

Complete Genomic Sequence of a Novel Natural Recombinant H6N2 Influenza Virus from Chickens in Guangdong, Southern China

Peirong Jiao, Runyu Yuan, Liangmeng Wei, Baoqin Jia, Lan Cao, Yafen Song, Lang Gong, Chenggang Xu, Tao Ren, and Ming Liao

MOA Key Laboratory for Animal Vaccine Development, Key Laboratory of Zoonoses Control and Prevention of Guangdong, College of Veterinary Medicine, South China Agricultural University, Guangzhou, China

Here, we reported the complete genome sequence of a novel H6N2 avian influenza virus (AIV) isolated from chicken in Guangdong, Southern China, in 2011 which was a natural recombinant virus between the H6N2 and H5N1 subtypes. It will help to understand the epidemiology and molecular characteristics of H6N2 influenza virus in Southern China.

Avian influenza (AI) is caused by type A influenza viruses, which result mainly in systemic or respiratory infection diseases of birds. Based on the antigenicity of hemagglutinin (HA) and neuraminidase (NA), avian influenza virus (AIV) can be divided into H1 to H16 and N1 to N9, respectively (5, 7, 9). In recent years, there have been several subtypes of AIVs (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, H5N1 highly pathogenic AIV derived its HA gene from A/Goose/Guangdong/1/96(H5N1)-like virus and its other seven genes from A/teal/HongKong/W312/97(H6N1)-like virus and caused disease outbreaks in chickens in Hong Kong (2) and caused 6 deaths among 18 people infected (3, 8). Therefore, it is necessary to examine the epidemiological research to understand the evolution and recombination of AIVs in Southern China.

In November 2011, we isolated a natural recombinant H6N2 influenza virus (A/Chicken/Guangdong/C273/2011 [C273]) from healthy chickens that was between the H6N2 and H5N1 subtypes, a strain which was rarely reported in Guangdong.

To investigate more information about this recombinant virus, we determined the complete genomic sequence with an ABI 3730 genetic analyzer. The 8 genome segments of the virus encoded 10 proteins (PB2, PB1, PA, HA, NP, NA, M1, M2, NS1, and NS2) with the amino acid lengths 759, 757, 716, 566, 498, 469, 252, 80, 230, and 121, respectively. The C273 virus possessed the low-pathogenic influenza A virus sequence PQIETR ↓ G at the cleavage site between HA1 and HA2. The receptor-binding pocket of HA1 retains the amino acid residues Q226 and G228 (H3 numbering), which preferentially bind to the avian influenza virus receptor. The consensus amino acid sequences revealed eight potential N-linked glycosylation sites in HA (26 or 27, 39, 182, 306, 311, 498, and 557) and nine potential N-linked glycosylation sites in NA (61 or 69, 70, 86, 146, 200, 234, 331, and 402). Amino acid residue 627 of PB2 was E rather than K and amino acid residue 701 of PB2 was D, showing residues which are characteristic of the avian influenza virus.

Phylogenetic analysis of the C273 surface genes HA and NA showed that they belonged to ST339-like [A/duck/Shantou/339/2000(H6N2)-like] viruses (6) and that their nucleotide homologies were 95.4% and 94.8% compared with the ST339-like virus, respectively. The internal genes showed that the PB1, NP, M, and NS genes belonged to ST339-like viruses, and nucleotide homologies were above 95.3%, 93.8%, 97.1%, and 96.8% compared with

the ST339-like virus, respectively. The PA gene belonged to HN573-like [A/duck/Hunan/573/2002(H6N2)-like] virus (6) and had nucleotide homology above 95.7% compared with the HN573-like virus. However, the PB2 gene of C273 was derived from the GS/GD96-like H5N1 virus lineage, and nucleotide homology was 90.7% compared with the A/Goose/Guangdong/1/96-like H5N1 virus. Thus, we isolated a natural recombinant H6N2 influenza virus from chicken that was between the H6N2 and H5N1 subtypes.

Therefore, continuing influenza virus surveillance in poultry is critical to understanding the genesis and emergence of coming pandemic strains in Southern China.

Nucleotide sequence accession numbers. The genome sequences of A/Chicken/Guangdong/C273/2011 (C273) have been deposited in GenBank under accession numbers [JQ924794](https://www.ncbi.nlm.nih.gov/nuccore/JQ924794) and [JQ924801](https://www.ncbi.nlm.nih.gov/nuccore/JQ924801).

ACKNOWLEDGMENTS

This work was supported by grants from the Natural Science Foundation of Guangdong Province (no. 10251064201000004 and no.10151064201000021), the National Natural Science Foundation of China (no. 31172343), the Science and Technology Projects of Guangdong Province (no. 2010B020307005), the Earmarked Fund for Modern Agro-Industry Technology Research System (nycyt-x-42-G3-03), and the High-level Talents in University Project of Guangdong Province.

REFERENCES

1. Cheung CL, et al. 2007. Establishment of influenza A virus (H6N1) in minor poultry species in southern China. *J. Virol.* 81:10402–10412.
2. Chin PS, et al. 2002. Molecular evolution of H6 influenza viruses from poultry in Southeastern China: prevalence of H6N1 influenza viruses possessing seven A/Hong Kong/156/97 (H5N1)-like genes in poultry. *J. Virol.* 76:507–516.
3. Claas EC, et al. 1998. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. *Lancet* 351:472–477.
4. Duan L, et al. 2008. The development and genetic diversity of H5N1 influenza virus in China, 1996–2006. *Virology* 380:243–254.
5. Fouchier RA, et al. 2005. Characterization of a novel influenza A virus

Received 20 April 2012 Accepted 23 April 2012

Address correspondence to Ming Liao, mliao@scau.edu.cn.

P.J. and R.Y. contributed equally to this work.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JVI.00963-12

- hemagglutinin subtype (H16) obtained from black-headed gulls. *J. Virol.* 79:2814–2822.
6. **Huang K, et al.** 2010. Establishment of an H6N2 influenza virus lineage in domestic ducks in southern China. *J. Virol.* 84:6978–6986.
 7. **Subbaral K, Joseph T.** 2007. Scientific barriers to developing vaccines against avian influenza viruses. *Nature* 7:267–278.
 8. **Subbarao K, et al.** 1998. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science* 279:393–396.
 9. **Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y.** 1992. Evolution and ecology of influenza A viruses. *Microbiol. Rev.* 56:152–179.
 10. **Xu KM, et al.** 2007. The genesis and evolution of H9N2 influenza viruses in poultry from southern China, 2000 to 2005. *J. Virol.* 81: 10389–10401.