Virus Genes (2010) 41:67–72
DOI 10.1007/s11262-010-0486-3

Complete genome sequence of highly virulent neurotropic Newcastle disease virus strain Texas GB

Anandan Paldurai · Sachin Kumar · Baibaswata Nayak · Siba K. Samal

Received: 15 March 2010 / Accepted: 15 April 2010 / Published online: 30 April 2010

Abstract Newcastle disease virus (NDV) strain Texas GB is a highly virulent neurotropic virus that is used as a standard vaccine challenge virus in the U.S. In this study, the complete genome sequence of strain Texas GB was determined and compared with the complete genome sequences of other NDV strains. The genome is 15,186 nucleotides (nt) long and consists of six genes in the order of 3′leader-N-P-M-F-HN-L-5′trailer. The genome contains a 55-nt leader sequence at the 3′ end and a 114-nt trailer sequence at the 5′ end. The intergenic sequences are 2, 1, 1, 31, and 47 nt between N/P, P/M, M/F, F/HN, and HN/L genes, respectively. The putative cleavage site of fusion protein showed amino acid sequence of R-R-Q-K-R↓F in position 112 to 117, which corresponds to those of virulent NDV strains. The phylogenetic analysis showed that strain Texas GB is closely related to the neurovirulent mesogenic strain Beaudette C (BC) and to NDV viruses isolated in China and Egypt than to other strains of NDV.

Keywords Strain Texas GB · Newcastle disease virus · BSL-3+ facility · Complete genome sequence · Phylogenetic analysis

Newcastle disease (ND) is a highly contagious and fatal disease of chickens leading to huge economic losses in the poultry industry worldwide [1]. The causative agent, Newcastle disease virus (NDV), is a member of the genus *Avulavirus* in the family *Paramyxoviridae* [1, 2]. NDV isolates display a spectrum of virulence in chickens, ranging from inapparent infection to 100% mortality. Based on their pathogenicity in chickens, NDV isolates are categorized into three main pathotypes, lentogenic (low virulence), mesogenic (intermediate virulence), and velogenic (high virulence) [1]. The velogenic strains cause severe outbreaks in poultry. Based on the intracloacal inoculation test, velogenic strains are further classified into viscerotropic, which induce mortality with hemorrhagic lesions in the intestinal tract and neurotropic, when nervous signs predominate without hemorrhagic lesions in the intestine [1, 3, 4].

NDV strain Texas GB is considered as the prototype neurotropic velogenic strain and is widely used as the standard challenge virus in vaccine efficacy studies in the U.S. Though, strain Texas GB is biologically one of the well characterized NDV strains to date [3–8], the complete genome sequence of this strain has not been reported. Only partial or full sequences of few genes dating from 1988 to 2004 are available [9–15]. The complete sequences of phosphoprotein and large polymerase genes, the intergenic sequences between N/P, P/M, HN/L genes, and the trailer sequence are not available. Since the current sequences of strain Texas GB were determined by different groups at different times, it is necessary to determine the complete genome sequence of strain Texas GB to understand its pathogenicity and neurovirulence. In this study, we report the complete genome sequence of the NDV strain Texas GB (APMV-1/chicken/U.S.(TX)/GB/1948).

The NDV strain Texas GB was isolated from a fatal ND outbreak in chicken with severe neurological involvement, in Texas, U.S.A., in 1948 [3, 4]. We received this strain from National Veterinary Services Laboratory, Ames, Iowa, U.S.A. We passaged the virus three times in 9-day-old specific pathogen free (SPF) embryonated chicken eggs. The pathogenicity of NDV strain Texas GB was

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

determined by mean death time (MDT) in 9-day-old embryonated SPF chicken eggs and by intracerebral pathogenicity index (ICPI) in 1-day-old SPF chicks. The MDT and ICPI values of strain Texas GB were 50 h and 1.925, respectively, consistent with a velogenic NDV strain. The work was performed in our USDA approved enhanced biosafety level 3 (BSL-3+) facility.

To determine the complete genome sequence of NDV strain Texas GB, the virus was grown in the allantoic cavities of 9-day-old embryonated SPF chicken eggs. Plaque purification of the virus was not performed to avoid the likely chance of picking up a minor variant that may not represent the actual viral genome. The viral genomic RNA was extracted from the infective allantoic fluid using RNeasy mini kit (Invitrogen). Primer sets were designed from the available sequences of strain Texas GB (accession numbers, AF144730, AF138899, AY505057 and U22293) and from the complete genome sequence of BC [16]. Reverse transcription and PCR were done using virus specific primers by Superscript-II reverse transcriptase and high fidelity Platinum *Pfx* polymerase (both from Invitrogen), respectively. The 3′-leader and 5′-trailer sequences of the strain Texas GB were determined using 3′-rapid amplification of cDNA ends (3′-RACE) and the 5′-RACE, respectively [17, 18]. The PCR-amplified products were directly sequenced using BigDye terminator v 3.1 matrix standard kit and 3130*xl* genetic analyzer data collection software v3.0 (Applied Biosystems Inc). The entire genome was sequenced at least four times from four independent RNA preparations to ensure a consensus sequence.

The genome of NDV strain Texas GB is 15,186 nt (GenBank accession number GU978777), a length that is present in most NDV strains. There are two other genome size classes reported among strains of NDV, which are 15,192 nt and 15, 198 nt [19–22]. The longer genome sequences are due to the presence of 6 and 12 additional nt in the 3′ untranslated region (3′-UTR) of N gene and in the open reading frame (ORF) of P gene, respectively (Fig. 1b). Similar to other NDV strains, the genome of strain Texas GB consists of six genes encoding six different proteins in the order of a nucleocapsid protein (N), a phosphoprotein (P), a matrix protein (M), a fusion protein (F), an attachment protein called the hemagglutinin-

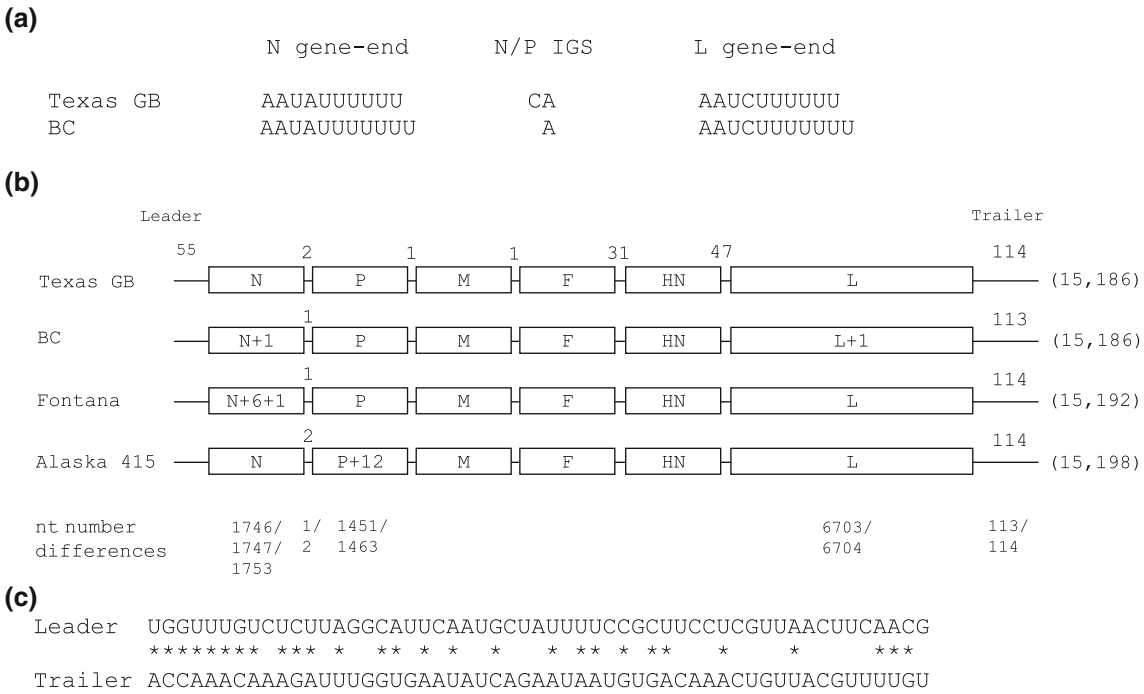

Fig. 1 **a** Comparison of nucleotide (nt) sequences of gene-end of N and L genes and IGS between N/P genes (genome sense), **b** gene map of NDV strains Texas GB, BC, Fontana and Alaska 415 (*numbers* on the *top* of gene map of strain Texas GB indicate nt sequence lengths of leader, intergenic sequences (IGS) between N/P, P/M, M/F, F/HN, HN/L genes and trailer, respectively; *numbers* at the *bottom* of the gene map of Alaska 415 indicate putative nt length differences in N genes, IGS between N/P, P genes, L genes and trailer sequences of the four NDV strains described; *numbers* given in the *parenthesis* at the end of each gene map reflects total genome length of each NDV strain; *numbers within* the N, P, L gene-boxes of genomes BC, Fontana and Alaska 415 indicate putative additions in length compared to N, P and L genes of strain Texas GB), **c** nt sequence complimentarity between 3′-leader and 5′-trailer (genome sense) sequences of strain Texas GB (stars indicate base pairs) and **d** phylogenetic tree of NDV strain Texas GB with 78 other available genome sequences of NDV strains was constructed using maximum parsimony method with bootstrap values calculated for 1000 replicates (strain Texas GB is indicated by a *solid diamond*)

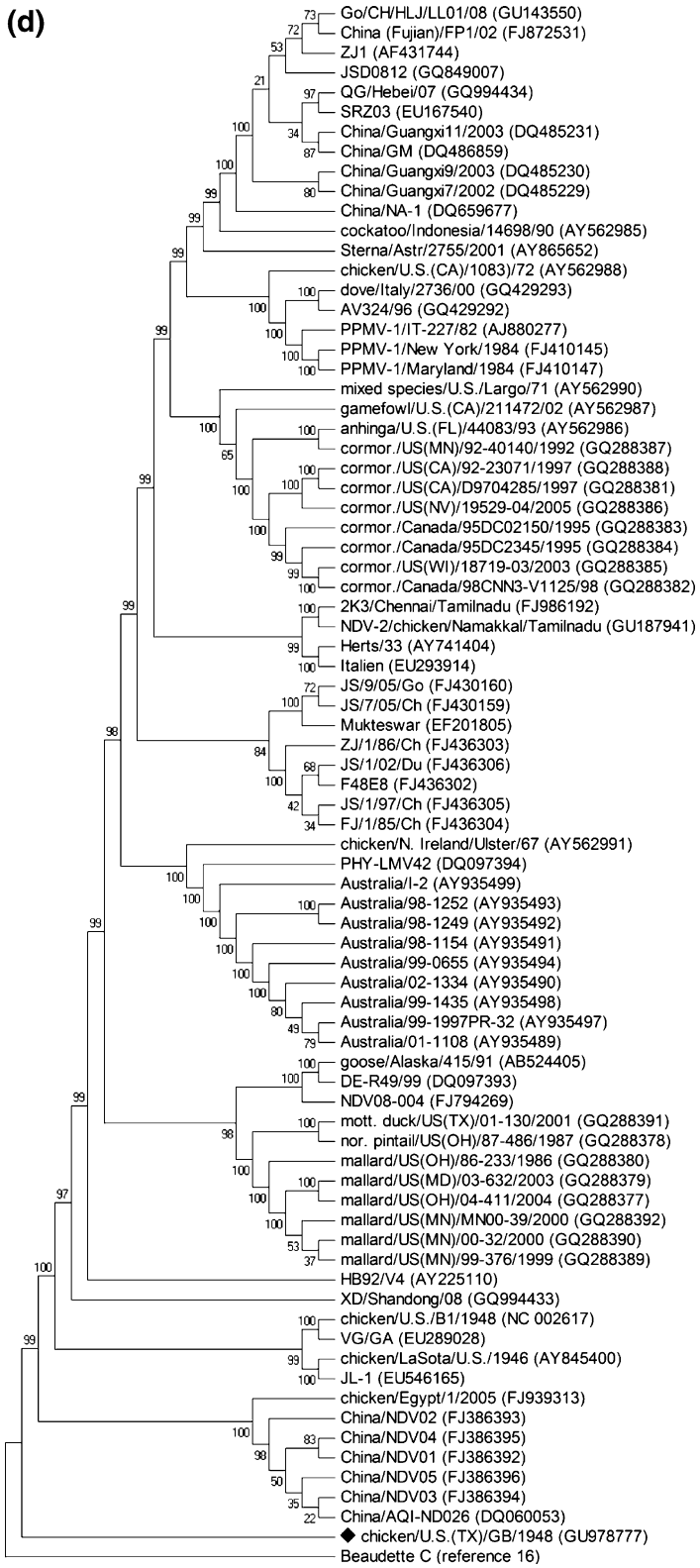

Fig. 1 continued

neuraminidase (HN), and a large polymerase protein (L) (3′leader-N-P-M-F-HN-L-5′trailer). The length, position, and characteristics of the six genes and their intergenic sequences (IGS) are summarized in Table 1, and the comparison of its proteins with other NDV strains in Table 2.

Table 1 Genomic features and protein characteristics of NDV strain Texas GB

Genes	Hexamer phasing position at gene-start[a]	Gene characteristics (nt)				Intergenic sequence (nt)	Deduced protein characteristics		
		Total length	5′-UTR	ORF	3′-UTR		Size (aa)	MW (kDa)	p*I*
N	2	1746	66	1470	210	2	489	53.1	5.492
P	4	1451	83	1188	180	1	395	42.2	6.341
P/V	4	1452	83	720	649	–	239	25.4	6.200
P/W	4	1453	83	666	704	–	221	24.1	9.533
M	4	1241	34	1095	112	1	364	39.6	9.494
F	4	1792	46	1662	84	31	553	58.9	8.453
HN	3	2002	91	1734	177	47	577	63.3	6.750
L	6	6703	11	6615	77	–	2204	248.6	6.861

The table describes the gene characteristics, hexamer phasing positions of individual genes along with coding and non-coding sequence lengths. Intergenic sequences and size and characteristics of putative viral proteins are also provided

[a] The nt position at the beginning of each gene-start sequence when the entire genome is viewed as multiple of six nt from the 3'-end. The hexamer phasing positions at the gene-start range from position 1 through 6

Table 2 Percent nucleotide sequence identity of the complete genome sequence and percent amino acid sequence identity of different proteins of strain Texas GB with other NDV strains

NDV strains	Lentogens		Mesogens		Velogens	
	LaSota	B1	BC	Anhinga	Fontana	Largo
Complete genome[a]	96.9	97.0	99.1	83.6	85.5	85.4
Viral proteins[b]						
N	99.2	98.8	99.8	92.2	93.0	93.0
P	97.5	93.7	98.7	79.0	84.3	82.8
P/V	95.0	91.2	98.3	69.5	78.7	74.9
P/W	93.9	93.9	97.7	60.9	75.1	69.8
M	97.3	97.0	98.9	89.3	90.4	90.4
F	96.6	96.4	98.6	88.6	90.1	87.9
HN	95.5	96.4	98.1	87.6	89.1	89.3
L	97.0	97.6	99.4	93.5	94.4	94.5

[a] Percent nucleotide identity

[b] Percent amino acid identity

The 3′-leader sequence of strain Texas GB consists of 55 nt, a length present in all NDV strains [5]. The leader sequence of strain Texas GB showed 100% identity with the leader sequence of strain BC, but there are 2, 3, 7, 8, 7, and 6 nt differences between the leader sequence of strain Texas GB and leader sequences of strains LaSota, B1, Anhinga, Fontana, Largo and Alaska 415, respectively. Comparison of the sequences of NDV strains Texas GB with those of NDV strains BC and LaSota showed differences in the stretch of 6 or 7 Uracil residues in the gene-ends of N and L genes and that alters the putative IGS between N/P genes to 1 or 2 residues and the putative trailer sequence to 113 or 114 nt, respectively. This leads to the putative length of N gene to 1,746 or 1,747 nt (in case of genomes with 15,186 nt) or 1,753 nt (in case of genomes with 15,192 nt) and the putative length of L gene to 6,703 or 6,704 nt (Fig. 1a, b). The putative length of P gene consists 1,451 (in genome lengths 15,186 and 15,192 nt) or 1,463 nt (in genome length of 15,198 nt). The P gene of strain Texas GB contains a putative editing site ^{2280}UUUUUCCC^{2287} (genome sense) that is identical in position to other NDV strains [2]. The 5′-trailer sequence of the strain Texas GB is 114 nt long and showed 5 nt differences from the trailer sequence of strain BC.

All the six major proteins of strain Texas GB invariably showed higher amino acid sequence identity with the cognate proteins of other NDV strains that are well characterized (Table 2). The comparison of complete genome sequences showed that strain Texas GB is more related to strain BC (99.1% nt identity) than to other NDV strains (Table 2; Fig. 1d). Comparison of the amino acid (aa) sequences between strains Texas GB and BC showed differences in all six proteins; one aa in N (K421E), four aa in P (P30S, K36R, A98T V104A and D336E), four aa in M (M132V, D152N, P216S and A336T), eight aa in F (S10P, A11V, S265G, E304G, T457I, T510I, A520V and A550T), 11 aa in HN (Q7R, V9A, A34V, M35V, I191V, N228S, V271A, S310G, E332G, L454P and A571V), and 14 aa in L (T75A, I89V, N265D, C698R, K889N, F1379Y, D1531N, N1643S, R1668K, F1706L, L1734P, S1758A, M1785I and I2067V). There was no aa difference between the unique C-terminal regions of V and W proteins of strains Texas GB and BC.

The F protein cleavage site is a well-characterized determinant of NDV pathogenicity in chickens [23, 24]. Virulent NDV strains typically contain a polybasic cleavage site (R-X-K/R-R↓F), which is recognized by intracellular proteases of most cells. The cleavage site of strain

Texas GB is ^{112}R-R-Q-K-R↓F^{117}, which contained four basic amino acids at positions 112–116, corresponding to those of virulent NDV strains [1, 2].

Phylogenetic tree analysis of complete genome sequence of strain Texas GB with 78 full length genomes (or genome sequences >15 kb) of other NDV strains was performed by maximum parsimony method using MEGA 4 software (Molecular Evolutionary Genetics Analysis) [25, 26]. Based on the available genotype classification system for NDV, strain Texas GB is classified under genotype II of class II viruses. Phylogenetic clustering of strain Texas GB with other genotype II strains including BC, LaSota, B1, VG/GA, was evident (Fig. 1d). It was also observed that strain Texas GB is more closely related to strain BC and viruses isolated from China [27] and Egypt [28] than to other NDV strains. The recently sequenced NDV isolates from a northern pintail, a mottled duck, and mallards in the U.S. [29] showed phylogenetic relatedness to strain Texas GB at the genome level than the virulent and avirulent NDV isolates from cormorants [29] (Fig. 1d). The high degree of nt identity and phylogenetic relatedness of velogenic strain Texas GB with a mesogenic strain BC suggest that subtle differences at the genome level may have a profound effect on the pathogenicity of a NDV strain. Availability of the complete genome sequence of strain Texas GB will be useful in further understanding of the NDV pathogenesis and neurovirulence.

Acknowledgments We thank Daniel Rockemann, Arthur Samuel, Flavia Militino Dias and all our laboratory members for their excellent technical assistance. We thank Andrea Ferrero for her help in BSL-3+ facility coordination. We also thank LaShae Green for proofreading the manuscript.

References

1. D.J. Alexander, Newcastle disease and other avian paramyxoviruses. Rev. Sci. Tech. **19**(2), 443–462 (2000)
2. 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)
3. S.K. Sinha, R.P. Hanson, C.A. Brandly, Comparison of the tropisms of six strains of Newcastle disease virus in chickens following aerosol infection. J. Infect. Dis. **91**(3), 276–282 (1952)
4. G. Schloer, R.P. Hanson, Relationship of plaque size and virulence for chickens of 14 representative Newcastle disease virus strains. J. Virol. **2**, 40–47 (1968)
5. D.J. Alexander. in *A Laboratory Manual for the Isolation and Identification of Avian Pathogens*, 4th ed, ed. by D.E. Swayne, J.R. Glisson, M.W. Jackwood, J.E. Pearson, W.M. Reed. (American Association of Avian Pathologists, Inc., Kennett Square, PA, 1998), pp. 156–163
6. D. King, Influence of chicken breed on pathogenicity evaluation of velogenic neurovirulent newcastle disease virus isolates from cormorants and turkeys. Avian Dis. **40**, 210–217 (1996)
7. D.J. King, Newcastle disease virus passage in MDBK cells as an aid in detection of a virulent subpopulation. Avian Dis. **37**(4), 961–969 (1993)
8. M. Banerjee, W.M. Reed, S.D. Fitgerald, B. Panigrahy, Neurotropic velogenic Newcastle disease in cormorants in Michigan: pathology and virus characterization. Avian Dis. **38**, 873–878 (1994)
9. U.M. Schaper, F.J. Fuller, M.D. Ward, Y. Mehrotra, H.O. Stone, B.R. Stripp, E.V. De Buysscher, Nucleotide sequence of the envelope protein genes of a highly virulent, neurotropic strain of Newcastle disease virus. Virology **65**(1), 291–295 (1988)
10. S. Palmieri, B.W. Mitchell, Comparison of three velogenic strains of Newcastle disease virus by RNA oligonucleotide fingerprinting. Avian Dis. **35**(2), 384–388 (1991)
11. M.D. Ward, F.J. Fuller, Y. Mehrotra, E.V. De Buysscher, Nucleotide sequence and vaccinia expression of the nucleoprotein of a highly virulent, neurotropic strain of Newcastle disease virus. Avian Dis. **44**(1), 34–44 (2000)
12. D.P. Locke, H.S. Sellers, J.M. Crawford, S. Schultz-Cherry, D.J. King, R.J. Meinersmann, B.S. Seal, Newcastle disease virus phosphoprotein gene analysis and transcriptional editing in avian cells. Virus Res. **69**(1), 55–68 (2000)
13. B.S. Seal, Analysis of matrix protein gene nucleotide sequence diversity among Newcastle disease virus isolates demonstrates that recent disease outbreaks are caused by viruses of psittacine origin. Virus Genes **11**, 217–224 (1996)
14. M.G. Wise, H.S. Sellers, R. Alvarez, B.S. Seal, RNA-dependent RNA polymerase gene analysis of worldwide Newcastle disease virus isolates presenting different virulence types and their phylogenetic relationship with other members of the *Paramyxoviridae*. Virus Res. **104**, 71–80 (2004)
15. B.S. Seal, Nucleotide and predicted amino acid sequence analysis of the fusion protein and hemagglutinin-neuraminidase protein genes among Newcastle disease virus isolates. Phylogenetic relationships among the Paramyxovirinae based on attachment glycoprotein sequences. Funct. Integr. Genomics **4**(4), 246–257 (2004)
16. S. Krishnamurthy, S.K. Samal, Nucleotide sequences of the trailer, nucleocapsid protein gene and intergenic regions of Newcastle disease virus strain Beaudette C and completion of the entire genome sequence. J. Gen. Virol. **79**(10), 2419–2424 (1998)
17. Z. Li, M. Yu, H. Zhang, H.Y. Wang, L.F. Wang, Improved rapid amplification of cDNA ends (RACE) for mapping both the 5′ and 3′ terminal sequences of paramyxovirus genomes. J. Virol. Methods **130**, 154–156 (2005)
18. A. Paldurai, M. Subbiah, S. Kumar, P.L. Collins, S.K. Samal, Complete genome sequences of avian paramyxovirus type 8 strains goose/Delaware/1053/76 and pintail/Wakuya/20/78. Virus Res. **142**(1–2), 144–153 (2009)
19. A. Czeglédi, D. Ujvári, E. Somogyi, E. Wehmann, O. Werner, B. Lomniczi, Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. Virus Res. **120**(1–2), 36–48 (2006)
20. Y. Huang, H.Q. Wan, H.Q. Liu, Y.T. Wu, X.F. Liu, Genomic sequence of an isolate of Newcastle disease virus isolated from an outbreak in geese: a novel six nucleotide insertion in the noncoding region of the nucleoprotein gene. Arch. Virol. **149**(7), 1445–14457 (2004). doi:10.1007/s00705-004-0297-8
21. H. Liu, F. Chen, Y. Zhao, D. Zheng, J. Li, T. Xu, L. Qi, Z. Wang, Genomic characterization of the first class I Newcastle disease virus isolated from the mainland of china. Virus Genes **40**(3), 365–371 (2010). doi:10.1007/s11262-010-0452-0
22. B.S. Seal, M.G. Wise, J.C. Pedersen, D.A. Senne, R. Alvarez, M.S. Scott, D.J. King, Q. Yu, D.R. Kapczynski, Genomic sequences of low-virulence avian paramyxovirus-1 (Newcastle disease virus) isolates obtained from live-bird markets in North

America not related to commonly utilized commercial vaccine strains. Vet. Microbiol. **106**(1–2), 7–16 (2005)
23. A. Panda, Z. Huang, S. Elankumaran, D. Rockemann, S.K. Samal, Role of fusion protein cleavage site in the virulence of Newcastle disease virus. Microb. Pathog. **36**(1), 1–10 (2004)
24. O.S. de Leeuw, G. Koch, L. Hartog, N. Ravenshorst, B.P.H. Peeters, Virulence of Newcastle disease virus is determined by the cleavage site of the fusion protein and by both the stem region and globular head of the haemagglutinin–neuraminidase protein. J. Gen. Virol. **86**, 1759–1769 (2005)
25. S. Kumar, J. Dudley, M. Nei, K. Tamura, MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief. Bioinformatics **9**, 299–306 (2008)
26. K. Tamura, J. Dudley, M. Nei, S. Kumar, MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24**, 1596–1599 (2007)
27. H.T. Chen, Genomic sequence of Newcastle disease virus. Direct submission in GenBank (2008), http://www.ncbi.nlm.nih.gov/nuccore
28. M.H.A. Mohamed, S. Kumar, A. Paldurai, M.M. Megahad, I.A. Ghanem, M.A. Lebdah, S.K. Samal, Complete genome sequence of a virulent Newcastle disease virus isolated from an outbreak in chickens in Egypt. Virus Genes **39**(2), 234–237 (2009)
29. P.J. Miller, L.M. Kim, C.L. Afonso, H.S. Ip, Evolutionary dynamics of Newcastle disease virus. Virology **391**, 64–72 (2009)