Detection of Antibodies against Avian Influenza Virus Subtypes H7 and H9 among Veterinarians in Guangdong Province, China

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Zoonotic transmission of pathogenic avian influenza virus (AIV) is a potential public health threat (1, 2), as the virus may acquire human-to-human transmissibility through mutations or by reassortment with seasonal influenza viruses (e.g., H3N2 and H1N1). The recent outbreak of novel H7N9 virus in China is an enigma (3). In contrast to outbreaks of human infections by other avian influenza viruses such as H5N1 (4) or H7N7 (5), this zoonosis by H7N9 was apparently not preceded by overt epizootics in domestic poultry or other avian species in the wild, and the source of H7N9 virus has not been definitively established.

Epidemiological and serological evidences have indicated that poultry farm and live market workers are high-risk groups (6–9). Due to the nature of their work environment and practices, veterinarians are also at high risk of exposure to avian influenza virus. An epizootic of avian influenza virus H7N7 in The Netherlands resulted in the death of a veterinarian (5). Detection of antibodies against subtypes H5, H6, and H7 have been reported in a seroprevalence survey of veterinarians in the United States (10).

Both industrial-scale production and backyard rearing of poultry are present in Guangdong Province, which ranks as the largest province for poultry production in China. To assess the risk of avian influenza virus infection for local veterinarians, we collected single serum samples anonymously from practicing veterinarians (n = 406; 144 from Guangzhou, 86 from Shenzhen, 99 from Fo Shan, and 77 from Hui Zhou) from May 2011 to April 2012. Their ages ranged from 20 to 65 years, and 90% are male. A total of 83 serum samples were collected from healthy individuals as unexposed controls. Collection procedures were performed as previously described (11) and with institutional review board (IRB) approval and individual consent. (This study protocol was reviewed and approved by the Institutional Review Board of the Guangdong Centers for Disease Control and Prevention.) The serum samples were identified only by their group, i.e., veterinarian or control. Hemagglutination inhibition (HI) assay was carried out as previously described (12). Briefly, serum samples were treated with receptor-destroying enzyme and preabsorbed with horse erythrocytes to remove nonspecific inhibitors. The virus antigens used in this study, low-pathogenicity avian influenza viruses (LPAIs) A/duck/Guangdong/1/1996 (H7N3) and A/chicken/Guangdong/V/2008 (H9N2), were isolated by us at the College of Veterinary Medicine (13). Allantoic fluids containing the viruses were clarified and partially purified by centrifugation (700 × g for 15 min) and then diluted to 4 hemagglutinating units (HAU) per 25 μl. Fifty microliters of 1% horse erythrocyte solution was added to the serum-antigen mix for HI titration.

The results shown in Table 1 are mean HI titers of three independent assays. There are two significant observations. First, HI antibodies against H7 and H9 were detected in serum samples from the veterinarian group only. Although the cutoff titers have not been established for H7 and H9, applying a conservative cutoff at 1:80, we determined positivity rates of 1.48% and 3.69%, respectively. None of the samples were positive for H7 AIV infection by HI assays using a ≥1:160 cutoff antibody titer. Second, the detection rate for H9 was significantly higher than that for H7. Interestingly, none of the positive samples had dual reactivity toward H7 and H9. Like similar samples in previous reports, these serum samples were nonreactive toward H5N1 (11).

Highly pathogenic avian influenza virus (HPAI) H5N1 and LPAI H9N2 have been established as enzootic viruses in China and other parts of the world (14, 15). As H9N2 is currently the most prevalent avian influenza virus in China (15), detection of HI antibodies against H9 in veterinarians is not unexpected. This result parallels the detection of antibodies against H9 in poultry workers in Northern China (16). In contrast, H7N3 is only occasionally isolated in China and is mainly confined to ducks (17).

<table>
<thead>
<tr>
<th>Virus antigen</th>
<th>Dilution</th>
<th>No. (% [95% CI]) of serum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>H7N3</td>
<td>&lt;1:20</td>
<td>348 [81]</td>
</tr>
<tr>
<td></td>
<td>1:20</td>
<td>36 [2]</td>
</tr>
<tr>
<td></td>
<td>1:40</td>
<td>16 [0]</td>
</tr>
<tr>
<td></td>
<td>1:80</td>
<td>6 [0]</td>
</tr>
<tr>
<td></td>
<td>1:160</td>
<td>0 [0]</td>
</tr>
<tr>
<td></td>
<td>≥1:80</td>
<td>6 [0]</td>
</tr>
<tr>
<td>H9N2</td>
<td>&lt;1:20</td>
<td>293 [79]</td>
</tr>
<tr>
<td></td>
<td>1:20</td>
<td>66 [4]</td>
</tr>
<tr>
<td></td>
<td>1:40</td>
<td>32 [0]</td>
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<tr>
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<td>1:80</td>
<td>9 [0]</td>
</tr>
<tr>
<td></td>
<td>1:160</td>
<td>0 [0]</td>
</tr>
<tr>
<td></td>
<td>≥1:160</td>
<td>6 [0]</td>
</tr>
</tbody>
</table>

*P < 0.05 (two-tailed t test).

NA, not applicable.

TABLE 1 Distribution of hemagglutination inhibition titer

Published ahead of print 18 September 2013

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Interestingly, Jia et al. and Hai-bo et al., while using a more contemporary H7 virus as an antigen, did not detect seroconversion (16, 17), perhaps because they set 1:160 as their cutoff. Nevertheless, in our study, the 1.48% positivity rate may be an underestimate, as we used a virus isolated in 1996 as an antigen (hence, a somewhat antigenic distant virus with a difference of more than 15 years between virus isolation and serum sample collection). Other possibilities to explain our discrepancy include differences in the nature of exposure of poultry workers and veterinarians, e.g., veterinarians have greater exposure to morbid animals, and the difference in the intrinsic properties of the viruses circulating in their respective locations.

The positivity rate for H9 being higher than that for H7 is interesting. Whether it is a result of more-extensive circulation of H9N2 in local poultry or this virus is more easily transmitted to humans (or a combination of both) remains to be determined. The absence of antibodies against H5 but positivity for H7 and H9 needs further investigation. Furthermore, to overcome the limitation on interpretation of single serum samples, a prospective study collecting sequential serum samples is in progress.

Prior to the recent zoonosis of H7N9, there were very few studies on the seroprevalence of the H7 subtype. Although none of the samples were positive for H7 AIV infection in this study, since our serum samples were collected within the last 2 years, this seroprevalence study may provide useful information regarding the emergence of the zoonotic H7N9 virus. In addition, more cross-species transmission of avian influenza virus to other mammalian species has recently been reported, with species including pigs (18) and pet animals, such as dogs (19) and cats (20).

No competing interests are declared.

REFERENCES


