Advances in Science and Technology, Vol. 4, No. 2, September / October 2010, pp 144 - 147

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

A.U. Opara[1] and Alex D.W. Acholonu[2]
[1]School of Medical Laboratory Sciences, College of Medicine and Health Sciences, Imo State University, Owerri, Nigeria
[2]Department of Biological Science, Alcorn State University, Alcorn State, MS 39096, USA
(Submitted: March 16, 2010; Accepted: October 20, 2010)

Abstract

The carrier of Newcastle Disease Virus (NDV) in ducks was investigated in three Owerri Local Government Areas (LGAs) – Owerri Municipal, Owerri North and Owerri West in Imo State, Nigeria between January and April 2006. A total of sixty apparently healthy ducks were tested in the study areas. Ducks cloacal swabs were obtained and inoculated into old embryonated hens' eggs via the allantoic route and incubated for 96 hours at 37°C in a humidified incubator. Only one of the 60 ducks (D14) tested positive for NDV and it was collected from the Owerri Municipal Council area. No positive results were recorded for Owerri North and Owerri West LGAs. The isolate that yielded positive result was from a white duck. From this study, the carrier rate in NDV in ducks in Owerri area of Imo State is 1.6% (1/60). The occurrence of NDV in ducks is a geographical record for Imo State. Further studies of this nature are recommended for future investigators.

Keywords: Newcastle Disease Virus, Ducks and 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-poultry plaque and Ranikhet disease in various areas of the world (Doyle, 1992; Adu, 1987; AL-Jumaily *et al.*, 1980). In Nigeria, Newcastle disease was first identified in 1953 (Echeonwu *et al.*, 1997) and with Newcastle Disease Virus (NDV) incriminated as the causative agent.

Newcastle Disease Virus (NDV) is a paramyxo-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 as deadly (lentogenic). The virus can infect all species of birds – both domestic and wild bird populations. The impact of the disease even in mild forms is a drastic reduction in the commercial production 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. A similar study in Owerri area of Imo State detected a carrier rate of 5% in pigeons in the area (Acholonu and Opara, 2007). Recovery of NDV from pigeons has only been reported by Hanson and Sinha (1952), Vitiak (1958), Adeshina and Oluwole (1989), Alfonso and Cowen (1995) and Boyvier and Shaw (1984). Laboratory and field observations indicate that ducks are important reservoirs and could be means of spread of NDV (Lancaster, 1963; Echeonwu *et al.*, 1997). The main methods of transmission of the disease from one location to another seem to be via bird contact, human activities, insects, rodents, cages, machinery equipment and infected eggs. 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).

In Owerri area of Imo State (see Figure 1), ducks are owned by families, but are not routinely caged, as are pigeons. Ducks are allowed to roam about unrestricted and this result in the exposure of poultry to competition for space, feed and water with ducks, some of which may harbor Newcastle Virus. These

Corresponding Author's E-mail: chiefacholonu@alcorn.edu

ducks shed the virus in their feces, which easily contaminate feeds for poultry. Ducks are, also reared intensively for money making, ritual practices, egg production and are also exchanged as prestige gift items between families during ceremonies. Meat and blood of such ducks, if infected, constitute health hazards to the population.

2.0 Materials and Methods

Viable hens' eggs used for virus isolation were selected through candling to determine their fitness. The eggs are prepared for use by disinfecting the site of inoculation of the specimen with 70% ethyl alcohol soaked in cotton wool. Cloacal samples derived from suspected ducks were 1:10ml with phosphate-buffered saline (PBS, Ph 7.4) for inoculation (0.05ml) through the allantoic route. The cloacal sample was first drawn into a 5ml sterile syringe with needle. The needle was then introduced into the allantoic cavity of the egg to a depth of 16mm via the egg membrane and 0.5ml of cloacal sample released into the allantoic cavity.

The point of egg inoculation was then sealed with colodium and incubated in a humidified incubator maintained at 37°C. Death of eggs within the first 24 hours was regarded as non-specific and was ignore. Eggs that died after 48hours were chilled and the allantoic fluid harvested and used for erythrocyte agglutination test.

2.1 Chicken Erythrocytes (RBC)

Red blood cell stock was obtained by bleeding healthy chicken (via wing vein) and collecting the blood in a sterile bottle containing acid citrate dextrose anticoagulant (ACD). The blood sample was washed three times in phosphate buffered saline solution (PBS) and prepared as a 10% stock of the chicken erythrocytes.

2.2 Spot Agglutination Test

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

2.3 Hemagglutination Titration (HA)

The hemagglutination (HA) test was done in U-bottom microtiter plates. Using sinlge channels pipette, 50 μl of the PBS was dispensed into U-bottom microtiter beginning from the first to the 12th. A two-fold serial dilution of the viral harvest was carried out. This involved placing an equal volume (50) of the allantoic harvest in the first well and mixing thoroughly 5 times with the pipette. About 50 of the mixed content of the first well was transferred to the second well and the process was repeated up to the 11th well. Finally, 50 μl of 0.5% chicken red blood cell was added to each well including the 12th (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 calculate the 4HA units used in the hemagglutination inhibition (HI) test.

2.4 Hemagglutination Inhibition Test: Beta Procedure (Constant Virus Plus Diluted Serum)

NDV antiserum was obtained from the Virology Division of the National Veterinary Research Institute (NVRI), from, Nigeria. Tests were performed according to the methods of Alan and Gough (1974). Four HA units of the stock viral harvest was used. Into each well of U-bottom microtiter plate was dispensed 25 μl of the 4HA dilution of the antigen was added to each well excluding the control wells. The plates were gently rotated to mix the reagents. About 25 of 0.5% washed reed 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 HI end point noted.

3.0 Results

Of the 60 cloacal swabs inoculated into embryonated eggs, only one (D14) yielded positive results for Newcastle Disease Virus (NDV). This

Table 1: Prevalence of NDV carriage among ducks from three LGAs in Owerri

Duck Type	Owerri Municipal		Owerri North LGA		Owerri West LGA		
	No. Examined	*No. Positive And %*	*No. Examined*	*No. Positive And %*	*No. Examined*	*No. Positive And %*	*Total (%)*
White	8	1 (12.5)	10	0 (0)	6	0 (0)	24 (4.16)
Black	11	0 (0)	16	0 (0)	9	0 (0)	36 (0)
Total	19	1 (5.6)	26	0 (0)	15	0 (0)	60 (1.6)

accounts for an NDV carrier rate of 1.6% in ducks screened in the three local government areas (LGAs). The positive isolate (D14) was derived from a sample collected from a white duck encountered in Owerri Municioal Council area. There were no positive results of NDV from ducks (black or white) tested in Owerri North and Owerri West LGAs (see Table 1 and Figure 2). Accordingly, the carrier rate of the disease in white ducks was 4.16% while the rate in ducks colored back was 0%.

Figure 1: Map of Nigeria showing the location of Imo State in which Owerri is situated.

Table 2: Hemagglutination (HA) and Hemagglutination inhibition (HI) test results of the NDV- positive allantoic sample (D14).

Allantoic Sample	HA titer	HI titer
D14	256	8

Table 2 represents the HA and HI titers of the

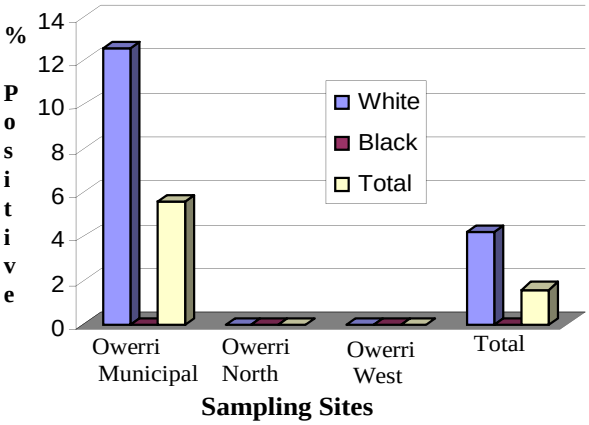

Figure 2: Prevalence of NDV carriage among ducks from three LGAs in Owerri

allantoic harvest of sample D14 with NDV infection. The HA titer for the isolate was 256 while the titer by specific antiserum against NDV hamagglutinin was 8.

4.0 Discussion

Newcastle Disease Virus (NDV) has been reported in various areas of the world implicating Newcastle disease virus as the causative agent. The virus can infect all species of birds both domesticated and wild bird population (Ritchard, 1985). Recovery of NDV from ducks has been reported by Hanson and Shina (1952), Vitak (1958) and Lancaster (1963).

Literature concerning Newcastle disease in Imo State is scarce and record of it s prevalence in Owerri is non-existent. Only recently, a 5% carrier rate of NDV in pigeons was reported in Owerri area of Imo State, Nigeria (Acholonu and Opara, 2007). No information is available concerning the disease in ducks in the area. So the occurrence of NDV of

ducks in Imo State, constitutes a geographical record. In this study, the carrier rate of NDV was investigated in ducks in Owerri area of Imo State. Carriage of NDV was detected in 1.6% of ducks tested, a rate lower than that reported for pigeons in the area. The hemagglutination inhibition (HI) titer by specific antiserum against NDV hemagglutinin in white pigeons was 16 (Acholonu and Opara, 2007). The HI titer reported for white ducks in this study is lower (8). In a study of Newcastle disease in Nigeria, the virus was isolated from 1 of 8 chickens, 2 of 21 ducks and I of 9 pigeons. Chickens were reported as the most susceptible poultry to NDV while ducks and geese are the least susceptible poultry (Echeonwu, Iroegbu and Emeruwa, 1997). The low prevalence of NDC in ducks (1.6%) recorded agrees with already established observation on the susceptibility of carrier birds to NDV.

The hazards of NDV carriage appear more threatening in ducks than pigeons when bird to man and bird to bird contacts are considered. Ducks are routinely not in cages but roam about unrestricted and constitute higher risks to poultry in the area especially when infected with NDV. Furthermore, ducks' meat is a common food item than meat of pigeons and other feral birds in Owerri area. Duck meat is routinely consumed and provide quick source of protein for natives. It is served frequently in canteens and stored in frozen state and prepared as delicacy on demand. These practices constitute serious health risk factors to the population, especially, if not hygienically or well prepared. Therefore, the method of preparation of duck meat for human consumption should be critically examined. It has been reported that NDV can live up to 12 months in frozen chicken carcass (see Okeke and Lamorde, 1988) and conversely, can do so in ducks.

References

Acholonu, A.D.W. and Opara, A.U. 2007, "The carrier rate of Newcastle Disease Virus in Pigeons in Owerri area of Imo State, Nigeria", J. Am. Soc. Trop. Med. Hyg. Program and Abstract.

Adeshina, A.G. and Oluwole, B.O. 1989, "Isolation of Newcastle Disease Virus from resident and migratory ducks", J. Am. Vet. Assoc. **171,** 949-963.

Adu, F.D. 1987, "Characterization of Nigerian Strain of Newcastle Disease Virus", L. Med. Lab. Sci. **6,** 8-11.

Allan, W.H. and Gough, R.E. 1974, "A standard hemagglutination inhibition test for Newcastle Disease (I) A comparison of macro methods", Vet Rec. **95,** 120-123.

AL-Jumaily, W.I.T., Al-Atar, A.R., Mansour, A.D., Jaid, J.D. and Abdullatif, H. 1980, "This incidence of salmonella and serological evidence of Newcastle disease in some wild birds from Bagdad area", J. Biol. Sci. Res. **20,** 213-219.

Alfonso, C.P. and Cowen, B.S. 1995, "Newcastle Disease Virus isolated from ducks and waterfowls in two wild life management areas of Penn State", J. Wile. Dis. **31,** 179-185.

Boyvier, D.E. and Shaw, D.L. 1984, "Newcastle Disease outbreak due to sub-types and possible transmission by ducks", Avian Dis. **27,** 357-366.

Doyle, T.M. 1992, "A hitherto unrecorded disease of fowls due to titer passing virus", J. Comp. Path. And Therapy **72,** 144-169.

Echeonwu, G.O.N., Iroegbu, C.O. and Emeruwa, A.C. 1997, " Occurrence of Velogenic Newcastle Disease (NDV) in apparently healthy pigeons (Colombia Livia domestica) in Northern parts of Nigeria", J. Med. Lab. Sci. **6,** 8-11.

Hanson, R.P. and Shina, S.K. 1952, "Epizootic of Newcastle Disease in Pigeons and study on transmission of the virus", Poultry Sci. **31,** 404-408.

Lancaster, J.E. 1963, "Newcastle Disease control by vaccination", Vet. Bull. **34,** 57-76.

Okeke, E.N. and Lamorde, A.G. 1988, "Newcastle Disease and its control on Nigeria" in Williams, A.O. aand Masiga, W.N. (eds): Viral Disease of Animals in Africa Technical Centre for Agriculture and Rural Co-operation (CTA), Organization of Africa Unity / Scientific, Technical and Research Commission (OAU/STRC), pp 283-300.

Ritchards, R.W. 1985, "Immunization of broiler breeder chickens against Newcastle disease with oil emulsion vaccine", J. Avian Dis. **23,** 597-607

Vitak, S.K. 1958, "The production of properties of Newcastle Disease Vaccine (Komarov strain) in Hampshire", Brit. Vet. **116,** 427-435.