Virus Genes (2009) 39:234–237
DOI 10.1007/s11262-009-0385-7

Complete genome sequence of a virulent Newcastle disease virus isolated from an outbreak in chickens in Egypt

Mahmoud H. A. Mohamed · Sachin Kumar · Anandan Paldurai · Mohamed M. Megahed · Ibrahim A. Ghanem · Mohamed A. Lebdah · Siba K. Samal

Received: 29 April 2009 / Accepted: 26 June 2009 / Published online: 10 July 2009

Abstract The complete genome sequence of a virulent Newcastle disease virus (NDV) isolated from chickens in Egypt was determined and compared to the sequence of NDV strains isolated from different parts of the world. The genome is 15,186 nucleotides (nt) long and consists of 6 genes in the order of 3′-N-P-M-F-HN-L-5′. The genome contains a 55-nt leader region at the 3′ end and a 114-nt trailer region at the 5′ end. Interestingly, the phylogenetic analysis showed that strain Egypt is closely related with the NDV strains isolated in China. In addition, the sequence of the fusion protein cleavage site of strain Egypt was identical to that of the NDV strain recently isolated in Mali. Determination of complete genome sequences of additional NDV strains from Africa is necessary to understand the epidemiology of currently circulating viruses in Africa.

Keywords Newcastle disease virus · African strain · Complete genome sequence · Phylogenetic analysis

Newcastle disease (ND) is one of the most important diseases of poultry worldwide. It is caused by Newcastle disease virus (NDV), a member of the genus *Avulavirus* in the family *Paramyxoviridae*. ND is endemic in many parts of the world (East Asia, Africa, and South America), and these may serve as sources of infection for sporadic outbreaks in disease-free countries around the world [1]. The disease is particularly devastating to village farmers in developing countries whose livelihood depends on chicken farming [2]. The NDV isolates are characterized by their pathogenicity in chickens and categorized into three main pathotypes, depending on severity of the disease; lentogenic isolates are of low virulence, mesogenic isolates are of intermediate virulence, while virulent isolates that cause high mortality are velogenic [3]. It is thought that continents having warm climates are reservoirs of virulent NDV strains [1]. NDV is a well-studied paramyxovirus, and complete genome sequences of many North American NDV strains are available; however, very little is known about the genome sequences of NDV strains isolated from different parts of Africa.

This study deals with the analysis of a complete genome sequence of an NDV isolated from an outbreak on a poultry farm in Al-Sharkia province in Egypt and designated as chicken/Egypt/1/2005. The infected birds showed severe neurological and/or respiratory symptoms. The virus was confirmed as NDV by hemagglutination inhibition assay using a known NDV antiserum. The pathogenicity of NDV strain Egypt was determined by mean death time (MDT), intracerebral pathogenicity index (ICPI), and intravenous pathogenicity index (IVPI) tests [4]. The results showed that NDV strain Egypt was a velogenic strain with an MDT value of 55 h, an ICPI value of 1.75, and an IVPI value of 2.5.

To understand the molecular characteristics of the virus, the complete genome sequence of NDV strain Egypt was determined. To our knowledge, this is the first complete genome sequence of an NDV strain isolated in Africa. In brief, the virus was grown in the allantoic cavities of 9-day-old embryonated specific pathogen free chicken eggs by standard procedures [4]. The viral genomic RNA was

M. H. A. Mohamed · S. Kumar · A. Paldurai · S. K. Samal (✉)
Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland, College Park, MD 20742, USA
e-mail: ssamal@umd.edu

M. H. A. Mohamed · M. M. Megahed · I. A. Ghanem · M. A. Lebdah
Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

extracted from virus purified from infected allantoic fluid. The 3′ leader and 5′ trailer sequences of the strain Egypt were determined using 3′-rapid amplification of cDNA ends (3′RACE) and the 5′-RACE, respectively [5, 6]. The primer sets designed from the available NDV genome sequences (GenBank accession numbers NC_002617, EF201805, AF077761, AY562988, M23407, X04719) were used to determine the complete genome sequence of strain Egypt. The PCR-amplified products and plasmid DNAs (cloned PCR products) were sequenced using Big-Dye terminator v 3.1 matrix standard kit and 3130xl genetic analyzer data collection software v3.0 (both from Applied Biosystems, California, USA). The entire genome was sequenced at least three times, and at least once from uncloned PCR product, to ensure a consensus sequence.

The genome of NDV strain Egypt is 15,186 nucleotides (nt) (GenBank accession number FJ939313), a length that is present in most NDV strains. Similar to other NDV strains, the genome consists of six open reading frames encoding six different proteins in the order of a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a fusion protein (F), an attachment protein called the hemagglutinin–neuraminidase (HN), and a large polymerase protein (L) (3′-N-P-M-F-HN-L-5′). The length, position, and characteristics of the six genes and their intergenic sequences (IGS) are summarized in Table 1a, and the comparison of its proteins with other NDV strains in Table 1b. NDV strain LaSota was isolated from an outbreak in 1946 (New Jersey), subsequently strain B1 (1948), strain Beaudette C (BC) (1959), and strain Fontana (1972) were isolated form different parts of United States [7–9].

The 3′ leader sequence of strain Egypt consists of 55 nt, a length present in all NDV strains [5]. The leader sequence of strain Egypt showed 100% identity with the leader sequence of strain BC, but there are 8 nt difference between the leader sequence of strain Egypt and leader sequences of strains LaSota, B1, and chicken/USA(CA)/1083(Fontana)/72. The P gene contains a putative editing site ^{477}AAAAAGGG^{484} (mRNA sense) that is identical in position to other NDV strains [10]. The 5′ trailer sequence of the strain Egypt was 114 nt long and showed a 4 nt difference from the trailer sequence of strain BC. The phylogenic analysis of the complete genome sequence of strain Egypt with other strains of NDV showed that strain Egypt clustered with the strains isolated from China (Fig. 1).

All the six major proteins of strain Egypt invariably showed higher amino acid sequence identity with the cognate proteins of other NDV strains that are well characterized (Table 1b). The comparison of complete genome sequences showed that strain Egypt is more related to strain BC than to other NDV strains (Table 1b). Interestingly, strain BC is a mesogenic strain, but strain Egypt is a velogenic strain; therefore, our results indicated that minimum changes in the nucleotide sequence of an NDV strain can have a profound effect on the pathogenicity of the virus.

The F protein cleavage site is a well-characterized determinant of NDV pathogenicity in chickens [11–13].

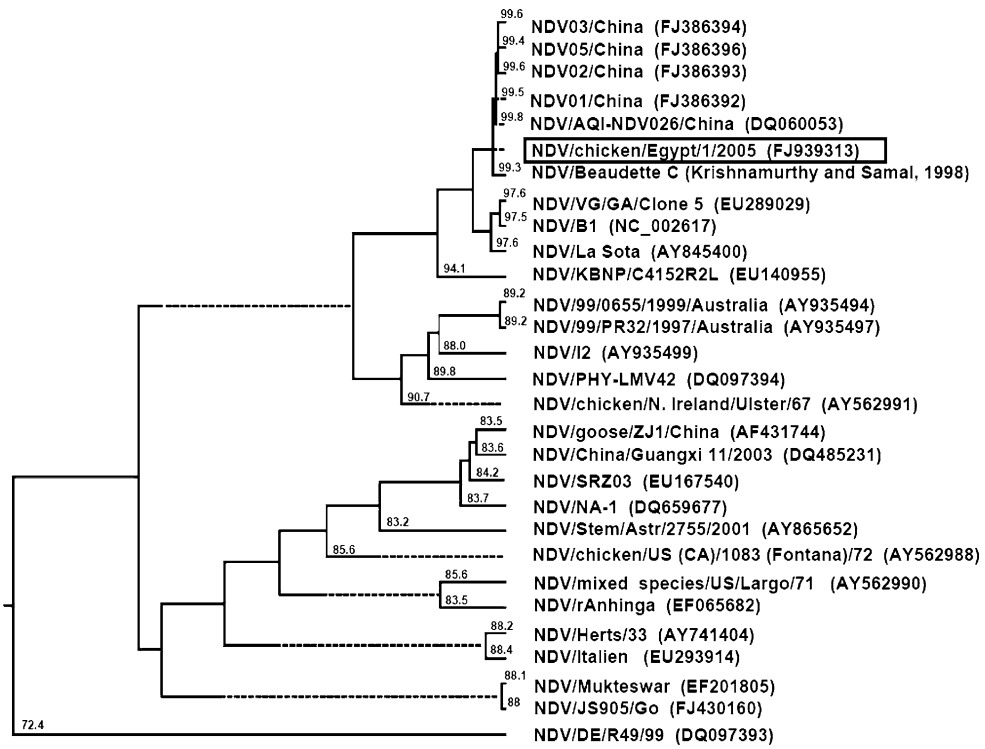

Fig. 1 Phylogenic analysis of the complete genome of NDV strains chicken/Egypt/1/2005. The phylogenetic trees were constructed using a DNASTAR software package (Lasergene8). All the accession numbers correspond to GenBank submissions of different strains of NDV. The values at the branches represent the percent nucleotide identity of NDV strain chicken/Egypt/1/2005 with other NDV strains

Table 1 Genomic features and protein characteristics of NDV strain chicken/Egypt/1/2005

(a)

Genes	Hexamer phasing position at gene-start	mRNA characteristics (nt)				IGS (nt)	Deduced protein characteristics		
		Total length	5′UTR	ORF	3′UTR		Size (aa)	MW (kDa)	pI
N	2	1746	66	1470	210	2	489	53.1	5.239
P	4	1451	83	1188	180	1	395	42.3	6.342
P/V	4	1452	83	720	649	–	239	25.5	6.200
P/W	4	1453	83	666	704	–	221	24.2	9.570
M	4	1241	34	1095	112	1	364	39.7	9.584
F	4	1792	46	1662	84	31	553	59.1	8.450
HN	3	2002	91	1734	177	47	577	63.3	6.841
L	6	6703	11	6615	77	–	2204	248.5	6.793

(b)

chicken/Egypt/1/2005	NDV strains[††]			
	La Sota	B1	BC	Fontana
Complete genome[*]	97.6	97.5	99.3	85.6
Nucleoprotein[†]	98.2	99.0	100	93.0
Phosphoprotein[†]	97.7	94.2	98.7	84.8
Matrix protein[†]	97.3	97.0	98.4	90.7
Fusion protein[†]	97.5	96.9	99.1	90.8
HN protein[†]	97.2	97.1	97.7	88.6
L protein[†]	97.7	97.9	98.1	94.6

(a) The table shows hexamer phasing positions along with individual genes, coding and non-coding, and IGS with their protein profiles. (b) Percent sequence identity of the complete genome sequence and percent aa sequence identity of different proteins encoded by NDV strain chicken/Egypt/1/2005 with other strains of NDV [LaSota, B1, BC, and chicken/USA (CA)/1083(Fontana)/72]

[*] Nucleotide level

[†] Amino acid level

[††] References for NDV genome sequences are La Sota (AY845400), B1 (NC_002617), BC [5] and Fontana (AY562988)

Virulent NDV strains typically contain a polybasic cleavage site (R-X-K/R-R↓F), which is recognized by most cells. The cleavage site of strain Egypt ^{112}R-R-Q-K-R↓F-I^{118} contained four basic amino acids at positions 112–116, corresponding to those of virulent NDV strains. In addition, the presence of the phenylalanine (F) residue at position 117 has been described as being a possible contributor to the neurological effects [10, 14]. Interestingly, the F cleavage site of strain Egypt is identical to that of NDV strains isolated from chickens and guinea fowl in the Mopti and Sikasso regions of Mali in 2008 [15]. Although the complete genome sequences of the Malian isolates are not available, based on F cleavage site sequence, our results indicate that the same strain may be circulating in the continent of Africa. Furthermore, the F cleavage site of Egypt strain was identical to most NDV strains isolated in different parts of China [16]. It is possible that several species of birds, mostly waders, are known to migrate between parts of Africa and their breeding grounds in Siberia, through communal stopover grounds in the African Rift Valley, the Middle East, and central Asia, where they are likely to come into contact with birds from the Far East [16, 17]. The phylogenetic analysis of complete genome sequence showed that the strain Egypt is closely related to the isolates from China, suggesting that the virus transmission could have occurred through migratory water birds from the Far East [16, 17].

Recently, it was shown that the L gene is also associated with the virulence of NDV [18]. Amino acid sequence of L gene of strain Egypt showed high degree of identity with strain La Sota, B1, and BC (Table 1b). However, there was lower amino acid identity with strain Fontana (94.6%). Whether this lower identity of L protein plays any role in the virulence of this virus needs to be explored.

This is the first report of a complete genome sequence of an NDV strain isolated in the continent of Africa. Our results demonstrated that high levels of nucleotide and amino acid sequence identity exist between the African and North American NDV strains. On the other hand, our findings raise questions concerning the validity of

phylogenic analysis using only partial F gene sequences. However, determination of the complete genome sequences of additional NDV strains isolated in different geographic regions of Africa is necessary to understand the genetic relatedness among NDV strains circulating in different parts of the world.

Acknowledgments We thank Daniel Rockemann and all our laboratory members for their excellent technical assistance. We also thank Ireen Dryburgh-Barry for proofreading the manuscript.

References

1. J. Herczeg, E. Wehmann, R.R. Bragg, P.M. Travassos Dias, G. Hadjiev, O. Werner, B. Lomniczi, Arch. Virol. **144**, 2087–2099 (1999)
2. T.D. Tu, K.V. Phuc, N.T. Dinh, D.N. Quoc, P.B. Spradbrow, Prev. Vet. Med. **34**, 205–214 (1998)
3. D.J. Alexander, *Newcastle Disease and other Avian Paramyxovirus Infections* (Iowa State University Press, Ames, 1991)
4. D.J. Alexander, *Newcastle Disease Diagnosis* (Kluwer Academic Publishers, Boston, MA, 1988)
5. S. Krishnamurthy, S.K. Samal, J. Gen. Virol. **79**(Pt 10), 2419–2424 (1998)
6. S. Kumar, B. Nayak, P.L. Collins, S.K. Samal, Virus Res. **137**, 189–197 (2008)
7. B.S. Seal, D.J. King, J.D. Bennett, J. Clin. Microbiol. **33**, 2624–2630 (1995)
8. G. Schloer, Infect. Immun. **10**, 724–732 (1974)
9. T.M. Goldhaft, Avian Dis. **24**, 297–301 (1980)
10. R. Lamb, G. Parks, in *Paramyxoviridae: The Viruses and Their Replication*, ed. by D.M. Knipe, P.M. Howley, D.E. Griffin, R.A. Lamb, M.A. Martin, B. Roizman, S.E. Straus (Lippincott Williams & Wilkins, Philadelphia, 2007)
11. Z. Li, T. Sergel, E. Razvi, T. Morrison, J. Virol. **72**, 3789–3795 (1998)
12. T. Morrison, C. McQuain, T. Sergel, L. McGinnes, J. Reitter, Virology **193**, 997–1000 (1993)
13. A. Panda, Z. Huang, S. Elankumaran, D.D. Rockemann, S.K. Samal, Microb. Pathog. **36**, 1–10 (2004)
14. J.A. Kattenbelt, M.P. Stevens, A.R. Gould, Virus Res. **116**, 168–184 (2006)
15. R. Servan de Almeida, O.F. Maminiaina, P. Gil, S. Hammoumi, S. Molia, V. Chevalier, M. Koko, H.R. Andriamanivo, A. Traore, K. Samake, A. Diarra, C. Grillet, D. Martinez, E. Albina, Vaccine **27**, 3127–3129 (2009)
16. C. Abolnik, R.F. Horner, S.P. Bisschop, M.E. Parker, M. Romito, G.J. Viljoen, Arch. Virol. **149**, 603–619 (2004)
17. T. Piersma, N.C. Davidson, Wader Study Group Bull. **64**, 187–197 (1992)
18. S.N. Rout, S.K. Samal, J. Virol. **82**, 7828–7836 (2008)