



Short communication

Avian-origin H3N2 canine influenza A viruses in Southern China

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ABSTRACT

This study reports four sporadic cases of H3N2 canine influenza in Southern China, which were identified from sick dogs from May 2006 to October 2007. The evolutionary analysis showed that all eight segments of these four viruses are avian-origin and phylogenetically close to the H3N2 canine influenza viruses reported earlier in South Korea. Systematic surveillance is required to monitor the disease and evolutionary behavior of this virus in canine populations in China.

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1. Introduction

The first documented canine influenza infection was probably caused by the early variants of the pandemic H3N2 influenza A virus (Kilbourne and Kehoe, 1975). However, the laboratory confirmed case of canine influenza was not reported until 2004, and that case was caused by an equine-origin H3N8 influenza A virus (Crawford et al., 2005). Infection experiments showed that this H3N8 virus could reproduce respiratory disease in dogs (Deshpande et al., 2009), and this disease is seemingly epidemic in dog populations in North America (Kruth et al., 2008; Payungporn et al., 2008). A report of similar H3N8 canine cases in United Kingdom (Daly et al., 2008) indicated that this virus had spread across the Atlantic boundary, possibly through pet dog exchange.

During the 2003–2004, highly pathogenic H5N1 avian influenza (HPAI) outbreaks in southeastern Asia, an H5N1 canine case was reported in Thailand (Songserm et al., 2006). The results from animal infection experiments suggested that H5N1 HPAIV could infect dogs but was not fatal. These experiments demonstrated also that this H5N1 virus could neither be transmitted between dogs nor between dogs and cats (Giese et al., 2008).

In 2007, another canine infection was reported in a pet dog in the Republic of Korea (Song et al., 2008). This case was caused by a H3N2 avian-origin canine influenza virus (CIV), which infected dogs successfully through nasal inoculation or contact (respiratory fluid exchange) under experimental conditions (Song et al., 2008). The serological survey in 829 serum samples (361 farmed dogs and 468 pet dogs) collected between June and December 2007 across Korea showed that the canine populations investigated had a serum conversion rate of 19% with anti-influenza viral antibody, and that one farm had a serum conversion rate in dogs of 100% (Lee et al., 2009). This surveillance result suggested strongly that H3N2 avian-origin CIV had been circulating in the canine population in Korea.

From May 2006 to October 2007, the Animal Clinics at the South China Agricultural University received four canine patients with severe respiratory syndrome (Table 1). Two of these dogs were from Guangzhou and the other two from Dongguan, a city located about 50 km southeast of Guangzhou. These dogs showed similar symptoms of coughing, sneezing, copious nasal discharge, and low fever (39.6–39.9 °C) when the dogs entered the clinics. The two cases in 2007 were treated with ribavirin and recovered from the disease.

Nasal swabs were collected from these sick dogs, and viral isolation was performed using 9- to 11-day-old embryonated SPF chicken eggs. Four influenza A viruses were isolated: A/canine/Guangdong/01/2006(H3N2), A/canine/Guangdong/02/2006(H3N2), A/canine/Guangdong/01/2007(H3N2), and A/canine/Guangdong/02/2007(H3N2). The genomes of these viruses were fully sequenced

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Table 1
Four avian-origin H3N2 canine influenza cases occurred in Southern China (2006–2007).

Case ID	Species	Dog residence	Date	Age	Sex	Symptom (s)	Treatment (s)	Disease history	Isolate ^a
1	Cocker Spaniel	Guangzhou	May 26, 2006	2-month-old	Male	Cough, sneeze, nasal discharge, body temperature: 39.7 °C	Cefoselis (22 mg/kg by weight) for 10 days	This pet dog was bought from pet market 3 days before sending to the hospital. The dog died after 2 weeks	A/canine/Guangdong/01/2006
2	Mini-Poodle	Guangzhou	August 17, 2006	6-month-old	Male	Cough, sneeze, nasal discharge, low appetite, body temperature: 39.6 °C	Cefoselis (22 mg/kg by weight) for 4 days	This pet dog died 4 days after being received at the animal clinics. This dog was raised in a flat in the city, and the history of its contacts with other dogs is not clear	A/canine/Guangdong/02/2006
3	Japanese Akita dog	Dongguan	April 13, 2007	18-month-old	Male	Productive cough, sneeze, nasal discharge, body temperature: 39.8 °C	Ribavirin (15 mg/kg by weight) for 5 days	This dog recovered one week after being received at the animal clinics. This dog was raised in a rural area as a guard dog, and it had limited contacts with other dogs	A/canine/Guangdong/01/2007
4	Chinese native dog	Dongguan	October 6, 2007	3.5-year-old	Female	Cough, sneeze, nasal discharge, body temperature: 39.9 °C	Ribavirin (15 mg/kg by weight) for 5 days	The dog recovered 10 days after being received at the animal clinics. This dog was raised in a rural area as a guard dog, and it has limited contacts with other dogs	A/canine/Guangdong/02/2007

^a The sequences for these isolated were deposited in GenBank with the accession numbers GU433345–GU433376.

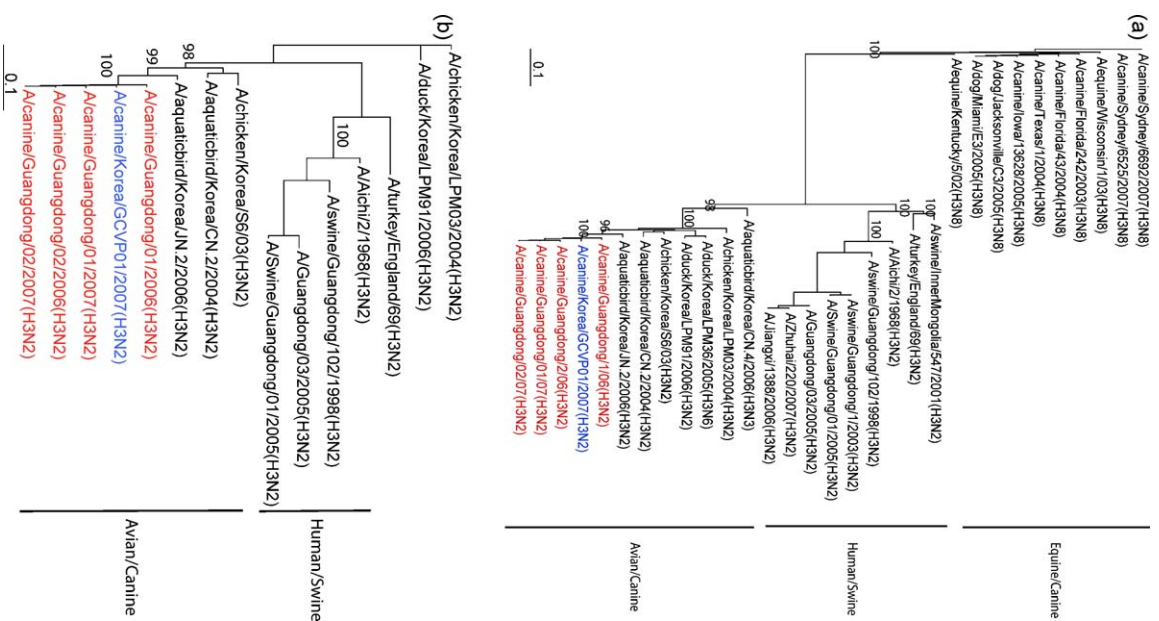


Fig. 1. Phylogenetic analysis and molecular characterization of H3N2 canine influenza viruses isolated from Southern China. (A) The phylogenetic tree for HA gene. (B) The phylogenetic tree for NA gene. The phylogenetic trees were constructed using maximum likelihood implemented in GARLI version 0.96, and the bootstrap values were generated using neighborhood joining methods implemented in PAUP* with 1,000 replications.

as described before (Wan et al., 2005) and deposited in GenBank with the accession numbers GU433345–GU433376.

Evolutionary analyses showed that all eight genes of these four viruses were phylogenetically close to the H3N2 AIVs as well as to the H3N2 CIV isolated in Korea in 2007 (Fig. 1 and Fig. S1). With high nucleotide sequence similarities between these four isolates and the AIVs (HA, 97.7%; NA, 97.8%; PB2, 97.2%; PB1, 97.5%; PA, 98.3%; NP, 96.1%; MP, 98.3%; NS, 94.7%), these H3N2 CIVs were most likely of avian-origin. No reassortments were observed in these H3N2 CIVs. In comparison with contemporary H3N2 AIVs present in Eastern Asia, the HA protein in these four isolates has six mutations in HA1 (T10A, D81N, L111I/V, A160T, D172N, W222L) (Fig. 2) and one mutation in HA2 (D489N). Among these mutations, the position 222 is located in the 220 loop, which is critical for receptor binding. Further experiments are required to test whether these mutations are required for H3N2 virus to jump from bird to dog.

From December of 2009 to January of 2010, based on hemagglutination inhibition assays, our passive serological survey

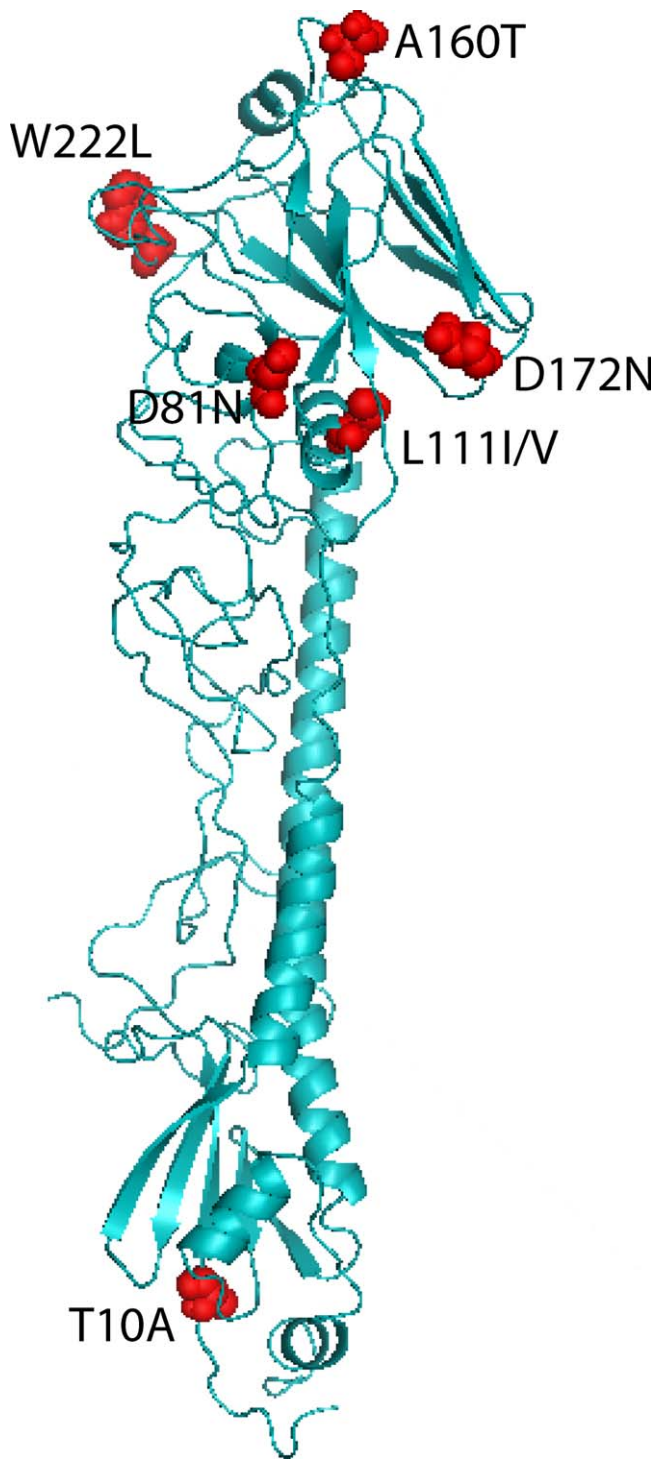


Fig. 2. The mutations in HA protein surface area of H3N2 avian-origin canine influenza viruses. The template of H3 three-dimensional structure used here is 1 HGD, which is downloaded from the Protein Data Bank (www.pdb.org).

in the pet dog population at Guangzhou area showed that about 6.7% of the 58 dog sera samples were positive. Although it is still very limited, this survey suggested it would be possible that H3N2 avian-origin CIV is circulating in the dog population in Southern China. A more systematic and active survey is still ongoing in the dog population, especially in the dog farms in Southern China.

In summary, we isolated four strains of H3N2 avian-origin CIVs in dogs in Southern China. Our results suggest that all eight genes of these viruses were phylogenetically close to one H3N2 Korean

CIV available in GenBank, which was likely to be circulating in Korean dog population. The sporadic cases in Southern China reported in this study and a limited serological survey indicate this H3N2 virus could have been circulating in canine population. This virus could have been transmitted between Korea and China through pet dog exchange. The emergence of these canine influenza cases in China could result also from the ecological changes in China, especially as the changing of socio-economic circumstances in the last 15–20 years in China have led to more people, particularly in urban areas, having companion dogs and dogs continuing to be raised for food, in some circumstances. A systematic surveillance of H3N2 CIV is required to monitor the disease and evolutionary behavior of this virus in canine populations, especially in eastern Asia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.meegid.2010.08.010](https://doi.org/10.1016/j.meegid.2010.08.010).

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