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Characterization of an H9N2 avian influenza virus from a *Fringilla montifringilla* brambling in northern China ☆

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ABSTRACT

Avian H9N2 influenza viruses circulating in domestic poultry populations are occasionally transmitted to humans. We report the genomic characterization of an H9N2 avian influenza virus (A/Brambling/Beijing/16/2012) first isolated from a healthy *Fringilla montifringilla* brambling in northern China in 2012. Phylogenetic analyses revealed that this H9N2 virus belongs to the BJ/94-like sublineage. This virus had a low pathogenicity for chickens and was able to replicate at a low level in mouse lung tissue. Transmission studies in ferrets showed that this H9N2 strain shed high levels of the virus in nasal and throat swabs. In vitro receptor binding assays, the virus bound only to α-2,6 linkage receptors and not to the avian-type α-2,3 linkage receptors, suggesting that H9N2 influenza viruses present potential public health risks. Therefore, attention should be paid to H9N2 influenza viruses and the close surveillance of H9N2 viruses in poultry.

Introduction

Avian influenza viruses are influenza A viruses belonging to the family of Orthomyxoviridae which are categorized into 16 hemagglutinin (HA) subtypes and 9 neuraminidase (NA) subtypes according to the antigenicity of the surface glycoproteins HA and NA (Tong et al., 2012; Webster et al., 1992). Among these subtypes, the H9N2 subtype is of great concern because it is endemic in the poultry populations across Asia and the Middle East and has occasionally been transmitted from poultry to mammalian species (Butt et al., 2005; Lin et al., 2000; Peiris et al., 2001). Furthermore, phylogenetic analyses revealed that H9N2 viruses were the donors of the internal genes of the H5N1 viruses in Hong Kong in 1997 (Guan et al., 1999) and the novel H7N9 viruses in mainland China in 2013 (Gao et al., 2013; Kageyama et al., 2013). Recent research also demonstrated that H9N2 viruses replicate efficiently in experimental mice without adaptation (Choi et al., 2004) and can be transmitted via respiratory droplets in ferrets after obtaining the internal genes of the 2009H1N1 pandemic (Kimble et al., 2011). However, to date, limited data are available to describe the relative infectivity and transmissibility of H9N2 viruses. Therefore, surveillance of the H9N2 virus in poultry is required for us to better understand the ecology and epidemiology of AIV and the potential risk that these viruses pose to human health.

In November 2012, we first isolated an H9N2 virus (A/Brambling/Beijing/16/2012) from a wild *Fringilla montifringilla* brambling in northern China, and phylogenetic analyses revealed that this H9N2 virus was the donor of the internal genes of the novel H7N9 viruses in mainland China in 2013 (Liu et al., 2013). *F. montifringilla* are very common wild birds in China. Huge numbers of them inhabit the plains, hills, mountains, and forests. The brambling is a migratory bird that flies south for wintering. They have many opportunities for close contact with human beings. These characteristics suggest that the viruses that infect these birds could have many opportunities to infect humans.

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The ferret model has been widely used to study the transmission of the H5, H7, and H9 subtypes of avian influenza viruses (AIV), as well as the 1918H1N1 and 1957H2N2 viruses (Belser et al., 2007; Herlocher et al., 2001; Maines et al., 2006, 2005; Salomon et al., 2006; Shinya et al., 2005; Yen et al., 2007). Ferrets are susceptible to infection with human influenza viruses and develop some symptoms of influenza that closely resemble those observed in humans. Importantly, the respiratory tract of ferrets expresses predominantly human-type receptors and is thus very similar to human respiratory epithelia (van Riel et al., 2007; Xu et al., 2010). However, no airborne transmission of wild H9N2 AIV has been reported in mammals (Wan et al., 2008). In this study, we report the genomic information of an H9N2 avian influenza virus first isolated from a brambling. Phylogenetic analyses reveal that it belong to the BJ/94-like sublineage. Because this H9N2 virus was the donor of the internal genes of the novel H7N9 viruses in mainland China in 2013 (Liu et al., 2013), we systematically examined the pathogenicity, transmissibility, and receptor binding specificity of this new ancestor virus. This comprehensive characterization of emerging host viruses is necessary to understand the evolution of H9N2 viruses and provides information that is important for preventing and controlling potential pandemics in the future.

Results

Phylogenetic and sequence analysis

The H9N2 viruses that have recently circulated in China are primarily descendants of the BJ/94-like and G1-like genotypes (Bi et al., 2010; Sun et al., 2010; Xu et al., 2007; Zhang et al., 2011). Phylogenetic analyses based on the HA protein showed that the A/Brambling/Beijing/16/2012 virus belongs to the BJ/94-like (A/chicken/Beijing/1/1994) sublineage (Fig. 1), which demonstrates that BJ/94-like viruses may be the primary epidemic H9N2 strains currently circulating in northern China.

The amino acid sequence of the HA cleavage site is RSSR/GLF. This sequence contains two basic amino acids but is still characteristic of a low-pathogenic AIV (Callan et al., 1997; Guo et al., 2000). Eight potential glycosylation sites were observed in the HA protein. A potential glycosylation site at amino acid residue 313 was observed in the new isolate, suggesting this mutation may affect virus-induced cell fusion and its receptor binding ability (Kaverin et al., 2004). The receptor-binding pocket of HA1 has the key amino acid L226 (H3 numbering), thereby providing H9N2 AIV with the ability to bind to the α2,6-linked sialic acid receptors, which enables it to infect and replicate within mammalian hosts

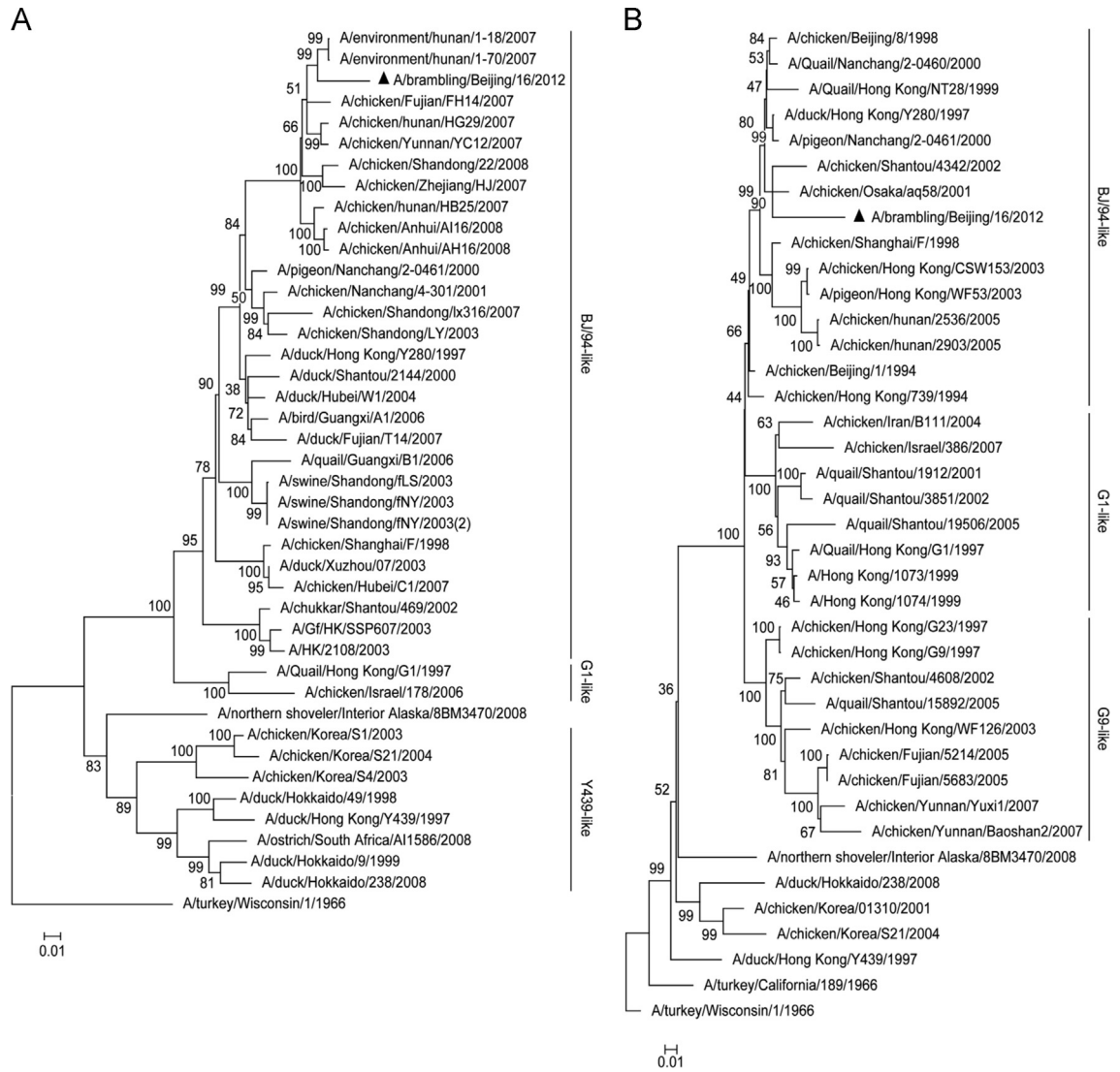

Fig. 1. The phylogenetic analyses for the HA and NA genes of the A/Brambling/Beijing/16/2012 virus in comparison with other H9N2 strains. (A) An analysis based on the HA gene. (B) An analysis based on the NA gene.