CHARACTERIZATION OF NEWCASTLE DISEASE VIRUS ISOLATES FROM CAGED BIRDS IN BANGLADESH


Department of Microbiology and Biotechnology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh -2202, Bangladesh.

ABSTRACT

Two isolates of Newcastle disease virus from two different caged birds, one from a parrot and another from a kakatua were characterized during the period from July 2001 to October 2002. In HA tests both the isolates hemagglutinated chicken red blood cells at 1:256 and showed agglutination pattern but the parrot isolate was found to be hemagglutinating type (HA) and avian erythrocytes and the kakatua isolate to bovine erythrocytes. In EID50 tests, the parrot isolate was found to be a rapid cluser and the kakatua isolate as slow cluser. Both the isolates were found to be net-susceptible in relation to haemagglutinating activity and embryotoxic infectivity. The mean death time (MDT) of the parrot and kakatua isolates were 35.6 hours and 117.6 hours and the intracerebral pathogenicity indices (ICPI) were 1.38 and 0.45 respectively. So, the parrot isolate was found to be virulent type 1 and the kakatua isolate as the lethally strain of Newcastle disease virus.

Key words: Caged bird, NDV, mean death time, intracerebral pathogenicity index, haemagglutination test, elution test.

INTRODUCTION

Newcastle disease (ND) is a highly contagious disease that attacks many species of domestic and wild birds (Garth et al., 2002). This disease is caused by a single stranded RNA virus known as avian paramyxovirus belonging to the family Paramyxoviridae (Scholtissek and Bittl, 1994; Garth et al., 2000). The disease is characterized by sudden appearance and rapid spread within a flock resulting in high morbidity and mortality. It may cause 100% mortality in young chicks and 80-90% in adults (Calnek et al., 1997). In Bangladesh, the caged birds are closely associated with domestic fowls and the occurrence of the disease in these type of birds may be active source of infection for domestic fowls. Suitable control programmes often appear difficult to take because of lack of knowledge about the strains of Newcastle disease virus (NDV) that occurs in caged birds. Therefore, an attempt was made to study the biological, physical and pathological properties of two isolates of NDV from caged birds in Bangladesh for the ease of taking control programmes of the disease.

MATERIALS AND METHODS

Four strains of NDV including two field isolates from caged birds and two reference strains were used in this study.

Among the field isolates, one was isolated from a 'parrot' and another from a 'kakatua' from the Dharra Zoo, Mirpur, Dhaka, during 1998 and 2000 respectively. The parrot isolate was termed as Parrot Newcastle disease virus (PDV) and the 'kakatua' isolate was termed as Kakatua Newcastle disease virus (KNDV). The two reference virus strains, lentiogenic 'Blackburg' (B.l.) and the mesogenic 'Kwajalein' (K.K.), were obtained from the repository of the Department of Microbiology and Biotechnology, BAU, Mymensingh.

A total of 300 fertile eggs of White Leghorn (WLH), 'Lohia' breed chicken was purchased from the BAU Poultry Farm. These eggs were incubated at 37°C under steady humidity of 85% for 9 to 11 days and the well developed viable embryos were used in the experiment. Two hundred-day-old chicks of 'Stutto' breed were purchased from the Mirpur Government Poultry Farm, Mirpur, Dhaka, which were used for the determination of intracerebral pathogenicity indices (ICPI) of the virus isolates / strains.

Chick embryo red blood corpuscle (ECRC) suspensions, guinea pig red blood corpuscle (GRC) suspensions, bovine red blood corpuscle (BCRC) suspensions, canine red blood corpuscle (CRC) suspensions, and equine red blood corpuscle (ERCBC) suspensions of different concentrations, 2%, 4%, 6%, 8% and 10% were prepared and boiled at 56°C for 30 minutes for PRR test. Five ml of each suspension were used and tested at 4°C and 37°C for 24 hours and the results were compared with those of a sample which was incubated at 4°C and used for three days each time. For slide HA test 2% suspension of EBC were used while for HI test 0.5% of RBC were used.

Slide and tube haemagglutination (HA) tests were carried out following the method described by Atta, (1971) and Stephen et al. (1975) to determine the presence of NDV in the amniotic fluids (AF) of the infected embryos and the HA titer of the viruses in AF.

The virus test (ET) was performed both at room temperature and at 4°C following the procedure described by Spalton et al. (1970).

The NDV isolates / strains were subjected to thermal treatment at 50°C and at 56°C for varying period of time to study the thermal stability of haemagglutinins and infectivity in chicken embryos. The test was carried out following the procedure described by Fiser and Thompson (1957). Chicken embryos were used for testing the infectivity of viruses after heat treatment. The embryo lethal dose 50% (ELD50) of virus isolates / strains was calculated following the standard method of Reed and Muench (1938). The same procedure was followed for each NDV isolate.
The calculation of mean death time (MDT) was performed in developing 10 days chick embryo following the procedure of Swaye et al. (1998). The same procedure was adopted for each test NDV isolate as per the following formula.

\[
\text{MDT} = \frac{\text{(Number dead at 'A' hours} \times \text{A' hours}) + \text{(Number dead at 'B' hours} \times \text{B' hours}) + \text{etc.}}{\text{Total number of dead}}
\]

Determination of ICP in day-old chicks was conducted following the procedure of Swaye et al. (1998). Same procedure was followed for each test NDV isolate as per the following formula.

\[
\text{ICP} = \frac{\text{(No. of observation as dead} \times \text{Numerical value}) + \text{(No. of observation as Symptom of illness} \times \text{Numerical value})}{\text{(No. of observation as normal} \times \text{Numerical value})}
\]

RESULTS AND DISCUSSION

The biological and physical properties such as HA activity, death pattern and heat sensitivity and the pathogenicity indices such as ELD₅₀, MDT, ICP of the NDV isolates from patten of birds were performed in this study. Thus, the isolates recovered from "Kakata" (KNDV) and "pattar" (PNDV) were studied in respect of these properties and the properties were compared with those of the selected standard strains B, (Blackburg) and K (Komatyn) of NDV. HA (Table 1) activity of the two strains (B, and K) and the two isolates of NDV were tested with the RBC of chicken, guinea pig, cattle and horse. It was observed that both the strains of NDV and the two virus isolates agglutinated RBC of chickens and guinea pig but bovine, RBCs were agglutinated only by B virus and equine RBCs were agglutinated only by KNDV and K virus, but not by PNDV and B virus (Table 1). Winslow et al. (1950) reported that the erythrocytes of man, mouse and guinea pig were agglutinated by all strains of NDV. The author also observed that RBC of cattle, goat, sheep, swine and horses were agglutinated to some but not all strains of NDV. On the other hand Clark and Naylor (1943) stated that while erythrocytes of all amphibian, reptiles and birds were agglutinable to some degree by NDV, some mammalian erythrocytes were unagglutinable. In the present study, the results of HA test correlated with the findings of Winslow et al. (1950) and Clark and Naylor (1943). Hanson and Splatin (1978) mentioned that the RBC of certain individual cattle might be agglutinated by one NDV strain while the cells of another individual were not. In this study, erythrocytes from only one individual were used which was found to be agglutinated by the virus strain B.

Table 1. Demonstration of biological, physical and pathogenic properties of NDV from caperl birds

<table>
<thead>
<tr>
<th>Virus isolates / strains</th>
<th>HA time (h)</th>
<th>ELD₅₀ (h)</th>
<th>25°C At 30°C</th>
<th>37°C At 50°C</th>
<th>Stability of EAA</th>
<th>Stability of EAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kakata (KNDV) 640 640</td>
<td>22 24</td>
<td>15 15</td>
<td>15 15</td>
<td>15 15</td>
<td>17.7 7.5</td>
<td>0.45</td>
</tr>
<tr>
<td>Pampu (PNDV) 640 640</td>
<td>2 8</td>
<td>15 15</td>
<td>15 15</td>
<td>15 15</td>
<td>7.6 5.8</td>
<td>1.58</td>
</tr>
<tr>
<td>Blackburg (B) 640 640</td>
<td>5 5</td>
<td>10 15</td>
<td>5 5</td>
<td>5 5</td>
<td>120 0.0</td>
<td>1.11</td>
</tr>
<tr>
<td>Komar (K) 320 320</td>
<td>13 22</td>
<td>15 15</td>
<td>5 5</td>
<td>5 5</td>
<td>84.3 1.1</td>
<td>1.11</td>
</tr>
<tr>
<td>cRBC</td>
<td>Guinea pig red blood corpuscle, hRBC = Guinea pig red blood corpuscle, bRBC = Bovine red blood corpuscle, cRBC = Equine red blood corpuscle, EAA = Embryo allantoic activity, E = Hemagglutination, ELD₅₀ = Embryo lethal dose fifty, MDT = Mean death time, ICP = Intracerebral pathogenicity Index.</td>
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</table>
Characterization of NDV from caged birds

The NDV test isolates were also investigated for the determination of their potency in relation to their HA activity using RBC of chicken and other species of animals which were found positive in slide HA test. It was observed that while complete HA with chicken RBC was detected within 30 minutes; such HA with RBC of guinea pig, cattle and mice could not be ascertained before one hour of incubation at room temperature. With chicken RBC, the HA titers recorded for B1 and K strain were 640 and 320 respectively in comparison to the HA titers of 640 and 160 respectively for KNDV and PNDV isolates (Table 1). Similar HA titers for all virus strains/isolettes were also observed with guinea pig RBC. With RBC of cattle, the HA titer of B1 was 640 which was similar to that of chicken and guinea pig RBC. With horse RBC, HA titers of KNDV and K virus were 640 and 320 respectively which were similar to those of RBC of chicken and guinea pig. The HA titer (640) of KNDV isolate was always similar to its HA titer of B1 strain.

The elution pattern of the two virus isolates and the two standard strains from chicken erythrocytes were studied hourly (interval Table 1). Spalatin et al. (1970) reported that elution pattern at higher temperature was too fast or erratic, but at 4°C the elution pattern was found to remain unaltered for more than two replicates. In this study, the elution patterns of the virus isolates/strains were studied both at room temperature and at 4°C to make a comparison between the elution patterns at both temperatures.

Spalatin et al. (1970) differentiated between rapid and slow eluters on the basis of two observations such as viruses which showed complete elution within 24 hours as contrast to the failure to do so and the failure to haemagglutinate after resuspension at 34 hours as contrast to haemagglutinate. The complete elution of the KNDV isolate occurred at 22 hours and 30 hours at room temperature (25°C) and at 4°C respectively. On the basis of the elution at room temperature the virus may be classified as rapid eluter but on the basis of elution at 4°C the virus may be classified as slow eluter. The PNDV isolate may be grouped as rapid eluter on the basis of its elution both at room temperature (25°C) and at 4°C (Table 1). B1 and K strains were found completely eluted after 3 and 13 hours of haemagglutination respectively at room temperature (25°C) and after 10 and 22 hours respectively at 4°C. These viruses may be grouped as rapid eluters which correlate with the report of Spalatin et al. (1970). From Table 1, it is observed that elution rate of the test viruses is too rapid at room temperature (25°C) compared to that at 4°C. This correlates with the report of Spalatin et al. (1970).

From the Table 1, it is also observed that KNDV virus retained its HA activity and embryoviricity for 30 minutes at 56°C whereas for 15 minutes at 56°C. On the other hand, PNDV isolate retained its HA activity for 15 minutes at 56°C but its embryoviricity was retained upto 30 minutes of the same temperature. At 56°C, the HA activity of the PNDV isolate retained for 5 minutes and embryoviricity for 15 minutes revealing that this isolate (PNDV) was less thermostable than the (KNDV) isolate.

Both B1 and K strains were found thermostable for 15 minutes at 56°C in respect of HA activity but embryoviricity of the B1 virus was found stable upto 30 minutes of the same temperature. At 56°C, both HA activity and embryoviricity of B1 strain were found stable for 5 minutes, and at the same temperature HA activity of K strain was found stable for 5 minutes but its embryoviricity up to 15 minutes (Table 1).

The pattern of the inactivation of embryoviricity and HA activity due to thermal treatment are somewhat different between the observations reported by Yamada (1981) and Dinggoa et al. (1970). Yamada (1981) studied leucocytopathogenicity of NDV by inactivation of HA and embryoviricity in case of heating at 56°C. In this study similar results were found in case of PNDV, B1 and K viruses. NDV strains are generally recognized as "heat stable" virus when they retain their activities for 30 minutes at 56°C. On this basis both the KNDV and PNDV isolates may be classified as heat unstable as the selected B1 and K strains are.

The results presented in the Table 1 revealed that the log(E/LD50) of 0.1 ml of allantoic fluids of KNDV and PNDV isolates were 10.6 and 10.6 respectively and those of B1 and K strains were 10.6 and 10.6 respectively. Thus it is observed that E/LD50 of K, KNDV and PNDV isolates has similarity to each other.

Among the test NDV isolates, KNDV was found to be lethagic and the rest of PNDV as velogenic on the basis of MDt, and on the basis of the reference B1 and K strains were found to be, as these are, lethagic and velogenic respectively (Table 1). Hanson and Brandly (1955) reported that the velogenic strains of NDV killed the embryos in about 60 hours, mesogenic strains killed in 60 to 90 hours while the lethagic strains killed in more than 100 hours. In this study, the MDT of the KNDV isolate was found to be 117.6 hours which is similar with that of lethagic strains. The MDT of PNDV isolate was found to be 57.6 hours. This result was quite different from that found by King and Seal (1998) who found that a parrot isolate of NDV as lethagic on the basis of MDT in embryos, by ICP and / or intravenous pathogenicity index. In the present study MDT of the parrot isolate (PNDV) was found to be (57.6 hours) like velogenic (Table 1). The MDT of B1 was found to be 120.8 hours in the present study which is similar with the Lethagic variant (Allens et al., 1978) while that of the K strain was 84.3 hours as was also recorded by Samuel et al. (1979). 115
Reports of Hanson and Brandly (1955) stated that meningococcal and velogenic strains of NDV usually kill all of the chicks within a period of 4 days and the tetragenic strains rarely kill the chicks under the conditions of the ICPI test. Swaine et al. (1998) reported that the NDV strains demonstrating ICPI values from 1.5 to 2.0 may be classified as velogenic, the strains demonstrating ICPI values from 0.0 to 1.5 may be classified as mesogenic and finally the strains demonstrating ICPI values from 0.0 to 0.5 may be classified as lentogenic strains. The results of the ICPI tests obtained in the present study (Table 1) indicated that lentogenic isolate with ICPI of 0.45 falls in the group of lentogenic strain of NDV and the PDNV isolate having ICPI of 1.58 may be classified as velogenic. The results of ICPI tests of E, K and K strains with ICPI of 0.0 and 1.11 proved those to be lentogenic and tetragenic respectively.

The ICPI of the 'kakao' isolate (KDNV) is found to be 0.45 which is almost similar to the ICPI of NDV lentogenic strain LaSota. Allan et al. (1978) reported that the ICPI and MDT of the LaSota strain are respectively 0.4 and 103 hours. In the present study both ICPI and MDT of the KDNV isolate are found to be greater than those of LaSota strain. Thus, it may be suggested that KDNV isolate is more neuro-pathogenic than the LaSota strain of NDV. The ICPI of the parrot isolate of NDV (PDNV) is noted to be 1.58 a characteristic of velogenic viruses, which is similar with the report of Allan et al. (1978) who observed that the ICPI and MDT of a parrot isolate of NDV "NY parrot 1974" was 1.8 and 31 hours respectively, whereas King and Seal (1998) found a parrot isolate to be lentogenic on the basis of its ICPI and MDT values. Thus the PDNV isolate is less pathogenic than the velogenic "NY parrot 7011972" strain, though as consideration of ICPI it may be grouped as velogenic strain. The ICPI of the B1 virus is recorded as 0.0 (Table 1) and the virus failed to kill any chicks or to produce any symptoms of the disease. However, ICPI value was lesser than that reported by Swaine et al. (1998) which can not be explained. As regards to the strain K, the ICPI is found to be 1.11, which is a characteristic of mesogenic viruses. As the report of Calnek et al. (1997), the ICPI of K strain is 1.4, but in this study, the K strain has been found less pathogenic which needs further investigation.

REFERENCES