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▣ BRIEF COMMUNICATION

# Prevalence and Genetic Characterization of *Toxoplasma gondii* in House Sparrows (*Passer domesticus*) in Lanzhou, China

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**Abstract:** The prevalence of *Toxoplasma gondii* infection in birds has epidemiological significance because birds are indeed considered as a good indicator of environmental contamination by *T. gondii* oocysts. In this study, the prevalence of *T. gondii* in 313 house sparrows in Lanzhou, northwestern China was assayed by the modified agglutination test (MAT). Antibodies to *T. gondii* were positive in 39 (12.46%) of 313 samples (MAT titer ≥ 1:5). Tissues of heart, brain, and lung from the 39 seropositive house sparrows were tested for *T. gondii* DNA, 11 of which were found to be positive for the *T. gondii* B1 gene by PCR amplification. These positive DNA samples were typed at 9 genetic markers, including 8 nuclear loci, i.e., SAG1, 5´- and 3´-SAG2, alternative SAG2, SAG3, GRA6, L358, PK1, c22-8 and an apicoplast locus Apico. Of them, 4 isolates were genotyped with complete data for all loci, and 2 genotypes (Type II variants; ToxoDB #3 and a new genotype) were identified. These results showed that there is a potential risk for human infection with *T. gondii* in this region. To our knowledge, this is the first report of *T. gondii* seroprevalence in house sparrows in China.

Key words: *Toxoplasma gondii*, sparrow, seroprevalence, PCR-RFLP typing, northwestern China

*Toxoplasma gondii* is an important protozoan parasite that infects most species of warm-blooded animals worldwide, including humans and birds [1-3]. It has a complex life cycle in that sexual development occurs only in the intestine of felines, but asexual replication can occur extra-intestinally in many vertebrate hosts. Humans become infected postnatally via ingesting *T. gondii* tissue cysts from undercooked meat, consuming food or drinking water contaminated with *T. gondii* oocysts, or by accidentally ingesting oocysts from the environment [4]. Birds as intermediate wild hosts are very important in the circulation of the parasite in wildlife, and *T. gondii* infection in birds is considered to be epidemiologically significant because of their dietary habit. The house sparrow is frequently commensal with humans and easily adaptable to urban and rural areas [5]. Therefore, the investigation of *T. gondii* infection in house sparrows is an effective way to estimate environmental contamination with *T. gondii* oocysts.

The genetic diversity of *T. gondii* varies in different geographical regions and different hosts. Isolates of *T. gondii* in South America, for example, are genetically and biologically different from those in North America and Europe [6-13], where the *T. gondii* population structure is highly clonal and composed mainly of 4 distinct lineages, i.e., Types I, II, III, and 12 [13]. We have previously identified limited genotypes in isolates from humans, cats, pigs, and sheep in China [14-17], but there is little genetic data on *T. gondii* isolates from wild birds [18]. In the present paper, we report the seroprevalence and genetic characterization of *T. gondii* isolates from house sparrows in Lanzhou, northwest China.

A total of 313 blood samples were obtained from house sparrows between September and November 2011 in Lanzhou. Blood samples were incubated at 37℃ for 2 hr and then centrifuged at 3,000 *g* for 5 min to separate the serum samples. The separated serum samples were stored at -20℃ until further analysis.

Antibodies to *T. gondii* were determined in house sparrow

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sera by the modified agglutination test (MAT) as described previously [19,20]. Briefly, sera were added to the "U" bottom of 96-well microtiter plates, and diluted 2-fold starting from 1:5 to 1:20. Birds sera with MAT titers of 1:5 or higher were considered positive for *T. gondii* antibodies based on a previous study [21], those sera with doubtful reactions were re-tested, and positive and negative controls were included in each test.

Heart, brain, and lung tissues from 39 seropositive house sparrows were used for DNA extraction. Genomic DNA was extracted from 5-10 g of heart, brain, or lung tissues by SDS/proteinase K treatment, column-purified (Tiangen™, Beijing, China) and eluted into 50 ml $H_2O$ according to the manufacturer's recommendations. A nested PCR targeting the *T. gondii* B1 gene was performed to detect possible infection with *T. gondii* [22]. DNA samples giving positive B1 amplification were then used for genetic characterization.

Genetic characterization of *T. gondii* isolates from sparrows was carried out using the multilocus PCR-RFLP method [16,18,23]. Multiplex PCR-amplified products were 1:1 diluted in sterile, double-distilled water, and then used for nested PCR amplifications with internal primers for each marker, separately. The nested PCR products were digested with restriction enzymes. The restriction fragments were resolved in 2.5% agarose gel, stained by the GoldenView™, and photographed using a gel documentation system (UVP GelDoc-ItTM Imaging System, Cambridge, U.K.).

Thirty nine (12.46%) out of 313 serum samples were detected positive for *T. gondii* antibodies by MAT (Table 1). However, most of them had low titers, with MAT titers 1:5 in 29 house sparrows, and with 1:10 or higher in 10 house sparrows. Of the 117 DNA samples, 11 were positive for the *T. gondii* B1 gene by PCR amplification (Fig. 1), including 6 from brains, 3 from lungs, and 2 from hearts. Moreover, among the 11 positive DNA samples, 2 samples were come from the same house sparrow (sample LZS232) but different tissues (brain and heart).

Four DNA samples showed complete genotyping results, 2 from brain (sample LZS232, LZS253), 1 from heart (sample LZS232), and 1 from lung (sample LZS107) (Fig. 2). Due to low DNA concentration, 7 of the 11 positive samples could not be completely genotyped and was therefore not used. Two genotypes (ToxoDB #3 and a new genotype) were identified from the 11 positive samples (Table 2).

Little is known of the prevalence of *T. gondii* in sparrows in China. Sparrows could be one of the important species during the cycle of *T. gondii* transmission. Sparrows especially house sparrows are normally distributed around the human environment. Due to their habit of feeding close to the ground, sparrows, like free-range chickens, are indeed considered as a good indicator of environmental contamination by *T. gondii* oocysts [4]. More importantly, comparing to chickens, sparrows can transmit *T. gondii* faster and wider because of their flying ability, hence are more important intermediate hosts of *T. gondii* than chickens.

Any meat from warm-blooded animals and birds has been traditionally considered a major source of *Toxoplasma* infection in Western countries [4]. Some local people in this study region like to eat the sparrow meat very much. The risk associated with the type of meat varies among different countries according to local eating habits and according to the *T. gondii*

**Table 1.** Seroprevalence of *Toxoplasma gondii* in house sparrows in Lanzhou, northwest China using the modified agglutination test (MAT)

| | No. tested | No. positive | Prevalence (%) | 95% CI |
|---|---|---|---|---|
| House sparrow | 313 | 39 | 12.46 | 11.25-13.67 |

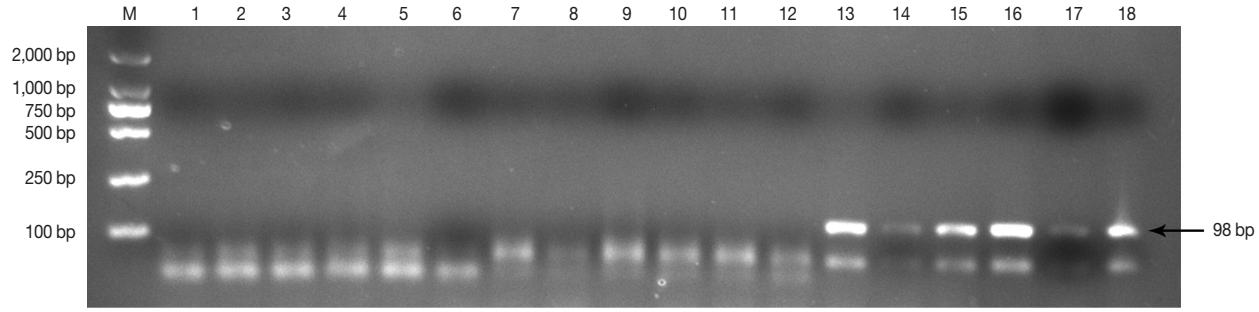


**Fig. 1.** Representative PCR products of *Toxoplasma gondii* B1 gene amplified from tissue DNA samples of house sparrows. Lanes 1-12 represent negative amplification, and lanes 13-18 represent positive amplification, respectively.

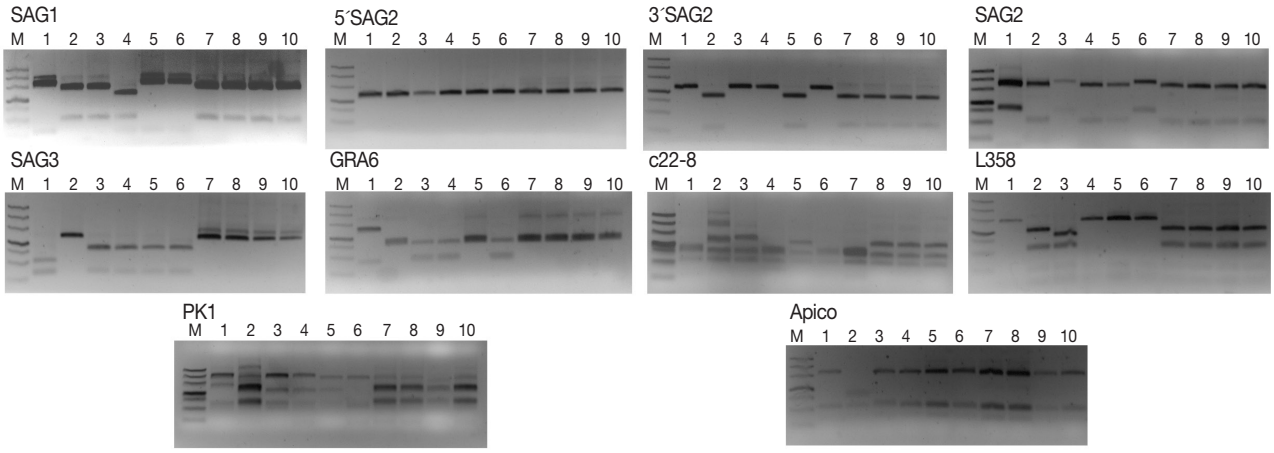


**Fig. 2.** PCR-RFLP analysis of *Toxoplasma gondii* isolates from house sparrows in Lanzhou, China based on 9 different loci. M represents a DNA marker. Lanes 1-10 represent GT1, PTG, CTG, MAS, TgCatCal, TgCaBr5, LZS253, LZS232 (brain), LZS232 (heart), and LZS107, respectively.

**Table 2.** Summary of genotyping of *Toxoplasma gondii* from house sparrows in Lanzhou, northwest China

| Isolate ID | Host | Tissue | Location | SAG1 | 5′+ 3′SAG2 | Alternative SAG2 | SAG3 | GRA6 | c22-8 | L358 | PK1 | Apico | Genotype |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GT1I | Goat | | United States | I | I | I | I | I | I | I | I | I | Reference, Type I, ToxoDB #10 |
| PTG | Sheep | | United States | II/III | II | II | II | II | II | II | II | II | Reference, Type II, ToxoDB #1 |
| CTG | Cat | | United States | II/III | III | III | III | III | III | III | III | III | Reference, Type III, ToxoDB #2 |
| MAS | Human | | France | u-1[a] | I | II | III | III | u-1[a] | I | III | I | Reference, ToxoDB #17 |
| TgCgCa1 | Cougar | | Canada | I | I | II | III | II | II | I | u-2[a] | I | Reference, ToxoDB #66 |
| TgCatBr5 | Cat | | Brazil | I | III | III | III | III | I | I | u-1[a] | I | Reference, ToxoDB #19 |
| LZS253 | House Sparrow | Brain | Lanzhou, Gansu | II | II | II | II | II | I | II | II | I | Type II variant, new genotype |
| LZS232 | House Sparrow | Brain | Lanzhou, Gansu | II | II | II | II | II | II | II | II | I | Type II variant, ToxoDB #3 |
| LZS232 | House Sparrow | Heart | Lanzhou, Gansu | II | II | II | II | II | II | II | II | I | Type II variant, ToxoDB #3 |
| LZS107 | House Sparrow | Lung | Lanzhou, Gansu | II | II | II | II | II | II | II | II | I | Type II variant, ToxoDB #3 |

[a]u-1 and u-2 represent unique RFLP genotypes, respectively.

prevalence in meat-producing animals. We previously reported that 4 of 178 bird DNA samples were positive for the *T. gondii* B1 gene by PCR amplification. Because serum samples of these 178 birds were not available for serological examination, the data could not indicate the seroprevalence of *T. gondii* infection in these wild birds in China [18]. Meanwhile, only breast muscle samples were available in that study [18]. Here, we collected heart, brain, and lung tissues for genetic characterization. In the present study, antibodies to *T. gondii* were examined by MAT, and found in 39 (12.46%) of 313 house sparrows at the cut of 1:5. Although with low titers, house sparrows are easily exposed to *T. gondii* through the ingestion of food or water contaminated with sporulated oocysts excreted by infected felids. Free-living animals such as wild birds and mammals were considered to be sentinels of environmental spreading of *T. gondii* oocysts, because of the increasing probability of sharing the same environment with humans [24].

Two genotypes (both are Type II variants) were identified in this study, one of them had a Type I allele at the Apico locus and is considered the Type II variant (ToxoDB #3), which is widely distributed in the world [25-27]. This was the third time that this Type II variant was identified in China, as this genotype was previously reported from sheep in Qinghai Province [17] and wild birds in Xinjiang Uygur Autonomous Region [18]. However, the other strain isolated from sample LZS253 had the Type I allele at the c22-8 locus in addition to the Apico locus, this genotype has not been reported before, and is considered a Type II variant (a new genotype). This is different from previous studies that showed the genotype ToxoDB #9 was predominant in cats and other animals in southern, south-

western, and central parts of China [14,17,18,28]. Therefore, the results of the present study and other recent studies indicate that ToxoDB #3 is the major clonal *T. gondii* genotype circulating in northwestern China [17,18].

To our knowledge, this is the first report of *T. gondii* seroprevalence in house sparrows in China. The present work also provided new genetic information about *T. gondii* infection in house sparrows in northwestern China. The prevalence of *T. gondii* infection in birds has epidemiological significance because infection of the ground-foraging birds like sparrows suggests soil contamination with *T. gondii* oocysts. These birds are potential reservoirs and good indicators for *T. gondii* transmission.

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