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Seroprevalence of Newcastle disease in indigenous chickens in Ilorin, Kwara State, Nigeria

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Abstract

Newcastle disease (ND) is a disease of high economic importance to poultry farmers in Nigeria. Its impact on poultry include illness of poultry, reduction in egg production, immunosuppression, and death. This study was carried out to determine the prevalence of Newcastle disease in indigenous (local) chickens from 2 poultry abattoirs in the Ilorin metropolis. A total of 400 blood samples were aseptically collected in plain bottles from the jugular veins of local chickens at slaughter using exsanguination and transported to the laboratory in batches. Sera samples were harvested from the blood by centrifugation at 3000 rpm for 10 minutes, after which they were stored at -20^{o}C before serological assay. The sera were subjected to Haemagglutination Inhibition (HI) test to check for the presence of Newcastle disease virus (NDV) antibody following a standard procedure with titer values for each sample recorded. The geometric mean of the HI antibody titer (GMT) and the percentages of detectable NDV HI antibody titer were calculated using descriptive statistics. Of the 400 serum samples examined, 53 (13.25%) were positive for ND antibodies, with titre value ≥ 1:16. The location from which the birds were selected had no significant relationship with the prevalence of ND antibodies as both Oja tuntun (11.9%), and Ipata market (14.6%) had a closely similar prevalence of antibodies ($p > 0.05$). The feather arrangement of birds did not also have any significant impact on the prevalence of antibodies ($p > 0.05$). However, in this study, we observed a higher prevalence of antibodies among hens (14.7%) than in cocks (12.5%) or growers (8.9%). The high prevalence of ND antibodies in indigenous chickens in the study area showed the endemicity of the disease in the study areas. With most of the chickens are not vaccinated amid non-compliance to vaccine administration for local chickens. There is a need for poultry farmers in the study location to be educated on the importance of vaccinating poultry birds against ND.

Keywords: Chicken, Newcastle disease, Poultry, Seroprevalence, Zoonotic disease

Introduction

Newcastle Disease (ND) is a highly contagious viral disease of birds that is caused by *avian paramyxovirus serotype 1* (APMV-1) viruses and is characterized by respiratory signs often associated with depression, nervous and digestive disorders as the predominant clinical manifestations (Rajesh, 2014). It affects birds such as chickens, ducks, guinea fowl, geese, turkeys, pheasants, and partridges as well as other birds such as ostriches, emus, and rhea (Brown & Bevins, 2017). Newcastle disease is caused by paramyxovirus belonging to the family Paramyxoviridae and genus Rubulavirus. The paramyxoviruses are RNA viruses often enveloped with two glycoproteins haemagglutinin-neuraminidase (HN) and glycoproteins F, for virus attachment and fusion (Ganer *et al*., 2014). The APMV-1 is a pleomorphic, enveloped, spherical virus with a helical symmetry measuring about 100-500 nm in diameter. The envelope has surface projections known as a fusion protein (F) and haemagglutinin-neuraminidase protein (HN) that are 8 nm long (Ganer *et al*., 2014). The nucleocapsid shows a 'herringbone' arrangement and measures about 18 nm across. The genome of Newcastle Disease Virus (NDV) is linear negative-sense single-stranded RNA of 15186 nt long but some strains have 15192 and 15198 nt long RNA genome (Rajesh, 2014). This virus can be cultured in chicken embryos and cell culture or grown in laboratory animals, but chickens remain the animal of choice for viral propagation (Rajesh, 2014).

The Newcastle disease virus can also infect humans, making it a zoonotic disease (Ganar *et al*., 2014). In the UK, the virus is placed in Hazard group 2 of the Advisory Committee on Dangerous Pathogens (HSE, 2004). The virus is thus considered to be a biological agent that can cause human disease and may be a hazard to employees although it is unlikely to spread to the community (Alexander, 2000). People that come in direct contact with birds infected with the virus are likely to develop a minimal short-term eye infection that is most times self-limiting (Anon, 2018). There are three different pathotypes of Newcastle disease viruses, this includes the velogenic, mesogenic, and lentogenic strains (OIE, 2020). The velogenic pathotype is endemic in Mexico, Central, and South America and is widely spread across Asia, Africa, and the Middle East, while in the US and Canada it is common among double-crested wild cormorants (OIE, 2020). The lentogenic pathotype is distributed worldwide and found across every geographical location, while the mesogenic pathotype is broadly spread, having a special adaptation to pigeons (i.e. pigeon paramyxovirus), but do not appear to readily infect other birds (OIE, 2020).

Newcastle disease outbreaks due to velogenic strains frequently occur in Low- and Medium-income countries in spite of the regular domestic poultry vaccination in those regions (OIE, 2020). Likewise, a host of new genotypes of NDV have been identified and the numbers reported have increased in recent years, suggesting continuous evolution of the virus, which results in the emergence of new genetic variants (Ganar *et al*., 2014). There is therefore an urgent need to utilize all available information on these variants, including their epidemiology, method of diagnosis, pathotypes, and genotypes, in any intervention measures designed to combat this disease.

Newcastle disease is endemic in Nigeria and cases are on the rise for the past decades (Ekiri *et al*., 2021). Since its first report in 1952, in Ibadan and its surroundings (Hill *et al*., 1953), the disease became widespread in both local and exotic chickens (Shittu *et al*., 2016; Okoroafor *et al*., 2020; Ekiri *et al*., 2021). The economic impact of the Newcastle disease outbreak was recently estimated, alongside other outbreaks such as Infectious bursal disease (IBD) and Avian influenza (AI), and reports showed that Newcastle disease is the most economically important, of all the diseases affecting poultry (Sadiq & Mohammed, 2017). This disease constituted a total of 58% of all poultry disease outbreaks and resulted in a total cumulative loss of 13 million Naira (Sadiq & Mohammed, 2017).

Newcastle disease (ND) has also been reported in other avian species. Haruna *et al*. (1993) reported an outbreak of ND in a flock of guinea fowl in Nigeria, affecting 1,029 birds of which 250 (24.3%) died. The features of the disease observed in that study included, paralysis of the legs and wings, coughing, sneezing, white diarrhoea, and complete cessation of egg production (Haruna *et al*., 1993). Newcastle disease is of great economic importance in Nigeria due to its high mortality and morbidity in both locally and commercially raised chickens (Shittu *et al*., 2016). Therefore, there is a need for a unified and concerted effort in determining the presence and spread of this disease across the country, which would be useful in the implementation of control strategies and intervention measures, and preventive economic losses associated with Newcastle disease. This study was designed to determine the seroprevalence of Newcastle disease (ND) in slaughtered local chickens in two abattoirs in Ilorin, Kwara State, Nigeria, to obtain useful data that will contribute to the national

database, for accessibility for the formulation of control strategies against this disease in Nigeria and elsewhere.

Materials and Methods

Study area

This study was carried out in Ilorin, the capital city of Kwara State, Nigeria. Located at latitude 8.48° N, and longitude 4.54° E, it is about 292km from Lagos and 490km from Abuja and has an estimated population of 777,667 (2006 Census) (Figure 1). It has a tropical savannah climate with wet and dry seasons and an annual temperature ranging from 33°C to 37°C and annual rainfall ranging from 990.3 mm to 1318 mm (Olubanjo, 2019). In Ilorin, there are major markets where locally raised crops such as yams, cassava, corn, sorghum, millet, rice, peppers, groundnuts, kola nuts, cotton, and livestock such as cattle, hides, and poultry are sold. The poultry industry in Ilorin, Nigeria is largely characterized by small-scale farming with most of the farmers owning birds less than a hundred in population. Also, poultry farming in Ilorin is often done on a part-time basis, with the farmers also involved in other farming activities (Banjoko *et al*., 2014). Two of the main markets - Oja tuntun (New Market) and Ipata old abattoir, where indigenous chickens are slaughtered on a large scale for commercial purpose, were selected. Oja tuntun is one of the most popular markets in Ilorin and is located at longitude 8°29'16.7"N and latitude 4°32'16.9"E. Similarly, the Ipata market can be found at longitude 8.4996° N, and latitude 4.5619° E. Birds slaughtered in these markets usually originate from villages within the state and other neighbouring states.

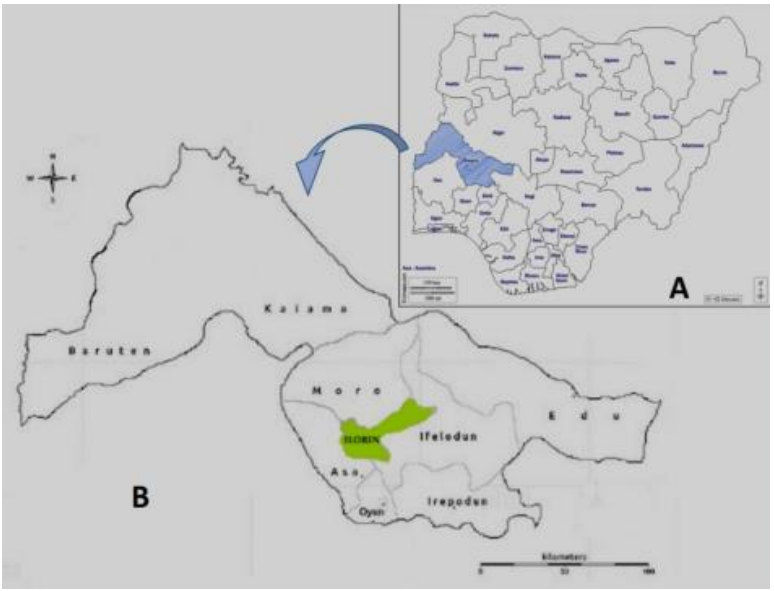

Figure 1: A- Map of Nigeria showing Kwara State. B- Map of Kwara State showing Ilorin, the study area

Sample size

Convenience sampling was used to survey the two markets. Sample size was determined using the formula by Cohen (1988): $n = z^2 p (1-p)/d^2$, where: n = number of samples, z = standard normal deviation at 95 % confidence interval, p = prevalence (was set at 50% as the prevalence in Ilorin was not known), d = desired absolute precision n = 200. Sample size per market surveyed each was 200, making a total of 400 samples collected from both markets.

Interviewer administered semi-formal questionnaires

An interviewer-administered semi-formal questionnaire was used to gather information concerning the vaccination status of the birds, feeding, general health, and vaccination status from the poultry sellers some of whom were also the farmers.

Sample collection

Immediately after slaughter, blood samples were collected from chickens through exsanguination into sterile plain bottles and were transported to the Mycoplasma Diagnostic and Research Laboratory Departments of Veterinary Microbiology Faculty of Veterinary Medicine University of Ilorin Nigeria in a cool box after appropriate labeling. The blood was then centrifuged at 3000rpm for 10 minutes to harvest the serum into a cryovial tube using a Pasteur pipette. The separated sera were stored at -20°C until the time of use for assay.

Sampling of chickens

Chickens were sampled based on the following: type; age and sex. They were also sampled based on their feather arrangement such as smooth, rough and frizzle.

Haemagglutination (HA) test and haemagglutination inhibition (HI) test

Preparation of washed RBC: Chicken blood was collected into a sterile anticoagulant tube containing Ethylenediaminetetraacetic acid (EDTA), using a sterile hypodermic syringe under sterile conditions. The blood was then centrifuged at 3000 rpm for 10 minutes

and the supernatant, with the white blood cell layer, was removed and discarded. The red blood cells were then washed 4 times using 1% normal saline with the removal of the supernatant at each wash. After the red blood cells were properly washed, 1% of Chicken RBC was then prepared by adding 99 ml of normal saline to 1ml of the washed RBC, the solution was stored in the refrigerator at -4^{o}C till use.

Haemagglutination test: Using a Multichannel pipette, 0.025ml volume of normal saline was put into each well in the clean microtitre plates. 0.025 ml volume of each sera sample was then added to the first column of each row and a 2-fold serial dilution was done with the last volume left in the tips discarded. 0.025 ml volume of the reconstituted virus (4HAU antigen) was added to each well on the microtitre plates (Abraham-Oyiguh *et al*., 2014). The mixture was shaken and incubated at room temperature for 25 minutes. After 25 minutes, 0.025 ml of 1% washed chicken RBC is added, and then it was gently mixed and then left for 25 minutes. The result was read and recorded by assessing the highest dilution of serum causing complete inhibition of 4 HA units of antigen (Abraham-Oyiguh *et al*., 2014).

Statistical analysis

All data were entered into Microsoft® Excel 2010 version after data cleaning was done. All data analysis was performed using Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc., USA). Associations were determined using the Chi-Square test. Also, the Geometric mean of the HI antibody titer (GMT) and percentages of detectable NDV HI antibody titer was calculated.

Results

Of the 400 samples examined in this study, Newcastle disease virus antibody was detected in 53 (13.2%) chicken sera (HI titer≥4 Log_2), with the Geometric Mean titer (GMT) value and modal titer being 3.0 and 7 respectively (Table 1). Results from this study showed the frequency of Newcastle disease virus antibody titre from the chicken sera samples (Figure 2). The ND antibody titres from all 400 sera revealed titre value 1 as the most occurring titre value. Also, results showed that the modal titre value was 7 (Figure 2).

The prevalence of infection was almost the same in both markets with 24 (11.9%) and 29 (14.6%) positive samples detected in Oja tuntun and Ipata markets respectively (Figure 3). Results showed that there was a higher GMT value of 7.8 and a modal titre of 12 among the samples collected from Oja tuntun. Of the types of chicken examined, hens were more infected, 35 (14.7%) and had the highest modal titre value of 12. Growers were the least infected, 6 (8.9 %), however, they had the highest GMT value of 8.1 (Figure 4). Chickens with smooth feathers were the most affected, 52 (%), while frizzle chickens had no infection, although, smooth-feathered chickens were more examined than other feather types chickens.

Discussion

The findings of this study revealed Newcastle disease seroprevalence of 13.25% in slaughtered indigenous chickens in Ilorin. This could partly be due to the endemic nature of the disease among indigenous chickens in the study location. Previous studies in Nigeria have shown the endemicity of Newcastle

Table 1: Modal Titre, percentage distribution and GMT of NDV antibody in local chicken

Features	Number Tested (%)	Number (%) positive	Modal titre	Mean HI titre	GMT
Bird type					
Chicken	400 (100)	53 (13.25)	7	9.2	3.0
Location					
Oja tuntun	202 (50.5)	24 (11.9)	12	8.4	7.8
Ipata Market	198 (49.5)	29 (14.6)	7	10.1	7.1
Chicken Type					
Growers	67 (16.75)	6 (8.9)	11	5.9	8.1
Cock	96 (24.0)	12 (12.5)	9	10.4	7.3
Hen	237 (59.25)	35 (14.7)	12	9.7	7.4
Feather arrangement					
Smooth	382 (95.5)	52 (13.6)	9	9.4	7.4
Rough	3 (0.75)	0 (0)	0	0	0
Frizzle	15 (3.75)	1 (6.6)	10	6.6	10

disease in the country. Ohore *et al.* (2002) and Jibril *et al*. (2014) recorded a prevalence of 73.3% and 32.5% respectively using Indirect Enzyme Immunosorbent Assay (ELISA) to survey the prevalence of ND virus antibodies among free-range local chickens in Ibadan and Zamfara respectively. While the prevalence of ND is higher in the studies of Ohore *et al.* (2002) and Jibril *et al*. (2014), the differences can be seen in the type of analysis used which was ELISA as compared to the HI test in the present study. In a more closely related study, Musa *et al*. (2009) observed a prevalence of 14.1% among indigenous chickens in Plateau State.

A total of 64 (16.6%) of the sera samples had a titre value less than <1:16, a value that is not sufficiently high enough to be significantly positive. This is likely due to previous exposure to the virus, even though the infection might not have been present at the time of sampling. Similar reports were given by Abraham-Oyiguh *et al*. (2014). Nevertheless, this still indicates the presence of Newcastle disease virus antibody among the chickens. Ameji *et al*. (2011) reported ND 25.6% seropositivity among chickens in Kogi State, Nigeria using a similar methodology. However, 74.5% of the positive chickens in that study had titre values <1:16. This was higher than what was obtained in this study. There was a higher seroprevalence in Ipata market with 14.6% for titre levels ≥1:16 and also for titre levels ≥1:128 (10.1%) compared to that of Oja tuntun which were 11.9% and 8.4% respectively. Although Oja tuntun had a higher GMT value (7.8) and modal titre (12), there was no statistical significance of prevalence in relation to the market location. Jibril *et al*. (2014) also highlighted the insignificance of farm or poultry location in the spread and prevalence of ND.

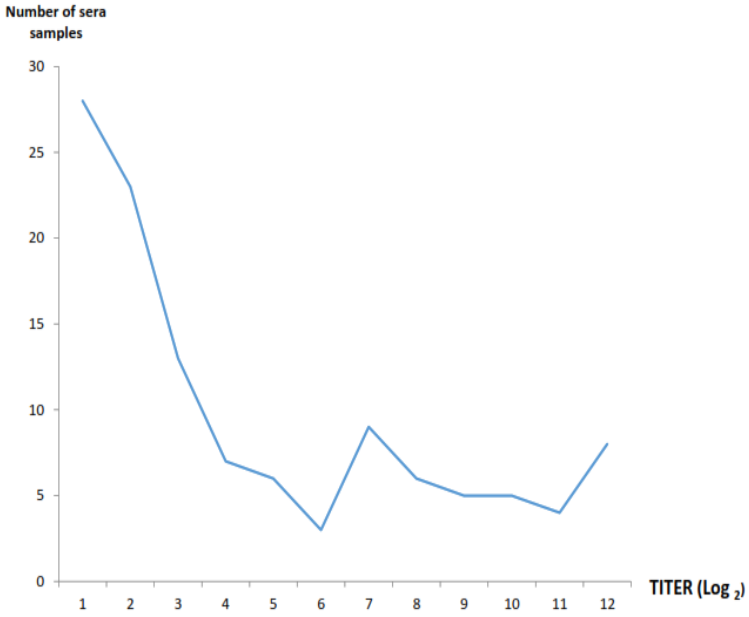

Figure 2: Frequency of NDV positive sera sample

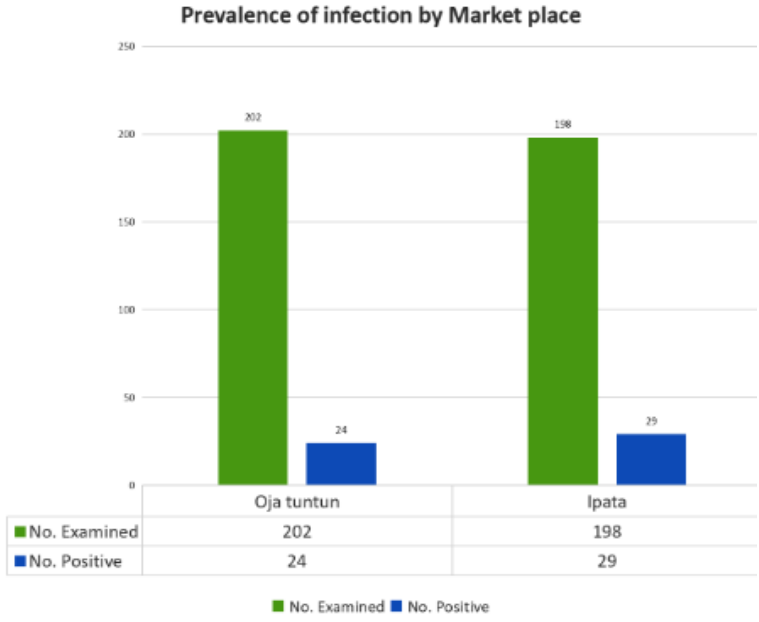

Figure 3: Frequency of NDV positive sera samples by chicken types by market sampled

This study also showed that there was no significant relationship between types of birds and infection. Despite the hens having a higher seroprevalence (14.7%), there was no statistically significant difference between the number of positive samples for all 3 types of chickens examined for this study. Hence, the type of chicken was not a significant risk factor predisposing the birds to Newcastle disease. Likewise, there was no significant difference in feather arrangements of the birds and ND seroprevalence in this study, as most of the birds examined had smooth feathers. In this study, a semi-formal interview with the poultry sellers revealed that none of the birds had been previously vaccinated, and were all kept close together in different cages. Some of the chickens were said to have been brought in

from the Northern part of Nigeria, where a high prevalence of ND had been previously reported (Shittu *et al*., 2016). This may be a major factor for the quick transmission of the disease since the disease is highly contagious and a major risk factor of infection is proximity to infected birds (Njagi *et al*., 2010). Other studies have highlighted factors such as confinement, hot temperatures, cold among birds, sick birds, winds, as major risk factors for Newcastle disease among indigenous chickens (Otim *et al*., 2007; Njagi *et al*., 2010). Also, the introduction of market birds to poultry and disposal of manure could influence the prevalence of Newcastle disease among indigenous chickens (Otim *et al*., 2007; Njagi *et al*., 2010).

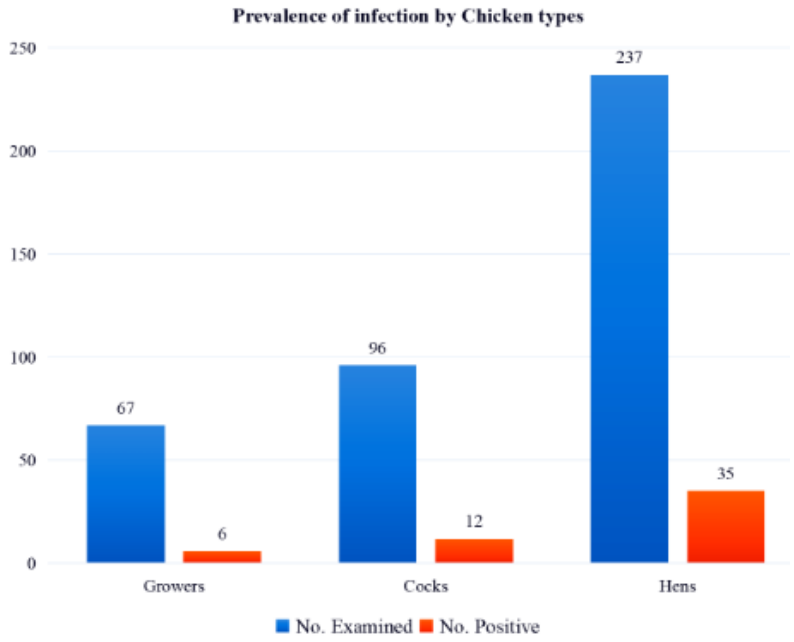

Figure 4: Frequency of Newcastle disease virus-positive sera samples by chicken types

Results from this study clearly showed the prevalence of ND in indigenous chickens in Ilorin using the haemagglutination inhibition test. Newcastle disease prevalence of 13.25% obtained in this study could be due to the lack of vaccination of the birds as gathered from the interview, as well as the living conditions of the birds that could lead to increased transmissibility of ND (Shittu *et al*., 2016). It is worthy to note that Newcastle disease is of high importance in Nigeria, as it was reported as the top viral disease with the most economic impact in Nigeria (Sadiq & Mohammed, 2017). FAO revealed that one of the major constraints to the achievement of an effective poultry disease control strategy is the ignorance of poultry keepers on appropriate procedures such as vaccination, pen spacing, breeding, and withdrawal of sick birds. Results from this study showed that a probable presence of Newcastle disease is prevalent in the study area, with lack of vaccination a major feature among these indigenous chickens. Therefore, there is a need for poultry farmers and other stakeholders to be sensitized and enlightened on the importance of vaccination for birds as well as other vital prevention and control measures that would stop the spread of Newcastle disease among chickens. In addition, effective intervention is needed from government agencies and policymakers, to make vaccination and veterinary care accessible, easier and affordable to chicken farmers.

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Nil

Conflict of Interest
The authors declare that there is no conflict of interest.

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