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COMPARATIVE MOLECULAR CHARACTERIZATION OF FIELD AND VACCINAL STRAINS OF NEWCASTLE DISEASE VIRUS

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ABSTRACT

Seven commercial vaccinal strains and three field isolates from various outbreaks of Newcastle disease were subjected to polypeptide analysis using Sodium Dodysel Sulphate (SDS-PAGE). On electrophoresis, the Newcastle Disaese Vaccine (NDV) proteins were settled into several peptide components related to structural elements of virus. Virus strains procured from different outbreaks, having variable mortality, fragmented into analogous peptide pattern of molecular weights of 181, 112, 89, 74.4, 63, 53, 24 and 12.5 KDa. In addition, the proteins responsible for conferring protection were consistently present in the three field isolates of NDV and were missing in some of the vaccinal strains. Moreover, the vaccinal strains of NDV showed marked variation among themselves in the peptide patterns. Some vaccinal strains had small kilobases of certain proteins. It was concluded that pivotal differences in the peptide patterns of lentogenic strains existed which could materialize to the justification of vaccination flop.

Key words: NDV strain, SDS-PAGE, polypeptide mapping, characterization.

INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease and still remains number one disease affecting poultry industry in Pakistan. It has attained an intricate state in the way that different isolates and strains of virus provoke tremendous variation in the severity of the outbreak. Because of this variation, vaccination programmes launched against ND in the past could not reach complete accomplishment for its control. Extensive prophylaxis and control programmes against ND using different types of vaccines are in practice but absolute success in controlling the disease could not be accomplished. It may be owing to the use of infective vaccines, antigenic variations amongst different ND virus strains or the fallacious administration. Multiple Newcastle Disease Virus (NDV) strains, viz, Lentogenic, Mesogenic and Velogenic based upon their pathogenicity for chicken embryo, had been reported (Kumanan and Venkatesan, 1991; Vijayarani *et al.*, 1992; Capua *et al.*, 2000; Hanson and Brandly, 1955).

The present investigations were designed to elucidate the antigenic diversities displayed among different field strains as well as vaccinal strains of NDV using polypeptide mapping by Sodium Dodycel Sulphate-Poly AcrylamideGel Electrophoresis (SDS-PAGE).

MATERIALS AND METHODS

Collection of samples

Twelve spleen samples were collected for the isolation of NDV from clinically diseased birds from different areas in and around Faisalabad. The samples were stored at -20^{0}C till further use. Eight commercially available vaccines of NDV were procured from market and processed in the same way.

Isolation of virus

Isolation of virus was carried out using the method described by Buxton and Frazer (1977). Each spleen sample (approximately 5 gm) was triturated in a sterilized pestle and mortar with sterilized sand and 5 ml physiological saline solution containing 1000 IU/ml penicillin and 1000 ug/ml streptomycin. The suspension was placed at room temperature for 30 minutes and centrifuged at 2000 RPM for 10 minutes, the supernatant was inoculated into embryonated eggs through allantoic cavity. Inoculated eggs were placed at 37^{0}C for 48 hours. After nine days, the eggs with live embryos were chilled, allantoic fluid aspirated and was stored at -20^{0}C till further use.

Confirmation of virus

The presence of NDV in the allantoic fluid was determined by spot haemagglutination and micro-agglutination tests, as described by Allan and Gough

(1974). The positive haemagglutinating allantoic fluids were confirmed through haemagglutination inhibition (HI) test with known NDV antiserum (Cadman *et al.*, 1997).

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis

a) Purification and concentration of NDVstrains

Eight millilitre of allantoic fluid having field strains and purified vaccinal strains was transferred to 30 ml centrifuge tube and spun at 5000 x g for 30 minutes at 4^0C in JA-20 Beckman rotor. The collected supernatant was subjected to the gradient centri-fugation, as described by Nagy and Lominiczi (1984). Two layers of sucrose solution (30 and 20%) were prepared under -20^0C for 2 hours. The test samples were poured on the solidified sucrose gradient gently and centrifuged at 15000 RPM for 3 hours at 4^0C in JA-20 Beckman rotor. A clear visible pellet of virus was obtained and washed with distilled water. Half of the pellet was resuspended in NTE (100 mM NaCl, 20 mM Tris-HCl, 2 mM EDTA, pH 6.8) buffer and other half of the pellet was resuspended in TE (20 mM tris HCl, 2mM EDTA, pH 6.8) buffer. One percent Sodium Dodycel Sulphate was added in both of the pellet suspensions.

b) Partial digestion

The samples were subjected for digestion with chymotrypsin (Leurquin Lab., France). The freeze-dried powder of chymotrypsin was resuspended in 5 ml distilled water. A 5 ml of chymotrypsin solution was added in both centrifuge tubes containing virus suspension and incubated in water bath at 37 ^{0}C. The 50 µl digested samples were collected from each tube at 5, 10, 20 and 30 minutes interval and boiled at 100 ^{0}C for 5 minutes in water bath to stop the reaction (Cleveland *et al.*, 1977).

c) Preparation of samples/markers for Gel Electrophoresis

Vertical gel electrophoresis (BRL, USA) with discontinuous buffer system (Laemmli, 1970) was adopted. A 3.5% of stacking gel, 7.5 and 12.5% separating gels were prepared for analysis of structural proteins. A 50 µl sample buffer was added in the protein sample (purified resuspended viral suspension). A 3 µl of 0.2 % bromophenol blue was added and kept in water bath at boiling temperature for 2 minutes and cooled at room temperature. Six protein markers were selected as M1 and M2. M1 comprised of lysozyme and M2 comprising of bovine serum albumin, chicken egg albumin, trypsin, pepsin and beta glycosidase. Two mg of each (M1 and M2) was mixed with 200 µl of sample buffer separately. Three µl of 0.2 % bromophenol blue was added to each tube. These samples were loaded (20 µl) into the gel slots with Hamilton syringe. Electrophoresis was carried out at room temperature at 200 V for 6 hours till bromophenol dye travelled to about 1 cm from the bottom of the separating gel.

d) Staining and destaining

After electrophoresis, the gel was removed from the glass plates and subjected to the staining solutions (Coomassie brilliant blue, 0.1%) overnight. The destaining was done for 3 hours with agitation in destaining solution (Methanol, Glacial acetic acid).

RESULTS

The present study was conducted for comparing polypeptide profile of prevalent field isolates of NDV with the commercial vaccinal strains using SDS-PAGE. The haemagglutination titers of NDV strains causing disease were ranged from 1:16 to 1:64 and haemagglutination inhibition titers was 1:256. The isolates from each outbreak were grouped as A, B and C based on their haemagglutination titers. In SDS-PAGE (12.5%) using TE (20 mM tris HCl, 2mM EDTA, pH 6.8) buffer, all the field strains of NDV were almost similar in their polypeptide pattern. The field isolates presented eight polypeptide bands of Rf range 0.171 to 0.857, having molecular weight ranging from 181 to 12.5 KDa. In NTE (100 mM. NaCl, 20 mM Tris-HCl, 2mM EDTA, pH 6.8) buffer, the field isolates of NDV had five polypeptide bands with Rf range of 0.266 to 0.733 and molecular weight of 100 to 13.5, respectively. All the field strains were found similar in peptide pattern and any peculiar difference was not observed.

The SDS-PAGE for NDV strains suspended in TE buffer of gels (5-20% gradient) revealed that the polypeptide bands of all the vaccinal and field strains of NDV ranged from 89 to 16 KDa (Fig.1). The purified NDV vaccinal strain, electrophoresed through 7.5 per cent gel, migrated quickly and protien bands were concentrated at the bottom (Fig. 2).

The three field strains electrophoresed through 12.5% gel along with protein markers (Fig. 3). The vaccinal strains were electrophoresed through 12.5% gel and pattern was observed along with markers, the polypeptide bands of all the vaccinal and field strains of NDV ranged from 182 to 16 KDa. In lane 1 and 2 some of vaccinal strains were missing the protien of 182 Kda (Fig. 4).

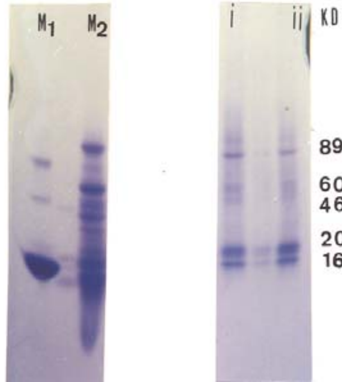

Fig 1: SDS-PAGE of NDV vaccinal strains suspended in TE buffer on gradient gel (5-20%)

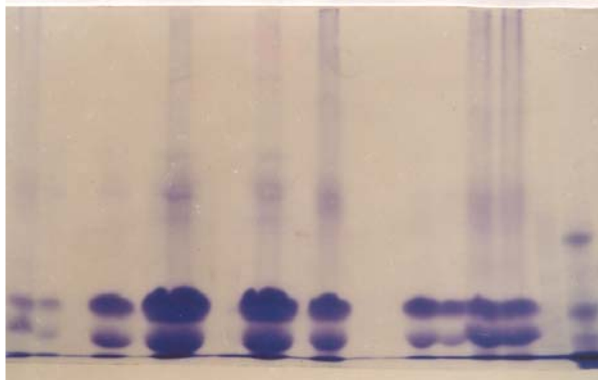

Fig 2: SDS-PAGE of NDV vaccinal strains elctrophoresed through 7.5% gel

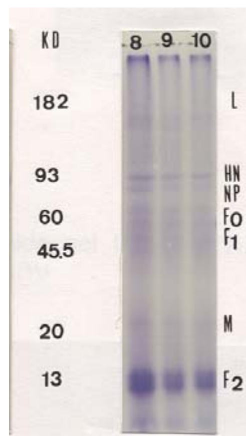

Fig 3: SDS-PAGE of isolated three field strains of NDV electrophoresed through 12.5% gel

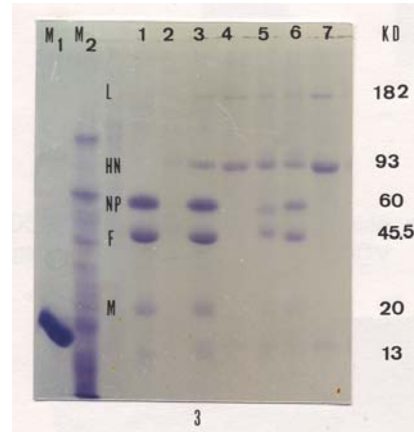

Fig 4: SDS-PAGE of NDV seven vaccinal strains elctrophoresed through 12.5% gel

DISCUSSION

Newcastle disease has an intricate state in the way that different isolates and strains of virus may provoke tremendous variation in the severity of the disease. Because of this factor vaccination programmes launched against ND in the past could not reach complete accomplishment for its control. Three field isolates obtained from various outbreaks, with variable mortality, did not indicate characteristic differences in the number and nature of protein fractions by SDS-PAGE. Seven major polypeptides (L, HN, NP, F_0, F1, M and F2) were present in all the field isolates as also reported by Nagy and Lominiczi (1984) and King and Seal (1997) and one contaminating protein "Actin" was identified, as also observed by Nagy and Lominizi (1984).

Regarding the number of proteins of virus with different mortality, Vijayarani *et al*. (1992) also observed almost similar peptide pattern. During replication of NDV, it is obligatory that the precursor glycoprotein F_0 to be cleaved into F1 and F2 for the progeny virus to be infectious (Rott and Klenk, 1988; Yu *et al*., 2002). The present study revealed that the virulent virus replicated in range of tissues and organs, their F_0 cleavage could be affected by wide range of proteases. The importance of F_0 cleavage was easily demonstrated, since viruses normally are unable to replicate or produce plaques in cell culture. While all viruses could replicate and produce infectious progeny in the allantoic cavity, the viruses pathogenic for chicken could replicate in wide rang of cell types in vitro. It was also reported by Lamb and Choppin (1978) that F1 polypeptide segment was only present in the infected cells. Other cleavage product F2 was poorly

examined by this technique of peptide mapping. The matrix protein of molecular weight of 20 KDa had no difference in the electrophoretic mobility of NP and M protein among influenza virus B, as reported by Nakamura *et al.* (1981). This matrix protein also elicited the formation of antibodies but not significant in inducing host immunity and has also been used for the typing of recombinant strains other than Paramyxo viruses (Peroulis-Kourtis *et al*., 2002).

The use of TE buffer in the present study for suspending NDV pellet, as described by Bolen *et al.* (1982), was useful and made equally high resolution as that of NTE buffer as described by Nagy and Lominiczi (1984). Different concentrations of gel used in this study for establishing the exact resolution percentage yielded sharp bands as also reported by Lamb and Choppin (1978). The purification of NDV strains by the sucrose gradient of 20-30% was likewise excellent in order to get purified virus pellet as reported by Wild *et el.* (1969).

It may be concluded that a pivotal difference in the peptide pattern of vaccinal strain existed that could be materialized to be the justification of vaccination failure. The absence of main immunogenic proteins may also weaken the vaccinal strains. However, the field isolates of NDV comprised of similar pattern along with the presence of immunogenic components. The present study also justified one of the major bottle necks in the way of successful protection of Newcastle disease.

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