



Full Genomic Characterization of a Lentogenic Newcastle Disease Virus Isolated from Farm-Reared Ostriches (*Struthio camelus*) in Northwest China

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ABSTRACT To our knowledge, our study is the first to report the whole-genome sequence of an ostrich-origin Newcastle disease virus (NDV) isolate, abbreviated as Ostrich/SX-01/06. Phylogenetic analysis revealed that this isolate belongs to the sub-genotype c in class II. The identification of the complete genome will provide useful information regarding ostrich diseases, especially NDV.

Newcastle disease (ND) is one of the most highly pathogenic viral diseases of avian species (1). ND virus (NDV), the causative pathogen, is also known as avian paramyxovirus type 1 (APMV-1) and belongs to the genus *Avulavirus*, family *Paramyxoviridae*, order *Mononegavirales* (2).

NDV has a wide host range, and it has been demonstrated in at least 250 species in most orders of birds (1). The African ostrich is the largest bird that also serves as a susceptible host of NDV (3). However, the roles of these birds in the ecological evolution of NDV are largely unknown. Furthermore, the complete genomic sequence of ostrich-origin NDV has yet to be published on GenBank.

Two tissue samples from different organs, including liver, lung, heart, spleen, and kidney, were collected from ostriches less than 4 months old at the end of October 2006. After three series of passages in 10-day-old specific-pathogen-free (SPF) chicken embryos, an NDV was isolated from an ostrich and designated NDV/Ostrich/China/Shaanxi-01/2006, abbreviated Ostrich/SX-01/06. Allantoic fluids were collected and stored in the Laboratory of Avian Diseases for use in subsequent analyses. In this study, isolate Ostrich/SX-01/06 was subjected to plaque purification three times by using an agar overlay on chicken embryo fibroblast cells. Total RNA was extracted from allantoic fluids by using RNAisoPlus reagent (TakaRa Biotechnology, Dalian, China). cDNA was synthesized using the PrimeScript first-strand cDNA synthesis kit (TakaRa Biotechnology) with random hexamers, according to the manufacturer's instructions. Ten overlapping fragments covering the full-length genome of the NDV isolate were amplified by reverse transcription-PCR (RT-PCR). The nucleotide and amino acid sequences of the isolate were assembled and aligned using the DNASTar software suite (version 3.1; DNASTar, Madison, WI, USA). This ostrich-origin NDV genome, with 15,192 nucleotides (nt) in length, comprised six open reading frames (ORFs) in the order 3'-NP-P-M-F-HN-L-5' and followed the "rule of six," which is a common rule relied on by other *Paramyxoviridae* members (4).

The pathogenicity index of intracerebral pathogenicity index (ICPI) is 0.1, indicating that Ostrich/SX-01/06 is a lentogenic NDV strain. However, the isolate contained a motif of -¹¹²R-R-Q-R-R-F¹¹⁷- at the F₀ cleavage site, which is a typical molecular characterization of a virulent (velogenic or mesogenic) strain. Coincidentally, several reported NDVs,

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especially the pigeon paramyxovirus type 1 (PPMV-1) isolates with typical virulent cleavage site motifs were not always confirmed by classical OIE assessment criteria for pathogenicity (5–8). Apparently, these findings suggested that the F cleavage site is required but is not the sole determinant of viral virulence for some NDV isolates to become virulent. Further studies should investigate whether the virulence of Ostrich/SX-01/06 can also be determined by other factors, which can be examined by using a platform of reverse genetic systems.

To determine the genetic relationship, a phylogenetic tree was constructed on the basis of the complete sequence (1,662 bp) (9, 10) using the MEGA 6.02 software with the Kimura two-parameter model and the maximum likelihood (ML) method (11). The results showed that Ostrich/SX-01/06 isolate was clustered in subgenotype c in genotype.

Accession number(s). The complete genome sequence of Ostrich/SX-01/06 has been deposited in GenBank under accession number [KU373026](https://www.ncbi.nlm.nih.gov/nuccore/KU373026).

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