Virological and Epidemiological Evidence of Avian Influenza Virus Infections Among Feral Dogs in Live Poultry Markets, China: A Threat to Human Health?

To the Editor— Since its first detection in March 2013, the novel H7N9 avian influenza virus (AIV) has quickly spread among poultry and people in China. As of 16 February 2014, a total of 348 laboratory-confirmed human H7N9 infections in China have been confirmed by the World Health Organization [1–3]. The H7N9 virus has spread widely with little sign of infection among poultry [4]. Epidemiologic studies have identified poultry exposure as an important risk factor for human infections with H5N1 and H7N9, especially for those individuals associated with live poultry markets (LPMs) [5–8]. As dogs in China have been shown to be infected with AIVs, we sought to investigate whether dogs living in close proximity to LPMs and H7N9-affected farms might have been infected with the novel H7N9 virus or other influenza viruses.

From August 2011 to August 2013, we studied a total of 2357 dogs that lived in close proximity to LPMs and poultry farms in the rural areas of Shanghai, Guangdong, Zhejiang, and Jiangsu provinces in China where novel H7N9 AIV had been previously detected (for Materials and Methods, see Supplementary Data).

Overall, 68.18% (n = 1607) of the 2357 stray dog samples were collected in rural areas, with the remaining samples collected in LPMs (Table 1). Of the 2357 nasal swab samples collected, 93 (3.9%) were positive for influenza A virus by real-time reverse transcription polymerase chain reaction (PCR), and 11 viruses were isolated from these samples (see Supplementary Data). Hemagglutination inhibition (HI) assays and hemagglutinin antigen–specific enzyme-linked immunosorbent assays against H7N9 viral antigens revealed no evidence of H7N9 infection. Results of the HI and microneutralization (MN) assays are reported in Table 1 and in the Supplementary Data. A total of 19 serum samples had HI antibody titers of ≥1:20 against H5 antigen (Table 1), and 3 of these 19 samples were also positive by MN assay. Dogs that were sampled in LPMs had a greater probability of having elevated HI antibodies against avian H9N2, avian H5N1, and canine H3N2 viruses (Table 2), compared with the dogs that were raised in poultry farms.

Our study supports this premise in that, although we failed to find evidence of previous H7N9 infections among the dogs, we found the world's first evidence of previous H5N1 and H9N2 infection among dogs by real-time PCR, HI, and MN assay. These findings were unexpected but biologically plausible. In LPMs and farms in rural China, stray dogs and cats have considerable contact with poultry or poultry products. This can occur indirectly through aerosol and fecal transmission or directly through the consumption of dead bird carcasses or entrails. LPMs are particularly problematic as they offer a mixing of animal species from often diverse geographical areas, frequent venues for contact with the public, and often nonhygienic behavior of workers who handle and process the birds for sale. Both rural farms and LPMs provide opportunities for wild aquatic birds, domestic poultry, stray dogs, and humans to closely interact and potentially share pathogens (Supplementary Figure 1). Additionally, compared

Table 1. Characteristics and Influenza Serological Assay Results of Feral Dogs Sampled With Nasal Swabs and Serum Specimen Collections, August 2011–August 2013, by Sampling Site

					No. (%) With Serological Evidence of Avian H9N2 Infection		No. (%) With Serological Evidence of Canine H3N2 Infection		No. (%) With Serological Evidence of Avian H5N1 Infection	
Location	Site Type	No. Sampled	No. (%) Female	No. (%) With ILI	HI Assay ≥1:20	MN Assay ≥1:80	HI Assay ≥1:20	MN Assay ≥1:80	HI Assay ≥1:20	MN Assay ≥1:80
Shanghai	Rural farm	266	88 (33.0)	39 (14.7)	4 (1.5)	0 (0.0)	62 (23.3)	21 (7.9)	2 (0.7)	0 (0.0)
Shanghai	LPM	224	69 (30.8)	22 (9.8)	10 (4.5)	1 (0.4)	74 (33.0)	30 (13.4)	4 (1.8)	0 (0.0)
Guangdong	Rural farm	401	169 (42.1)	75 (18.7)	10 (2.5)	2 (0.5)	107 (26.7)	56 (14.0)	2 (0.5)	0 (0.0)
Guangdong	LPM	99	52 (52.5)	17 (17.2)	7 (7.0)	1 (1.0)	31 (31.3)	12 (12.1)	2 (2.0)	2 (2.0)
Jiangsu	Rural farm	562	299 (53.2)	166 (29.5)	10 (1.8)	0 (0.0)	91 (16.2)	29 (5.2)	1 (0.2)	0 (0.0)
Jiangsu	LPM	158	33 (20.9)	55 (34.8)	8 (5.1)	1 (0.2)	35 (22.2)	8 (5.0)	3 (1.9)	1 (0.6)
Zhejiang	Rural farm	478	186 (38.9)	134 (28.0)	11 (2.3)	1 (0.2)	112 (23.4)	48 (10.0)	2 (0.4)	0 (0.0)
Zhejiang	LPM	169	45 (26.6)	31 (18.3)	9 (5.3)	1 (0.5)	33 (19.5)	9 (5.3)	3 (1.8)	0 (0.0)

Abbreviations: H3N2, A/canine/Guangdong/2/2011(H3N2); H5N1, A/chicken/Guangdong/178/04(H5N1); H9N2, A/chicken/Guangdong/V/2008(H9N2); HI, horse red blood cell hemagglutination inhibition assay; ILI, influenza-like-illness; LPM, live poultry market; MN, microneutralization assay.

with pets, feral dogs or cats living in LPMs or rural farms would logically have more potential to come into contact with sick or dead avian species. Moreover, feral dogs and cats may be immunocompromised due to poor diet, harsh environmental conditions, open wounds, etc, which may make them more susceptible to cross-species pathogen transmission. Hence, such feral animals may increase the risk of the emergence and transmission of novel influenza A viruses and serve as a threat to both veterinary health and human public health. These data support the importance of periodically conducting influenza A surveillance among feral dogs in China. As humans live in very close contact with dogs in many areas of the world, we posit that surveillance for novel viruses among feral dogs living in close proximity to LPMs or poultry farms could serve as an early warning system of viral threats to humans.

Table 2. Risk Factors for Elevated Antibody Against Influenza A Viruses by Horse Red Blood Cell Hemagglutination Inhibition Assay (Titer ≥1:20) Among Feral Dogs Sampled in Shanghai, Guangdong, Zhejiang, and Jiangsu Provinces, China

	A/Chicken/Guangdong/V/2008(H9N2)		A/Canine/Guangdong/2/2011 (H3N2)		A/Chicken/Guangdong/178/2004(H5N1)	
Risk Factor	No. (%)	OR (95% CI)	No. (%)	OR (95% CI)	No. (%)	OR (95% CI)
Location						
Shanghai	14 (2.9)	0.9 (.5–1.9)	136 (27.8)	1.3 (1.0–1.8)	6 (1.2)	1.6 (.5–5.2)
Guangdong	17 (3.4)	1.1 (.6–2.1)	138 (27.6)	1.3 (1.0–1.7)	4 (0.8)	1.0 (.3–3.9)
Jiangsu	18 (2.5)	0.8 (.4–1.5)	126 (17.5)	0.7 (.6–1.0)	4 (0.6)	0.7 (.2–2.7)
Zhejiang	20 (3.1)	Reference	145 (22.4)	Reference	5 (0.8)	Reference
Farm type						
Live poultry market	34 (5.2)	2.6 (1.6–4.3)	173 (26.6)	1.3 (1.1–1.6)	12 (1.8)	4.6 (1.8–11.7)
Farm	35 (2.1)	Reference	372 (21.8)	Reference	7 (0.4)	Reference
Sex						
Male	36 (2.5)	0.7 (.4–1.2)	305 (21.5)	0.8 (.7–1.0)	11 (0.8)	0.9 (.4–2.3)
Female	33 (3.5)	Reference	240 (25.5)	Reference	8 (0.9)	Reference
Influenza-like illness						
Yes	30 (5.6)	2.7 (1.6–4.3)	207 (38.4)	2.7 (2.2–3.4)	10 (1.9)	3.8 (1.5–9.4)
No	39 (2.1)	Reference	338 (18.6)	Reference	9 (0.5)	Reference

Dogs were studied during the period August 2011 to August 2013.

Abbreviations: CI, confidence interval; OR, odds ratio.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Shuo Su,[1,a] Pei Zhou,[1,a] Xinliang Fu,[1,a] Lifang Wang,[1] Malin Hong,[1] Gang Lu,[1] Lingshuang Sun,[1] Wenbao Qi,[1] Zhangyong Ning,[1] Kun Jia,[1] Ziguo Yuan,[1] Heng Wang,[1] Changwen Ke,[3] Jie Wu,[3] Guihong Zhang,[1] Gregory C. Gray,[2] and Shoujun Li[1]

[1]College of Veterinary Medicine, South China Agricultural University, Guangzhou, People's Republic of China; [2]Department of Environmental and Global Health, College of Public Health and Health Professions, and Emerging Pathogens Institute, University of Florida, Gainesville; and [3]Guangdong Centers for Disease Control and Prevention, Guangzhou, People's Republic of China

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[a]S. S., P. Z., and X. F. contributed equally to this work.
Correspondence: Shoujun Li, PhD, Infectious Disease Study, South China Agricultural University, 483 Wushan St, Guangzhou 510642, China (shoujunli@scau.edu.cn).

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