Journal of Virology

Genetic Characterization of a Novel Recombinant H5N2 Avian Influenza Virus Isolated from Chickens in Tibet

Suhong Zhao, Langsizhu Suo and Meilin Jin
J. Virol. **2012, 86(24):13836. DOI: 10.1128/JVI.02595-12.**

JVI Journals.ASM.org

GENOME ANNOUNCEMENT

Genetic Characterization of a Novel Recombinant H5N2 Avian Influenza Virus Isolated from Chickens in Tibet

Suhong Zhao,[a,b] Langsizhu Suo,[a] and Meilin Jin[a,b]

Unit of Animal Infectious Disease, National State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, People's Republic of China,[a] and Laboratory of Animal Infectious Disease, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, People's Republic of China[b]

In this report, a novel H5N2 avian influenza virus (AIV) was isolated from chickens in Tibet in 2010, western China. Phylogenetic analysis demonstrated that it was a natural reassortant between H9N2 and H5N1 subtypes. It is of note that this virus has an HP genotype with HA, PB2, M, and NS genes homologous to those of A/peregrine falcon/Hong Kong/2142/2008(H5N1)-like HPAIV isolated from dead wild birds. Publishing this genome information will contribute to the investigation of avian influenza epidemiology and to further research of AIV's biological properties.

Avian influenza viruses (AIV) are members of the *Orthomyxoviridae* family (1), possessing 16 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes (3). Particular emphasis is placed on the H5 and H7 subtypes because of their potential to become highly pathogenic (HP) by obtaining a multibasic cleavage site in the HA protein (11). H5N2 HPAIV is known to have caused three large outbreaks in poultry in America and Europe (8). Recently, such American lineage H5N2 viruses have been continuously discovered in Asia (4, 5). Most recently in 2012, the first occurrence of HP H5N2 virus in Taiwan was reported (2), which raised more public concerns about this subtype to prepare for the next AIV outbreak.

Tibet is a desirable site to monitor influenza virus in China, as it is located on the Central Asia flyway of migratory birds and also shares a complex ecosystem with Qinghai Lake (9). During one surveillance in Tibet in 2010, a novel H5N2 AIV, named A/chicken/Tibet/LZ01/2010(H5N2), was isolated from a chicken farm; it was also the first reported H5N2 isolate from the Qinghai-Tibet plateau.

To understand its molecular characteristics better, whole-genome sequence analysis was conducted on this purified virus. The results showed that the HA gene was most closely related to the HPAI H5N1 strain A/peregrine falcon/Hong Kong/810/2009(H5N1) with a high level of identity of 98.3%, while the NA gene showed highest similarity (98.6%) to A/chicken/Shandong/B2/2007(H9N2). In the phylogenetic tree, three internal segments (PB2, M, and NS), like HA, all grouped with that HPAI H5N1 strain A/peregrine falcon/Hong Kong/2142/2008, an antigenic variant of clade 2.3.4 previously discovered in dead wild birds (10). However, the other internal segments were clustered into the A/chicken/Tibet/S1/2009(H9N2)-like branch with highest identity above 99%, showing a phylogenetic pattern similar to that of NA. The above-described results indicated that LZ01 (H5N2) probably originated from natural reassortments between Hong Kong wild-bird-like H5N1 and local preexisting poultry-like H9N2.

LZ01(H5N2) had a multiple basic amino acid motif (RERRR KRG) at the cleavage site of the HA protein, which made it meet the OIE's molecular criterion for HPAIV (7), although it showed low virulence *in vitro* by the intravenous pathogenicity index (IVPI) test. Receptor binding sites of HA retained Q-222 and G-224 (H5 numbering), revealing an avian-specific receptor-binding preference (12). Moreover, six potential N-glycosylation sites were found at positions 26, 27, 39, 170, 181, and 302 of the HA1 protein. The NA protein exhibited a 3-amino-acid deletion in the stalk motif (positions 63 to 65), like most H9N2 viruses from mainland China. Fortunately, neither the E627K nor the D701 mutation could be detected in the PB2 protein.

Briefly, we documented an uncommon H5N2 reassortant virus from Tibet, which was another natural HPAIV with low virulence *in vitro* (6). Considering the special geographical position of Tibet, this result should highlight the need for continuous surveillance of the dynamic evolution of such H5N2 reassortment as well as H5N1 HPAIVs.

Nucleotide sequence accession numbers. The genome sequences of A/chicken/Tibet/LZ01/2010(H5N2) have been deposited in GenBank under accession numbers JX565016 to JX565023.

ACKNOWLEDGMENT

This work was supported by the 973 Program (2011CB505000).

REFERENCES

1. **Alexander DJ.** 2000. A review of avian influenza in different bird species. Vet. Microbiol. **74**:3–13.
2. **Chan YJ.** 2012. The threat of highly pathogenic avian influenza H5: will H5N2 infections occur in humans? J. Chin. Med. Assoc. **75**:420–421.
3. **Fouchier R, et al.** 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J. Virol. **79**:2814–2822.
4. **Jiao P, et al.** 2012. Complete genome sequence of an H5N2 avian influenza virus isolated from a parrot in Southern China. J. Virol. **86**:8890–8891.
5. **Lee JH, et al.** 2009. Isolation and genetic characterization of H5N2 influenza viruses from pigs in Korea. J. Virol. **83**:4205–4215.
6. **Londt BZ, Banks J, Alexander DJ.** 2007. Highly pathogenic avian influenza viruses with low virulence for chickens in vivo tests. Avian Pathol. **36**:347–350.
7. **Office International des Epizooties.** 2004. Manual of diagnostic tests and vaccines for terrestrial animals, 5th ed. Office International des Epizooties, Paris, France.
8. **Okamatsu M, et al.** 2007. Low pathogenicity H5N2 avian influenza outbreak in Japan during the 2005–2006. Vet. Microbiol. **124**:35–46.

Received 25 September 2012 **Accepted** 26 September 2012

Address correspondence to Meilin Jin, jinmeilin@mail.hzau.edu.cn.

doi:10.1128/JVI.02595-12

9. **Prosser DJ, Cui P, John Y.** 2011. Wild bird migration across the Qinghai-Tibetan plateau: a transmission route for highly pathogenic H5N1. PLoS One **6**:e17622. doi:10.1371/journal.pone.0017622.
10. **Smith GJ, et al.** 2009. Characterization of avian influenza viruses A (H5N1) from Wild Birds, Hong Kong, 2004–2008. Emerg. Infect. Dis. **15**:402–407.
11. **Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y.** 1992. Evolution and ecology of influenza A viruses. Microbiol. Rev. **56**:152–179.
12. **Yena HL, et al.** 2009. Changes in H5N1 influenza virus hemagglutinin receptor binding domain affect systemic spread. Proc. Natl. Acad. Sci. U. S. A. **106**:286–291.