Virus Genes (2013) 46:554–557
DOI 10.1007/s11262-013-0882-6

# Short communication: isolation and phylogenetic analysis of an avian-origin H3N2 canine influenza virus in dog shelter, China

**Shuo Su · Ziguo Yuan · Jidang Chen · Jiexiong Xie · Huatao Li · Zhen Huang · Minze Zhang · Guohao Du · Zhongming Chen · Liqing Tu · Yufei Zou · Junhao Miao · Hui Wang · Kun Jia · Shoujun Li**

Received: 19 September 2012 / Accepted: 15 January 2013 / Published online: 26 January 2013


**Abstract** A H3N2 canine influenza virus, A/canine/Guangdong/3/2011 (H3N2), was isolated from roaming dogs in rural China. Sequence and phylogenetic analysis of eight gene segments revealed that the A/canine/Guangdong/3/2011 (H3N2) was most similar to a recent H3N2 canine influenza virus isolated in cats from South Korea, which originated from an avian strain. To our knowledge, this is the first report of an avian-origin H3N2 CIV which was isolated from roaming dogs in China. The epidemiologic information provided herein suggests that continued study is required to determine if this virus could be established in the roaming dog population in rural China and pose potential threats to public health.

**Keywords** H3N2 · Canine influenza virus · Roaming dog

Influenza A virus can infect a wide variety of hosts, from birds to mammals, and can exhibit varying degrees of host adaptation [1]. Soon after a case of H3N2 canine influenza virus (CIV) was reported in eastern Asia, infections in different types of dog populations were widely reported [2–5]. The first case of H3N2 CIV in China was reported in Guangdong province in 2006, and almost all of the reported outbreaks of canine influenza occurred in pet dogs from Animal Clinics, which indicates that the avian-origin H3N2 CIV might be circulating in the pet dog population in southern China [3–5]. However, the laboratory-confirmed canine influenza outbreak in roaming dogs was not reported until now. In the past several decades, many influenza epidemics and pandemic strains were reported to have emerged from southern China. This area is critical for the emergence of novel influenza A viruses. In order to monitor the epidemics of CIVs and to identify the potential H3N2 canine influenza threats in southern China, we performed an active surveillance in different dog populations in southern China.

During September 2011 to February 2012, a total of 900 samples, including 360 nasal swabs and 540 blood samples, were collected from six dog farms. The six cities included Guangzhou, Huizhou, Zhuhai, Shenzhen, Shantou, and Zhanjiang city. In addition, 500 nasal swab samples were collected from pet dogs in 60 different pet hospitals, and 100 nasal swabs were collected from roaming dogs in six dog shelters located in the same cities. When we collected nasal swab samples from dog shelters in a rural area northwest of Huizhou, we found that a dog showed similar symptoms of coughing, copious nasal discharge, sneezing, and a low fever of 39.7 °C. The case was treated with ribavirin and the dog recovered from the disease. A total of 960 nasal swabs were collected from different dog populations (including the sick dog). Isolation of viruses from nasal swabs in Madin–Darby canine kidney cells and sequencing were carried out as previously described [2, 4]. As a result, five H3N2 CIVs were successfully isolated. Three of the five CIVs were isolated from dog farms; the other two CIVs were isolated from pet hospitals and shelters, respectively. As we knew little about the canine influenza outbreak in roaming dogs, this study mainly focuses on the roaming dog isolates. RT-PCR was employed to amplify the full-length protein coding regions

Shuo Su, Ziguo Yuan, and Jidang Chen contributed equally to this study.

S. Su · Z. Yuan · J. Chen · J. Xie · H. Li · Z. Huang · M. Zhang · G. Du · Z. Chen · L. Tu · Y. Zou · J. Miao · H. Wang · K. Jia · S. Li (✉)
College of Veterinary Medicine, South China Agricultural University, 483 Wushan Road, Tianhe District, Guangzhou 510642, Guangdong, China
e-mail: shoujunli@scau.edu.cn

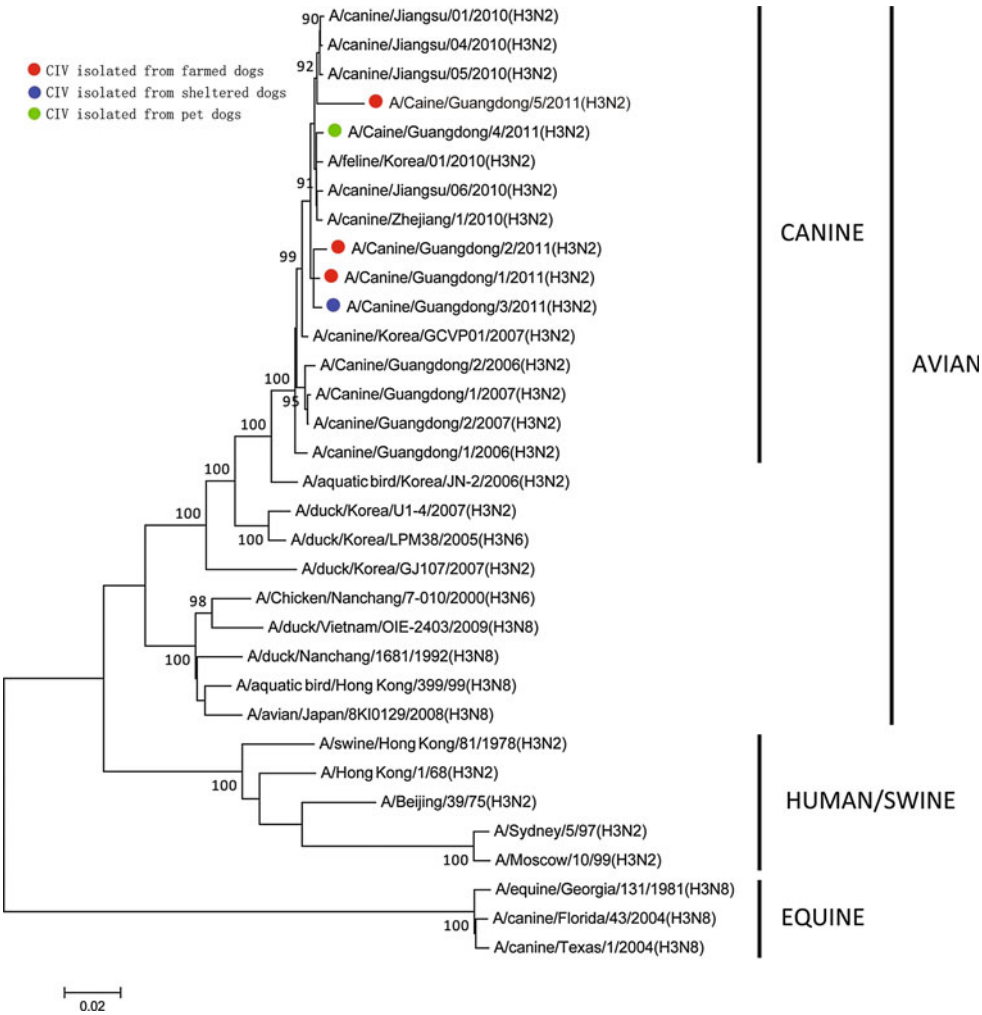


**Fig. 1** Phylogenetic trees for the A/canine/Guangdong/3/2011(H3N2) HA gene and those of other influenza A viruses. The analysis was based on the nucleotide sequences in the open reading frames of the HA gene. The trees were generated with the MEGA program (version 5.0) by using neighbor-joining analysis

of all eight viral RNAs for sequencing analysis. Viral RNA was extracted using Trizol reagents (GIBCO-BRL), and reverse transcription was performed using influenza virus oligonucleotide universal primer: 5′-AGC AAA AGC AGG-3′. A series of primers were designed to amplify eight genes for sequencing. PCR products were purified with the Agarose Gel DNA Purification Kit (TaKaRa, Dalian), cloned into the pMD18-T vector, and sequenced (TaKaRa, Dalian). Accordingly, the nomenclature for the only roaming dog isolates was A/canine/Guangdong/3/2011 (H3N2).

The nucleotide sequence identity between each segment of A/canine/Guangdong/3/2011(H3N2) and its potential progenitor identified in the public database varied from 97 to 99 %. From the phylogenetic trees, we can see that the A/canine/Guangdong/3/2011 (H3N2) was grouped together with the newly isolated H3N2 CIVs in dogs and cats from Korea and China. In addition, phylogenetic analysis showed that the roaming dog isolates were most closely related to the feline isolate in Korea [6]. A/canine/Guangdong/3/2011(H3N2) and other 2011 canine H3N2 virus isolates formed a subclade that was separated from the clades of avian H3 influenza viruses (Fig. 1). The HA and NA genes of A/canine/Guangdong/3/2011 (H3N2) were in the same clade with the avian H3N2 viruses from Korea, while other countries' avian influenza viruses were in different clades (Figs. 1, 2). All eight gene segments from our 2011 isolates were closely related to H3N2 CIVs isolated from Zhejiang and Jiangsu provinces of southern China in 2010, with the representative strains of A/canine/Jiangsu/6/2010 and A/canine/Zhejiang/1/2010.

We compared the deduced amino acid sequences of the HA1 gene from the A/canine/Guangdong/3/2011 against the A/canine/Guangdong/2/2007(H3N2) strain (pet dog isolates); none of the mutations were found in the proposed antigenic sites, receptor-binding sites, or potential glycosylation sites. Significantly, continued evolution was detected in H3N2 CIVs in southern China [2]. Our early 2007 canine H3N2 isolates possessed a PEKQTR/G motif in the HA cleavage site. The mutation K326R was located at the cleavage site in A/canine/Guangdong/3/2011 (H3N2). Some obvious mutations were found in A/canine/Guangdong/3/2011 (H3N2),

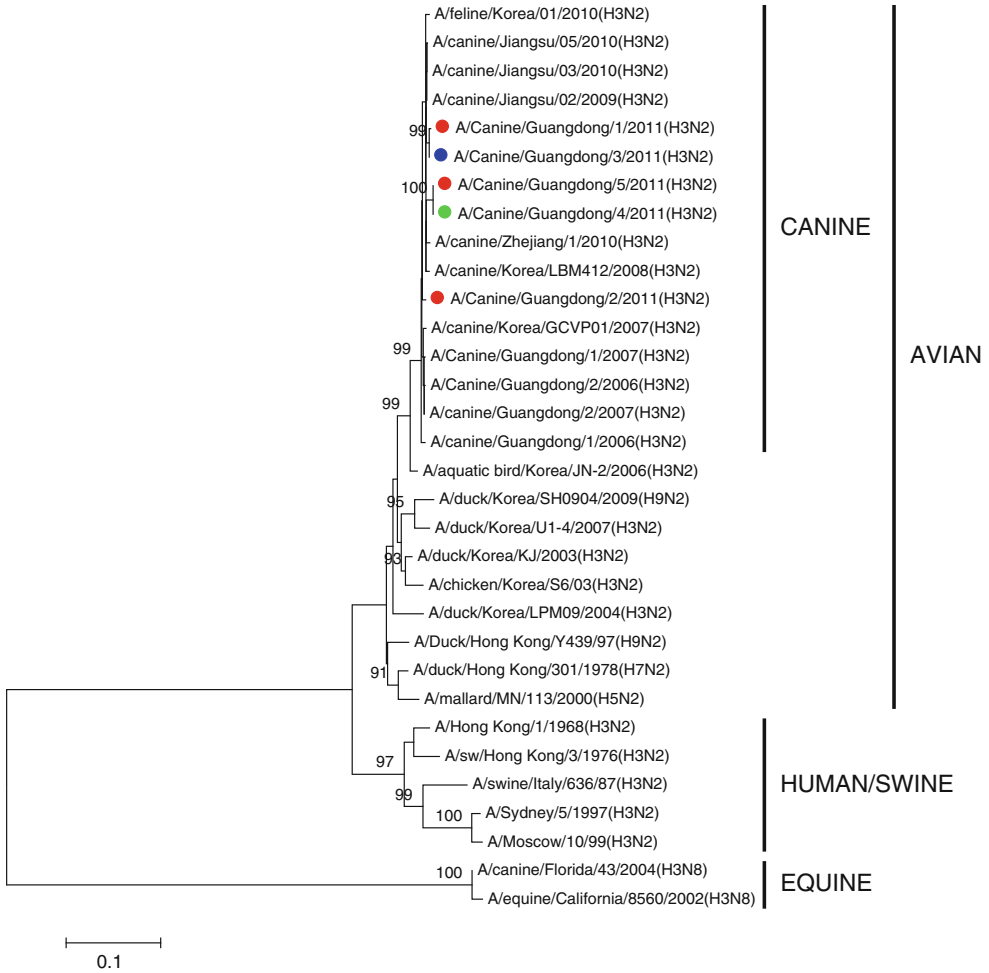


**Fig. 2** Phylogenetic trees for the A/canine/Guangdong/3/2011 (H3N2) NA gene and those of other influenza A viruses. The analysis was based on the nucleotide sequences in the open reading frames of the NA gene. The trees were generated with the MEGA program (version 5.0) by using neighbor-joining analysis

compared with the A/canine/Guangdong/2/2007(H3N2) isolates: F59L, T212A, I260M, H261R, R411K, A451T, R326K.

This is the first report of an avian-origin H3N2 CIV which was isolated from roaming dogs in rural China. In this area, the close interactions of dogs with farmers and surrounding wild life provide frequent opportunities for cross-species virus transmissions. Roaming dogs carrying influenza viruses are not only potential transmission to farm watchdogs or pet dogs when they are close contact with each other in rural, pose a threat to human health. Both the 1957 and 1968 pandemic influenza viruses had emerged in this area [7]. For the above described reasons, the appearance of H3N2 CIV among dogs contributes to concerns on both veterinary and human health. The most likely route of roaming dog infection, how long these dogs were infected, and whether the dogs were capable of transmitting the virus to humans are all questions that remain unanswered. Continued surveillance for H3N2 CIV cases in different dog populations, and further seroprevalence investigations are needed to assess the risk of dogs CIV infection, given that H3N2 CIV continues to circulate and evolve among dogs in southern China.

*Nucleotide sequence accession number*. The GenBank accession numbers of A/canine/Guangdong/3/2011(H3N2) are JX195356 to JX195363.

**Acknowledgments** This work is supported, in part, by Grants from the National Natural Science Foundation (No. 30972233) of China, the National Special Research Programs for Non-Profit Trades (Agriculture) (Grant No. 201303042), the National Key Basic Research Program (Project 973) of China (Grant No. 2011CB504700-G), the Natural Science Foundation of Guangdong Province (Nos. 8251064201000008) and the Scientific and Technological Planning Project of Guangdong Province (Grant No. 2010B020307006).

## References

1. H. Yu, R.H. Hua, Q. Zhang, T.Q. Liu, H.L. Liu, G.X. Li, G.Z. Tong, J. Clin. Microbiol. **46**(3), 1067 (2008). doi:10.1128/JCM.01257-07
2. S.J. Li, J.H. Shi, P.R. Jiao, G.H. Zhang, Z.W. Zhong, W.R. Tian et al., Infect. Genet. Evol. **10**(8), 1286–1288 (2010)

3. Y. Lin, Y.B. Zhao, X.J. Zeng, C.P. Lu, Y.J. Liu, Veterinar. Microbiol. (2012). doi:10.1016
4. S. Su, N. Cao, J.D. Chen, F.R. Zhao, H.T. Li, M.X. Zhao, Y.J. Wang, Z. Huang, L.G. Yuan, H. Wang, G.H. Zhang, S.J. Li, S.J. Li, J. Virol. **86**, 10238 (2012). doi:10.1128/JVI.01588-12
5. Q.Y. Teng, X. Zhang, D.W. Xu, J.W. Zhou, X.G. Dai, Z.G. Chen, Z.J. Li, Veterinar. Microbiol. (2012). doi:10.1016/j.vetmic.10.006
6. D.S. Song, D.J. An, H.J. Moon, M.J. Yeom, H.Y. Jeong, W.S. Jeong, S.J. Park, H.K. Kim, S.Y. Han, J.S. Oh, B.K. Park, J.K. Kim, H. Poo, R.G. Webster, K. Jung, B.K. Kang, J. Gen. Virol. **92**, 2350–2355 (2011)
7. G.H. Zhang, W.L. Kong, W.B. Qi, L.P. Long, Z.X. Cao, M. Liao, X.F. Wan et al., Infect. Genet. Evol. **11**((5), 1174–1177 (2011)