

## Prevalence of *Toxoplasma gondii* in Dogs in Zhanjiang, Southern China

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**Abstract:** Toxoplasmosis, caused by *Toxoplasma gondii*, is a parasitic zoonosis with worldwide distribution. The present study investigated the prevalence of *T. gondii* in dogs in Zhanjiang city, southern China, using both serological and molecular detection. A total of 364 serum samples and 432 liver tissue samples were collected from the slaughter house between December 2012 and January 2013 and were examined for *T. gondii* IgG antibody by ELISA and *T. gondii* DNA by semi-nested PCR based on B1 gene, respectively. The overall seroprevalence of *T. gondii* IgG antibody was 51.9%, and *T. gondii* DNA was detected in 37 of 432 (8.6%) liver tissue samples. These positive DNA samples were analyzed by PCR-RFLP at 3'- and 5'-SAG2. Only 8 samples gave the PCR-RFLP data, and they were all classified as type I, which may suggest that the *T. gondii* isolates from dogs in Zhanjiang city may represent type I or type I variant. This study revealed the high prevalence of *T. gondii* infection in dogs in Zhanjiang city, southern China. Integrated measures should be taken to prevent and control toxoplasmosis in dogs in this area for public health concern.

**Key words:** *Toxoplasma gondii*, dog, prevalence, genotyping, Zhanjiang (China)

*Toxoplasma gondii* is an obligate intracellular protozoan parasite that has a worldwide distribution and infects a wide range of warm-blooded vertebrates, including humans and dogs [1]. Domestic cats and other felids are the only known definitive hosts of this parasite since they can excrete oocysts into the environment. According to recent statistical data, *T. gondii* prevalence has been found in one third of the world population [2]. Humans get infected with *T. gondii* through ingesting undercooked meat containing tissue cysts or water or food contaminated with *T. gondii* oocysts, or by occasionally ingesting oocysts from the environment [3]. The dog, an intermediate host for *T. gondii*, is important in the epidemiology of this parasite because they can serve as sentinels of environmental contamination with oocysts and can be used to demonstrate the infection pressure to other hosts, including humans [4,5].

*T. gondii* isolates in North America and Europe present a

highly clonal population structure mainly consisted of 4 lineages, namely type I, II, III, and 12 [6-9], which have genetic and biological differences from that in South America [10-12]. Several studies have documented the genotype of *T. gondii* isolates from different animals in China [13-15], but information about genotyping of *T. gondii* isolates from dogs in China is limited. Therefore, the current study was conducted to determine the prevalence of *T. gondii* infection in dogs from Zhanjiang, southern China, and to study the genotypes of the *T. gondii* isolates.

A total of 364 blood samples and 432 liver tissue samples were collected from dogs in Zhanjiang city, southern China, between December 2012 and January 2013. Dogs were randomly selected from the dog farms. These dogs are farmed for meat and are mainly consumed by the local people. One blood sample or 1 liver tissue sample was collected from a dog. However, the blood samples did not correspond to the tissue samples by the number due to the constraint of field condition. When the dog was slaughtered, tissue samples were collected, and blood samples were drawn from jugular vein into a sterile, plain centrifuge tube. Then, the tissue samples were saved in the microtubes at 4°C. The blood samples were

•Received 2 June 2015, revised 17 July 2015, accepted 20 July 2015.

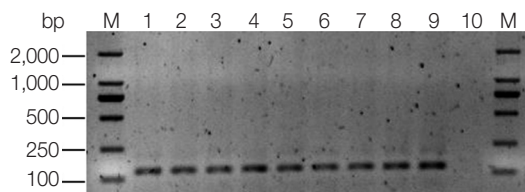
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left to clot at room temperature for 6 hr and centrifuged at 3,000 rpm for 10 min. The separated sera were stored at -20°C until needed for ELISA. This study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (permit code, LVRIAEC2012-007). Dogs were handled in strict accordance with the Animal Ethics Procedures and Guidelines of the People's Republic of China.

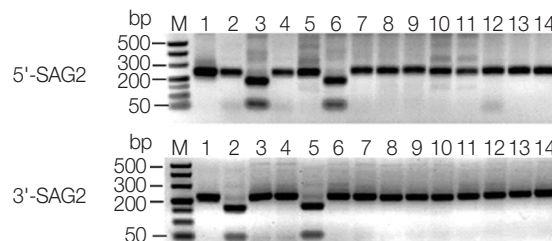
IgG antibodies to *T. gondii* were determined using a commercially available ELISA kit (Haitai, Zhuhai, Guangdong Province, China) according to the manufacturer's recommendations. A serum sample was considered positive when the value was 1.1 times higher than the mean value of positive control, negative control, and blank control. Genomic DNA was extracted from these liver tissues using TIANamp Genomic DNA kit (TianGen™, Beijing, China) according to manufacturer's recommendations and previous descriptions [15]. Then, a semi-nested PCR based on B1 gene was employed to detect the *T. gondii* DNA following the previously described method [16]. B1 gene positive DNA samples were submitted to nested PCR amplification of 3'- and 5'- SAG2 [17,18], followed by diges-



**Fig. 1.** Representative PCR products of *T. gondii* B1 gene by semi-nested PCR from liver tissue DNA samples of dogs. M represents a DNA marker. Lane 1 represents positive control. Lanes 2-9 represent positive amplification. Lane 10 represents negative control.

tion with restriction enzymes *Mbo*I and *Hha*I, respectively. The products were resolved in 2.5-3.0% agarose gel to display restriction fragment length polymorphisms (RFLP) using a gel document system (UVP GelDoc-It™ Imaging System, Cambridge, UK). Six *T. gondii* strains, namely GT, PTG, CTG, MAS, TgCatCa1, and TgCatBr5, were used as references.

Of the 364 serum samples of dogs in Zhanjiang city, southern China, 189 (51.9%) reacted positively. A previous study reported a seroprevalence of 70.9% in 175 stray dogs housed in shelters at Umuarama city, Brazil [19]. Alvarado-Esquivel et al. [20] reported a high seroprevalence of 67.3% in dogs in Veracruz, Mexico. The overall seroprevalence (51.9%) of *T. gondii* in dogs in Zhanjiang city was lower than that of Umuarama and Veracruz, but much higher than that observed in other parts of China, such as Guangzhou [21], Lanzhou [22], Kunming [23], and Shenyang [24]. High prevalence of *T. gondii* infection in dogs in Zhanjiang city reported here indicates a high environmental contamination with oocysts. Cats are the only definitive hosts of *T. gondii*, playing a significant role in the transmission of this parasite [1]. During the present investiga-



**Fig. 2.** PCR-RFLP analyses of *T. gondii* isolates from dogs in Zhanjiang, southern China based on 3'-SAG2 and 5'-SAG2. M represents a DNA marker. Lanes 1-14 represent GT1, PTG, CTG, MAS, TgCatCa1, TgCaBr5, TgDZJ1, TgDZJ2, TgDZJ3, TgDZJ4, TgDZJ5, TgDZJ6, TgDZJ7, and TgDZJ8, respectively.

**Table 1.** Summary of genotyping of *T. gondii* isolates from dogs in Zhanjiang city, southern China

Isolates ID	Host	Location	5'-SAG2	3'-SAG2	(5'+3') SAG2	Genotype
GT1	Goat	United States	I	I	I	Reference, Type I, ToxoDB #10
PTG	Sheep	United States	II	II	II	Reference, Type II, ToxoDB #1
CTG	Cat	United States	III	I	III	Reference, Type III, ToxoDB #2
MAS	Human	France	I	I	I	Reference, ToxoDB #17
TgCgCa1	Cougar	Canada	II	II	II	Reference, ToxoDB #66
TgCatBr5	Cat	Brazil	III	I	III	Reference, ToxoDB #19
TgDZJ1-8	Dog	Zhanjiang, Guangdong	I	I	I	May be type I

tion, we observed that cats were present on the farm and had free access to the dogs and their feed. This may represent a major risk factor for *T. gondii* infection in the dog farm.

*T. gondii* DNA was detected in 37 of the 432 (8.6%) liver tissue samples by using semi-nested PCR targeting the B1 gene. The target fragment was about 130 bp in length (Fig. 1). Four of these positive PCR products were randomly selected and sent to sequencing. Sequence comparison and analysis revealed 100% homology with the published *T. gondii* B1 gene sequence (GenBank accession no. AF179871). Genotyping of positive DNA samples was performed by employing PCR-RFLP technique. Due to low DNA concentration, only 8 of 37 positive DNA samples gave the PCR-RFLP data on 3'- and 5'-SAG2, and they were identified as type I (Fig. 2). The results of genotyping of these isolates and 6 references are summarized in Table 1.

ELISA is among the most commonly used methods for investigation of IgG antibody. IgG antibodies usually appear within 1-2 weeks of acquisition with *T. gondii* infection, peak within 1-2 months, decline at various rates, and usually persist for life. Because of its high sensitivity and specificity, low cost, and ease of practice, ELISA is widely used for diagnosis of *T. gondii* infection. In the present study, seroprevalence of IgG antibodies (51.9%) does not keep in agreement with the prevalence of *T. gondii* DNA (8.6%). Actually, IgG antibody based seroprevalence mainly reflects that exposure of dogs to *T. gondii* infection in the investigated geographic area may be very common, while DNA detection reveals the presence of viable *T. gondii* in dogs and that they may be mostly acute infections. New detection methods such as IgG antibody avidity test and more studies are needed to explore the *T. gondii* infection details in dogs in Zhanjiang city. Anyway, what we could confirm in this study was that the dog farm is seriously contaminated with *T. gondii* oocysts. Urgent measures should be taken to prevent infection from spreading.

Limited data about genotyping of *T. gondii* isolates from dogs in China is available. A previous study reported the genotype of *T. gondii* isolates from dogs in Henan province and considered it as a type I variant [25]. The present result shared the same type at the (3'+5') SAG2 loci with *T. gondii* isolates from dogs in Henan [25]. This may suggest that *T. gondii* isolates from dogs in Zhanjiang city may belong to type I or a type I variant. However, further studies of sampling more dog samples from wider geographical locations are needed to draw a valid conclusion.

The present survey showed that *T. gondii* prevalence in dogs in Zhanjiang city, southern China is high. The dog meat is consumed in this region by the local people, and *T. gondii* is considered as an important food-borne parasite. Thus, dogs can serve as a transport host for *T. gondii* to humans. Therefore, it is essential to implement integrated measures to prevent and control *T. gondii* infection in dogs. Moreover, it is urgent to improve the eating habit of the local people and implement *T. gondii*-inspection during dog slaughtering and processing.

## ACKNOWLEDGMENTS

The project support was provided in part by the National Natural Science Foundation of China (grant no. 31228022) and the Science Fund for Creative Research Groups of Gansu Province (grant no. 1210RJA006). Assoc. Prof. Chunlei Su at Department of Microbiology, the University of Tennessee, Knoxville, USA is thanked for providing reference *Toxoplasma gondii* DNA samples used in the present study.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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