



LETTER



SARS-CoV-2 Serological Survey of Cats in China before and after the Pandemic

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Dear Editor,

SARS-CoV-2 or COVID-19 has become pandemic and spread to more than 200 countries with over 24 million human infected cases (WHO 2020). The origin of SARS-CoV-2 remains unknown, though bat, pangolin, and snake were reported to be the potential animal reservoirs (Ji *et al.* 2020; Ward *et al.* 2020; Chen *et al.* 2020). Companion animals including dogs and cats were recent sporadically reported to be infected by SARS-CoV-2 with or without clinical symptoms (Li 2020a, b; Sailleau *et al.* 2020). Recently, both Chinese and American groups reported that cats were highly susceptible to SARS-CoV-2 after artificial inoculation and could spread the virus via respiratory droplets (Shi *et al.* 2020; Bosco-Lauth *et al.* 2020). Coincidentally, Zhang *et al.* (2020) reported that 15 of 102 (14.7%) cats in Wuhan City during SARS-CoV-2 outbreak showed serological positive using an in-house indirect enzyme linked immunosorbent assay (ELISA) (Zhang *et al.* 2020). These reports raise a huge public concern as the infected cats could play a role in transmission of SARS-

CoV-2. The high percentage of seropositivity of cats in Wuhan, the epicentre of the SARS-CoV-2 epidemic, could be due to the large number of infected human cases (more than 14 folds than that in other cities) (Table 1) where cats may have been more frequently exposed to SARS-CoV-2 patients or contaminated environment. As yet, SARS-CoV-2 serological prevalence of cats in other Chinese cities remains unknown. Therefore, a serological survey including more cities with numbers of SARS-CoV-2 human cases in China will be valuable for elucidating the role of cats in transmission of the viruses and relieve public concerns. In this study, 630 cat serum samples collected before November 2019 and 423 cat serum samples collected during SARS-CoV-2 outbreak (from February 2020 to April 2020) in 20 cities in China for detecting the prevalence of SARS-CoV-2 specific antibodies.

In a recent study, we conducted a survey of SARS-CoV-2 in 35 different species of animals, in which 87 cats including 66 pet cats and 21 stray cats were tested serological negative using a commercial ELISA Kit (Deng *et al.* 2020). There were no seropositive cat samples from Wuhan City in our study, which is totally different from the high serological prevalence of cats in Wuhan in Zhang's report. To further explore seropositivity among cats in other cities, 1053 cats were sampled before and after the SARS-CoV-2 outbreak from 20 cities of different provinces in China where the SARS-CoV-2 epidemic situations were classified into A, B, C, and D according to official reported numbers of human cases (Table 1). All cat serum samples were collected from pet hospitals. SARS-CoV-2 specific antibodies were detected using ELISA as previously described (Deng *et al.* 2020).

630 cat serum samples collected before the outbreak of SARS-CoV-2 were tested to be serologically negative, which is consistent with Zhang's report (Zhang *et al.* 2020). To our surprise, 423 cat serum samples (including 48 samples in Wuhan and 42 samples in other 3 cities of Hubei Province) collected during SARS-CoV-2 pandemic

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Table 1 Detailed information about the cat serum samples in different cities and ELISA results.

Class	Provinces (reported human cases)	City	Reported human cases	Cat numbers		ELISA results (ELISA ₄₅₀ readout) ^a
				Before Nov 2019	After Nov 2019	
A	Hubei (68128)	Wuhan	50333	0	48	— (0.005 to 0.195)
		Xiaogan	3518	0	16	— (0.007 to 0.124)
		Jingmen	928	0	20	— (0.005 to 0.132)
		Huanggang	2907	0	6	— (0.005 to 0.138)
B	Henan (1276)	ZhengZhou	157	23	9	— (0.006 to 0.085)
		Luoyang	31	92	26	— (0.009 to 0.125)
	Guangdong (1579)	Guangzhou	499	0	89	— (0.005 to 0.790)
		Shenzhen	460	0	4	— (0.005 to 0.013)
C	Heilongjiang (892)	Harbin	224	7	10	— (0.007 to 0.034)
	Beijing (593)	Beijing	593	145	37	— (0.005 to 0.101)
	Sichuan (561)	Chengdu	166	0	9	— (0.007 to 0.078)
	Chongqing (579)	Chongqing	579	17	10	— (0.005 to 0.081)
	Shandong (787)	Qingdao	65	5	5	— (0.005 to 0.023)
		Yantai	47	0	9	— (0.005 to 0.100)
	Anhui (991)	Hefei	174	0	6	— (0.007 to 0.039)
D	Tianjin (189)	Tianjin	189	249	33	— (0.005 to 0.144)
	Jilin (102)	Changchun	47	3	32	— (0.005 to 0.116)
	Hainan (168)	Haikou	39	89	15	— (0.005 to 0.097)
	Guizhou (147)	Guiyang	36	0	14	— (0.005 to 0.195)
	Yunnan (184)	Kunming	53	0	25	— (0.005 to 0.186)
Total	14	20	61045	630	423	—

The cutoff was set as 0.26+ the mean value of OD₄₅₀ of negative controls according to the manufacture's manual.

^aThe OD₄₅₀ readout of positive controls ranged from 0.582 to 0.722.

were also serologically negative to SARS-CoV-2 (Table 1). To confirm this result, a real-time PCR specific for SARS-CoV-2 recommended by Chinese Center for Disease Control and Prevention was also applied to all 1053 samples and the results were consistent with the serologic test (Deng *et al.* 2020). The discrepancy of results between the two research groups could be due to the source of cat samples since all of our samples were collected from pet hospitals. Besides, a SARS-CoV-2 double-antigen sandwich ELISA based on S1 protein of the virus was applied in our study. The potential cross-reaction with other coronavirus including infectious bronchitis virus (IBV), porcine epidemic diarrhea virus (PEDV), porcine transmissible gastroenteritis virus (TGEV), porcine delta-coronavirus (PDCoV), mouse hepatitis virus (MHV) and rat coronavirus (RCV) were tested with corresponding positive serum samples were evaluated in our study. The results showed that it has good specificity without cross-reaction with other coronaviruses from different animal species. By contrast, an indirect ELISA base on virus receptor-binding domain (BRD) protein were used in Zhang's study (Zhang *et al.* 2020). The indirect ELISA showed good specificity since it has no serological cross-

reactivity between the SARS-CoV-2 and type I or II feline infectious peritonitis virus (FIPV).

Both Chinese and American research groups individually performed the artificial inoculation of SARS-CoV-2 to cats of different ages and proved cats were susceptible to SARS-CoV-2 (Shi *et al.* 2020; Bosco-Lauth *et al.* 2020). In their study, no clinical symptoms were observed after infection for adult cats. By contrast, juvenile cats (70-day to 3-month old) died at 3 days post-inoculation with severe histological lesions in the nasal and tracheal mucosa epithelium and lungs. However, the experimentally infected cats only had viral replications in their upper airways but not in the lower airways or other organs even with a high dose of virus (10⁵ plaque-forming units) inoculation. In this study, no minimum infection dose was reported, which will provide valuable information to evaluate the virus load in natural environment where cats may get infected.

It is interesting to note that one Belgium cat was reported to be infected by her SARS-CoV-2 positive owner and showed hard breathing, vomiting, and diarrhea. Among the 15 SARS-CoV-2 serologically positive cats in Zhang's report, the owners of 3 cats coincidentally were confirmed to

be SARS-CoV-2 positive (Zhang *et al.* 2020). Besides pet cats, one tiger and five lions were reported to be infected with SARS-CoV-2 in the Bronx zoo, USA, and infection was assumed to be caused by a SARS-CoV-2 positive zoo employee (Brownlie and Sibley 2020). Collectively, the above results demonstrated that cats can be infected with SARS-CoV-2 only by close contact with patients who were actively shedding the virus or the contaminated environments where the virus titer was relatively high. However, the reverse transmission from cats to human in natural condition is not yet known. As for the other species of animals for passing SARS-CoV-2 to human was not reported except for the minks in Netherlands (Oreshkova *et al.* 2020).

The SARS-CoV-2 retrospective survey among cats is important to explain the role of cats play in the transmission of the virus, since cats could be infected by SARS-CoV-2 without clinical symptoms and have a narrow window for virus shedding. In this case, molecular techniques such as qRT-PCR may lead to false negative results. Under this circumstances, serological diagnosis result is more useful since the SARS-CoV-2 specific antibodies in cats could be detected during a long period of time after infection. The serologically negative result of 1053 cats from 20 cities in our study indicates that cats play a limited role in transmission during SARS-CoV-2 pandemic and as the social distancing animals the transmission of SARS-CoV-2 among cat populations could be minimum. However, this preliminary conclusion was made based on a relative limited number of cat serum samples. A large number of cat populations should be needed for further investigation to confirm the above conclusion.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal and Human Rights Statement This study was approved by the Animal Care and Ethics Committee of National Research Center for Veterinary Medicine with IACUC Number 2020001.

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