

Detection of anti-*Toxoplasma gondii* antibodies in wild free-living birds and mammals from the northwest region of São Paulo state, Brazil

Detecção de anticorpos anti-*Toxoplasma gondii* em aves e mamíferos silvestres de vida livre da região noroeste do estado de São Paulo, Brasil

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ABSTRACT

Toxoplasmosis is a protozoonosis caused by an obligate intracellular parasite named *Toxoplasma gondii*, which can infect humans and a large number of homeothermic animal species with worldwide distribution. The present study aimed to detect anti-*T. gondii* antibodies from serological samples of free-living wild animals from the northwest region of São Paulo state, Brazil. Thirty-two samples (eight from birds and 24 from mammals) were analyzed by the modified agglutination test (MAT) using 5 cut-off points for birds and 25 for mammals. Seropositivity was observed in 25% (2/8) of birds, including the species *Rupornis magnirostris* (roadside hawk) and *Caracara plancus* (southern caracara), and 29.2% (7/24) animals were seropositive among mammals, including one hoary fox (*Lycalopex vetulus*), two maned wolves (*Chrysocyon brachyurus*), one black howler monkey (*Alouatta caraya*), two crab-eating foxes (*Cerdocyon thous*) and one gray brocket deer (*Mazama gouazoubira*). The results obtained with the present study indicate the exposure to *T. gondii* of free-living wild animals from the northwest region of São Paulo state and, therefore, that they probably play a role in the transmission and maintenance of *T. gondii* in the environment they inhabit. Thus, identification of the infection in several animal species in the region indicates the environmental contamination of the area. Studies of this nature may help to understand the importance of the prevention and control of this disease in Brazil.

Keywords: Toxoplasmosis. MAT. Wildlife. Zoonosis. Serology. Occurrence.

RESUMO

A toxoplasmose é uma protozoonose causada por um parasita intracelular obrigatório denominado *Toxoplasma gondii*, que pode infectar os humanos e um vasto número de espécies animais homeotérmicas, apresentando distribuição mundial. O presente estudo objetivou a detecção de anticorpos anti-*T. gondii* a partir de amostras sorológicas de animais silvestres de vida livre da região noroeste do estado de São Paulo. Foram analisadas 32 amostras (oito de aves e 24 de mamíferos) por meio do teste de aglutinação modificado (MAT), utilizando ponto de corte 5 para as aves e 25 para os mamíferos. Soropositividade foi observada em 25% (2/8) das aves, incluindo as espécies *Rupornis magnirostris* (gavião-carijó) e *Caracara plancus* (carcará); entre os mamíferos, 29,2% (7/24) foram soropositivos incluindo uma raposa-do-campo (*Lycalopex vetulus*), dois lobos-guará (*Chrysocyon brachyurus*), um bugio-preto (*Alouatta caraya*), dois cachorros-do-mato (*Cerdocyon thous*) e um veado-catingueiro (*Mazama gouazoubira*). Os resultados obtidos com o presente estudo indicam a exposição dos animais selvagens de vida livre a *T. gondii* na região noroeste do estado de São Paulo e, portanto, que provavelmente apresentam papel na transmissão e manutenção de *T. gondii* no meio ambiente em que vivem. Assim, a identificação da infecção em várias espécies de animais na região indica a contaminação ambiental da área. Estudos dessa natureza podem ajudar no entendimento sobre a prevenção e o controle dessa importante doença no Brasil.

Palavras-chave: Toxoplasmose. Teste de aglutinação. Animais selvagens. Zoonose. Sorologia. Ocorrência.

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Toxoplasma gondii is a protozoan that has a wide geographical distribution in Brazil and the world and is capable of infecting humans and other homeothermic animals, both domestic and wild. This parasite has a heteroxenous life cycle and felids are the definitive hosts, playing a key role as they are the only ones that can excrete fecal oocysts. Transmission can occur by ingestion of infective oocysts in soil, water, and food, by ingestion of cysts containing bradyzoites (carnivorism) and, congenitally, by the transplacental transmission of tachyzoites (Dubey et al., 1995; Dubey, 2010; Weiss & Dubey, 2009).

Several studies show variation in *T. gondii* seroprevalence in different animal species (from 23 to 83%, up to 90%) and these differences may be associated with geographic, climatic, cultural, and socioeconomic factors (Dubey et al., 2012; Fialho et al., 2009; Gennari et al., 2004; Minervino et al., 2010; Vitaliano et al., 2014).

This type of study is relevant because it contributes to an indication of the environmental contamination and the dimension of the toxoplasmosis problem in a region, as well as the participation of the hosts in the maintenance of the life cycle.

The present study aimed to detect the presence of anti-*T. gondii* antibodies in different species of wild free-living birds and mammals in the northwest region of the state of São Paulo, Brazil.

The study area was the northwest region of the state of São Paulo, Brazil, in a perimeter of approximately 150 km around the municipality of São José do Rio Preto (Coordinates: 20°48'29"S 49°22'52"W). Blood samples were obtained from six species of wild birds (eight samples) and nine species of wild mammals (24 samples), in a total of 32 samples (Table 1).

All included animals were rescued by the State Environmental Police and the Federal Highway Police after being run over or injured because of burnings that are common in the region due to the planting of some crops, such as sugar cane. It was not possible to get data on the exact location where the animals were rescued. These injured animals were referred to a veterinary hospital where first aid and clinical conduct were provided. The responsible veterinarian collected blood for emergency animal examinations and the unused blood volume was made available for the development of this study.

Serum samples were tested using the modified agglutination test (MAT) to detect antibodies against *T. gondii* (Dubey & Desmots, 1987). The cut-off used for the serological test was 25 for mammals (Dubey, 1997) and 5 for birds (Dubey et al., 2016). All positive samples were two-fold serially diluted for titration.

The frequency of occurrence of antibodies against *T. gondii* observed in free-living birds and mammals from the northwest region of the state of São Paulo are summarized

Table 1. Anti-*Toxoplasma gondii* antibodies in free-living wild birds from the northwest region of São Paulo state, Brazil, using the modified agglutination test (MAT \geq 5)

Species	N. negative	N. positive	Total	MAT titers
Accipitriformes				
<i>Rupornis magnirostris</i>	1	1	2	120
Falconiformes				
<i>Caracara plancus</i>	0	1	1	20
Cathartiformes				
<i>Coragyps atratus</i>	1	0	1	<5
Cariamiformes				
<i>Cariama cristata</i>	2	0	2	<5
Pelecaniformes				
<i>Tigrisoma lineatum</i>	1	0	1	<5
<i>Nycticorax nycticorax</i>	1	0	1	<5

in Tables 1 and 2, respectively. Seropositivity was observed in 25% (2/8) of birds, including *Rupornis magnirostris* (roadside hawk) and *Caracara plancus* (southern caracara) species. Among mammals, 29.2% (7/24) were seropositive including *Lycalopex vetulus* (hoary fox), *Chrysocyon brachyurus* (maned wolf), *Alouatta caraya* (black howler monkey), *Cerdocyon thous* (crab-eating fox), and *Mazama gouazoubira* (gray brocket deer) species. No statistical analysis was performed due to the limited number and convenience of the samples.

Serological evidence of contact with *T. gondii* had previously been reported in free-living animals of the *C. plancus*, *M. gouazoubira*, and *L. vetulus* species in the study by Vitaliano et al. (2014); in *A. caraya* and *C. thous* by Da Silva et al. (2014), and *C. brachyurus* by Vitaliano et al. (2014), Da Silva et al. (2014), and Oliveira et al. (2016), also in the state of São Paulo, highlighting the circulation of the agent in the state. For the first time, in the northwest region of the state of São Paulo, the presence of anti-*T. gondii* antibodies are reported in *R. magnirostris*. Isolation of *T. gondii* in this bird of prey species has been reported in Minas Gerais state (Rêgo et al., 2018). Gonçalves et al. (2013) had already reported the presence of *T. gondii* in this species, although it was in the central west region of the state of São Paulo.

The fact that the birds of prey species were seropositive is probably because they preyed on infected animals, and carnivorous species may have become infected by ingesting prey tissues.

These results are important because the northwest region of the state of São Paulo is a major producer of poultry products and wild fauna can contribute to the maintenance of diseases for poultry (Gonçalves et al., 2013).

Thus, monitoring the health of wild birds and mammals can contribute to the assessment of the presence of diseases of economic interest. Still, according to Gonçalves et al. (2013), in the case of carnivorous birds, because they feed on previously contaminated prey (through indirect contact), they serve as “sentinel” birds with great potential for an epidemiological reading of the studied disease.

Concerning non-reactive bird species such as *Coragyps atratus*, *Cariama cristata*, and *Nycticorax nycticorax*, the eating habits could contribute to infection by *T. gondii*, but the small number of samples did not allow for a more detailed analysis. This fact could be emphasized by other studies that identified *T. gondii* infection in the aforementioned species (Chen et al., 2015; Gennari et al., 2017; Vitaliano et al., 2014).

Regarding the seropositive mammals, the reactive specimens are carnivorous or predator species, except for *A. caraya* and *M. gouazoubira*.

In the case of the *A. caraya* species, because it is an arboreal species but has a foraging habit and feeds mainly on shoots and leaves, its infection may be attributed to environmental contamination, probably by oocysts released into the environment by infected felids. The species (*A. caraya*) has already been reported to be infected by *T. gondii* (Garcia et al., 2005).

Furthermore, the species *M. gouazoubira* is a terrestrial species and is considered predisposed to *T. gondii* infection, since the prevalence of *T. gondii* for terrestrial species was shown to be significantly higher than for arboreal animals. Also, the feeding habits of the species suggest a propensity to infection with *T. gondii* oocysts found in the environment, which are excreted from wild felids and may contaminate water sources and wash up on stream banks,

Table 2. Anti-*Toxoplasma gondii* antibodies in free-living wild mammals from the northwest region of São Paulo state, Brazil, using the modified agglutination test (MAT \geq 25)

Species	N. negative	N. positive	Total	MAT titers
Carnivora				
<i>Lycalopex vetulus</i>	1	1	2	200
<i>Chrysocyon brachyurus</i>	1	2	3	50, 100
<i>Cerdocyon thous</i>	2	2	4	200, 1600
Primates				
<i>Alouatta caraya</i>	3	1	4	25
<i>Sapajus nigritus</i>	3	0	3	<25
Artiodactila				
<i>Mazama gouazoubira</i>	1	1	2	25
Marsupialia				
<i>Didelphis albiventris</i>	2	0	2	<25
Xenarthra				
<i>Tamandua tetradactyla</i>	3	0	3	<25
Rodentia				
<i>Hydrochoerus hydrochaeris</i>	1	0	1	<25

where they may remain infectious for years (Aston et al., 2013; Carme et al., 2002).

It is important to consider that, in the present study, MAT serologic titers varied between 20 and 120 in birds and between 25 and 1600 in mammals. High titers are generally associated with recent infections. However, in this case, it is not possible to make any conclusions because just one sample was collected and antibody titers can remain high for several months (Dubey, 2010), so single titer values by MAT indicates only exposure to *T. gondii*.

The validity of serologic tests for *T. gondii* antibodies in wild birds and wild mammals is not well understood and many variables can be applied such as age, the number of animals studied, and animal species. Validation for MAT is well recognized in chickens (Dubey et al., 2016) and pigs (Dubey, 1997). Nonetheless, MAT is one of the most used serological tests for the diagnosis of toxoplasmosis in humans and other animals, particularly because it requires no conjugates (Dubey, 2010).

Using another serological test, the indirect fluorescent antibody test, Vitaliano et al. (2010) observed that experimentally infected *C. plancus* had a peak of IgG anti-*T. gondii* between 15- and 30-days post-inoculation (dpi), with a decreasing or even absence of antibody titers just around 60dpi. This could suggest that negative birds could have been previously exposed to the parasite and confirmation would only be possible by isolating the parasite from their tissues.

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The data obtained in the present study show *T. gondii* infection in several animal species in the northwest region of São Paulo state and indicate the environmental contamination of the area. Studies of this nature may help to understand the importance of the prevention and control of this disease in Brazil.

Conflict of Interest

The authors declare no conflicts of interest.

Ethics Statement

All biological material collection herein was authorized for scientific purposes by the Biodiversity Information and Authorization System (SISBIO) from the Brazilian Federal Environmental Agency (IBAMA) under certificate number 43854-4 and was approved by the Research Ethics Committees at the universities involved in this study (Rio Preto University Center [UNIRP] under authorization number 04/2014PP and São José do Rio Preto Medical School [FAMERP] under authorization number 2014/05302-8).

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