Short communication

Avian-origin H3N2 canine influenza virus circulating in farmed dogs in Guangdong, China

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Abstract

Since 2006, more and more cases of the infectious H3N2 canine influenza virus (CIV) in pet dogs have been reported in southern China. However, little is known about the prevalence situation of H3N2 CIV infections in farmed dogs in China. This is the first systematic epidemiological surveillance of CIV in different dog populations in southern China. Two virus strains A/Canine/Guangdong/1/2011(H3N2) and A/ Canine/Guangdong/5/2011(H3N2) were isolated from canine nasal swabs collected at one dog farm in Guangzhou and the other farm in Shenzhen. Sequence and phylogenetic analysis of eight gene segments of these viruses revealed that they were most similar to the newly isolated canine H3N2 viruses in dogs and cats from Korea and China, which originated from avian strain. This indicates that H3N2 CIV may be a common pathogen for pet and farmed dog populations in southern China at present. Serological surveillance has shown that the infection rate of this avian-origin canine influenza in farmed dogs and in pet dogs were 12.22% and 5.3%, respectively; as determined by the ELISA. The data also suggested that transmission occurred, most probably by close contact, between H3N2 CIV infected dogs in different dog populations in recently years. As H3N2 outbreaks among dogs continue in the Guangdong province (located very close to Hong Kong), the areas where is densely populated and with frequent animal trade, there is a continued risk for pets H3N2 CIV infections and for mutations or genetic reassortment leading to new virus strains with increased transmissibility among dogs. Further in-depth study is required as the H3N2 CIV has been established in different dog populations and posed potential threat to public health.

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1. Introduction

Influenza A virus can infect a wide variety of hosts, from birds to mammals, and exhibits varying degrees of host adaptation (Crawford et al., 2005; Wright and Webster, 2001). Given the world-wide interaction between humans, pigs, birds and other mammalian species, there is a high potential for cross-species transmission of influenza viruses in nature (Brown, 2000). Although waterfowl are considered to be the natural host for many influenza A viruses, many subtypes of influenza A viruses have host specificity, and they are not pathogenic for humans. Dogs are one of the most popular pets around the world, with an estimated global population of over 400 million (Coppinger et al., 2001). As companion animals, dogs have a special status in modern human life. The close interactions of dogs with human and surrounding wild life provide frequent opportunities for cross-species virus transmissions, so dogs carrying influenza viruses can pose a great threat to human health.

Influenza viruses in dogs can be caused by 2 subtypes of influenza A virus: H3N2 and H3N8. The H3N8 CIV is known as an equine-derived H3N8 influenza virus which was first identified in the United States in 2005 (Payungporn et al., 2008). H3N2 subtype influenza viruses appeared in humans and caused a major pandemic in 1968. Subsequently, H3N2 influenza viruses were isolated regularly from mammals all over the world (Nerome et al., 1981). Epidemics of avian origin H3N2 CIV among dogs have been found in Asian countries, including South Korea and China (Lee et al., 2009; An et al., 2010). 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 (Li et al., 2010; Lin et al., 2012; Crawford et al., 2005; Zhao et al., 2011). In December 2011, an avian-origin H3N2 CIV [A/canine/Guangdong/2/2011 (H3N2)] was first isolated from a farmed dog with outbreak of severe respiratory diseases in Guangdong Province, southern China (Su et al., 2012). The dog showed similar symptoms of sneezing, copious nasal discharge, coughing and low fever 39.5°C when the dogs entered the clinics. The case
close related and clustered in the same clade with the avian
HA and Neuraminidase (NA) genes of the two H3N2 CIVs were
most closely related to the Korea feline isolate. The Hemagglutinin
et al., 2012). In this manner, four H3N2 canine influenza viruses
carried out according to reported protocols (Li et al., 2010; Su
by canine kidney (MDCK) cells and subtyping experiments were
Control Center.
study had been approved by Guangdong Province Animal Disease
Care International- accredited facility. Our animal research in this
CDC's Institutional Animal Care and Use Committee and in an Asso-
ciation for Assessment and Accreditation of Laboratory Animal
Care International- accredited facility. Our animal research in this
study had been approved by Guangdong Province Animal Disease
Control Center.

The isolation of viruses from canine nasal swabs in Madin–Darby
canine kidney (MDCK) cells and subtyping experiments were
carried out according to reported protocols (Li et al., 2010; Su
2012). In this manner, four H3N2 canine influenza viruses
[A/canine/Guangdong/1/2011(H3N2), A/canine/Guangdong/3/
2011(H3N2), A/canine/Guangdong/4/2011(H3N2), and A/Canine/
Guangdong/5/2011(H3N2) were successfully isolated. Of these,
A/canine/Guangdong/1/2011(H3N2) and A/canine/Guangdong/5/
2011(H3N2) were isolated from canine nasal swabs collected at
one dog farm in Guangzhou and the other farm in Shenzhen. RT-
PCR was performed to amplify the full-length coding regions of
all 8 viral RNAs for sequencing analysis. Viral RNA was extracted
by using Trizol (GIBCO-BRL), and reverse transcription was per-
formed using an influenza virus oligonucleotide universal primer,
5'-AGCAGG-3'. A series of primers were designed to am-
plify the 8 genes for sequencing. PCR products were purified using
the Agarose Gel DNA Purification Kit (TaKaRa, Dalian), cloned into a
pMD18-T vector, and sequenced (TaKaRa, Dalian). The GenBank
accession numbers of the farmed dog isolates in this study were
JX195341 to JX195347, JX414244 to JX414251. The genotype of
A/canine/Guangdong/1/2011 (H3N2) and A/canine/Guangdong/5/
2011 (H3N2) were determined initially by pair wise comparisons
of the nucleotide sequences of each gene segment to the sequences
of reference influenza viruses available in GenBank with the highest level of sequence identity to A/canine/
Guangdong/1/2011 (H3N2) and A/canine/Guangdong/5/2011
(H3N2) for each gene segment. A phylogenetic tree was con-
structed using MEGA 5 software with the neighbor-joining meth-
Method; bootstrap values were calculated on 1000 replicates for
alignment.

The phylogenetic trees were mainly divided into three sub-
groups that correlated with avian lineages, human and swine line-
ages, and equine lineages. As shown in the eight constructed
phylogenetic trees, the two viruses grouped with the newly iso-
lated canine H3N2 viruses in dogs and cats from Korea and China.
Additionally, compared with these isolates from an earlier study
reported by Li et al. (2010), all eight genes of the two viruses were
most closely related to the Korea feline isolate. The Hemagglutinin
(HA) and Neuraminidase (NA) genes of the two H3N2 CIVs were
closely related and clustered in the same clade with the avian

Table 1

<table>
<thead>
<tr>
<th>Name of the CIV</th>
<th>The same of the strain identity of HA, PA, NA, M, and NS genes from A/canine/Guangdong/1/2011 (H3N2) compared with sequences of the most closely related strains available in GenBank.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/canine/Guangdong/1/2011(H3N2)</td>
<td>JX195341 to JX195347, JX414244 to JX414251. The genotype of A/canine/Guangdong/1/2011 (H3N2) and A/canine/Guangdong/5/2011 (H3N2) were determined initially by pair wise comparisons of the nucleotide sequences of each gene segment to the sequences of reference influenza viruses available in GenBank with the highest level of sequence identity to A/canine/Guangdong/1/2011 (H3N2) and A/canine/Guangdong/5/2011 (H3N2) for each gene segment. A phylogenetic tree was constructed using MEGA 5 software with the neighbor-joining method; bootstrap values were calculated on 1000 replicates for alignment.</td>
</tr>
</tbody>
</table>
H3N2 viruses from Korea, while avian viruses isolated from another countries were in different clade. Furthermore, the two genes of the two H3N2 canine strains seemed to be both derived from the A/aquatic bird/Korea/JN-2/2006 (H3N2) strain (Figs. 1 and 2). The internal genes of A/canine/Guangdong/1/2011(H3N2) and A/canine/Guangdong/5/2011(H3N2) were most closely related to the A/canine/Korea/GCVP01/2007(H3N2), and they all clustered closely with various Chinese avian influenza viruses (Supplementary 1–6). Similarly, we compared the deduced amino acid sequences of the HA1 gene from the two isolates against the canine and feline isolates from Korea, China and the most similar avian strain, A/aquatic bird/Korea/JN-2/2006. None of the mutations are found in proposed antigenic sites, receptor-binding sites, potential glycosylation sites and cleavage site, although novel mutations were found in other sites (Fig. 3). All these analysis revealed that few changes happened in CIVs after years of spreading.

We sought to gain more insight into the epidemiology of avian-origin H3N2 CIV in different dog populations in Guangdong province, serologic study was performed to assess the prevalence of CIV in different dog populations. All serum samples were treated with a receptor-destroying enzyme (RDE) and absorbed with erythrocytes in order to remove nonspecific inhibitors before analysis with the primary assay. All samples were tested with hemagglutination inhibition (HI) and NP-specific ELISA assay according to the manufacturer’s instructions as reported in previous studies (An et al., 2010; Lee et al., 2009; Zhao et al., 2011). Previous findings suggested that the NP-based ELISA method is a better tool for the serological diagnosis of influenza H3N2 virus infections in dogs (Lee et al., 2009; Zhao et al., 2011), thus, ELISA was our prior choice for the measurement of anti-influenza virus antibodies to reflect the positive rate of dogs in different dog population.

Fig. 1. Phylogenetic trees for the A/canine/Guangdong/1/2011(H3N2) and A/canine/Guangdong/5/2011(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.
The serological screening revealed that 66 sera (12.2%) and 48 sera (5.3%) were found positive by NP-specific ELISA in farmed dogs and pet dogs, respectively. Results of the NP-ELISA and HI tests are reported in Table 2. Pet and farmed dogs were examined by ELISA and HI for avian-origin H3N2 CIV antibodies from six cities in Guangdong, China. Farmed dogs from Shenzhen was detected with the highest seroprevalence when tested by NP-specific ELISA (31.1%), followed by Guangzhou (20%) and Huizhou (13.3%). The dog farms with high seropositive rate are mainly located at Shenzhen and Guangzhou (20% and 31.1%) (Table 2).

This is the first epidemiology survey to assess the risk of H3N2 CIV transmission among dogs in China. Two of the four strains were isolated from the pet dog and free-roaming dog in the Animal Clinics of South China Agricultural University, and the other two were isolated from farmed dogs on Guangzhou and Shenzhen dog farms. This indicates that H3N2 CIV may currently be a common pathogen for pet and farmed dog populations in southern China. Serological surveillance has shown that the infection rate of this avian-origin canine influenza in farmed dogs and pet dogs were 12.22% and 5.3%, respectively; as determined by the ELISA. The data also suggested that transmission occurred, most probably in populations between H3N2 CIV infected dogs and uninfected dogs from different dog populations by close contact, in recently years, dog-to-dog transmission is the most likely route of infection. Our findings strengthened the previous data by showing that after long-term adaptation in dogs, the avian-origin H3N2 CIV have already circulated in different dog populations (Li et al., 2010; Zhao et al., 2011). However, how long these dogs infected, whether all the infection had clinical manifestations, and whether the dogs were capable of transmitting the virus to humans are all questions that remain unanswered. As H3N2 outbreaks among dogs continue in the Guangdong province (located very close to Hong Kong), the areas where is densely populated and with frequent animal trade, there is a continued risk for pets H3N2 CIV infections and for mutations or genetic reassortment leading to new virus strains with increased transmissibility to and among dogs. As a companion dog, it...
is in close contact with humans, although no direct transmission of H3N2 CIV from dogs to humans has been reported, it is possible that the CIV may generate a new pandemic outbreak, which poses a great threat to our human life, these findings highlight the importance of monitoring dogs in pet hospitals and on dog farms; furthermore, the development of a canine H3N2 vaccine is extremely urgent. Further in-depth study is required as the H3N2 CIV has been established in different dog populations and posed potential threat to public health.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2012.11.018.

References


Table 2

CIV seropositivity rates in farmed and pet dogs in different cities in Guangdong Province, Southern China.

<table>
<thead>
<tr>
<th>Farmed dogs</th>
<th>Pet dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study sites</td>
<td>No. dogs</td>
</tr>
<tr>
<td>GuangZhoua</td>
<td>90</td>
</tr>
<tr>
<td>Shenzhenb</td>
<td>90</td>
</tr>
<tr>
<td>Huizhoub</td>
<td>90</td>
</tr>
<tr>
<td>Zhuhai</td>
<td>90</td>
</tr>
<tr>
<td>Shantou</td>
<td>90</td>
</tr>
<tr>
<td>JiangMen</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>540</td>
</tr>
<tr>
<td>Study sites</td>
<td>No. dogs</td>
</tr>
<tr>
<td>GuangZhou</td>
<td>150</td>
</tr>
<tr>
<td>Shenzhen</td>
<td>150</td>
</tr>
<tr>
<td>Huizhou</td>
<td>150</td>
</tr>
<tr>
<td>Zhuhai</td>
<td>150</td>
</tr>
<tr>
<td>Shantou</td>
<td>150</td>
</tr>
<tr>
<td>JiangMen</td>
<td>150</td>
</tr>
<tr>
<td>Total</td>
<td>900</td>
</tr>
</tbody>
</table>

a Cases of severe respiratory disease at these dog farms were discovered.
b The trade zones of the large-scale breeding of poultry, pigs in Southern China.


