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Research Article
Seroprevalence of Newcastle Disease Virus in Chickens in Six Local Government Areas of Benue State, Nigeria

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Abstract

Objective: This study was conducted to determine the seroprevalence of Newcastle Disease (ND) antibodies in apparently healthy chickens in commercial poultry farms, village households and Live Bird Markets (LBMs) in six Local Government Areas (LGAs) of Benue state, Nigeria. **Methodology:** A total of 1,370 sera were collected and screened for ND antibodies by Haemagglutination Inhibition (HI) test and enzyme linked immunosorbent assay (ELISA). **Results:** The overall seroprevalence by HI was 66.5% (911/1,370) with a mean HI antibody titre of 5.87±0.11 $\log_2$ with the highest seroprevalence of 88.7% (189/213) recorded in Otukpo LGA and 75.1% (232/309) in commercial chickens. About 43.1% (591/1370) of the birds had protective antibody titre of ≥4 $\log_2$ while 32.1% (440/1370) of seropositive birds had antibody titre ≥7 $\log_2$. The overall seroprevalence of ND using ELISA was 98.3% (226/230) with a mean titre of 3.65±0.03 $\log_{10}$ and the highest seroprevalence recorded in chickens from Katsina-Ala, Oju and Otukpo LGAs (100%) and birds from commercial farms and LBMs (100%). **Conclusion:** The study concludes that birds in Benue state were exposed to ND virus and the virus was circulating in apparently healthy chickens and this could be considered an important epidemiological factor in the spread of the disease in the state.

Key words: Chicken, live bird market, Newcastle disease, seroprevalence, village household

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Newcastle Disease (ND) is one of the most important viral disease of poultry worldwide[1]. It is an economically important disease and a major threat to the poultry industry worldwide[2]. The disease is caused by Newcastle Disease Virus (NDV) which belongs to the avian paramyxovirus type 1 (APMV-1) serotype, genus *Avulavirus* and family Paramyxoviridae[3]. Newcastle disease viruses occurs in three pathotypes: Lentogenic, mesogenic and velogenic, reflecting increasing levels of virulence[4]. The most virulent (velogenic) strains are further subdivided into neurotropic and viscerotropic NDVs[4]. According to variation in strains of NDV, the rate of mortality and morbidity in a flock varies from 90-100% along with decrease in egg production[5,6]. The transmission of NDV occurs through newly introduced birds, selling of sick birds, exposure to faecal and other excretions from infected birds and contact with contaminated feed, water, equipment and clothing[7]. The disease is characterized by respiratory, nervous, gastrointestinal and reproductive impairment[8,9]. Vaccines are being used to control and prevent ND. Currently, many inactivated and live ND vaccines are available around the world[10,11]. Newcastle disease is endemic in Nigeria with frequent outbreaks occurring in commercial poultry causing annual epidemics in susceptible flocks[12]. Besides commercial poultry the disease also affects village poultry and it remains a constant threat to backyard poultry[13,14]. Village poultry production is an important economic activity for rural dwellers in Nigeria. Village poultry are kept for meat and egg and sold to earn extra income. Unfortunately, ND is a major hindrance to the realization of the full potential of village poultry due to high mortality it causes in Nigeria[15]. Live bird markets can be high risk areas for disease transmission due to high concentration and the interaction of a wide variety of birds brought from different sources. When the viruses causing avian diseases especially ND are established in a LBM they can spread to other markets and farms through contaminated equipment, birds, people and vehicles[16]. In view of the devastating effects of ND and its significant economic importance in the poultry industry, a serological survey was conducted to determine the prevalence of the disease in apparently healthy chicken flocks in Benue state in order to generate information necessary to formulate appropriate control measures in the state.

MATERIALS AND METHODS

Study area: The study was conducted in Benue state located in the North central zone of Nigeria. The state lies within longitude 7°47′ and 10°0′ East, latitude 6°25′ and 8°8′ North of the equator. The state shares boundary with Nasarawa, Taraba Cross River, Enugu and Kogi states and the Republic of Cameroun. The state is divided in to three geopolitical zones made up of 23 LGAs.

Sampling units: Three sampling units made up of chickens from village households, commercial poultry farms and LBMs were used for the study.

Sampling methods: Purposive sampling was used to select six LGAs with two LGA selected from each of the three geopolitical zones of the state. Four villages and four households were selected from each of the LGAs. A total of 24 villages, 12 commercial farms and 9 LBMs were selected and sampled based on the consent and readiness of farmers to participate in the study. Five or more birds were sampled randomly per household, 10-20 birds from each commercial farm and LBM respectively. Samples were collected from Gboko, Kwande, Katsina Ala, Makurdi, Oju and Otukpo LGAs of Benue state, Nigeria from May-July, 2013.

Collection of blood: About 1-2 mL of blood was collected from the brachial vein of each bird. The blood was allowed to stand and clot at room temperature from which sera were decanted into serum vials, transported on ice packs to the laboratory and stored at -20°C. A total of 1,370 sera were collected.

Detection of Newcastle disease antibodies: The LaSota strain of NDV and antibody positive serum were obtained from the National Veterinary Research Institute (NVRI), Vom and used as positive antigen and control serum, respectively. Haemagglutination (HA) and HI tests were conducted according to the method described in OIE[17]. Sera with HI antibody titre $\geq 1 \log_2$ and $\geq 4 \log_2$ were considered positive and protective respectively based on OIE[17] manual.

Sera were also tested for antibodies using a commercial enzyme-linked immunosorbent assay (ELISA) kit for ND (IDEXX Laboratories USA) according to the recommended procedure by the manufacturer. Results were read using a microplate reader and absorbance values were measured at 650 nm. Sample/positive ratios (S/P) were calculated according to the recommended procedure. Serum samples with S/P $\leq$ 0.2 were considered negative, whereas those with S/P $\geq$0.2 were considered positive for ND antibodies.

Data analysis: The data obtained from serology were analyzed by descriptive statistics using Statistical Package for Social Sciences version 17 program (SPSS Inc., Chicago,

IL, USA). The frequency, percentages of sera with ND antibodies and the mean HI and ELISA antibody titre level for the different sampling units were calculated. The association of antibody titre level between the different sampling units were assessed by cross tabulation and chi square values calculated. Values of $p \leq 0.05$ were considered significant.

RESULTS

The overall seroprevalence by HI test of birds with detectable antibody titre was 66.5% (911/1370) with a mean titre of 5.87±0.11 (Table 1). About 43.1% (591/1370) of the birds had HI protective antibody titre ≥ 4 $\log_2$ and a mean titre of 8.02±0.08. The overall percentage of birds with HI titre ≥ 7 $\log_2$ was 32.1% (440/1370). The percentage of chickens within LGAs that had protective HI antibody titre was 36.5% (225/616) for Makurdi LGA and 20.5% (23/112) for Katsina-Ala (Table 1). A total of 230 sera were tested for ND antibodies by ELISA. The overall seroprevalence using ELISA was 98.3% (226/230) with a mean titre of 3.65±0.03 $\log_{10}$ (Table 2). The seroprevalence within LGAs by ELISA was 94.7% (36/38) for Gboko and 100% (34/34) for Otukpo LGAs (Table 2). Within sampling units, commercial poultry had 75.1% (232/309) of birds with ND protective HI antibody titre of ≥ 4 $\log_2$ while poultry from LBMs had 12.9% (49/380) (Table 3). The percentage of birds with antibody titre of ≥ 7 $\log_2$ was 54.4% (168/309) for commercial chickens, 35.7% (243/681) for village chickens and 7.6% (29/380) for LBMs (Table 3).

DISCUSSION

The study revealed that chickens in Benue state were exposed to ND virus which might be either through vaccination or the field virus similar to reports in other States in Nigeria[18-20]. However, the study showed a lower percentage of birds with protective antibody titre level compared to a similar study in Plateau state, Nigeria[21]. The observed differences in the percentage of birds with protective antibody titre level could be due to ecological variations in NDV activity and a reflection of environmental impact on the viability of NDV and epidemiology of ND as reported by Orajaka *et al.*[22].

The lower HI seroprevalence compared to ELISA is likely because HI detect only the haemaglutinating antibodies while ELISA detects all types of functional antibodies[23]. Similarly,

Table 1: Seroprevalence, protective antibody and titre ≥ 7 $\log_2$ of Newcastle disease by haemagglutination inhibition test in chickens in six local government areas of Benue state, Nigeria

Local government area	HI seroprevalence (No. of positive/No. of tested) (%)	Percentage of birds with HI protective antibody (No. of positive/No. of tested)	Percentage of birds with HI titre ≥ 7 $\log_2$ (No. of positive/No. of tested)
Gboko	88.2 (127/144)	68 (98/144)	51.4 (74/144)
Katsina-Ala	57.1 (64/112)	20.5 (23/112)	17.9 (20/112)
Kwande	70.7 (128/181)	50.3 (91/181)	37.0 (67/181)
Makurdi	55.0 (339/616)	36.5 (225/616)	25.8 (159/616)
Oju	61.5 (64/104)	51.0 (53/104)	40.4 (42/104)
Otukpo	88.7 (189/213)	47.4 (101/213)	36.6 (78/213)
Overall	66.5 (911/1370)	43.1(591/1370)	32.1 (440/1370)

Table 2: Seroprevalence of Newcastle disease antibodies by ELISA, HI and ELISA mean antibody titres in chickens in six local government areas of Benue state, Nigeria

Local government area	Seroprevalence by ELISA (No. of positive/No. of tested) (%)	Mean ELISA titre ($\log_{10}$)	Mean HI titre ($\log_2$)
Gboko	94.7 (36/38)	3.57±0.07	6.41±0.27
Katsina-Ala	100 (25/25)	3.57±0.07	4.20±0.44
Kwande	97.6 (41/42)	3.61±0.07	6.50±0.29
Makurdi	98.5 (65/66)	3.81±0.05	5.79±0.18
Oju	100 (25/25)	3.68±0.07	7.20±0.39
Otukpo	100 (34/34)	3.48±0.06	5.34±0.25
Overall	98.3 (226/230)	3.65±0.03	5.87±0.11

Table 3: Seroprevalence of Newcastle disease antibodies in chickens within sampling units in Benue state, Nigeria

Sampling unit	Seroprevalence by ELISA (No. of positive/No. of tested) (%)	Percentage of birds with HI titre ≥ 4 $\log_2$ (No. of positive/No. of tested)	Percentage of birds with HI titre ≥ 7 $\log_2$ (No. of positive/No. of tested)
Commercial chickens	100 (50/50)	75.1 (232/309)	54.4 (168/309)
LBM chickens	100 (60/60)	12.9 (49/380)	7.6 (29/380)
Village chickens	96.7 (116/120)	45.6 (310/681)	35.7 (243/681)
Overall	98.3 (226/230)	43.1 (591/1370)	32.1(440/1370)

LBM: Live bird market

Adu *et al.*[24] in a study using HI test postulated that some birds habouring NDV may have low or no HI antibody levels due to continuous depletion of the HI antibodies by NDV resulting in detection by ELISA due to its higher sensitivity. Similarly, McNulty *et al.*[25] demonstrated the effectiveness of ELISA in detecting infection with avirulent NDV strains which were often demonstrated by the presence of low HI antibody titre in birds.

The low HI seroprevalence reported in some LGAs might be due to the high proportion of village chickens sampled which are not routinely vaccinated. However, the high seroprevalence reported in some LGAs may be due to continuous exposure to antigen either from vaccinal or field NDV as supported by the high prevalence of birds with HI titre $\geq 7 \log_2$ especially in Gboko LGA. The study also disclosed variability in the percentage of birds with HI protective antibody titre level between LGAs. The lowest percentage was recorded in Katsina-Ala and Makurdi LGAs. The mean HI antibody titre level was also lowest in birds sampled from Katsina-Ala LGA. This implies that birds from Katsina-Ala will be more vulnerable to challenge with a virulent strain of NDV. The percentage of birds with HI titre $\geq 7 \log_2$ was highest in Gboko LGA and this indicates that there was an active ongoing infection at the time of sampling or recent vaccination. The distribution of the percentage of birds with HI protective antibody titre level by sampling units showed that it was highest in commercial chickens. This could be attributed to the routine ND vaccination conducted by most commercial poultry farmers. Chickens from the LBMs recorded low HI antibody prevalence compared to the village household poultry which could be due to either differences in age and exposure to NDV or the composition of poultry within the village households and the LBMs. Field observations during the study revealed that younger village chickens (especially young cocks) formed the largest population of birds in the LBMs, indicating less exposure to NDV compared to older hens forming the bulk of household chickens with comparatively high HI protective antibody titre level as a result of previous long exposure and recovery from NDV.

CONCLUSION

Based on these results, the study concludes that a high percentage of apparently healthy chickens in Benue state, Nigeria have been exposed to NDV without protective antibodies. Furthermore, most of the chickens are at risk of ND if exposed to virulent strain of NDVs particularly those in Katsina-Ala LGA and LBMs which could result in ND outbreak. Hence, there is need for routine surveillance by trained government personnel for ND in village household and LBMs in the state. The Ministry of Agriculture Benue state should organize and maintain annual vaccination campaigns against ND in village poultry. Further studies should be carried out to isolate and characterize the circulating NDVs in Benue State to provide more information that could be used to plan for an effective control of ND in the state.

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