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Abstract

Yobe State is located in Nigeria; it lies between latitudes 11°56' and 13°20'E and longitudes 9°38' and 12°25'N and shares boundary with Niger Republic. The state is known for rearing of livestock and local poultry production. Viruses are known to cause most of the important respiratory diseases of poultry, these diseases results in morbidity, mortality and decline in production. We conducted a survey to detect Avian Influenza (AI), Newcastle Disease (ND) and Infectious Bronchitis (IB) viruses in domestic and captive migratory wild birds using Nested Polymerase Chain Reaction (nPCR), we sampled 159 domestic and 34 wild migratory birds from poultry slaughter slabs located in four Local Governments Areas of Yobe state. Tissue samples were collected processed and tested using nPCR. The prevalence of ND virus was 30.8% and of IB virus was 2.5% in domestic birds. The prevalence of ND and IB viruses was highest (33.1% and 2.9% respectively) among local chickens when compared to other domestic birds. ND, IB, and AI viruses were not detected in wild migratory birds. Some of these viruses are zoonotic and of economic importance in poultry production, an effective control and prevention strategy should be established.

Keywords: Domestic bird, wild migratory birds, avian influenza virus, infectious bronchitis virus, Newcastle disease virus, Yobe state, Nigeria
Introduction

Viruses cause most of the important respiratory diseases of poultry and these diseases generally result in morbidity, mortality, decline in production functions, decrease in body weight and poor feed conversion. Viral diseases are characterised by little or no response to conventional antibiotic therapy, however, a number of these diseases can be prevented by vaccination and good management practices (Abdu P.A. 2007; FAO 2008).

The potential ways of spread of viral infections include movement of contaminated fomites, feed or water and introduction of carrier bird to susceptible flocks. Migratory wild birds, use of vaccines and mix poultry populations can equally help in spread of viral infections (Adene D.F. 2007; CIDRAP 2008).

Direct economic consequences of outbreaks of respiratory viral infections on the poultry industry generally include the loss of birds as a result of death, disruption of production and trade in poultry and poultry products from affected areas, increase of price of retail of poultry and poultry products as well as cost of logistics on the part of authorities to put control measures in place (Owoade A.A 2006; CIDRAP 2009). The World Bank estimated in October 2006 that about 1.5 billion was needed to combat Highly Pathogenic Avian Influenza (H5N1) globally. However, by October 2008, $1.5 billion has been disbursed; yet, the infection was far from being contained in many nations of the world. In Nigeria, over 1.3 million birds have been culled and over $5.4 million has been paid as compensation to farmers due to Highly Pathogenic Avian Influenza H5N1 between 2006 and 2008 (Adene D.F. 2007; CIDRAP 2008; CIDRAP 2009).

The mutagenic tendencies of some respiratory viral agents of avian species make them a potential threat to human health especially in some developing countries where there is intimate interaction between birds and humans. Some of these viral agents are known to remain in evolutionary stasis in their avian hosts. Such evolutionary stasis is often indicated by low genetic mutation rates. When disease determinants supports cycles of infection, the viral agents may mutate and adapt to a new hosts (Grain 2006; Greger M 2006).

Avian Influenza and Newcastle Disease viruses are of public health concerns with clinical manifestations ranging from inflammations to systemic infections among humans (Alexander D.J. 2003). Infectious Bronchitis viruses however, have not been reported to be a public health threat to humans yet. However, the virus continues to be an economically significant problem by causing severe decline in egg production (Cavanagh D. and Naqi S.A. 2003; Chousalkar K.K et al., 2009).

Live bird markets provides avenue for mixing of birds of different species. These markets exist in different locations within and outside Yobe state. Biosecurity level in rural poultry production and live bird market systems is minimal or in some cases non-existing, this may lead to spread of multiple infections (Permin A, 1997).

Despite serological evidence of AI and ND viruses in domestic birds in some parts of Nigeria (Owoade A.A et al., 2002; Saidu L. et al., 2004; Ocholi R.A et al., 2006; Owoade A.A. et al., 2006; Abdu P.A. 2007; Abubakar M.B et al., 2008) there is dearth of information on these respiratory tract viral infectious agents in local poultry in north-eastern Nigeria. There is no documented evidence describing the presence of IB virus in both domestic and wild birds in the area under study.

This study investigated the presence of AI, ND and IB viruses in domestic and wild birds using nested polymerase chain reaction (nPCR).

Materials and Methods

Study area

Yobe State is located in north-eastern Nigeria. It is located between latitudes 11°56' and 13°20'E and longitudes 9°38' and 12°25’N of the Northern hemisphere. It shares international boundary with Niger Republic in the extreme north and interstate boundaries with Borno State in the east, Bauchi and Jigawa States in the west and Gombe State in the south.

Hadejia-Nguru and Dogona Wetlands is located in Yobe state, it lies between Sudanian Savanna to the south and the drier Sahel to the north. It is part of the Chad Basin National Park which covers 938 km²; some of the land is permanently flooded, while other parts are flooded only in the wet season.
(August and September). The annual rainfall ranges between 200–600 mm, during the period May–September. The wetlands are protected by five Forest Reserves, and a Wildlife Sanctuary (Ramsar, 2008). The wetland supports over 25 bird species, a wide range of fish species and is an important source of drinking water for local cattle (Ramsar, 2008).

**Sampling**

Gashua, Nangere, Nguru and Potiskum Local Government Areas (LGAs) out of the seventeen LGAs of the State were selected at random by balloting, four out of six Live bird markets and five out of ten slaughter slabs in the selected LGAs were identified and selected by balloting (Snoeck C.J et al., 2009).

Thirty five captive wild birds were sampled from Nguru LGA by convenient sampling technique (Singh K et al., 2005). Cloacal and tracheal swabs were collected aseptically using sterile cotton swabs, with double ends (one for each bird). Each of the cotton ends were cut and deposited into Eppendorf tubes containing 1.5 ml of Hanks viral transport medium.

Samples were placed in a sterile transparent plastic bag and stored in a customized cool box containing ice packs. Samples were preserved at −20°C for less than 48 h before transportation to Biotechnology Research Laboratory, Faculty of Veterinary Medicine, and University of Ibadan, Nigeria for analysis as recommended (Thrusfield M, 1997).

**Sample processing**

The samples were prepared for viral genome extraction. Using QIAGEN kits manual (Hilden, Germany), 140 µl of each field sample was pulse vortexed, centrifuged and dispensed into a set of new sterile 1.5 ml Eppendorf.

Total RNA was extracted directly using QIAamp viral RNA mini spin protocol (Hilden, Germany). The extracted RNA was subjected to reverse transcription polymerase chain reaction using superscript III reverse transcriptase (Life Technologies, California, US).

PCR amplification was carried out for each of the viruses as described by (WHO, 2002; OIE, 2008). AIV oligonucleotide primers: ChenF(M52C) and ChenR(M253R) were used as forward and reverse primers respectively for AIV matrix assay (Fouchier and Wallensten, A, 2005). NDV forward primer F0P1 and F0P2 reverse primer first round while forward primer F1P1 and reverse primer F1P2 were used for nested NDV PCR assay (Kho C.L et al., 2000). Infectious bronchitis virus (IBV) was assayed using forward primer N784 and reverse primer N1145 for first round PCR while forward primer N791 and reverse primer N1129 were used for nested PCR (Akin A et al., 2001). A 1kb plus DNA marker was used to determine the DNA segments size of each virus.

**Results and Discussion**

All the birds examined were about 22 weeks of age and apparently healthy at ante mortem, there was no record of vaccination and general health management. The live bird markets in the study areas served as feeder for the attached slaughter slabs. The overall sanitary conditions in the live bird markets and the slaughter slabs were observed to be associated with inadequate water supply, lack of proper drainage system and minimal control of movement of people on and around the slaughter slabs.

A total of 159 domestic birds were sampled from four LGAs, 87.4% were local chickens, and 10.1% were turkeys and 2.5% Muscovy ducks. The Average number of birds slaughtered per day in the study area was 401, average number of birds slaughtered in the various LGAs are, Potiskum 199 (49.6%), Nguru 95 (23.7%), Gashua 59(14.7%) and Nangere 48(12%) (See table 1).

The prevalence of NDV in domestic birds slaughtered in the study area was 30.8%, a local chicken was 33.1% and in turkeys was 18.8%. NDV was not detected in ducks (N=4) slaughtered in the study area.

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The prevalence of IBV in local chickens slaughtered in the study area was 2.9%. IBV was not detected in Turkeys and ducks slaughtered in the study area. AIV was not detected in domestic birds slaughtered in the study area (See table 2).

A total of 34 wild birds, consisting of seven species were captured. The highest was Goose.
DETECTION OF AVIAN INFLUENZA, NEWCASTLE DISEASE AND …

(Anser anser) 32.4% and the least was peacock (Afropavo congensis) 2.9%. NDV, AIV and IBV were not detected in wild birds (See table 3).

Table 1: Distribution Of Domestic Birds Sampled From Slaughter Slabs By Local Government Areas

<table>
<thead>
<tr>
<th>Location</th>
<th>Average slaughter/day</th>
<th>Number of birds sampled No (%)</th>
<th>Chicken</th>
<th>Duck</th>
<th>Turkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gashua</td>
<td>59</td>
<td>26 (44.1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nangere</td>
<td>48</td>
<td>20 (41.7)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nguru</td>
<td>95</td>
<td>40 (37.9)</td>
<td>0</td>
<td>4 (4.2)</td>
<td>0</td>
</tr>
<tr>
<td>Potiskum</td>
<td>199</td>
<td>73 (28.6)</td>
<td>4 (2.0)</td>
<td>12 (6.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>401</td>
<td>(34.7)</td>
<td>4 (0.9)</td>
<td>16 (3.9)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Prevalence Of NDV, AIV And IBV In Domestic Chickens Slaughtered In The Study Area

<table>
<thead>
<tr>
<th>Domestic Bird</th>
<th>No. Sampled</th>
<th>AIV</th>
<th>NDV</th>
<th>IBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local chickens</td>
<td>139</td>
<td>0 (0.0)</td>
<td>46 (33.1)</td>
<td>4 (2.9)</td>
</tr>
<tr>
<td>Turkey</td>
<td>16</td>
<td>0 (0.0)</td>
<td>3 (18.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Duck (Muscovy)</td>
<td>4</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>159</td>
<td>0</td>
<td>49 (30.8)</td>
<td>4 (2.5)</td>
</tr>
</tbody>
</table>

AIV= Avian Influenza Virus, NDV =Newcastle Disease Virus, IBV =Infectious Bronchitis Virus

Table 3: Distribution of captured wild birds in Yobe State Nigeria and the prevalence of NDV, AIV and IBV among the various species

<table>
<thead>
<tr>
<th>Wild bird</th>
<th>Number sampled</th>
<th>AIV (%)</th>
<th>NDV (%)</th>
<th>IBV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goose (Anser anser)</td>
<td>11</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Common scoter (Melanitta nigra)</td>
<td>4</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Little bittern (Ixobrychus minutus)</td>
<td>1</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Peacock (Afropavo congensis)</td>
<td>7</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Purple gallinule (Porphyrio porphyrio)</td>
<td>3</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Speckled pigeon(Columba guinea)</td>
<td>6</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>White stork(Ciconia ciconia)</td>
<td></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Domestic birds in Yobe state are commonly seen to be raised in semi-intensive settings mainly in the rural communities where there was little or no veterinary cares and birds scavenge for feed most part of the day. This type of management practice is common in most African and Asian countries. This practice is known to encourage easy acquisition and spread of infectious agents (Permin A, 1997; Adene D.F, 2007; Gueye E.F. 2007).

The birds presented for sale at the poultry markets and the slaughter slabs for slaughter were sourced from different locations both within and outside the state; occasionally the birds are sourced across the international borders of Chad and Niger Republics. It is common to see birds in the study area hanged on handles of bicycles and motorcycles as they are transported to live bird markets. These practices not only put stress on the birds but may encourage the spread of infectious agents through aerosol or contact from infected to susceptible birds on transit. Similarly, zoonotic infections can be transmitted from infected individual(s) to susceptible humans or birds (CIDRAP, 2008). Furthermore, infected birds from one locality can serve as source of infection to susceptible bird’s population in another locality (CIDRAP, 2008; J. Vet. Adv., 2012, 2(10):481-487
Saidu L. et al., 2004). Research have shown that most birds found in live bird markets in Nigeria harbours one or another disease (Saidu L. et al., 2004).

Newcastle disease virus had the highest prevalence in local chickens and turkeys (Table 2). This may be supported by the findings of Saidu et al., (2004) that local chickens are more susceptible to NDV infection than other domesticated birds. NDV has been described as the most important virus in poultry causing huge economic losses in poultry farms in developed and developing countries (Snoeck et al., 2009). The prevalence of NDV in this study was low when compared to 92% reported by Saidu et al., (2004). Although serology is less sensitive when compared with PCR techniques (Singh et al., 2005), the wide difference in prevalence may be as a result of the variations in geographical locations, usage of varied ND vaccines and the study design. Saidu et al., (2004) worked in north central area of Nigeria which is popular for intensive commercial poultry rearing. Indiscriminate usage and mishandling of vaccines on commercial farms may lead to contamination of equipment and the immediate environment, this increase possibility of transmission to neighbouring farms

IBV was detected in local chicken only. The prevalence of 2.9% found in this study is low when compared to 26% reported in commercial poultry in south western Nigeria (Owoade A.A et al., 2006). However this study confirms Ducatez et al (2009) findings that IBV infections seemed to be less common in live bird markets in northern Nigerian and in backyard poultry in Niger Republic,. Seroprevalence evidence in Nigeria suggests increase in new variant serotypes of IBV currently circulating in Nigeria and Niger Republic (Ducatez M.F et al., 2009). This may portend danger as IBV is known to cause significant economic losses because of reduced productivity other than bird mortality (Cavanagh D. and Naqi S.A, 2003).

Although Ocholi et al., (2006) listed the study area among the sites in Nigeria that had AI (H5N1) outbreak in 2006, and Gaidet et al., (2007) reported the presence of LPAI in wild migratory birds sampled from wetlands areas of Nigeria, which includes Yobe State, these reports are contrary to the findings of this study. This may be related to the less number of sample investigated when to the previous studies. This may also mean that AI response and control measures put in place by Nigerian Government during the outbreak of AI in year 2006 (Ocholi R.A et al., 2006) might have aided the containment of the disease in the area.

None of the three viruses was detected in Muscovy ducks and wild birds. This is contrary to the report by Mai H.M et al., (2004) who obtained 6.7% infection rate of Newcastle disease virus among local ducks in a North-central Nigeria. The absence may be attributed to the fewer number of ducks examined in this study. HPAI (H5N1) virus is known to circulate among ducks without serious effect but the ducks may serve as source of infection for other birds species and human (Henning et al., 2010).

Generally, the viral agents detected in this study are known to infect birds and cause respiratory diseases or diseases associated with respiratory system in infected flock(s) (Owoade A.A et al., 2006). Infections with either of these viruses have been reported to lead to reduced feed intake and utilization and diverse pathological conditions resulting ultimately in reduction of production functions and death of the infected birds; consequent to these are economic loses to the farmer in particular and to the poultry industry at large (Alexander D.J. 2003; Van Gils J.A et al., 2007).

Great losses have been experienced from avian epizootics such as AI outbreaks in different parts of the world in recent times (CIDRAP, 2008). The cost of control measures including vaccination represents a continuing loss to the industry and increase cost to the consumer. In many developing countries NDV is endemic and pose an important limiting factor in the development of commercial poultry production and the establishment of trade links (Sonaiya E.B. and Swan 2008; Iroegbu C.U and Nchinda, 1999.). Infection with IBV in small and medium scale poultry farms has led to severe declines in quality and quantity of egg thereby, leading to losses from production inefficiencies (Cavanagh D. and Naqi S.A. 2003).

There is a growing prospect for large scale poultry farming in Yobe State to complement the
existing wide spread backyard production of local chickens. Thus, measures have to be taken to ensure economically successful poultry production in the area. Some of these measures include strict enforcement of biosecurity in all poultry farms and live birds markets, depopulation of all flocks infected with AIV and vaccination of all poultry flocks against common viral agents in the area (Alexander D.J. 2003; Cavanagh D. and Naqi S.A. 2003; Ahmed Z; Naeem K and Hameed A. 2007). Further studies should be under taken to identify the various strains and serotypes circulating in the area.

Acknowledgement

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