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SURVEY OF ANTIBODIES TO NEWCASTLE DISEASE VIRUS IN APPARENTLY HEALTHY ADULT NIGERIAN INDIGENOUS CHICKENS *(Gallus domesticus)* IN IBADAN USING ELISA

*[1] Ohore, O.G., [2] Ozegbe, P.C., [1] Emikpe, B.O., [2] Okojie V.E.

[1] Department of Veterinary Pathology and [2] Veterinary Anatomy, Faculty of Veterinary Medicine. University of Ibadan, Ibadan

The prevalence of antibodies to Newcastle disease virus (NDV) in Nigerian indigenous chickens raised in Ibadan was surveyed using the indirect enzyme-linked immunosorbent assay (ELISA). Sera from 161 chickens from 3 are as of Ibadan viz University campus, Agbowo and Oremeji were analysed. The prevalence rate obtained ranged between 52.5% and 83.4% with an overall prevalence of 73.3%. The extent and implication of NDV activity in the Nigerian indigenous chicken as well as the advantages, sensitivity and usefulness of ELISA in serological investigation were discussed.

INTRODUCTION

Newcastle diseases (ND) is an acute infectious and highly contagious viral disease of poultry avian species, (1). ND was first diagnosed in Eastern Nigeria in 1951 and confirmed Hill et al; (2) in 1953 and since then, the disease has continued to be the most devastating disease of poultry in Nigeria. (3,4,5). Despite the availability of locally produced vaccines and the protective immunity conferred by recommended vaccination regime,(6), the number of reported outbreaks of ND remain high. (7). Cases of outbreaks in vaccinated birds have also been reported (5) and these were attributed to antigentic differences between the vaccines used and the field ND viral strains and the resultant insufficient immunogenic protection (8).

Previous serological survey of ND virus haemagglutination inhibition (NDVHI) antibody in exotic birds in parts of Nigeria showed that 22% had detectable (NDVHI antibodies (6). Adene and Njoku (9) reported low NDVHI antibody titre (0-10) for imported day-old exotic chicks, while Abdu and Garba (10) reported higher titres for those hatched in one of the hatcheries in the country.

According to Akinwunmi et al (11), 124 million of 134 million chickens in Nigeria are indigenous local ckicken. They are usually kept on free range management system and are normally not vaccinated (12). Adene (13) also reported that the rural poultry account for over 70% of Nigerian poultry, hence they are very important in the epidemiology of poultry diseases. To properly evaluate their role, several studies had been done on many important poultry diseases which affect these birds such as Marek's disease (Adene 14), infectious bursal disease (15,16,17, 18), Newcastle disease (4,12, 17,18), egg drop syndrome (18), Fowl typhoid (19, 20) and brucellosis (21,22).

In most of the studies done on the seroprevalence of Newcastle disease in Nigerian indigenous chickens, haemagglutination inhibition technique were employed. In order to properly evaluate the prevalence, there is need to employ other diagnostic techniques hence the use of ELISA technique which has been found to be efficient, accurate, easier and more sensitive in the diagnosis and seromontoring of poultry diseases (23, 24). The following study was thus undertaken to investigate the extent of NDV activity among Nigerian indigenous chicken in Ibadan and also to determine the usefulness of ELISA technique in the detection of humoral antibodies to Newcastle disease in the indigenous chicken.

MATERIALS AND METHODS

The test sera were obtained by jugular venopuncture of adult indigenous chickens kept on free range management by small holder/backyard rearers in 3 areas of Ibadan. 3-4 samples were collected per household in these areas, and all the chickens had no history of any vaccination but they were apparently healthy. A total of 161 samples were randomly obtained. Sera were heat inactivated at 56oC for 30mins and stored at 20oc until analysed.

The Newcastle disease virus (Lasota strain) was used as antigen in the ELISA test the lyophilized virus was obtained from the Nigerian Veterinary Research Institute, VOM and used at a protein concentration of 5.0mg/ml following the determination of the viral protein concentration as described by Warburg and Christian (25). The virus was diluted in carbonate – bicarbonate buffer pH 9.6.

Rabbit anti-chicken IgG horseradish peroxidase labeled conjugate (Zymed Inc. California) was used as conjugate. It was diluted 1:2,000 in PBS containing 0.5% Tween 20 and 1% Bovine serum albumin (PBST-BSA) following a chequer board titration.

The substrate/chromogen was prepared by dissolving 0.82g of sodium acetate in 100ml of distilled water and adjusting the pH to 6.0 with 0.5m citric acid. This solution was divided into 25mls aliquots. To each 25mls aliquots, 4il of 30% hydrogen peroxide and 250il of tetramethy lbenzidine (TMB) in dimethylsulphoxide (10mg/ml) were added prior to use.

The ELISA procedure was conducted essentially by adaptation of the method described by Oyejide et al (26) for infectious Bronchitis with some modifications. Following the determination of the optimal working dilutions for antigen, serum and conjugate by chequer-board titration, polystyrene micro ELISA

Corresponding Author

plates with 96 flat bottom wells were coated with 100il of NDV antigen of 5.0mg/ml protein concentration in carbonate-bicarbonate buffer. The plates were incubated overnight at + 40oC. Excess antigens were washed off in 2 washes with PBST using automatic microplate washer (SLT Labinstruments ® Austria).

100il of test sera diluted to 1:500 in PBST-BSA was added to duplicate wells of the paltes and incubated at 37°C for 30 minutes. The plates were rocked manually at every 10minutes interval during the incubation. Thereafter, the plates were washed 3 times with PBST and flipped to dry.

100il of conjugate was added to each well at 1:2,000 dilution in PBST-BSA and the plates were incubated at 37°C for 30 minutes, and manually shaken every 10 minutes. Excess conjugate was removed in 3 washed with PBST, the 100 il of freshly prepared substrate/chromogen was added to each well and the plates were incubated at 37°C for 15 minutes. Plates were immediately read at 450nm wavelength in microplate reader (SLT Labinstruments ® Austria).

In each plate, control wells consisting of specific pathogen free chicken serum, hyperimmune serum to ND diluted to 1:500 with PBST-BSA, as well as blank wells consisting of PBST-BSA alone were included. The positive test samples were those whose optimal density (OD) values were either equal to or above 1.5 times the OD of negative control.

RESULTS

Sampling Area/Location	No. of samples tested	No +ve with ELISA	Prevalen rate %	Mean OD of +ve (± SD)
1. University of Ibadan Campus	64	54	83.4	0.218 ± 0.060
2. Agbowo	57	43	75.4	0.198 ± 0.030
3. Oremeji	40	21	52.5	0.207 ± 0.140
Total	161	118	73.3	

Table 1: Prevalence of Antibodies to NDV in Indigenous Nigerian Chickens in Ibadan,

Using ELISA

Of the thee different areas sampled viz: University Campus, Agbowo and Oremeji prevalence rates of 83.4%, 75.4% and 52.5% were obtained respectively. The combined prevalence of NDV antibodies among 161 indigenous chickens sampled in Ibadan was found to be 73.3%.

The mean optical density readings obtained for positive reactors for the different areas were 0.128 ± 0.060 (U.I), 0.198 + 0.030 (Agbowo) and 0.207 ± 0.140 (Oremeji).

DISCUSSION

The antibodies observed in these birds is in response to exposure to field strains of Newcastle disease virus because the birds were not vaccinated against the disease.

The prevalence of NDV antibodies in this study ranged between 52.5% and 83.4%. Previous data on the prevalence of antibodies to NDV with the use of HI test showed that 22% of unvaccinated exotic chickens had demonstrable antibodies (6), while 51.4%, (18 out of 35) was found for local/indigenous chickens in Ibadan in particular and 41.04% in Nigeria (12). In this study, however, a higher prevalence of 73.3% was observed among indigenous chickens in Ibadan. This higher prevalence may be attributed to the higher sensitivity of the ELISA method compared to the HI test.

Adu et al (12), using haemagglutination test postulated that some birds may habour the virus in the absence of antibodies, or presence of very low levels of antibody. Thus the higher prevalence rate observed in our study could be as a result of this group of birds with low antibody titres which could not be detected by HA test but detectable by the ELISA.

The variation in the prevalence of NDV antibodies in the 3 areas studied could be attributed to the level of poultry production in these areas. For example the university community high poultry production especially in the Teaching and Research Farm in since samples were collected from households in this vicinity, it was possible that the indigenous chickens reared here, were constantly exposed to the virus through outbreaks in the exotic breeds, hence the higher prevalence than Oremeji area with a few commercial poultry units. Since the movement of the indigenous chickens is not controlled they can possibly constitute infected carriers as suggested by Adene et al, (15), and hence perpetuate the disease especially among susceptible exotic breeds. There is therefore the need for adequate fencing of poultry farm premises (18).

The result of this study shows that the ELISA test could be a useful tool in the assessment of NDV activity in a poultry population and would also be useful in the assessment of humoral response to vaccination. The extent of ND virus activity in apparently healthy Nigerian indigenous chicken in significantly high hence the need to take into cognizance the role of rural birds in the epidemiology of the disease.

Further research on the varied pathtypes of ND associated with indigenous chickens is envisaged to properly aid in the control of ND in Nigeria.

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