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Short communication

Isolation and characterization of a novel H10N2 avian influenza virus from a domestic duck in Eastern China

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ABSTRACT

During the surveillance for avian influenza viruses (AIVs) in live poultry markets (LPMs) in Eastern China, in 2013, an H10N2 AIV was isolated from a domestic duck. Phylogenetic analysis showed that this strain received its genes from H10, H1 and H7 AIVs of wild birds in China. The virulence of this strain was examined in chickens and mice, and was found to be low pathogenic in chickens but demonstrated moderate pathogenicity in mice. These results suggest that active surveillance of AIVs in LPMs should be used in an early warning system for avian influenza outbreaks.

Influenza A virus belongs to the family *Orthomyxoviridae*, and based on the antigenic properties of the hemagglutinin (HA) and neuraminidase (NA) glycoproteins, influenza A viruses are classified into 18 HA and 11 NA subtypes (Tong et al., 2013; Zhu et al., 2013). Most subtypes of influenza A virus have been identified in aquatic birds, including domestic ducks, which are considered a natural reservoir for influenza A viruses (Kawaoka et al., 1988). Though domestic ducks do not usually display the symptoms when they are infected with avian influenza viruses (AIVs), they provide an environment for the reassortment of low pathogenic avian influenza (LPAI) viruses which can serve as progenitors of highly pathogenic avian influenza (HPAI) viruses (Liu et al., 2003). The H10N7 subtype of AIV has caused disease outbreaks in poultry world-wide since the 1990s (Abolnik et al., 2010; Karunakaran et al., 1983) and in humans in Egypt and Australia since 2004 (Arzey et al., 2012). In December, 2013, a novel H10N8 AIV associated with a human death has emerged in Eastern China (Chen et al., 2014). The persistent introduction of H10 AIVs into the human population raises the possibility of the emergence of a human influenza pandemic virus (Montomoli and Maria, 2014; To et al., 2014).

Since live poultry markets (LPMs) are considered a major source of AIV dissemination and sites for potential influenza virus reassortment as well as interspecies transfers (Cardona et al., 2009; Liu et al., 2003), active surveillance of AIVs in LPMs should be used in an early warning system for avian influenza outbreaks. During the routine surveillance for avian influenza in LPMs in Zhejiang Province, Eastern China, in December 2013, an H10N2 AIV, A/duck/Zhejiang/6D20/2013(H10N2) (ZJ-6D20), was isolated from an apparently healthy domestic duck. A total of 117 samples from poultry in these LPMs, and we have isolated 8 strains of AIVs, subtypes H2 ($n = 1$), H3 ($n = 2$), H5 ($n = 4$) and H10 ($n = 1$). To understand better about the genetic relationship between this H10N2 strain from Eastern China and from birds in Asia, all eight gene segments of ZJ-6D20 were sequenced and compared with those available in GenBank.

For virus isolation, each cloacal swab material from domestic ducks was inoculated into embryonated chicken eggs as described elsewhere (Wu et al., 2012). RNA extraction was achieved using the Viral RNA mini kit (Qiagen), according to the manufacturer's instructions. All segments were amplified with primers as described elsewhere (Hoffmann et al., 2001). Fragment sequencing was carried out using Big Dye Terminator V.3.0 Cycle Sequencing Ready Reaction kit (ABI), according to the manufacturer's instructions. The sequences were analyzed using BioEdit version

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7.0.9.0 DNA analysis software. Phylogenetic trees were constructed with MEGA5 software version 5.05, applying the neighbor-joining method with bootstrap analysis (1000 replicates) (Tamura et al., 2011). The sequence data obtained in this study have been deposited in GenBank under accession Nos. KP063194-201.

Phylogenetic analysis of all eight genes, polymerase basic protein 2 (PB2), polymerase basic protein 1 (PB1), polymerase acidic protein (PA), HA, nucleocapsid protein (NP), NA, matrix protein (M), and nonstructural protein (NS), showed that this strain clustered in the AIV Eurasian lineage, Figs. 1 and S1 (Supplementary material). Phylogenetic analysis showed that the HA gene of ZJ-6D20 was very closely related to H10 viruses circulating in Eastern Asia from 2009 to 2012 (Fig. 1). It was showed that the HA of ZJ-6D20 shared a higher sequence similarity with H10 AIVs coming from wild birds than other H10 AIVs isolated from poultry. The HA gene phylogeny indicate that ZJ-6D20 and the novel 2013 H10N8 influenza virus which caused human infection had different ancestors for this gene.

Homologous comparison in GenBank database with BLAST showed that the PB2, PB1, PA and NP genes of ZJ-6D20 were most closely related to A/chicken/Jiangsu/RD5/2013(H10N9). The HA and NA genes of ZJ-6D20 were most closely related to A/wild bird/Korea/A12/2010(H10N1) and A/duck/Zhejiang/0224-6/2011 (H1N2), respectively. The M gene of ZJ-6D20 was most closely related to A/duck/Wenzhou/47/2013(H7N7), and the NS gene of ZJ-6D20 had the highest nucleotide similarity to A/duck/Hunan/S11205/2012(H10N3), Fig. 2 and Table S1 (supplementary material). Previous reports showed that the HA genes of H10 AIVs that have been introduced to poultry in Eastern China, and these H10 viruses all underwent frequent reassortment with multiple virus subtypes, including H7 and H9 (Chen et al., 2014; Hai-bo et al., 2012b; Su et al., 2013). According to the above-described analysis, ZJ-6D20 was a reassortant virus and received its genes from H10, H1 and H7 subtype viruses of poultry and wild birds in Eastern Asia. To our knowledge, this is the first report of the genomic sequence and phylogenetic analysis of H10N2 AIV isolated from poultry in China. These results also add more evidence for the active evolution and segment reassortment mechanism of H10 subtype AIVs in Eastern China.

Based on the deduced amino acid sequence of the HA, the cleavage site pattern, PEIMQGR, of ZJ-6D20 displayed features of a monobasic cleavage site. It is known that the addition of multiple

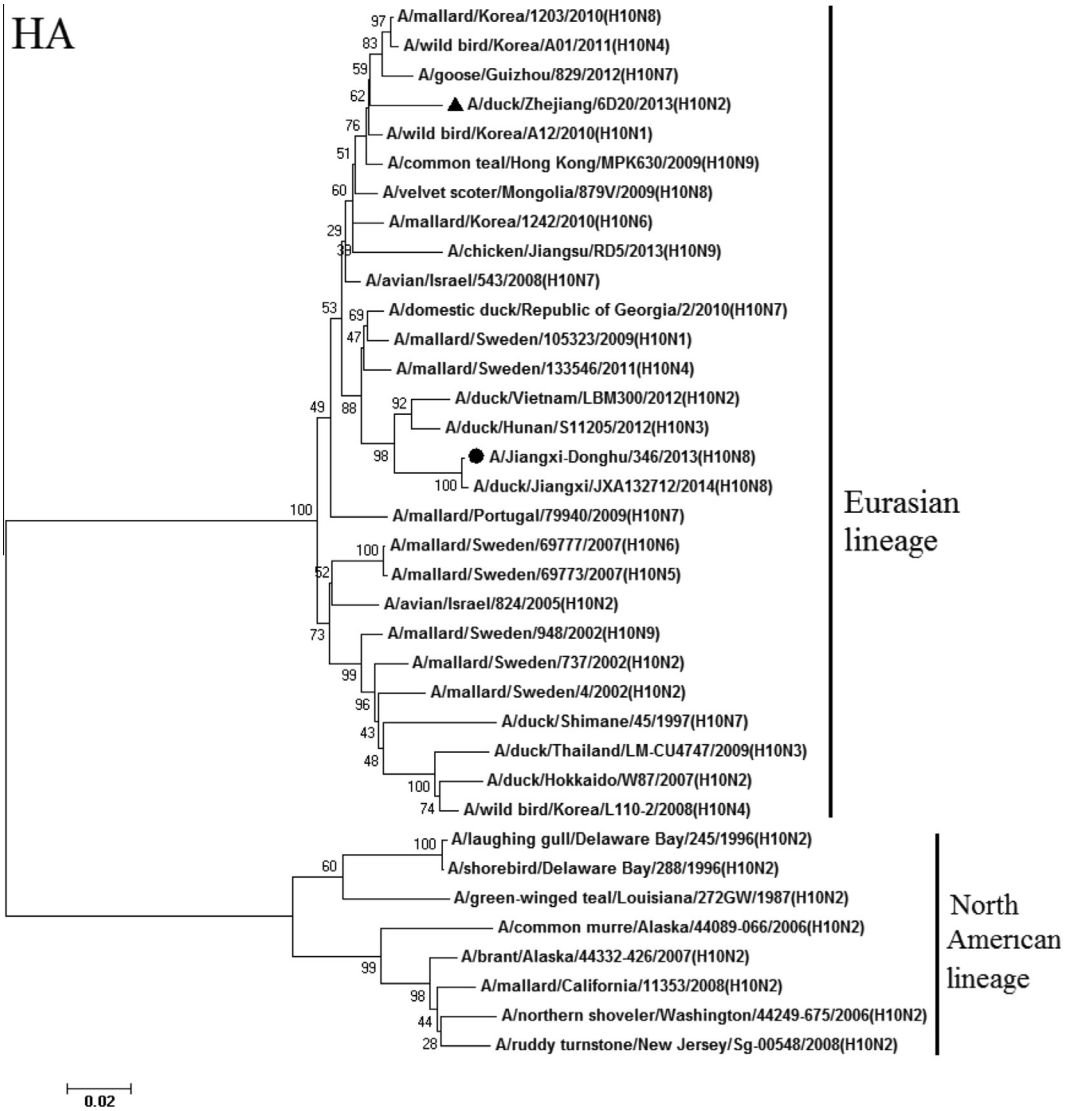

Fig. 1. Phylogenetic trees of HA (positions 49–1611) and NA (positions 1–1410) genes of the novel H10N2 avian influenza virus. The tree was created by the Neighbor-Joining method and bootstrapped with 1000 replicates using the MEGA5 software version 5.05. The H10N2 virus characterized is highlighted by a triangle, and the novel 2013 H10N8 influenza virus which caused human infection highlighted by a dot. The scale bar represents the distance unit between sequence pairs.