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REVIEW ARTICLE

An Overview of Avian Influenza A H10N8 Subtype Viruses

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ABSTRACT

The first avian influenza A subtype H10N8 virus able to infect humans and induce mortality was identified in China at the end of 2013. No such case was observed in other parts of the world. Similar to H7N9, H10N8 viruses are less pathogenic in poultry but could induce severe illness or even death in humans. H10N8 infection poses a potential threat to public health. There is currently no effective means to prevent or treat H10N8 infections. Moreover, it remains unclear how H10N8 viruses are transmitted to humans, and substantial public concerns have been raised about whether H10N8 infections will lead to an outbreak in humans. This article briefly describes the current research on H10N8 subtype viruses.

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INTRODUCTION

Avian influenza virus (AIV) belongs to the *Orthomyxoviridae* family and is a member of type A influenza viruses. AIV is an enveloped, negative-sense, single-stranded RNA virus. The viral genome consists of eight segmented genes, encoding viral structural and non-structural proteins (NS), respectively, including haemagglutinin (HA), neuraminidase (NA), matrix protein M1, ion channel protein M2, nucleoprotein (NP), and RNA polymerase complex proteins (PB1, PB2, and PA) (Lamb and Krug, 2001; El Zowalaty *et al.*, 2013; Sarachai *et al*., 2014; Sun *et al.*, 2014; Rafique *et al.*, 2015).

Currently, sixteen HA subtypes (H1-H16) and nine NA subtypes (N1-N9) of Influenza A viruses have been identified in wild aquatic birds, the natural reservoir of low pathogenic avian influenza viruses (LPAIV). LPAIV primarily circulate in wild birds and are typically considered species-specific (Peng *et al.*, 2013). However, gene mutations or reassortment might generate a novel AIV strain acquiring the ability to cross species barriers to directly infect humans or other mammals (He *et al*., 2009; He *et al*., 2012). Prior to 2013, human cases of infection with AIVs were identified, including infections with H5N1, H7N2, H7N3, H7N7, H9N2 and H10N7, respectively (Chan *et al*., 2013; Chen *et al.*, 2014; WHO, 2014). Since 2013, in addition to H10N8 virus, three other subtypes of AIVs (H7N9, H6N1 and H5N6) were found to be able to infect humans (Tan *et al*., 2014; Tan *et al*., 2015; Li *et al*., 2016).

The first human case of H10N8 infection in China in December 2013: The human-infecting H5N1 virus was first isolated in Hong Kong in 1997 (Guan and Smith, 2013). Since then, other subtypes of human-infecting avian influenza viruses (AIVs) have been documented (To *et al.*, 2014). In December 2013, Chen and colleagues reported the first human case of H10N8 infection in Nanchang City, Jiangxi Province, China (Chen *et al.*, 2014; Wan *et al.*, 2014). The infected patient was a 73-year-old woman who visited a live poultry market (LPM) four days before the onset of illness. The clinical manifestations included fever, cough and influenza-like symptoms, and she was admitted to a hospital on November 30, 2013. Unfortunately, the patient conditions progressively deteriorated, and the woman eventually died of multiple organ failure on December 6, 2013 (Chen *et al.*, 2014; Wan *et al.*, 2014).

Prior to infection, the patient had severe underlying medical conditions, such as hypertension, coronary heart disease, and myasthenia gravis. She underwent a thymectomy in the previous year (Chen *et al.*, 2014), and she might be susceptible to pathogen infections. On the 4th day of admission, tracheal aspirate specimens were collected from the patient. The specimens were tested at the Nanchang Center for Disease Control and Prevention, and the virus was isolated and identified as an avian influenza A virus (Chen *et al.*, 2014). The typed virus was subsequently sent to the Chinese National Influenza Centre in Beijing and subtyped as a novel influenza A H10N8 virus. This virus was subsequently named

A/Jiangxi-Donghu/346/2013(H10N8) or JX346 for short (Chen *et al.*, 2014).

On January 19 and February 9, 2014, a 55-year-old woman and a 75-year-old man infected with H10N8 viruses, respectively, were identified in Nanchang city, Jiangxi, China (Liu *et al.*, 2015). The 55-year-old woman recovered from the illness, whereas the 75-year-old man died of multiple organ dysfunctions. All the three individuals infected with H10N8 viruses visited the LPM prior to the onset of clinical symptoms (García-Sastre and Schmolke, 2014; Liu *et al.*, 2015).

Origin of influenza A H10N8 subtype viruses: An avian H10N8 subtype virus was first isolated in quails in Italy in 1965 (De Marco *et al.*, 2004), and thereafter, avian H10N8 viruses were detected from wild birds or poultry in Australia, Sweden, Canada, US, Korea and Japan (To *et al.*, 2014). Viral genome sequencing and phylogenetic analysis revealed that the human-infecting H10N8 (JX346) subtype virus isolated in China represents a novel reassortment among different AIV subtype viruses (Chen *et al.*, 2014). Interestingly, all the genes of the JX346 virus are of avian origin. A previous study suggested that the HA gene of H10N8 (JX346) was likely derived from an H10N3 virus isolated from Hunan ducks in China in 2012, whereas the NA gene of JX346 might have derived from an H10N8 virus isolated from a mallard in Korea in 2010 or an H10N8 virus isolated in wild birds in Japan in 2008 (Chen *et al.*, 2014). Interestingly, Qi *et al.* 2014 suggested that the NA gene of H10N8 (JX346) might have derived from an H3N8 virus in duck in Vietnam in 2012.

It has been suggested that the H10 and N8 subtype viruses initially infected wild birds, generating a novel H10N8 virus strain (Chen *et al.*, 2014). Thereafter, the H10N8 virus might infect poultry and reassort with an H9N2 virus present in infected birds, thereby resulting in a novel reassortant H10N8 virus with an acquired ability of infecting humans. Epidemiological surveillance revealed that H9N2 viruses circulated in poultry and occasionally infected humans (Cheng *et al.*, 2011). Similar to H5N1 and H7N9, the six internal genes of the H10N8 (JX346) virus also originated from H9N2 viruses (Chen *et al.*, 2014; García-Sastre and Schmolke, 2014; To *et al.*, 2014). Previous study showed that an H10N8 virus [A/Chicken/Jiangxi/102/2013/(H10N8)] was isolated in chicken from the LPM, where the 73-year-old patient visited a few days prior to the illness (Qi *et al.*, 2014). The human H10N8 virus (JX346) and chicken H10N8 virus (Jiangxi/102) share the same genetic origins in six genes (HA, NA, PA, M, NP and NS genes) but differ in PB1 and PB2 genes (Qi *et al.*, 2014). Xu *et al.*, 2015 reported that H10N8 viruses were isolated from the LPM A where the 73-year-old woman visited before the onset of her illness. Detailed analysis revealed that the nucleotide sequences of the H10N8 viruses isolated from LPM A are more than 99% identical in sequence to those of the human-infecting H10N8 isolate (Xu *et al.*, 2015). These results indicate that the LPM is the most likely source of H10N8 infection for the 73-year-old female patient.

However, currently, the precise origin of the H10N8 virus cannot be definitively determined, reflecting a lack of sufficient epidemiological data on H10N8 infection in poultry and wild birds. Whether the human-infecting H10N8 isolates were generated prior to introduction into the LPM or arose among the poultry in the LPM remains unclear. Before the human H10N8 (JX346) virus was isolated, only two avian H10N8 virus strains were detected in China (Zhang *et al.*, 2011; Jiao *et al.*, 2012). One virus, named A/Environment/Dongting Lake/ Hunan/3-9/2007(H10N8), was isolated from a water sample in the wetlands of the Dongting Lake in Hunan Province in 2007 (Zhang *et al.*, 2011). The other virus, designated A/Duck/Guangdong/E1/2012(H10N8), was isolated from a duck in a LPM in Guangdong Province in 2012 (Jiao *et al.*, 2012).

A recent study reported that eight H10N8 viruses were isolated from ducks and chickens in a LPM in China during the period from 2009 to 2013 (Deng *et al.*, 2015). Ma *et al.* (2015) also reported that they isolated 124 H10N8 or H10N6 viruses from chickens in South China (main in Jiangxi Province) during 2002 to 2014. The sequences of HA gene segments from these H10N8 viruses shared highly homologous to those of the H10N8 (JX346) isolate (Fig. 1). Interestingly, Phylogenetic analysis of H10 and related viruses showed that the six internal genes of the H10N8 viruses were generated through multiple reassortment derived from H9N2 viruses (Deng *et al.*, 2015; Ma *et al.*, 2015). In 2014, an AIV H10N8 strain was isolated from feral dogs exposed to poultry in the LPM in Guangdong Province, China (Su *et al.*, 2014), suggesting that poultry and some mammals should be actively monitored for H10N8 infections. Currently, most H10N8 viruses reported after December 2013 were isolated either from individuals who visited the LPM or poultry and animals in or near the LPM. Increasing evidence suggests that H10N8 viruses primarily circulate in the LPMs (Ma *et al.*, 2015; Xu *et al.*, 2015); however, the infected birds typically have no apparent signs of illness or mortality. Moreover, the detection of H10N8 viruses on poultry farms seems uncommon (Su *et al.*, 2015).

Zhang *et al.* (2014) reported that two H10N8 viruses were isolated from apparently healthy poultry in several LPMs in Nanchang City, during January 2014. Liu *et al.* (2015) also reported that eight H10N8 viruses were isolated in samples collected from LPMs in Nanchang City, Jiangxi Province between December 2013 and February 2014. These authors observed that the sequences of H10N8 virus (A/chicken/Jiangxi/77/2014, A/chicken/Jiangxi/B15/2014 and Ev/JX/03489/2013) were highly homologous to the sequences of H10N8 (JX346) virus. For example, the HA gene from the the H10N8 virus (A/chicken/Jiangxi/77/2014 and A/chicken/Jiangxi/B15/2014) and the NA protein sequence from the H10N8 virus (Ev/JX/03489/2013) shared 99.5% and 100% identity with that from the human-infecting H10N8 virus (JX346), respectively. These results suggest that LPM was the likely source of human infections with H10N8 viruses.

The HA and NA genes of AIVs can be classified as either Eurasian or North American lineages (To *et al.*, 2014). The eight genes of the H10N8 Hunan strain belong to the Eurasian lineage (Zhang *et al.*, 2011). Interestingly, although the HA gene of the H10N8 (JX346) virus belongs to the Eurasian lineage, the NA gene of the JX346 belongs to the North American N8 lineage (Chen *et al.*,

2014). The human H10N8 (JX346) isolate and the duck H10N8 Hunan strain differ in HA, NA and six internal genes. The HA and NA genes of JX346 were derived from H10N3 and H10N8 viruses, respectively, whereas those of the H10N8 Hunan strain were derived from H10N5 and H6N8 viruses, respectively (Zhang *et al.*, 2011; Chen *et al.*, 2014). The six internal genes of the H10N8 (JX346) virus were all derived from H9N2 viruses (Chen *et al.*, 2014; García-Sastre and Schmolke, 2014), whereas those of the H10N8 Hunan strain were primarily derived from H5 or H7 subtype viruses; only the NS1 gene was likely derived from a duck infected with H9N2 viruses (Zhang *et al.*, 2011).

Amino acid substitutions in the viral proteins of human-infecting H10N8 (JX346) virus: Viral genome sequencing and phylogenetic analysis revealed that amino acid substitutions (A135T and S138A) occurred in the H10N8 (JX346) HA protein, and these changes facilitate the adaptation of an AIV to mammalian cells (de Wit *et al.*, 2010). The NA protein of an AIV can be divided into four regions: head, stalk, membrane-spanning region and cytoplasmic tail (Shtyrya *et al.*, 2009). The length of the NA stalk might affect the virulence of an AIV (Sun *et al.*, 2013). The H9N2 virus contains a shorter NA stalk and has an increased virulence in chickens (Shtyrya *et al.*, 2009; Chan *et al.*, 2013; Sun *et al.*, 2013), whereas the H5N1 virus has a shortened NA stalk region and an enhanced virulence in mammalian hosts (Matsuoka *et al.*, 2015). Although a deletion in the NA stalk was detected from H5N1 and H7N9 viruses (Matrosovich *et al.*, 1999; Gao *et al.*, 2013), however, such a deletion has not been detected in the H10N8 (JX346) or the H10N8 Guangdong strains (Jiao *et al.*, 2012; To *et al.*, 2014). Similar to H7N9, a PDZ domain at the C-terminus of NS1 protein in H10N8 (JX346) virus was deleted, generating a truncated NS1 protein (Chen *et al.*, 2014). In contrast, the H10N8 Guangdong strain does not have any amino acid deletions in the NS1, NA or HA proteins (Jiao *et al.*, 2012).

The NS1 protein of H10N8 (JX346) contains a P42S substitution, whereas the M1 protein has two amino acid substitutions (N30D, T215A), all of which might increase viral virulence in mice (Jiao *et al.*, 2008; Fan *et al.*, 2009). The H10N8 (JX346) PB1 protein has L473V and L598P substitutions, responsible for efficient viral adaptation and replication in mammalian cells (Chen *et al.*, 2014). The H10N8 (JX346) PB2 protein contains an E627K substitution (PB2-E627K), which has been observed in the PB2 proteins of H1N1, H5N1, H7N7, H7N9, and H9N2 viruses (Taubenberger *et al.*, 2005; de Wit and Fouchier, 2008; To *et al.*, 2013; Schrauwen and Fouchier, 2014; Chen *et al.*, 2015). This PB2 E627K substitution has been associated with the enhanced adaptation of viruses to mammalian cells, increased virulence and improved spread of viruses between mammals (Hatta *et al.*, 2001; Chen *et al.*, 2014). The M2 protein of the H10N8 (JX346) virus contains an S31N substitution, suggesting that the JX346 virus might be resistant to M2 ion channel inhibitors (Chen *et al.*, 2014). The NA protein of the H10N8 (JX346) virus does not contain H247Y and R292K substitutions, and *in vitro* assays have shown that the JX346 is sensitive to neuraminidase inhibitors (Chen *et al.*, 2014; To *et al.*, 2014).

Mammals infected with H10 or N8 subtype viruses: The first H10 subtype AIV (H10N7) was isolated from chickens in Germany in 1949 (Feldmann *et al.*, 1988). One of the features of avian influenza H10 subtype viruses is that these viruses infected wild birds, domestic poultry and certain mammals, and occasionally infected humans (To *et al.*, 2014). For example, three human cases of H10N8 infection were observed in Nanchang City, Jiangxi Province, China (Chen *et al.*, 2014; García-Sastre and Schmolke, 2014). The H10N7 subtype viruses infected minks and caused mild damage to the lungs (Englund, 2000). The first H10N7-infecting human case occurred in Egypt in 2004 (Pan American Health Organization, 2004), and the second case occurred in Australia in 2010 (Arzey *et al.*, 2012). H10N5 viruses could infect pigs (Wang *et al.*, 2012), whereas H10N4 viruses infected minks, causing severe lung disease (Klingeborn *et al.*, 1985). The cleavage site in the HA precursor of the H10N4 virus does not contain multiple basic amino acid residues (Zohari *et al.*, 2010); hence, H10N4 is classified as a LPAIV. To date, human infections with H10N4 or H10N5 subtype virus have not been reported.

Some N8 subtype viruses could also infect mammals. For example, H4N8 virus could infect pigs (Su *et al.*, 2012), whereas H3N8 virus could infect not only pigs but also horses, dogs, and seals (Webster *et al.*, 1981; To *et al.*, 2014). It should be noted that the H3N8 subtype virus was implicated in the 1889 "Russian Flu" (Trombetta *et al.*, 2015). Prior to the end of 2013, not a single case of human infection with H10N8 subtype virus was reported. Currently, patients infected with an H10N8 subtype virus were only observed in China (Chen *et al.*, 2014; García-Sastre and Schmolke, 2014).

A person infected with an H6N1 subtype virus was observed in Taiwan in June 2013 (Yuan *et al.*, 2013). In May 2014, the World Health Organization reported the first human case of an avian H5N6 infection (WHO, 2014). Therefore, in addition to H10N8, other AIV subtype viruses that infected humans include H5N1, H5N6, H6N1, H7N2, H7N3, H7N7, H7N9, H9N2 and H10N7 (Chan *et al.*, 2013; Chen *et al.*, 2014; WHO, 2014). Among these, H6N1, H7N2, H7N3, H7N7, H9N2 and H10N7 only caused mild clinical symptoms with some exceptions. For example, the H7N7 subtype resulted in one death among 89 infected individuals in the Netherlands (Fouchier *et al.*, 2004), and the H9N2 virus caused relatively severe symptoms only in a bone-marrow transplant patient (Cheng *et al.*, 2011). Interestingly, during one year period of 2013, it was reported that three AIV subtypes (H7N9, H10N8 and H6N1) infect humans, respectively (Morens *et al.*, 2013; Yuan *et al.*, 2013; To *et al.*, 2014).

Virulence and receptor-binding preference

Virulence of H10N8 viruses: The presence of a multibasic cleavage site in an HA protein is a critical parameter determining the virulence of an AIV in poultry (Senne *et al.*, 1996). The cleavage site of the H10N8 (JX346) HA precursor contains only a single basic amino acid, arginine (PELIER*G; *denotes the cleavage site in the H10N8 HA precursor) (Chen *et al.*, 2014), implying that H10N8 (JX346) is a LPAIV (Zohari *et al.*, 2010; Chen *et al.*, 2014; To *et al.*, 2014). Interestingly, the

cleavage site in the HA precursor of the H10N8 Guangdong strain also contains only a single arginine (PEIVQER*G), and the H10N8 Guangdong isolate is less pathogenic in chickens (Jiao *et al.*, 2012; To *et al.*, 2014).

One common feature between the H10N8 and H7N9 subtype viruses is that both strains are classified as LPAIVs. Although H10N8 and H7N9 viruses are less pathogenic in poultry, their infection of humans could result in death (Su *et al.*, 2015), suggesting that an LPAIV becomes more pathogenic when it crosses host-species barriers to infect a new host. Indeed, after several passages of an avian H10N8 strain in the lungs of mice, the passaged H10N8 viruses became more virulent in mice, leading to death of the infected animals (Zhang *et al.*, 2011). Chen *et al.* (2015) demonstrated that the human H10N8 virus efficiently replicated in the lungs of a mouse model, inducing acute and persistent cytokine expression and resulting in high pathogenicity in mice, whereas the chicken H10N8 isolate (A/Chicken/Jiangxi/102/2013) was less pathogenic in the same mouse model (Chen *et al.*, 2015).

Until recently, some human-infecting AIVs, including H5N1, H7N7, H7N9 and H10N8 subtypes, have resulted in death of patients. The six internal genes of the H5N1, H7N9 and H10N8 were all derived from H9N2 viruses (Chen *et al.*, 2014; García-Sastre and Schmolke, 2014). The H9N2 viruses evolve continuously and diversely in poultry displaying both genetic and antigenic variations (Choi *et al.*, 2004), suggesting that the internal genes of H9N2 viruses might influence the adaptation of an AIV to human cells, and that these genes are likely associated with the enhanced virulence of viruses.

Receptor-binding preference of H10N8 viruses: Two types of receptors bind to the HA proteins of influenza A viruses. One is the avian-type α2,3 galactose-linked sialic acid (α2,3-SA) receptor (van Riel *et al.*, 2006), and the other is the human-type α2,6-SA receptor. High levels of α2,6-SA receptor and lower levels of α2,3-SA receptor are present on the surfaces of epithelial cells in the human upper respiratory tract. In contrast, the α2,3-SA receptor is abundantly present on the surfaces of epithelial cells in the intestinal tract of birds and is also located in human lower respiratory tract (Shinya *et al.*, 2006; Cheng *et al.*, 2012).

Receptor-binding specificity is primarily determined through a receptor-binding site in an HA protein. Mutations located in or near the receptor-binding site of an HA protein might affect binding to avian-type or human-type receptors (Matrosovich *et al.*, 2000; Vachieri *et al.*, 2014). The H10 protein from H10N8 (JX346) binds strongly to avian-type receptors, but weakly to human-type receptors (Wang *et al.*, 2015; Zhang *et al.*, 2015), suggesting that the HA protein of the human H10N8 isolate is poorly adapted to bind to the human-type α2,6-SA receptor, and additional amino-acid substitutions are required to shift to human-type receptor preference. Wang *et al.* (2015). further showed that the arginine residue at position 137 (R137) located within the HA receptor-binding site of H10N8 (JX346) is critical for preferential binding to avian-type receptors (Wang *et al.*, 2015), whereas Skehel and colleagues demonstrated that a lysine (K) residue at position 137 (K137) in the H10 protein from avian H10N2 virus has some human receptor-binding capacity (Vachieri *et al.*, 2014). Taken together, these studies suggested that the H10 protein of H10N8 viruses is not efficiently adapted for the human type α2,6-SA receptor, and additional mutations in the H10 protein are required to switch receptor-binding preference to human receptors.

Based on the currently available experimental data, it is clear that the human-infecting H10N8 (JX346) virus possesses preferential binding to avian-type receptors, indicating that JX346 virus behaves like a typical AIV. The H10N8 (JX346) isolate and H5N1 (Hong Kong 1997 isolate) virus are similar in that both viruses bind strongly to avian receptors, but weakly to human receptors (Wang *et al.*, 2015). In contrast, the H7N9 virus (A/Anhui/1/2013) has dual receptor-binding capacities because the virus binds strongly to both avian and human receptors (Wang *et al.*, 2015). Similar to the H10 protein of the human H10N8 isolate, the H10 proteins of eight H10N8 viruses isolated from chickens and ducks, respectively, bound strongly to avian-type α2,3-SA receptors, but weakly to human-type α2,6-SA receptors (Deng *et al.*, 2015). Thus, it is important to identify mutations in the HA protein of avian H10N8 virus that could simultaneously increase binding to the α2,6-SA receptor and decrease binding to the α2,3-SA receptor or completely switch receptor-binding preference from avian to humans.

Clinical manifestations and laboratory examinations

Clinical manifestations: The incubation period for human infections with H10N8 viruses was approximately four days (Chen *et al.*, 2014), similar to those of the H5N1 and H7N9 infections (Cowling *et al.*, 2013). Early in H10N8 infection, patients might show mild symptoms of infection, such as a fever, cough, sputum, sore throat, shortness of breath or poor appetite (Chen *et al.*, 2014). Although antiviral and antibacterial drugs and glucocorticoids have been used to treat H10N8-infected patients, the conditions of these patients deteriorated progressively and rapidly. Examining the blood of the infected patients revealed severe lymphocytopenia, slightly increased numbers of neutrophils and leukocytes, and elevated levels of C-reactive protein, creatinine, and lactate dehydrogenase, but reduced levels of total IgG and complement C3 (Chen *et al.*, 2014; Liu *et al.*, 2015). The levels of cytokines and chemokines were substantially increased in the sera of the infected individuals, particularly in fatal cases of infection (Liu *et al.*, 2015).

Two patients had severe pneumonia, acute respiratory distress syndrome, septic shock, acute kidney failure, and eventually died of multi-organ failure (Chen *et al.*, 2014; Liu *et al.*, 2015). However, seventeen individuals who had close contact with the patients did not show flu-like symptoms. Virus isolation and antibodies against H10N8 tested negative for all close contacts examined, suggesting that H10N8 viruses have not required the ability to efficiently spread among humans. Co-infections with bacteria, fungi or other subtypes of AIVs were not detected in blood culture tests (Chen *et al.*, 2014).

Two out of the three patients infected with H10N8 died (Chen *et al.*, 2014), and the mortality rate was 67%, much higher than the 36% mortality rate in the case of human H7N9 infections (Yu *et al.*, 2013). However, this figure might not reflect the actual status because there are currently no sufficient numbers of H10N8-infected human

cases to allow reliable statistical estimations. It is likely that some people infected with H10N8 viruses might only exhibit mild symptoms. If those infected individuals did not visit a hospital for an examination, they may have been missed. Therefore, screening for antibodies against H10N8 in individuals within the affected area might facilitate a better evaluation of the prevalence of H10N8 infections in human populations.

Laboratory examinations: It is difficult to distinguish the respiratory symptoms caused by an H10N8 infection from those induced by other respiratory pathogens (To *et al.*, 2014). Therefore, diagnosing an H10N8 infection requires specific laboratory tests, such as virus isolation, detection of viral RNAs or proteins, sequencing viral gene segments or whole genome, and measuring antibody responses against H10N8 viral proteins (El Zowalaty *et al.*, 2013).

Real-time reverse transcriptase polymerase chain reaction (RT-PCR) is typically applied as a quick diagnostic method for detecting AIVs, including H10N8. Virus isolation and the detection of antibodies against H10N8 viruses can be subsequently performed to verify cases of H10N8 infection. Quantitative RT-PCR is a sensitive, specific assay for H10N8 viral RNAs (El Zowalaty *et al.*, 2013). Reverse-transcription loop-mediated isothermal amplification assays for detecting H10N8 subtype viruses have recently been developed (Bao *et al.*, 2015; Luo *et al.*, 2015), and these assays can be useful for the surveillance of H10N8 virus infection in live poultry, environment and LPMs.

Similar to H5N1 and H7N9, H10N8 viruses infected humans through the respiratory tract, causing severe symptoms in the lower respiratory tract. The H5N1 virus was isolated through throat swabs (de Jong *et al.*, 2006), whereas H7N9 viruses were detected in the sputa or aspirates from the lower respiratory tract (Gao *et al.*, 2013). The collection of sputum or tracheal aspirate specimens from the trachea has been recommended for the detection of human H10N8 infections. Because human infections with H5N1, H7N9 and H10N8 viruses lead to death, the enhanced surveillance of AIV infection in poultry, wild birds and humans is required to monitor the mutations and evolution trends of AIVs. The first case of a human infected with an H6N1 virus was observed in Taiwan in June 2013 (Yuan *et al.*, 2013), therefore, the screening of H6N1 infection should also be included as a part of surveillance programmes.

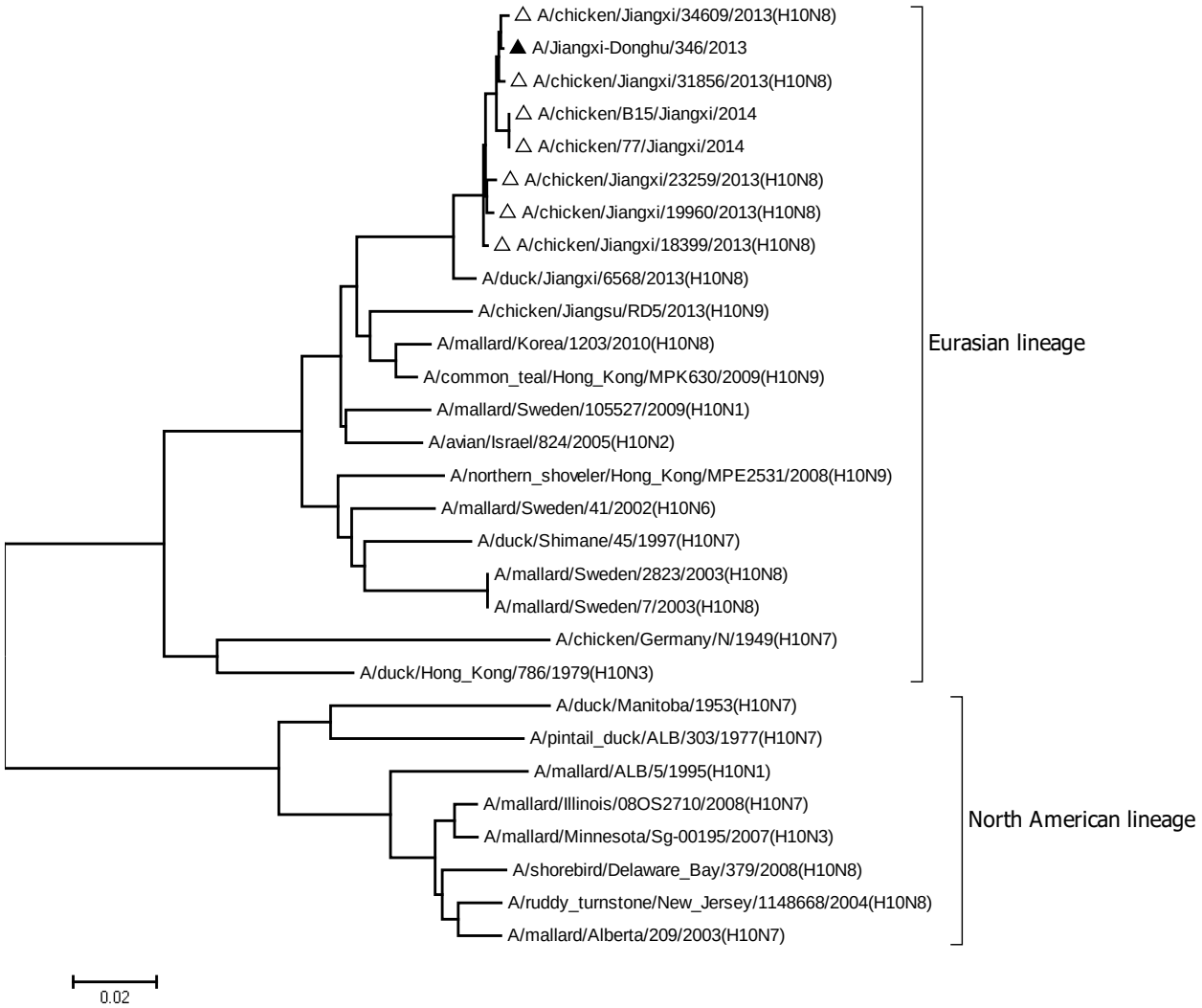

Fig. 1: Phylogenetic tree of hemagglutinin gene segments of influenza A virus isolates (H10N8) from Jiangxi province, China,with those from other closely related subtypes of influenza A viruses. The human-origin H10N8 virus isolate, A/Jiangxi/346/2014(H10N8), is marked with ante-black triangle, whereas the rest of the H10N8 isolates marked with ante-white triangles.

Conclusions: Cases of human infections with AIVs occurred occasionally in the past. However, more cases of human infections with AIVs have been reported since 2013. Between 2013 and 2014, Chinese researchers reported the first fatal case of human infection with an avian H10N8 virus. Subsequent surveillance revealed that the viruses did not acquire the ability for a sustained human-to-human transmission. Currently, the exact origin of human infections with H10N8 viruses remains unclear, although LPMs are the most likely source of human infections. There are currently no efficacious vaccines and antiviral drugs for prevention and treatment of human infections with H10N8 virus.

It is highly important to identify genetic and phenotypic determinants of AIVs that affect virus virulence, reassortment, host adaptation, fitness and transmission between humans, and to examine common biological features of AIVs contributing to cross-species transmission, particularly poultry-to-human transmission. Much effort should be made to determine the mutations or combinations of mutations in HA protein required for switching receptor-binding preferences from avian- to human-type receptors, and to understand the structural and functional basis for receptor-binding specificity of human or avian HA proteins.

Considering the fact that H10N8, H5N1 and H7N9 viruses all possess internal genes from H9N2 viruses, researchers should focus on determining the mechanisms by which the internal genes contribute to the enhanced adaptation of avian influenza viruses to humans. Whether an H10N8 virus can be mutated or reassorted to generate a new virus with a potential to cause a pandemic influenza warrants further investigation. Research should be conducted to explore the origin of human infections with H10N8, the routes of the infections, and the interactions between H10N8 viruses and host factors. These studies will ultimately facilitate the development of novel avenues for global surveillance, diagnosis, prevention and treatment of human infections with AIVs.

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