

Characterization and Sequencing of an H6N6 Avian Influenza Virus Isolated from Sansui Sheldrake Ducks in Guizhou, Southwestern China

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Here, we report the complete genome sequence of an H6N6 avian influenza virus (AIV) isolated from Sansui Sheldrake ducks in Guizhou Province, China, in 2014. Phylogenetic analysis showed that the H6N6 virus was a reassortant virus derived from three different H6 subtype lineages. The finding of this study will help us understand the epidemiology and the evolutionary characteristics of H6 subtypes of AIV in ducks in southwestern China.

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Waterfowls are known as a giant genetic reservoir for avian influenza virus (AIV), and to date, all 16 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes of AIV have been isolated from waterfowl species (1). The H6 subtypes of AIV are the most abundantly detected influenza subtypes in wild and domestic aquatic avian species, and they have a much broader host range than any other AIV subtypes (2, 3). Recent studies concerning human H5N6 infection have demonstrated that the isolated H5N6 viruses are the avian-originated viruses and belong to novel reassortants between H5N1 and H6N6 viruses (4, 5). Therefore, it is important to enhance the epidemiological surveillance of the H6N6 subtypes of AIV in ducks to understand the emergence of novel reassortants with potential threat to human health.

In this study, an H6N6 subtype AIV strain, A/duck/Guizhou/013/2014 (H6N6), was isolated from apparently healthy Sansui Sheldrake ducks in Guizhou Province, China, in 2014. All eight gene segments of the H6N6 AIV were sequenced by DNA direct sequencing. Editing and analysis of the sequence data were performed with Lasergene 7.1 and MEGA 6.0.

The full lengths of the polymerase basic 2 (PB2), PB1, polymerase acidic (PA), HA, nucleoprotein (NP), NA, matrix (M), and nonstructural (NS) genes were 2,341, 2,341, 2,233, 1,744, 1,565, 1,465, 1,027, and 890 nucleotides, respectively. Phylogenetic analysis showed that the nucleotide sequence identities of the HA and NA genes with that of the H6N6 isolates A/duck/Jiangsu/022/2009 (H6N6) and A/duck/Fujian/8089/2007 (H6N6) were 97% and 98%, respectively. The PB2 and PB1 genes were most closely similar to the foreign isolates A/duck/Yamagata/06/2014 (H6N6) and A/duck/Vietnam/LBM6/2011 (H6N6), with which they share 97% and 98% nucleotide homology, respectively. The other four genes were found to be more similar to those of southern China H6N6 strains (up to 97% to 99% nucleotide homology). In addition, the phylogenetic trees revealed that all eight gene segments of this isolate belonged to the Eurasian avian lineage, but the HA and PB2 genes belonged to the sequence type 2853 (ST2853)-like and

HN573-like lineages, respectively, whereas the other genes belonged to ST339-like lineage. These results indicate that the H6N6 virus was a reassortant virus resulting from three different H6 subtype lineages.

Amino acid sequence analysis showed that the cleavage site motif between HA1 and HA2 was PQIETR ↓ G; this is a typical characteristic of low-pathogenicity AIV (6). Similar to H5 HA, the receptor binding sites in the HA protein were Q224 and G226 (H6 numbering), which would preferentially bind to alpha-linked (2, 3) sialic acid receptor (7, 8). Analysis of the NA sequences revealed that there was no mutation at the H275Y position, indicating that the virus may be sensitive to neuraminidase inhibitor drugs (9). The PB2 amino acid residues at positions 627 and 701 still remained the characteristics of AIV (E627 and D701), so it could not replicate in mammalian hosts (10, 11). In addition, there were no mutations at positions L26, I27, A30, S31, and G34 in the M2 protein, suggesting that this strain is not amantadine resistant (12). These findings will help us understand the epidemiology and the evolutionary characteristics of H6 subtype AIVs circulating in ducks in southwestern China.

Nucleotide sequence accession numbers. The complete genome sequences of A/duck/Guizhou/013/2014 (H6N6) have been deposited in GenBank under the accession numbers [KU762356](https://www.ncbi.nlm.nih.gov/nuccore/KU762356) to [KU762363](https://www.ncbi.nlm.nih.gov/nuccore/KU762363).

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