Veterinary Parasitology 203 (2014) 318–321

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short Communication

Prevalence and genetic characterization of *Toxoplasma gondii* infection in bats in southern China

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ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form 8 April 2014
Accepted 14 April 2014

Keywords:
Toxoplasma gondii
Bats
Prevalence
Genetic characterization
Southern China

ABSTRACT

Toxoplasma gondii can infect a wide variety of warm-blooded animals, including bats. Limited information on *T. gondii* infection in bats is available in China. The objective of the present study was to determine prevalence and genetic diversity of *T. gondii* infection in bats in southern China. A total of 608 bats representing 12 species, including 120 *Aselliscus stoliczkanus*, 59 *Myotis chinensis*, 11 *Miniopterus schreibersii*, 53 *Rhinolophus affinis*, 32 *Rhinolophus pusillus*, 81 *Hipposideros armiger*, 28 *Hipposideros fulvus*, 32 *Cynopterus brachyotis*, 14 *Cynopterus sphinx*, 45 *Eonycteris spelaea*, 109 *Hipposideros larvatus*, and 24 *Taphozous melanopogon*, were collected from Yunnan and Guangxi provinces, southern China. They were examined for the presence of *T. gondii* DNA by amplification of the B1 gene using a nested PCR, and the positive samples were genotyped at 11 genetic loci (SAG1, 5′- and 3′-SAG2, alternative SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico) using multilocus polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technology. Fifty-nine (9.7%) of these bats were detected positive by PCR but only five of these positive DNA samples were completely typed at all loci; of which 4 samples, 2 from *A. stoliczkanus*, and 2 from *H. larvatus*, belonged to ToxoDB Genotype #10, and the other one from *H. larvatus* was identified as ToxoDB Genotype #9 (http://toxodb.org/toxo/). To our knowledge, this is the first report of molecular detection and genetic characterization of *T. gondii* infection in bats in China. The results show that these bats are potential reservoirs for *T. gondii* transmission, which may pose a threat to human health.

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1. Introduction

Toxoplasma gondii can infect a wide variety of warm-blooded animals, including bats. Although bats represent about 20% of all classified mammal species in the world, reports on *T. gondii* infection in bats are limited. The prevalence of *T. gondii* infection was reported to be 10.39% in insectivorous bats in Britain (Dodd et al., 2014), and 29.3% in insectivorous bats in Myanmar (Sun et al., 2013). Two

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http://dx.doi.org/10.1016/j.vetpar.2014.04.016
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cases of toxoplasmosis have recently been documented in captive bats in Australia (Sangster et al., 2012), and two *T. gondii* strains have been isolated in bats in Brazil (Cabral et al., 2013). *T. gondii* in bats in Myanmar were closely related to or belong to clonal type I (Sun et al., 2013). In China, Yuan et al. (2013) provided serological evidence of *T. gondii* infection in *Megaderma lyra* (26.5%), *Rousettus leschenaultia* (13.6%), *Cynopterus sphinx* (13.6%), *Vespertilio superaus* (20%) and *Pipistrellus javanicus* (15.8%). However, the genotypes of *T. gondii* in bats in China are unknown. The objective of the present study was to determine the presence and genotypes of *T. gondii* in bats in China using a molecular approach.

2. Materials and methods

2.1. Bat collection

The study was approved by the ethics committee of Military Veterinary Institute, Academy of Military Medical Sciences, and conducted in Yunnan (97.83°–105.6° N, 21.58°–28.22° E) and Guangxi (105.08°–111.54° N, 21.78°-25.96° E) provinces, southern China during 2010–2011. Bats were captured at roosts with hand nets, and identified to species in the field.

2.2. DNA extraction and PCR detection

The sampled bats were euthanized, organs (lung, heart, liver, spleen, stomach, intestine or kidney) were taken out individually from a single bat, and genomic DNA was extracted using the TIANamp genomic DNA kit (Tiangen, Beijing, China) from individual tissues or pooled tissues when necessary. *T. gondii* infection in bats was tested by a nested PCR targeting the B1 gene as described elsewhere (Sun et al., 2013).

2.3. Genetic characterization of T. gondii

Genetic characterization of *T. gondii* of the positive samples was carried out using the multilocus PCR-RFLP method, including 11 genetic markers (i.e., SAG1, 5′- and 3′-SAG2, alternative SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico) as previously described (Su et al., 2010; Jiang et al., 2013). Briefly, the target sequences were amplified by multiplex PCR using external primers for all 11 markers. Multiplex PCR amplified products were diluted 1:1, and then used for nested PCR amplifications with internal primers for each marker, respectively. The nested PCR products were digested with restriction enzymes, and the restriction fragments were resolved in 2.5–3% agarose gel. The reference strains, including GT1, PTG, CTG, MAS, TgCgCa1, TgCatBr5, TgCatBr64, TgRsCr1 and TgWtdSc40 were included as the positive controls.

3. Results and discussion

A total of 608 bats, belonging to 12 species of 8 genera, were collected in this study. There were 10 species including *Aselliscus stoliczkanus*, *Myotis chinensis*, *Miniopterus schreibersii*, *Cynopterus brachyotis, C. sphinx*, *Hipposideros armiger*, *Hipposideros fulvus*, *Rhinolophus affinis*, *Rhinolophus pusillus* and *Eonycteris spelaea* sampled from Yunnan province, and 2 species including *Hipposideros larvatus* and *Taphozous melanopogon* sampled from Guangxi province (Table 1). Among these bats, *C. sphinx* and *E. spelaea* are frugivorous, others are insectivorous.

Of the 12 bat species tested for *T. gondii* infection by nested PCR, 10 were positive, with infection rates ranging from 1.2% to 45.8%. No *T. gondii* DNA was detected in the two insectivorous bat species *M. schreibersii* and *C. brachyotis*, which may be due to the small sample sizes. Among 133 bats collected from Guangxi, 27 (20.3%) were positive, with a high prevalence of 45.8% in *T. melanopogo*. In contrast, among 475 bats collected from Yunnan, 32 (6.7%) were infected with *T. gondii*. A higher prevalence was found in insectivorous bats (55/549, 10.0%) than that in frugivorous bats (4/59, 6.8%) (Table 1).

The positive DNA samples were further genotyped, and 10 (16.9%) were successfully genotyped at six or more genetic loci; of which 5 were completely genotyped, 2 from *A. stoliczkanus*, 3 from *H. larvatus* (Table 2). Due to low DNA

Table 1
Prevalence of *Toxoplasma gondii* infection in bats from Yunnan and Guangxi provinces, southern China, detected by PCR amplification of the B1 gene.

Region	Bat species	No. of examined (%)[a]	No. of positive	Prevalence (%)
Yunnan	*Aselliscus stoliczkanus*	120 (19.7)	10	8.3
	Myotis chinensis	59 (9.7)	11	18.6
	Miniopterus schreibersii	11 (1.8)	0	0
	Rhinolophus pusillus	32 (5.3)	1	3.1
	Rhinolophus affinis	53 (8.7)	4	7.5
	Hipposideros armiger	81 (13.3)	1	1.2
	Hipposideros fulvus	28 (4.6)	1	3.6
	Cynopterus brachyotis	32 (5.3)	0	0
	Cynopterus sphinx	14 (2.3)	1	7.1
	Eonycteris spelaea	45 (7.4)	3	6.7
	Subtotal	475 (78.1)	32	6.7
Guangxi	*Hipposideros larvatus*	109 (17.9)	16	14.7
	Taphozous melanopogon	24 (3.9)	11	45.8
	Subtotal	133 (21.9)	27	20.3
Total		608	59	9.7

[a] The percent accounts for the total bats.

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