

Serologic Evidence of Pandemic Influenza Virus H1N1 2009 Infection in Cats in China

Shuo Su, Liguo Yuan, Huatao Li, Jidang Chen, Jiexiong Xie, Zhen Huang, Kun Jia, Shoujun Li

College of Veterinary Medicine, South China Agricultural University, Guangzhou, Guangdong Province, People's Republic of China

Infection of domestic cats with (H1N1) pandemic 2009 (pdm09) influenza A virus has recently been documented. In this paper, we report for the first time the sporadically current seroprevalence of (H1N1) pdm09 influenza A virus infection in cats in China. Thirteen of 1,080 sera were found positive by nucleoprotein (NP)-specific enzyme-linked immunosorbent assays (ELISAs) in different cat populations in southern China. It is very important to stress further surveillance of pandemic (H1N1) 2009 influenza A virus in cats in southern China.

The (H1N1) pandemic 2009 (pdm09) human influenza A virus has infected not only humans; there has also been interspecies transmission from infected humans to other animals, including pigs, poultry, dogs, ferrets, and so on (1, 2). Until recently, it was widely acknowledged that cats are rarely susceptible to natural infection with influenza A viruses; reports about cats with circulating influenza virus antibodies are even more rare and occur in unusual epidemiologic situations (3). However, since the recent upsurge of infections by circulating subtype H1N1 and H5N1 influenza A viruses, cases of natural infection in cats have apparently increased (4). Thus, the role of cats in the transmission and spread of influenza viruses is being reconsidered. Recent reports have shown that cats can also be infected with (H1N1) pdm09 human influenza A virus (5, 6). Until now, no such serological or etiological studies about pandemic (H1N1) 2009 influenza A virus infections have been carried out in cats in China. We initiated this serologic study to determine whether evidence of pandemic (H1N1) 2009 influenza A virus transmission could be found in cats in China.

A total of 1,080 feline blood samples were collected from 40 different pet hospitals and animal shelters in Guangdong Province and Guangxi Province, southern China, between February and December 2011. Of these blood samples, 680 were collected from pet cats in pet hospitals and 400 were collected from free-roaming cats in animal shelters. In addition, 415 serum samples were collected from pet dogs and pet cats in Guangdong Province in 2006. These sera were separated by centrifugation at 3,000 rpm for 15 min. The serum samples were transferred to new Eppendorf tubes and stored at -20°C until they were tested for antibodies against

influenza A virus. All samples were tested by hemagglutination inhibition (HI) assays and by nucleoprotein (NP)-specific enzyme-linked immunosorbent assays (ELISAs), according to the manufacturer's instructions as previously reported (7). Previous findings suggest that the NP-based ELISA method is the better tool for the serological diagnosis of influenza virus infections in dogs and cats (3). The 1,495 serum samples described above were analyzed for virus-specific antibodies by using ELISAs (Animal Genetics Inc., South Korea) that can detect antinucleoprotein (NP) antibodies based on competition principles. HI titers ≥ 40 are considered to represent seropositivity and to indicate previous infection. The (H1N1) pdm09 human influenza A viruses used in this study were A/Guangdong/1057/2010. The following antigens were also used for HI testing: H1N1 swine influenza virus (A/Swine/Guangdong/L6/2009/H1N1) for H1 and H3N2 swine influenza virus (A/Swine/Guangdong/01/2005) for H3. These viruses were isolated by the College of Veterinary Medicine, South China Agricultural University. The comparison of categorical variables between cat serum samples was

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Address correspondence to Shoujun Li, shoujunli@scau.edu.cn.

S.S. and L.Y. contributed equally to this article.

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TABLE 1 Survey structure in cats in southern China where the seroepidemiological study was conducted

Location (yr of data collection) ^a	Total no. of cats	Survey result		No. of females (%)	Category (no. of cats)
		Age in yr (no. of cats)	Avg age (yr)		
Guangdong (2011)	668	≤ 3 (430)	1.67	301 (45.6)	Pet cats (408) Free-roaming cats (236)
		> 3 (238)	6.95		
Guangxi (2011)	412	≤ 3 (309)	1.39	163 (39.5)	Pet cats (272) Free-roaming cats (164)
		> 3 (103)	8.11		
Guangdong (2006)	415			200 (48.2)	Pet cats (105) Pet dogs (300)

^a Survey sites in southern China where the seroepidemiological study was conducted.

TABLE 2 Seroprevalence of influenza A and pandemic H1N1 2009 virus in different cat populations in southern China

City	Free-roaming cats			Pet cats		
	No. examined	% virus seroprevalence (no. of infected cats/total no. of cats)		No. examined	% virus seroprevalence (no. of infected cats/total no. of cats)	
		ELISA	HI ^a		ELISA	HI ^a
Guangdong	200	2.0 (4/200)	0.5 (1/200)	340	1.17 (4/340)	0.29 (1/340)
Guangxi	200	1.5 (3/200)	1 (2/200)	340	0.58 (2/340)	0.58 (2/340)
Total	400	1.75 (7/400)	0.75 (3/400)	680	0.88 (6/680)	0.44 (3/680)

^a An HI titer ≥ 40 was considered to represent seropositivity and to indicate previous infection.

performed with a chi-square test where appropriate. Statistical significance was defined as $P < 0.05$. The data were analyzed with SPSS for Windows (Release 18.0, standard version; SPSS Inc., Chicago, IL).

A total of 1,495 serum samples were examined by ELISA and HI for pandemic (H1N1) 2009 antibodies (Table 1). The serological screening revealed that 7 sera (1.75%) and 6 sera (0.88%) were found to be positive by NP-specific ELISA in samples from free-roaming cats and pet cats, respectively ($P = 0.251$). Results of the NP ELISA and HI tests are reported in Table 2. Screening by HI assay showed that 6 samples were positive (titers ≥ 40) for pandemic (H1N1) 2009 influenza A virus (Table 2); of those HI-positive samples, 4 had titers of 40 to 80 and only 2 had HI antibody titers ≥ 160 . Based on Table 1 and the age and the type of cat population, they were all not statistically associated with pandemic (H1N1) 2009 influenza A virus infection. All samples were also tested with other antigens (H1 and H3) by the HI test, and they were all negative. The investigation also showed that the seroprevalences in female animals were 0.43% (ELISA) and 0% (HI) and that the seroprevalences in male animals were 1.78% (ELISA) and 0.97% (HI) (see Table 3). Pandemic (H1N1) 2009 influenza A virus seropositivity was significantly ($P < 0.05$) associated with cats of different genders by HI assays ($P = 0.040$) and NP-specific ELISAs ($P = 0.043$). In addition, a total of 415 serum samples collected in 2006 had no HI and NP ELISA antibodies against A/Guangdong/1057/2010.

In this paper, we report for the first time the sporadically current seroprevalence of (H1N1) pdm09 influenza A virus infection in cats in China. A total of 13 of 1,080 sera were found positive by NP-specific ELISA in different cat populations in southern China, which is a rate significantly lower than that in America. The seroprevalence of pandemic (H1N1) 2009 influenza A virus infections in pet cats in America in 2009 was 21.8% (17/78) as determined by HI assays (5). The data thus suggest that transmission occurred, most probably by close contact, between pandemic (H1N1) 2009 influenza A virus-infected owners and their pets in recent years; human-to-cat transmission is the most likely route of infection. However, on the basis of the low number of positive specimens

TABLE 3 Seroprevalence of influenza A (H1N1) virus in cats of different genders in southern China determined using the ELISA and HI tests

Gender	% virus seroprevalence (no. of infected cats/total no. of cats)	
	ELISA	HI
Male	1.78 (11/616)	0.97 (6/616)
Female	0.43 (2/464)	0 (0/464)

identified in this study, it would be unrealistic to suggest that cats are particularly susceptible to pandemic (H1N1) 2009 influenza A virus infection. What the most likely route of free-roaming cats infection is, how long these cats were infected, whether the infection had clinical manifestations, and whether the cats were capable of transmitting the virus to humans are all issues that remain unresolved. In this study, both of the provinces of southern China participating in the investigation have been confirmed with seropositivity for pandemic (H1N1) 2009 influenza A virus; in the past several decades, many influenza epidemics and pandemic strains were reported to have emerged from southern China. Both the 1957 and 1968 pandemic influenza viruses emerged from this area. This area is critical for emergence of novel influenza A viruses; as pandemic (H1N1) 2009 outbreaks among humans continue in the region, there is a continued risk for cat pandemic (H1N1) 2009 virus infections and for mutations or genetic reassortment leading to new virus strains with increased transmissibility to and among cats. The pet cats are in close contact with humans, and although no direct transmission of pandemic (H1N1) 2009 influenza A virus from cats to humans has been reported, it is possible. Therefore, cats in areas of influenza A (H1N1) pdm09 endemicity should be scrutinized (8). Thus, it is very important to stress further surveillance of pandemic (H1N1) 2009 influenza A virus infections in cats; in future studies, these results can be used to compare the results of seroepidemiological investigations among cats living in southern China that are infected by pandemic (H1N1) 2009 influenza A virus.

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REFERENCES

- American Veterinary Medical Association. 2010. NY dog infected with H1N1; study shows virus not in pork. American Veterinary Medical Association, Schaumburg, IL. <https://www.avma.org/News/JAVMANews/Pages/100201e.aspx>.
- Dundon WG, Benedictis PD, Viale E, Capua I. 2010. Serologic evidence of pandemic (H1N1) 2009 infection in dogs, Italy. *Emerg. Infect. Dis.* 16: 2019–2021.
- Paltrinieri S, Spagnolo V, Giordano A, Martin AM, Luppi A. 2007. Influenza virus type A serosurvey in cats. *Emerg. Infect. Dis.* 13:662–664.
- Butler D. 2006. Thai dogs carry bird-flu virus, but will they spread it? *Nature* 439:773.
- McCullers JA, Van De Velde LA, Schultz RD, Mitchell CG, Halford CR,

- Boyd KL, Stacey SC. 2011. Seroprevalence of seasonal and pandemic influenza A viruses in domestic cats. *Arch. Virol.* 156:117–120.
6. Sponseller BA, Strait E, Jergens A, Trujillo J, Harmon K, Koster L, Jenkins-Moore M, Killian M, Swenson S, Bender H, Waller K, Miles K, Pearce T, Yoon KJ, Nara P. 2010. Influenza A pandemic (H1N1) 2009 virus infection in domestic cats. *Emerg. Infect. Dis.* 16:534–537.
 7. An DJ, Jeoung HY, Jeong W, Chae S, Song DS, Oh JS, Park BK. 2010. A serological survey of canine respiratory coronavirus and canine influenza virus in Korean dogs. *J. Vet. Med. Sci.* 72:1217–1219.
 8. Lin D, Sun S, Du L, Ma J, Fan L, Pu J, Sun Y, Zhao J, Sun H, Liu J. 2012. Natural and experimental infection of dogs with pandemic H1N1/2009 influenza virus. *J. Gen. Virol.* 93:119–123.