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CHARACTERIZATION OF NEWCASTLE DISEASE VIRUS ISOLATES FROM CAGED BIRDS IN BANGLADESH

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ABSTRACT

Two isolates of Newcastle disease virus from two different caged birds, one from a parrot and another from a kakatoa were characterized during the period from July 2001 to October 2002. In HA tests both the isolates haemagglutinated chicken and guineapig erythrocytes but the parrot isolate was found refractory to bovine and equine erythrocytes and the kaktoa isolate to bovine erythrocytes. In elution tests, the parrot isolate was found to be a rapid eluter and the kakatoa isolate as slow eluter. Both the isolates were found to be heat–unstable in relation to haemagglutinating activity and embryo–infectivity. The mean death time (MDT) of the parrot and kakatoa isolates were 57.6 hours and 117.6 hours and the intracerebral pathogenicity indices (ICPI) were 1.58 and 0.45 respectively. So, the parrot isolate was found as the velogenic strain and the kakatoa isolate as the lentogenic 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 (Garib *et al.*, 2003). The disease is caused by a single stranded RNA virus known as avian paramyxovirus belonging to the family *Paramyxoviridae* (Scholtissek and Rott, 1964; Garib *et al.*, 2003). The disease is characterised by sudden appearance and rapid spread within a flock resulting in high morbidity and mortality. It may cause 100% mortality in young chickens 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 'kakatoa' from the Dhaka Zoo, Mirpur, Dhaka, during 1999 and 2000 respectively. The parrot isolate was termed as Parrot Newcastle disease virus (PNDV) and the 'Kakatoa' isolate was termed as Kakatoa Newcastle disease virus (KNDV). The two reference virus strains, lentogenic 'Blacksburg (B_1)' and the mesogenic 'Komarov (K)', were obtained from the repository of the Department of Microbiology and Hygiene, BAU, Mymensingh.

A total of 300 fertile eggs of White leghorn (WLH) breed chicken was purchased from the BAU Poultry Farm. These eggs were incubated at 37^{0}C under steady humidity of 85% for 9 to 11 days and the well developed and viable embryos were used in the experiment. Two hundred day-old chicks of 'Sonali' 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.

Chicken red blood corpuscle (cRBC) suspensions, guineapigs red blood corpuscle (gRBC) suspensions, bovine red blood corpuscle (bRBC) suspensions, and equine red blood corpuscle (eRBC) suspensions of different concentration (2% and 0.5%) were prepared. A considerable volume of stock RBC suspension was made and preserved at 4^{0}C and used for three days each time. For slide HA test 2% suspensions of RBC while for tube HA test 0.5% of RBC were used.

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

The elution test (ET) was performed both at room temperature and at 4^{0}C following the procedure described by Spalatin *et al.* (1970).

The NDV isolates / strains were subjected to thermal treatment at 50^{0}C and at 56^{0}C for varying period of time to study the thermostability of haemagglutinins and infectivity in chicken embryos. The test was carried out following the procedure described by Foster and Thompson (1957). Chicken embryos were used for testing the infectivity of viruses after heat treatment. The embryo lethal dose fifty (ELD_{50}) of virus isolates / strains was calculated following the standard method of Reed and Muench (1938). The same procedure was followed for each test NDV isolate.

The calculation of mean death time (MDT) was performed in developing 10 days chicken embryos following the procedure of Swayne *et al.* (1998). The same procedure was adopted for each test NDV isolate as per the following formula.

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

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

$$ICPI = \frac{\text{(No. of observation as dead} \times \text{Numerical value) + (No. of observation as Symptom of illness} \times \text{Numerical value) + (No. of observation as normal} \times \text{Numerical value)}}{\text{Total number of observation}}$$

RESULTS AND DISCUSSION

The biological and physical properties such as HA activity, elution pattern and heat sensitivity and the pathogenicity indices such as ELD_{50}, MDT, ICPI of the NDV isolates from psittacine birds were performed in this study. Thus, the isolates recovered from 'kakatoa' (KNDV) and parrot (PNDV) were studied in respect of these properties and the properties were compared with those of the selected standard strains B_1 (Blacksburg) and K (Komarov) of NDV.

HA (Table 1) activity of the two strains (B_1 and K) and the two isolates of NDV were tested with the RBC of chicken, guineapig, cattle and horse. It was observed that both the strains of NDV and the two virus isolates agglutinated RBC of chickens and guineapig but bovine, RBCs were agglutinated only by B_1 virus and equine RBCs were agglutinated only by KNDV and K virus, but not by PNDV and B_1 virus (Table 1). Winslow *et al.* (1950) reported that the erythrocytes of man, mouse and guineapig were agglutinated by all strains of NDV. The author also observed that RBC of cattle, goat, sheep, swine and horses were agglutinated by some but not by all strains of NDV. On the other hand Clark and Nayler (1943) stated that while erythrocytes of all amphibian, reptiles and birds were agglutinable to some degree by NDV, some mammalian erythrocytes were inagglutinable. In the present study, the results of HA tests correlated with the findings of Winslow *et al.* (1950) and Clark and Nayler (1943). Hanson and Spalatin (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 cattle were used which was found to be agglutinated by the virus strain B_1.

Table 1. Demonstration of biological, physical and pathological properties of NDV from caged birds

Virus isolates / strains	HA titre				Elution time (h)		Thermostability (minutes)				Log_{10} ELD_{50}/ 0.1 ml	MDT (h)	ICPI
	cRBC	gRBC	bRBC	eRBC	At 25°C	At 4°C	At 50°C Stability of HAA	At 50°C Stability of EI	At 56°C Stability of HAA	At 56°C Stability of EI			
Kakatoa NDV (KNDV)	640	640	–	640	22	30	30	30	15	15	$10^{7.32}$	117.6	0.45
Parrot NDV (PNDV)	160	160	–	–	2	8	15	30	5	15	$10^{7.67}$	57.6	1.58
Blacksburg (B_1)	640	640	640	–	3	10	15	30	5	5	$10^{8.68}$	120.8	0.0
Komarov (K)	320	320	–	320	13	22	15	15	5	15	$10^{7.84}$	84.3	1.11

cRBC = Chicken red blood corpuscle, gRBC = Guineapig red blood corpuscle, bRBC = Bovine red blood corpuscle, eRBC = Equine red blood corpuscle, HAA = Haemagglutination activity, – = No haemagglutination, EI = Embryo-infectivity, ELD_{50} = Embryo lethal dose fifty, MDT = Mean death time, ICPI = Intracerebral pathogenicity index.

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 guineapig, cattle and mice could not be ascertained before one hour of incubation at room temperature. With chicken RBC, the HA titres recorded for B_1 and K strain were 640 and 320 respectively in comparison to the HA titres of 640 and 160 respectively for KNDV and PNDV isolates (Table 1). Similar HA titres for all virus strains / isolates were also observed with guineapig RBC. With RBC of cattle, the HA titre of B_1 was 640 which was similar to that of chicken and guineapig RBC. With horse RBC, HA titres of KNDV and K viruses were 640 and 320 respectively which were similar to those of RBC of chicken and guineapig. The HA titre (640) of KNDV isolate was always similar to the HA titre of B_1 strain.

The elution pattern of the two virus isolates and the two standard strains from chicken erythrocytes were studied at hourly interval (Table 1). Spalatin *et al.* (1970) reported that elution pattern at higher temperature was too fast or erratic, but at 4^0C the elution pattern was found to remain unaltered for more than two replica test. In this study, the elution patterns of the virus isolates / strains were studied both at room temperature and at 4^0C 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 that showed complete elution within 24 hours as contrast to the failure to do so and the failure to haemagglutinate after resuspension at 24 hours as contrast to haemagglutinate. The complete elution of the KNDV isolate occurred at 22 hours and 30 hours at room temperature (25^0C) and at 4^0C 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 4^0C 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 (25^0C) and at 4^0C (Table 1). B_1 and K strains were found completely eluted after 3 and 13 hours of haemagglutination respectively at room temperature (25^0C) and after 10 and 22 hours respectively at 4^0C. These viruses may be grouped as rapid eluters which correlate with the report of Spalatin *et al.* (1970). From the Table 1, it is observed that elution rate of the test viruses is too rapid at room temperature (25^0C) compared to that at 4^0C. This correlates with the report of Spalatin *et al.* (1970).

From the Table 1, it is also observed that KNDV virus retained it's HA activity and embryo activity for 30 minutes at 50^0C whereas for 15 minutes at 56^0C. On the other hand, PNDV isolate retained its HA activity for 15 minutes at 50^0C but its embryo infectivity was retained upto 30 minutes at the same temperature. At 56^0C, the HA activity of the PNDV isolate retained for 5 minutes and embryo infectivity for 15 minutes revealing that this isolate (PNDV) was less thermostable than the (KNDV) isolate.

Both B_1 and K strains were found thermostable for 15 minutes at 50^0C in respect of HA activity but embryo infectivity of the B_1 virus was found stable upto 30 minutes at the same temperature. At 56^0C, both HA activity and embryo infectivity of B_1 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 embryo infectivity up to 15 minutes (Table 1).

The pattern of the inactivation of embryo infectivity and HA activity due to thermal treatment are somewhat different between the observations reported by Yamada (1981) and Digioia *et al.* (1970). Yamada (1981) studied lentogenic strains of NDV and observed that the HA activity were lost earlier than the embryo infectivity in case of heating at 56^0C. In this study similar results were found in case of PNDV, B_1 and K viruses. NDV strains are generally recognised as "heat stable" virus when they retain their activities for 30 minutes at 56^0C. On this basis both the KNDV and PNDV isolates may be classified as heat unstable as the selected B_1 and K strains are.

The results presented in the Table 1 revealed that the $\log_{10}ELD_{50}$ / 0.1 ml of allantoic fluids of KNDV and PNDV isolates were $10^{7.32}$ and $10^{7.67}$ respectively and those of B_1 and K strains were $10^{8.68}$ and $10^{7.87}$ respectively. Thus it is observed that ELD_{50} of K, KNDV and PNDV isolates has similarity to each other.

Among the test NDV isolates, KNDV was found to be lentogenic and that of PNDV as velogenic on the basis of MDT, and on the same basis the reference B_1 and K strains were found to be, as these are, lentogenic and mesogenic 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 lentogenic 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 lentogenic 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 lentogen on the basis of MDT in embryos, by ICPI, and / or intravenous pathogenicity index. In the present study MDT of the parrot isolate (PNDV) was found to be (57.6 hours) like velogens (Table 1). The MDT of B_1 was found to be 120.8 hours in the present study which is similar with other reports (Allan *et al.*, 1978) while that of the K strain was 84.3 hours as was also recorded by Samuel *et al.* (1979).

Reports of Hanson and Brandly (1955) stated that mesogenic and velogenic strains of NDV usually kill all of the chicks within a period of 8 days and the lentogenic strains rarely kill the chicks under the condition of the ICPI test. Swayne *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 1.0 to 1.5 may be classified as mesogenic and finally the strains demonstrating ICPI values from 0.2 to 0.5 may be classified as lentogenic strains. The results of the ICPI tests obtained in the present study (Table 1) indicated that KNDV isolate with ICPI of 0.45 falls in the group of lentogenic strain of NDV and the PNDV isolate having ICPI of 1.58 may be classified as velogenic. The results of ICPI tests of B_1 and K strains with ICPI of 0.0 and 1.11 proved them to be lentogenic and mesogenic respectively.

The ICPI of the 'kakatoa' isolate (KNDV) 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 KNDV isolate is found to be greater than those of LaSota strain. Thus, it may be suggested that KNDV isolate is more neuro-pathogenic than the LaSota strain of NDV. The ICPI of the parrot isolate of NDV (PNDV) 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 70181 1972" was 1.8 and 51 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 PNDV isolate is less pathogenic than the velogenic "NY parrot 70181 1972" strain, though in consideration of ICPI it may be grouped as velogenic strain. The ICPI of the B_1 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 Swayne *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.

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