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Short Communication

SEROPREVALENCE OF NEWCASTLE DISEASE VIRUS IN PIGEONS (COLUMBIDAE: COLUMBIDIFORMES) FROM ZARIA NIGERIA

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ABSTRACT

Newcastle disease (ND) outbreak even in the face of vaccination is a common problem in Nigeria. Three Newcastle Disease (ND) panzootic were recorded in the past and one of such was caused by feral pigeon fecal contamination of poultry feed materials. The study was designed to investigate the presence of ND antibodies in pigeons slaughtered in three markets in Zaria Nigeria. A total of 1500 Pigeons sera samples were tested across the selected markets, namely: Sabon-gari, Samaru and Tudun-wada markets. Five hundred blood samples were collected from each market. An overall seroprevalence of 22.33% (335/1500) was recorded in this study. Haemagglutination inhibition test revealed that pigeons from Sabon-gari market had the highest seroprevalence of 29% (145/500). Female pigeons had higher seroprevalence of 27.66% (130/470) compared to male pigeons 19.90% (205/1030). Younger pigeons of <10 weeks old are more prone to ND infection with a seroprevalence of 34.21% (52/152) compared to pigeons >10 weeks old 20.99% (238/1348). Also, only in Samaru market was there a significant association between ages (p = 0.00) and sexes (p = 0.00) of pigeons and seroprevalence of ND. Therefore, free routine ND vaccination campaign should be launched in the study areas with more emphases targeting the market species in order to mitigate the epidemic cycle of the virus. "Moreover, market poultry farmers should be enlightened on the economic significance of the disease and the need to maintain strict biosecurity measures on their market farms." (“Module-trait relationships calculated by WGCNA. Factors of ...”)

Keywords: Seroprevalence; Newcastle Disease, Pigeons, Haemagglutination Test, Zaria

INTRODUCTION

Newcastle disease (ND) is a devastating disease of poultry worldwide, responsible

for millions of dollars in assets lost from the poultry sector due to morbidity, mortality, outbreak containment measures, and trade restrictions (OIE., 2020; Ibrahim *et al*., 2020). Newcastle disease is caused by virulent isolates of Newcastle disease virus (NDV) and is reportable to Office International des Epizooties (OIE)(Deist *et al.*, 2020;OIE., 2020). Newcastle disease virus is an enveloped virus within the *Avulavirus* genus, Paramyoxoviridae family, Mononegavirales order (ICTV, 2019; Yang *et al*., 2021).

Newcastle disease virus infection has been described in pigeons in a worldwide panzootic of paramyxovirus 1 (PMV-1) infection, causing a frequently fatal disease primarily associated with neurological signs (Dimitrov *et al*. 2017). Pathogenicity tests, F protein sequencing, and monoclonal antibody binding studies were utilized to characterize the NDV strains from infected pigeons, dubbed “pigeon PMV-1” (PPMV-1) (Mayahi *et al*. 2017). Outbreaks have been described in ostriches (order Struthioniformes) while Columbiformes especially pigeons are prone to the disease with Avian paramyxovirus type 1 (APMV-1) strains maintained in pigeon populations having some antigenic differences from other APMV-1 isolates, often called "pigeon paramyxovirus type 1" (Shan *et al*. 2021).The NDV strains can infect over 200 bird species, and pigeons, chickens, turkeys, and pheasants have been impacted most by ND (OIE, 2018). Ducks have been shown to be immune to disease development despite being prone to infection(Onwuchokwe *et al*. 2018)

Newcastle disease virus has rarely been used in disease eradication frameworks and continues to be a threat to Nigerian poultry flocks, particularly local unvaccinated pigeons. However, there have been few reports of NDV isolations in epizootiological surveys of wild birds in Nigeria. Given the possibility of poultry species being contaminated by pigeon carrier of NDV, it is worthwhile to investigate the pathogenesis of the disease in pigeons.

Pigeons are slaughtered on a daily basis in Zaria but the status of NDV and public health issues as well as the role of the birds in the spread of NDV in communities has not been documented. Such data is required for the country's strategic planning for ND control. The purpose of this study was to look for serological evidence of NDV infection in pigeons slaughtered in markets in Zaria, Kaduna State, Nigeria because demonstration of antibodies from the birds may be sufficient for epidemiologists to plan control measures.

MATERIALS AND METHODS

Ethics approval and consent to participate

Ethical approval was waived by the Ahmadu Bello University Animal Ethics Committee as blood samples were collected from birds at slaughter and because the butchers handle the birds in the live market themselves.

Study area: The study area in Kaduna State, Nigeria is Zaria (latitude 11 º5′ 7.9476″ N and longitude 7º 43′ 11.8020″ E) while Samaru (Ganga Uku) Market, Tudunwada market, and Sabon-gari Market were the sampling locations(Figure 1).

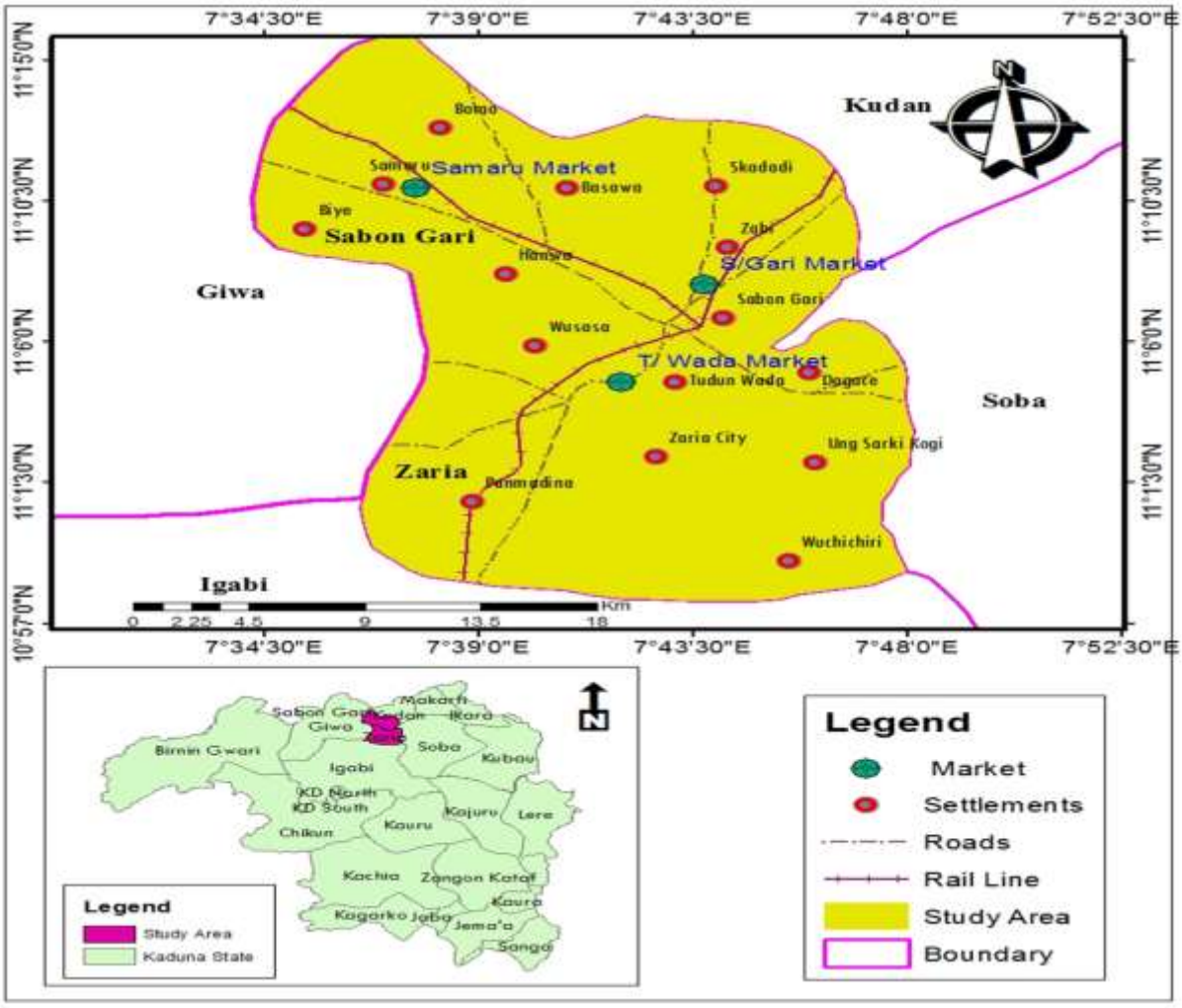


Figure 1: Map of Zaria showing sampling locations

Selection of study markets: Random sampling procedure was adopted to select three markets out of about 10 markets in Zaria. The markets selected are Samaru (Ganga Uku) Market, Tudunwada Market and Sabon-Gari Market.

Study animals and sample size determination: All pigeons (N >10,000) slaughtered in the selected markets constitute the study population. Sample size was be determined using the formula

$$N = \frac{z^2 pq}{d^2}$$ (Thrusfield, 2007)

n = Desired sample size (when N is greater than 10,000)

z = Standard normal deviate, set at 1.96, which corresponds to the level of the 95% confidence level

d = Degree of accuracy desired usually set at 0.05.

Substituting P for target population

P= the population in the target population 31.2 % (Halle *et al.* 1999))

q = 1- P = 0.688

Minimum sample size from each market will be 329.

$$N = \frac{z^2 pq}{d^2} = 329$$

$$N = \frac{1.96^2 \times 0.312 \times 0.688}{0.05^2}$$

$$N = 329.4$$

Therefore, a total number of 1500 pigeons, comprising 500 pigeons from each of the three markets make up the sample size for the study.

Study design: A cross-sectional study was carried out using the HI test performed on sera collected from pigeons to ascertain their immediate immune status. Blood sample used for detection of NDV was collected from slaughtered pigeons selected by systematic random sampling. Sex and ages of the pigeons were recorded. Age classification was done primarily by observing the beaks of the pigeons, which were then classified as young (<10 weeks old), and as old (>10 weeks as old).

Blood sample collection: Three to five ml of blood collected from each pigeon at the point of slaughter was transferred into sterile plain sample bottles and stored in a cooler containing ice block. The blood samples were then transported to the Public Health and Preventive Medicine laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna state where the samples were analyzed and stored at -4ºC. Test materials composed of pigeon sera which were harvested from clotted blood. Clotted blood samples were centrifuged at 3000 revolutions per minute (rpm) for five minutes before sera were harvested/decanted into sterile sample bottles and stored at -20ºC. Antibodies against NDV were determined by using the haemagglutination inhibition test. Newcastle disease vaccine (LaSota strain) obtained from the Public Health and Preventive Medicine Laboratory; Faculty of Veterinary Medicine at Ahmadu Bello University Zaria was used as an antigen after reconstitution of 200 dose vial in 8ml of distilled water. A 0.5% washed pigeon red blood cells (RBC) suspension as described by Wosu (1984) was prepared and used as the indicator.

Haemagglutination (HA) test: The HA test was done by the micro test method using two-fold serial dilutions of 50μl of reconstituted vaccinal virus (antigen); and 50μl of the 0.5% pigeon RBC was added to each well. Equivalent volume of pigeon RBC suspension was added to wells containing phosphate buffered saline(PBS) alone and then was served as control. The plate was gently tapped to mix the contents and after 45 min of incubation at room temperature, the end point of the HA was read. The titre was taken as the reciprocal of the highest dilution giving a 100% agglutination of the 0.5% pigeon RBC. This amount of virus represented one haemagglutination (HA) unit. The HA test is used to titrate the antigen used in the experiment to determine the HA unit which is the lowest dilution that will cause complete agglutination of the red blood cells. Value for 4 HA unit of the control virus was then calculated according to OIE (2012) as follows: Aliquots of 0.025 ml of PBS were dispensed into each well of the plastic U-bottomed microtiter plate and 0.025ml of virus suspension (La Sota strain reconstituted in 2mls PBS) was added to the first well. Doubling dilutions of 0.025 ml of the virus was made across the plate and 0.025 ml of the 1% (v/v) of the chicken RBC was dispensed into each well. The plate was tapped gently to mix its content and the RBCs allowed equilibrating for about 40 minutes at room temperature. Haemagglutination was determined by placing the plate on microtiter equipment and observing the absence or presence of tear-shaped

streaming of RBCs. The titration was read to the highest dilution giving complete HA i.e., no streaming. This represented 1 HA unit. Afterwards the value of 4HA unit was calculated thus: If 1 HA unit in the haemagglutination titration was the minimum amount of virus that caused complete agglutination of the red blood cells, the last well that showed complete agglutination was the one containing 1 HA unit. The HA titer of the antigen was found to be 512, dilution factor to prepare 4 HA unit is calculated thus, 512/4 = 128. Therefore, 1ml of the original viral suspension was diluted in 127ml PBS. The HA titer of the test sample was the reciprocal of the dilution that produced one HA unit. i.e., since the last micro well was 512, HA titer = 1/512.

Haemagglutination inhibition (HI) test: The HI test was performed using beta-technique (constant virus and varying serum) against 4 HA units of virus that was computed from the results of the HA titration. Doubling dilutions (50 µl) of the different chicken sera extract were reacted with 50 µl of 4 HA units of the antigen suspension per well. The mixture was tapped gently to mix and allowed to stand for 30 min at room temperature for antigen-antibody reactions to take place. Antigen control wells also included 50μl of 0.5% washed chicken RBC that were added to all the wells and tapped to mix, incubated, and read after 45 min. The titers were taken as the reciprocal of serum dilutions that gave 100% inhibition of the pigeon RBC.

Data analyses: Data collected were analyzed using Epi-info (version 7.0) software. Descriptive and analytical statistics was used to summarize the data obtained. Differences and extent of association between the investigated variables in the seroprevalence of ND was carried out using chi-square tests and odds ratio. Test results were considered significant when Chi-square test *p*-value was < 0.05.

RESULTS AND DISCUSSION

A cross-sectional study was carried out using the HI test performed on sera collected from pigeons to ascertain their immediate immune status. These sera revealed the presence of the antibodies in the blood of the pigeons. The overall seroprevalence of ND in the three selected markets in Zaria Kaduna State Nigeria was 22.33% (Table 1). This was considerably lower than the 49% seroprevalence obtained in the study of Munmun *et al*. (2015). The differences in the seroprevalence could be as a result of the study area, as our study was carried out in selected markets while study of Munmun *et al*. (2015) was carried out in Veterinary Teaching Hospital in Bangladesh and India.

Table 1: Prevalence of Newcastle Disease Virus from markets studied

Markets	Examined (no.)	Positive (no.)	Prevalence (%)
Sabon-gari	500	145	29
Samaru	500	80	16
Tudun-Wada	500	110	22
Total	1500	335	22.33

Secondly, some of the pigeons in Munmun *et al*. (2015)were already vaccinated indicating the presence of antibodies. This, however, was not the case with our pigeons as there are no records of them ever being vaccinated. It was also higher than the seroprevalence of 7.5% obtained in the study of Owoya *et al*. (2020). These findings are particularly worrisome as Newcastle Disease is on A List of the World Organization for Animal Health (OIE, 2012) which is notifiable disease. Birds in the Columbiformes order, which includes pigeons and doves, can be infected with NDV (Wakamatsu *et al.* 2006). Pigeons could closely associate with humans when searching for food near human habitations, which may lead to transmission of zoonotic diseases.

Sex-specific seroprevalence of NDV in markets studied is shown in Table 2. The exact cause of apparent sex difference in Newcastle disease virus carriage is still unknown. In relation to sex, only pigeons from Samaru market indicated an association between the sexes and the seroprevalence of ND with a *P*-value of 0.00. Further, female pigeons from the same market are three times (OR = 3.00, C.I = 1.783 – 5.048) more prone to the infection than the male pigeons (Table 2). The fact that pigeons are rarely vaccinated in this part of the country, antibodies detected may be because of the previous infection or an ongoing subclinical infection(Aziz-ul-Rahman *et al*. 2019). Our findings are in agreement with those of Munmun *et al*. (2015) where female birds had higher seroprevalence than male birds.

Table 2: Sex-specific seroprevalence of Newcastle Disease Virus in markets studied

Markets	Examined (no.)		Positive (no.)		Prevalence (%)		χ^2	*p-value*	*OR (CI)*
	Male	Female	Male	Female	Male	Female			
Sabon-gari	280	220	85	60	30.36	27.27	0.569	0.451	M = 0.860 (0.582- 1.271) F = 1.162 (0.780 – 1.710)
Samaru	400	100	50	30	12.5	30.00	18.229	0.000	M = 0.333 (0.198 – 0.561) F = 3.00 (1.783 – 5.048)
Tudun-Wada	350	150	70	40	20.00	26.67	2.720	0.099	M = 0.688 (0.440 – 1.075) F = 1.455 (0.931 – 2.274)
Total	1030	470	205	130	19.90	27.66			

Newcastle Disease can occur at any age, but as a bird grows older, it develops a strong immunity to this disease. In general, young birds are more vulnerable to ND. This is true for our study as the overall seroprevalence of ND of pigeons <10 weeks is higher (34.21 %) as compared to birds of >10 weeks old (20.99 %). Similarly, there was an association between age of pigeons and the seroprevalence of ND in Samaru market with a *P* value of 0.00. In all markets, pigeons <10 weeks old are more prone to infection than older pigeons (Table 3). For instance, in Samaru market, pigeons <10 weeks old are nine times (OR= 9.088, CI= 3.583 – 23.049) more susceptible to ND than older pigeons (OR=0.110, CI= 0.043 – 0.279). Our findings are in disagreement with those of Munmun *et al*. (2015) where older birds are more at risk of ND infection than younger birds.

Table 3: Age-specific seroprevalence of Newcastle Disease Virus in markets studied

Markets	Examined (no.)		Positive (no.)		Prevalence (%)		χ^2	*p-value*	*OR (CI)*
	<10 wk	>10wk	<10wk	>10wk	<10wk	>10wk			
Sabon-gari	100	400	30	115	30.00	28.75	0.061	0.805	<10wk = 1.062 (0.658-1.715) >10wk = 0.942 (0.583-1.521)
Samaru	20	480	12	68	60.00	14.17	30.01	0.000	<10wk = 9.088 (3.583-23.049) >10wk=0.110 (0.043-0.279)
Tudun-Wada	32	10	468	100	31.25	21.37	1.705	0.192	<10wk = 1.673 (0.767-3.647) >10wk = 0.598 (0.274-1.303)
Total	152	890	510	283	34.21	20.99			

CONCLUSION

This study has established an overall seroprevalence of 22.33% of Newcastle Disease Virus (NDV) among pigeons slaughtered in selected markets in Zaria, Nigeria. Female pigeons and younger birds are more vulnerable to ND than males and older birds. Routine vaccination is required to avoid disease outbreaks where pigeons are sold in the selected markets in Zaria. It is also recommended that proper cooking of the local chicken sold at these markets in Zaria is strongly encouraged to avoid

disease outbreak in humans as ND is zoonotic. Further study should be done on virus detection and isolation from feces, tracheal and nasal swabs to determine whether pigeons are reservoir and shedding the virus to the environment.

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Conflict of Interest
We declare that there is no conflict of interest.

Authors' contributions
IAB conceived and designed the study and carried out hemagglutination test, YW interpreted results and drafted the manuscript and SK collected samples from pigeons. All authors read and approved the final manuscript.

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