SEROPREVALENCE OF NEWCASTLE DISEASE VIRUS IN LOCAL CHICKENS SLAUGHTERED IN SELECTED MARKETS IN ZARIA, KADUNA STATE

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Abstract: The incidence of Newcastle disease (ND) in Nigeria is high and is a persistent cause of mortality and (or) morbidity among chickens causing huge economic losses. It has been observed that local chickens are being slaughtered daily in Zaria but, the ND status and public health problems are unknown. This study was carried out to evaluate the prevalence of Newcastle disease virus antibodies, sex and age-related prevalence in local chickens slaughtered in the selected markets in Zaria, Kaduna State between February and March 2019. The antibody detection was done by using Hemagglutination test (HA) and Hemagglutination Inhibition Test (HAI). A total number of three hundred (300) blood samples were taken across three selected markets, namely: Sabon-Gari, Samaru and Tudun-Wada markets. A hundred (100) blood samples were collected from each market. An overall seroprevalence of 37.67% (113/300) was recorded in this study. A comparison was made between sex and between ages. Result obtained showed female chickens (45.39%) had higher prevalence compared to male chickens (30.43%) and the association was significant (P=0.005). Likewise, higher seroprevalence was recorded in younger chickens (53.33%) than in older chickens (30.95%) with a significant association (P=0.015). it is therefore recommended that awareness program to enlighten the public on proper cooking of the chicken and effect of NCD is instituted. Vendors should also be educated on the need to screen and quarantine infected chickens from the stock to be slaughtered.

Keywords: Hemagglutination test, local chickens, markets, Newcastle disease, seroprevalence

Introduction

Newcastle disease is a viral disease of poultry caused by a single-strand, non-segmented, negative-sense RNA virus known as Avian paramyxovirus 1(APMV-1) (Deist *et al*., 2020). All avian paramyxoviruses are part of the genus *Avulavirus*, subfamily Paramyxovinae, family Paramyxoviridae, order Mononegavirales (ICTV, 2019). A Newcastle disease virus occurs in three pathotypes: lentogenic, mesogenic, and velogenic, reflecting increasing levels of virulence (Dimitrov *et al*., 2017). Newcastle disease is the most important viral disease of poultry in the world (OIE, 2018). It occurs in most countries and has a devastating effect on commercial poultry production. It is acute, rapidly spreading, contagious, nervous and respiratory disease of birds (Giovanni *et al*., 2011).

The Nigerian poultry industry comprises about 180 million birds making Nigeria the country with the second-largest chicken population in Africa after South Africa producing 650,000 tons of eggs and 300,000 tons of poultry meat in 2013(ASL 2050, 2018).

Poultry production in Nigeria amounts up to 454 billion tons of meat and 3.8 million eggs per year, with a standing population of 180 million birds. About 80 million chickens are raised in extensive systems, 60 million in semi-intensive systems and the remaining 40 million in intensive systems (ASL 2050, 2018).

Local chicken production is recognized as an important activity in all villages of developing countries (Minga *et al.*, 1989). The benefits of local chicken production are however constraint due to the presence and endemicity of Newcastle disease. It is the major constraint to the production of local chickens in many developing countries (Alexander, 1991). Ekue *et al.* (2002) reported that Newcastle disease is the principal limiting factor in rural poultry production. The disease affects poultry of all ages and species (Alexander, 1998). It constitutes the single most dreaded fatal disease of the local chickens and may kill up to 80% of the household poultry (Bell, 1992). These adverse effects of Newcastle disease on the local chickens eventually limit the economic dependence of the rural household (Bell, 1992). Similar effects of Newcastle disease have been reported in local ducks and local guinea fowls in Jos, Nigeria (Mai *et al.*, 2004). The spread of this highly infectious Newcastle disease virus could be as a result of close contact of the exotic flocks with the local chickens.

It hinders the progress of poultry management because it affects all breeds of chickens. Newcastle disease virus (NDV) has rarely been applied in the framework of disease eradication and remains a threat to poultry flock in Nigeria and local unvaccinated chickens (Ekue *et al.*, 2002).

It has been observed that local chickens are being slaughtered daily in Zaria but, the status of NDV and the public health problems are unknown. The role of the birds in the maintenance of the disease in localities is obscure. Such information is necessary for strategic planning for NDV control in the country.

It has been observed that local chickens are being slaughtered daily in Zaria; therefore, the need to check the status of the NDV is necessary to effectively control the spread of the animal disease at international, national and farm level since it poses a public health problem. This study was therefore designed to investigate the seroprevalence of Newcastle disease virus in local chickens slaughtered in markets in Zaria, Nigeria.

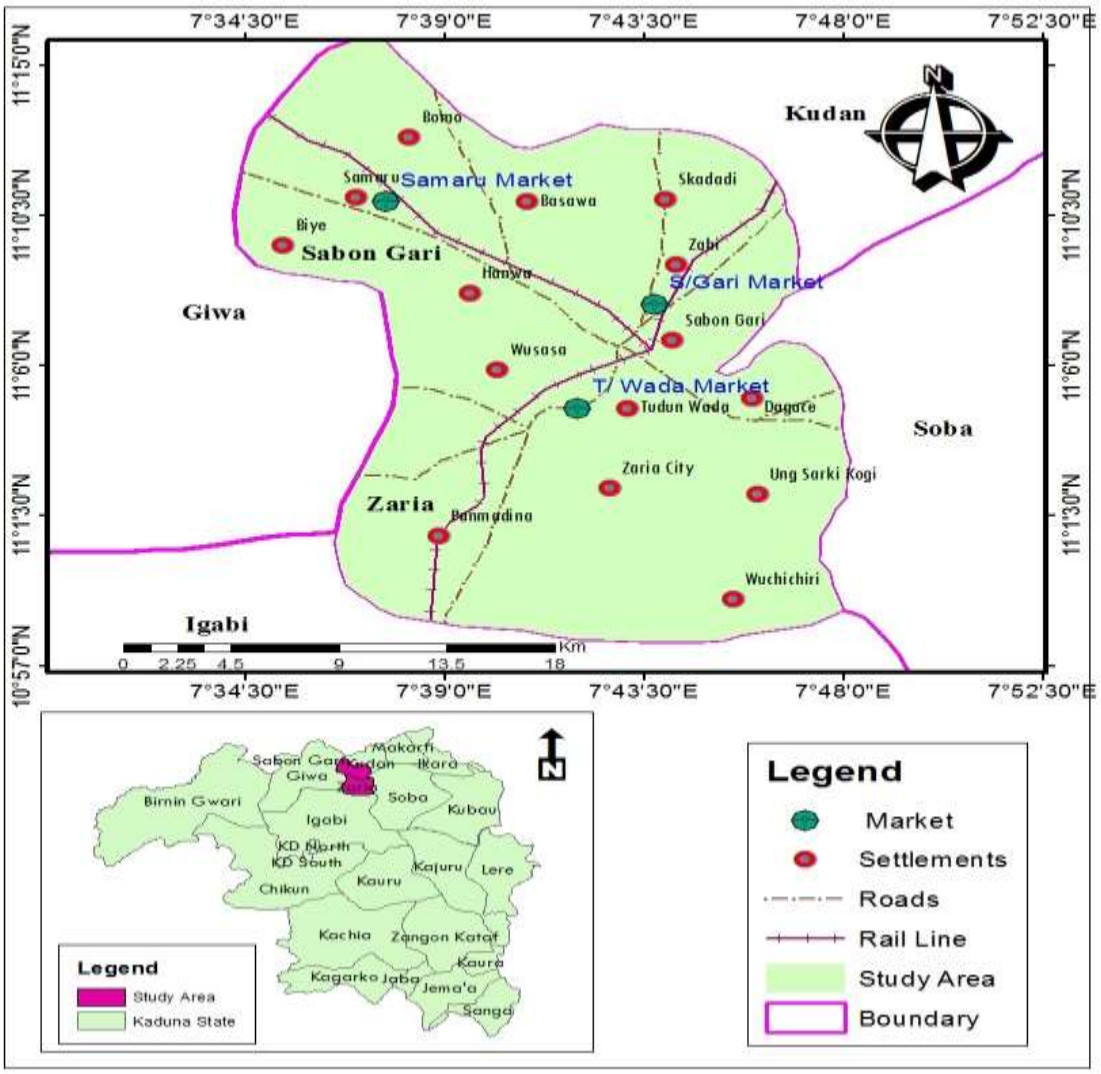
Materials and Methods

Study area

The study area is Zaria which is located at longitude (7º 43' 11.8020" E) and latitude (11º 5' 7.9476" N). The climate of Zaria is characterized by a clear distinction between dry and rainy seasons which may last from late October to early April and mid-April to early October, respectively. It shares common borders with Zamfara, Katsina and Kano to the north. Bauchi and Plateau to the east; Nasarawa to the south, Niger to the west and Abuja to the southwest.

Sampling locations

The sampling was carried out from three selected markets in Zaria, based on their size and observations of a large slaughtering of local chickens daily. These markets are Sabon-Gari market, Tudun-Wada market and Samaru markets (Fig. 1).



Source: Geography Department, ABU, Zaria
Fig. 1: Map of Zaria showing sampling locations

Sample size determination
Sample size will be determined using the formula

$N = z^2pq/d^2$ (Thrusfield, 2007)

Where: 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 (14.72%) (Alkali *et al*., 2017)
q = 1- P = 0.8528
N is Therefore 193 This represents the minimum sample size to be collected.
300 blood samples were finally collected to minimise sampling error.

Sample/Sera collection
3 – 5 ml of blood was collected from each chicken 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 research was done and stored at –4ºC. Test materials composed of chicken 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 –20ºC. 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. It was reconstituted into 200 dose vials in 8 ml of distilled water and 0.5% washed chicken red blood cells (RBC) suspension as described by Wosu (1984) was prepared and used as the indicator.

Hemagglutination (HA) test
Hemagglutination (HA) test was done by the micro test method using two-fold serial dilutions of 50 μl of reconstituted vaccinal virus (antigen), and 50 μl of the 0.5% chicken RBC was added to each well. An equivalent volume of chicken 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 endpoint of the HA was read. The titre was taken as the reciprocal of the highest dilution giving a 100% agglutination of the 0.5% chicken

RBC. This amount of virus represented one hemagglutination (HA) unit.

The principle behind the test is in the titration of antigen used in the experiment to determine the HA unit i.e., 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 (OIE, 2012). Briefly, aliquots of 0.025 ml of 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 2 mls PBS) was added to the first well. Doubling dilutions of 0.025 ml of the virus were made across the plate and 0.025 ml of the 1% (v/v) of the chicken red blood cell (RBC) was dispensed into each well. The plate was tapped gently to mix its content and the RBCs allowed equilibrating for about 40 min at room temperature. HA was determined by placing the plate on microtitre 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 a 1 HA unit. Afterwards, the value of 4HAU 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 titre 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 128 ml PBS.

The HA titre of the test sample was the reciprocal of the dilution that produced one HA unit. i.e., since the last microwell was 512,

HA titre = 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 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 50 µl of 0.5% washed chicken RBC that was 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 chicken RBC.

Data analyses

Chi-square test was used to check for the association between the disease and the selected markets, age and gender. The value of $p<0.05$ was considered significant.

Results and Discussion

The first report of Newcastle disease (ND) in Nigeria was in 1952 (Hill *et al*., 1953); thereafter, several cases have been reported in commercial, rural scavenging, captive and free-living wild birds, making it enzootic across the entire country (Nawathe *et al*., 1975; Fatumbi and Adene, 1979; Onunkwo and Momoh, 1981; Adu *et al*., 1985; Ojeh and Okoro, 1992; Echeonwu *et al*., 1993; Haruna *et al*., 1993; Nwanta *et al*., 2008; Ibu *et al*., 2009). Reports have shown that ND was ranked first among other diseases affecting the poultry industry (ASL 2050, 2018). Economic and financial losses as a result of incessant ND outbreaks in Nigeria are not being regularly quantified. An estimated 78,526 outbreaks of the disease were reported in 2008 across Nigeria with an estimated financial burden of 8.9 billion Naira for local chickens alone (ASL 2050, 2018).

This present study reveals a seroprevalence of 37.67% of ND (Table 1). This seroprevalence is higher (31.2 and 32.3%) than that reported by Halle *et al.* (1999) and Saidu *et al.* (1999) in Zaria, respectively. Although this study was carried out during the dry season, it was observed by Saidu *et al.* (1999) that ND occur throughout the year but seem to peak during the dry season. This was in tandem with our findings and this could explain the differences in the prevalence observed. The harsh harmattan wind and the cold chilly weather predisposes the birds to ND (Saidu *et al*., 1999). During this period, the birds are particularly vulnerable. Similarly, the significant seropositive rate of NCD in these markets in our investigation is indicative of the continuous infection pressure. This might be because of the free-ranging management system that allows the uninterrupted cycle of infection as the virus passes from one to the other. The chickens are also prone to acquire infection from wild birds. The local open markets where huge numbers of chickens are gathered might also serve as continuous foci of infection.

Musa *et al.* (2009) reported a higher seroprevalence of 51.90% in four local governments in Jos, Plateau state which was higher than our study. This high seroprevalence could be due to the climatic variations in Jos and Zaria as Jos is known to be one of the coldest cities in Nigeria. An overall seroprevalence of 14.72% was reported by Alkali *et al.* (2017) in Sokoto which is lower as compared to our study. The fact that local chickens are rarely vaccinated in this part of the country, antibodies detected may be because of previous infection or an ongoing subclinical infection (Alkali *et al.*, 2017). In the African context, the prevalence of 14% was observed by Courtecuisse *et al.* (2005) while Zeleke *et al.* (2005) reported 19.78% seroprevalence in non-vaccinated village chickens in the Niger Republic and southern Ethiopia respectively. Another seroprevalence of ND reported in Nigeria include a prevalence of 32.5% recorded in Zamfara by Jibril *et al.*(2014), El-Yuguda *et al.* (2007) reported a seroprevalence of 46% in village chickens in Borno State and Ameji *et al.* (2011) reported 25.5% prevalence in live bird markets of Kogi State, Nigeria.

Table 1: Seroprevalence of Newcastle disease virus in selected Markets in Zaria, Kaduna State

Location	Number Examined	Number Positive	Number Negative	%
Sabon-Gari	100	32	68	32
Samaru	100	48	52	48
Tudun-Wada	100	33	67	33
Total	**300**	**113**	**187**	**37.67**

Table 2: Sex specific seroprevalence of Newcastle disease virus in Selected Markets in Zaria, Kaduna State

Location	Number sampled		Number positive		%	
	Male	Female	Male	Female	Male	Female
Sabon-Gari	55	45	14	18	25.45	40.00
Samaru	46	54	20	28	43.48	51.85
Tudun-Wada	60	40	15	18	25.00	45.00
Total	**161**	**141**	**49**	**64**	**30.43**	**45.39**

Chi-square value = 0.376, P value = 0.005

Further, this study showed that females had higher seroprevalence (45.39%) compared to males (30.43%) as presented in Table 2. There was a significant association (P =0.005) between the sex and seroprevalence of ND. Sex could, therefore, be said to be a risk factor in this study. Female chickens are kept for reproduction and they are not as

easily sold as the male. This means they stay longer in the cages or are left to free-range longer than the male thereby exposing them more to infection and reinfection. This could account for the reason females had a higher prevalence than males in our study. The result of this study is similar to the findings of Alkali *et al*. (2017) who recorded a seroprevalence of (15.32%) in females and (14.02%) in males and Kelm *et al.* (2005) who recorded a seroprevalence of 25.7% in females and 22.9% in males. This is as opposed to the findings of Aschalew *et al*. (2005) in Ethiopia who recorded a higher seroprevalence rate among males (21.74%) than females (19.16%). Higher seroprevalence of 31.63% and 32.63% had been recorded amongst male and female chickens respectively by Tadesse *et al*. (2005).

Table 3: Age specific seroprevalence of Newcastle disease virus in selected Markets in Zaria, Kaduna State

Location	Number sampled		Number positive		Prevalence (%)	
	Below 28 weeks	28 weeks and above	Below 28 weeks	28 weeks and above	Below 28 weeks	28 weeks and above
Sabon-gari	31	69	14	18	45.16	26.09
Samaru	31	69	20	28	64.52	40.58
Tudun-Wada	28	72	14	19	50.00	26.39
Total	**90**	**210**	**48**	**65**	**53.33**	**30.95**

Chi-square value = 1.028, P-value = 0.015

Concerning the age of the birds, higher seroprevalence was recorded in chickens below 28 weeks (53.33%) and lower in chickens above 28 weeks (30.95%) as shown in Table 3. Like sex, there was also a significant association (P = 0.015) between the age of the birds and the seroprevalence of ND. Similarly, Alkali *et al.* (2017) reported a higher prevalence in young birds (13.66%). It has been suggested that age-specific seroprevalence might result from different breeding behaviours of each sexor inherent differences in immune response or antibody persistence (Ely *et al*., 2013).

Conclusion and Future Thought

This study has shown a seroprevalence of 37.67% of ND in Local chickens slaughtered in selected markets in Zaria. Age and Sex-specific seroprevalence was measured where females had higher seroprevalence than males and younger birds were more seropositive than older birds. Further, accumulation of antibody responses throughout life probably generates differences in the epidemiology of ND among host species with different lifespans. A more complete understanding of ND dynamics will require long-term screening of active infection and serological immunity in other well-studied animal populations in nature.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. Lastly, local chicken vendors at markets in Zaria should be encouraged to screen their flock and quarantine infected local chickens.

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Conflict of Interest

The authors declare no conflict of interest.

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