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Short Communication

Molecular and Serological Detection of *Toxoplasma gondii* in South China Tiger

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Key words
Toxoplasmosis, The South China tiger, PCR, ELISA

ABSTRACT

Toxoplasmosis is one of the most important zoonotic diseases with serious health risks for wild animals. The South China tiger, the most endangered tiger subspecies endemic to China, remain only a little more than 200 today, was susceptibly infected with *Toxoplasma gondii*. In this study, we used polymerase chain reaction to detect DNA, and enzyme-linked immunosorbent assay to test for *T. gondii* antibodies. Antibodies (S/P>0.31) to *T. gondii* were found in 20 (26.32%) of the 76 tigers, while all blood samples tested through nested PCR were negative. This is the first investigation of *T. gondii* infection in South China tiger.

Toxoplasmosis is one of the most common parasitic infections in humans and in domesticated and wild animals all over the world. This disease causes severe neurologic, ocular, and systemic diseases inneonates and those with weakened immune systems (Dubey and Beattie, 1988; Dubey and Su, 2009). Toxoplasma infection poses serious health concerns for wildlife. In the past, outbreaks were predominately sporadic and self-limited. Recently, however, major outbreaks of the disease have led to irretrievable losses in wildlife. Toxoplasmosis represents a potential risk to biodiversity, modifying the behavior and structure of animal populations while driving some species to complete extinction (Daszak *et al*., 2000; Williams *et al*., 2002). Toxoplasmosis is caused when *Toxoplasma gondii* parasitizes in cells, infecting various parts of the host's body and damaging tissues and organs. The condition eventually leads to a decline in the host's immune function and an increased prevalence.

The Felidae family plays a prominent role in the epidemiology of *T. gondii* infection because felids release millions of oocysts in a short period of time, often in one to two weeks through their feces. This causes pollution in soil, food, and water. *T. gondii* oocystsin feline feces contaminate surroundings and cause sporulation in terrestrial and aquatic environments.

There are many stray cats in the zoos in China. In addition, the living space of captive wild animals is limited, and there is more daily contact with each other, so captive wild animals in zoos are at high risk of becoming infected and spreading toxoplasmosis. Beijing, Shanghai, Chengdu, Fuzhou, and other Chinese cities have various levels of captive wild animals infected by Toxoplasma gondii. Even the Siberian tiger breeding base in Heilongjiang and the giant panda breeding base in Chengdu have been infected by *T. gondii* (Yang, 2020).

The South China tiger, the most endangered tiger subspecies endemic to China, has long been extinct in the wild. It has been classified as Critically Endangered (CR) by the World Conservation Union (IUCN). There remain only a little more than 200 of this species today, and all are in captivity. Tigers become can become infected by *T. gondii* through ingestion of bradyzoites in raw meat that contains tissue cysts, and oocysts on rare occasion. Infection can also occur by consuming food or water contaminated with oocysts shed by stray cats, or through vertical transmission of tachyzoites from mother to fetus.

With the rise of urban zoos, human populations and captive wildlife are interacting more frequently. Because it is difficult to recognize specific clinical symptoms or representative signs of zoonosis in wild animals, continuous surveys of the prevalence of toxoplasma infection in wild animals are important from a public health perspective (Cutler *et al*., 2010). On the other hand, little is known

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about diseases in the South China tiger, and strategies are lacking that control and prevent infectious diseases in this critically endangered species (Daszak *et al*., 2000; King *et al*., 2004). This short communication describes a study to evaluate toxoplasma infection in captive South China tigers in China.

Materials and methods

Seventy blood samples of captive South China tigers were collected from nine zoos (Shanghai, Hangzhou, Chengdu, Chongqing, Guangzhou, Meihuashan, Linyi, Suzhou, Nanchang) in China (Fig. 1). Sera were separated by centrifugation at 2,000 g for 5 min. The sera were stored at -20°C for toxoplasma antibodies detection, and the blood samples were processed for genomic DNA extraction using a Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), according to the manufacturer's instructions.

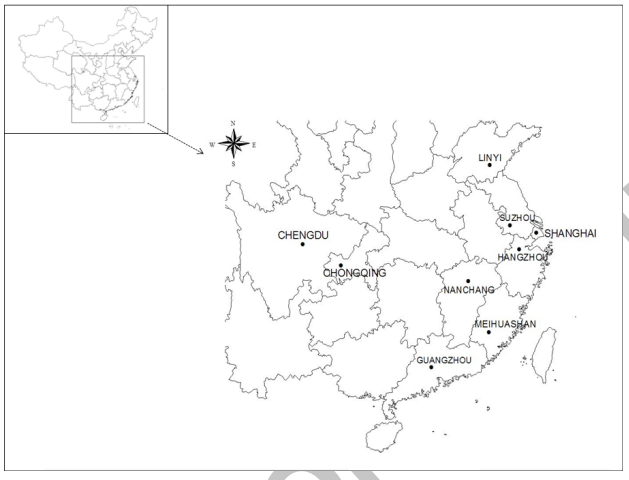

Fig. 1. Map of surveyed cities in China, where the blood samples of tigers were collected. The black dots indicate the sample collection areas.

T. gondii was detected by Nested PCR targeting B1 DNA around the *XhoI* and *Pm*lI restriction sites, according to a previously described method (Grigg and Boothroyd, 2001). PCR primers used for amplification are as follows: Pml/S1, 59-TGTTCTGTCCTATCGCAACG (positions 128 to147); Pml/S2, 59-TCTTCCCAGACGTGGATTTC (positions 152 to 171); Pml/AS1, 59-ACGGATGCAGT-TCCTTTCTG (positions 688 to 707); and Pml/AS2, 59-CTCGACAATACGCTGCTTGA (positions 663 to 682), yielding a 504bp product. PCR was carried out in the final volume of 20μL containing 10μLPCR mix (2×) (Takara Dalian, China), 0.4 μL of each forward and reverse primer (10μmol/L), 2μL DNA (200 ng/mL) and 7.2 μL DNase/RNase-free water. The amplified PCR products were separated by electrophoresis in 1.5% agarose gels.

Serum antibodies against *T. gondii* were screened using the EVL Toxo test ELISA kit (European Veterinary Laboratory, Woerden, The Netherlands), according to the manufacturer's recommendations. The serum samples and controls were diluted to 1:500 and tested in duplicate. The optical density (OD) was measured at 450 nm with an ELISA plate reader (Thermo Fisher, Waltham, MA, USA). The S/P (samples/positive control) ratio for each sample was calculated according to the formula: S/P = ($OD_{450}$of the sample - OD_{450} of negative control)/ (OD_{450}ofpositive control - OD_{450} of negative control). Samples with S/P ratio lower than 0.31 were considered negative for *T. gondii* antibodies. If the S/P ratios were greater than or equal to 0.31, the samples were considered positive.

The prevalence of *T. gondii* infection in South China tigers of different sexes, ages, regions, and sampling times was analyzed using the Chi Square Test in SPSS (version 18.0, SPSS Inc. Chicago, IL, USA). A probability (p) value of< 0.05 was considered statistically significant.

Results and discussion

The present study is the first epidemiological investigation of *T. gondii* infection in South China tiger in China. Antibodies (S/P>0.31) to *T. gondii* were found in 20(26.32%) of the 76 tigers, sampled from all nine sampling regions: Shanghai, Hangzhou, Chengdu, Chongqing, Guangzhou, Meihuashan, Nanchang, Suzhou and Linyi. The seropositive rates in Chongqing (66.7%) and Chengdu (50.0%) were significantly higher than those in the other sampling regions. The difference in the frequency of S/P ratio on *T. gondii* infection in male (X =0.26, SD=0.16) and female (X =0.22, SD=0.13) tigers was not significant. The antibody-positive rate of sub-adult tigers (80.0%) was higher compared with young (16.0%) and adult tigers (26.1%). Sampling time had an impact on the results the positive rate of serum samples was much higher in October and December than in other months (January, February, April, July, November). Variance in test results on the basis of region, age, or time of the sample for the detected parasite showed significant differences. Epidemiological survey results for the detection of *T. gondii* are available in Table I.

The prevalence of *T. gondii* in the Chongqing and Chengdu tigers was noticeably higher compared to tigers in other places in China. This may be related to sporulation and survival of coccidial oocysts in the environment due to temperature and humidity.

We found that the offspring of antibody-positive female tigers were also antibody-positive. This is consistent with the conclusion of Sharma *et al*. (2019). *T. gondii* infection can transmit tachyzoites from mother to fetus through vertical transmission (Sharma *et al*., 2019).

Table I. General characteristics and prevalence of *Toxoplasma gondii*in South China tigers in Eastern China (n=76).

Variables	Antibody level					Seropositive	Nested PCR
	N	Mean±SD	95% CI		P value	% ELISA	n(%)
			Lower limit	Upper limit			
Region							
Shanghai	60	0.29±0.159	0.21	0.36	0.01	2(3.33)	0
Hangzhou	13	0.27±0.12	0.20	0.34		3(23.08)	0
Chengdu	4	0.29±0.16	0.03	0.544		2(50.00)	0
Chongqing	6	0.359±0.18	0.16	0.55		4(66.67)	0
Guangzhou	9	0.269±0.13	0.16	0.36		4(44.44)	0
Meihuashan	6	0.109±0.16	-0.07	0.27		1(16.67)	0
Linyi	2	0.12±0.00	0.11	0.13		0(0)	0
Suzhou	1	-0.04±\	\	\		0(0)	0
Nanchang	15	0.19±0.09	0.13	0.24		1(6.67)	0
Gender							
Male	40	0.26±0.16	0.21	0.32	0.19	13(32.50)	0
Female	36	0.22±0.13	0.18	0.26		7(19.44)	0
Age							
≤1 year	25	0.20±0.18	0.13	0.28	0.02	4(16.00)	0
1-3 year	5	0.41±0.10	0.28	0.54		4(80.00)	0
>3 year	46	0.24±0.13	0.20	0.28		12(26.09)	0
Time							
January	6	0.10±0.16	-0.07	0.28	0.01	1(16.67)	0
February	2	0.12±0.00	0.11	0.14		0(0)	0
April	1	0.21±\	\	\		0(0)	0
July	6	0.15±0.12	0.02	0.28		0(0)	0
October	19	0.30±0.13	0.24	0.37		6(31.58)	0
November	25	0.22±0.12	0.17	0.27		4(16.00)	0
December	17	0.31±0.15	0.23	0.39		9(52.94)	0
Total	76	0.24±0.15	0.21	0.28		20(26.32)	0

T. gondii can infect a variety of mammals. Though felines are the primary hosts for the parasite, other animals feed on the egg sacs of felines and become infected (Dubey, 2008; Munhoz *et al*., 2017). At present, captive African lions (*Panthera leo*), Lynxes (*Lynx canadensis*), and Siberian tigers (*Panthera tigris altaica*) are susceptible to *T. gondii* (Chen *et al*., 2014). Evidence of infection mostly discovered through serological detection has been found for 31 of the world's 39 felid species (Dubey, 2016).

It is necessary to prevent and control the spread of *T. gondii* during daily feeding. First, we should control the number of stray cats, optimize the layout of zoos, and reduce contact between stray cats and South China tigers. Additional measures will include strengthening disinfection management, interrupting the transmission route of *T. gondii*, and ensuring the health and safety of infected South China tigers by quarantining them even after treatment, which in turn will guard the health of other wild animals in the zoo. Preventing cross infection and repeat infection in zoos is crucial in managing this dangerous condition.

In our study, all blood samples tested through nested PCR were negative for the presence of *T. gondii*. Lack of positive results from nested PCR in seropositive tigers was most likely related to *T. gondii* infection in tigers in the past. Moreover, the difference between the results may derivefrom the fact that *T. gondii* is temporarily present in blood, and it is therefore possible that it is not readily detected by nested PCR.

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Statement of conflict of interest

The authors have declared no conflicts of interest.

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