Gene 553 (2014) 84–89

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Genomic characterisation of two virulent Newcastle disease viruses isolated from crested ibis (*Nipponia nippon*) in China

Huafang Hao [a,1], Shengli Chen [b,1], Pengpeng Wu [a], Jie Wang [a], Xuji Duan [a], Enqi Du [a], Xinglong Wang [a], Zengqi Yang [a,*]

[a] College of Veterinary Medicine, Northwest A & F University, Yangling, 712100, Shaanxi, People's Republic of China
[b] The State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, 1 Xujiaping, Chengguan District, Lanzhou, 730046, Gansu, People's Republic of China

ARTICLE INFO

Article history:
Received 8 September 2013
Received in revised form 20 September 2014
Accepted 29 September 2014
Available online 1 October 2014

Keywords:
Crested ibis
Nipponia nippon
Newcastle disease virus
Genomic sequence
Phylogenetic analysis

ABSTRACT

This paper describes the complete genomic sequences of two virulent Newcastle disease virus (NDV) isolates, Shaanxi06 (prevalent genotype VIId) and Shaanxi10 (novel sub-genotype VIi), from sick crested ibises. The genomes of both isolates were 15,192 nt long and consisted of six genes in the order of 3′-NP-P-M-F-HN-L-5′. The genomes of the two isolates were highly similar to other reference NDV strains. However, some unique features were found in the HN protein of Shaanxi06 and the F gene end of Shaanxi10. Shaanxi06 and Shaanxi10 shared the same virulent motif $^{112-}$R-R-Q-K-R-F^{-117} at the F protein cleavage site, which coincided with previous pathogenicity test results. Phylogenetic analysis revealed that both isolates were clustered within class II NDV, with Shaanxi06 in genotype VII and Shaanxi10 in genotype VI. Both isolates shared high homology with the prevalent genotype NDV strains that circulate in fowls and waterfowls. This study is the first to provide genomic information about a novel sub-genotype VIi NDV strain and another genotype VIId virus, which will be useful for subsequent investigations.

1. Introduction

The crested ibis (*Nipponia nippon*) is a globally endangered bird which is found only in Shaanxi Province in Central China and has been reintroduced in Sadogashima in Japan. This endangered avian species is included in the State Protection List of China and is considered a national treasure. The ibis population has increased from seven to over 1500 birds through 30 years of intensive conservation efforts. However, the species remains in danger because of some adverse factors, such as habitat loss, small population size, limited range, winter starvation, persecution and disease (Li et al., 2009). Researchers gradually realised that Newcastle disease (ND) poses a deadly threat to the survival of ibis species (Chen et al., 2013; Fan et al., 2001).

ND is a primary infectious disease in poultry because of its worldwide distribution and devastating effects. ND virus (NDV), a member of the avian paramyxovirus serotype 1 (APMV-1) of genus *Avulavirus* within the *Paramyxoviridae* family, is the causative agent of ND (Mayo, 2002). The enveloped virus has a negative-sense single-stranded RNA genome which is approximately 15.2 kb long; this genome contains six genes in the order of 3′-NP-P-M-F-HN-L-5′, which encode six proteins, namely, nucleocapsid protein, phosphoprotein, matrix protein, fusion protein, haemagglutinin–neuraminidase and large polymerase protein (Alexander, 2003). Two additional proteins, V and W, are generated by an RNA-editing event that occurs during the transcription of the P gene (Steward et al., 1993). NDV isolates are categorised as highly pathogenic (velogenic), intermediate (mesogenic) and apathogenic (lentogenic) strains based on their pathogenicity to chickens (OIE, 2004). Although the molecular basis for NDV virulence involves multiple genes, the F protein cleavage site is an absolute prerequisite for determining NDV pathogenicity in chickens (Panda et al., 2004; Peeters et al., 1999).

Phylogenetic analysis based on the complete genome or partial sequence of the F gene revealed that NDVs can be divided into two major classes (I and II) (Czeglédi et al., 2006). Previously, class I NDVs comprised at least nine genotypes, whereas class II NDVs consisted of genotypes I–IX. On the basis of partial F gene sequence, genotypes VI and VII are further divided into sub-genotypes (VIa–VIi and VIIa–VIIf) (Ballagi-Pordány et al., 1996; Miller et al., 2010; Tsai et al., 2004). A

Abbreviations: aa, amino acid(s); APMV-1, avian paramyxovirus-1; cDNA, DNA complementary to RNA; F, fusion protein; F, phenylalanine; G, guanine; GE, gene end; GS, gene start; HA, hemagglutination; HN, hemagglutinin–neuraminidase; ICPI, intracerebral pathogenicity index; IGS, intergenic sequence; IVPI, intravenous pathogenicity index; L, large protein; L, leucine; M, matrix protein; MDT, mean death time; ND, Newcastle disease; NDV, Newcastle disease virus; NP, nucleoprotein; nt, nucleotide(s); ORF(s), open reading frame(s); P, phosphoprotein; pI, isoelectric point; RACE, rapid amplification of cDNA ends; SPF, specific pathogen-free; UTR(s), untranslated region(s).

* Corresponding author at: College of Veterinary Medicine, Northwest A&F University, Yangling, 712100, People's Republic of China.
E-mail address: yzq8162@163.com (Z. Yang).

[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.gene.2014.09.059
0378-1119/

Table 1
Genomic features and protein characteristics of Shaanxi06 isolate.

Genes	Hexamer phasing position at gene start[a]	Gene characteristics (nt)						Intergenic sequence (nt)	Deduced protein characteristics		
		Gene start (from–to)	Total length	5′-UTR	Coding sequence (from–to)	3′-UTR	Gene end (from–to)		Size (aa)	MW (kDa)	pI
NP	2	56–65	1753	66	122–1591	216	1798–1807	2	489	53.4	5.47
P	4	1810–1819	1451	83	1893–3080	180	3250–3260	1	395	42.1	6.82
V	–	–	–	–	–	–	–	–	239	25.4	6.82
W	–	–	–	–	–	–	–	–	227	24.8	9.75
M	4	3262–3271	1241	34	3296–4390	112	4493–4502	1	364	39.8	9.56
F	4	4504–4513	1792	46	4550–6211	84	6285–6295	31	553	58.9	8.29
HN	3	6327–6336	2002	91	6418–8133	195	8319–8328	47	571	62.7	7.74
L	6	8376–8385	6703	11	8387–15001	77	15069–15078	–	2204	248.5	7.06

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

recent study has proposed that class I NDVs comprise a single genotype while class II NDVs contain genotypes I–XV (Diel et al., 2012).

NDV has a wide host range and can infect at least 250 species within most orders of birds. This virus can be a serious disease agent not only in poultry birds but also in wild birds, including double-crested cormorants (Rue et al., 2010), white pelicans (Wobeser et al., 1993) and crested ibis (Chen et al., 2013; Fan et al., 2001; Qu et al., 2003). Our previous study firstly described the pathogenic and phylogenetic characteristics of two crested ibis NDV isolates, Shaanxi06 (velogenic) and Shaanxi10 (mesogenic), with the former in genotype VIId and the latter in a novel genotype VIi (Chen et al., 2013). The present study describes the genomic characteristics of these two NDV isolates from crested ibis.

2. Materials and methods

2.1. Virus

Two NDV strains (Shaanxi06 and Shaanxi10) were isolated from sick crested ibises from February 2006 to January 2010. The viruses were plaque purified thrice on primary chicken embryo fibroblasts and then propagated on 10-day-old SPF chicken embryos (Merial-Vital Laboratory Animal Technology, Beijing). Allantoic fluids that exhibited a high HA titre were divided into working stocks and were stored at −70 °C for sequence analysis.

2.2. RNA extraction and RT-PCR

Viral RNA genome extraction and first-strand cDNA synthesis were performed as previously described (Chen et al., 2013). Eight pairs of specific primers were designed to amplify the complete genomic cDNA. PCR amplification was performed with 200 ng of cDNA as a template in a 50 μL reaction volume containing 10 pmol of each primer and 2.5 U of Pfu polymerase (TransStart™ FastPfuDNAPolymerase, TransGenBiotech, Beijing, China). The PCR conditions were as follows: 95 °C for 5 min; 32 cycles (95 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min 30 s); and a final 10 min elongation step at 72 °C. The amplified products were separated by electrophoresis in 1% agarose gel.

2.3. Amplification of the 3′ and 5′ end sequences of the viral genome

The 3′ and 5′ end sequences of both viral genomes were amplified by rapid amplification of cDNA ends as previously reported (Qiu et al., 2011).

2.4. Cloning and sequencing of PCR products

The purified RT-PCR products were cloned into the pMD18-T vector (TaKaRa Biotechnology, Dalian, China) and then transformed into calcium-competent *Escherichia coli* DH5α cells (TransGenBiotech, Beijing, China). Recombinant plasmids which contain the product of each PCR fragment were purified and then sequenced in both directions. Each PCR fragment was sequenced at least thrice on an ABI 3730 genetic analyser (Beijing Genomics Institute, Beijing, China).

2.5. Sequence and phylogenetic analyses

Nucleotide sequence editing, analysis and amino acid sequence prediction were conducted using the SEQMAN program of the DNASTAR software suite (version 3.1; DNASTAR, Madison, WI, USA). NDV sequences which represent different genotypes and sub-genotypes were downloaded from GenBank. Nucleotide similarity was processed using the ClustalW multiple alignment algorithm of the DNASTAR software suite (version 3.1; DNASTAR, Madison, WI, USA). On the basis of the complete genome, a phylogenetic tree was constructed using the MEGA software (Version 5.2) with the Kimura two-parameter model and the neighbour-joining algorithm with 1000 bootstraps (Tamura et al., 2011). The evolutionary distances among genotype VI sub-

Table 2
Genomic features and protein characteristics of Shaanxi10 isolate.

Genes	Hexamer phasing position at gene start[a]	Gene characteristics (nt)						Intergenic sequence (nt)	Deduced protein characteristics		
		Gene start (from–to)	Total length	5′-UTR	Coding sequence (from–to)	3′-UTR	Gene end (from–to)		Size (aa)	MW (kDa)	pI
NP	2	56–65	1753	66	122–1591	217	1798–1807	2	489	53.2	5.48
P	4	1810–1819	1451	83	1893–3080	180	3250–3260	1	395	42.1	7.25
V	–	–	–	–	–	–	–	–	239	25.2	5.70
W	–	–	–	–	–	–	–	–	155	16.4	6.37
M	4	3262–3271	1241	34	3296–4390	113	4493–4502	1	364	39.8	9.64
F	4	4504–4513	1792	46	4550–6211	84	6285–6295	31	553	59.0	8.01
HN	3	6327–6336	2002	91	6418–8133	198	8318–8328	47	571	62.8	7.56
L	6	8376–8387	6704	11	8387–15001	78	15069–15078	–	2204	248.4	6.93

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