Seroprevalence of Newcastle Disease Virus in Slaughtered Pigeons from Selected Markets in Zaria, Kaduna State 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. Hemagglutination 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.

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). ND is caused by virulent isolates of Newcastle disease virus (NDV) and is reportable to the OIE 'Office International des Epizooties' (Deist et al., 2020; OIE., 2020). NDV is an enveloped virus within the *Avulavirus* genus, Paramyxoviridae family, Mononegavirales order. (ICTV, 2019; Yang et al., 2021)

Newcastle disease virus (NDV) 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 gotten 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). NDV strains can infect over 200 bird species. Pigeons, Chickens, turkeys, pheasant, 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 stifles progress in pigeon poultry management because it affects all pigeon breeds. Newcastle disease virus (NDV) 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 disease's pathogenesis in pigeons.

Pigeons are slaughtered on a daily basis in Zaria, but the status of NDV and public health issues are unknown. The role of birds in the spread of disease in communities is unknown. Such data is required for the country's strategic planning for NDV control.

The purpose of this study is to look for serological evidence of ND virus infection in pigeons in local markets in Zaria, Kaduna State, Nigeria. The demonstration of the presence of antibodies and may be sufficient for epidemiologists to plan control measures.

Materials And Methods

Study area

The study area Zaria, Kaduna state, Nigeria is located between latitude (11 °5' 7.9476" N) and longitude (7° 43' 11.8020" E). The markets where samples were collected from are: Samaru (Ganga Uku) Market, Tudun wada market and Sabon-gari Market. Fig 1 below shows the sampling location.

Laboratory analyses

The experiment was carried out in the Department of Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria Nigeria.

Sampling: Random sampling procedure was adopted to select three markets in Zaria, Blood sample was collected from slaughtered pigeons using systematic random sampling. The markets include; Samaru (Ganga Uku) Market, Tudun wada Market and Sabon-Gari Market. The sex and age of the pigeons were recorded. The age classification was done primarily by observing the beaks of the pigeon and the age classification used was <10 weeks as young and >10 weeks as old.

Sample Size Determination

Sample size will be determined using the formula

$$N = z^2 pq / d^2$$

(Thrusfield, 2007)

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

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

d = The 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))

$q = 1 - P = 0.688$

Sample size will be **329**. Therefore, a total number of 1500 pigeons' blood will be collected from three markets in Zaria for the detection of NDV.

Sera collection

Three to five ml of blood was collected from each pigeon at the point of slaughter, 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 analysed and stored at -4°C. Test materials composed of pigeon sera which were harvested from clotted blood. The clotted blood was centrifuged at 3000 revolutions per minute (RPM) for five minutes, the sera were harvested/decanted into sterile sample bottles and stored at -20°C. Antibodies against Newcastle disease virus were determined by using the hemagglutination inhibition test. Newcastle disease vaccine virus (LaSota strain) obtained from the Public Health and Preventive Medicine laboratory, Faculty of Veterinary Medicine, Ahmadu Bello university, Zaria, Kaduna State was used as an antigen after reconstitution of 200 dose vial in 8ml of distilled water. 0.5% washed pigeon red blood cells (RBC) suspension as described by Wosu (1984) was prepared and used as the indicator.

Hemagglutination (HA) test

The Hemagglutination (HA) test was done by the micro test method using two-fold serial dilutions of 50 μ l of reconstituted vaccinal virus (antigen), and 50 μ l of the 0.5% pigeon RBC was added to each well. Equivalent volume of pigeon RBC suspension was added to wells containing 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 hemagglutination (HA) unit.

Hemagglutination (HA) test

It 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. The method is as follows; (OIE, 2012):

Aliquots of 0.025 ml of Phosphate-buffered saline (PBS) was dispensed into each well of the plastic U-bottomed microtiter plate and 0.025 ml 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. HA was determined by placing the plate on a 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 4HAU was calculated thus:

If 1 HA unit in the hemagglutination 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 titre 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 = $1/512$

Hemagglutination inhibition (HI) test

The Hemagglutination Inhibition test (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 μ l) of the different chicken sera extract were reacted with 50 μ l 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 50 μ l 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 will be analyzed using Epi-info (version) 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 will be considered significant if the chi-square test p -value was < 0.05 .

Results And Discussion

Table 1: Prevalence of Newcastle Disease Virus from three market in Zaria, Kaduna State Nigeria.

Markets	Number Examined	Number positive	Prevalence (%)
Sabon-gari	500	145	29
Samaru	500	80	16
Tudun-Wada	500	110	22
Total	1500	335	22.33

Table 2: Sex-Specific Seroprevalence of Newcastle Disease Virus in Selected Markets in Zaria, Kaduna State Nigeria.

Markets	Number Examined Male (Number positive)	Number Examined Female (Number Positive)	Prevalence Male (%)	Prevalence Female (%)	Chi-square Value	P value	Odds Ratio (CI)
Sabon-gari	280 (85)	220 (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 (50)	100 (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 (70)	150 (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 (205)	470 (130)	19.90	27.66			

Table 3: Age Specific Seroprevalence of Newcastle Disease Virus in Selected Markets in Zaria, Kaduna State Nigeria.

Markets	Number Examined < 10 Weeks (Number positive)	Number Examined > 10 Weeks (Number Positive)	Prevalence < 10 Weeks (%)	Prevalence > 10 Weeks (%)	Chi-square Value	P value	Odds Ratio
Sabon-gari	100 (30)	400 (115)	30.00	28.75	0.061	0.805	<10 = 1.062 (0.658 – 1.715) >10 = 0.942 (0.583 – 1.521)
Samaru	20 (12)	480 (68)	60.00	14.17	30.010	0.000	<10 = 9.088 (3.583 – 23.049) >10 = 0.110 (0.043 – 0.279)
Tudun-Wada	32 (10)	468 (100)	31.25	21.37	1.705	0.192	<10 = 1.673 (0.767 – 3.647) >10 = 0.598 (0.274 – 1.303)
Total	152 (52)	1348 (283)	34.21	20.99			

A cross-sectional study was carried out using the hemagglutination inhibition test performed on the 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. 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 the A List of the World Organization for Animal Health (OIE) which is notifiable disease. Birds in the Columbiformes order, which includes pigeons and doves, can be infected with NDV (Wakamatsu et al. 2006). Pigeons are wild and freely to move which closely associated with human when they searching for food near to house compound which may cause zoonotic disease.

The sex and age of the pigeons from all the markets were checked using chi square test statistics if they have an association with the seroprevalence. The exact cause of this 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.

ND 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. (2016) where older birds are more at risk of ND infection than younger birds.

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.

Declarations

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Conflict of Interest

We declare that there is no conflict of interest.

Funding

No funding for this research.

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.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are included in the manuscript.

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.

List of Abbreviations

APMV 1

C.I

HA

ICTV

ND

NDV

OIE

PPMV 1

RBC

RPM

PBS

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Figures

Figure 1

Map of Zaria showing sampling locations