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The carrier rate of Newcastle disease virus in ducks in Owerri area of Imo State, Nigeria

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ABSTRACT

The carrier rate of Newcastle disease virus (NOV) in ducks was investigated in three Owerri Local Government Areas (LGAs) – Owerri municipality, Owerri North and Owerri West in Imo State, Nigeria between January and April 2006. Sixty apparently healthy ducks were tested in the area using a stock of 20 ducks from each LGA. Cloacal swabs were obtained from embryonated hen's egg via the allantoic route and incubated for 96 hours at 37^{o}C in a humidified incubator. Only one of the 60 ducks (D14) tested positive for NDV and it was detected in 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 NDV in ducks in Owerri area of Imo State is put at 1.6%.

INTRODUCTION

Newcastle disease is an acute, rapidly spreading, nervous and respiratory disease of birds of all ages. The disease has been referred, variously, to 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). In Nigeria Newcastle disease was first identified in 1953 (Echeonwu, et al 1997) and has Newcastle Disease Virus (NDV) as the causative agent.

Newcastle Disease Virus (NDV) is an 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 domesticated 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 (Opara, 2005). Recovery of NDV from ducks has also been reported by Hanson and Shina (1952), Vitak (1958), Adeshina and Oluwole (1989), Alfonso and Cowen (1995) and Bouyvier and Shaw (1984). Laboratory and field observation still 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 to 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, (1998).

MATERIALS AND METHODS

In Owerri area of Imo State, ducks are owned by families, but are not routinely caged, as are pigeons. Ducks are allowed to roam about unrestricted and these results in the exposure of poultry to competition for space, feed and water with ducks, some of which may harbour Newcastle disease virus. These ducks shed the virus in their faeces, which easily contaminate feeds for poultry. Ducks are, also reared intensively for money making, ritual practices, egg production and also exchanged as prestige gift items between families during ceremonies. Meat and blood of such ducks if infected constitute health hazards to the population.

Viable hen's eggs used for Newcastle disease virus isolation were selected through candling to determine their fitness. The eggs were prepared for use by disinfecting site for inoculation of specimen with 70% ethyl alcohol soaked in cotton wool. Cloacal samples derived from suspected ducks were diluted 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 and needle. The needle as 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.

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

Chicken erythrocytes (RBC)

Red blood cell stock was obtained by bleeding healthy chicken (via the 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.

Spot agglutination test

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

Haemagglutination titration (HA)

The haemagglutination (HA) test was done in U-bottom microtitre plates. Using single channel pipette, 50ul of the PBS was dispensed into V-bottom microplates 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 (50ul) of the allantoic harvest in the first well and mixing thoroughly 5 times with the pipette. About 50ul 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, 50ul of 0.5% chicken red blood cells 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 titre was recorded. This was used to calculate the 4HA units used in the Haemagglutination inhibition (HI) test.

Haemagglutination inhibition (HI) test: beta procedure (constant virus diluted serum)

NDV antiserum was obtained from the Virology Division of the National Veterinary Research Institute (NVRI), VOM, Nigeria. Test was performed according to the methods of Alan and Gough **(1974).** Four HA units of the stock viral harvest was used. Into each well of a U-bottom microtitre plate was dispensed 25ul of PBS. About 25ul of NDV antiserum was dispensed into the first well and the last (control) of a row of 12 microwells. The 12th well was the serum control. About 25ul of the 4HA dilution of antigen was added to each well excluding the control wells. The plates were gently rotated to mix the reagents. About 25ml of 0.5% washed red blood cells was added to all the wells. The reagents were mixed and incubated at room temperature for 45 minutes. The settling pattern was observed and the HI end point noted.

RESULTS

Of the 60 cloacal swabs inoculated into embryonated eggs, only one (D14) yielded positive results for Newcastle disease virus (NDV). This 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 sample collected from a white duck encountered in Owerri Municipal council area. There were no positive results of NDV from ducks (black or white) tested in Owerri North and Owerri West LGAs (Table 1). Accordingly, the carrier rate of disease in white ducks was 4.16% while the rate in ducks coloured black was 0%.

Table 2 represents the HA and HI titres of the allantoic harvest of sample D14 with NDV infection. The HA titre for the isolate was 256. The titre by specific antiserum against NDV haemagglutinin was 8.

Carrier rate of Newcastle disease virus in ducks

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

	No. Positive/ No. tested (%) from			
	Owerri Municipality	**Owerri North**	**Owerri West**	**Total**
White (n = 24)	1/8 (12.5)	0/10 (0.0)	0/6 (0.0)	1/24 (4.16)
Black (n = 36)	0/11 (0.0)	0/16 (0.0)	0/9 (0.0)	0/36 (0.0)

Table 2. Haemagglutination (HA) and haemagglutination inhibition (HI) test results of the NDV – positive allantoic sample (D14)

Allantoic sample	HA titre	Hi titre
D14	256	8

DISCUSSION

Newcastle disease has been reported in various areas of the world implicating Newcastle disease virus (NDV) as the causative agent. The virus can infect all species of birds both domesticated and wild bird populations (Ritchards, 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 records of its incidence in Owerri area is rare. Only recently, a 5% carrier rate of NDV in Pigeons was reported in Owerri area of Imo State, Nigeria (Opara, 2005). No information is available concerning the disease in ducks in the area. 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 haemagglutination inhibition (HI) titre by specific antiserum against NDV haemagglutinin in white pigeons was 16 (Opara, 2005). The HI titre reported for white ducks in this study is isolated 1 of 8 chickens, 2 of 21 ducks and 1 of 9 pigeons (Echeonwu, et al 1997). Chickens are reported as the most susceptible poultry to NDV while ducks and geese are the least susceptible poultry (Echeonwu, et al 1997). The low prevalence of NDV in ducks (1.6%) recorded agrees with already established observations on the susceptibility of carrier birds to NDV. The prevalence of NDV among the white (4.16%) and the black ducks (0%) appear significantly variable ($P<0.05$). A similar observation indicating higher NDV carriage in white than black pigeons was reported in Owerri area of Imo State (Opara, 2005) and should be a subject for further study.

The hazards of NDV carriage appear more threatening in ducks than in pigeons when bird to man and bird to bird contacts are considered. Ducks are routinely not in cage but roam about

unrestricted and constitute higher health risks to poultry in the area especially when infected with NDV. Furthermore, ducks meat is common food items than meat of pigeons and other feral birds in Owerri area. Duck meat is routinely consumed and provide quick source of protein for natives. 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 well prepared. Therefore, 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. Okeke and Lamorde, (1988), and conversely, can do so in ducks.

REFERENCES

Adu, F. D (1987) Characterization of Nigerian strain of Newcastle disease virus. *J. Med. Lab. Sci*. 6:8-11,

Allan, W. H.and Gough, R.E. (1974). A standard haemagglutination inhibition tests for Newcastle disease (1) 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). The incidence of salmonella and serological evidence of Newcastle in some wild birds from Baghdad area. *J. Biol. Sci. Res*. 20:213-219.

Adeshina, A. G. and Oluwole, B. O. (1989). Isolation of Newcastle disease virus from resident and migratory ducks. *J. Am. Vet. Med. Assoc.* 171:949-963,

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,

Bouvier, 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 titre passing virus. *J. Como. Path. And Ther*. 72:144-169.

Echeonwu, G. O. N., Iroegbu, C. O. and Emeruwa, A. C. (1997). Occurrence of velogenic Newcastle disease virus (NDV) in apparently healthy pigeons (Columbia 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

Kawaoka, Y. and Chambers, T. M (1988). Is the gene of Newcastle disease virus in shore birds and gulls different from that of wild ducks? *Virol. Jour*. 163:27-250.

Lancaster, J. E (1963). Newcastle disease control by caccination. *Vet. Bull.* 34:57-76.

Okeke, E. N., Lamorde, A.G. (1998). Newcastle Disease and its control in Nigeria. In: Viral Disease of animals in Africa. (Williams, A. O. and Masiga, W. N. eds) Technical Centre for Agricultural and Rural Co-operation. OAU-Scientific, Technical Research Commission (STRC) : 283-300.

Opara, A. U. (2005). The carrier rate of Newcastle disease virus in pigeons in Owerri area of Imo State, Nigeria. *J. Med. Lab. Sci.* 14:43-47.

Ritchards, A.M. (1985). Immunization of broiler- breeder chickens against Newcastle disease with oil emulsion vaccine. *Jour. Avian Dis.* 23:597-607.

Vitak, S. K (1958). The production of properties of Newcastle disease vaccine (Komarov strain) in Hampshire *Vrit. Vet.* 116:427- 435.