

## ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2010  
AJCEM/200977/21013  
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ISBN 1595-689X VOL 11(2)  
[-http://www.ajol.info/journals/ajcem](http://www.ajol.info/journals/ajcem)

AFR. J. CLN. EXPER. MICROBIOL 11(2): 95-101

### **DETECTION OF SERUM ANTIBODY LEVELS AGAINST NEWCASTLE DISEASE IN LOCAL CHICKENS IN BAUCHI METROPOLIS, BAUCHI STATE, NIGERIA**

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#### ABSTRACT

Poultry diseases are one of the main factors constraining poultry practice in most developing countries. Newcastle disease (ND) is a highly contagious and commonly fatal viral poultry disease caused by Newcastle disease virus (NDV). Detection of antibodies to Newcastle disease virus in 300 blood samples from local chickens slaughtered at Muda Lawal Market Bauchi was carried out using the haemagglutination inhibition test (HI). This was to determine the immune status of local birds to NDV in Bauchi Metropolis. 169 (56.3%) birds tested positive with antibody titre ranging from 2 to 512. The geometric mean titre was 19.7. This low antibody titre reveals the epizootic nature of the virus in the study area and is suggestive of an inter-epidemic phase or early phase of infection pointing a finger to possible economic losses in the event of an outbreak, alongside the role of local chickens in the transmission cycle of NDV to other avian species. Vaccination of local chickens where possible is advocated for.

**Key words:** Poultry diseases, Newcastle disease, haemagglutination inhibition, antibody titre

#### Introduction

Poultry keeping is the dominant form of poultry production in the developing world. The practice of traditional poultry system is higher because it entails less or minimal human involvement with birds scavenging in the backyard for food. It entails no investments beyond the cost of the foundation stock, a few handfuls of local grain, and possibly simple night shades, and little or no veterinary medical attention [1]. As in many tropical and subtropical countries in Asia, Africa and South America, a large population of small traditional chicken flocks exists alongside large industrialized poultry farms. This may mean that chickens in the same vicinity may be in direct or indirect contact with each other [2].

Poultry diseases such as Newcastle disease (ND) are one of the main factors constraining this

poultry practice in most developing countries. ND is the most important infectious disease affecting local chickens. Outbreaks of the disease have a tremendous impact on backyard poultry farming, where these birds are a significant source of protein and this disease is endemic. Its spread is normally either via newly introduced birds, selling or giving away sick and carrier birds. The usual source of infection is usually other chickens.

Newcastle disease (ND) is a highly contagious and commonly fatal viral poultry disease affecting mainly domestic and wild avian species [3]. The disease is caused by Newcastle disease virus (NDV) which belongs to the *Paramyxoviridae* family and genus *Rubulavirus*

[4]. The Paramyxoviruses isolated from avian species have been classified by serological testing into nine serotypes designated; APMV – 1 to APMV-9. NDV has been designated APMV – 1 [5]. APMV -1 strain is classified into three pathotypes based on their virulence in chickens which are: lentogenic, mesogenic and velogenic [6,7]

The disease is characterized by respiratory symptoms such as coughing, gasping, sneezing and rales. Other signs include: dropping wings, dragging legs, swelling of the tissues around the eye and neck, twisting of the neck, circling and cessation of egg production [8]. Human infection via exposure to infected birds can cause mild conjunctivitis and influenza-like symptoms and in severe cases, it can lead to some lasting impairment of vision [9].

Newcastle disease, being an epizootic problem in different parts of Nigeria [10,11,12,13] can have serious negative impact on the economics of these areas and the country at large. These losses will be due to losses in productivity and death of poultry. Its presence can limit trade and the development of intense poultry production resulting to major constraint to the availability of protein for human consumption.

Establishment of the disease status in Bauchi metropolis is therefore of great importance to avoid the economic losses highlighted above. This objective triggered this research which was aimed at detecting antibodies to Newcastle disease virus infection from local chickens at Muda Lawal Central Market, Bauchi (a

converging point for the sale and slaughter of local chickens from different localities in the State).

## **Methods**

### **Study location**

Samples were collected at slaughter house in Muda Lawal Market Bauchi, Bauchi State, Nigeria.

### **Sample collection and processing**

300 blood samples were collected randomly from unvaccinated local chickens at slaughter point. Samples were taken from the wing veins of chickens. They were allowed to clot. Sera was separated, transported in a refrigerated box and subsequently stored at -20°C in Virology laboratory, Federal College of Veterinary and Medical Laboratory Technology, National Veterinary Research Institute (NVRI), Vom). The antigen used was obtained in the same laboratory.

### **Washing of RBC's**

5ml of chicken blood was collected aseptically in a disposable syringe and transferred to a sample bottle containing 1 ml of sodium citrate (4% solution) as an anticoagulant. The blood was centrifuged at 1500 rpm for 15 minutes. The plasma and buffy coat was pipetted off. After washing thrice with phosphate buffer saline (PBS), 10% and 1% suspensions in PBS were made to be used in spot test and HI test.

Spot test was carried out using clean white tile. A drop of viral antigen was placed at the centre of the tile. A drop of 10% chick red blood cell was added to the antigen, mixed together and

rocked gently. The mixture was observed for haemagglutination. This test was used to identify potency of the antigen [7].

### Test procedure

The test was performed as described by Allan and Gough [18]. Briefly, after making two fold serial dilution of test serum up to 10th well, 4 HA unit of Newcastle disease virus was added up to 11th well and kept at 25 - 30°C for 25-30 minutes. A 1% chicken RBC's suspension was added into each well. The samples showing peculiar central button shaped settling of RBC's were recorded as positive and the maximum dilution of each sample causing

haemagglutination inhibition was the end point. The HI titer of each serum sample was expressed as reciprocal of the serum dilution.

### Results

Results from 300 samples screened show that 169 samples (56.3%) were positive for Newcastle disease virus antibodies while 131 samples (43.7%) were negative as shown in table 1. 130 (77.4%) of samples showed specific immunity levels as presented in table 2. Haemagglutination inhibition antibody titre ranged from 2 to 512. The geometric mean titre was 19.7. These are shown in Table 3.

**Table 1: NDV antibody screening results.**

Nos of samples	Nos positive	Percentage positive	Nos negative	Percentage negative
300	169	56.3	131	43.7

**Table 2: Serum samples showing specific or non-specific immunity to ND by HI test**

Positive samples	Specific immunity	% Non-specific immunity	Non-Specific immunity
168	130	77.4	38

**Table 3: NDV antibody titres of positive samples**

Nos of samples	Titres									
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	GMT
168	23	15	31	18	10	16	21	27	7	19.7

GMT- Geometric Mean Titre (Brugh, 1978).

### Discussion

ND is regarded as the most economically important disease that devastates village poultry

in Nigeria [14] as it causes death of millions of birds (particularly young birds) and economic losses through the slaughter of sick birds [15]. Mortality rate as high as 80% has been recorded in chickens [16].

Out of 300 samples screened in this study, 169 were positive representing 56.3%. This result indicates considerable presence of Newcastle disease virus among local chickens sampled. As these birds have no history of vaccination against the disease, demonstration of antibodies to NDV in them is an evidence of natural infection with the virus. Our finding only buttresses the findings of other researchers who reported the presence and epizootic nature of NDV in some northern and middle belt States of Nigeria [10,11,13,17]. These similarities in ND prevalence may be due to similarities in live style and poultry practises in these areas. Inter-State trade of poultry and poultry products is also a common feature especially on known market days.

Allan and Gough [18] suggested a ND–HI titre greater than 8 as indicative of specific immunity. This means that 130 (77.4%) birds had specific immunity to NDV. Schmidt and Schmidt [19] considered HI titer of 32 and above to be protective against NDV. In this study, 81 (47.93%) birds had antibody titres greater than

32 but the geometric mean titre was 19.7 which gave a clearer picture of the poor immune protective level against NDV infection. Since [20] low ND-HI antibody prevalence is suggestive of an interepidemic 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. The wider range of NDV titres in birds is likely due to natural infection which is known to produce higher antibody titres than vaccination [21].

In spite of vigorous vaccination schedules, ND is still a threat to the poultry industry in developing countries [22] especially Nigeria. Vaccination is an effective method of controlling ND in both commercial and village poultry, but is rarely given priority in rural communities of Nigeria where majority of local poultry are kept [13,16]. Limitations to vaccination of local chickens are basically a vaccine dose/vial issue. Most vaccines come in doses for 100 birds or more but local chicken farmers have just a couple of birds. Also, the availability and means of maintenance of vaccines in cold chain in rural communities coupled with poor enlightenment is difficult and most times not available. So ‘why bother’, most of the farmers ask.

However, the advent of the heat stable ND V<sub>4</sub> vaccine [3,23,24,25] offers village poultry keepers the opportunity and hope of still being in business. The V<sub>4</sub> vaccine technology, though not yet fully adopted by village poultry owners in Nigeria, has been tested with good results [15,26]. The cost-benefit analysis following the

use of the V<sub>4</sub> in village chicken in Kaduna State, Nigeria showed that it was highly beneficial to adopt this technology in protecting village poultry flocks than leaving them unvaccinated/unprotected [26].

### Conclusion

The high titre of HI antibodies to NDV local chickens recorded in this research is suggestive of a possible epizootic of the disease in Bauchi metropolis. Although the presence of antibody to NDV in unvaccinated local chicken is mostly associated with contact with field strain of the virus which occasionally confers immunity to

them, it may not protect in times of outbreak. Infected birds could also serve as reservoir and source of transmission of the virus to exotic

### References

1. Dessie T, Ogle B: A Survey of Village Poultry Production in the Central Highlands of Ethiopia. *Part I*. Master's Thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden 1996.
2. Hassanzadeh M, Bozorgmeri Fard MH: A Serological Study of Newcastle Disease in Pre- and Post-Vaccinated Village Chickens in North of Iran. *International Journal of Poultry Science* 2004, 3(10): 658-661
3. Spradbrow PB: Village Poultry and Preventive Veterinary Medicine. *Preventive Veterinary Medicine* 1990, 8: 305-307.
4. Alexander DJ: Newcastle disease and other avian Paramyxoviridae Infection.

poultry and other avian species in that area due to their close proximity. Were possible, we

recommend that alternative routes of vaccinating local birds via incorporation of thermostable vaccines in their food [25] should be practiced.

### Acknowledgements

The authors wish to acknowledge the Management of Federal College of Veterinary and Medical laboratory Technology, NVRI, Vom for creating an enabling environment for the laboratory analysis. Also Staff of the Virology department of the establishment are acknowledged for their assistance

- In: Calnik BW, Barries H, Beard CW, McDougald I, Saif UM ed *Disease of Poultry* 1997, pp 541-569 Ames Iowa State University Press.
5. Alexander DJ: Newcastle disease. In YM Saif, Barnes HJ, Lisson JR, Fadly AM, McDougald LR, Swayne DE (ed) *Disease of Poultry* 2003, pp 64-87. Iowa State University Press.
6. Gerlach H: Paramyxovirus. In Harrison, G.J., Harrison, L.R. (Ed). *Clinical Avian Medicine and Surgery* 1986, pp 421-426 (Philadelphia, WB Saunders Co).
7. Office Internationale des epizootics (OIE): Manual of Standards for diagnostic test and vaccines draft test for comment by experts in member countries. *OIE Manual* 2004, <http://www.oie.int>.

8. Wakamatsu N, King DJ, Kapozynski DR, Seal BS, Brown CC: Experimental Pathogenesis for chickens, turkeys and pigeons of exotic Newcastle disease virus from an outbreak in California during 2002–2003. *Veterinary Pathology* 2006, 43: 926–933.
9. Beard CW, Hanson RP: Newcastle disease. In Hofsad, M. S., Barnes, H. J., Clanek, B. W., Reid, W. M., Yoder, H. W. (ed). *Disease of poultry*, 1984, 8<sup>th</sup> ed. 452–470 Iowa State University Ames.
10. Ezeokoli CD, Umoh JU, Adesiyun AA, Abdu PA: Prevalence of Newcastle disease virus antibodies in local and exotic chicken under different management system in Nigeria. *Bulletin of Animal Health Production, Africa* 1984, 2(3): 253–257.
11. Adu FD, Edo U, Sokale B: Newcastle disease. The immunological status of Nigerian local chicken. *Tropical Veterinarian* 1986, 4: 149–152.
12. Nwanta JA: Field vaccination trials with Newcastle Disease Vaccine (NDV4HR) in local chicken in Kaduna State, Nigeria. *Ph.D Dissertation*, 2003, Ahmadu Bello University, Zaria.
13. Olabode AO, Lamorde AG, Shidali NN, Chukwedo AA: Newcastle disease in village chickens in Nigeria. Australian Center for International Agricultural Research. *Proceedings of an International Conference on the thermostable ND and Control. Malaysia* 1992.
14. Abdu PA, George JB, Umoh JU: A study on chicken mortality in Zaria, Nigeria. *World's Poultry Congress* 1985, Amsterdam, The Netherlands, pp 151.
15. Nwanta JA, Umoh JU, Abdu PA, Ajogi I, Alli-Balogun JK: Management of losses and Newcastle disease in rural poultry in Kaduna State, Nigeria. *Nigerian Journal of Animal Production* 2006, 3(2): 274–285.
16. Fatummbi OO, Adene DF: Susceptibility of the Nigerian local chicken to fulminating Newcastle disease outbreak. *Nigerian Veterinary Journal* 1979, 8(2): 30–32.
17. Baba SS, El-Yuguda AD, Baba MM: A serological evidence of mixed infection with Newcastle disease and Egg drop syndrome 1976 viruses in Village chicken in Borno State, Nigeria. *Tropical Veterinarian* 1998, 16: 137 – 141.
18. Allan WH, Gough RE: A Standard HI test for Newcastle disease virus. A comparison of Macro and Micro Methods. *Veterinary Records* 1974, 95: 120-123.
19. Schmidt U, Schmidt D: Connection between Haemagglutination–inhibition antibodies and immunity after vaccination against Newcastle disease. *Archives of Experimental Veterinary Medicine* 1955, 9: 505–516.
20. Awan M, Otte J, James AD: The epidemiology of Newcastle disease in

- rural poultry: A review. *Avian Pathology* 1994, 23: 405–23.
21. Luc PV, Hong NT, Chinh VT: Level of anti–Newcastle disease virus antibodies in industrial poultry at various ages and seasons. *Agricultural Food Industry* 1992, 9: 348–50.
  22. Siddique M, Sabri MA, Khan MZ: Outbreaks of Newcastle disease in vaccinated flocks in and around Faisalabad. *Pakistan Veterinary Journal* 1986, 6: 41–45.
  23. Copland JW: Newcastle Disease in poultry: A new food pellet vaccine. *Australian Center for International Agricultural Research, Canberra*. 1987.
  24. Ibrahim MA, Abdu PA: Ethnoagraveterinary prospective of poultry Management, Health and Production among Hausa/Fulani of Rural Nigeria. In *Proceedings of 29<sup>th</sup> Annual General Meeting of Nigerian Veterinary Medicine Association, Kaduna*, 1992, pp 172–181.
  25. Echeonwu GON, Ireogbu CU, Echeonwu BC, Ngene A, Olabode AO, Okeke OI, Ndako J, Paul G, Onovoh EM, Junaid SA, Nwankiti OO: Delivery of thermostable Newcastle Disease Vaccine to chickens with broken millet grains as the vehicle. *African Journal of Biotechnology* 2007, 6(23): 2694-2699.
  26. Nwanta JA, Umoh JU, Abdu PA, Ajog I, Egege SC: Comparison of the cost of Unvaccinated and Oral vaccinated local chickens with a Malaysian thermostable Newcastle disease vaccine (NDV4HR) in Kaduna State, Nigeria. *Bulletin of Animal Health and Production in Africa* 2005, 53: 202–210.
  27. Brugh MA, Jr: A simple method for recording and analysing serological data. *Avian Diseases* 1978, 22: 362-365