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Detection and molecular characterization of Newcastle disease virus in broiler chickens from Bangalore, Karnataka

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Abstract

The collected field samples were subjected for molecular detection of Newcastle disease virus. NDV was confirmed through RT-PCR assay targeting F gene and phylogenetic tree was constructed using MEGA-10 software. The current study revealed that samples were showing presence of Newcastle disease virus genome in the pooled tissue samples. Overall, the present study confirmed the NDV of pathotype velogenic, class II, genotype XIII is circulating in the backyard broiler chickens.

Keywords: Newcastle disease virus, RT-PCR, genotype XIII

Introduction

Newcastle disease is an acute contagious disease affecting most of the avian species. It causes heavy morbidity and mortality to the poultry industry. Newcastle disease is the fatal disease in poultry which is notifiable to the World Organization for Animal Health (OIE) (OIE *et al.*, 2018) ^[1]. NDV caused by virulent forms of Avian Paramyxovirus 1 (APMV1). The Newcastle disease virus belongs to genus Avian Aulavirus of the subfamily Avulavirinae, family Paramyxoviridae and order Mononegavirales (Amarasinghe *et al.*, 2019) ^[2]. NDV has a wide host range including wild birds approximately 241 species of 27 orders, out of 27 orders, out of known 50 orders of birds (Madadger *et al.*, 2013) ^[3].

The viral envelope is composed of two glycoproteins the haemagglutinin-neuraminidase (HN) and the fusion (F) proteins. HN protein is responsible for the inducing haemagglutination of red blood cells and binding to the host cell receptors. HN protein activates the Fusion protein which is responsible for the fusion of the viral envelope to the cell membrane (Acheson *et al.*, 2011) ^[4]. High molecular variability and association with virulence, the F gene has been widely used as a target for the genotype classification of NDV (Diel *et al.*, 2012; Dimitrov *et al.*, 2019) ^[5, 6]. Two major groups are classified i.e class I and class II viruses based on the complete sequence of the F gene. Class I belongs to single genotype whereas the more genetically diverse class II viruses are currently divided into 20 (more than) distinct genotypes separated by nucleotide distances above 10% (Dimitrov *et al.*, 2019) ^[6]. NDV outbreaks occurs worldwide and endemic in many countries in Asia and Africa (Suarez *et al.*, 2020; WAHIS 2019) ^[7, 8]. Though endemic are reports available in India but information Karnataka state is still scarce. In this study molecular detection of Newcastle disease outbreaks were studied from unorganized broiler farms of Bengaluru district.

Materials and Methods

Study area and Sample collection

Samples were collected from Bengaluru district of Karnataka state during the period between September to December 2018. Cloacal swabs from live birds and pooled tissue such as intestines, spleen and lungs from dead birds were collected from backyard farms. Swab samples were collected in virus transport medium containing with antibiotics. Samples were placed immediately in ice packs and transported to concerned laboratory and stored at 80 °C until further use.

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RNA isolation and cDNA Conversion

The pooled tissue samples were mixed in PBS (pH 7.2) and triturated thoroughly to make 10% homogenate suspensions. RNA was extracted by using Trizol method (Invitrogen, USA) following the manufacturer's instructions. The purity of RNA was checked by measuring the absorbance at 260 and 280 nm with a Nanodrop UV spectrophotometer (Thermo Scientific, USA). The extracted viral RNA was used to synthesize complementary DNA (cDNA) by employing a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) following the manufacturer's instructions. The cDNA product was then stored at -20°C until further use.

NDV Fusion gene amplification by PCR

The cDNA was further amplified by PCR using NDV F gene specific published primer pairs listed in table 1. The RT-PCR reaction was optimized in a standard 25 μl reaction mixture containing 3.0 μl of cDNA, 12.5 μl of SapphireAmp Fast PCR Master mix 2X (Takara Bio, Japan), 1.0 μl each of Forward and Reverse primer (10 pmol/ μl), and 7.5 μl of nuclease free water. The amplifying conditions of PCR were initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing 52°C for 30 sec and extension 72°C for 45 sec, and final extension at 72°C for 7 min in a Thermal cycler (Verity PCR, Applied Biosystem).

Table 1: NDV Fusion gene amplification by PCR

Primers	Primer sequence	Size	Reference
NDV/Forward	5'-TCAACATATACACCTCATCCCAGACAGG-3'	387 bp	Hasan <i>et al.</i> , 2010
NDV/Reverse	5'-CTGCCACTGCTAGTTGGGATAATCC-3'		

The PCR products were analyzed by electrophoresis in 1.5% agarose gel stained with ethidium bromide (0.5 $\mu\text{g}/\text{ml}$) and observed in Gel Doc System (Bio-Rad, USA).

Sequencing

Thermo scientific GeneJET Gel Extraction kit, was used for purification of the amplified products from the excised gel as per manufacturer's instructions. The purified DNA was sequenced using the sequencing primers (10pm concentration) by M/s Eurofins Genomics India Pvt Ltd., Bangalore. The obtained sequences were aligned and analysed.

Phylogenetic analysis

The current study sequence was compared with some reference NDV strains that represent the NDV different

genotypes and subgenotypes. Multiple alignment and comparison of the study sequences and GenBank references were performed using MUSCLE v3.8.31. Phylogenetic and molecular evolutionary analyses were conducted using MEGA (Molecular Evolutionary Genetics Analysis) version 6.0 (Tamura *et al.*, 2013). Phylogenetic tree was constructed by the neighbor joining method with maximum likelihood substitution model at 1000 bootstraps replicates.

Results

NDV detection in the tissues by RT-PCR

The NDV genome was identified through RT-PCR in the pooled tissue samples of NDV infected broiler chickens. RT-PCR amplification revealed 387 bp amplicons were observed in all the NDV infected birds.

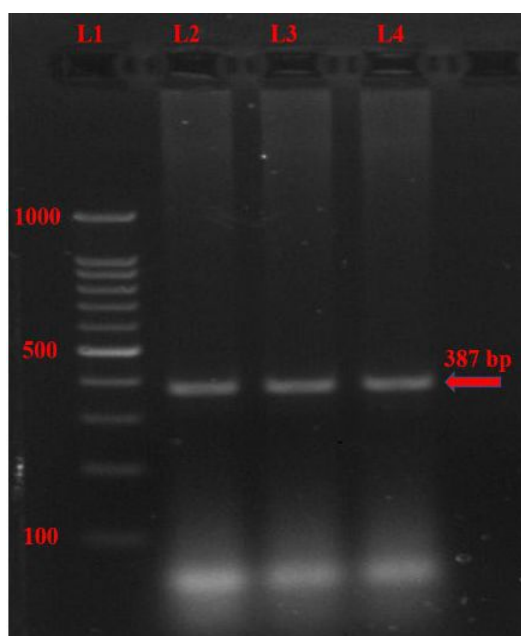


Fig 1: RT-PCR amplification of NDV in the tissue samples. (Lane L1: 100 bp DNA ladder; L2-L4: pooled tissue samples).

Phylogenetic analysis

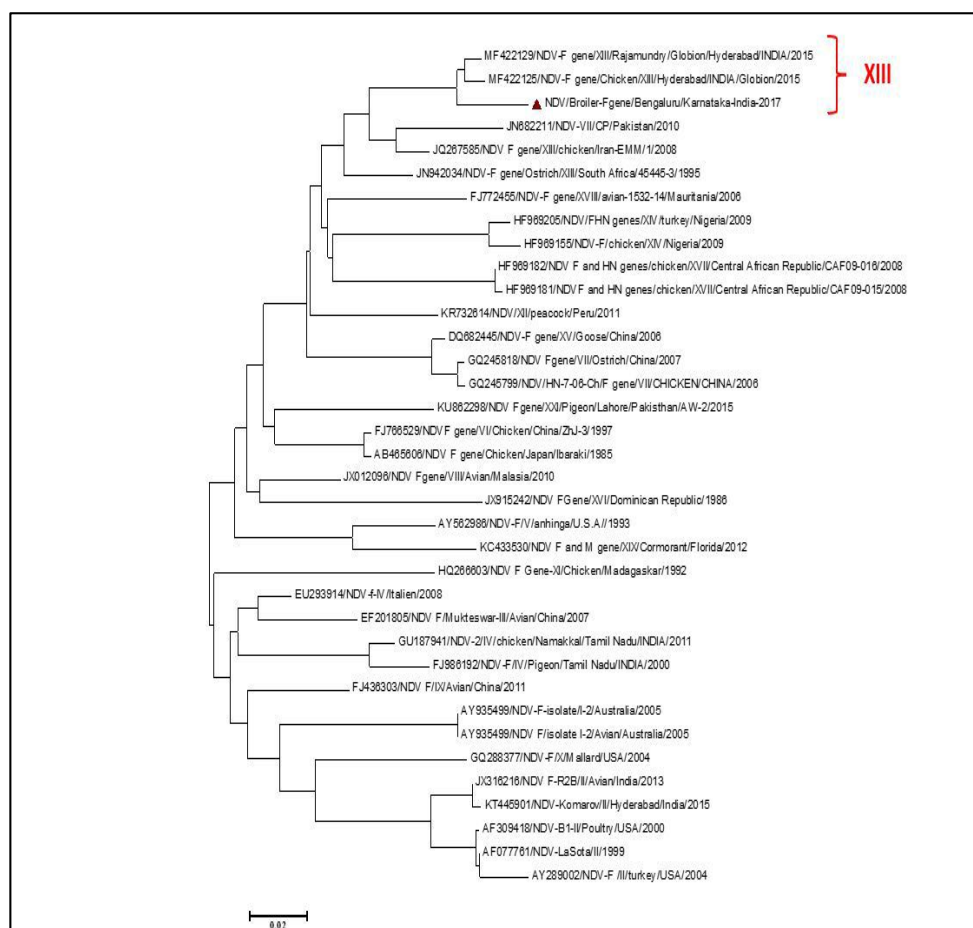


Fig 2: Phylogenetic tree construction of the present isolate using MEGA 10. (Present isolate was indicated with brown arrow head)

Phylogenetic tree construction revealed the present isolate was classified under Class II, Genotype XIII. Within this genotype, it was specifically placed in the sub-genotype XIII.2.2. The present isolate was more closely related with the MF422129 and MF 422125 isolates from the Globion, Hyderabad, India.

Discussion

The gross lesions observed in this study were suggestive of ND such as petechial haemorrhage on the tip of the proventricular papilla and caecal tonsils, hemorrhages and consolidation of lungs and multiple button like ulcers in the intestinal mucosa (Shanmuganathan *et al.*, 2023) [9]. The clinical signs of a highly virulent NDV infection in chickens can be divided into 2 pathotypes: Virulent strains that cause diarrhoea and frequent hemorrhagic intestinal lesions are called viscerotropic velogenic NDVs. On the other hand, strains that cause respiratory and neurotropic signs are called neurotropic velogenic NDVs (Alexander and Allan, 1974; Balachandran *et al.*, 2014) [10, 11]. Based on observation of clinical symptoms in digestive organs, it is known that the ND infection was of velogenic type. The onset of clinical signs in NDV infected chickens could vary depending on the age and genetic endowment of the birds and dose and route of infection, genotype, host adaptability and pathogenicity of the virus (Alexander, 2000) [12]. Strains of NDV are highly variable in their pathogenicity for chickens and amino acid residues at the fusion protein cleavage site (FPCS) is postulated as the primary determinant of NDV virulence (OIE, 2018; Samal *et al.*,

2011) [1, 13]. Virulent strains of NDV are defined by the World Organization for Animal Health (OIE) as viruses that have an intracerebral pathogenicity index (ICPI) of 0.7 or higher (2.0 is maximum) or a fusion protein cleavage site (FPCS) with multiple basic amino acids and phenylalanine at position 117 (OIE, 2018) [1].

Phylogenetic tree construction revealed the present isolate was classified under Class II, Genotype XIII. Within this genotype, it was specifically placed in the sub-genotype XIII.2.2. The present isolate was more closely related with the MF422129 and MF 422125 isolates from the Globion, Hyderabad, India. Similarly, NDV isolates recovered from different outbreaks in chicken flocks in Assam during 2014-15 were genotypically and pathotypically characterized. Nucleotide sequence analysis of fusion (F) and hemagglutinin protein genes showed a close similarity with genotype XIII strains of NDV (Nath *et al.*, 2016) [14]. The significant genetic distance between the Bengaluru 2017 isolate (Genotype XIII) and common vaccine strains (Genotype II) suggests "genotype mismatch." The tree showed that Genotype XIII is the predominant genotype circulating in the Indian subcontinent, indicating a stable local evolution rather than a new introduction from distant continents.

Conclusions

In conclusion, the present outbreak was caused by class II genotype XIII of NDV strain. The emergence of newer strains and failure of the vaccination are the major reasons of its outbreak in developing countries.

References

1. World Organisation for Animal Health (OIE). OIE—listed diseases, infections and infestations in force in 2018. Paris: OIE; 2018. Available from: <http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2018>
2. Amarasinghe GK, Bejerman N, Kim-Blasdell BR. Taxonomy of the order *Mononegavirales*: update 2017. *Arch Virol*. 2017;162(8):2493-2504.
3. Madadgar O, Karimi V, Nazaktabar A, Kazemimanesh M, Ghafari MM, Dezfouli SMA, et al. A study of Newcastle disease virus obtained from exotic caged birds in Tehran between 2009 and 2010. *Avian Pathol*. 2013;42(1):27-31.
4. Acheson NH, Kolakofsky D, Richardson C. Paramyxoviruses and rhabdoviruses. In: Acheson NH, editor. *Fundamentals of Molecular Virology*. Hoboken (NJ): Wiley; 2011. p. 173-187.
5. Diel DG, da Silva LH, Liu H, Wang Z, Miller PJ, Afonso CL. Genetic diversity of avian paramyxovirus type 1: proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. *Infect Genet Evol*. 2012;12:1770-1779.
6. Dimitrov KM, Abolnik C, Afonso CL, Albina E, Bahl J, Berg M, et al. Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infect Genet Evol*. 2019;74:103917.
7. Suarez DL. Newcastle disease, other avian paramyxoviruses, and avian metapneumovirus infections. In: Swayne DE, editor. *Diseases of Poultry*. 14th ed. Hoboken (NJ): Wiley-Blackwell; 2020. p. 111-166.
8. World Animal Health Information System (WAHIS). World Animal Health Information Database. Terrestrial: Newcastle disease, January-June 2019. Paris: OIE; 2019. Available from: https://www.oie.int/wahis_2/public/wahid.php
9. Shanmuganathan S, Praveen V, Manikandan R, Ramasamy B. Gross and histopathological identification of Newcastle disease virus (NDV) in broiler chickens from Bangalore, Karnataka. *Pharma Innovation J*. 2023;12(7S):210-213.
10. Alexander DJ, Allan WH. Newcastle disease virus pathotypes. *Avian Pathol*. 1974;3:269-278.
11. Balachandran P, Srinivasan P, Sivaseelan S, Balasubramaniam GA, Gopala Krishna Murthy TR. Isolation and characterization of Newcastle disease virus from vaccinated commercial layer chicken. *Vet World*. 2014;7(7):457-462.
12. Alexander DJ. Newcastle disease and other avian paramyxoviruses. *Rev Sci Tech*. 2000;19:443-462.
13. Samal S, Kumar S, Khatkar SK, Samal SK. A single amino acid change, Q114R, in the cleavage-site sequence of Newcastle disease virus fusion protein attenuates viral replication and pathogenicity. *J Gen Virol*. 2011;92:2333-2338.
14. Nath B, Barman NN, Kumar S. Molecular characterization of Newcastle disease virus strains isolated from different outbreaks in Northeast India during 2014-15. *Microb Pathog*. 2016;91:85-91.