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▣ BRIEF COMMUNICATION

# Prevalence and Genetic Characterization of *Toxoplasma gondii* in Pet Dogs in Central China

Wei-Feng Qian[1,*], Wen-Chao Yan[1], Tian-Qi Wang[1], Kai Zhai[1], Li-Fang Han[1], Chao-Chao Lv[2]

[1]College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471003, China; [2]PuLike Biological Engineering Co., Ltd, Luoyang 471000, China

**Abstract:** The prevalence and genotype of *Toxoplasma gondii* infection in dogs in Henan Province, Central China was investigated. A total of 125 blood samples were collected from pet dogs during April to June 2013, and all samples were examined by indirect hemagglutination antibody test (IHA) and nested PCR. The overall *T. gondii* prevalence in pet dogs was 24.0% (30/125), with 20.8% (26/125) in IHA and 10.4% (13/125) in PCR, respectively. No statistical associations were found between animal gender and age and the prevalence of *T. gondii* infection. Thirteen positive DNA samples were genotyped using 11 PCR-RFLP markers, including SAG1, (3'+5') SAG2, alt.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico. Of these, only 2 samples were genotyped with complete data for all loci, and a novel genotype (type III at SAG3 and GRA6 loci, and type I at other loci) was identified. This is the first report of genetic characterization of *T. gondii* infection in dogs in China.

Key words: *Toxoplasma gondii*, prevalence, genotype, dogs, China

*Toxoplasma gondii*, an obligate intracellular protozoan parasite, can infect all warm-blooded animals and humans. Humans and animals can acquire *T. gondii* infection through consuming food or water contaminated with *T. gondii* oocysts or ingesting uncooked meat harboring tissue cysts of *T. gondii* [1]. Commonly, primary infections in healthy humans and animals are asymptomatic. However, infections acquired during pregnancy in women and animals may cause abortion, fetal abnormalities, and prenatal death. Dogs, the most popular companion animal, however, have been associated as a potential risk factor for *T. gondii* infection in humans due to mechanical transmission of oocysts, although dogs do not shed *T. gondii* oocysts [2].

*T. gondii* has subpopulation structures in different geographical regions. Most *T. gondii* isolates from humans and animals in North America, Europe, and Africa have been grouped into 1 of 3 clonal lineages (type I, II, and III) [3]. The fourth clonal lineage (type 12) has been described and is the most common type in wildlife in North America [4]. In contrast, *T. gondii* isolates in South America are diverse [4]. In China, attention has been recently focused on genetic characterization of *T. gondii* isolates from domestic and wild animals, such as pigs, cats, chickens, birds, bats, and voles [5-21]; however, there is so far no genetic data on *T. gondii* from dogs in China. In China, previous serological surveys show that the prevalence of *T. gondii* infection in pet dogs was 10.0% in Shenyang [9], 10.8% in Lanzhou [10], 13.2% in Beijing [11], and 17.5% in Guangzhou [12]. In the present paper, we report the prevalence and genetic characterization of *T. gondii* isolates from pet dogs in Henan Province, Central China.

Venous blood samples were collected from 125 pet dogs, 48 from 1 pet hospital in Zhengzhou and 77 from 2 pet hospitals in Luoyang, 2 biggest cities in Henan Province, Central China during April to June 2013. These blood samples were centrifuged, sera and clotted blood were used for detection of antibodies and DNA of *T. gondii*, respectively. Pet dog owners were asked for details of the dog breeds, age, gender, living conditions, and medical history using a structured questionnaire.

Antibodies to *T. gondii* in sera were examined by indirect hemagglutination antibody test (IHA) with a commercially available kit (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science, China) according to the manufacturer's instructions. The serum sample was considered positive if a layer of agglutinated erythrocytes was observed in

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* Corresponding author (qwf2012@yeah.net)

wells with dilutions of 1:64 or higher. Positive and negative controls were included in each test.

Genomic DNA was extracted from clotted blood samples using the Universal Genomic DNA Kit (Beijing Zoman Biotechnology Co., Beijing, China) according to the manufacturer's recommendations. A nested PCR targeting the *T. gondii* B1 gene was performed to detect infection with *T. gondii* as described previously [13]. DNA samples giving positive B1 gene amplification were further genotyped using the PCR-RFLP method based on genetic markers SAG1, (3'+5') SAG2, alt. SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico as described previously [3].

Chi-square analysis was performed to assess the correlation between the prevalence of *T. gondii* and gender and age of animals using SPSS version 11.5 (Statistical Package for the Social Sciences) for Windows (SPSS Inc., Chicago, Illinois, USA).

The results showed that the overall *T. gondii* prevalence in pet dogs was 24.0% (30/125) (Table 1), with 20.8% (26/125) in IHA and 10.4% (13/125) in PCR, respectively. The prevalence was within the reported range of 3.2-30.9% in China [9]. The IHA titers were 1:64 in 5 dogs, 1:128 in 9, 1:256 in 8, 1:512 in 3, 1:1024 in 1, respectively. The prevalence of *T. gondii* was 17.6% (6/34), 25.8% (16/62), and 27.6% (8/29) in pet dogs of below 1-year-old, 1-3-year-old, and above 3-year-old, respectively, and 25.0% (18/72) and 22.6% (12/53) in females and males. These differences in *T. gondii* prevalence between the 2 age or gender groups were not significant (for age, $\chi^2 = 1.07$ and $P > 0.05$; for gender, $\chi^2 = 0.09$ and $P > 0.05$).

Due to low DNA concentration, only 2 B1 gene positive DNA samples (TgDogHN1 and 2) from pet dogs in Luoyang

**Table 1.** Prevalence of *Toxoplasma gondii* infection (examined by IHA and PCR) in pet dogs in Henan Province, Central China

| Variable | Category | No.examined | No. of positive samples | | Subtotal[a, b] | *P*-value |
|---|---|---|---|---|---|---|
| | | | IHA | PCR | | |
| Age (year) | <1 | 34 | 5 | 2 | 6 (17.6%) | >0.05 |
| | 1-3 | 62 | 14 | 8 | 16 (25.8%) | |
| | >3 | 29 | 7 | 3 | 8 (27.6%) | |
| Gender | Female | 72 | 17 | 9 | 18 (25.0%) | >0.05 |
| | Male | 53 | 9 | 4 | 12 (22.6%) | |
| Region | Zhengzhou | 48 | 8 | 4 | 9 (18.8%) | >0.05 |
| | Luoyang | 77 | 18 | 9 | 21 (27.3%) | |
| Subtotal[a] | | 125 | 26 (20.8%) | 13 (10.4%) | 30 (24.0%) | |

[a]No. positive samples among examined samples.
[b]No. of positive samples (positivity in percentage) detected by IHA or PCR.

**Table 2.** Multilocus genotyping of *Toxoplasma gondii* from pet dogs in Henan Province, Central China

| Isolate ID | Host | Location | SAG1 | 5'+3' SAG2 | alt. SAG2 | SAG3 | BTUB | GRA6 | C22-8 | C29-2 | L358 | PK1 | Apico | Genotype |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GT1, reference | Goat | USA | I | I | I | I | I | I | I | I | I | I | I | Type I, ToxoDB #10 |
| PTG, reference | Sheep | USA | II/III | II | II | II | II | II | II | II | II | II | II | Type II, ToxoDB #1 |
| CTG, reference | Cat | USA | II/III | III | III | III | III | III | III | III | III | III | III | Type III, ToxoDB #2 |
| MAS, reference | Human | France | u-1[a] | I | II | III | III | III | u-1[a] | I | I | III | I | ToxoDB #17 |
| Completely genotyped isolates | | | | | | | | | | | | | | |
| TgDogHN1, 2 | Dog | Luoyang, Henan | I | I | I | III | I | III | I | I | I | I | I | new genotype |
| Partially genotyped isolates | | | | | | | | | | | | | | |
| TgDogHN3 | Dog | Zhengzhou, Henan | I | nd[b] | nd | III | I | nd | nd | nd | nd | nd | nd | |
| TgDogHN4 | Dog | Zhengzhou, Henan | I | nd | nd | III | I | nd | nd | I | nd | nd | nd | |
| TgDogHN5 | Dog | Zhengzhou, Henan | I | I | nd | III | nd | III | nd | nd | I | nd | nd | |
| TgDogHN6 | Dog | Zhengzhou, Henan | I | nd | nd | nd | nd | III | nd | nd | nd | nd | I | |
| TgDogHN7 | Dog | Luoyang, Henan | I | I | I | III | nd | nd | nd | nd | nd | nd | nd | |
| TgDogHN8, 12 | Dog | Luoyang, Henan | I | I | nd | nd | nd | nd | nd | nd | nd | nd | nd | |
| TgDogHN10 | Dog | Luoyang, Henan | I | nd | nd | III | nd | III | nd | nd | nd | nd | nd | |
| TgDogHN9, 11, 13 | Dog | Luoyang, Henan | I | nd | nd | III | nd | nd | nd | nd | nd | nd | nd | |

[a]u-1 represents unique RFLP genotypes.
[b]nd represents no data.

gave complete genotyping results at all 11 gene loci, and 11 of the 13 positive samples were genotyped at 5 or less genetic loci (Table 2). A new genotype was identified, which did not match to the identified RFLP genotypes listed in the *Toxoplasma* Genomics Resource database ToxoDB (www.toxodb.org). This genotype had the type III alleles at both SAG3 and GRA6 loci, and type I alleles at all other 9 loci, thus, the genotype is considered a type I variant. Genotype ToxoDB#9, the predominant genotype in China, was not found in the present study.

The novel genotype (type I variant) in this study was most closely related to genotype ToxoDB#225 previously reported in chickens and cats [7,14] in China and another type I variant found in cattle in Jilin Province, China [15]. They all revealed type I at the SAG1, (3'+ 5') SAG2, alt.SAG2, BTUB, c22-8, c29-2, L358, PK1, and Apico loci. ToxoDB#225 revealed type III at the SAG3 locus and type I at the GRA6 locus; another type I variant revealed type I at the SAG3 locus and type III at the GRA6 locus; the novel genotype in this study revealed type III at both SAG3 and GRA6 loci, seemed to be a recombination of ToxoDB#225 and another type I variant.

So far, there are 16 genotypes of *T. gondii* found in China; genotype ToxoDB#9 and ToxoDB#10 (type I) are the most common [16]; ToxoDB#3 (type II variant) and other 13 genotypes are occasionally or rarely found in humans or animals [7,15,17-20]. Genotype ToxoDB#9 is the predominant lineage in mainland China, which circulates in cats, pigs, and humans [5-7]. Genotype ToxoDB#10, namely the clonal type I, has been found from several hosts, including pigs [5,17], humans [17,22], voles [20,21], Plateau pika [20], cats [22], and wild birds [23]. Combined with the previous reports, 4 type I variants have now been found in China, including ToxoDB#213 from pigs [24], ToxoDB#225 from chickens and cats [7,14], 1 from cattle [15], and a new genotype from pet dogs in the present study. This suggests that the clonal type I and type I variant are widespread in China, with a wide variety of hosts including avians besides mammals.

There is only 1 report on the *T. gondii* genotypes in Henan Province, with ToxoDB#9 and type I found in pigs in Zhengzhou and Nanyang cities [5]. These 2 genotypes were not detected from pet dogs in this study. A limitation of the present study is that the sample size is not large. To obtain more accurate information about the genetic characteristics of *T. gondii* in dogs, more samples from different regions should be included.

This is the first report on genotypes of *T. gondii* in dogs in China. These results provide new genetic information about *T. gondii* in dogs and have implications for better understanding of the genetic diversity of *T. gondii* in China. The observations are based on sampling of limited animal numbers and regions, thus, further studies on more samples collected from different regions are needed to understand the genetic diversity of *T. gondii* from dogs in China.

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## CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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