Research Note

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Toxoplasma gondii antibodies in wild rodents and marsupials from the Atlantic Forest, state of São Paulo, Brazil

Anticorpos contra *Toxoplasma gondii* em roedores e marsupiais da Mata Atlântica, estado de São Paulo, Brasil

Solange Maria Gennari[1]*; Maria Halina Ogrzewalska[2]; Herbert Sousa Soares[1]; Danilo Gonçalves Saraiva[1]; Adriano Pinter[3]; Fernanda Aparecida Nieri-Bastos[1]; Marcelo Bahia Labruna[1]; Matias Pablo Juan Szabó[4]; Jitender Prakash Dubey[5]

[1]Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo - USP, São Paulo, SP, Brasil

[2]Laboratório de Hantaviroses e Rickettsioses, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz - FIOCRUZ, Rio de Janeiro, RJ, Brasil

[3]Superintendência de Controle de Endemias, São Paulo, SP, Brasil

[4]Faculdade de Medicina Veterinária, Universidade Federal de Uberlandia - UFU, Uberlandia, MG, Brasil

[5]Animal Parasitic Diseases Laboratory, Beltsville Agriculture Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, USA

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Abstract

Toxoplasma gondii is a protozoan parasite that infects a large spectrum of warm-blooded animals, including humans. Small rodents and marsupials play an important role in the epidemiology of *T. gondii* because they are sources of infection for domestic and feral cats. Serum samples from 151 rodents and 48 marsupials, captured in the Atlantic Forest, São Paulo State, southeastern Brazil, were analyzed for the presence of *T. gondii* antibodies. Antibodies detected by the modified agglutination test (MAT ≥ 25) were found in 8.6% (13/151) of the rodents and 10.4% (5/48) of the marsupials, with titers ranging from 25 to 6400 and from 25 to 3200, respectively for the rodents and marsupials. Three of the eight species of rodents (*Akodon* spp., *Oligoryzomys nigripes* and *Rattus norvegicus*), and one from the four marsupial species (*Didelphis aurita*) presented positive animals. *T. gondii* was described for the first time in the rodent *Oligoryzomys nigripes*.

Keywords: Atlantic Forest, Brazil, marsupials, rodents, *Toxoplasma gondii*.

Resumo

Toxoplasma gondii é um protozoário parasita que infecta animais de sangue quente, incluindo seres humanos. Pequenos roedores e marsupiais têm papel importante na epidemiologia do *T. gondii*, pois são fontes de infecção para os felídeos domésticos e selvagens. Amostras de soro de 151 roedores e 48 marsupiais, capturados na Mata Atlântica, Estado de São Paulo, Sudeste do Brasil, foram analisadas para a pesquisa de anticorpos anti-*T. gondii*. Os anticorpos foram detectados pelo Teste de Aglutinação Modificada (MAT ≥ 25), com 8,6% (13/151) dos roedores e 10,4% (5/48) dos marsupiais soropositivos, com títulos variando de 25 a 6.400 e de 25 a 3.200, respectivamente, para os roedores e os marsupiais. Três das oito espécies de roedores (*Akodon* spp., *Oligoryzomys nigripes* e *Rattus norvegicus*) e uma das quatro espécies de marsupiais (*Didelphis aurita*) apresentaram animais positivos. A presença de anticorpos anti-*T. gondii* foi descrita pela primeira vez no roedor *Oligoryzomys nigripes*.

Palavras-chave: Mata Atlântica, Brasil, marsupiais, roedores, *Toxoplasma gondii*.

***Corresponding author:** Solange Maria Gennari. Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo - USP, Avenida Prof. Orlando Marques de Paiva, 87, Cidade Universitária, CEP 05508-270, São Paulo, SP, Brasil.
e-mail: sgennari@usp.br

Introduction

Toxoplasma gondii is a protozoan parasite that infects a large spectrum of warm-blooded animals, including humans (DUBEY, 2010). Rodents play an important role in the life cycle of *T. gondii* because they are one of the important sources of infection for domestic and feral cats. Studies of cat predation on wildlife suggest that rodents comprise about two-thirds of the prey consumed, although this may vary according to the season, rodent abundance, and the availability of other prey (MARSHALL et al., 2004). Antibodies to *T. gondii* in small wild rodents from Brazil were reported in *Proechimys* sp., *Rattus rattus*, *Nectomys rattus*, *Akodon cursor,* and *Thrichomys laurentius* (COSTA et al., 2012; SIQUEIRA et al., 2013). In urban area *R. rattus* presented antibodies to *T. gondii* (FERRARONI & MARZOCHI, 1980; COLA et al., 2010), and viable parasites were isolated by bioassay and detected by PCR in *Rattus norvegicus* and *Mus musculus* (MURADIAN et al., 2012).

Marsupials in Americas are considered resistant to clinical toxoplasmosis (CANFIELD et al., 1990). The occurrence of *T. gondii* antibodies in marsupials in Brazil were reported in different species of *Didelphis* and *Monodelphis* (FERRARONI & MARZOCHI, 1980; YAI et al., 2003; SIQUEIRA et al., 2013).

The Atlantic Forest is known of its biodiversity. Originally occupied 1,300,000 km^2, however, nowadays, only 7% of this area remained. Despite its devastation, the Atlantic Forest is a rich biome with about 850 species of birds and 270 species of mammals (Brasil, 2014). This study aims to investigate the occurrence of anti-*T. gondii* antibodies in wild rodents and marsupials captured in the Atlantic Forest, in the state of São Paulo, southeastern region of Brazil.

Material and Methods

The use of the wild animals was approved by the Brazilian Institute of Natural Resources (IBAMA No.22212-1) and the project was in accordance with the Ethical Principles in Animal Research by Brazilian College of Animal Experimentation.

Study area

This study encompassed eight localities: three forest areas in the southern part of the São Paulo metropolitan area (SPMA), within the municipalities of São Bernardo do Campo, Diadema and Santo André; three forest areas in the northern part of the SPMA (municipalities of Arujá, Mairiporã and Nazaré Paulista), one forest area (Cantareira State Park) in the northern part of the SPMA, within São Paulo municipality. The detailed description of these areas is found elsewhere (OGRZEWALSKA et al., 2012). The last area was located in the municipality of Peruíbe, the largest area (79,830 ha) of preserved forest represented by an Ecological Reserve (Estação Ecológica de Juréia-Itatins). The Reserve is located by the seaside, 20 m above sea level and all other areas are located between 765 and 1000 m above sea level, and as a Reserve area have a governmental protection and very few contact with humans and domestic animals. All the areas have a subtropical climate (average annual temperature of 18°C, rain index of 1400 mm).

Collection of samples

Inside the forest fragment the trapped animals were anaesthetized (1 mL/Kg ketamine chloride via intramuscular), identified to species (BONVICINO et al., 2008; PAGLIA et al., 2012), submitted to blood collection, marked with metal rings with individual number, and released at the capture site. Blood samples were taken from mandibular (rodents) and tail (marsupials) veins (OGRZEWALSKA et al., 2012). Samples were collected from April 2010 to January 2011.

Diagnostic test

Sera were tested for the presence of antibodies to *T. gondii* by modified agglutination test (MAT) as previously described (DUBEY & DESMONTS, 1987). Sera were screened at 1:25 (SIQUEIRA et al., 2013; YAI et al., 2003) and the positive samples were end titrated using 2-fold serial dilutions.

Results and Discussion

Samples were obtained from 151 rodents and 48 marsupials, from eight rodent and four marsupial species. The occurrence of *T. gondii* antibodies was 8.6% (13/151) for rodents and 10.4% (5/48) for marsupials. From the rodents, three different species were positive: *Akodon* spp. (10.5%, 11/105), *Oligoryzomys nigripes* (5.5%, 1/18), *Rattus norvegicus* (100%, 1/1), and from the marsupials only the species *Didelphis aurita* had *T. gondii* antibodies (12.5%, 5/40). Rodents were captured in all investigated areas, however, the positive animals were only found in the Municipality of Arujá (5.5%), Santo André (21.7%) and São Bernardo do Campo (17.5%). Marsupials were captured in six investigated areas and positive animals were observed in Municipality of Diadema (25%), São Bernardo do Campo (16.6%) and São Paulo (60%). Table 1 presents the occurrence of antibodies to *T. gondii* per rodent and marsupial species per municipality.

Antibody titers ranged from 25 to 6400 for the rodents and from 25 to 3200 for the marsupials (Table 2).

The occurrence of *T. gondii* antibodies in marsupials in Brazil were also previously reported in *Didelphis marsupialis* and *Didelphis albiventris,* captured in the Brazilian Savanna (YAI et al., 2003), and in *Didelphis aurita*, *Monodelphis domestica*, *Metachirus nudicaudatus*, *Marmosa murina*, *Marmosa demerarae* and *Marmosa* sp. collected in northeastern region of the Atlantic Forest, in the state of Pernambuco (FERRARONI & MARZOCHI, 1980; SIQUEIRA et al., 2013).

Toxoplasma gondii antibodies are reported here for the first time in the rodent *Oligoryzomys nigripes*. This species is abundant in the Atlantic Forest and Araucaria forest areas throughout Southern Brazil and the Southern part of the gallery forests of the Cerrado, extending to the Pampas on the southern edge of Brazil. This species has a generalist habit being found within

Table 1. Occurrence (%) (examined/positive) of anti-*Toxoplasma gondii* antibodies in rodent and marsupial species per municipality. State of São Paulo, 2010-2011, Brazil.

SPECIES	MUNICIPALITIES %								
	S. B. do Campo	Diadema	Santo André	Arujá	Mairiporã	Nazaré Paulista	São Paulo	Peruíbe	(Total/ Positive)
RODENTS									
Akodon spp.	17.6 (34/6)	(8/0)	22.2 (18/4)	7.6 (13/1)	(6/0)	(6/0)	(12/0)	(8/0)	10.5 (105/11)
Oligoryzomys nigripes	16.6 (6/1)	(4/0)	(4/0)	(2/0)	0	0	(2/0)	0	5.5 (18/1)
Oecomys spp.	0	0	0	0	0	0	0	(1/0)	(1/0)
Oxymycterus spp.	0	0	0	0	0	0	0	(2/0)	(2/0)
Euryoryzomys russatus	0	0	0	(2/0)	0	(1/0)	(2/0)	(17/0)	(22/0)
Hylaeamys megacephalus	0	0	0	(1/0)	0	0	0	0	(1/0)
Rattus norvegicus	0	0	100 (1/1)	0	0	0	0	0	100 (1/1)
Nectomys squamipes	0	0	0	0	0	0	(1/0)	0	(1/0)
TOTAL	17.5 (40/7)	(12/0)	21.7 (23/5)	5.5 (18/1)	(6/0)	(7/0)	(17/0)	(28/0)	8.6 (151/13)
MARSUPIALS									
Didelphis aurita	16.6 (6/1)	(4/1)	(3/0)	0	(5/0)	0	60 (5/3)	(17/0)	12.5 (40/5)
Monodelphis spp.	0	0	0	0	0	0	0	(3/0)	(3/0)
Metachirus nudicaudatus	0	0	0	0	0	0	0	(3/0)	(3/0)
Micoureus demerarae	0	0	0	0	0	0	0	(2/0)	(2/0)
TOTAL	16.6 (6/1)	25 (4/1)	(3/0)	(0/0)	(5/0)	0	60 (5/3)	(25/0)	10.4 (48/5)

Table 2. Occurrence of antibodies to *Toxoplasma gondii* and antibody titer in rodent and marsupial species. São Paulo state, 2010-2011, Brazil.

Species	Tested	Positive	%	MAT titer (No. positives)
RODENTS				
Akodon spp.	105	12	11.4	25 (8), 50 (1), 100 (1), 1600 (1), 6400 (1)
Oligoryzomys nigripes	18	1	5.5	25 (1)
Oecomys spp.	1	0	*	*
Oxymycterus spp.	2	0	*	*
Euryoryzomys russatus	22	0	*	*
Hylaeamys megacephalus	1	0	*	*
Rattus norvegicus	1	1	100.0	100 (1)
Nectomys squamipes	1	0	*	*
MARSUPIALS				
Didelphis aurita	40	5	12.5	25 (2), 50 (1), 200 (1), 3200 (1)
Monodelphis spp.	3	0	*	*
Metachirus nudicaudatus	3	0	*	*
Micoureus demerarae	2	0	*	*
Total	**199**	**19**	**9.5**	

*Negative.

forests and open vegetation formations and has a great capacity for adaptation to anthropic environments, including agricultural areas dwellings and barns entering in contact with domestic animals (PÜTTKER et al., 2008; DALMAGRO & VIEIRA, 2005) which make this specie important in the epidemiology of *T. gondii*.

Although 52% of all marsupials represented by all four species were captured in the Ecological Reserve, at Peruíbe, none had antibodies to *T. gondii*. Similar results were observed for the rodents, with all the 25 rodents from the Reserve been seronegative to *T. gondii*. In the Reserve exist various species of wild cats, *Leopardus pardalis, Puma concolor, Panthera onca, Felis pardalis, Puma yagouaroundi* (PARDINI & DEVELEY, 2004; MARTINS et al., 2008), that might play role in the zoonotic cycle of *T. gondii* being the define host, as suggested by Cañón-Franco et al. (2013). However the absence of positive animals indicate that this environment is on equilibrium and emphasizes the importance of the anthropogenic pressure on the Atlantic rainforest, since all positive animals were captured on areas under high anthropogenic effect, where interactions between small mammals and domestic cats are easily observed. Such impact should be further investigated.

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