



## RESEARCH ARTICLE

### Newcastle Disease Virus Shedding Among Healthy Commercial Chickens and its Epidemiological Importance

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#### ABSTRACT

Newcastle disease virus (NDV), shedding among apparently healthy commercial chickens reared intensively in Nsukka and its environs were studied. Samples were collected from birds that were not vaccinated against Newcastle disease (ND), and also from those that were vaccinated but given an interval of 28 days between vaccination and sample collection. Cloacal and tracheal swabs were collected from each bird and a total of 1800 birds were sampled from 72 farms located in the area. The birds in the area were monitored for 15 days post sampling. The samples were examined for NDV by isolation in embryonated chicken eggs through the allantoic cavity route. Result showed an isolation frequency of 3.2% and this could be termed as the prevalence of NDV in clinically healthy chickens in Nsukka area as the birds remained apparently healthy for more than 15 days. We therefore conclude that there is virus shedding among healthy commercial chickens in Nsukka and its environs and this should be considered an important epidemiological factor in the spread of the disease. Healthy carriers can serve as short term reservoirs and transmit the disease to other birds.

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#### INTRODUCTION

Newcastle disease virus (NDV), also known as Avian Paramyxovirus serotype I (APMV-1) is an RNA virus belonging to the genus *Avulavirus* in the family *Paramyxoviridae* (Mayo, 2002). The virus is an important avian pathogen causing significant disease outbreaks in poultry worldwide (Alexander and Senne, 2008; Shahzad *et al.*, 2011). It is economically significant since it causes high morbidity and mortality, reduces egg production, deteriorates egg quality and impairs live performance (Orsi *et al.*, 2009; Yan *et al.*, 2011). NDV isolates have been grouped into velogenic, mesogenic and lentogenic pathotypes based on virulence, clinical signs and death in infected birds, mean death time (MDT) of chicken embryos upon allantoic fluid inoculations, and intracerebral pathogenicity index (ICPI) in day old chicks, (Anonymous, 2008; Orsi *et al.*, 2009). In chickens, lentogenic strains produce mild or inapparent respiratory infections; the mesogenic strains produce low mortalities, acute respiratory disease and neurologic signs in some birds while the velogenic strains which can be either neurotropic velogenic NDV (NVNDV) or viscerotropic

velogenic NDV (VVNDV) cause disease with high mortality (Huang *et al.*, 2004; Piacenta *et al.*, 2006).

It is very clear that persistent virus infections or carrier states in virus infections are responsible for a wide range of human and animal diseases (Abe *et al.*, 2007). In Newcastle disease (ND) the strain of the virus may influence its persistence or carrier state in tissues as strains with low virulence may be carried for a longer period. In Nigeria, NDV has been noted to be widespread due to rapid expansion of the poultry industry, high stocking densities and inadequate biosecurity measures. These have created conditions conducive for the spread and maintenance of the endemicity which the disease has assumed with all strains of the virus being common (Okwor and Eze, 2010). The incubation period in ND infection after natural exposure varies from 2-15 days with the average of about 5 days (Kahn *et al.*, 2005). Experimental data using Nigerian velogenic strain of NDV have shown an incubation period of 2-5 days depending on age and immune status of the birds (Okwor *et al.*, 2007). The usual source of infection for healthy commercial and village chickens are infected chickens which are suffering from the disease and shedding the virus and those that are incubating the virus and shedding

them at the same time (Nwanta *et al.*, 2008). Virus excretion commences before clinical signs occur, therefore, those with longer incubation period poses greater danger.

Commercial flocks of poultry are important source of protein to the Nigerian populace and ND has been a limiting factor in their production. Virus shedders among healthy flocks could be a major epidemiological factor in this disease. Therefore this study was carried out to investigate the extent of virus shedding among clinically healthy commercial flocks.

## MATERIALS AND METHODS

**Sampling of Birds:** Exotic chickens, reared intensively on commercial basis were studied. These included broilers, layers, pullets and cockerel. Farms in Nsukka located in Southeast Nigeria and its environs were sampled. Twenty to thirty birds were sampled in a farm. Samples were collected from apparently healthy flocks. History of vaccinations against ND was taken and samples were collected from birds that did not receive ND vaccine for 28 days and beyond. In other words, only birds not vaccinated against ND for 28 days and beyond were sampled and sampling was delayed until 28 days post vaccination in those that had received the vaccine prior to visit.

Cloacal and tracheal swabs were collected from each bird and pooled separately in universal bottles containing 1.5ml of antibiotics (10,000 IU/ml Penicillin, 10mg/ml Streptomycin and 0.25 mg/ml Gentamycin) reconstituted with phosphate buffered saline (PBS) and adjusted to pH 7.0-7.4. Samples were transported in cold flask to the laboratory where it was stored at  $-35^{\circ}\text{C}$  until virus isolation. The farms that were sampled were monitored for 15 days and the health status of the birds noted.

**Virus Isolation:** Virus was isolated in embryonated chicken eggs using the allantoic cavity route. The pooled samples in PBS were centrifuged at 1000 g for 20 minutes and the undiluted supernatant used for the isolation, which was done according to the standard method described by Alexander (2003). About 0.2ml of the undiluted supernatant was inoculated into each of three 9-11 day old embryonated chicken eggs. After 72 hours of incubation, the eggs were chilled and the allantoic fluid was harvested. The allantoic fluid harvested was used in hemagglutination test to establish the presence of a hemagglutinating virus. Positive samples were subjected to hemagglutination inhibition test (Anonymous, 2008) using NDV specific antisera to confirm the presence of NDV.

## RESULTS AND DISCUSSION

Most of the farms visited vaccinated against ND. This informed our reason for delaying sample collection for up to 28 days post vaccination in vaccinated flocks. This was done to minimize the possibility of isolation of vaccinal viruses among the flocks and to increase the chances of isolation of field viruses. Because of the wide spread vaccinations against ND, screening for antibodies against ND was not included in this study as this will not reflect the extent of infection by field viruses but will cover greatly, antibodies developed as a result of

vaccination. Seventy two flocks were sampled in 45 farms that were visited. From 72 flocks, a total of 1800 birds were sampled. Out of these, NDV was isolated in 58 (3.2%) birds while the other birds remained apparently healthy for 15 days.

The result of this study will help in evaluating the prevalence of ND among exotic birds reared intensively in Nsukka and its environs. It will also help in establishing their role in the epidemiology of ND. This study showed 3.2 % virus isolation in healthy birds in this region for the birds that remained clinically healthy up to day 15 post sampling which is the upper limit of incubation of the virus. Similar study carried out by Orsi *et al.* (2010) in Brazil showed a prevalence of 6.8 - 58.4% within some geographical regions. However, they did not distinguish between vaccinated birds & birds that were not immediately vaccinated as the higher prevalence was got in regions where vaccination was widely done. Alexander and Senne (2008) noted that vaccines protect birds from clinical disease while virus replication and excretion may occur. In this study, vaccinated birds were excluded and that may be responsible for the low figure that was obtained. Nevertheless, the prevalence could be meaningful as shedding of NDV by these birds for a long time will definitely pose a risk and influence the spread of NDV.

An important factor in the epidemiology of ND in commercial chickens is the introduction of the virus in a flock. Moreover, the persistence of the virus in a flock can influence the course of the disease and the spread or distribution of the virus to neighboring farms. Nwanta *et al.* (2008) were of the opinion that most cases of ND in village chickens can be attributed to chickens that are shedding the virus. These are usually chickens that are incubating the disease as virus excretion commences before clinical signs occur. Birds that have recovered from clinical infection or vaccinated birds may be shedders. Vaccinated birds may show no clinical signs at all on challenge with virulent virus but will become infected and excrete the virulent virus for up to 2 weeks (Miller *et al.*, 2009). Birds incubating the virus will spread the virus to the healthy non infected ones.

Experimental and field data review suggests that permanent carrier or shedding state in ND is rare in chickens (Alexander *et al.*, 2006). These authors noted that though this may be true for chickens, an opinion was expressed that more lasting carrier state may occur in turkeys and other avian species. For instance, Lima *et al.* (2004) wrote that Japanese quail (*Coturnix coturnix japonica*) might be a carrier of NDV suggesting that these species may play an important role in the epidemiology of ND in regions with commercial poultry production. Sparrows have been found to be reservoirs for NDV (Silva *et al.*, 2006). Waterfowls, ducks, geese and teals maintain NDV strains. The virulent strain circulating in wild environment can be transmitted to commercial poultry flocks (Hlinak *et al.*, 2006). Moreover, persistent infection of cells has been seen in cell culture and some tissue explants have remained morphologically intact and able to support the replication of NDV for 6 months (Zaffuto *et al.*, 2008). It therefore means that other avian species which are carriers and shedders of this virus are potential source of infection to chickens.

Most field outbreaks of ND in Nigeria are caused by the velogenic viscerotropic strain of the virus (Okwor and Eze, 2010; Okwor and Eze, 2011). These outbreaks are usually fulminating resulting in high mortalities within short periods of time. Ibu *et al.* (2009) was able to isolate only lentogenic strains from feral birds in Nigeria. Though attempts are under way to characterize these isolates, we believe that the three strain of NDV are present in chickens in Nigeria.

**Conclusions:** A prevalence of 3.2% of NDV in non-vaccinated apparently healthy commercial chickens in Nsukka and environs was found. These viruses are shed by these healthy birds and they remain healthy for up to 15 days and this can significantly influence the epidemiology of this disease. Farmers in Nigeria have paucity of information about disease prevention. Birds move freely, human beings and vehicle have no restrictions in their movements and biosecurity measures are not applied in many farms. It is therefore important that biosecurity measures be implemented and the movement of animal, man and instruments be controlled to minimize spread of the virus shed by both healthy carriers and sick birds.

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