BAOJ Microbiology

Rafia Maqbool, et al, BAOJ Microbio 2017 3: 1
3: 025

Research Article

Detection and Pathotyping of Newcastle Disease Virus from Poultry in Kashmir

Rafia Maqbool[1*], Shakil A Wani[1], Asifa Wali[2], Aazima Shah[3], Zahid A Kashoo[1], Parvaiz S Dar[1], Mohd Y Ganaie[1], Salik Nazki[1] , Sabia Qureshi[1] and Isfaqul Hussain[1]

[1]Division of Veterinary Microbiology & Immunology, Faculty of Veterinary Science & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUASTK), India

[2]Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUASTK), India

[3]Division of Veterinary Pathology, Faculty of Veterinary Science & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUASTK), India

Abstract

Newcastle disease (ND) caused by virulent strain of avian paramyxovirus-1(vAPMV-1) is a highly contagious disease of poultry. ND remains a major problem in existing or developing poultry industries. There are different pathotypes of NDV in chickens: velogenic (high virulence), mesogenic (moderate virulence), and lentogenic (low virulence).The present study was carried out on 5 suspected outbreaks to detect and pathotype the Newcastle disease virus from poultry in Kashmir. The detection was carried out by amplification of 356 bp of Fusion protein gene including F_0 cleavage site by RT-PCR which is regarded as a major determinant of pathogenicity. All the 5 suspected outbreaks were positive for ND. Restriction digestion by *Alu*I of the amplified PCR product yielded ~284bp and ~72bp products in all the outbreaks. The pathern of digestion was similar to that of mesogenic R2B strain indicating the pathotype of circulating virus in Kashmir. The technique of RT-PCR followed by restriction enzyme digestion can been exploited to pathotype NDV.

Keywords: Fusion Protein Gene; Reverse Transcriptase PCR; Newcastle Disease Virus; Restriction Digestion

Introduction

Newcastle disease (ND), a poultry disease that causes severe outbreaks resulting in huge economic losses, is caused by *Newcastle disease virus* (NDV) [1]. NDV is a single stranded negative sense, non-segmented RNA virus, which is a virulent strain of avian paramyxovirus type 1 (APMV-1) serotype of the genus *Avulavirus* belonging to subfamily *Paramyxovirinae*, family *Paramyxoviridae*, order *Mononegavirales* [2,3]. The disease has a worldwide prevalence including in India and is seen affecting many species of birds causing huge economic losses to poultry industry due to high morbidity and mortality associated with virulent strains of the virus [4,5,6]. There are 9 serotypes of APMV, but all isolates of Newcastle Disease Virus (NDV) belong to serotype 1 (APMV-1) [1], therefore NDV is synonymous with APMV-1. The APMV-1 viral genome, approximately 15Kb, is composed of 6 genes encoding 6 structural proteins- fusion (F), nucleoprotein (NP), matrix (M), phosphoprotien (P), RNA polymerase (L), and hemaglutinin-neuraminidase (HN) [7,8,9]. Two additional proteins are encoded by RNA editing of the P protein, namely V and W. The V protein is known to have an inhibitory effect on the alpha/beta interferon response in avian host [10].

There are different pathotypes of NDV in chickens: velogenic (high virulence), mesogenic (moderate virulence), and lentogenic (low virulence) [11]. The pathogenicity of NDV is determined primarily by cleavage of F protein by host cellular proteases [12,13]. Molecular pathotyping for detection and differentiation of avian paramyxovirus-1 isolates by the RT-PCR amplification followed by Restriction Fragment Length Polymorphism (RFLP) using restriction enzyme is one of rapid diagnostic method which can be used to determine of NDV pathotype [14,15].

Materials and Methods

A total of 5 suspected outbreaks of Newcastle Disease virus infection in Kashmir valley were attended. The samples collected from the dead birds included tracheal swabs, cloacal swabs, caecal tonsils, spleen, brain, proventriculus and Payer's patches. The samples were preserved in 50% glycerol saline at -20° C or directly in TRIzol (Sigma, USA) at - 80° C. RNA was extracted from the suspected samples by manual method (TRIzol). Complementary DNA (cDNA) synthesis was carried out using random hexamer primers and Revert-Aid First-Strand cDNA Synthesis kit. A primer set targeting hypervariable region of Fusion protein gene, as described by Nanthakumar *et al.* (2000) was used to detect NDV

***Corresponding author:** Rafia Maqbool, Division of Veterinary Microbiology & Immunology, Faculty of Veterinary Science & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology Of Kashmir (SKUASTK), India, Tel: +919419055539; E-mail: rafia.maqbool89@gmail.com

Sub Date: March 1, 2017, **Acc Date:** March 28, 2017, **Pub Date:** March 28, 2017.

Citation: Rafia Maqbool, Shakil A Wani, Asifa Wali, Aazima Shah, Zahid A Kashoo, et al. (2017) Detection and Pathotyping of Newcastle Disease Virus from Poultry in Kashmir. BAOJ Microbio 3: 025.

by RT-PCR.PCR assay was performed in a 25 µl total reaction volume in a 0.2 ml PCR tube on Master cycler gradient PCR machine (Eppendorf, USA) with Nuclease Free Water 13.5µl, 10x PCR Buffer 2.5µl, 25mM $MgCl_2$ 2.5 µl, 25mM dNTP mix 0.2 µl, Forward primers (5pM)0.5 µl, Reverse primers (5 pM) 0.5 µl, Taq DNA Polymerase 0.3 µl, cDNA (2 µg/20 µl) 5 µl. At the end of the run, the amplification was checked by Agar gel electrophoresis. Digestion of RT-PCR-amplified product with restriction enzymes *Alu*I was carried out in order to characterize Newcastle disease viruses of varying pathogenicityat 37^0 C for 4hrs followed by overnight incubation at 4^0C. The digested product was analyzed in an analytical 3% agarose gel on UV trans-illuminator. For 20ul reaction, PCR product 10**µl**, NFW 7**µl**, Buffer 2**µl** and Enzyme 1**µl** was used.

Result and Discussion

Suspected tissues from all the 5 outbreaks on RT- PCR amplified a fragment of hyper variable region of F gene with the expected size (~356 bp) and confirmed the presence of NDV in the samples (Figure 1). RT-PCR systems are usually being used to amplify a specific portion of the genome that contains the F_0 cleavage site for detection of NDV and for assessing virulence by restriction digestion or amino acid sequencing of F_0 cleavage site [16]. The PCR products on restriction digestion by *Alu*1 yielded ~282bp and ~72bp products (Figure 2) in all the outbreaks showing the presence of a single type of strain present in all the positive outbreaks. RT-PCR and Restriction Digestion have been reported by Nanthakumar et al 2000 and on pigeon paramyxovirus serotype-1(PPMV-1) isolates by Naveen *et al.*, 2013 [17,18]. The pattern of digestion of the PCR products was similar to the pattern of digestion by mesogenic R2B vaccine strains as reported by Nanthakumar *et al.* 2000 [17]. Endonuclease digestion with *Alu* I has also been used to differentiate vaccine and field strains [19]. Future studies involving a higher number of vaccine and field strains will be carried out to determine whether the *Alu I* cleavage difference could be used to differentiate field strains additional to sequence analysis.

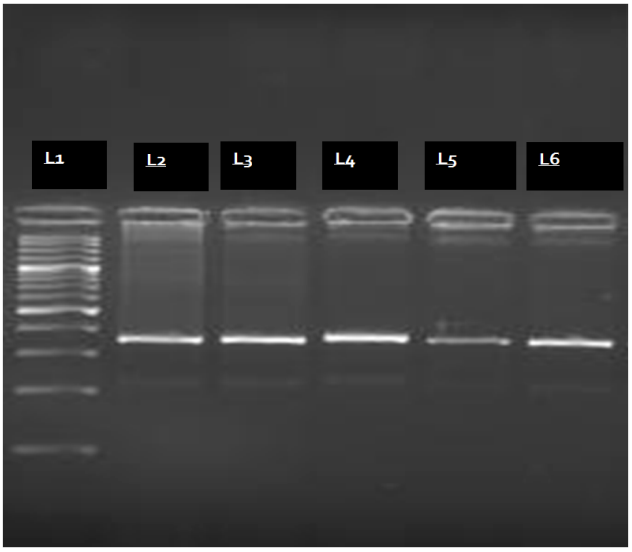

Fig1: Fusion protein gene fragment (~356 bp) corresponding to the hypervariable region was amplified in the positive samples. L1 = 100 bp DNA ladder, L2-L6 positive disease outbreaks.

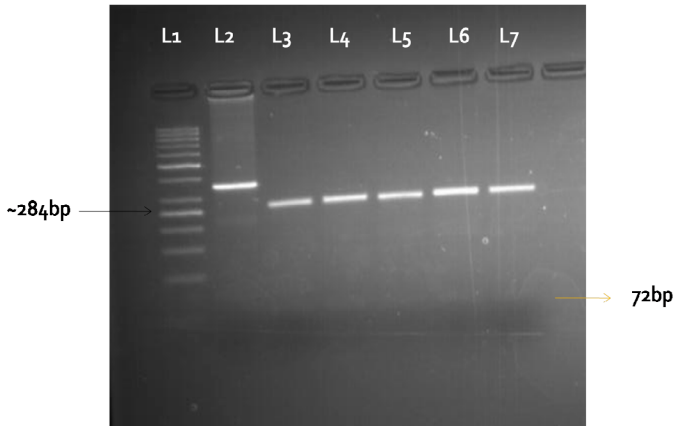

Fig 2: Electrophoresis of restriction enzyme *Alu*I digested 356bp PCR product of field isolates in 3%gel. L1,50bp DNA ladder, L2, undigested PCR product as control L3-L7, field isolates

Acknowledgment

The ICAR- NAIP and NAE(anaerobic bacteriology) projects are duely acknowledged.

References

1. Alexander DJ (2003) Newcastle disease and other Paramyxoviridae infections. In: Diseases of Poultry. Saif YM, Barnes HJ, Glisson JR, Fadly AM, Mc Dougald LR. and Swayne DE. (eds.). 11th edition. Iowa State University Press, Ames, IA 63-87.
2. Abolnik C, Horne RF, Bisschop SP, Parker ME, Romito M, et al. (2004) A phylogenetic study of South African Newcastle disease virus strains isolated between 1990 and 2002 suggests epidemiological origins in the Far East. Archives of Virology 149(30): 603-619.
3. Pedersen JC, Senne DA, Woolcock PR, Kinde H, King DJ, et al. (2004) Phylogenetic relationships among virulent Newcastle disease virus isolates form the 2002-2003 outbreaks in California and other recent

outbreaks in North America. Journal of Clinical Microbiology 42(5): 2329-2334.

4. Kim LM, Suarez DL, Afonso CL (2008) Detection of a broad range of class I and II Newcastle disease viruses using a multiplex real-time reverse transcription polymerase chain reaction assay. Journal of Veterinary Diagnostic Investigation 20(4): 414–425.
5. Lee YJ, Sung HW, Choi JG, Lee EK, Yoon H, et al. (2008) Protection of chickens from Newcastle disease with a recombinant baculovirus subunit vaccine expressing the fusion and hemagglutinin- neuraminidase proteins. Journal of Veterinary Science 9(3): 301–308.
6. Miller PJ, Decanini EL, Afonso CL (2010) Newcastle disease: evolution of genotypes and the related diagnostic challenges. Infection, Genetics and Evolution 10(1): 26–35.
7. de Leeuw O, Peeters B (1999) Complete nucleotide sequence of Newcastle disease virus: evidence for the existence of a new genus within the subfamily Paramyxovirinae. Journal of General Virology 80(pt 1): 131-136.
8. Aldous EW, Alexander DJ (2001) Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). Avian Pathology 30(2): 117–128.
9. Oberdörfer A, Werner J, Veits T, Mebatsion TC, Mettenleiter TC (2003) Contribution of the length of the HN protein and the sequence of the F protein cleavage site to Newcastle disease virus pathogenicity. Journal of General Virology 84(pt 11): 3121-3129.
10. Huang Z, Panda A, Elankumaran S, Govindarajan D, Rockemann DD, et al. (2004) The hemagglutinin-neuraminidase protein of Newcastle disease virus determines tropism and virulence. Journal of Virology 78(8): 4176–4184.
11. Beard CW, Hanson RP (1984) Newcastle disease In: Disease of Poultry. Hofstad MS, Barnes HJ, Calnek BW, Reid WM and Yoder HW (eds.), 8th edition. Iowa State University Press, Ames, IA 452-470.
12. Peeters BP, de Leeuw OS, Koch G, Gielkens AL (1999) Rescue of Newcastle disease virus from cloned cDNA: Evidence that cleavability of the fusion protein is a major determinant for virulence. Journal of Virology 73(6): 5001-5009.
13. de Leeuw OS, Hartog L, Koch G, Peeters BPH (2005) Effect of fusion protein cleavage site mutations on virulence of Newcastle disease virus: nonvirulent cleavage site mutants revert to virulence after one passage in chicken brain. Journal of General Virology 84(pt 2): 475–484.
14. Heine H, Trinidad L (2006) Rapid identification and pathotyping of virulent IBDV, NDV and AIV isolates. A report for the rural industries research and development corporation. Australia Government 15-18.
15. Haryanto A, Purwaningrum M, Verawati S, Irianingsih SH, Wijayanti N (2015) Pathotyping of local isolates newcastle disease virus from field specimens by RT-PCR and restriction endonuclease analysis. Procedia Chemistry 14: 85-90.
16. Nanthakumar T, Kataria RS, Tiwari AK, Butchaiah G, Kataria JM (2000) Pathotyping of Newcastle disease viruses by RT-PCR and restriction enzyme analysis. Veterinary Research Communications 24(4): 275-286.
17. Creelan JL, Graham DA, McCullough SJ (2002) Detection and differentiation of pathogenicity of avian paramyxovirus serotype-1 from field cases using one- step reverse transcriptase- polymerase chain reaction. Avian Pathology 31(5): 493-499.
18. Naveen KA, Singh SD, Kataria JM, Barathidasan R, Dhama K (2013) Detection and differentiation of pigeon paramyxovirus serotype-1 (PPMV-1) isolates by RT-PCR and restriction enzyme analysis. Tropical Animal Health Production 45(5): 1231-1236.
19. Gohm DS, Thur B, Hofmann MA (2000) Detection of Newcastle disease virus in organs and faeces of experimentally infected chickens using RT-PCR. Avian Pathology 29(2): 143-152.