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Antigenic Differences in Newcastle Disease Viruses Isolated in Iran

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Abstract: The samples included 30 velogenic strains of NDV, isolated from diseased chicks during 3 years period, and 2 vaccinal strains were cultured on chicken embryo and then concentrated and purified by ultra centrifugation. All samples were electrophoresed on 12.5% SDS-PAGE gels for staining and Western blotting using anti-NDV antibody. Six different antigens with molecular weight 79, 75, 64, 40, 36 and 27 were detected in viral proteins and in some of the isolates a 48-49 kDa antigen were observed instead of a 64 kDa protein that had similarities to fusion protein in ND viruses. Western blot analysis of the antigenic patterns did not produce concise, discrete groupings, but did emphasize some relationships between virus properties and antigenicity.

Key words: Newcastle disease virus, antigenic structure, viral proteins, western blotting, fusion protein

Introduction

Newcastle disease virus (NDV) is a member of the genus *Avulavirus*, in the family *Paramyxoviridae*, which is composed of enveloped helical nucleocapside (Murphy *et al.*, 1999).

Single stranded minus sense RNA genome of ND virus encoded six major proteins. The NP (nucleocapsid protein), P (phospho protein) and L (large protein) are located in nucleocapside. The two glycoproteins, F (fusion) and HN (Hemagglutinin-Neuraminidase) as well as non-glycosylated M (matrix protein) are associated with the viral envelope (Aldous and Collins, 2001).

Fusion and entry of NDV require two glycoproteins F and HN. Like all the paramyxoviruses, the F protein is synthesized initially as a precursor, F_0 , and then cleaved into F_1 and F_2 by a furin-like enzyme of the host cells (Ballagi and Wehmann, 1996, Epsilon and Henav, 1987). Cleavage of F is required for NDV to function in hemolysis, cell-to-cell fusion, and early event in infection. Proteinal pattern of different NDV isolates usually is similar but a number of previous studies have demonstrated a minor difference in molecular weight and proteinal pattern of NDV isolates (Kumanan and Mustay-Ahamed, 1994).

Materials and Methods

Viruses: To obtain pure virus stocks each 30, virulent isolates and two different vaccine strains (B1 and La Sota) of NDV were used. The viruses were isolated from different parts of Iran over a period of 5 years (1999 to 2003) and confirmed by RT-PCR and restriction endonuclease mapping (Hemmatzadeh and Alinejad, 2003).

All the viruses were inoculated into allantoic cavity of 7 days old of chicken embryonated eggs and harvested after 3 to 5 days. Following HA examination, the allantoic fluid of eggs was clarified by centrifugation at 600g for 20 min, and ultra-centrifuged (Beckman-Avanti J-251,

Rotor:JA-25-50) at 70,000 g for 2 h through a 30% sucrose cushion. The pellet was then resuspended in 1 ml TSE (0.01 M Tris-hydrochloride [pH 7.2], 0.001 M EDTA, 0.15 M NaCl), and stored in aliquots at -80°C . Protein content of purified samples were measured by Bradford method (Hay *et al.*, 2002) and adjusted to 2 mg/ml.

SDS-PAGE: All the samples were run in discontinuous 5 and 12.5% of polyacrylamide gels under denaturing and reducing conditions in duplicate gels (one of the gels were stained and another one used for western blotting). The SDS-PAGE gels were stained by Coomassie Brilliant Blue and molecular weight of the protein bands were measured.

Western blotting: The gels were transferred to nitrocellulose (NC) membrane (Schleicher & Schuell BA-S 83, 0.2 μm , Keene, NH) then blocked at room temperature (3% BSA/0.05% Tween-20/PBS) for 1 h and washed 3 \times 5 min in PBS-T. The membranes were overlaid with dilution 1/8 of positive antisera obtained from diseased chicks (previously tested by ELISA and HI) and incubated for 1 h at 37°C and washed 3 \times 5 min in PBS-T. As indicator of bounded antibodies the membranes were overlaid with a dilution 1/100 of horseradish peroxidase (HRP) conjugated rabbit anti-chicken antibody and incubated and washed as previously mentioned and developed with a TMB (3,3', 5,5'-tetramethylbenzidine) substrate solution (Sigma, Chemical. Co. St Louis, Mo.) (Sambrook *et al.*, 1989). The color reaction was allowed to proceed for 10 min and then stopped with several washes with distilled water. The membranes were air dried between filter paper in the dark before being read. At last the membranes were scanned and molecular weight of proteins was measured in comparison to pre-stained MW marker. The resultant images have been shown in Fig. 1.

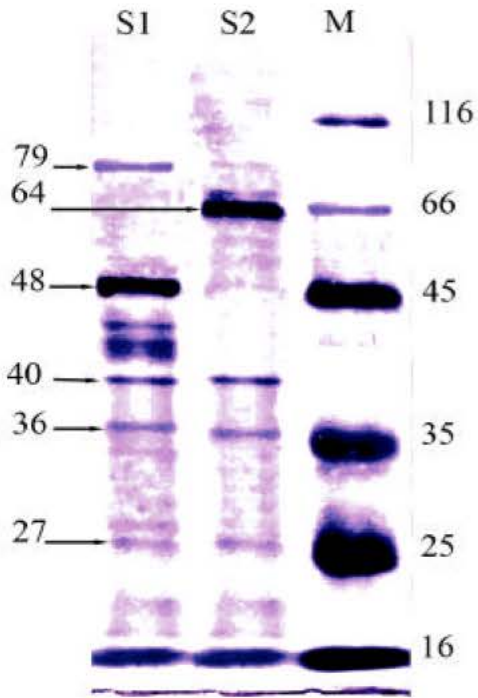


Fig. 1: Comparison of the antigenic pattern of the NDV. S1: NDVs with different Fusion protein (48kDa), S2: NDVs with normal fusion protein (64 kDa), M: Molecular weight marker.

Results

Following electrophoresis and staining over all, a total of 6 polypeptides (Mr 79, 75, 64, 40, 36 and 27 kDa) were identified in most of the strains and a 48 kDa protein in 3 of them. Electrophoretic patterns of major proteins demonstrate no variation between velogenic and vaccinal strains. Most notably, 64 kDa polypeptide was present in 27 of strains and other proteins were identified in all the strains.

Based on RT-PCR and mean death time assays findings, NDV strains that have different F protein in Western blotting were shown different restriction endonuclease pattern in F gene PCR product. At blotting by using antisera obtained from diseased birds (previously tested by HI test), 79, 64, 40, 36 and 27 kDa) for 27 same viruses, 79, 48, 40, 36 and 27 kDa proteins for 3 different strains were identified (Fig. 1).

Discussion

The study of variation in viral proteins is the basis of most studies on identification of protective antigens and designing the serological diagnostic tests. This study was done to identify the antigenic variation of isolated velogenic and vaccinal strains of Newcastle disease viruses in Iran. (Umino and Kohama, 1984).

As shown in Fig. 1 significant variation was determined

in 64 and 48 kDa in different isolates, these findings were not notified in different researches through the world previously.

Molecular studies done by Kumanan and Mustay Ahamed (1994), Swain and Verma (1997) and Ballagi and Wehmann (1996) and Hemmatzadeh and Alinejad (2003) have identified diversity in different molecular weight of F gene of NDV isolates in different areas. Panshin *et al.* (2000) demonstrated a significant diversity in NDVs antigens by using a panel of monoclonal antibodies.

In the present study the analysis of viral isolates revealed the presence of 79, 64, 40, 36 and 27 kDa proteins. These proteins have major role in viral structure, glycol protein 79 and 64kDa has been shown to mediate neutralization of NDVs and 48 kDa protein did not mention by another researcher.

In velogenic strains F1 protein is derived from the cleavage of F0 to F1 and F2, and in non-virulent strains especially vaccinal stains, f protein doesn't cleave to other proteins. (Panda *et al.*, 2004, Reynolds and Maraqa, 2000)

This study present evidence indicating that there is remarkable heterogenicity among the field viral isolates of NDV and this methodology is of potential value for molecular epidemiology studies.

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Hemmatzadeh *et al.*: Antigenic Differences in NDV.

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