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Polypeptide Mapping of Newcastle Disease Vaccine Virus

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Abstract

Seven commercially available Lentogenic vaccinal strains of Newcastle disease virus (NDV) were analysed for protein profiling by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Concentration of the NDV was done through sucrose gradient centrifugation. Out of seven lentogenic vaccines, only three contained six (6) polypeptides with molecular weights of 182, 93, 60, 45.5, 20 and 13.5 KDa approximately, three had four polypeptides with M.W. of 63, 42.5, 21 and 13 KDa and one showed only one peptide of 93 KDa approximately.

Key words: Newcastle disease, Polypeptide mapping

Introduction

Newcastle disease, despite of extensive vaccination programmes, along with the adaptation of hygiene and stock managemental techniques, is still among the top ranking fatal diseases of poultry. Measures to curtail it by vaccination have partially failed in Pakistan. Outbreaks of ND were recorded even in the poultry farms which were isolated and where proper vaccination/measure has been adopted, indicating that the control of ND was by no mean perfect. This was highly suggestive of some factors responsible for the outbreaks of this disease. These may include, environmental conditions, imported vaccines, lack of knowledge to administer and antigenic differences.

Antigenic differences have been reported among virulent and non-virulent strains of NDV using RNA oligonucleotide finger-printing (McMillan and Hanson, 1982), hemagglutination-elution pattern (Spalation *et al.*, 1970), antipeptide antibodies (Hodder *et al.*, 1993), SDS-PAGE (Nagy and Lominiczi, 1984), double diffusion (Guo *et al.*, 1987) and two dimensional electrophoresis (McMillan and Hanson, 1982). The present study was to illustrate the antigenic variation among different Lentogenic vaccinal strains of NDV using polypeptide mapping.

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Materials and Methods

Lentogenic Vaccines

Seven different marketed vaccines (Lentogenic Strain) were procured, these were:

- Delvax ND LaSota (Mycofarm Internatinal)
- Newcastle disease vaccine LaSota (VRI, Karachi)
- V.P. Vaccine Nobilis LaSota (Intervet Int., Holland)
- Newcastle disease vaccine (Vine-land lab, USA)
- Bio LaSota (Bioteke, Italy)
- Bio b1 (Bioteke, Italy)
- Sotasec (Rhone Merieux, France)

Concentration and Purification of Virus

Each vaccine was reconstituted in 5 ml Phosphate Buffer Saline (PBS) and centrifuged at 5000 x g for 20 minutes at 4°C in 30 ml tube in JA-20 Beckman rotor. The supernatant was collected and subjected to sucrose gradient centrifugation (Nagy and Lominiczi, 1984). Thirty percent sucrose solution (6 ml) was poured in 30 ml tube and kept at –20°C for two hours. A 20 percent sucrose solution (4 ml) was layered on it and kept at –20°C for one hour. A 5 ml volume of supernate fluid was layered on the sucrose gradient solution and centrifuged at 15,000 rpm for 3 hours at 4°C in JA-20 Beckman rotor (J2-HS model). A clear visible pellet of virus was obtained at the bottom of the tube, which was suspended in NTE buffer (100 mM NaCl, 20 mM tris-HCl, 2mM EDTA, pH 6.8) in Eppendorf tube (1ml) and stored at –70°C for further use.

Preparation of Samples

The samples were prepared by adding 50µl of 2x sample buffer to 50µl of protein sample (purified re-suspended viral suspension) in 1ml Ependrof tube. A three µl of 0.2% bromophenol blue was added and kept in water bath at boiling temperature for 2 minutes and cooled at room temperature.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Vertical gel electrophores is (BRL, USA) with discontinuous buffer system (Laemmli, 1970) was adopted. A 3.5% stacking gel and 12.5% separating gel were used for the analysis of structural proteins. Structural proteins of virus were dissociated by heating in boiling water with 2x sample buffer containing tris. HCl (pH 6.8), SDS, glycerol, bromoplenol blue and 2

mercaptoettanol. Electrophoresis was performed at room temperature at 200 volts for 6 hours. Gels were stained with Coomassie Brilliant Bule, destained by acetic acid + methanol and photographed. Relative morbility (Rf) for each polypeptide was calculated by the formula (Weber and Osborn, 1969):

$$Rf = \frac{\text{Distance of protein migration}}{\text{Distance of dye migration}}$$

A graph between Rf and log MW for the standards was plotted for the determination of viral peptides.

Results and Discussion

Newcastle disease has attained a complicated situation in the way that different isolates and strains of virus may induce enormous variation in the severity of disease. Different vaccination programes are in practice against Newcastle disease but a complete success could not be achieved as yet. No work has been done on the aspect regarding the monitoring of antigenic variation among different Newcastle disease virus strains in Pakistan that may be one of the possible causes of disease recurrence.

All the seven vaccinal lentogenic strains fragemented by SDS-PAGE showed different protein profiles. Among several protein fractions, the peptide of MW 182 KDa was found to be present in lane 3,4,5,6 and 7 of lentogenic strain, that resembled the Hemagglutinin-Neuraminidase (HN) protein in their electrophoretic mobility as reported by Bollen et al. (1982). The vaccine of Lasota strain in lane 2 showed only haemagglutinin (HN) protein that may show haemagglutination but may not be protective as described by Nagai et al. (1989). In few strains of NDV the HN protein is also produced as a precursor that requires cleavage to become biologically active and this proteolytic activation of the HN glycoprotein play role in virulence of the virus (Garten *et al.,* 1980).

The protein fractions with molecular weight of approximately 60 KDa was found to be present in lane 1,3,5 and 6 of lentogenic strain that is parallel in MW of Nucleoprotein (NP) as reported by Parker and Collier (1990).

The protein fractions with MW of 45 KDa were present in lentogenic vaccinal strain (lanes 1,3,5 and 6) that resemble the fusion protein (F) in their electrophoretic mobility as identified by Bollen et al. (1982). It is further reported that the ability to induce cell fusion for causing plaques determined the virulence of virus for which the presence of HN and F proteins are highly essential (Nagai *et al.*, 1980).

All the lentogenic strains were not cleaved into F1 and F2 because in lentogenic strains, cleavage can occur only with protease recognizing a single arginine amino acid. That were present in pairs in virulent viruses as described by Rott, 1979. Therefore in our studies it may be concluded that the vaccine lacking in their HN and F peptide bands may not be protective as reported in the birds.

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