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Detection of Newcastle Disease Antibodies amongst Local Chicken Slaughtered in Live Bird Markets in Kaduna, Nigeria

[1]Y. S. Wungak, [2]A. Alhassan, [3]D. B. Judith, *[3]I. Bitrus, [2]I. B. Shallangwa and [1]H. G. Ularamu
[1]Foot and Mouth Disease FMD Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria
[2]Federal College of Animal Health Production Technology, Vom, Plateau State, Nigeria
[3]Regional Laboratory for Animal Influenza and Newcastle Disease, National Veterinary Research Institute, Vom, Plateau State, Nigeria
[*Corresponding Author: E-mail: usfilmalgwi@yahoo.com]

ABSTRACT
Newcastle Disease Virus (NDV) constitutes a major constraint to the poultry production system in Nigeria. This study was carried out to investigate the seroprevalence of NDV antibodies in local chickens (*Gallus domesticus*) slaughtered in five different live bird markets (LBMs) in Kaduna metropolis, Nigeria. Three hundred blood samples were collected and screened for antibodies against NDV using Haemagglutination Inhibition (HI). An overall seroprevalence rate of 23% (95%:CI18.5-28.0) was recorded in this study. Seroprevalence based on different LBMs revealed a higher prevalence of 28.3% (95% : CI 18.0-40.7) in Sabo market, followed by Sokoto road market 26.7% (CI:16.7-38.9), Kawo market 18.3% (95% CI: 10.0-29.6), Railway market 13.3% (CI:6.4-23.5), and the lowest prevalence was recorded in central market 11.7% (CI:5.2-21.7). The difference in seroprevalence among the LBMs was not statistically significant ($p > 0.05$). Higher seroprevalence of NDV antibodies in female birds 30.5 % (CI: 24.1-37.6) was recorded compared to male 13.9% (CI: 8.3-21.4). The study indicated that NDV is endemic in the population. LBMs location had no significant influence on the seroprevalence of NDV in the study area. The difference in seroprevalence between sexes was statistically significant ($p < 0.05$). Therefore, this study demonstrated the need for a regular strategic vaccination programme against NDV in local chickens in Kaduna metropolis.
Keywords: Kaduna metropolis, Live birds' markets, Local chicken, Newcastle disease virus, Seroprevalence

INTRODUCTION
Newcastle disease (ND) is caused by virulent strains of avian paramyxovirus, family paramyxoviridae. The paramyxoviridae isolated from avian species have been classified by serological and phylogenetic analysis into twenty-one serotypes designated as APMV-1 to APMV-21 (Amarasinghe *et al.,* 2019; ICTV 2019). The disease is in most countries and has a devastating effect on commercial poultry production. In Nigeria and in many developing countries, Newcastle Disease Virus (NDV) is a major viral disease of economic importance in the poultry and rated as one of the greatest constraints to the meaningful development of rural poultry husbandry, causing devastating loss to the poultry industry (Solomon *et al.,* 2012; Unigwe *et al*., 2020). The disease is an acute, contagious, rapidly spreading, with nervous and respiratory signs. The clinical manifestations are known to vary based on the tropism and virulence of NDV involve, species of the birds, immune status of the bird, age of the host, and environmental conditions (Oladele *et al.,* 1996). All ages and breed of birds of different species are susceptible to ND and 90% mortality may result from the acute or virulence form of the disease (OIE, 2021).

The disease is known to be common during harmattan period of the year. The clinical manifestation of the disease is associated with inappetence, greenish, yellowish diarrhea, coughing and sneezing, somnolence, dullness, drop tailed and wings feathers, ruffled feathers, facial oedema, nasal and ocular discharge, thin shelled eggs and drop in egg production, muscular twitching, complete paralysis, incoordination, sitting on the hock, star gazing and opisthotonos, weakness and sudden deaths (Sa'idu *et al*., 2006).This study was design to detect NDV antibodies in local chickens slaughtered from different live bird markets in Kaduna metropolis. This would help in improving the existing knowledge and understanding of the virus in chicken population for better preventive and control strategies.

MATERIALS AND METHODS
Study Area
The study was conducted in live bird markets (LBMs) in Kaduna metropolis Kaduna State. The State has human population of more than 6 million based on the 2006 census, with about 80% of individuals actively engaged in animal and crop farming.

Sample Collection
In a convenient sampling technique, 4ml blood samples was collected at slaughter from local chickens brought from different parts of the State to be sold at the five largest poultry live bird markets (LBMs) within Kaduna metropolis namely; Sokoto road, Railway market, Kawo market, Central market and Sabo market. A total of 300 samples were collected, 60 from each of the live bird markets with 123 samples from male and female (n = 177). Samples were properly labeled and transported in cool box to the Regional Laboratory for Animal Influenza and Newcastle Disease, National Veterinary Research Institute (NVRI), Vom. The samples were arranged in a slanting position on a test tube rack for about 3 hours. Serum samples obtained were decanted in a properly labeled 2 ml cryovials and stored at -20 ºC until required for analyses.

Haemagglutination Inhibition (HI) Test

Antibody titer for NDV was determined from each serum sample using the OIE, (2021) haemagglutination inhibition (HI) test protocol. Briefly, 0.025 ml of PBS was dispensed into all wells of a plastic 96-well microtiter plate (v-bottomed wells) and 0.025 ml of sera sample was placed in the first wells (A1-E1). Positive and negative control serum with a known HI titer was added to two respective wells of the microtiter plates (F1 and G1). With the aid of a multichannel micro pipette, twofold dilutions of the sera were made across the plate (A1–A12). The last 0.025 ml was discarded and 0.025 ml of antigen containing 4 HAU was added to all the wells except row H which serves as back titration. Newcastle disease virus (very virulent Kudu strain) was used as antigen. Back titration was carried out; thus, 0.025 mL of antigen suspension containing 4 HAU was added into each of the first two wells of row H (4 HAU control from H1–H6), and twofold dilution was made from H2 to the H6 and the last 0.025 ml was discarded in order to obtain 4, 2, 1, 0.5, 0.25, and 0.25 HAU. The plates were mixed by tapping gently and were placed at 20°C for 30 minutes and 0.025 ml of 1% washed chicken-RBC was added to each well. Mixing was done gently by tapping and the plates were placed on the bench for 30 minutes and observed for HI. Validity of the results was assessed against a negative control serum which gave a titre of not less than 2^2 ($\log_2 2$) and a positive control serum which the HI titre was within one dilution. The test was conducted at the Regional Laboratory for Animal Influenza and Newcastle Disease, NVRI, Vom

Data Analysis

The data were stored in Microsoft Excel® spreadsheet. Descriptive statistics was carried out using Microsoft Excels spreadsheet and proportions were obtained using open Epi.Version 2.3.1 Statistical tool (Open Source Epidemiological Statistics for Public Health calculation). Chi-square was used to measure the strength of association between locations (markets) and sex and the seroprevalence of NDV. Values of $p < 0.05$ were considered significant.

RESULTS

The result in Table 1 revealed an overall seroprevalence of 23% (95%: CI 18.5-28.0). Seroprevalence based on different locations showed a higher prevalence of 28.3% (95%: CI 18.0-40.7) in Sabo Market, followed by Sokoto road 26.7% (95% CI:16.7-38.9), Kawo market 18.3 % (95% CI:10.0-29.6,), Railway market 13.3% (96% 6.4-23.5), and the lowest prevalence was recorded in central market 11.7% (95%CI: 5.2-21.7). The difference in seroprevalence among the different locations was not statistically significant P>0.05 (Table1).

Table 1: Seroprevalence of NDV in different LBMs in Kaduna metropolis using HI

LBMS LOCATION	NUMBER EXAMINED	NUMBER OF POSITIVE	NUMBER OF NEGATIVE	PROPORTION (%)	CRITICAL INTERVAL
Sokoto road	60	16	44	26.7	16.7-38.9
Railway market	60	8	52	13.3	6.4-23.5
Kawo market	60	11	49	18.3	10.0-29.6
Central market	60	7	53	11.7	5.2-21.7
Sabo market	60	17	43	28.3	18.0-40.7
Total	**300**	**69**	**231**	**23%**	**CI 18.5-28.0**

HI = Haemagglutination Inhibition

Chi-square = 8.73, P value = 0.068 (p>0.05), degree of freedom = 4

Seroprevalence based on sex showed a higher prevalence of NDV antibodies in female 30.5% (95% CI: 24.1-37.6) compared to the males 13.9% (95% CI: 8.3-21.4). The difference in seroprevalence between the sex was statistically significant P<0.05 (Table 2).

Table 2: Seroprevalence of NDV virus in LBMs in Kaduna Metropolis based on sex using HI

SEX	NUMBER EXAMINED	NUMBER OF POSITIVE	NUMBER OF NEGATIVE	PROPORTION (%)	CRITICAL INTERVAL
Male	123	15	108	13.9	8.3-21.4
Female	177	54	123	30.5	24.1-37.6
Total	**300**	**69**	**231**		

HI = Haemagglutination Inhibition

Chi-square = 12.7 P value = 0.0001 P<0.05 Degree of freedom = 1

DISCUSSION

The present study detected the presence of antibodies against NDV in three hundred sera samples collected from local chickens in five live bird markets in Kaduna metropolis, Kaduna State Nigeria using the HI test. This indicates that Newcastle disease virus infection is endemic in the area, and the markets are serving as mixing point of infected birds with susceptible ones as some of these birds are taken back home to be reared. The sellers and buyers as well as those processing the meat may also be veritable vehicle of transmission and spread of the disease. This is therefore, a great threat to rural and commercial poultry production in Kaduna State. The implication of the spread and the carrier status of the rural household chickens could be of importance considering the fact that free ranged chickens were reported to constitute almost 50% of chicken population in Nigeria and are capable of scavenging around the environment spreading the NDV and other avian viral diseases to vaccinated and unvaccinated healthy exotic birds (FAO, 2018; Bitrus *et al.,* 2020; Sulaiman *et al*., 2021). This study recorded an overall seroprevalence of 23% in the study area. This prevalence rate is higher than the prevalence reported by Abraham-Oyiguh *et al.* (2014) where they reported a seroprevalence of 17% in live bird markets in Abuja. However, this prevalence is lower than the 32.5 % prevalence rates reported by Jibril *et al.* (2014) in a study conducted on local chickens of live bird markets and households in Zamfara State, Nigeria. Ameji *et al.* (2011) also reported a prevalence rate of 25.6% in live bird markets in Kogi State. This finding of NDV seropositivity in these apparently healthy birds suggests that the birds have either recovered from clinical ND or are having subclinical infections (Adu *et al*., 1986; Ameh *et al*., 2016).This study recorded variation in seroprevalence of NDV antibodies between the different live bird markets in the study area with highest prevalent rates of 28.3% in Sabo Market, followed by Sokoto road with 26.7% prevalence, Kawo market 18.3 %, Railway market 13.3%, and the lowest prevalence was recorded in Central market 11.7%.The difference in seroprevalence among the different locations was not statistically significant P>0.05. These findings agreed with studies conducted by Musa *et al*. (2009) and Jibril *et al*. (2014) who reported a locational variation in NDV antibodies in the studies they carried out in local chickens from live bird markets and households in Zamfara State, Nigeria and rural household chickens in plateau state, Nigeria respectively.

Ameji *et al*. (2011) and Abraham-Oyiguh *et al*. (2014) also reported differences in seropositivity of NDV antibodies due to different locations in birds from live bird markets in Kogi State and Abuja respectively. This study also shows a higher prevalence rate among the female chickens of 30.5% more than male chickens with 13.9%. The difference in seropositivity was statistically significant (p< 0.05). These findings differ from the findings reported by Aschalew *et al*. (2005) in a similar study conducted in Ethiopia, where a higher prevalence rate was detected among males (21.74%) than among females (19.16%). However, the result of this study corroborated with the findings of Asfaw-Geresu *et al*. (2016) in a study carried out in Ethiopia where, a slightly higher prevalence of 30.53% among female chickens was obtained when compared with a prevalence of 14.29 in male chickens. Alkali *et al*. (2017) in a study they conducted in Local Chickens from Sokoto, Nigeria reported higher prevalence in (15.32%) female birds compared to males (14.02%). The difference in seroprevalence between the two sex may be attributed to sample size, as more female were sampled than the male.

CONCLUSION

Newcastle disease virus (NDV) antibodies were detected in local chickens sold in the five live birds markets studied in Kaduna metropolis which are likely to serve as host/carrier of NDV to commercial flocks as well as local poultry population. Further studies are required to determine the strains circulating in order to control them appropriately. Therefore, the need to conduct regular, strategic vaccination programme against ND for local chickens in Kaduna metropolis, Kaduna State is advocated.

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