A Comparative Study of New Castle Disease Virus Isolated From Poultry with Standard Velogenic Strain

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Abstract

Newcastle disease [avian paramyxovirus serotype 1 (APMV-1)] is highly prevalent in poultry thought India. The present study was undertaken to extrapolate the pathogenicity of a field isolate of Newcastle disease virus (NDV) in comparison with velogenic BOM-1 isolate (AY 294125). The field NDV isolate and the BOM-1 isolate were propagated in SPF eggs and the infected allantoic fluids were used for characterization of pathotype. The Mean Death Time (46 hours), Intracerebral Pathogenicity Index (1.74), Intravenous pathogenicity Index (2.01) were enumerated to find out the pathotype of the virus. The field NDV isolate was able to agglutinate mammalian erythrocytes except equine RBCs, stable at 56°C for 60 mins and proved to be a rapid eluter. It formed plaques (5.39 x 10⁶ PFU ml) in chick embryo fibroblast with characteristic cytopathic effects of velogenic NDV comparable with BOM-1 isolate. The viral proteins that were detected by SDS-PAGE were VN (76 KD), F (66 KD), NP (55 KD) and M (44 KD). The total protein in the field virus isolate was determined to be 17.85 mg%, which was lower than that of the total proteins in BOM-1 isolate (45 mg%). The field virus contained 28.83 mg% of total lipids and 4.98 mg% total carbohydrates, which were more or less similar to that of BOM-1 isolate and that of allantoic fluid. On the basis of all the biological and the chemical tests conducted, the field virus isolate included in this present study was characterized as velogenic viscerotropic strain of NDV.

Keywords: New castle disease virus; Pathotype; Velogenic viscerotropic strain; Specific pathogen free embryonated eggs; Chick embryo fibroblast; Cytopathic effect

Introduction

Newcastle disease (ND) is a severe avian disease of high prevalence and great economic importance in India. It is caused by avian paramyxovirus serotype 1 (APMV-1), which shows variable pathogenicity and severity depending on the virulence of its pathotypes [1]. ND is a single greatest constraint on the production of village poultry [2-4]. Serological surveys indicate the presence of the virus in village poultry in countries throughout Asia and Africa, and where virus isolation has been attempted; virulent strains have been found [5]. Geographical distribution of NDV is often confused due to the indiscriminate use of live vaccines in most of the countries throughout the world [6]. Therefore, zone wise proper characterization of the strains of NDV with effective and validated pathotyping is required for planning of an effective control programme against this disease. In this perspective, the present study was carried out using several pathotype determining tests which were conducted to characterize a field NDV isolate of poultry in comparison with a standard velogenic strain of NDV (“BOM-1 isolate” bearing accession No. AY294125).

Materials and Methods

Viruses

NDV was isolated from a severe outbreak at a poultry farm (mortality-89.13%) in the Konkan (latitude-17°N and longitude- 73°19’E) region of Maharashtra in the year 2007. The spleen and liver of the dead birds were aseptically collected in sterile antibiotic supplemented medium (MEM-199, Sigma) and dispatched in chilled condition to the Department of Microbiology, Bombay Veterinary College. The samples were aseptically triturated with Phosphate buffer solution and a 10% suspension was made. Thereafter, the solution was passed though syringe filter (pore size 0.22 µm) and 0.1 ml of the filtrate was inoculated aseptically in 10 days old embryonated Specific pathogen free (SPF) eggs (Venkateshwara Hatcheries Ltd., Pune). The eggs were incubated at 37°C for virus propagation. After the death of the embryos allantoic fluid was aseptically collected, pooled, and stored in -20°C for further use. BOM-1 isolate (accession No. AY294125) was obtained from Department of Microbiology, Bombay veterinary college. R_B-Mukteshwar and LaSota Standard Vaccine Stains were purchased from Hester pharmaceuticals Ltd, India.

Detection of viral virulence

The infected allantoic fluid was subjected to Haemagglutination test and further embryo infective dose of the virus was estimated. The mean death time (MDT) of the virus was calculated in SPF chick embryos of ten days age according to the method of Hanson and Brandley [7]. The intra cerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI) were enumerated according to the method of Allan et al. [8] in SPF chicks (Gallus domesticus). The chicks used in the experiment of ICPI and IVPI were day-old and eight-weeks-old.
develop on sterile tissue culture grade coverslips in growth medium.

Temporal development of the CPE of field isolate keeping the BOM-I effects (CPE) was carried out. The test was conducted for studying the neuraminidasae activity of the field virus isolate was assessed by testing the haemagglutination elution patterns in which the time required by the virus to completely elute from the agglutinated chicken erythrocytes was recorded at 4°C. The ability to agglutinate mammalian erythrocytes was checked by using 1% suspension of erythrocytes from guinea pig, sheep, goat, cow, rabbit, horse and 0.5% suspension of pig erythrocytes. Plaque formation on primary chick embryo fibroblast cell culture was used to differentiate between the strains of the virus. Plaque assay was carried out as per method of Barahona and Hanson [9] for the demonstration and titration of NDV in primary chicken embryo fibroblast (CEF) culture in a 24 well tissue culture plate. Tenfold dilution (10⁻¹ to 10⁻¹³) of the suspension of erythrocytes from guinea pig, sheep, goat, cow, rabbit, and plaques were observed for their differential characteristics under an inverted light microscope.

Comparative cytopathogenicity study on primary chick embryo fibroblast culture (PCEF)

Comparative observations of CPE in different time intervals in Chick embryo fibroblast monolayers infected with Field Virus isolate and BOM-1 isolate.

| Test viruses | H.A titer | EID₅₀ u/ml | MDT hours | ICPI | IVPI | Thermostability of Haemagglutinins | Rate of Elution | Agglutination of mammalian RBC | PFU in CEF (PFU/ml) |
|--------------|-----------|------------|------------|------|------|-----------------------------------|----------------|-------------------------------|-------------------|
| Field virus isolate | 64       | 10⁻⁷/⁻¹⁴ | 46         | 1.74 | 2.01 | inactivated at 60°C within 15 minutes | rapid eluter | Agglutinates mammalian RBC except equine RBC | 5.39 x 10⁶ |
| BOM-I isolate | 512       | 10⁻⁷/⁻³  | 38         | 1.8  | 2.63 | inactivated at 60°C within 30 minutes | rapid eluter | Agglutinates mammalian RBC except equine RBC | 6.35 x 10⁶ |

Table 1: Showing the comparison of the biological characteristics of field virus isolate and BOM-I isolate.

| SI no. | Time intervals of observation | Cytopathic effects in CEF from 0 hours PI to 96 hours PI |
|--------|------------------------------|--------------------------------------------------------|
| 1      | 0 hour                       | No CPE observed                                        |
| 2      | 12 hours                     | No CPE observed                                        |
| 3      | 24 hours                     | Rounding of cells, small cytoplasmic vaculations        |
| 4      | 36 hours                     | Variable sizes of plaques, discrete clumping of cells.  |
| 5      | 48 hours                     | Formation of Syncitia, Number and diameter of plaques increased. |
| 6      | 60 hours                     | Mild coalescence of plaques.                           |
| 7      | 72 hours                     | Large syncitia observed, Disintegration of cell sheet started. |
| 8      | 84 hours                     | Large cell clumps, Disorganization of monolayer.        |
| 9      | 96 hours                     | Absence of cellular morphology and cell organization    |

Table 2: Comparative observations of CPE in different time intervals in Chick embryo fibroblast monolayers infected with Field Virus isolate and BOM-I isolate.

The molecular weights of the electrophoretic protein fractions of freeze dried field NDV isolate were studied by sodium dodecyl sulphate-polyacrylamide gel electrophoresis as per the method of Moore and Burke [10]. The total proteins, lipids and carbohydrates of the freeze dried viruses were estimated by modified Lowry’s method [11], Knight method [12] and Ada and Gottschalk method [13] respectively. Further, all of the above stated chemical properties of the field virus isolate of NDV were compared with BOM-1 isolate, R,B and LaSota. All the chemicals used in these tests were purchased from Bangalore Genie and S.D. Fine Chemicals limited.

Results

Comparison of pathogenicity and biological properties of the viruses

The results of haemagglutinating titers, embryo infective dose, MDT, ICPI, IVPI, thermostability of haemagglutinin, rate of elution, agglutination of mammalian erythrocytes and plaque forming unit per ml of the propagated field virus isolate and the BOM-1 isolate are shown in Table 1.

The field virus isolate and the BOM-1 Isolate was capable to agglutinate the erythrocytes of guinea pig, sheep, goat, cow, rabbit, and pig except equine RBCs. The field virus isolate and BOM-1 isolate exhibited elution after 33 minutes and 29 minutes respectively. After resuspension of erythrocytes at 24 hours, elution was recorded after 75 minutes and 55 minutes respectively for Field virus and BOM-1 isolate. The field virus isolate was found to be capable of producing plaques of diverse morphology on chick embryo fibroblast similar to those produced by the velogenic strain of BOM-1 isolate. The plaques
generated by the field virus isolate were large and irregular in shape whereas some showed minute clearings of the cells. The results of the comparative study of replication of the field virus isolate and BOM-I (control) on the basis of cytopathic effects in CEF culture stained with May-Grüenwald Giemsa and Crystal violet stain (Figures 1-6).

The characteristic cytopathic effects which were recorded in the infected CEF monolayer from 0 hours PI to 96 hours PI are shown in Table 2. However, the uninfected CEF monolayer which was maintained as negative control throughout the experiment remained intact till 96 hours PI.

**Comparative chemical characteristics of the viruses**

The field NDV isolate, BOM-I isolate, R_B and LaSota showed four prominent protein fractions (Figure 7) in SDS-PAGE. In all the NDV strains subjected to present protein fraction analysis study, three major proteins viz. haemagglutinin-neuraminidase (HN), fusion (F) protein and nucleoprotein (NP) were identified. The molecular weights of these proteins were 76 KD, 66 KD and 55 KD respectively. The matrix (M) protein was identified as minor protein (MW 42 KD), which is in accordance with Kumanan et al. [14].

The estimation of total proteins, lipids and carbohydrates of NDV field isolate in comparison with BOM-1 isolate, LaSota, R_B and uninfected allantoic fluid is shown in (Figure 8).

**Discussion**

The values of MDT, ICPI and IVPI suggest that the field virus isolate was a velogenic strain of NDV. Further these results are in conformity with the positive control (velogenic BOM-1 isolate) and meet the specifications of OIE [15]. In both ICPI and IVPI tests all chicks except the controls showed characteristic symptoms resembling velogenic viscerotropic strain of NDV. The chicks subjected to ICPI revealed torticollis, paralysis of legs and weakness prior to death. Whereas,
pinpoint haemorrhages in the proventriculus and caecal tonsils were observed in both of the groups (intracerebrally inoculated groups in ICPI and intravenously inoculated groups in IVPI). These findings are in conformity with Pant et al. [16] and Vijayarani et al. [17]. Further MDT, ICPI and ICPI values indicate that the field virus isolate was less virulent than BOM-1 isolate. From the results of thermostability of haemagglutinin, the field virus isolate was found to be thermostable as velogenic BOM-1 isolate of NDV. For instance, the velogenic isolates characterized by Roy et al. [18] remained thermostable for at least 90 minutes whereas the lentogenic isolates, characterized by King and Seal [19], lost their haemagglutinating activity within 15 minutes. On the basis of haemagglutination elution patterns, the virus can be classified as a “rapid eluter”, indicating higher neuraminidase activity. As the rate of elution appeared to be independent of other viral properties, it can serve as a marker for differentiating strains of NDV [20]. Diversities in plaque morphology in NDV infected PCEF monolayers is a distinct characteristic of velogenic stain of NDV as observed by Schloer and Hanson [21].

On the basis of the results of pathotype specific tests viz. MDT, ICPI, IVPI, agglutination of mammalian RBCs, thermostability of haemagglutinins, rate of elution and ability to form plaques in CEF, the field isolate of NDV in the present study can be classified as a velogenic Viscerotropic strain. The field isolate was found to be a less virulent than the standard velogenic isolate BOM-1. The present study clearly demonstrates that MDT, ICPI and IVPI are most efficient tools for strain differentiation of NDV in epidemiological point of view. The comparative pathobiology of field NDV isolate and BOM-1 isolate in gallinaceous poultry was clearly correlated with biochemical properties of virus and cytopathogenicity in PCEF.

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