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Comparative Analysis of Newcastle Disease Virus Shedding from Naturally Infected Breeds of Poultry in Maiduguri, Nigeria

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ABSTRACT

Newcastle Disease (ND) has been considered as a threat to poultry industry worldwide. Despite different strategies aimed at controlling ND in Nigeria, the severe form of the disease continues to occur even in vaccinated poultry farms. The disease is transmitted primarily through contact with infected or carrier birds. Newcastle disease virus (NDV) shedding through either cloacal or oropharyngeal route play a critical role in the spread of NDV. However, there is paucity of information on the comparative contribution of these routes to NDV shedding. In this study, a total of 256 swab samples were collected from cloacal (n = 128) and oropharyngeal (n = 128) routes from broilers, layers, village chickens, ducks and turkeys that were naturally exposed to NDV. Haemagglutination (HA) and haemagglutination inhibition (HI) tests were carried out to detect the presence of the virus. The results of NDV shedding from cloacal and oropharyngeal routes were compared. The result showed a higher prevalence (42.2%) of NDV shedding from cloacal route when compared with oropharyngeal route (26.6%). In addition, village chickens showed a higher prevalence (43.8%) of NDV shedding when compared with all other breeds sampled. Furthermore, birds at 6 weeks shed higher NDV (66.6%) than the birds of other ages. Therefore, the result of this study showed that the prevalence of NDV shedding was higher in village chickens via cloacal route. There is a need to quantify the amount of NDV shedding in both cloacal and oropharyngeal routes from these breeds of poultry so as to evaluate the viral infective dose.

Keywords: Broilers; Cloacal and Oropharyngeal routes; Layers; Newcastle disease virus

INTRODUCTION

Newcastle disease (ND), caused by virulent Newcastle disease virus (vNDV), is a severe and often fatal infection in naive chickens and is a threat to poultry worldwide (Miller and Koch, 2013). Newcastle disease viruses belong to the family; *Paramyxoviridae,* genus; *Orthoavulavirus,* species; *Avian orthoavulavirus 1* (Kuhn *et al*., 2019). Based on the disease produced in chickens under laboratory conditions, NDV isolates have been placed in five pathotypes; Viscerotropic velogenic, Neurotropic velogenic, Mesogenic, Lentogenic, and asymptomatic enteric strains (Dimitrov *et al*., 2017).

Laying birds that are sick and shedding the virus as well as those that are incubating the virus are the usual source of infection for healthy commercial chickens (Roy and Venugopalan, 2005). Virus shedders in healthy flocks may play a significant epidemiological role in this infection. It has been shown that virus excretion begins before the appearance of clinical symptoms, therefore, viruses with longer incubation periods can cause more severe disease outcome (Alexander and Senne, 2003). In general, all avian species are susceptible to ND infection, but the chickens are the most affected in terms of severity of the disease (Alexander and Senne, 2003). In chickens, 100 % mortality has been previously reported (Aldous and Alexander, 2001). Although ducks and quails had been known to be resistant to the disease, studies have however reported their susceptibility with lower morbidity and mortality than in chickens (Eze *et al*., 2014; Susta *et al*., 2018).

In Nigeria, ND outbreaks have been reported in free-range village and exotic chickens, guinea-fowls, wild and captive birds, quail, dove, mallard duck, ostrich, turkey, vulture, eagle, sparrows, crows, parrot (Shittu *et al*. 2016; Bello *et al*., 2018). Newcastle Disease Virus shedding either through oropharyngeal or cloacal route of birds is of epidemiological significance toward the spread of ND. The spread of NDV begins with the shedding of the virus by the infected birds and the subsequent inhalation of contaminated aerosol by

susceptible birds (Brown and Bevins, 2017; Hamisu *et al.,* 2022). Ingestion of NDV-contaminated feed and water or inhalation of NDV-contaminated air are the two primary modes by which ND is transmitted among birds. (Alexander *et al.,* 1984). Although live vaccines administered through aerosol can establish respiratory infection; but there is limited experimental evidence that infected birds transmit the virus to other susceptible birds in this way, even over short distances (Abdisa and Tagesu, 2017). The success of transmission through inhalation of excreted droplets depends on various environmental factors such as temperature, humidity, and stocking density. Infected birds shed the virus through exhaled air, respiratory discharges, and feces during the incubation, clinical, and convalescent stages for a limited period. But it is highly probable that viruses like the pigeon variant and others that do not induce major respiratory symptoms in birds are primarily spread through contact with contaminated fecal matter (Capua and Alexander, 2009).

Although several studies on virus shedding revealed that virus shedding do occur from both oropharyngeal and cloacal routes, even in vaccinated birds, however, data about which of these routes significantly contribute to higher NDV shedding in a naturally exposed avian species, are generally lacking. In this study, virus shedding from naturally infected avian species was investigated, and the results between different routes and breeds were compared.

MATERIALS AND METHODS

Study Area

This study was conducted in Maiduguri metropolis, the capital city of Borno State, Nigeria, lying within latitude 10^{o}N to 13^{o}N and longitude 11.04^{o}E and 14.04^{o}E located on north-eastern part of Nigeria. The average annual rainfall is 650mm (Ishaku and Majid, 2010).

Sample Collection

Samples were collected from a total of 128 birds of different breeds of poultry: 40 broiler chickens, 24-layer chickens, 24 village chickens, 20 turkeys and 20 ducks from 5th December 2021 to 17th January 2022. The layers and broilers were sampled from different farms with reported outbreaks of ND. However, the village chickens, turkeys and ducks were sampled from live bird markets in Maiduguri Monday market; Shagari Low-cost A; and Lake Chad Basin within Maiduguri. Physical examination was conducted, and data associated with age and breeds of the birds were collected from the farm managers. The broilers were between 5, 6, and 7 weeks, while the layers were 15, 17 and 22 weeks old.

Cloacal and oropharyngeal swabs were collected from each of the bird, and placed separately in 1 ml viral transport medium. All samples were transported on ice to the Virology Laboratory, Department of Veterinary Microbiology, University of Maiduguri, and stored at -20^{o}C refrigerator.

Virus Detection

All samples collected were subjected to Hemagglutination test (HA) to determine the presence of a hemagglutinating virus. All positive samples for HA were then further subjected to Hemagglutination inhibition (HI) test according to standard protocol (Hierholzer *et al*., 1969; Kaufmann *et al*., 2017) using NDV specific antisera to confirm the presence of the virus.

Preparation of 1% Chicken RBC

Blood was taken from an NDV-free, unvaccinated chicken and pooled in an equal volume of Alsever's solution. The blood was centrifuged and washed three times with Phosphate Buffer Saline (PBS). Then, 1% RBC (packed cell v/v) suspension was prepared using PBS.

Statistical Analysis

Data generated were presented in form of Graphs and Tables. Data were cleaned and analyzed using the Microsoft Excel (v2010) and SPSS-IBM, USA (v25.0). Chi-squared test of association was employed to determine the relationship between the variables (breeds, routes, age) and the NDV shedding. Value of $P<0.05$ was considered statistically significant throughout the study.

RESULTS

The overall breed-specific frequency of NDV in the broilers was 39.7% (31/78), which is higher than that of the layers 40% (20/50) (Table 1). A total frequency of 43.8% of the NDV was shed by village chickens, whereas turkeys shed a total prevalence of40% (16/40). There is no NDV shedding detected from ducks. No statistically significant association ($P>0.05$) between breeds and being positive for NDV with the exception of village chickens (Table 1).

In relation to routes, Cloacal route has the highest number of positive 42.2% (54/128) when compared with oropharyngeal route 26.6% (34/128). The results also revealed a statistically significant association (χ^2= 6.926; $P<0.05$*) between routes and NDV shedding (Figure 1).

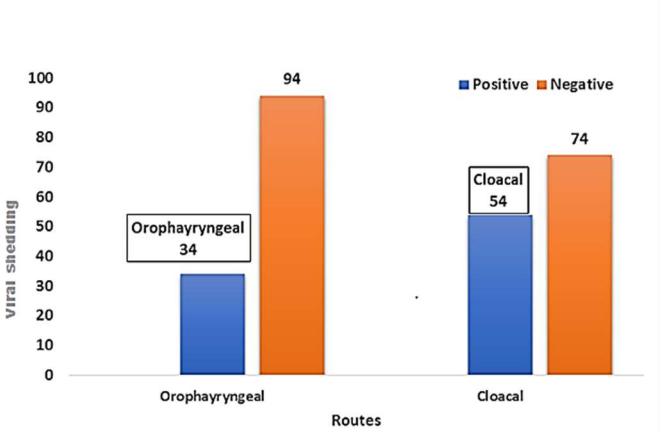

Figure 1: Comparison of viral shedding between the Cloacal and Oropharyngeal routes among different breads of birds in Maiduguri Metropolis

The results of this study further revealed that there is a significant association ($P>0.05$) between age and NDV shedding among broilers and layers (Table 2).

Table 1: Prevalence of Newcastle disease virus shedding among different breeds of bird in Maiduguri Metropolis

Breeds	Serological Status		Inferential Statistics		
	Positive (%)	Negative (%)	χ^2	P-value	OR (95%CI)
Broilers	31 (39.7)	47 (60.3)			
Layers	20 (40.0)	30 (60.0)			
Village chickens	21 (43.8)	27 (56.3)	25.081	0.000*	39.74 (28.88–50.60)
Turkeys	16 (40.0)	24 (60.0)			
Ducks	0 (0.0)	40 (100.0)			
Total	88 (34.4)	168 (65.6)			

*Statistically significant

Table 2: Age-Distribution of Newcastle disease virus shedding positive among Broiler chickens and Layers in Maiduguri Metropolis

Species	Age (weeks)	TNT(%)	TNP(%)	% Prevalence	χ^2	P-value
Broiler	5	20 (15.6)	10(50)	50	30.914	p=0.0001*
	6	30 (23.4)	20(66.6)	66.66		
	7	28(21.8)	0(0)	0		
Layers	14	14 (10.9)	4(28.5)	14.2		
	17	26 (20.3)	11 (32.30)	42.30		
	22b	10(7.8)	6(60)	60		
Total		**128**	**51 (39.84)**	**39.84**		

TNT: Total Number Tested and TNP: Total Number Positive; *statistically significant

DISCUSSION

The global impact of ND on the poultry industry is substantial. The disease causes reduced productivity, high bird mortality rates, and severe trade restrictions due to the perceived risk of disease spread (Alexander, 1995). Despite vaccination efforts, the virulent Newcastle disease virus (vNDV) responsible for ND remains widespread, and reports from various countries indicate that it continues to spread even among vaccinated flocks (Martinez *et al*., 2018). This is facilitated by the virus's presence in the environment, which makes it easy for the transmission of the virus to susceptible birds. Therefore, there is a renewed interest in investigating the NDV shedding in the environment (Ayala *et al.*, 2020).

The findings of this study showed that NDV shedding via the cloacal route is higher than its shedding through the oropharyngeal route (Figure 1). This may be attributed to the higher tropism of the virus to the digestive tract than respiratory tract. Previous study had attributed the degree of NDV strain tropism to rate of replication and virus shedding. For instance, Lentogenic strains have low tissue tropism and low virus shedding than velogenic strain (Zhang *et al*., 2018). Our finding is in agreement with Haque *at al*. (2010) who reported higher prevalence of NDV from cloacal swab samples compared with samples from oropharyngeal route.

The finding of this study further showed that broiler chickens demonstrated higher prevalence of NDV shedding than layers. In relation to breed, the finding of this study revealed that village chickens showed higher virus shedding than ducks and turkeys. Chickens are known to be more susceptible to NDV than ducks (Alexander and Senne, 2003). It is therefore not surprising that the prevalence of NDV shedding in chickens is higher than in ducks as reported in this study. Okoroafor *et al*. (2020) reported lower NDV prevalence in turkeys than in village chickens, which shows the severity of the virus in village chickens than in turkeys, but turkeys are likely to be the source of the infection for other species, especially chickens. Other researchers have compared NDV shedding from oropharyngeal and cloacal routes of different breeds of poultry. For instance, Panus *et al*. (2015) reported higher NDV shedding in cloacal of chickens and ducks. Furthermore, Saepulloh and Darminto (2005) reported that there was no NDV shedding from oropharynx swabs of ducks, and concluded that ducks tend to excrete the virus via cloaca. This is in disagreement with our finding which showed that there was no NDV shedding in both the cloacal and oropharyngeal routes of ducks. The inability to detect NDV from the cloaca of ducks in our study could be due to the less sensitive nature of the HI test used when compared with the highly sensitive real time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) employed by Panus *et al*. (2015). The present study reported that 40% of NDV shedding was detected in both cloacal and oropharyngeal swabs of turkeys. This is in variance with 68% and 57.2% recorded from turkeys in the Nigerian cities of Zaria and Maiduguri (Sa'idu *et al*., 2004; Sadiq *et al*., 2011) respectively. Therefore, the co-rearing of different species of poultry can facilitate the introduction and spread of NDV among poultry species and breeds as suggested by Sa'idu *et al*. (2004) and Ramey *et al*. (2017).

The results of this study also revealed that there is a significant association (P>0.05) between age and NDV shedding. Six-weeks old broilers tend to have high percentage of NDV shedding among the sampled birds. Perhaps, this might be associated with the fact that around six weeks of age, the bursa of Fabricius regresses in broilers, and as an important primary lymphoid organ, this will lead to immunosuppression and the resultant NDV shedding (Glick, 1983). In addition, Sharifi and Talebi (2022) reported that immunosuppresive activities caused by co-infection with infectious bursal disease virus (IBDV) could also lead to the shedding of the NDV at 6 weeks. Regarding the 22-week layers having almost 60% shedding of NDV, this could be due to a combination of factors. At the point of lay, layers undergo physiological changes, which can be stressful,

leading to immunosuppression that may lead to vaccination failure or increased disease during production due to the complex neuroendocrine response to stressors (Hoerr, 2010; Campbell *et al*., 2019), and hence higher NDV shedding.

Conclusion

This study reported higher frequencies of NDV shedding from village chickens when compared with all other birds sampled in the study. Higher NDV frequency was detected from cloacal route than oropharyngeal route. The study further revealed statistically significant associations between age, species of bird and route of sample collection, and the frequency of NDV shedding ($P<0.05$). There is need to quantify the NDV shed from both the oropharyngeal and cloacal routes in order to explore the NDV infectivity dose from shedding.

Conflict of Interest

The authors declared that they have no conflict of interest.

Author's Contribution

MUS and TMH designed the work and wrote the first draft of the manuscript; AS and NMH analyzed the data; YMS, ADEY and MBA reviewed the manuscript; EDM and MMW collected the samples and carried out the laboratory work.

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