Emergence of a Novel Reassortant H5N6 Highly Pathogenic Avian Influenza Virus of Clade 2.3.2.1c from domestic poultry in China

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Abstract

H5N6 avian influenza virus (AIV) has posed a threat to poultry and human health. Here, we isolated a new clade 2.3.2.1 H5N6 virus, not like most reported H5N6 AIVs of clade 2.3.4.4. Our analysis revealed a complex pattern of its evolution in which the virus was derived as a result of genetic reassortment among clade 2.3.2.1 and clade 2.3.4.4 H5 and H6N6 AIVs. Moreover, the results suggested that H5N6 clade 2.3.2.1 AIVs had multiple reassortment pattern. It is necessary that to strengthen continuing surveillance to prevent infection in human and poultry.

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Abstract:

H5N6 avian influenza virus (AIV) has posed a threat to poultry and human health. Here, we isolated a new clade 2.3.2.1 H5N6 virus, not like most reported H5N6 AIVs of clade 2.3.4.4. Our analysis revealed a complex pattern of its evolution in which the virus was derived as a result of genetic reassortment among clade 2.3.2.1 and clade 2.3.4.4 H5 and H6N6 AIVs. Moreover, the results suggested that H5N6 clade 2.3.2.1 AIVs had multiple reassortment pattern. It is necessary that to strengthen continuing surveillance to prevent infection in human and poultry.

H5 subtype highly pathogenic avian influenza (HPAI) viruses has become endemic among domestic poultry in China since the first detection in 1996(Chen, 2009; Swayne, 2012). Based on the phylogenetic analysis of HA sequences, H5 avian influenza viruses (AIVs) has evolved into clades 0˜9(Zhao et al., 2012). Clades 2.3.4.4 and 2.3.2.1 of H5N1 subtype HPAIVs have become dominant clade in China(J. Li et al., 2018). Currently, in the process of constant evolution, H5 subtypes AIVs of clade 2.3.4.4 are now reasserted with various NA subtypes such as the emerging reassortant H5N2, H5N5, H5N6, and H5N8 viruses. Notably, H5N6 AIVs are now one of the major subtype reassortant viruses and pose a potential threat to human

health. In the reports of AIV surveillance in China from 2014 to 2016, Bi et al found the H5N6 has replaced H5N1 as a dominant AIV subtype in southern China(Bi et al., 2016). Thus far, 24 human H5N6 cases have been reported(Y. Li et al., 2020). Importantly, nearly all of the H5N6 HA sequences mentioned in these previous reports fell within clade 2.3.4.4. They arose from reassortments with the main pattern identified: H5 HA genes, H6N6 NA genes, and various internal genes of low pathogenic AIVs (eg.H5-origin, H6-origin, H9-origin, H3-origin)(Sun et al., 2018).

In the previous phylogenetic analysis of 505 H5N6 AIVs performed by Bi et al, the HA gene of one H5N6 strain, A/duck/Vietnam/LBM360c1-4-1/2013, belonged to clade 2.3.2.1(Bi et al., 2016). A novel reasortment H5N6 AIVs of clade 2.3.2.1c, A/Streptopelia decaocto/Jiangxi/G6/2016, was detected from a wild bird in China(Zhang, Li, Zhu, Chang, & Xu, 2019). Here, we isolated one HPAI H5N6 virus (A/chicken/Guangxi/16/2019, GX16) from chicken in China in 2019, with the HA gene divided into clade 2.3.2.1c. Remarkable, not all clade 2.3.2.1 viruses belong to H5N1, and it also involves several H5N6 reassortments. Moreover, the diverse host range of these variants detected in poultry and wild bird species, and the distinct geographic regions, demonstrate the current expansion of H5N6 reassortments of clade 2.3.2.1.

Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Gmbh, Hilden, Germany) according to the manufacturer's instructions. The complete genome sequences of the virus GX16 was amplified using RT-PCR with SuperScript-III One-Step RT-PCR System with Platinum® Taq DNA Polymerase (Invitrogen, Waltham, MA, USA) with primers described previously(Hoffmann, Stech, Guan, Webster, & Perez, 2001). The gene-specific RT-PCR amplicons were purified from gels using the QIAquick Gel Extraction Kit (Qiagen, Gmbh, Hilden, Germany) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequences generated in this study were deposited in GenBank (accession nos.–).

Nucleotide identity analysis showed that the GX16 virus was closely related, according to its hemagglutinin (HA) gene segment, to A/Streptopelia decaocto/China/4/2016(H5N6), with a nucleotide sequence identity of 98.36%; the identity of 98.30% with A/pigeon/Zhejiang/112090/2014(H5N1) virus was also recorded. The neuraminidase (NA) gene of the GX16 virus shared 98.70% nucleotide identity with the H5N6 viruses isolated from Kumamoto and Korea (Supplementary file). Six internal segments—polymerase basic (PB2), PB1, polymerase acidic (PA), nucleoprotein (NP), matrix protein (MP), nonstructural protein (NS)—revealed a nucleotide identity of 98.42%–99.49% with the H5N6 and H5N1 viruses (Supplementary file).

To understand the evolutionary pattern of clade 2.3.2.1 H5N6 viruses, the phylogenetic tree was constructed based on the HA sequences of H5 AIVs from reference sequences of WHO and from GenBank Influenza Virus Database and GISAID database. The GX16/H5N6 virus belongs to the clade 2.3.2.1 with other six H5N6 viruses in these databases. As shown in Figure, there were three clusters in clade 2.3.2.1c: Nanjing-like, including the Hunan(HN232)/2015, Hunan(HN234)/2015, Yunnan/2015, and Hubei/2015; Vietnam-like, including the Vietnam/2013; Alberta-like, including GX16/2019 isolate and China non-duck originated isolate A/Streptopelia decaocto/China/4/2016. There were 7 kinds of possible evolutionary approaches of the novel clade 2.3.2.1 H5N6 viruses found, in which the pattern of GX16/2019 was obviously different from other 5 isolates in China.

Interestingly, the genetic distance between the seven viruses was disproportionate to the date of the virus was isolated. The Alberta-like was more closed to Vietnam-like, compared to Nanjing-like in which most of H5N6 isolates clustered (Supplementary file). It is important that each cluster has isolate can infect human in clade 2.3.2.1.

The phylogenetic tree of N6 genes showed that there were 2 groups: Group 1 of H5N6 2.3.4.4d with aa58–68 deletion and Group 2 of clade 2.3.4.4 H5N6 and H6N6 with no deletion. Isolates that infected human mainly came from Group 1. Among above seven clade 2.3.2.1 H5N6 isolates, the NA genes of Vietnam/2013 and Hubei/2015 were derived from H6N6 with no deletion in their regions respectively. The NA gene of GX16/2019 isolate was originated from Japan-like clade 2.3.4.4 H5N6 (Supplementary Figure).

The remaining six internal genes of the GX16/2019 virus were reassorted with clade 2.3.2.1c Alberta-like

H5N1 viruses (NP, MP, and NS) and clade 2.3.4.4d Japan-like H5N6 viruses (PB2, PB1, and PA), and revealed a close phylogenetic relatedness with these viruses frequently found in Asia in 2014–2019 (Supplementary Figure). However, the NP gene of Hunan(HN234)/2015 which was reported previously originated from the H7N3 virus.

The HA gene of the GX16/2019 virus possessed multiple basic amino acids, "PQRERRRKR/GLF", in the cleavage site of the HA indicating high pathogenicity of this virus. The GX16 virus exhibited D101N, S137A, S158N, S159N, T160A, S114R, and T151I amino acid substitutions (H3 numbering) at its HA protein have been reported previously to be related to virulence, transmission, and host specificity. The NA coded protein of the GX16 virus was found 11aa deletion in position 58–68, indicating that the virus could exhibit increased virulence in mice. Further, the GX16 possessed several mutations, like L89V and G309D in PB2, D3V and D622G in PB1, N383D in PA, N30D, I43M and T215A in M1, or. P42S in NS1, suggesting that the virus could exhibit increased virulence and transmission in mammals.

Summary, we have isolated a new clade 2.3.2.1 H5N6 virus and revealed a complex pattern of its evolution. The evidence demonstrates that clade 2.3.2.1 H5N1 AIVs could reassort with circulating H5N6 and H6N6 AIVs. Clearly, clade 2.3.2.1 H5N6 viruses are capable of receiving gene segments from different subtypes and different clades.

Avian influenza viruses are a threat to human health, as they could cross the species barrier and infect humans occasionally with severe outcome. The genetic diversity of H5 viruses is increasing, due to continued circulation and reassortment in poultry, posing a constant risk for public health and requiring regular risk assessments. Our study underlines the importance of active surveillance in the timely detection of new AIV reassortants, including influenza itself and its hosts from the perspective of epidemiology, virology and ecology.

Conflict of Interest: All authors have no conflict of interest.

References

Bi, Y., Chen, Q., Wang, Q., Chen, J., Jin, T., Wong, G., . . . Gao, G. F. (2016). Genesis, Evolution and Prevalence of H5N6 Avian Influenza Viruses in China. *Cell Host Microbe, 20* (6), 810-821. doi:10.1016/j.chom.2016.10.022

Chen, H. (2009). H5N1 avian influenza in China. *Sci China C Life Sci, 52* (5), 419-427. doi:10.1007/s11427-009-0068-6

Hoffmann, E., Stech, J., Guan, Y., Webster, R. G., & Perez, D. R. (2001). Universal primer set for the full-length amplification of all influenza A viruses. *Arch Virol, 146* (12), 2275-2289. doi:10.1007/s007050170002

Li, J., Hou, G., Wang, Y., Wang, S., Cheng, S., Peng, C., & Jiang, W. (2018). Protective efficacy of an inactivated chimeric H5 avian influenza vaccine against H5 highly pathogenic avian influenza virus clades 2.3.4.4 and 2.3.2.1. *J Gen Virol, 99* (12), 1600-1607. doi:10.1099/jgv.0.001140

Li, Y., Li, M., Li, Y., Tian, J., Bai, X., Yang, C., . . . Chen, H. (2020). Outbreaks of Highly Pathogenic Avian Influenza (H5N6) Virus Subclade 2.3.4.4h in Swans, Xinjiang, Western China, 2020. *Emerg Infect Dis, 26* (12), 2956-2960. doi:10.3201/eid2612.201201

Sun, W., Li, J., Hu, J., Jiang, D., Xing, C., Zhan, T., & Liu, X. (2018). Genetic analysis and biological characteristics of different internal gene origin H5N6 reassortment avian influenza virus in China in 2016. *Vet Microbiol, 219* , 200-211. doi:10.1016/j.vetmic.2018.04.023

Swayne, D. E. (2012). Impact of vaccines and vaccination on global control of avian influenza.*Avian Dis, 56* (4 Suppl), 818-828. doi:10.1637/10183-041012-Review.1

Zhang, T., Li, R., Zhu, G., Chang, J., & Xu, B. (2019). First Detection of a Novel Reassortant Avian Influenza A(H5N6) Clade 2.3.2.1c Virus, Isolated from a Wild Bird in China.*Microbiol Resour Announc, 8* (36). doi:10.1128/MRA.00797-19

Zhao, G., Gu, X., Lu, X., Pan, J., Duan, Z., Zhao, K., . . . Liu, X. (2012). Novel reassortant highly pathogenic H5N2 avian influenza viruses in poultry in China. *PLoS One, 7* (9), e46183. doi:10.1371/journal.pone.0046183

Figure Legend

Figure. Genesis of H5N6 AIVs. Virus particles are shown as colored ovals containing horizontal bars that represent the eight gene segments (from top to bottom: PB2, PB1, PA, HA, NP, NA, M, and NS). To illustrate the history of reassortant events, segments in descendant viruses are colored according to their corresponding source viruses.