

Complete Genome Sequence Analysis of an H6N1 Avian Influenza Virus Isolated from Guangxi Pockmark Ducks

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We report here the complete genomic sequence of a novel H6N1 avian influenza virus strain, A/Duck/Guangxi/GXd-5/2010(H6N1), isolated from pockmark ducks in Guangxi Province, Southern China. All of the 8 gene segments of A/Duck/Guangxi/GXd-5/2010(H6N1) are attributed to the Eurasian lineage; the amino acid motif of the cleavage site between HA1 and HA2 was P-Q-I-E-T-R-G. These are typical characteristics of the low-pathogenicity avian influenza virus. This study will help to understand the epidemiology and molecular characteristics of avian influenza virus in ducks.

The avian influenza virus genome consists of eight separate RNA segments, HA, NA, NP, M, PB1, PB2, PA, and NS, which encode 12 viral structural and nonstructural proteins (6, 7). Avian influenza viruses are divided into subtypes based on the two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). So far, 16 HA and 9 NA subtypes have been identified (5, 8, 9). In recent years, there have been several subtypes of avian influenza virus (H5, H6, and H9 subtypes) circulating and evolving in Southern China (1, 4, 10), which might provide an opportunity for these virus subtypes to recombine.

In 1997, highly pathogenic avian influenza virus A/Hong Kong/156/97(H5N1) derived its HA gene from A/Goose/Guangdong/1/96(H5N1)-like virus and the other seven genes from A/teal/HongKong/W312/97(H6N1)-like virus. It caused disease outbreaks in chickens in Hong Kong (2), consequently resulting in 6 deaths among 18 infected people (3).

In this study, an H6N1 strain, named A/Duck/Guangxi/GXd-5/2010(H6N1), was first isolated from a pockmark duck in Guangxi, Southern China, in 2010. Nucleotide sequences of A/Duck/Guangxi/GXd-5/2010(H6N1) were amplified through reverse transcription-PCR (RT-PCR). The amplified products were purified and cloned into the pMD18-T vector (TaKaRa) and then sequenced (TaKaRa, Dalian, China). Sequences were assembled and manually edited to generate the final genome sequence.

The complete genome of the strain consists of eight segments of negative-sense single-stranded RNA molecules, including PB2, PB1, PA, HA, NP, NA, M, and NS. The full lengths of each segment are 2,341, 2,341, 2,233, 1,744, 1,565, 1,463, 1,027, and 838 nucleotides, respectively. The amino acid sequence at the cleavage site in the HA molecule is P-Q-I-E-T-R-G, which is characteristic of low-pathogenicity avian influenza virus.

Sequence analysis showed that five segments (PB2, PB1, PA, NP, and NA) had close relationships with those of the H5 subtype and the others (HA, M, and NS) had the highest homologies with those of the H6 subtype. HA shared the highest sequence homology (97.7%) with A/duck/Shantou/1080/2007(H6N2) (GenBank accession number [CY109746](#)). NA shared the highest sequence homology (99.1%) with A/Hubei/1/2010(H5N1) (GenBank accession number [CY098760](#)). PB2 shared the highest sequence homology (99.6%) with A/Hubei/1/2010(H5N1) (GenBank accession number [CY098755](#)). PA shared the highest sequence homology (96.5%) with A/Guangxi/1/2009(H5N1) (GenBank accession number [CY098743](#)). M shared the highest sequence

homology (98.2%) with A/chicken/Eastern China/49/2010 (H6N6) (GenBank accession number [JF965223](#)). NS shared the highest sequence homology (98.5%) with A/duck/Hubei/2/2010(H6N6) (GenBank accession number [CY110953](#)). The homologies of the PB1 with the sequences of A/Hubei/1/2010(H5N1), A/HongKong/156/97(H5N1), A/Chicken/Hong Kong/G9/97(H9N2), and A/Teal/HongKong/W312/97(H6N1) (GenBank accession numbers [CY098756](#), [AF036362](#), [AF156416](#), and [AF250477](#), respectively), were 95.5%, 90.6%, 90.5%, and 90.7%, respectively. The homologies of the NP with the sequences of A/Hubei/1/2010(H5N1), A/HongKong/156/97(H5N1), A/Quail/Hong Kong/G1/97 (H9N2), and A/Teal/HongKong/W312/97(H6N1) (GenBank accession numbers [CY098759](#), [AF036359](#), [AF156407](#), and [AF250480](#), respectively), were 99.5%, 91.5%, 91.2%, and 91.3%, respectively. These results are useful for analyses of epidemiology and evolutionary characteristics of avian influenza virus.

Nucleotide sequence accession numbers. The GenBank accession numbers of PB2, PB1, PA, HA, NP, NA, M, and NS for A/Duck/Guangxi/GXd-5/2010(H6N1) are [JX304761](#), [JX304760](#), [JX304759](#), [JX304754](#), [JX304757](#), [JX304756](#), [JX304755](#), and [JX304758](#), respectively.

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