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Prevalence and Genetic Characterization of *Toxoplasma gondii* in Bats in Myanmar

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We detected *Toxoplasma gondii* in 29.3% (95% confidence interval [CI], 25.5% to 33.1%) of 550 insectivorous bats collected in Myanmar. The genotyping of these positive samples revealed they were closely related to or belong to clonal type I, which is highly virulent in mice, showing that these bats are potential reservoirs for *T. gondii* transmission.

Toxoplasma gondii is an important intracellular protozoan parasite that infects almost all warm-blooded animals, including humans. The infection can cause serious diseases in the developing fetus and immunocompromised individuals (1). The life cycle of *T. gondii* includes the felids as the definitive hosts and other animals and humans as the intermediate hosts (2). The infections are obtained by the ingestion of oocysts in the environment, by the consumption of tissue bradyzoites in infected intermediate hosts, or by congenital transmission (3).

Bats are important to public health because they may serve as natural reservoirs for many pathogens, such as rabies virus, Hantavirus, Marburg virus, and others, and have become important infection sources for a variety of etiologic agents (4). In addition to viruses, bacteria, fungi, and protozoa have been found in bats and can potentially be transmitted to humans (5–7). The ability of bats to fly and their social life contribute to the maintenance, evolution, and spread of pathogens (8).

Although bats represent approximately 20% of the known species of mammals, only two cases of toxoplasmosis have recently been described in captive bats in Australia (9), and several *T. gondii* strains have been isolated in Kazakhstan (10) and in Brazil (11, 12). The prevalence of *T. gondii* infection was reported to be 13.4% in British insectivorous bats (N. Dodd, J. Lord, D. Brooks, and G. Hide, presented at the XII International Congress of Parasitology, Melbourne, Australia, 2010). *T. gondii* has three main clonal lineages, designated types I, II, and III, based on multilocus restriction fragment length polymorphism (RFLP) analysis. Type I is highly virulent in mice, while type II and III strains are less virulent (13). The genetic characterization of *T. gondii* may vary in different animals or geographic regions. This study was conducted to determine the prevalence and genotypes of *T. gondii* in bats in Myanmar.

Bat collection and species. The study was approved by the Ethics Committee of the Veterinary Institute, Academy of Military Medical Sciences. A total of 550 insectivorous bats were captured in caves and residential areas in Sedon and Wutao counties, southern Myanmar, close to Tengchong County, Yunnan Province, China, in 2008. These bats belonged to 6 species of 5 genera, including *Miniopterus fuliginosus*, *Rhinolophus ferrumequinum*, *Myotis chinensis*, *Hipposideros fulvus*, *Hipposideros armiger*, and *Megaderma lyra*. The dominant bat species was *M. fuliginosus* (64.2%), followed by *R. ferrumequinum* (29.5%), *H. fulvus* (2.0%), *M. chinensi* (1.8%), *H. armiger* (1.5%), and *M. lyra* (1.1%) (Table 1).

TABLE 1 Prevalence of *T. gondii* in different bat species from Myanmar

Bat species	No. (%) of bats examined[a]	No. of bats positive	Prevalence, % (95% CI)[b]
Miniopterus fuliginosus	353 (64.2)	137	38.8 (33.7–43.9)
Rhinolophus ferrumequinum	162 (29.5)	8	4.9 (1.6–8.3)
Myotis chinensis	10 (1.8)	9	90.0 (55.5–99.8)
Hipposideros fulvus	11 (2.0)	0	0 (0)
Hipposideros armiger	8 (1.5)	1	12.5 (0.3–52.7)
Megaderma lyra	6 (1.1)	6	100 (54.1–100)
Total	550	161	29.3 (25.5–33.1)

[a] Percentage of the total bats collected.
[b] A significant difference was found in prevalence of *T. gondii* in different bat species, which was analyzed by Fisher's exact test. The 95% confidence intervals (CI) are also indicated.

DNA extraction, nested PCR, and PCR-RELP. The sampled bats were euthanized, organs (lung, heart, liver, spleen, stomach, gut, and kidney) from single bats were pooled, and genomic DNA was extracted using the TIANamp genomic DNA kit (Tiangen, Beijing, China). *T. gondii* infection in bats was tested by a nested PCR targeting the B1 gene as described elsewhere (14). Genotyping was conducted using 11 genetic markers for PCR-RFLP (i.e., SAG1, SAG2, alter.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico), as previously described (13, 15). Genomic DNA of the *T. gondii* RH strain was used as a positive control, and molecular-grade water was used as a negative control, both of which were included in each PCR run. The primers used in this study are listed in Table 2.

Prevalence of *T. gondii* infection in bats. Of the 6 bat species tested, 5 were positive for *T. gondii* infection, and *H. fulvus* was

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TABLE 2 Nested PCR primers used for nested PCR and nested PCR-RELP of *T. gondii* in bats

Marker	Sequence (5′→3′)[a]	
	External primers	Internal primers
B1	F: GGAACTGCATCCGTTCATGAG R: TCTTTAAAGCGTTCGTGGTC	F: TGCATAGGTTGCAGTCACTG R: GGCGACCAATCTGCGAATACACC
SAG1	F: GTTCTAACCACGCACCCTGAG R: AAGAGTGGGAGGCTCTGTGA	F: CAATGTGCACCTGTAGGAAGC R: GTGGTTCTCCGTCGGTGTGAG
5′-SAG2	F: TCTGTTCTCCGAAGTGACTCC R: TCAAAGCGTGCATTATCGC	F: GAAATGTTTCAGGTTGCTGC R: GCAAGAGCGAACTTGAACAC
3′-SAG2	F: TCTGTTCTCCGAAGTGACTCC R: TCAAAGCGTGCATTATCGC	F: ATTCTCATGCCTCCGCTTC R: AACGTTTCACGAAGGCACAC
alter.SAG2	F: GGAACGCGAACAATGAGTTT R: GCACTGTTGTCCAGGGTTTT	F: ACCCATCTGCGAAGAAAACG R: ATTTCGACCAGCGGGAGCAC
SAG3	F: CAACTCTCACCATTCCACCC R: GCGCGTTGTTAGACAAGACA	F: TCTTGTCGGGTGTTCACTCA R: CACAAGGAGACCGAGAAGGA
BTUB	F: TCCAAAATGAGAGAAATCGT R: AAATTGAAATGACGGAAGAA	F: GAGGTCATCTCGGACGAACA R: TTGTAGGAACACCCGGACGC
GRA6	F: ATTTGTGTTTCCGAGCAGGT R: GCACCTTCGCTTGTGGTT	F: TTTCCGAGCAGGTGACCT R: TCGCCGAAGAGTTGACATAG
C22-8	F: TGATGCATCCATGCGTTTAT R: CCTCCACTTCTTCGGTCTCA	F: TCTCTCTACGTGGACGCC R: AGGTGCTTGGATATTCGC
C29-2	F: ACCCACTGAGCGAAAAGAAA R: AGGGTCTCTTGCGCATACAT	F: AGTTCTGCAGAGTGTCGC R: TGTCTAGGAAAGAGGCGC
L358	F: TCTCTCGACTTCGCCTCTTC R: GCAATTTCCTCGAAGACAGG	F: AGGAGGCGTAGCGCAAGT R: CCCTCTGGCTGCAGTGCT
PK1	F: GAAAGCTGTCCACCCTGAAA R: AGAAAGCTCCGTGCAGTGAT	F: CGCAAAGGGAGACAATCAGT R: TCATCGCTGAATCTCATTGC
Apico	F: TGGTTTTAACCCTAGATTGTGG R: AAACGGAATTAATGAGATTTGAA	F: GCAAATTCTTGAATTCTCAGTT R: GGGATTCGAACCCTTGATA

[a] F, forward primer; R, reverse primer.

negative (Table 1). Among 550 bats, 161 (29.3%; 95% confidence interval [CI], 25.5% to 33.1%) were infected. The infection rate was high in several bat species, e.g., 6 of 6 *M. lyra* bats (100%; 95% CI, 54.1% to 100%), and 9 of 10 *M. chinensis* bats (90%; 95% CI, 55.5% to 99.8%). Low prevalences of *T. gondii* infection were found in *R. ferrumequinum* (4.9%; 95% CI, 1.6% to 8.3%) and *H. armiger* (12.5%; 95% CI, 0.3% to 52.7%). Due to the limited numbers of samples from several bat species (*M. chinensis*, *H. fulvus*, *H. armiger*, and *M. lyra*) in this study, the prevalence of *T. gondii* infection in the bats should be investigated further.

Genotyping of *T. gondii* in bats. From these 161 positive samples, only 19 (11.8%) were successfully genotyped at 9 or more genetic loci, most likely due to a low DNA concentration in these samples. The typing results are summarized in Table 3. Based on the data obtained in the present study, the majority (15/19 [78.9%]) of *T. gondii* strains from bats in Myanmar did not match the identified RFLP genotypes listed in ToxoDB (www.toxodb.org) and were thus atypical and mixed novel genotypes. However, the other three samples, including TgBatMm11 from *M. fuliginosus* and TgBatMm15 and TgBatMm19 from *M. chinensis*, matched the known ToxoDB genotype 10, in which TgBatMm11 was the clonal type I, but the other two were not confirmed type I because there were some missing markers. The sample TgBatMm18 from *M. chinensis* may belong to ToxoDB 10 or 27 at 10 loci, except for the c29-2 locus, for which no PCR product was found.

Among the new genotypes, 2 (TgBatMm6 and-9) could be fully genotyped at all 11 loci, showing alleles of type I at 10 loci; 3 (TgBatMm7, -12, and -16) showed alleles of type I at 9 loci; 3 (TgBatMm5, -15, and -17) were classified into clonal type I at 8 loci; 4 (TgBatMm2, -8, -10, and -11) were grouped into clonal type I at 7 loci; and the other 3 (TgBatMm1, -3, and -4) showed alleles

TABLE 3 Multilocus genotyping of *T. gondii* isolates in bats from Myanmar by PCR-RFLP analysis

Host and isolate ID	PCR-RFLP genotype by genetic marker[a]											ToxoDB genotype[d]
	SAG1	5′ + 3′ SAG2[b]	alter.SAG2	SAG3	BTUB	GRA6	c22-8	c29-2[c]	L358	PK1	Apico	
Miniopterus fuliginosus												
TgBatMm1	ND	II	I	I	ND	I	I	u-1	II	I	I	Novel 1
TgBatMm2	ND	I	I	I	I	ND	I	u-1	II	I	I	Novel 2
TgBatMm3	II/III	I	I	I	I	ND	ND	u-1	ND	I	I	Novel 3
TgBatMm4	II/III	I	I	I	ND	I	I	u-1	II	II	I	Novel 4
TgBatMm5	ND	I	I	I	I	I	I	u-1	I+II	I	I	Novel 5
TgBatMm6, -9	I	I	I	I	I	I	I	u-1	I	I	I	Novel 6
TgBatMm7	I	I	I	I	I	I	I	u-1	ND	I	I	Novel 7
TgBatMm8	II/III	I	I	ND	I	I	I	u-1	II	I	I	Novel 8
TgBatMm10	ND	I	I	I	I	III	I	I	u-1	ND	I	Novel 9
TgBatMm11	I	I	I	I	I	I	I	I	I	I	I	10
TgBatMm12	I	I	ND	I	I	III	I	I	II	ND	I	Novel 10
Megaderma lyra												
TgBatMm13	I	I	I	I	I	III	I	ND	I	I	I	Novel 11
TgBatMm14	I	I	I	I	I	I	I	u-1	II	ND	I	Novel 12
Myotis chinensis												
TgBatMm15	I	I	I	I	I	I	I	I	ND	I	I	10 (?)
TgBatMm16	I	I	I	I	I	I	I	ND	II	I	I	Novel 13
TgBatMm17	I	I	I	I	I	III	I	I	II	I	ND	Novel 14
TgBatMm18	I	I	I	I	I	I	I	ND	I	I	I	10, 27 (?)
TgBatMm19	I	I	I	I	I	I	I	I	I	ND	ND	10 (?)

[a] Genotypes of *T. gondii* were determined according to PCR-RFLP analysis of 12 genetic loci, each locus usually producing three different genotypes, which were grouped into types I, II, or III, based on the three clonal types of reference strains (ToxoDB 10, 1, and 2). ND, no amplification detected due to a low DNA concentration in the sample.
[b] SAG2 marker based on the 5′ and 3′ ends of the gene sequence.
[c] u-1 is the new allele different from the clonal type I, II, and III alleles.
[d] A question mark indicates that the genotype of *T. gondii* in the sample may represent "others" that are not listed in ToxoDB due to some missing genetic markers.

of type I at 6 loci (Table 3). These data demonstrated that the *T. gondii* strains in bats in Myanmar were closely related to the clonal type I lineage.

Public health significance of *T. gondii* in bats. Few studies have been conducted on *T. gondii* infections in bats. The results of this study demonstrated that *T. gondii* infections are widely prevalent in insectivorous bats in Myanmar. *T. gondii* can cause infection by three transmission routes, including horizontal transmission and vertical or congenital transmission. *T. gondii* oocysts can be mechanically transmitted by certain types of insects to insectivorous bats (16, 17). Recent studies have shown that congenital transmission may play an important role in sustaining *T. gondii* infection in some species, including sheep and small mammals (18, 19). This transmission mode may be the main infection route in bat species, which needs further investigation. Differences in feeding habits among bat species could explain the different prevalences of *T. gondii* in bat species.

Previous studies have shown that bats can share the same genotypes of *T. gondii* found in domestic and wild animals (12). In the present study, *T. gondii* strains in bats in Myanmar were shown to be closely related to or to belong to clonal type I. As no previous data on the genotypes from different hosts in the studied region have been collected, the role of bats in the epidemiology of *T. gondii* infections in humans or domestic animals is unknown. However, the clonal type I lineage identified in bats has been found in animals and humans in North America, Europe, and Asia (20–22). In China, clonal type I has also been found, although it is not the predominant genotype (23). However, there is only one report on the *T. gondii* genotype in Yunnan Province, near the China-Myanmar border, with ToxoDB 19 found in pigs (24). Therefore, we cannot determine whether *T. gondii* in bats from Myanmar is transmitted to China by bats. The genetic diversity of *T. gondii* in Myanmar and along the China-Myanmar border should be further investigated to determine the public health significance of *T. gondii* in bats. To our knowledge, this study is the first report on molecular detection of *T. gondii* infection in bats in Asia.

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