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Research Article

Molecular Detection and Viral Isolation of Newcastle Disease Viruses from Chickens in Benue State, Nigeria

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Abstract: The present research work was carried out to detect and isolate Newcastle Disease Virus (NDV) from apparently healthy chickens and evaluate their role in Newcastle disease outbreak.A survey on the prevalence of Newcastle Disease Virus (NDV) was conducted in six Local Government Areas (LGAs) of Benue State, Nigeria. A total of 250 cloacal and tracheal swabs were collected from apparently healthy chickens from commercial farms, Live Bird Markets (LBMs) and village households. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was used to detect Newcastle Disease Viruses (NDV) while viral isolation was performed using embryonated chicken eggs. The overall detection rate for ND Viruses (NDVs) was 12% (30/250). Newcastle disease viruses were detected in all sampling units with the highest viral detection of 16.1% (9/56) from chickens in LBMs. The overall NDV isolation rate was 20% (6/30), isolates were from Kwande and Makurdi LGAs with village chickens from LBMs (55.6%) recording the highest isolation rate. Chickens from LBMs, Kwande and Makurdi LGAs may serve as sources of NDVs to those in commercial farms and village households. The vaccination of village chickens should be considered as important in the control of ND in Benue State, Nigeria.

Keywords: Chicken, live bird market, Newcastle disease virus, Nigeria, RT-PCR, village households

INTRODUCTION

Newcastle Disease (ND) is a highly contagious viral disease affecting domestic and wild birds (Seal *et al*., 2000; Alexander, 2003). However, chickens appear to be the most susceptible to ND whereas aquatic birds, geese and ducks, are relatively resistant (Alexander, 2003). Newcastle disease is economically significant as it causes high morbidity and mortality, reduces egg production, deteriorates egg quality and impairs live performance (Orsi *et al*., 2009). Due to the severe nature of ND and its related consequences, NDV is included in “LISTED” agents (reportable disease) by the Office International des Epizootics (OIE), (2008, 2012) (Aldous and Alexander, 2001; Boynukara *et al*., 2013). Newcastle Disease Virus (NDV) is a linear, non-segmented single stranded, enveloped, negative sense RNA virus which belongs to the genus Rubula virus and family *Paramyxoviridae* (Barbezange and Jestin, 2005). The whole genome is 15 kb and encodes six major proteins, namely: RNA polymerase (L), haemagglutinin-neuraminidase (H/N), fusion protein (F), matrix protein (M), Phosphoprotein (P) and Nucleoprotein (NP) (de Leeuw and Peeters, 1999; Aldous and Alexander, 2001). Virulence of NDV is mainly determined by the amino acid sequence surrounding the Fusion (F) protein cleavage site (de Leeuw *et al*., 2005).

Nigeria poultry population is estimated to be 137.6 million, with backyard and village poultry population constituting 84% (115.8 million) and 16% (21.7 million) of exotic poultry, with a higher percentage of this poultry raised for subsistence production (FDLPC (Federal Department of Livestock and Pest Control Services), 2006). Village poultry keeping is regarded as an important livelihood opportunity for poor households in Nigeria (Adene, 2004). This sector contributes to the improvement of the micro-economy of households, as the income from the sales of birds is used for food, children’s school fees and unexpected expenses such as medicines (Sonaiya, 1990; Moreki *et al*., 2001).

Previous studies have revealed that ND is endemic in both village and commercial poultry in Nigeria, with

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frequent epidemics recorded in highly susceptible flocks (Orajaka *et al*., 1999). Outbreaks of ND have been reported to occur more in farms that kept exotic birds together with village chickens and other poultry species like ducks and turkeys (Abdu *et al*., 2005). Live bird markets are essential for marketing poultry in many developing countries and they are a preferred place for people to purchase poultry for consumption throughout the world (Cardona and Kuney, 2002). In Nigeria, about 90% of poultry marketing is by sales of live birds with less than 2% comprising processed or frozen chicken (Muhammed, 2008). Live bird markets can be high-risk areas for disease transmission due to high concentration and interaction of a wide variety of birds coming from different sources.

Laboratory diagnosis of ND depends on the detection of the infectious agent by virus isolation in embryonated eggs or cell cultures because the wide use of vaccines hampers the interpretation of serological results (Aldous and Alexander, 2001; OIE, 2008). At present, virus isolation is the prescribed test for international trade and remains the method of choice for confirmatory diagnosis or as the “gold standard” method for the validation of other techniques (Alexander and Senne, 2008; Terregino and Capua, 2009). Recently, molecular methods such as Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and amino acid sequencing are used for diagnosis of NDV and the amino acid sequence of the cleavage site of F protein of NDV strains considered a virulence criterion (Panda *et al*., 2004; Alexander and Senne, 2008). Reverse transcription-polymerase chain reaction has already been established to identify NDV in allantoic fluid and in poultry vaccines (Jestin and Jestin, 1991; Stauber *et al*., 1995; Oberdorfer and Werner, 1998). Application of one-step RT-PCR to various NDV samples, including wild-type virulent isolates and avirulent vaccine strains, demonstrated the potential of the technique for rapid identification of NDV isolates (Wang *et al*., 2001; Creelan *et al*., 2002). In this study RT-PCR was used to enable the detection of NDVs from field samples and viral isolation was conducted.

MATERIALS AND METHODS

Sampling method: Three sampling units made up of chickens from commercial poultry farms, LBMs and village households were used for the study. Purposive sampling was used to select six LGAs for the study with two LGAs from each of the three geopolitical zones of Benue State, Nigeria. Twelve commercial farms, 24 villages and nine LBMs were selected from the LGAs based on advice from Avian Influenza Control Project (AICP) Desk Officer of each LGA and the consent and readiness of poultry farmers to participate in the study.

Collection of samples: A total of 250 cloacal and tracheal swabs were collected from apparently healthy chickens from commercial farms, LBMs and village households. Swabs were collected from chickens in Gboko, Katsina-Ala, Kwande, Makurdi, Oju and Otukpo LGAs of Benue State, Nigeria from May to July 2013. Similar swabs were pooled (five swabs per tube) and placed in viral transport medium tubes containing 1 mL PBS solution and antibiotics (10,000 IU/mL penicillin, 1 mg/mL streptomycin sulphate, 1 mg/mL gentamicin sulphate). Samples were immediately placed in a flask with ice packs and transported to the laboratory and stored at -70°C until required for RNA extraction.

Extraction of viral RNA: The genomic viral RNA was extracted from the cloacal and tracheal swabs using the QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany) according to the manufacture’s protocol.

Reverse transcription-polymerase chain reaction: The RT-PCR of the extracts was performed using a Qiagen One-Step RT-PCR kit according to the manufacturer’s instructions. The Forward and Reverse primers sequence for the NDV matrix (M) gene detection APMV-1F5’AGTGATGTGCTCGGACCT TC-3’ and APMV-1R5’CCTGAGGAGAGGCATTT GCTA-3’ respectively was used. The matrix gene segment was reverse transcribed and amplified using Applied Biosystem (AB) the rmocycler PCR system (Model, 9902) which amplified a 121 bp fragment of the matrix gene. Reverse transcription was achieved by incubation with reverse transcriptase at 50°C for 20 min which was stopped upon incubation at 95°C for 15 min. RT-PCR was performed by denaturing cDNA at 94°C for 45 sec, annealing at 60°C for 45 sec and extending the fragment at 72°C for 45 sec. The above program was repeated for 40 cycles with a final extension at 72°C for 5 min and PCR products maintained at 4°C.

Agarose gel electrophoresis: The PCR products were separated in 1.5% agarose gel in TAE buffer stained with ethidium bromide and compared with molecular DNA marker (100 bp) and visualized by Ultraviolet (UV) transillumination.

Newcastle disease viral isolation: A 140 μL of supernatant from swabs positive by RT-PCR were inoculated into three 9-11 day old Specific Pathogen Free (SPF) embryonated chicken eggs through the allantoic cavity. Egg inoculation, incubation, candling and virus harvesting were conducted in accordance with the OIE Manual of Standards for Diagnostic Tests and Vaccines (OIE, 2012). Allantoic Fluid (AF) was

Table 1: Distribution of Newcastle disease virus detection by RT-PCR in chickens in six local government areas of Benue State, Nigeria

Local government area	Number of swabs tested	Number of swabs that tested positive by RT-PCR	Virus detection rate % (no. positive/no. tested)
Gboko	29	7	24.1 (7/29)
Katsina-Ala	30	4	13.3 (4/30)
Kwande	37	12	32.4 (12/37)
Makurdi	76	3	3.9 (3/76)
Oju	24	1	4.2 (1/24)
Otukpo	54	3	5.6 (3/54)
Total	250	30	12 (30/250)

Table 2: Distribution of Newcastle disease viruses isolated from chickens in six local government areas of Benue State, Nigeria

Local government area	Number of swabs tested	% of swabs positive for Newcastle disease viruses
Gboko	7	0 (0/7)
Katsina-Ala	4	0 (0/4)
Kwande	12	25 (3/12)
Oju	1	0 (0/1)
Otukpo	3	0 (0/3)
Makurdi	3	100 (3/3)
Total	30	20.0 (6/30)

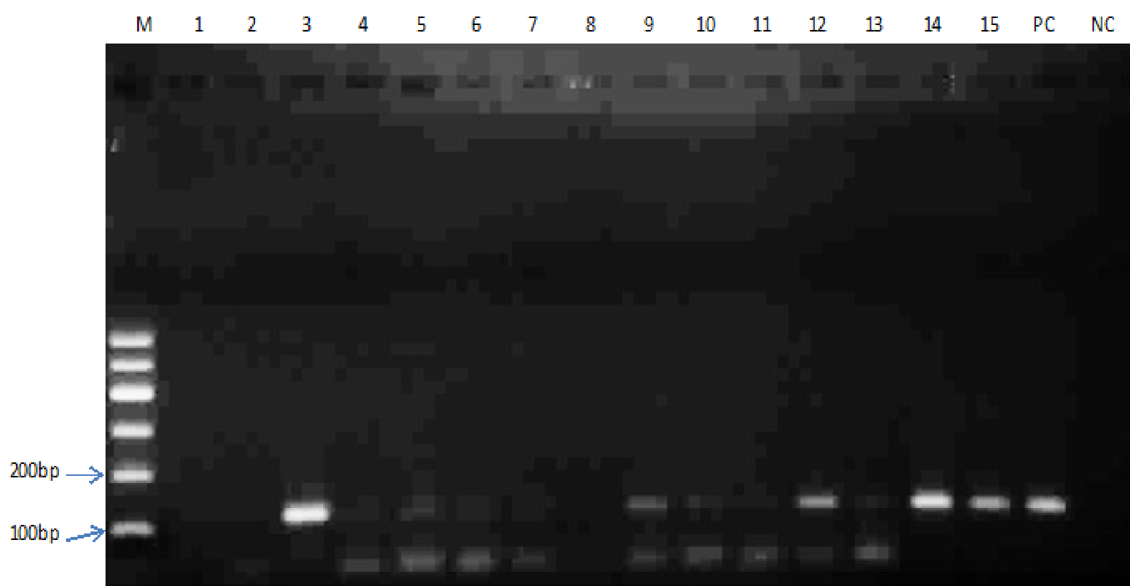

Fig. 1: Agarose gel electrophoresis of RT-PCR products for M gene detection of ND virus, Expected amplicon size was 121 bp. M = 100 bp molecular DNA marker, lane 3 = Village chicken from Kwande, lane 9 = Village chicken from Ikobi village Otukpo, lane 12 = Broiler from commercial farm Otukpo, lane 14 = Village chicken from AdakaMakurdi, lane 15 = Village chicken from Wurukum LBM Makurdi, PC= Positive control NC = Negative control

harvested from the eggs and NDV screened by Haemagglutination (HA) test. The HA positive AF were confirmed through Haemagglutination Inhibition (HI) test with known NDV antiserum. Virus stocks were stored at -70°C until further used.

RESULTS

The overall NDV detection rate by RT-PCR was 12% (30/250). By LGAs NDV detection rate was 24.1% (7/29) for Gboko, 32.4% (12/37) for Kwande and 3.9% (3/76) for Makurdi (Table 1, Fig. 1). The overall NDV isolation rate was 20% (6/30). Swabs from chickens in Oju, Otukpo, Gboko and Katsina-Ala LGAs were negative for NDVs while those from Kwande 25% (3/12) and Makurdi 100% (3/3) were positive for NDVs (Table 2). From the three sampling units, chickens from LBMs recorded the highest 16.1% (9/56) viral detection rate, while those from commercial poultry farms 13.6% (6/44) and village households had 10% (15/150). Also, chickens from commercial farms (0/6) yielded no NDV isolates, while those from village household had 6.7 % (1/15) and LBMs had 55.6 % (5/9) NDV isolation rate (Table 3).

DISCUSSION

The study revealed that chickens from all the sampled LGAs in Benue State were infected with NDV. This is an indication of an active on going NDV

Table3: Distribution of Newcastle disease viruses isolated from chickens in village households and live bird markets in Makurdi and Kwande local government areas of Benue State, Nigeria

Sampling units	% of Newcastle disease viruses detected by RT-PCR	% of Newcastle disease Viruses isolated	Origin of Newcastle disease Viruses isolated (no.)
Commercial chickens	13.6 (6/44)	0 (0/6)	None was isolated
Village household chickens	10.0 (15/150)	6.7 (1/15)	Makurdi (1)
Chickens in live bird market	16.1 (9/56)	55.6 (5/9)	Makurdi (2) Kwande (3)
Total	12.0 (30/250)	20.0 (6/30)	

infection of chickens in the study areas with differences in the activity of the virus. Kwande and Gboko LGAs had the highest virus detection rates of 32.4 and 24.1% respectively likely due to the large number of chickens sampled being village chicken raised extensively with poor biosecurity. Similar work by Semaka *et al*. (2013) in Benue State gave a higher NDV detection rate of 48.5% (94/194). This higher rate could be due to the fact that they sampled both healthy and sick birds. Previous study by Ibu *et al*. (2009) in wild and captive birds in Central Nigeria gave a virus detection rate of 7.9% which was lower than the rate in this study. This difference in detection rate could be attributed to differences in the species, susceptibility and management system of the birds sampled. The NDV detection rate from LBMs in this study was 16.1%. Another study in Yobe State, Nigeria by Garba *et al.* (2012) recorded a much higher virus detection rate of 30.8% from local poultry slaughtered in LBMs. Newcastle disease viruses were isolated from two LGAs, Kwande and Makurdi, and all the isolates were from village chickens. This could be due to the fact that village poultry is a mixture of birds of different ages, sex, and species from different households. These birds are raised under free range management system where they scavenge and interact with each other and this provides a favourable condition for NDVs to circulate and cause infection. The result of the ND viral isolation showed that chickens in LBMs had the highest percentage of birds infected with NDVs. This could be attributed to mixing of birds from different sources, different species and health status in the LBMs with possibility that some birds incubating or shedding NDV could transmit the virus to susceptible birds. It was observed from this study that most of the LBMs sellers buy poultry from village households thus the birds were from a similar pool (source). Hence, poultry from these LBMs are likely to spread the NDV to poultry in village households when new additions are sourced from the LBM and vice versa. Live bird markets are common sources of NDVs sometimes through the random sale of infected birds, but more often through salvage sales (Huchzermeyer, 1993; Sa'idu *et al*., 1994; Abdu *et al*., 2002).

The result of the study showed that only six NDVs were isolated from the 30 samples positive by RT-PCR. The inability to grow and isolate the other samples might be attributed to the non-viability of the NDV in the samples coupled with the capability of the PCR-based tests to detect the presence of a pathogen regardless of its viability (Giovanni *et al*., 2011). It can be concluded from this study that live infected apparently healthy chickens are the most likely means of introduction of NDVs into poultry populations. Village chickens and LBMs are major sources of NDVs with high risk of re-introduction of NDV to village household flock when sourcing breeder stock from the LBM. The fact that it was possible to isolate NDVs from apparently healthy chickens indicates that there was virus replication and shedding that could result in virus transmission and ND outbreaks in poultry in the study area. Vaccination of village poultry should be considered as an important tool in the control of ND. Further research should be carried out to characterize the NDVs circulating in Benue State and study the risk factors responsible for the high NDVs detection rates in Kwande and Gboko LGAs of Benue State Nigeria.

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