Serological Evidence of *Toxoplasma gondii* Infection in Five Species of Bats in China

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Abstract

Toxoplasma gondii is an obligate intracellular protozoan parasite that can infect almost all warm-blooded animals and humans with a worldwide distribution. Bats are reservoirs for an increasing number of emerging zoonotic viruses, such as henipaviruses and severe acute respiratory syndrome coronavirus (SARS-CoV). However, little is known of *T. gondii* infection in bats. The objective of the present study was to determine the seroprevalence of *T. gondii* infection in bats in China. A total of 217 serum samples from 5 species of bats were collected between April, 2010, and August, 2011, from 4 provinces in China. Antibodies to *T. gondii* were determined using the modified agglutination test (MAT, 1:25 or higher). Antibodies to *T. gondii* were found in 26.5% (18/68) *Megaderma lyra*, 13.6% (12/88) *Rousettus leschenaulti*, 13.6% (3/22) *Cynopterus sphinx*, 20% (4/20) *Vespertilio superaus*, and 15.8% (3/19) *Pipistrellus javanicus*. Antibody titers ranged from 1:25 to 1:400, with titers of 1:200 detected in 4 of the 5 bat species. The present study suggests the likely occurrence of *T. gondii* infection in bats in China, and these bats are new putative hosts for *T. gondii*, which may pose a threat to human health.

Key Words: *Toxoplasma gondii*—Toxoplasmosis—Bat—China—Seroprevalence—Modified agglutination test.

Introduction

THE PROTOZOAN *Toxoplasma gondii* infects almost all warm-blooded animals, including humans (Montoya and Liesenfeld 2004, Dubey 2010, Wendte et al. 2011, Zhou et al. 2011, Elmore et al. 2012, Wu et al. 2012). Bats are of biological interest because they are reservoirs for an increasing number of emerging zoonotic viruses, including henipaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV), lyssaviruses, including rabies viruses, and filoviruses (Calisher et al. 2006).

Little is known of *T. gondii* infection in bats. There are 2 old reports of *T. gondii* infection in insectivorous bats of the genus *Myotis* from France. Akinchinda and Doby (1968) demonstrated *T. gondii* in the brain of one *Myotis bechsteinii* by isolating viable *T. gondii* by mouse bioassay. The bat was

negative for the Sabin–Feldman dye test. Doby et al. (1974) reported that 35 bats belonging to 15 species were examined, and they detected *T. gondii* antibodies (Sabin–Feldman dye test titers ranged between 1:10 and 1:40) in one *Myotis daubentonii*. The objective of the present study was to examine the seroprevalence of *T. gondii* infection in bats in China.

Materials and Methods

Naturally infected bats

Between April, 2010, and August, 2011, a total of 217 bats were caught by using mist nets, which represented 5 genera and 5 species. From these, 124 (57.1%) were females and 93 (41.9%) were males; 98 (45.2%) of them were juveniles and 119 (54.8%) were adults. Of these, 68 samples were bats from Guangdong Province (GD), 29 from Guangxi Zhuang

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Nationality Autonomous Region (GX), 108 were from Hubei Province (HB), and 12 were from Anhui Province (AH) (Table 1). Blood samples were collected from the interfemoral vein, and serum samples were separated and stored at -20°C prior to use.

Characteristics of the bats

Five bat species were examined in the present study, namely *Megaderma lyra*, *Rousettus leschenaultia*, *Cynopterus sphinx*, *Vespertilio superaus*, and *Pipistrellus javanicus*. The characteristics are different among 4 bat species. *M. lyra* has a relatively large body size (65–95 mm) and its weight ranges between 40 and 60 grams. It has no tail. Fur color is bluish grey except on the underside, which is brownish grey. Its ears are big. *R. leschenaultia* has an elongated dog-like muzzle, large eyes, and broad wings; the average length is 126 mm. The average weight is 94.8 grams. *C. sphinx* has a relatively long snout. Its upper parts are brown to grey brown with paler under parts. The fur is very fine and silky. *V. superaus* has a body length of 6–7 cm, a tail of 4.3–4.5 cm, and a wing length of 5 cm. *P. javanicus* is a small brown bat with a flat head (head-body length 44.7 mm; dry weight 5.6 grams), a broad muzzle, and short ears with a short tragus rounded at the tip. The fur is dark brown, dark at the base and slightly paler ventrally.

Serological examination

Serum samples of bats were tested for *T. gondii* antibodies by the modified agglutination test (MAT), as described pre-

viously (Dubey 1997, Wu et al. 2011, Yan et al. 2011). Briefly, MAT was performed with a suspension of *Toxoplasma* tachyzoites fixed with formalin as antigen. Bat sera were added to the U-bottom of the 96-well microtiter plates and diluted 2-fold in phosphate-buffered saline (PBS, pH 7.2) starting from 1:25 to 1:1600. After adding the MAT antigen, the plates were shaken for 2 min and then incubated at 37°C overnight without shaking. Bat sera with MAT titers of 1:25 or higher were considered as positive. Positive and negative control sera were included in each test. In our experience we have found that individuals with a positive immunoglobulin G (IgG) titer of 25 or greater will continue to test positively on repeated follow-up examinations.

Statistic analysis

T. gondii antibody prevalence was calculated by using the binomial exact method; antibody prevalence between bat species and sexes were compared by using the chi-squared test and Fisher exact test in SPSS for Windows (Release 17.0 standard version, SPSS Inc., Chicago, Illinois). The differences were considered to be statistically significant when the *p* value was less than 0.05.

Results and Discussion

In the present investigation, we assayed the prevalence of *T. gondii* antibodies in bat serum samples from different regions in China using the MAT because this assay is sensitive and specific for detection of *T. gondii* antibodies in various

TABLE 1. SEROPREVALENCE OF *TOXOPLASMA GONDII* IN BATS IN PEOPLES REPUBLIC OF CHINA

Species	<i>Megaderma lyra</i>	<i>Rousettus leschenaulti</i>	<i>Cynopterus sphinx</i>	<i>Vespertilio superaus</i>	<i>Pipistrellus javanicus</i>
Common name	Big-eared bat	Fulvous fruit bat	Greater short-nosed fruit bat	Asian particolored bat	Javan pipistrelle
Habitat	Caves or tunnel in humid forest	Caves in forests	Caves in tropical forests	Caves in mountainous regions	Caves in forests
Diet	Insects, small mammals and birds	Fruit juice, figs	Fruits, include guava, banana and dates	Flying insects	Flies, ants and other small insects
Source ^a					
GD	20	26	11	6	5
GX	8	14	3	3	1
HB	38	42	7	9	12
AH	2	6	1	2	1
No. examined	68	88	22	20	19
No. seropositive (prevalence %) ^b	18 (26.5) (17–39) ^c	12 (13.6) (7–23) ^c	3 (13.6) (3–35) ^c	4 (20) (6–44) ^c	3 (15.8) (3–40) ^c
Positive males	4	1	1	2	1
Positive females	14	11	2	2	2
MAT titers ^b					
1:25	0	1	1	0	0
1:50	3	3	0	2	1
1:100	6	4	0	2	1
1:200	7	2	2	0	1
1:400	2	2	0	0	0

^aLocation where bats were trapped. GD, Guangdong Province; GX, Guangxi Zhuang Nationality Autonomous Region; HB, Hubei Province; AH, Anhui Province.

^bModified agglutination test titers ≥ 25 were classified as positive.

^cThe intervals in the bracket were the seroprevalence confidence limits rounded to the nearest whole number. The 95% confidence limits were calculated using the formula Mean $- 1.96 \times$ Standard Error and Mean $+ 1.96 \times$ Standard Error for the left- and right-hand side confidence limits.

animals (Dubey et al. 1995, Dubey 1997, 2010). By using the chi-squared test, the *T. gondii* seroprevalence was not significantly different among bats from different regions (p value=0.116). The overall seroprevalence of *T. gondii* in bats in the examined regions of China was 18.4% (40/217) (Table 1). The results showed that the prevalence in female bats (25.0%, 31/124) was significantly higher than that in male bats (9.7%, 9/93) ($p < 0.05$). The *T. gondii* seroprevalence in bats of 5 genera, namely *Megaderma*, *Rousettus*, *Cynopterus*, *Vespertilio*, and *Pipistrellus* was 26.5% (18/68), 13.6% (12/88), 13.6% (3/22), 20% (4/20), and 15.8% (3/19), respectively, and there were no significant differences among them ($p > 0.05$). Although difficult to interpret without knowing the ages of the bats tested, the higher seroprevalence in *Megaderma* (26.5%) than in other bat genera may be related to diet. In daily life, *Megaderma* mainly feeds on mice and sparrows, which are hosts of *T. gondii*. The other 4 bat genera are insectivorous and frugivorous bats.

For most serological surveys of *T. gondii* infection in animals, a MAT dilution of 1:25 is used as the cutoff (Dubey 2010). In the present study, a MAT titer of 1:200 was recorded in bats from 4 of the 5 species examined, indicating that the bats had possible acute infection. *T. gondii* seropositivity in insectivorous and frugivorous bats suggests that the caves where bats live are contaminated with *T. gondii* oocysts. As a result, bats may play an important role in the transmission of *T. gondii* in China where toxoplasmosis is a significant human health problem (Zhou et al. 2011)

Results of the present investigation demonstrated the high seroprevalence of *T. gondii* infection in bats in China, indicating that these bats are new putative hosts for *T. gondii*, which may pose a threat to human health. Bats flying outside at night pose little risk. However, bats appearing in the daytime, which is often a sign of disease in bats, or those roosting in accessible locations where people are exposed, should be considered a potential public health threat. Further studies are warranted to isolate *T. gondii* from bats, consequently virulence assessment in mice and genotyping of *T. gondii* isolates from bats will also be carried out in the next experiments.

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Author Disclosure Statement

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References

- Akínchina G, Doby JM. Première observation microscopique directe de *Toxoplasma gondii* Nicolle et Manceau 1909. C. R. Seances Acad Sci 1968; 266:2431–2433.
- Calisher CH, Childs JE, Field HE, Holmes KV, et al. Bats: Important reservoir hosts of emerging viruses. Clin Microbiol Rev 2006; 19:531–545.
- Doby JM, Desmonts G, Beaucournu JC, Akinchina GT. Recherche immunologique systématique de toxoplasmose chez les petits mammifères sauvages en France. Folia Parasitol (Praha) 1974; 21:289–300.
- Dubey JP. Validation of the specificity of the modified agglutination test for toxoplasmosis in pigs. Vet Parasitol 1997; 71: 307–310.
- Dubey JP. *Toxoplasmosis of Animals and Humans*, 2nd ed. Boca Raton, FL: CRC Press Inc., 2010:1–313.
- Dubey JP, Thulliez P, Weigel RM, Andrews CD, et al. Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows. Am J Vet Res 1995; 56:1030–1036.
- Elmore SA, Jenkins EJ, Huyvaert, KP, Polley L, et al. *Toxoplasma gondii* in circumpolar people and wildlife. Vector Borne Zoonotic Dis 2012; 12:1–9.
- Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet 2004; 363: 1965–1976.
- Wendte JM, Gibson AK, Grigg ME. Population genetics of *Toxoplasma gondii*: New perspectives from parasite genotypes in wildlife. Vet Parasitol 2011; 182:96–111.
- Wu SM, Huang SY, Fu BQ, Liu GY, et al. Seroprevalence of *Toxoplasma gondii* infection in pet dogs in Lanzhou, Northwest China. Parasit Vectors 2011; 4:64.
- Wu S.M, Ciren D, Huang SY, Xu MJ, et al. First report of *Toxoplasma gondii* prevalence in Tibetan pigs in Tibet, China. Vector Borne Zoonotic Dis 2012; 12:654–656.
- Yan C, Yue CL, Qiu SB, Li HL, et al. Seroprevalence of *Toxoplasma gondii* infection in domestic pigeons (*Columba livia*) in Guangdong Province of southern China. Vet Parasitol 2011; 177:371–373.
- Zhou P, Chen Z, Li HL, Zheng H, et al. *Toxoplasma gondii* infection in humans in China. Parasit Vectors 2011; 4:165.

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