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Short communication

Novel reassortant H10N7 avian influenza viruses isolated from chickens in Eastern China

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ABSTRACT

Background: Since 2004, the H10N7 subtype avian influenza virus (AIV) has caused sporadic human infections with variable clinical symptoms world-wide. However, there is limited information pertaining to the molecular characteristics of H10N7 AIVs in China.
Objective: To more fully characterize the genetic relationships between three novel H10N7 strains isolated from chickens in Eastern China and the strains isolated from birds throughout Asia, and to determine the pathogenicity of the H10N7 isolates *in vivo*.
Study design: All eight gene segments from the Chinese H10N7 strains were sequenced and compared with AIV strains available in GenBank. The virulence of the three isolates was determined in chickens and mice.
Results: Three H10N7 subtype avian influenza viruses were isolated from chickens in live poultry markets in Eastern China in 2014: (1) A/chicken/Zhejiang/2C66/2014(H10N7) (ZJ-2C66), (2) A/chicken/Zhejiang/2CP2/2014(H10N7) (ZJ-2CP2), and (3) A/chicken/Zhejiang/2CP8/2014(H10N7) (ZJ-2CP8). Phylogenetic analysis indicated that the viruses contained genetic material from H10, H2, H7, and H3 AIV strains that were circulating at the same time. The reassortant H10N7 viruses were found to be minimally pathogenic in chickens and moderately pathogenic in mice. The viruses were able to replicate in mice without prior adaptation.
Conclusion: These results suggest that H10N7 surveillance in poultry should be used as an early warning system for avian influenza outbreaks. The novel strains identified here may post a threat to human health in the future if they continue to circulate.

1. Background

Since 1997, the H5, H7, and H9 subtypes of avian influenza virus (AIV) have caused outbreaks in poultry and humans around the world and gained significant attention [1,2]. However, the information concerning the molecular characteristics of other subtypes AIVs, such as the H10 subtype, has been limited. However, details about the molecular characteristics of other AIV subtypes, such as the H10, are limited. Since 2004, the H10N7 AIV has caused sporadic human infections with clinical symptoms such as conjunctivitis and a mild respiratory syndrome in Egypt and Australia [3]. In December, 2013, a novel H10N8 AIV emerged in Eastern China and was linked to a human death [4]. The persistent introduction of H10 AIVs into humans suggests the possible emergence of a pandemic human influenza virus [5,6].

Live poultry markets (LPMs) are considered a major source of influenza virus dissemination and potential influenza virus reassortment. Chickens from LPMs may play an important role in expanding the host range for AIVs, and could generate new influenza viruses with pandemic potential. Emerging strains of AIV are routinely cataloged in LPMs in China. In 2014, three novel H10N7 AIVs were isolated from chickens in Zhejiang Province, Eastern China.

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2. Objective

The objective of this study was to understand the genetic relationships between the H10N7 strains isolated from chickens in Eastern China [A/chicken/Zhejiang/2C66/2014(H10N7) (ZJ-2C66), A/chicken/Zhejiang/2CP2/2014(H10N7) (ZJ-2CP2), and A/chicken/Zhejiang/2CP8/2014(H10N7) (ZJ-2CP8)], and strains isolated from birds in Asia, and to determine the pathogenicity of these isolates in animals.

3. Study design

Cloacal swabs ($n = 251$) were collected from apparently healthy chickens in three LPMs. The viruses were isolated from each sample and was inoculationed into embryonated chicken eggs as described elsewhere [7]. RNA was extracted using the viral RNA mini kit (Qiagen), according to the manufacturer's instructions. The viral segments were amplified with previously described primers [8] and fragment sequencing was performed using the 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 7.0.9.0 DNA analysis software. Phylogenetic trees were constructed using molecular evolutionary genetics analysis (MEGA) software version 5.05, applying the neighbor-joining method and Tamura–Nei model with bootstrap analysis (1000 replicates) [9]. The sequence data obtained in this study have been deposited in GenBank (accession nos. KP412438–KP412461).

To determine viral pathogenicity in chickens, 6-week-old specific-pathogen-free chickens ($n = 10$) were inoculated intravenously with $10^{6.0}$ egg infectious dose 50 (EID_{50}) of virus in a 0.2 mL volume of phosphate buffered saline, and mortality was observed over a 10 day period. Next, 6-week-old female BALB/c mice ($n = 15$) were intranasaly inoculated with $10^{6.0}$ EID_{50} of virus. Mice were sacrificed ($n = 3$) at 3, 6, and 9 days post-inoculation (dpi). Virus from the lungs, brains, hearts, and livers were titered in embryonated chicken eggs. Survival was monitored in the remaining mice ($n = 6$) for 14 days following inoculation. The animal studies were carried out according to the recommendation of the Office International des Epizooties [10] and approved by First Affiliated Hospital, School of Medicine, Zhejiang University.

4. Results

Phylogenetic analysis of the eight segments, polymerase basic protein 2 (PB2), polymerase basic protein 1 (PB1), polymerase acidic protein (PA), hemagglutinin (HA), nucleocapsid protein (NP), neuraminidase (NA), matrix protein (M), and nonstructural protein (NS), showed that ZJ-2C66, ZJ-2CP2, and ZJ-2CP8 belonged to the Eurasian lineage (Fig. 1 and Fig. S1). The HA genes of ZJ-2C66, ZJ-2CP2, and ZJ-2CP8 were very closely related to H10 viruses circulating in Eastern Asia from 2009 to 2013 (Fig. 1). However, based on the phylogeny of the HA gene, they had different ancestors from the novel 2013H10N8 virus responsible for a human death.

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Blast analysis showed that the PB2, PB1, and HA genes of ZJ-2C66, ZJ-2CP2, and ZJ-2CP8 were most closely related to A/duck/Zhejiang/6D20/2013(H10N2). The PA, NP, NA, and M genes of these strains were most closely related to A/duck/Zhejiang/12/2011(H7N3), A/duck/Zhejiang/D1-6/2013(H3N8), A/duck/Zhejiang/468/2013(H2N7), and A/duck/Wenzhou/775/2013(H7N2), respectively. The NS gene of ZJ-2C66 was most closely related to A/duck/Zhejiang/D1-6/2013(H3N8), while the NS genes of ZJ-2CP2 and ZJ-2CP8 were most similar to A/duck/Zhejiang/6D20/2013(H10N2) (Fig. 2 and Table S1). Previous reports demonstrated that H10 AIVs have

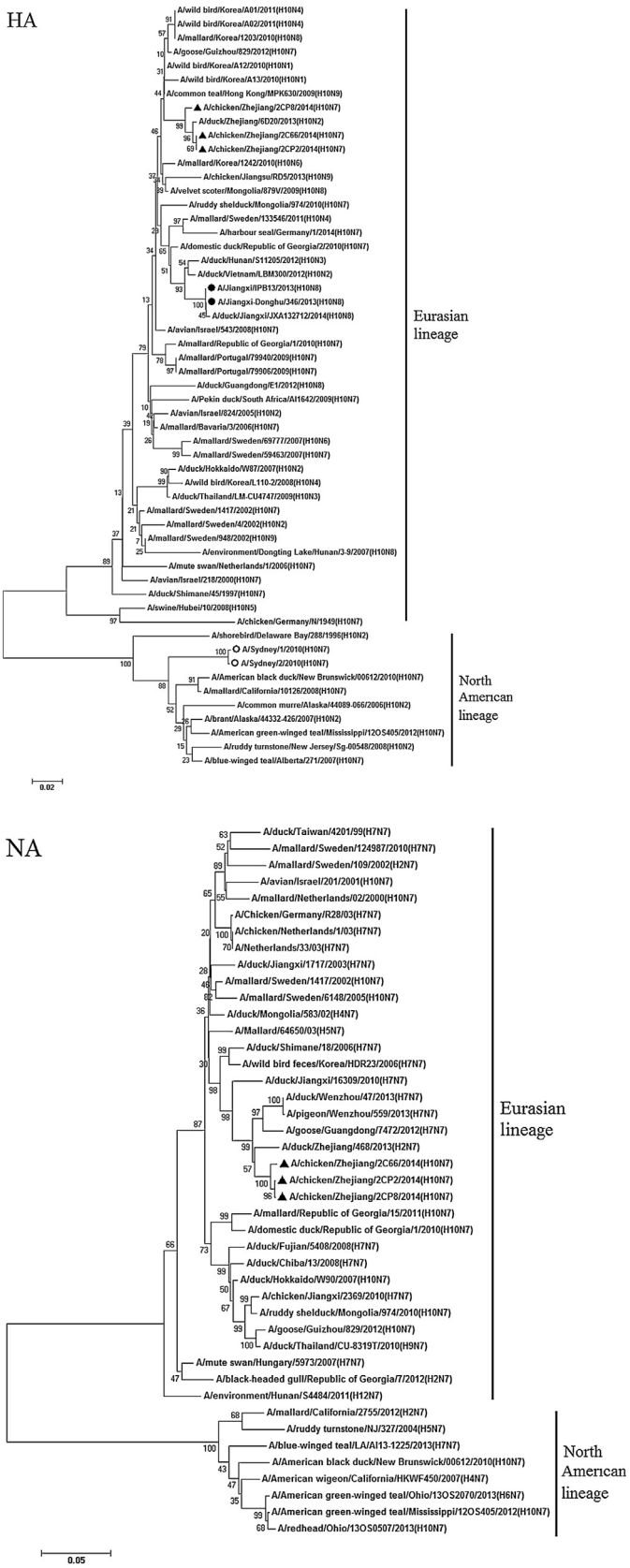

Fig. 1. Phylogenetic trees showing the genetic relationships between ZJ-2C66, ZJ-2CP2, and ZJ-2CP8, and other Eurasian avian influenza virus strains for HA (positions 1168–1641) and NA (positions 1–1416). The tree was constructed using MEGA software version 5.05, applying the neighbor-joining method and Tamura–Nei model with bootstrap analysis (1000 replicates). ZJ-2C66, ZJ-2CP2, and ZJ-2CP8 are indicated using a triangle, the 2013H10N8 influenza virus (caused human infection) is indicated by a dot, and the 2010H10N7 (human virus) is indicated by a circle. The scale bar at the bottom of the tree represents the nucleotide substitutions per site.