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Short Communication

PCR BASED DETECTION OF NEWCASTLE DISEASE VIRUS FROM RESPIRATORY DISEASE COMPLEX SUSPECTED POULTRY SAMPLES

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ABSTRACT

The present study reports the use of reverse transcriptase-polymerase chain reaction (RT-PCR) for the detection of Newcastle disease virus (NDV) infection in poultry directly from the suspected tissue samples. The samples were collected from birds showing signs of respiratory illness and necropsy revealing lesions of RDC (respiratory disease complex). The samples included tissues like trachea, lung and spleen. RT-PCR was carried out using species-specific primers for NDV. On agarose gel electrophoresis the expected band size of 356bp was observed for 22 samples out of 53 RDC suspected samples. On the basis of present investigation, it may be concluded that tissues may be used for rapid screening and detection of NDV in poultry.

Key words: RT-PCR, NDV, respiratory disease complex, poultry

Newcastle disease (ND) is one of the most serious infectious diseases affecting birds, particularly poultry, worldwide and has been the cause of serious economic losses. NDV potentially infects most species of birds and for susceptible poultry it is highly contagious and usually fatal (Alexander, 2003). The causative agent, *Newcastle disease virus* (NDV; order Mononegavirales, family Paramyxoviridae, subfamily Paramyxovirinae, genus *Avulavirus*; also known as Avian paramyxovirus 1), is a diverse group of single-stranded, negative-sense, non-segmented RNA viruses of approximately 15.2 kb (Lamb *et al*., 2005).

In the past, the diagnosis of ND has been based on serological tests like complement fixation test, haemagglutination inhibition test, virus neutralization test, single radial immunodiffusion test, immunoperoxidase assay, enzyme-linked immunosorbent assay, plaque neutralization, agar gel precipitation test, virus isolation etc. However these methods are more laborious and time consuming. In the recent past, molecular techniques like reverse-transcription polymerase chain reaction (RT-PCR) have been frequently used all over the world to detect NDV in the field samples targeting F gene/Fusion protein (Hoffmann *et al*., 2009). NDV detection by RT-PCR offers the possibility of subsequent sequencing and pathotyping of the virus isolate and help in understanding molecular epidemiology of pathogen. Direct nucleotide sequencing of the amplified product from the fusion protein cleavage site was reported to be a useful tool for pathotyping of NDV (Collins *et al*., 1993; Seal *et al*., 1995). The technique of RT-PCR and restriction enzyme analysis was standardized by Nanthakumar *et al*. (2000) to detect Newcastle disease viruses by RT-PCR with primers for the NDV flanking fusion (F) gene.

A total of 53 respiratory disease complex (RDC) suspected samples from broiler chickens were collected. The tissue samples including trachea, lungs and spleen were collected from birds showing typical signs of RDC viz. rales, coughing, nasal discharge, sinusitis etc. Samples collected from birds were brought for disease investigation at Disease Investigation Lab, College of Veterinary Sciences, LUVAS, Hisar. Post-mortem examination of dead birds revealed pathological findings such as tracheal exudates, severe tracheitis, head swelling, conjunctivitis, congested lung, pericarditis etc. La Sota vaccine strain was used as a positive control while trachea from a healthy non-vaccinated bird was taken as negative control.

The total RNA from tissue samples, vaccine strain and the un-vaccinated healthy bird was extracted with the TRIzol reagent (Daniela *et al*., 2000). The cDNA was synthesized using 50µg random hexamer primers, 100µg heat denatured viral RNA, 40 units RNase inhibitor, 1µl 10mM dNTPs, 2µl 0.1 M DTT, 4µl 5X RT buffer and 300 units MMLV-RT (MBI, Fermentas) in 20µl reaction mixture. After allowing the random primers to anneal at 25ºC for 5 min, reverse transcription was carried out at 42ºC for 1 h. The enzyme was heat inactivated at 70ºC for 5 min. The oligonucleotide primers viz. forward- 5'-GCAGCTGCAGGATTGTGGTG-3'

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(nucleotide position 158-177) and reverse - 5'-TCTTTGAGCAGGAGGATGTTG-3' (nucleotide position 493-513) (Nanthakumar *et al*., 2000) were used for the amplification of 356 bp region corresponding the cleavage activation site of F gene of NDV. PCR was carried out in 0.5ml PCR tubes in a final reaction of 25 µl volume. Each reaction mixture contained 2X PCR Master Mix (TopTaq Green Master Mix, Thermo Scientific), 10 pmol/ µl each of NDV forward and NDV reverse primers and 2µl of the cDNA template and the final volume was adujusted by nuclease free water (NFW). The following conditions were used for RT-PCR: initial denaturation at 95ºC for 5 min, 35 cycles of denaturation at 95ºC for 50 sec, annealing at 55ºC for 50 sec, elongation at 72ºC for 60 sec and final elongation at 72ºC for 10 min. The samples were amplified in MyCycler (Biorad, USA).The amplified products were separated by agarose gel electrophoresis (AGE) in 1X Tris-borate-EDTA buffer. The gel was stained with ethidium bromide (0.5 µg/ml of gel) and 10 µl of the amplified products were loaded in the wells along with the 100bp DNA ladder (GeneRuler, ThermoScientific, USA). The DNA fragments were observed by ultraviolet transilluminator and photographed in a gel documentation system (Alpha Imager, Germany).

The NDV was detected in 22 (41.50%) out of 53 RDC suspected field samples and in the positive control using RT-PCR. All the positive samples yielded a single band of 356 bp during AGE while no amplification was observed for negative control (Fig. 1). This technique using F glycoprotein therefore, can easily be used for the detection of NDV in clinical samples, without necessitating virus isolation and propagation in cell cultures or embryonated eggs. In the present study, the samples were collected from RDC suspected broiler chickens showing symptoms characterized by respiratory distress, severe tracheitis, coughing, nasal exudate, conjunctivitis, etc. The primary objective of the present study was to detect NDV in tissue samples from the field cases. Further, involvement of other pathogens could also be studied. The technique of RT-PCR along with restriction enzyme analysis and sequencing will be useful to know the type of strains circulating in a particular geographical region and in further classification of NDV field isolates into varying pathogenic strains viz. velogenic, mesogenic or lentogenic. It may also help to generate epidemiological data regarding the changes in antigenicity/ pathogenicity of the field isolates as well as in formulation of a vaccination strategy for effective control of the disease under field conditions.

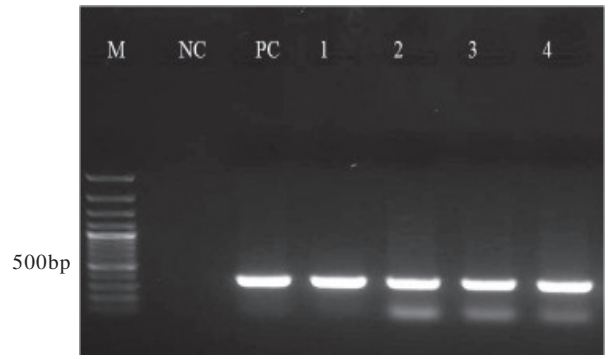

Fig 1. RT-PCR amplification of partial region of F-gene (356bp) for Newcastle disease virus
Lane-M: DNA ruler (100bp); Lane NC=Negative control; Lane-PC=Positive control Lane-1-4=Positive samples.

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