RESEARCH ARTICLE

Molecular Detection of Newcastle Disease Virus from Field Outbreaks in Assam, India

Pubaleem Deka[1]*, Sangeeta Das[2], Pankaj Deka[3], Mrinal K. Nath[1], Ritam Hazarika[4], Gunajit Das[5], Mihir Sarma[5]

Abstract

A study was carried out to detect the Newcastle disease virus (NDV) from various field outbreaks in Assam. Nine hundred ninety-two clinical and post -mortem samples during 2018-2021 were collected from the backyard and commercial poultry farms and subjected to molecular diagnosis of NDV by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). NDV could be detected in 58.78% tissue samples and 22.55% cloacal swabs collected from suspected poultry cases by RT-PCR. Organ wise study suggests that highest NDV (92.94%) was detected in trachea followed by spleen (90.62%), lung (86.95%), caecal tonsils (77.08%), proventriculus (60.81%), brain (48.91%), intestines (31.50%) and kidney (23.07%). Hence, the collection of specific tissue samples from field outbreaks for the detection of NDV is of paramount importance. In addition, RT-PCR can be considered a highly sensitive detection method for rapid and confirmative diagnosis of Newcastle disease virus.

Keywords: Newcastle disease virus, Poultry, primers, RT-PCR.

Ind J Vet Sci and Biotech (2022): 10.21887/ijvsbt.18.3.22

Introduction

Newcastle disease (ND) is a economically significant poultry disease and is distributed worldwide, causing big losses in the poultry industry. ND is a viral disease affecting more than 240 avian species (Alexander, 2000) and is caused by *Avian orthoavulavirus1* (AOAV-1) classified under the genus *Orthoavulavirus* within subfamily *Avulavirinae* of the family *Paramyxoviridae* (Dimitrov *et al.,* 2019). Newcastle disease has been reported in domestic and wild birds from different parts of India (Nath *et al.,* 2016; Rabari *et al*., 2017; Das *et al.,* 2021 and Gaurav *et al.,* 2021)

Based on the clinical signs, poultry pathogens affecting the respiratory system such as avian influenza, infectious bronchitis, and infectious laryngotracheitis can be confused with NDV infection (Piacenti *et al.,* 2006). The differential diagnosis of NDV from other associated diseases is essential for strategic disease-control programmes. Clinical diagnosis of NDV based on history, clinical signs and post-mortem lesions may establish a strong indicator of suspicion, therefore laboratory confirmation must be done. Virus isolation is regarded as the gold standard method for the definitive diagnosis of ND (Alexander, 2000). For confirmation of ND, the World Organisation for Animal Health standards Commission prescribes NDV isolation in 9th to 11th day-old embryonated chicken eggs and identification using Haemagglutination Inhibition (HI) test with an NDV-monospecific antiserum (OIE, 2012). However, molecular techniques like PCR have been frequently used worldwide to detect NDV in field samples targeting F protein which is a rapid and reliable test (Kant *et al*., 1997; Gohm *et al*., 2000). The technique of RT-PCR using F glycoprotein is used for the detection of NDV in clinical samples, without necessitating virus isolation and propagation in cell cultures or embryonated eggs, even when the virus is present in very minute quantity and has lost its infectivity (Singh *et al*., 2005). The aim of the present investigation was molecular detection of Newcastle disease virus from various clinical and post-mortem specimens collected from poultry in Assam.

[1]Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

[2]Department of Veterinary Microbiology, LUVAS, Hisar, Haryana, India

[3]Department of Veterinary Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Asam, India

[4]Department of Animal Biotechnology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assdam, India

[5]Livestock Research Station, Assam Agricultural University, Mandira, India

Corresponding Author: Pubaleem Deka, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India, e-mail: pubaleemdeka2@gmail.com

How to cite this article: Deka, P., Das, S., Deka, P., Nath, M.K., Hazarika, R., Das, G., Sarma, M. (2022). Molecular Detection of Newcastle Disease Virus from Field Outbreaks in Assam, India. Ind J Vet Sci and Biotech. 18(3), 97-99.

Source of support: Nil

Conflict of interest: None.

Submitted: 21/11/2021 **Accepted:** 28/06/2022 **Published:** 10/07/2022

Materials and Methods

Sample collection

Total 992 suspected cases (757 tissue samples and 235 cloacal swabs) of ND were collected from the backyard and commercial poultry from different field outbreaks in Assam during 2018-2021. Post mortem samples including spleen, lungs, brain, proventriculus, kidney, caecal tonsils, heart muscle, liver, intestines and trachea were collected in virus transport media (VTM) and transported to the laboratory for detection of the virus (OIE, 2016). All the samples were transported to the laboratory maintaining 4°C on ice and were either processed immediately on the same day or stored at -20°C until further processing. Simultaneously, cloacal swabs were collected in sterile cotton swabs (Himedia, India) containing VTM from clinically affected chicken.

Preparation of Antigen

As per, OIE Terrestrial manual (2016), the tissue samples were triturated in sterile sand and PBS (pH 7.0-7.4), using sterile pestle and mortar and prepared as 10% (w/v) suspensions. The triturated material was collected in sterile 15 mL centrifuge tube (Tarsons, India) and centrifuged at 1000 x g for 10 min. The supernatant was decanted into cryo-vial with added antibiotics (1000 U/mL penicillin, 100 U/mL myostatin, 100 U/mL neomycin and 50 U/mL polymyxin) for prevention of bacterial contamination. Following the method of Okwor *et al*., (2012) cloacal swabs were collected using sterile cotton swabs (Himedia®, India) containing PBS (pH 7.2) and antibiotics (1000 U/mL penicillin, 100 U/mL myostatin, 100 U/mL neomycin and 50 U/mL polymyxin) was vortexed to ensure thorough mixing. Then, the supernatant fluid of the swabs was obtained by centrifugation at 1000 x g for about 20 min. The supernatant fluid was transferred into cryo vials and stored at -20°C for further use.

Molecular Detection of NDV by RT-PCR

All the apparatus used for RNA isolation were treated with 0.1% Diethyl pyrocarbonate (DEPC) (Cat No: E17-5G, Thermo scientific) to make them RNase free. All the necessary chemicals used for RNA isolation, cDNA synthesis and RT-PCR, were available at DBT-Twinning project on NDV, Department of Microbiology, College of Veterinary Science, Khanapara, Guwahati-22. The viral genomic RNA was extracted using Qiagen RNA Extraction Reagent (Cat no: G3ID, GCC Pvt. Biotech Ltd.) as per the method described by the manufacturer's protocol. Viral RNA concentration and purity of the extracted samples was determined using a Nanodrop ND-1000 spectrometer V 3.5 (Thermo Fisher Scientific, USA) at 260/280nm. For the synthesis of cDNA, the reaction mixture was subjected to 25°C for 5 min in a thermal cycler, followed by 42°C for 1 h and 72°C for 10 min. Finally, the samples were kept at 4°C until withdrawn from the thermal cycler. After cDNA synthesis, the *F* gene was amplified as per the method described by Haryanto *et al.* (2016) using DNA template, PCR Master Mix and specific primers. The oligonucleotide primers were forward- 5′ TTGATGGCAGGCCTCTTGC 3′ and reverse 3′ GGAGGATGTTGGCAGCATT 5′ (Kant *et al*.,1997). A total of 5 µL of each PCR product per 1 µL of 6X Loading dye was subjected to agarose gel electrophoresis. For this, 1.0% agarose gel was prepared in 1X TAE buffer (40- mM Tris-acetate, 2-mM ethylenediaminetetraacetic acid (EDTA) stained with ethidium bromide (0.5 µg/mL) and the gel was run at 100 V for 40 min. To determine the size of the amplicons, an ethidium bromide–stainable 50-bp DNA ladder (Cat no: #SM0241, Thermo scientific, USA) was subjected to gel electrophoresis as marker. The gel was photographed using a gel documentation system (Bio-Imaging Systems MiniLumi).

Results and Discussion

The results of the present study reveals that overall prevalence of NDV was found to be 50.20% (498/992) of which 22.55% (53/235) and 58.78% (445/757) represents cloacal swabs and other tissues samples respectively. Organ wise study suggests highest NDV (79/85, 92.94%) was detected in trachea, followed by spleen (87/96, 90.62%), lung (80/92, 86.95%), caecal tonsils (74/96, 77.08%), proventriculus (45/74, 60.81%), brain (45/92, 48.91%), intestines (23/73 31.50%) and kidney (12/52, 23.07%).

For effective detection of NDV from tissue samples, the technique of RT-PCR has been used by different workers (Kant *et al*., 1997; Nanthakumar *et al*., 2000). Recently, Gaurav *et al*.

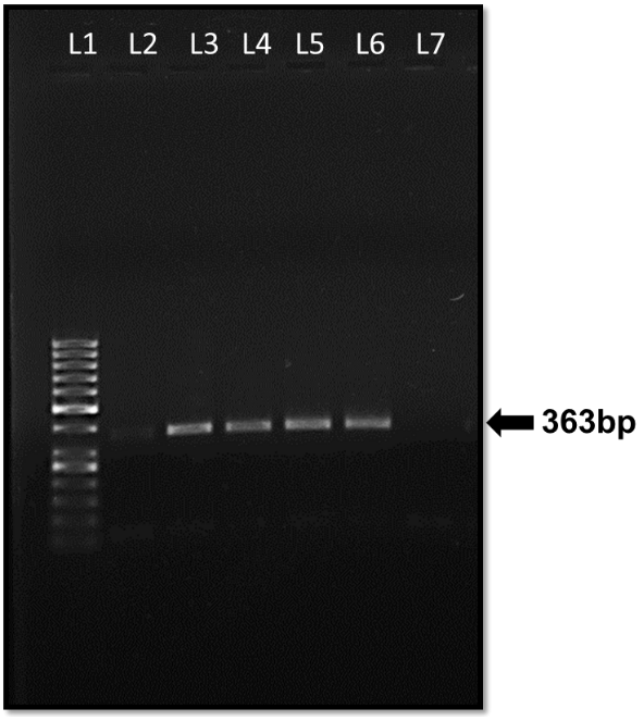

Fig. 1: Amplified gene products in tissue samples for *Fusion* (*F*) gene-specific for Newcastle disease virus (363 bp).
L1- 50bp marker, L2-L5- Tissue samples, L6- Positive control, L7- Non-Template Control

(2021) and Das *et al*. (2021) used this technique to detect NDV from Barn owls and broilers in Assam, India. In this study, a higher rate of NDV detection from the trachea (92.94%) might be due to the presence of maximum virus load in the trachea during infection and fewer bacterial contaminants. As NDV commonly spreads through aerosol and ingestion, the virus may have a chance to shed rapidly through the trachea. Similar result was observed by Haque *et al.* (2010) and Singh *et al*. (2005) where they reported that tracheal tissues may be considered as the best source of NDV during sample collection both from the clinical and experimental cases. During the present investigation, NDV could be detected in the spleen (90.62%) because spleen is a lymphoid organ and during viremic conditions, there is a chance of infiltration of NDV from the blood to the spleen. Thus, virus concentration and infection rate may be higher in the spleen and may be the best source of virus compared to other organs collected during post-mortem sampling. This is in agreement with Haque *et al.* (2010). A study conducted by Hussein *et al*. (2019) suggests that the tissue tropism of NDV depends upon the pathotype of the virus and the route of inoculation. The virus is replicated intensively in trachea if the virus is transmitted via intranasal route. However, the virus is transported to other tissues like proventriculus, caecal tonsils, kidney etc. via direct contact inside the intestinal lumen or by primary viremia. The virus can be detected in brain during secondary viremia which is attributed to the generalized infection or the circulatory nature of the macropahges. However, in the current study, NDV could not be detected from samples like heart muscle (n=45, 0.00%) and liver (n=52, 0.00%) which might be due to the nature of the viral strain and its pathogenesis.

Conclusion

From the study it can be concluded that tracheal tissue may be considered as a sample of choice for molecular detection of NDV from field outbreaks. Hence, RT-PCR is considered a highly sensitive detection method and can be introduced for rapid and confirmatory diagnosis of ND outbreak.

Acknowledgement

We acknowledge the DBT Twinning Project "An integrated omics approach to characterize circulating Newcastle disease virus and intervention strategies to control Newcastle disease in Northeast India" BT/PR24911/NER/95/903/2017 Dated 26/06/2017, Department of Veterinary Microbiology, College of Veterinary Science, AAU, Khanapara, Guwahati-22, Assam for providing research facility to undertake this study.

References

Alexander, D. J. (2000). Newcastle disease and other avian paramyxoviruses. *Revue scientifique et technique (International Office of Epizootics)*. 19(2), 443–462.

Das, S., Deka, P., Deka, P., Malik, A., Ansari, T., Rapthap, L. & Hazarika, R. (2021). Isolation and molecular detection of virulent Newcastle disease virus from an outbreak in broilers in Assam. *The Pharma Innovation journal*.10(11S), 2512-2515.

Dimitrov, K.M., Claudio, C.A.L., Albina, A.E., Berg, J.B.M., Briand, F.X., Brown, I.H. *et al*. (2019). Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infection, Genetics and Evolution*. 74,103917.

Gaurav, S., Deka, P., Das, S., Deka, P., Hazarika, R., Kakati, P., Kumar, A. & Kumar, S. (2021). Isolation of genotype VII avian orthoavulavirus serotype 1 from barn owl from Northeast India. *Avian Pathology*. 28,1-13.-

Gohm, D. S., Barbara, T. & Hofmann, M. A. (2000). Detection of Newcastle disease virus in organs and faeces of experimentally infected chickens using RT-PCR. *Avian Pathology*. 29,143-152.

Haque, M. H., Hossain, M. T., Islam, M. T., Zinnah, M. A., Khan, M. S. R. & Islam, M. A. (2010). Isolation and detection of Newcastle Disease virus from field outbreaks in broiler and layer chickens by reverse transcriptase-polymerase chain reaction. *Bangladesh Journal of Veterinary Medicine*. 8 (2), 87–92.

Haryanto, A., Wati. V., Pakpahan, S. & Wijayanti, N. (2016). Molecular Pathotyping of Newcastle Disease Virus from Naturally Infected Chicken by RT-PCR and RFLP Methods. *Asian Journal of Animal Science*. 10, 39-4.

Hussein, E. A., Hair-Bejo, M., Omar, A. R., Arshad, S. S., Hani, H., Balakrishnan, K. N., ... & Aini, I. (2019). Velogenic newcastle disease virus tissue tropism and pathogenesis of infection in chickens by application of in situ PCR, immunoperoxase staining and HE staining. *Microbial pathogenesis*, *129*, 213-223.

Kant, A., Koch, G., Roozelaar, D.J. V., Balk, F. & Huurne, A. T. (1997). Differentiation of virulent and non-virulent strains of Newcastle disease virus within 24 hours by polymerase chain reaction. Avian Pathol., 26: 837-849.

Nath, B., Barman, N.N. & Kumar, S. (2016). Molecular characterization of Newcastle disease virus strains isolated from different outbreaks in Northeast India during 2014-15, *Microbial Pathogenesis*. 91, 85-91.

Nanthakumar, T., R.S. Kataria, A.K. Tiwari, G. Butchaiah and J.M. Kataria, 2000. Pathotyping of Newcastle disease viruses by RT-PCR and restriction enzyme analysis. *Veterinary Research Communication*. 24, 275-286.

OIE, (2012). Newcastle Disease (Infection with Newcastle Disease Virus), Manual of Diagnostic Tests and Vaccines for Terrestrial Animals: (Mammals, Birds and Bees), vol.1, 555–574.

OIE, (2016). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris: *Office International des Epizooties*.

Okwor, E.; Didacus, C. E. & M, Umeh. (2012). Newcastle Disease Virus Shedding Among Apparently Healthy Commercial Chickens and its Epidemiological Importance. *Pakistan Veterinary Journal*. 32, 354-356.

Piacenti, A. M., King, D. J., Seal, B. S., Zhang, J. & Brown, C. C. (2006). Pathogenesis of Newcastle disease in commercial and specific pathogen-free Turkeys experimentally infected with isolates of different virulence, *Veterinary Pathology*. 43(2), 168–178.

Rabari, P.D., Jhala, M.K., Bhanderi, B.B. & Mathakiya, R.A. (2017). F Gene Sequencing and Analysis of Indian New Castle Disease Virus. *International Journal of Agricultural Sciences*. 9(17), 4156-4158.

Singh, K., Jindal, N., Gupta, S. L., Gupta, A. K., & Mittal, D. (2005). Detection of Newcastle disease virus genome from the field outbreaks in poultry by reverse transcription-polymerase chain reaction. *International Journal of Poultry Science*, *4*(7), 472-475.