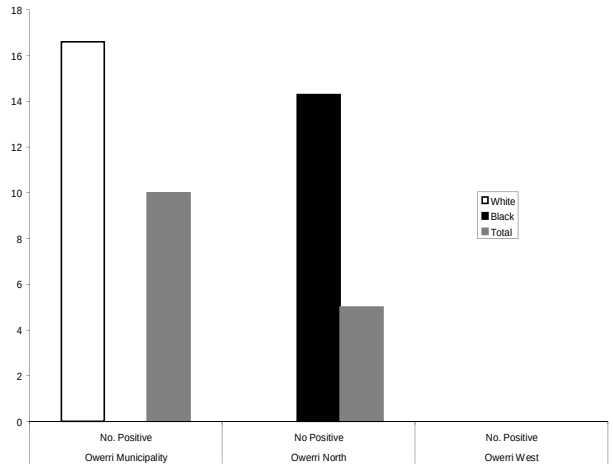

Figure 2: Graph showing carrier rate of NCV in Pigeons in Owerri zone.

reported a rate of 1 out of 19 pigeons (5.26%) in Kaduna State, Nigeria.

In 3 local government areas sampled in this study, 10% carrier rate was recorded for pigeons in Owerri municipality, 5% rate for Owerri north LGA and none for Owerri west. Large number of refuse dumps and other pollutants litter for days/weeks the Owerri municipality without evacuation. Similarly, the sub-urban status of Owerri North LGA and its close proximity to Owerri municipality may account for incidence of NDV in the local government area. Further research needs to be done to ascertain the role of environmental pollution in relation to the to the 3 local government areas mentioned. The higher incident of NDV recorded for white pigeons than for black ones should also be a subject for further study. Aerosolized fecal dust and contaminated beddings are considered sources of infections to humans (Lancaster, 1963). During this study no attempt was made to investigate incidence of Newcastle disease in chickens or humans in the area sampled. Control and eradication of Newcastle disease appear feasible among poultry flocks, carrier birds and humans. Solid structures and adequate fencing are important to reduce competition for food, water and space by pigeons. Other approaches include strict quarantine; culling and disposal of all infected and exposed birds. It has been reported that NDV can live up to 12 months in frozen chicken carcass (Okeke and Larmode, 1988). Domestic pigeons should be vaccinated against Newcastle disease virus. Evidence has shown that pigeons respond to such vaccination (Echeonwu *et al.* 1997). Also poultry workers who work around birds suspected of being infected with NDV, should shower, change clothes, remove shoes and disinfect any items that were exposed prior to handling healthy birds (Wisman, 2002). Available literature shows that Newcastle disease occurrence in pigeons from Imo State is a geographical record.

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