SHORT COMMUNICATION

Pathology and Molecular Diagnosis of Velogenic Viscerotropic Newcastle Disease Virus in Desi Chicken of Chhattisgarh

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Abstract

Newcastle disease (ND) is an economically important viral disease of birds caused by *Avian Paramyxovirus* type 1. In the present study, mortality of 56.50 to 74.50% due to ND in four desi chickens farms located in Durg district of Chhattisgarh was noticed which were not vaccinated against NDV, The gross and histopathological lesions were suggestive of viscerotropic velogenic form of ND. Tissue samples of trachea and lung collected from 18 desi birds belonging to four desi flocks were confirmed as ND by RT-PCR amplification of 535 bp of fusion protein gene of NDV. In the present study, velogenic viscerotropic NDV was confirmed in all four desi flocks by gross and microscopic examination and RT-PCR assay.

Key words: Newcastle, Velogenic Viscerotropic, Chicken

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Introduction

Newcastle disease (ND) is an economically important and highly infectious viral disease of domestic poultry and other species of birds which is caused by *Avian paramyxovirus* Type 1 of genus *Avulavirus* belonging to the family *Paramyxoviridae* (Cattoli *et al.,* 2011). Viral genome consists of six open reading frames (ORFs) which encode for six major structural proteins namely, nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and RNA-dependent RNA polymerase (L) (Ganar *et al*., 2014). Amino acid composition of F protein cleavage site is the main determinant of NDV virulence and tissue tropism (Hamid *et al.,* 1991).

Severity of disease produced varies with virulence of virus. On the basis of virulence and clinical signs, ND virus is characterized into different pathotypes, *viz*. velogenic viscerotropic (Doyle's form), neurotropic velogenic (Beach's form), mesogenic (Beudette's form), lentogenic (Hitchner's form) and asymptomatic enteric form (Beard and Hanson, 1984). Morbidity and mortality in a flock varies according to the strain involved, spanning from peracute disease with almost 100% morbidity to subclinical disease with no lesions and mortality (Aldous *et al.,* 2003).

Molecular technique like reverse transcription - polymerase chain reaction (RT-PCR) have been frequently used worldwide to detect NDV in the field samples (Aris *et al.,* 2016; Asma *et al.,* 2016).

In backyard poultry, the outbreak of ND occurs with high mortality due to negligence of vaccination and improper biosecurity measures which may affects the livelihoods of poor rural households and also pose a potential threat to commercial birds. Outbreak of ND in four desi chicken farms was observed in Durg district of Chhattisgarh. Hence, it was felt necessary to study the disease with respect to its pathology and molecular diagnosis.

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Materials and Methods

Outbreak of ND in four desi chicken flock was observed in Durg district of Chhattisgarh which was not vaccinated against NDV. These desi flocks were having average 2125 birds, between 5 to 10 wks of age with 66.00% mortality. The ailing birds were examined for clinical signs, if any. Dead birds were subjected to detailed post mortem examination and gross pathological lesions were recorded.

Histopathology: Tissue samples of trachea, lung, proventriculus, intestine and spleen was collected in 10%

buffered formalin and processed for histopathological study by paraffin embedding technique. Sections of 5-6 µ thickness were cut and stained with routine haematoxylin and eosin (H and E) staining (Luna, 1968).

Detection of NDV by RT-PCR: Tissue samples of trachea and lung were collected from five birds belonging to each three desi flocks and three birds from one flock which showed gross lesions suspected of ND and preserved at - 20°C for detection of viral RNA by RT-PCR. Total RNA was extracted from trachea and lung tissues using trizol reagent as per standard procedure (Ogali *et al.,* 2018). The cDNA synthesis was carried out by using Hi- cDNA synthesis kit. RT-PCR was carried out to amplify the 535 bp sequence of fusion protein gene by using forward (5′-ATG GGC (C/T) CC AGA C (C/T)CT TCT AC-3′) and reverse primer (5′-CTG CCA CTG CTA GTT GTG ATA ATC C-3′) (Liu *et al.,* 2007). For amplification, 3µL of cDNA was incubated in total volume of 20 µL reaction mixture containing 10 µL PCR master mix, 1 µL of each forward and reverse primer and 5 µL of nuclease free water. PCR was carried out following initial denaturation at 95°C for 2 min and then 30 cycles at 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min and a further extension at 72°C for 10 min. The PCR products were separated on 1.5% agarose-gel and visualized in Geldoc (Biorad).

Results and Discussion

Mortality in all four flocks ranged between 56.50 to 74.50%. Clinical signs recorded in birds include reduced feed intake, depression, loss of body weight and respiratory distress characterized by labored breathing, coughing, sneezing and nasal discharge. Greenish watery diarrhea soiling vent feathers and dehydration were consistent findings. In most of the birds, eyelids were congested leading to conjunctivitis. Birds were also found dead suddenly without showing any symptoms. These findings of clinical signs during field outbreaks of ND are almost in agreement with Momin and Singh (2018).

Gross pathology

Gross pathological lesions observed in all the desi flocks were typically of velogenic viscerotropic ND. The carcass was dehydrated and emaciated with deep congestion of breast musculature. The characteristic lesions of pin-point haemorrhages at tips of proventricular glands (Fig.1), necrotic haemorrhagic elongated button ulcers throughout the intestine (Fig.2) and haemorrhagic necrotic lesions in caecal tonsils (Fig.3) were observed. Spleen parenchyma showed multiple foci of necrosis and pin-point haemorrhages. The lungs were mostly congested, edematous and hemorrhagic which might be due to the degenerative and necrotic changes of the vascular endothelium caused by NDV. Haemorrhagic tracheitis with catarrhal exudates was noticed in many chickens (Fig.4). Similar lesions were reported by Pazhanivel *et al.* (2013) and Khorajiya *et al.* (2015). Lesions of disseminated multiple foci of necrosis and pin-point haemorrhages observed in the spleen parenchyma are in agreement with Gowthman (2013). All above lesions were noticed in most of the necropsied birds with little variation in the frequency of occurrence of lesions.

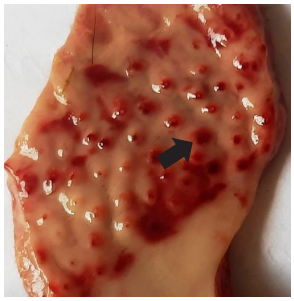

Fig. 1. Pin-point haemorrhages at tips of proventricular glands

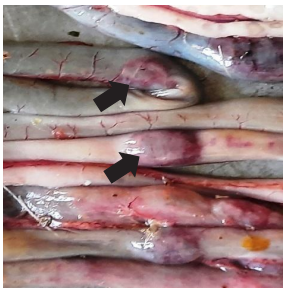

Fig. 2. Elongated haemorrhagic button ulcers throughout intestine

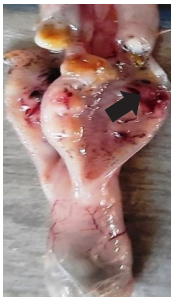

Fig. 3. Haemorrhagic and necrotic caecal tonsils

Fig. 4. Haemorrhagic tracheitis

Histopathology

Histopathological lesions observed in different visceral and lymphoid organs during the present study are in accordance with Khorrajiya *et al.* (2015) and indicated that NDV cause significant pathological lesions in proventriculus, intestine, trachea, lungs (Fig. 5) as well as lymphoid organs like caecal tonsils and spleen. Overall nature of histopathological lesions also suggested that present outbreaks caused by NDV were similar to that of classical velogenic viscerotropic ND.

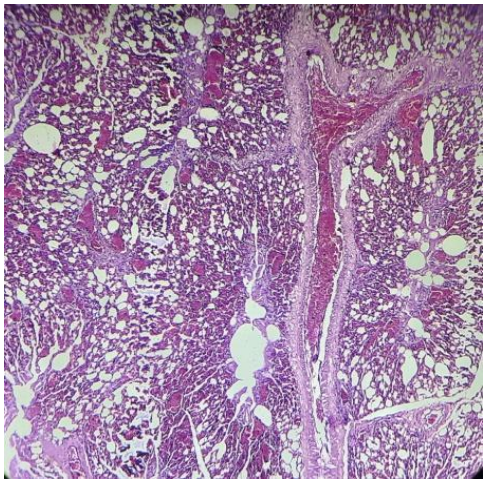

Fig. 5. Bronchoneumonia (H&E, 10x)

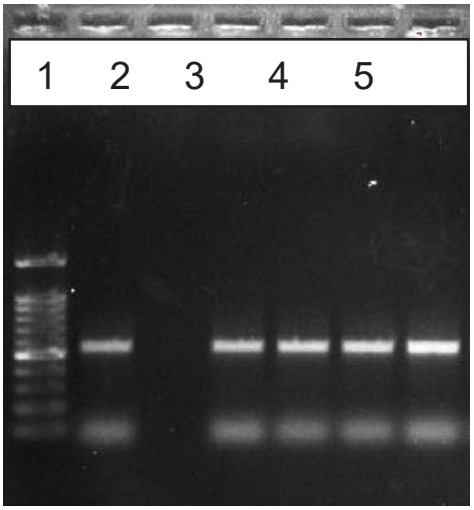

Fig. 6. Agarose gel photograph showing RT-PCR products of amplified NDV. Lane 1: 100bp DNA ladder, Lane 3: Negative control, Lane 2, 4, 5, 6, 7: positive field sample (535bp),

Detection of NDV by RT-PCR: Tissue samples of trachea and lung collected from five birds belonging to each three desi chicken flocks and three birds from one flock were confirmed as ND by RT-PCR. Amplification of partial fusion protein gene of NDV revealed 535 bp product for all four desi flocks (Fig. 6). These findings are in accordance with Ogali *et al.* (2018) who also detected NDV from tracheal and cloacal swab collected from backyard chickens. RT-PCR technique has been routinely used for diagnosis of NDV and detecting NDV in clinical samples without necessitating virus isolation. Conventional methods routinely used for diagnosis of NDV infection are laborious, time consuming and less sensitive. Hindrance for virus isolation is that, most of very virulent field isolates do not replicate in common tissue culture, whereas for virus neutralization test, the field strains need to be adapted to grow *in vitro* (Singh *et al.*, 2005).

Conclusion

In the present study, velogenic viscerotropic NDV was confirmed in all four desi flocks by gross and microscopic examination and RT-PCR assay. The outbreaks in desi farm occurred due to negligence of vaccination.

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