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

Shuo Su,a,b Jidang Chen,a Kun Jia,a,b Salah Uddin Khan,c Shuyi He,a Xinliang Fu,a Malin Hong,a,b Lingshuang Sun,a,b Wenbao Qi,a Gregory C. Gray,a,b Shoujun Li,a,b

College of Veterinary Medicine, South China Agricultural University, Guangzhou, Guangdong Province, People’s Republic of Chinaa; Key Laboratory of Comprehensive Prevention and Control for Severe Clinical Animal Diseases of Guangdong Province, Guangzhou, Guangdong Province, People’s Republic of Chinab; Department of Environmental and Global Health, College of Public Health and Health Professions, and Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USAc

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-reassortant A(H1N1)pdm09 virus, comprising a mixture of gene segments of human, swine, and avian origins, quickly became well established in humans and pigs (1, 2). This virus was first found to infect humans in China in 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 nonhuman 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 humans (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 but with low infection rates (9).

In China, the A(H1N1)pdm09 virus was first isolated from dogs in Beijing in November 2009 (9). We sought to examine the evidence that healthy dogs without clinical signs were experiencing A(H1N1)pdm09 virus infections in Guangdong Province, which is one of 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 infection 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 32 pet hospitals and 4 dog farms (dogs raised for commercial purposes). In each city, we selected the largest dog farm. We chose dogs from veterinary clinics based upon several factors, including the location of the veterinary clinic (to provide geographical diversity), volume (we chose veterinary clinics that had treated >3,000 canine patients during the last year), and history (we chose dogs with no history of canine influenza vaccination or clinical signs of influenza infection in the last 3 months). At each site, individual dogs were selected by a random-number procedure among the apparently healthy dogs without clinical signs of influenza infection. We recorded the demographic information of the dogs. We also studied 66 archived serum samples from dogs and cats collected in 2008 from 2 pet hospitals in Guangdong Province (15). Our sampling processes were assisted by local authorities and licensed veterinarians. The animal research in this study was reviewed and approved by the Guangdong Province Animal Disease Control Center.

All samples were tested by hemagglutination inhibition (HI) assays according to the recommended procedures (16). We studied the serum samples 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); an H9N2 avian influenza virus, A/chicken/Guangdong/V/2008(H9N2) (GenBank accession no. 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), an H3N2 canine influenza virus (CIV) that was recently circulating in dogs in China. The study sera that had titers of ≥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 of ≥1:40 were considered positive. Elevated HI assays were as high as 1:1,024.

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

We performed descriptive statistics to explore the potential
TABLE 1 Prevalence of elevated levels of antibodies against A(H1N1)pdm09 virus among dogs in 4 cities, Guangdong Province, China, 2012*  

<table>
<thead>
<tr>
<th>City</th>
<th>No.</th>
<th>MN*</th>
<th>HP*</th>
<th>No.</th>
<th>MN</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guangzhou</td>
<td>75</td>
<td>14</td>
<td>26</td>
<td>165</td>
<td>26</td>
<td>56</td>
</tr>
<tr>
<td>Shenzhen</td>
<td>75</td>
<td>18</td>
<td>26</td>
<td>165</td>
<td>16</td>
<td>47</td>
</tr>
<tr>
<td>Huizhou</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>165</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>Zuhai</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>165</td>
<td>16</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>32</td>
<td>52</td>
<td>660</td>
<td>72</td>
<td>185</td>
</tr>
</tbody>
</table>

* An HI titer of 1:40 and an MN titer of 1:20 were considered elevated.

TABLE 2 Prevalence of elevated antibody titers against an avian influenza H9N2, a canine influenza H3N2, and A(H1N1)pdm09 virus among dogs in a hemagglutination inhibition assay, Guangdong Province, China, 2012*  

<table>
<thead>
<tr>
<th>HI assay virus</th>
<th>No. of dogs</th>
<th>No. of dogs with antibody titer of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;1:20</td>
</tr>
<tr>
<td>H9N2</td>
<td>960</td>
<td>921</td>
</tr>
<tr>
<td>H3N2</td>
<td>960</td>
<td>403</td>
</tr>
<tr>
<td>H1N1</td>
<td>960</td>
<td>495</td>
</tr>
</tbody>
</table>

* H9N2, A/chicken/Guangdong/V2008(H9N2); H3N2, A/canine/Guangdong/2/2011(H3N2); H1N1, A/Guangdong/1057/2010 (H1N1), a pdm09 virus.

None of the 66 dog and cat serum samples from 2008 had elevated antibodies against influenza A(H1N1)pdm09, human seasonal H1N1, or H9N2 avian influenza virus by the HI assay. Eight samples were positive by the HI assay against H3N2 CIV.

This study is the first to identify a relatively high prevalence of elevated antibodies against influenza A(H1N1)pdm09 among dogs in China compared to that in the studies conducted earlier in different parts of the world (5, 6, 9, 18). 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, might have led to a higher prevalence of infection in the dogs. Previous studies of dogs for influenza A(H1N1)pdm09 infections have not found the high seroprevalences that we found. For instance, Dundon et al. (6) studied dogs in Italy during 2010 and found that <1% of those studied had evidence of (H1N1)pdm09 infection.

However, like our results, a similarly high seroprevalence (22.5%) of influenza A(H1N1)pdm09 infection was found among U.S. cats, and ~10% of the cats were noted to have signs of acute respiratory illness (19). Moreover, during a 2010 (H1N1)pdm09 outbreak in a cat colony in Italy, researchers identified higher seroprevalence (55%) of A(H1N1)pdm09 infection and a higher mortality rate (28%) (7, 18). Our findings highlight the limitations of relying upon specimens from dogs with clinical signs of influenza infection for customary studies of A(H1N1)pdm09 in dogs. The relatively higher seroprevalence identified in our study compared to that in the study in Italy may be explained by several factors. The dogs in southern China might have been at a higher risk of infection due to their exposure to dense populations of humans with high influenza A(H1N1)pdm09 attack rates (20). 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 to be particularly high (21). The difference in seroprevalences might also be explained by the 2-year temporal difference between the dogs sampled in Italy and the dogs sampled in China (6). Finally, dog farming is a unique practice in the Guangdong Province, where many dogs are in close contact. This promotes the rapid transmission of infectious agents within the dog farm. Although transmission experiments have shown that human A(H1N1)pdm09 virus can infect dogs, the transmission was thought to be inefficient among dogs (9). However, our findings can be considered evidence that long-term adaptation of the A(H1N1)pdm09 virus in local dogs may have led to more efficient risk factors for serological assay outcomes. The bivariate χ² test of independence or Fisher’s exact test was used to examine the association between the demographic characteristics and the serological outcomes. Covariates that had a P value of <0.25 in the bivariate analyses were entered into a multivariable logistic regression model. Forcing age into the model, we performed backward elimination of the covariates, keeping the covariates in the model which had a P value of <0.05. The final covariates were tested for goodness of fit.

From February through July 2012, we obtained samples from 960 dogs (240 dogs in each of the 4 cities). The median age of the dogs was 4 years (range, 1 to 11 years), and 57.4% were male. Most (68.8%) of them were raised as pets. There was no statistically significant difference in the average age or sex between pet and farm-raised dogs. Overall, 24.7% (n = 237) of the 960 dog serum samples had elevated antibodies against influenza A(H1N1)pdm09 by either the HI or the MN test. Comparing the four cities and the two serological methods, we consistently identified a higher prevalence of dogs with elevated levels of antibody titer against A(H1N1)pdm09 virus in Guangzhou and Shenzhen (Table 1). A total of 92 (88%) of the 104 MN assay-positive samples were also HI assay positive. When considered as a binary outcome (elevated or not), the two tests had a moderate to high agreement (κ = 0.46; 96% confidence interval [CI], 0.39 to 0.53). The seroprevalence estimated by the HI assay was significantly higher than the estimate derived from the MN assay (24.7% versus 10.8%, respectively; P < 0.01). The dogs that were raised as pets were about twice as likely to have elevated antibodies against influenza A(H1N1)pdm09 among U.S. cats, and ~10% of the cats were noted to have signs of acute respiratory illness (19). Moreover, during a 2010 (H1N1)pdm09 outbreak in a cat colony in Italy, researchers identified higher seroprevalence (55%) of A(H1N1)pdm09 infection and a higher mortality rate (28%) (7, 18). Our findings highlight the limitations of relying upon specimens from dogs with clinical signs of influenza infection for customary studies of A(H1N1)pdm09 in dogs. The relatively higher seroprevalence identified in our study compared to that in the study in Italy may be explained by several factors. The dogs in southern China might have been at a higher risk of infection due to their exposure to dense populations of humans with high influenza A(H1N1)pdm09 attack rates (20). 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 to be particularly high (21). The difference in seroprevalences might also be explained by the 2-year temporal difference between the dogs sampled in Italy and the dogs sampled in China (6). Finally, dog farming is a unique practice in the Guangdong Province, where many dogs are in close contact. This promotes the rapid transmission of infectious agents within the dog farm. Although transmission experiments have shown that human A(H1N1)pdm09 virus can infect dogs, the transmission was thought to be inefficient among dogs (9). However, our findings can be considered evidence that long-term adaptation of the A(H1N1)pdm09 virus in local dogs may have led to more efficient
transmission among the 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 (22, 23). We hypothesize that there was a relatively high A(H1N1)pdm09 transmission rate 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 had previous infection with A(H1N1)pdm09 than were farm dogs.

Our study had several limitations. We collected sera only during a 6-month period in 2012, so our study may not be representative of other time periods. Although a previous report found little evidence for cross-reactivity between antibodies against the canine H3N2 and human H3N2 viruses (24), as we did not test the dog sera against human H3N2, we cannot rule out that activity against the canine H3N2 virus is confounded by antibodies against the human H3N2 virus. We also note that there have been reports of human H3N2 virus infection among dogs in China and Japan (24).

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

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REFERENCES


