



Novel Reassortant H3N2 Avian Influenza Virus Isolated from Domestic Ducks in Eastern China in 2016

Wenqiang Sun, Jiaxin Li, Jiao Hu, Daxiu Jiang, Zhichuang Ge, Chaonan Xing, Xiaoquan Wang, Min Gu, Xiaowen Liu, Shunlin Hu, Xiufan Liu

Animal Infectious Disease Laboratory, School of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu, China; Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonosis, Yangzhou University, Yangzhou, Jiangsu, China; and Key Laboratory of Prevention and Control of Biological Hazard Factors (Animal Origin) for Agri-Food Safety and Quality, Ministry of Agriculture of China, Yangzhou University, Yangzhou, Jiangsu, China

ABSTRACT H3 subtype avian influenza virus (AIV) poses a great threat to public health, and so investigating its epidemiology is of great importance. A novel reassortant H3N2 AIV strain was isolated from a live poultry market in eastern China. The strain's genes originated from H1N1, H3, and H7 AIVs. Thus, the genome information of the H3N2 isolate will help to investigate further the epidemiology of H3 subtype AIVs in China.

Avian influenza viruses (AIVs) have a wide range of hosts, from birds to mammals (1–7). Notably, multiple subtypes of influenza viruses (H3, H4, H6, H9, H10, etc.) have also circulated and evolved in live poultry markets (LPMs), which are considered to be recombinant sources of AIVs in China (8–10). Meanwhile, highly pathogenic AIVs (HPAIVs) and low pathogenic AIVs (LPAIVs) frequently undergo reassortment with each other (7–9, 11–13). In particular, H3 subtype AIVs can be detected in LPMs in China (9). As we know, H3 subtype AIVs have a wide host range, including birds, humans, pigs, dogs, and horses (3, 4, 14–17). H3 subtype AIVs can serve their gene segments to other HPAIVs and also reassort with other AIV subtypes (10, 18–20). So, the monitoring of H3 subtype AIVs is of great significance to public health.

An H3N2 strain of AIV was isolated from a duck in September 2016 in Jiangsu, China, and named A/duck/Jiangsu/YZ916/2016(H3N2). RT-PCR was performed by universal primers (21), followed by sequencing (Nanjing Genscript). The DNA sequences were edited with Lasergene version 7.1 software (DNASTAR), and genetic evolution analysis of the isolate was performed with MEGA7 software.

The complete genome of the isolate included eight genes, polymerase basic 2 (PB2), polymerase basic 1 (PB1), polymerase acidic (PA), hemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), matrix (M), and nonstructural (NS) proteins. The open reading frame lengths of these segments are 2,280, 2,277, 2,151, 1,701, 1,497, 1,401, 982, and 838 nucleotides, respectively. PEKQTR↓G at the cleavage site of the isolate indicated that the virus was an LPAIV. The potential glycosylation sites of the isolate revealed that there were five potential N-linked glycosylation sites in HA (positions 22, 38, 165, 285, and 483) and six in NA (positions 61, 69, 86, 146, 200, and 402). Moreover, there were amino acid residues, Q226 and G228, at the receptor binding site in the HA protein, which indicated that the isolate preferentially binds to a receptor of avian origin. In PB2, there were no mutations for E627K and D701N, which are associated with mammalian host adaptation (22).

Genetic evolution analysis of the eight genes of the isolate indicated that the HA gene showed a high similarity (97%) with isolate A/duck/Jiangsu/J2186/2014(H3). The NA gene showed high homology (99%) with isolate A/chicken/Ganzhou/G243/

Received 5 October 2017 Accepted 30 October 2017 Published 30 November 2017

Citation Sun W, Li J, Hu J, Jiang D, Ge Z, Xing C, Wang X, Gu M, Liu X, Hu S, Liu X. 2017. Novel reassortant H3N2 avian influenza virus isolated from domestic ducks in eastern China in 2016. *Genome Announc* 5:e01237-17. <https://doi.org/10.1128/genomeA.01237-17>.

Copyright © 2017 Sun et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Xiufan Liu, xfliu@yzu.edu.cn.

2016(H3N2). The PB2 gene showed the highest homology (98%) with isolate A/duck/Jiangxi/21224/2013(H7N3). The PB1 gene showed the highest homology (99%) with isolate A/chicken/Huzhou/3916/2013(H7N3). The PA gene showed the highest homology (99%) with isolate A/duck/Mongolia/520/2015(H1N1). The NP gene showed the highest homology (99%) with isolate A/duck/Jiangxi/21710/2013(H7N3). The M gene showed the highest homology (99%) with isolate A/muscovy duck/Vietnam/LBM348/2013(H3N8). The NS gene was most closely related to isolate A/environment/Korea/W478/2014(H7N7) with 99% homology. Overall, the isolate was a novel reassortant H3N2 AIV originating from H1N1, H3, and H7.

H3 subtype AIVs can pose a great threat to public health, and thus the genome information reported here can help further epidemiological investigations of H3 subtype AIVs in China.

Accession number(s). This genome sequence has been deposited in GenBank under the accession numbers [MG021165](#) to [MG021172](#).

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (31502076), by the Jiangsu Provincial Natural Science Foundation of China (BK20150444), by the Natural Science Foundation of the Higher Education Institutions of Jiangsu Province, China (15KJB230006), by a Special Financial Grant from the China Postdoctoral Science Foundation (2016T90515), by the National Key Research and Development project of China (2016YFD0501601 and 2016YFD0500202), by the National Key Technologies R&D Program of China (2015BAD12B01-3), by the earmarked fund for Modern Agro-Industry Technology Research System (nycytx-41-G07), and by a project funded by the Priority Academic Development Program of Jiangsu Higher Education Institutions (PAPD).

REFERENCES

- Pollett S, Nelson MI, Kasper M, Tinoco Y, Simons M, Romero C, Silva M, Lin X, Halpin RA, Fedorova N, Stockwell TB, Wentworth D, Holmes EC, Bausch DG. 2015. Phylogeography of influenza A(H3N2) virus in Peru, 2010–2012. *Emerg Infect Dis* 21:1330–1338. <https://doi.org/10.3201/eid2108.150084>.
- Liu T, Xie Z, Luo S, Xie L, Deng X, Xie Z, Huang L, Huang J, Zhang Y, Zeng T, Wang S. 2015. Characterization of the whole-genome sequence of an H3N6 avian influenza virus, isolated from a domestic duck in Guangxi, southern China. *Genome Announc* 3(5):e01190-15. <https://doi.org/10.1128/genomeA.01190-15>.
- Su S, Li HT, Zhao FR, Chen JD, Xie JX, Chen ZM, Huang Z, Hu YM, Zhang MZ, Tan LK, Zhang GH, Li SJ. 2013. Avian-origin H3N2 canine influenza virus circulating in farmed dogs in Guangdong, China. *Infect Genet Evol* 14:444–449. <https://doi.org/10.1016/j.meegid.2012.11.018>.
- Abente EJ, Anderson TK, Rajao DS, Swenson S, Gauger PC, Vincent AL. 2016. The avian-origin H3N2 canine influenza virus that recently emerged in the United States has limited replication in swine. *Influenza Other Respir Viruses* 10:429–432. <https://doi.org/10.1111/irv.12395>.
- Usui T, Soda K, Tomioka Y, Ito H, Yabuta T, Takakuwa H, Otsuki K, Ito T, Yamaguchi T. 2017. Characterization of clade 2.3.4.4 H5N8 highly pathogenic avian influenza viruses from wild birds possessing atypical hemagglutinin polybasic cleavage sites. *Virus Genes* 53:44–51. <https://doi.org/10.1007/s11262-016-1399-6>.
- Liu L, Zeng X, Chen P, Deng G, Li Y, Shi J, Gu C, Kong H, Suzuki Y, Jiang Y, Tian G, Chen H. 2016. Characterization of clade 7.2 H5 avian influenza viruses that continue to circulate in chickens in China. *J Virol* 90: 9797–9805. <https://doi.org/10.1128/JVI.00855-16>.
- Huang K, Zhu H, Fan X, Wang J, Cheung CL, Duan L, Hong W, Liu Y, Li L, Smith DK, Chen H, Webster RG, Webby RJ, Peiris M, Guan Y. 2012. Establishment and lineage replacement of H6 influenza viruses in domestic ducks in southern China. *J Virol* 86:6075–6083. <https://doi.org/10.1128/JVI.06389-11>.
- Yuan R, Wang Z, Kang Y, Wu J, Zou L, Liang L, Song Y, Zhang X, Ni H, Lin J, Ke C. 2016. Continuing reassortant of H5N6 subtype highly pathogenic avian influenza virus in Guangdong. *Front Microbiol* 7:520. <https://doi.org/10.3389/fmicb.2016.00520>.
- Bi Y, Chen Q, Wang Q, Chen J, Jin T, Wong G, Quan C, Liu J, Wu J, Yin R, Zhao L, Li M, Ding Z, Zou R, Xu W, Li H, Wang H, Tian K, Fu G, Huang Y, Shestopalov A, Li S, Xu B, Yu H, Luo T, Lu L, Xu X, Luo Y, Liu Y, Shi W, Liu D, Gao GF. 2016. Genesis, evolution and prevalence of H5N6 avian influenza viruses in China. *Cell Host Microbe* 20:810–821. <https://doi.org/10.1016/j.chom.2016.10.022>.
- Li Q, Zhao Q, Gu M, Zhu J, Gu X, Zhao G, Liu Q, Wang X, Liu X, Liu X. 2013. Genome sequence of a novel reassortant H3N2 avian influenza virus from domestic mallard ducks in eastern China. *Genome Announc* 1(2): e0022112. <https://doi.org/10.1128/genomeA.00221-12>.
- Teng Q, Hu T, Li X, Li G, Li Z. 2012. Complete genome sequence of an H3N2 avian influenza virus isolated from a live poultry market in eastern China. *J Virol* 86:11944. <https://doi.org/10.1128/JVI.02082-12>.
- Zhao T, Qian YH, Chen SH, Wang GL, Wu MN, Huang Y, Ma GY, Fang LQ, Gray GC, Lu B, Tong YG, Ma MJ, Cao WC. 2016. Novel H7N2 and H5N6 avian influenza A viruses in sentinel chickens: a sentinel chicken surveillance study. *Front Microbiol* 7:1766. <https://doi.org/10.3389/fmicb.2016.01766>.
- Wu ZQ, Zhang Y, Zhao N, Yu Z, Pan H, Chan TC, Zhang ZR, Liu SL. 2017. Comparative epidemiology of human fatal infections with novel, high (H5N6 and H5N1) and low (H7N9 and H9N2) pathogenicity avian influenza A viruses. *Int J Environ Res Publ Health* 14:263. <https://doi.org/10.3390/ijerph14030263>.
- Liu T, Xie Z, Wang G, Song D, Huang L, Xie Z, Deng X, Luo S, Huang J, Zeng T. 2015. Avian influenza virus with hemagglutinin-neuraminidase combination H3N6, isolated from a domestic pigeon in Guangxi, southern China. *Genome Announc* 3(1):e01537-14. <https://doi.org/10.1128/genomeA.01537-14>.
- Lyoo KS, Kim JK, Kang B, Moon H, Kim J, Song M, Park B, Kim SH, Webster RG, Song D. 2015. Comparative analysis of virulence of a novel, avian-origin H3N2 canine influenza virus in various host species. *Virus Res* 195:135–140. <https://doi.org/10.1016/j.virusres.2014.08.020>.
- Su S, Chen JD, Qi HT, Zhu WJ, Xie JX, Huang Z, Tan LK, Qi WB, Zhang GH. 2012. Complete genome sequence of a novel avian-like H3N2 swine influenza virus discovered in southern China. *J Virol* 86:9533. <https://doi.org/10.1128/JVI.01315-12>.

17. Choi JG, Kang HM, Kim MC, Paek MR, Kim HR, Kim BS, Kwon JH, Kim JH, Lee YJ. 2012. Genetic relationship of H3 subtype avian influenza viruses isolated from domestic ducks and wild birds in Korea and their pathogenic potential in chickens and ducks. *Vet Microbiol* 155:147–157. <https://doi.org/10.1016/j.vetmic.2011.08.028>.
18. Li Q, Zhong L, Zhao Q, He L, Duan Z, Chen C, Chen Y, Gu M, Wang X, Liu X, Liu X. 2013. Genome sequence of a novel reassortant H3N6 avian influenza virus from domestic mallard ducks in Eastern China. *Genome Announc* 1(2):e0022312. <https://doi.org/10.1128/genomeA.00223-12>.
19. Ma J, Shen H, Liu Q, Bawa B, Qi W, Duff M, Lang Y, Lee J, Yu H, Bai J, Tong G, Hesse RA, Richt JA, Ma W. 2015. Pathogenicity and transmissibility of novel reassortant H3N2 influenza viruses with 2009 pandemic H1N1 genes in pigs. *J Virol* 89:2831–2841. <https://doi.org/10.1128/JVI.03355-14>.
20. Yang L, Zhu W, Li X, Bo H, Zhang Y, Zou S, Gao R, Dong J, Zhao X, Chen W, Dong L, Zou X, Xing Y, Wang D, Shu Y. 2017. Genesis and dissemination of highly pathogenic H5N6 avian influenza viruses. *J Virol* 91:e02199-16. <https://doi.org/10.1128/JVI.02199-16>.
21. Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. 2001. Universal primer set for the full-length amplification of all influenza A viruses. *Arch Virol* 146:2275–2289. <https://doi.org/10.1007/s007050170002>.
22. Ngai KL, Chan MC, Chan PK. 2013. Replication and transcription activities of ribonucleoprotein complexes reconstituted from avian H5N1, H1N1pdm09 and H3N2 influenza A viruses. *PLoS One* 8:e65038. <https://doi.org/10.1371/journal.pone.0065038>.