BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF INDIAN ISOLATES OF NEWCASTLE DISEASE VIRUS FROM PIGEONS

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Received July 14, 2004, accepted April 18, 2005

Summary. – Five Newcastle disease virus (NDV) isolates from pigeons were characterized by biological and molecular methods. Four of the five isolates were found to be velogenic with high intracerebral pathogenicity indices (ICPI). The fusion protein cleavage site (FPCS) sequences of these isolates had multiple basic amino acids RRQKRF at positions 112–116 and a phenyl alanine at position 117 characteristic of velogenic isolates. Three of these velogenic isolates were phylogenetically related to mesogenic vaccine virus strain and the fourth one to a few exotic velogenic isolates. The lentogenic isolate obtained in this study was identical with the LaSota strain.

Key words: fusion protein cleavage site; Newcastle disease virus; pigeons; phylogenetic analysis

Introduction

NDV, also called Avian paramyxovirus 1 (Rubulavirus genus) has a worldwide distribution and a broad host range. It causes Newcastle disease (ND) that has been first observed in poultry in 1926 (Doyle, 1927). Although ND is considered to be primarily a disease of poultry, subsequent reports have indicated the possibility of natural or experimental infections in 236 different species of birds (Kaleta and Baldauf, 1988). The ability of NDV to infect avian species other than poultry has been highlighted by panzootics in racing pigeons (Vindevogel and Duchatel, 1988). India has a large pigeon population, which includes both domesticated pet and racing pigeons and free-living wild pigeons. These birds are not regularly vaccinated against NDV.

India is endemic to ND and different pathotypes of NDV have been isolated and characterized from variety of avian hosts (Kumanan *et al*., 1992, 2003; Roy *et al.*, 2000; Nanthakumar *et al*., 2000). Although a number of NDV isolates have been obtained from pigeons (Roy *et al*., 2000; Nanthakumar *et al*., 2000), they were not characterized enough at molecular level. In the present study we describe biological and molecular characteristics of five Indian pigeon isolates of NDV from 1998–2002.

Materials and Methods

NDV isolates obtained from pigeons over a period of five years were propagated in allantoic cavity of SPF embryonated chicken eggs. The identity of the isolates was confirmed by hemagglutination (HA) and hemagglutination-inhibition (HAI) tests using a NDV specific antiserum.

Biological characterization. Mean death time (MDT), ICPI and ability to agglutinate equine erythrocytes were determined by standard procedures.

RT-PCR. Total RNA was isolated from virus infected amnio-allantoic fluid (AAF) as described earlier (Chomczynski and Sac-

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Abbreviations: FPCS = fusion protein cleavage site; HA = hemagglutination; HAI = hemagglutination-inhibition; ICPI = intracerebral pathogenicity index; MDT = mean death time; ND = Newcastle disease; NDV = ND virus

chi, 1987). NDV genomic RNA was amplified in total RNA by RT-PCR using degenerative oligonucleotide primers for FPCS according to the method of Seal *et al.* (1995). The RT-PCR products were subjected to agarose gel electrophoresis, purified using spin columns (Qiagen, Germany) and sequenced in an automated sequencer (ABI prism, version 3, Applied Biosystems, USA). The sequence data of the five isolates were submitted to GenBank and aligned by Clustal W programme version 1.82 along with those of other pigeon isolates and vaccine strains (LaSota, Mukteshwar and K used in India) available in GenBank. Phylogenetic tree was drawn using Molecular Evolutionary Genetic Analysis Software, version 1.02 (Sudhir *et al.*, 1993).

Results

The five Indian NDV isolates from pigeons originated from 1998–2002. Two isolates originated from dead pigeons with nervous symptoms, two from apparently healthy pigeons and one from a pigeon with diarrhea. The biological characteristics of the five isolates were as follows: the HA titers were 256–1024, HAI titers 512–1024, MDT 40–90 hrs, and ICPI 0.57–1.95. Except the isolate 2K6, none of the isolates agglutinated equine erythrocytes (Table 1).

The RT-PCR amplified a 254 bp product with all the five isolates. The amino acid FPCS sequences of the five isolates were compared with those of three Indian vaccine strains and other pigeon isolates available in the GenBank (Fig. 1). Although minor variations were noticed in the analyzed sequences, four of the five isolates had an identical motif, RRQKRF at the positions 112–117. Just the isolate 2K6 had a different sequence, GRQGRL instead of this motif. Moreover, the entire FPCS sequence of the 2K6 was found to be identical with that of the vaccine strain LaSota.

Phylogenetic analysis placed the isolates 2K3, 2K7 and 2K18 in one cluster along with the mesogenic K strain. The isolate 2K6 was found to be related to LaSota strain. The isolate 2K27 forming a separate branch was found to be related to exotic isolates from USA and Taiwan (Fig. 2).

Discussion

Regarding the virulence, MDT and ICPI values of three of the five isolates correlated well, while the isolates 2K7 and 2K27 were found to have a prolonged MDT (72 hrs). Nevertheless, based on ICPI (over 1.85) and sequence data (multiple basic amino acids and F at positions 112–116 and F at 117 (OIE, 2001)), four (2K3, 2K7, 2K18 and 2K27) of the five isolates were characterized as velogenic. The presence of velogenic isolates in pigeons has been reported a real source of infection for commercial poultry flocks (Vindevogel and Duchatel, 1988). Although an earlier Indian pigeon isolate causing ND in chicken had an ICPI of 1.4 only (Roy *et al*., 2000), all the four velogenic isolates reported in this study had very high ICPIs indicating that they may not require a prior adaptation for induction of the disease in chicken as it has been reported by Alexander *et al*. (1985) and Buonavoglia *et al*. (1991). Of the four velogenic isolates two were isolated from dead pigeons with neural signs and one from a live pigeon with diarrhea. Interestingly, the velogenic isolate 2K7 originated from an apparently healthy pigeon. Earlier serological investigations have also revealed the possibility of NDV infections in pigeons without clinical signs (Vindevogel *et al*., 1982). Such a symptomless carriers excreting a virulent virus may pose a serious threat to the susceptible avian population in India.

The lentogenic isolate, 2K6 that had identical amino acid FPCS sequence with the lentogenic vaccine strain LaSota was found to be related to this strain phylogenetically too. This indicated clearly that isolate 2K6 originating from an apparently healthy pigeon could be a reisolate of the vaccine strain. Similarly, the isolates 2K3, 2K7 and 2K18 were found to be closely related to the mesogenic K strain. However, these isolates had higher ICPIs compared to mesogenic isolates. It is possible that these isolates might have originated from the mesogenic vaccine strain and gained virulence for chicken following repeated field passages over a period of time. In contrary to the four isolates phylogenetically related to lentogenic and mesogenic vaccine strains used in India, the more recent velogenic isolate 2K27 was found to be closely related to a few exotic strains from USA and Taiwan.

Table 1. Biological and molecular characteristics of Indian pigeon isolates

Isolate	Samples used for isolation	History	Year of isolation	HA titer	HAI titer	MDT (hrs)	ICPI	Agglutination of equine erythrocytes	FPCS motif
2K18	Spleen and brain	Neural signs and death	1998	1024	512	48	1.85	Nil	RRQKRF
2K3	Spleen and brain	Neural signs and death	2000	256	1024	40	1.95	Nil	RRQKRF
2K6	Droppings	Apparently healthy	2001	1024	512	90	0.57	2	GRQGRL
2K7	Droppings	Apparently healthy	2001	256	512	72	1.95	Nil	RRQKRF
2K27	Cloacal swab	Diarrhea	2002	1024	512	72	1.95	Nil	RRQKRF

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AF506767/Belgium          .......... .......... .......... .......... ..........   .......... .......... .......
AY008328/USA              .......... .AT....... ...V...... .......A.. ..........   .......... .......... .......
AY390291/CHINA            .......... ......EK.. .......... .......... ..........   .......... .......... .......
AF474244/2K3/INDIA        ..E......E ..T....... .........G .......A.. ..........   K......... .......KYP .......
AY324318/2K6/INDIA        ..E......E ..T....G.. G.L......G .......A.. ..........   K......... .......... .......
AF510738/2K7/INDIA        .........E ..T....... .........G .......A.. ..........   K......... .......... .......
AF204754/2K18/INDIA       .....G...E ..T....... .........G .......A.. ..T.......   K.....V... N..F...... .L...T.
AY378322/2K27/INDIA       .....G...E .......... ......V... .......A.. ..........   .......... .......... .......
AY445669/SOUTHAFRICA      .......... ......V..K .......... .......... ..........   .......... .......KYP .......
AF506768/BELGIUM          .......... .......... .......... .......... ..........   .......... .......... .......
AJ415880/FRANCE           .........E ..T....G.. G.L......G .......A.. ..........   K......... .......... .......
AYOO8330/USA              .......... ......E... .......... .......... ..........   .......... .......... .......
AY008324/USA              .......... .AT..R.... ...V...... I......A.. ..........   .R........ .......... .......
AY428960/USA              ..E....... .....RV..K .......... .......... ..........   .......... .......... .......
AY372145/TAIWAN           ......K... .......... ......V... .......A.. ..........   .......... .......... .......
AY372144/TAIWAN           .........E ..T....G.. G.L......G .......A.. ..........   K......... .......... .......
AF325434/ARGENTINA        ..E....... .......G.. ......V... .......... ..........   .......... .......... .......
AF520970/ITALY            .......... ......RG.. .......... .......... ..........   .......... .......... .......
AF520968/ITALY            .......... .......G.. .......... .......... ..........   .......... .......... .......
AJ306305/FRANCE           .......... .......... .......... ....I..... ..........   .......... .......... .......
AY390314/CHINA            .........E ..T....G.. G.L......G .......A.. ..........   K......... .......... .......
AY444501/USA              .......... ......E... .......... .......... ..........   .......... .......... .......
U22292/LASOTA             ..E......E ..T....G.. G.L......G .......A.. ..........   K......... .......... .......
AF204755/MUKTESHWAR       ..E......E .LT....... .......... .......A.. ..........   L.........I .......... .......
K STRAIN                  ..E......E ..T....... .........G .......A.. ..........   K......... .......... ....G..
CONCENSUS                 [95]LGDSIRRIQG SVSTSGGRRQ KRFIGAIIGS VALGVATSAQ ITAAAALIQA   NQNAANLIRL KESIAATNEA VHEVTDG[171]
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Fig. 1

Alignment of predicted partial amino acid FPCS sequences of NDV isolates

The sequences cover the part of FPCS at positions 95–171. The region at positions 109–119 is underlined.

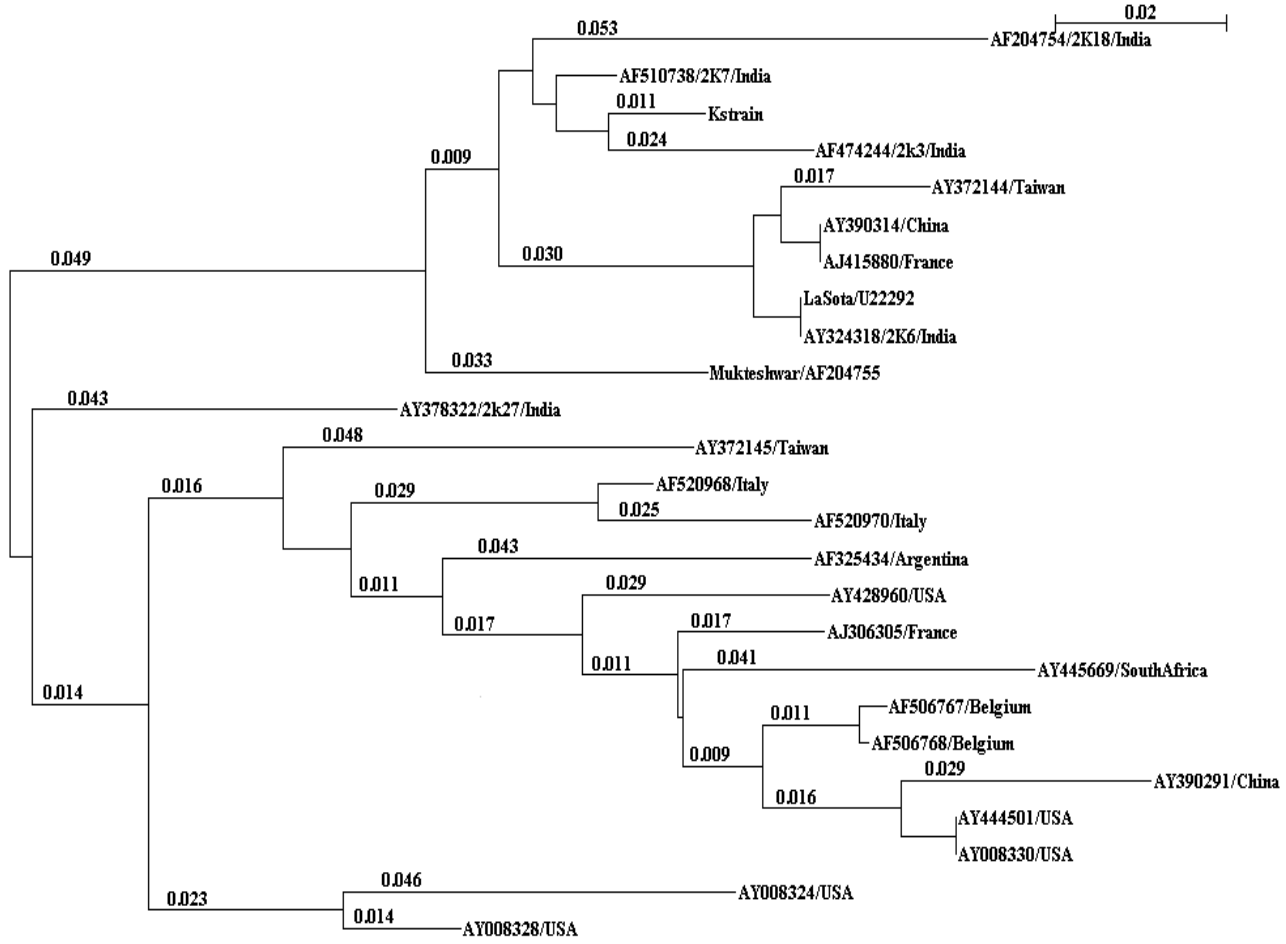

Fig. 2

Phylogenetic tree of NDV isolates

Five Indian NDV isolates from pigeons (AY324318, AF510738, AF474244, AF204754 and AY378322) were compared with other NDV isolates on the basis of the amino acid sequences of their FPCS.

That part of India, where from the isolate 2K27 was obtained, has got quite a few bird sanctuaries, which are visited regularly by a variety of migratory birds from all over the world. There is a distinct possibility that these birds might have been the source of this isolate.

Summing up, this study (i) demonstrates that the velogenic NDV isolates have not only been obtained from dead and ailing birds but also from apparently healthy birds and (ii) neccessitates the need of initiating proper control measures in pigeons.

Acknowledgement. The authors thank the Indian Council of Agricultural Research, Government of India, New Delhi, India for funding this work.

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