Evidence for Subclinical Influenza A(H1N1)pdm09 Virus Infection among Dogs, Guangdong Province, China

Shuo Su,1,* Pei Zhou,1,* Kun Jia,1,* Salah Uddin Khan,2 Shuyi He,1 Xinliang Fu,1 Malin Hong,1 Lingshuang Sun,1 Wenbao Qi,1 Gregory C. Gray,2 and Shoujun Li1†

1College of Veterinary Medicine, South China Agricultural University, Guangzhou, Guangdong Province 510642, People’s Republic of China.
2Department of Environmental and Global Health, College of Public Health and Health Professions, and Emerging Pathogens Institute, University of Florida, Gainesville, FL 32610, USA

* These authors contributed equally to the work.
† corresponding author: Fax:+86-20-85280240; E-mail address: shoujunli@scau.edu.cn
Abstract

During 2012, we identified dogs sampled with elevated antibodies (≥1:40) against A(H1N1)pdm09 virus: HI assay (24.7%) and MN assay (10.8%). This high seroprevalence of A(H1N1)pdm09 among dogs without clinical signs of influenza support the premise that dogs may play a role in human influenza ecology in China.

Key words: Dogs; epidemiology; canine influenza; zoonoses; A(H1N1)pdm09
Influenza A viruses have been found to infect numerous mammalian hosts. The susceptibility of the species is dependent upon the characteristics of the virus and the host (1,2). Numerous subtypes of influenza A viruses, including influenza A(H1N1)pdm09, have been documented to cross the species barrier. The triple reassortment A(H1N1)pdm09 virus, comprised of a mixture of gene segments from human, swine, and avian origin, quickly became well-established in humans and pigs (1,2). This virus was first detected to infect humans in China during 2009 (3). Since then, it has continued to circulate among humans along with a number of other seasonal influenza viruses. The influenza A(H1N1)pdm09 virus has also been detected in a number of non-human species, including pigs, poultry, dogs and cats (4-10).

As one of the most common companion animals in China, dogs are potentially at risk of acquiring human pathogens because of their close proximity and frequent contact with man (11). There are several studies suggesting that multiple subtypes of influenza viruses, including A(H1N1)pdm09, are infecting dogs (6-9,12-14). It has also been shown that dog-to-dog transmission of A(H1N1)pdm09 can occur, although with low infection rates (9).

In China, the A(H1N1)pdm09 virus was first isolated from dogs in Beijing during November 2009(9). We sought to examine evidence that healthy dogs without clinical signs of influenza in China were experiencing A(H1N1)pdm09 virus infections in Guangdong Province, one of the China’s most densely populated provinces.
During 2012, we conducted a seroepidemiological study among domestic and farm-raised dogs in four cities in Guangdong Province. We sought to study dogs without clinical signs of influenza that might have contact with large diverse populations of humans and animals. We selected the cities based on their large and dense human, poultry, and pig populations which we reasoned may be prone to cross-species influenza transmission. In these cities we studied dogs from a total of 32 pet hospitals and 4 dog farms (dogs raised for commercial purpose). In each city we selected the single largest dog farm. We selected dogs from veterinary clinics based upon several factors: veterinary clinic location for geographical diversity, veterinary clinics which had treated more than 3,000 canine patients during the last year, and dogs with no history of canine influenza vaccination or clinical signs of influenza in the last three months. At each site individual dogs were selected by a random-number procedure among the apparently healthy dogs without clinical signs of influenza. We recorded demographic information regarding the dogs. In addition, we also studied archived sera from dogs and cats collected during 2008 from 2 pet hospitals in Guangdong Province. Our sampling processes were assisted by local authorities and licensed veterinarians. The animal research in this study was reviewed and approved by Guangdong Province Animal Disease Control Center.

All samples were tested by hemagglutination inhibition (HI) and according to recommended procedures (15). We studied the sera for HI antibodies against four viruses: The pdm09 virus A/Guangdong/1057/2010 (H1N1); a human seasonal H1N1
influenza virus A/Brisbane/59/2007(H1N1), a H9N2 avian influenza virus
A/chicken/Guangdong/V/2008(H9N2) (Genbank Accession:JQ639786) as it is similar
to A/Chicken/Beijing/1/94(H9N2) (the most prevalent subtype detected among
poultry in southern China) and A/canine/Guangdong/2/2011(H3N2), a recently
circulating H3N2 canine influenza virus (CIV) in dogs in China. Study sera which had
titers ≤1:20 in endpoint serum dilution against all four specific antigens used in this
study were considered to be negative. Sera from the infected and vaccinated dogs with
antibody titers ≥1:40 were considered positive. Elevated HI assays were as high as
1:1024.

For the MN assay we followed procedures recommended by the World Health
Organization (17). We ran the dog sera with the MN assay only against the
A/Guangdong/1057/2010(H1N1) virus, a A(H1N1)pdm09-like virus. Again a titer of
≥1:40 was considered as evidence of previous infection.

We performed descriptive statistics to explore potential risk factors for serological
assay outcomes. Bivariate χ² test of independence or Fisher’s exact test were used to
examine the association between the demographic characteristics and serological
outcome. Covariates that had a p<0.25 in the bivariate analyses were entered in an
entered into a multivariable logistic regression model. Forcing age into the model, we
performed backward elimination of the covariates, keeping covariates in the model
which had a p<0.05. Final covariates were tested for goodness-of-fit.
From February-July 2012, we sampled 960 dogs, 240 dogs in each of the 4 cities. The median age of the dogs was 4 years (range 1-11 years) and 57.4% were male. Most (68.8%) were raised as pets. There was no statistically significant difference between the average age or gender between pet and farm-raised dogs. Overall, 24.7% (n=237) of the 960 dog sera had elevated antibodies against influenza A(H1N1)pdm09, by either the HI or MN tests. Comparing the four cities and both serological methods, we consistently identified a higher prevalence of dogs with elevated level of antibody titer against A(H1N1)pdm09 virus in Guangzhou and Shenzhen cities (Table 1). A total 92 (88%) of the 104 MN-positive samples were also HI positive. When considered as a binary outcome (elevated or not), the two tests had a moderate to high agreement [Kappa 0.46; 95% CI 0.39 – 0.53]. Seroprevalence estimated by HI assay was significantly higher compare to the estimates from MN assay (24.7% vs. 10.8%, p<0.01). Dogs that were raised as pets were about twice as likely to have elevated antibodies against influenza A(H1N1)pdm09 virus infection, compared to the dogs that were raised in farms (OR=1.7; 95% CI 1.3 – 2.6).

All 1026 (including the 66 samples collected in 2008) serum samples were also studied with HI assays against the human seasonal H1N1; the canine H3N2 and the avian H9N2 viruses. Eleven dogs (95% CI: 6 – 20) had elevated antibody titers by HI against season human H1N1 and none had evidence of previous infection with H9N2. Interestingly, only nine dogs had an elevated antibody titer for both influenza A(H1N1)pdm09 and seasonal H1N1 infection (data not shown). A total of 181 (19%)
sera were positive by HI assay against H3N2 CIV (Table 2). Twenty nine out of 181 sera from the dogs had an elevated antibody titer for both influenza A(H1N1)pdm09 and H3N2 CIV. None of 66 dog and cat sera from 2008 had elevated antibodies against influenza A(H1N1)pdm09; human seasonal H1N1 and H9N2 AIV by the HI assay. Eight samples were positive by HI assay against H3N2 CIV.

This study is the first to identify a relatively high prevalence of elevated antibody against influenza A(H1N1)pdm09 among dogs in China compared to the studies conducted earlier in different part of the world (5,6,9,20). We hypothesize that the sustained transmission of the influenza A(H1N1)pdm09 virus in the human population in our study area, as well as close and prolonged exposure of the dogs to the clinically ill individuals, could have led to a higher prevalence of infection in dogs. Previous studies of dogs for influenza A (H1N1)pdm09 infections have not found the high seroprevalence we found. For instance, Dundon et al studied dogs in Italy during 2010 and found that <1% of dogs had evidence of (H1N1) pdm09 infection (5,6).

However, like our results, a similarly high seroprevalence (22.5%) of influenza A(H1N1)pdm09 infection was found among US cats, and ~10% of the cats were noted to have signs of acute respiratory illness (21). Moreover, during a 2010 (H1N1)pdm09 outbreak in a cat colony in Italy, researchers identified a higher seroprevalence (55%) A(H1N1)pdm09 infection and mortality (28%) (7). Our findings highlight the limitations of relying upon specimens from dogs with clinical signs of influenza for customary studies of A(H1N1)pdm09 infection in dogs. The relatively higher
seroprevalence identified in our study compared to the study in Italy could be explained by several factors. The dogs in southern China could have been at a higher risk of infection due their exposure to dense populations of humans with high influenza A(H1N1)pdm09 attack rates (22). Southern China is among the most densely populated regions in the world, where people and domestic animals often live in close proximity and where the risk of novel virus generation is thought be particularly high (23). The difference in seroprevalence might also be explained by the two-year temporal difference between dogs sampled in Italy vs. dogs sampled in China (6). Finally, dog farming is a unique practice in Guangdong province where many dogs are in close contact. This promotes rapid transmission of infectious agents within the dog farm. Although transmission experiments have shown that human A(H1N1)pdm09 can infect dogs, transmission was thought to be inefficient between dogs (9). However, our findings could be considered as evidence that long-term adaptation of A(H1N1)pdm09 in local dogs may have led to more efficient transmission between dogs and between dogs and humans. Our high prevalence findings are also supported by research from South Korea, where sustained transmission of avian origin influenza A (H3N2) has been documented among farm dogs (24,25). We hypothesize that relatively high A(H1N1)pdm09 transmission may have occurred between humans and dogs during the peak period of virus infection in the human population. This hypothesis is supported by our observation that pet dogs
were more likely to have evidence of previous infection with A(H1N1)pdm09 that were farm dogs.

Our findings were subject to several limitations. In this study, we collected sera only during a six-month time period in 2012 and thus our study may not be representative of other time periods. Although a previous report found little evidence for cross-reactivity between antibodies against canine H3N2 and human H3N2 (26), as we did not test the dog sera against human H3N2, we can not rule out activity against canine H3N2 might be confounded by antibodies against human H3N2. We also note that reports of human H3N2 virus infection among dogs in China and Japan have been previously reported (26).

In summary, these study data suggest that dogs without a history of clinical signs of influenza in these four Chinese cities had a relatively high prevalence of evidence of previous subclinical influenza A(H1N1)pdm09 infection. The seropositivity was highest among pet dogs which likely had more diverse and frequent exposures to humans than did farm dogs. Further observational and experimental studies of various influenza A viral infections among dogs are necessary for us to understand what roles dogs plan in the ecology of influenza A.
Conflict of interest statement

The funding agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare that they have no conflict of interest.

Acknowledgements

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and human seasonal H3N2 influenza viruses in dogs in China Veterinary Microbiology
Table 1. Prevalence of elevated antibodies against A(H1N1)pdm09 among dogs in 4 cities, Guangdong Province, China, 2012.

<table>
<thead>
<tr>
<th>City</th>
<th>Number</th>
<th>Farm dogs</th>
<th></th>
<th>Pet dogs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Seroprevalence (%)</td>
<td></td>
<td>Seroprevalence (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MN</td>
<td>HI</td>
<td></td>
<td>MN</td>
</tr>
<tr>
<td>Guangzhou</td>
<td>75</td>
<td>14 (18.6%)</td>
<td>26 (34.7%)</td>
<td>165</td>
<td>26 (15.7%)</td>
</tr>
<tr>
<td>Shenzhen</td>
<td>75</td>
<td>18 (24.0%)</td>
<td>26 (34.7%)</td>
<td>165</td>
<td>16 (9.7%)</td>
</tr>
<tr>
<td>Huizhou</td>
<td>75</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>165</td>
<td>14 (8.5%)</td>
</tr>
<tr>
<td>Zhuhai</td>
<td>75</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>165</td>
<td>16 (9.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>32 (10.7%)</td>
<td>52 (17.3%)</td>
<td>660</td>
<td>72 (10.9%)</td>
</tr>
</tbody>
</table>

MN=microneutralization assay; HI= hemagglutination inhibition assay; a HI titer ≥1:40 and a MN titer ≥1:40 was considered as elevated.
Table 2. Prevalence of elevated antibody titers against an avian influenza H9N2, a canine influenza H3N2, and A(H1N1)pdm09 among dogs by hemagglutination inhibition (HI) assay, Guangdong Province, China, 2012

<table>
<thead>
<tr>
<th>HI assay virus</th>
<th>Number</th>
<th>&lt;1:20</th>
<th>1:20</th>
<th>1:40</th>
<th>1:80</th>
<th>≥1:160</th>
<th>titers ≥1:40 (%)</th>
<th>titers ≥ 1:160 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H9N2</td>
<td>960</td>
<td>921</td>
<td>39</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>H3N2</td>
<td>960</td>
<td>403</td>
<td>376</td>
<td>93</td>
<td>36</td>
<td>52</td>
<td>181 (18.9)</td>
<td>52 (5.4)</td>
</tr>
<tr>
<td>H1N1</td>
<td>960</td>
<td>495</td>
<td>229</td>
<td>133</td>
<td>70</td>
<td>23</td>
<td>236 (24.6)</td>
<td>23 (2.4)</td>
</tr>
</tbody>
</table>

H9N2= A/chicken/Guangdong/V/2008(H9N2) ; H3N2= A/canine/Guangdong/2/2011(H3N2); H1N1= A/Guangdong/1057/2010 (H1N1), a pdm09 virus