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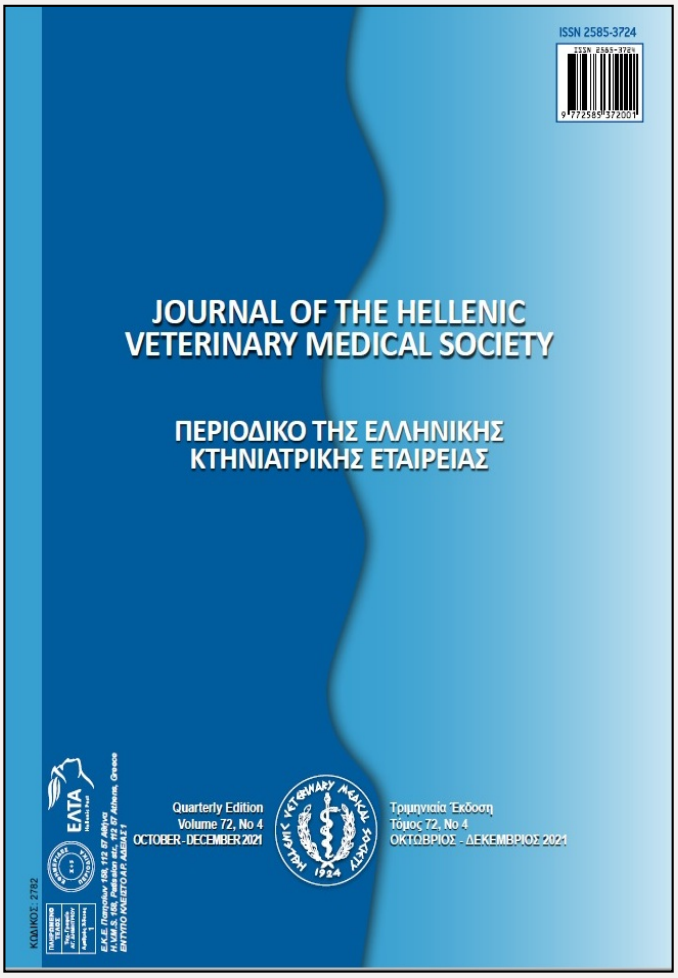

Detection of antibodies to Toxoplasma gondii in neotropical primates from São Paulo state, Brazil

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Research article
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Detection of antibodies to *Toxoplasma gondii* in neotropical primates from São Paulo state, Brazil

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ABSTRACT: Toxoplasmosis is a life-threatening disease in neotropical primates. The aim of the present study was to determine the frequency of antibodies to *Toxoplasma gondii* in neotropical primates from São Paulo state (SP), Brazil. The modified agglutination test (MAT, cut-off: 25) was used in 49 neotropical primates upon or after their admission to Associação Mata Ciliar (Jundiaí, SP, Brazil). Eight of the 49 animals (16.3%) were seropositive. The genus *Sapajus* had the highest antibody titer (12,800), followed by the genus *Callithrix* (3,200). No association ($p > 0.05$) was found between seroprevalence and genera (*Alouatta*, *Callicebus*, *Callithrix*, and *Sapajus*), sex or age. The three positive primates of the genera *Allouata*and the one of the genera C*allithrix* died, whereas the two seropositive *Sapajus* were alive. Further studies on the epidemiology of *T. gondii* infection are necessary in a larger sample size of captive and wild neotropical primates.

Keywords: Brazil; MAT; neotropical primates;*Toxoplasma gondii*

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INTRODUCTION

Toxoplasmosis is a zoonotic disease with worldwide distribution affecting homeothermic animals, including humans. Its aetiological agent, *Toxoplasma gondii*, is an obligatory intracellular protozoan parasite, having wild and domestic felids as definitive hosts. Intermediated hosts are mainly infected by ingestion of food or water contaminated with oocysts or by consumption of infective tissue cysts in meat or viscera (Tenter et al., 2000; Dubey, 2010). In some areas of Brazil *T. gondii* is estimated to have one of the highest human seroprevalences (Sepúlveda-Arias et al., 2014).

In New World primates, toxoplasmosis is a critical and life-threatening disease (Salant et al., 2009), and according to Catão-Dias et al. (2013) it is the most prevalent cause of death from acute infectious disease in captive neotropical primates. Wild animals may remain in rescue and recovery centers for long periods or indefinitely and are subject to stressful situations which can lead to a weakened immune system, thus becoming more susceptible to opportunistic infections and diseases including fatal toxoplasmosis (Gyimesi et al., 2006; Bouer et al., 2010). The aim of the present study was to assess seroprevalence of *T. gondii* in sera of neotropical primates at or after admission to Associação Mata Ciliar (AMC), a Rescue Center located in São Paulo state (SP), Brazil.

MATERIALS AND METHODS

Geographical area of the study and samples

This study was developed at AMC, in the city of Jundiaí, SP, Brazil. AMC was established in 1987 with the purpose of welfare and conservation of animal species as well as their habitat. Between April 2015 and December 2017, blood samples were collected from 49 neotropical primates from SP upon (n = 29 free-living) or from 1 week after (n = 20 captive) admission to AMC. Information on genus (taxonomy), sex (female or male) and age group (juvenile or adult) were also obtained. After centrifugation at 10,000 rpm for 3 minutes, serum samples were stored at -20 °C until analysis.

All samples used were surplus of clinical sera. All procedures within the scope of this study followed the ethical standards of the relevant national and institutional guidelines.

Serological testing for antibodies to *T. gondii*

Serum samples were assayed for *T. gondii* IgG antibodies by the modified agglutination test (MAT, cut-off: 25), as described by Dubey& Desmonts (1987). Samples were tested in serial dilutions on the basis two, starting at 1:25 (Valentini et al., 2004), and positive and negative control samples were included in each testing plate.

Data analysis

Exact binomial 95% confidence intervals (CI) were established for prevalence values. The Fisher's exact test was used to compare seroprevalence values between categories of the independent variables genus, sex and age. Pairwise comparisons between categories of the same independent variable incorporated Bonferroni's correction (Petrie and Watson, 2013). A probability (p) value < 0.05 was considered as statistically significant. Analyses were performed with the SPSS 26.0 program for Windows.

RESULTS

Eight (16.3%; CI: 7.3–29.7) out of 49 neotropical primates were seropositive to *T. gondii*, 5 (10.2%) had a titer of 25 (2 *Alouatta guariba*, 2 *Callicebus personatus* and 1 *Sapajusapella*); 1 (2.0%) a titer of 50(*A. guariba*); 1 (2.0%) a titer of 3,200 (*Callithrix penicillate*); and 1 (2.0%) a titer of 12,800 (*S.apella*).

Of the seropositive animals 62.5% (5/8) entered AMC due to urban rescue, 25% (2/8) were run over by vehicles and 12.5% (1/8) were free-living orphans.

Five of the 8 seropositive animals (62.5%) presented physical alterations. Sixty percent (3/5) of these animals had prostration or apathy, with one of them also presenting foamy nasal discharge, dyspnea and bloating, and another one having jaundice. However, 40% (2/5) of these animals did not show clinical signs compatible with toxoplasmosis, as one animal had a spine fracture and the other one a mandible fracture. No association was found between seroprevalence and all the variables analyzed (Table 1).

DISCUSSION

The frequency of primates positive to antibodies to *T. gondii* at or from 1 week after the admission in the AMC was 16.3% (IC 7.3-29.7).In neotropical primates, toxoplasmosis poses a conservation concern, since it may be the cause of acute disease and high mortality (Casagrande et al., 2013; Pardini et al., 2015; Nishimura et al., 2019). Clinical manifestations include general malaise, dyspnea, hypothermia, foamy or serosanguinolent nasal secretion, and abdominal distension (Epiphanio et al., 2003; Casagrande et al., 2013).

Table 1. *Toxoplasma gondii* infection in neotropical primates admitted to Associação Mata Ciliar, Brazil, according to genus, gender and age

Variable/categories	Nº of primates tested (%)	Number of seropositive (%)	CI (%)
Genus/species			
Alouatta guariba	31 (63.3)	3 (9.7)	2.0-25.7
Callicebus personatus	4 (8.2)	2 (50.0)	6.8-93.2
Callithrix spp.	12 (24.5)	1 (8.3)	0.2-38.5
Sapajus apella	2 (4.1)	2 (100)	15.8-64.3
		$p \geq 0.114^{*}$	
Sex			
Female	18 (36.7)	2 (11.1)	1.4-34.7
Male	27 (55.1)	5 (18.5)	6.3-38.1
ND	4 (8.2)	1 (25.0)	0.6-80.6
		$p = 0.684^{\dagger}$	
Age			
Juvenile	11 (22.5)	1 (9.1)	0.2-41.3
Adult	36 (73.5)	7 (19.4)	8.2-36.0
ND	2 (4.1)	0 (0.0)	0.0-84.2
		$p = 0.659^{\S}$	
Total	49 (100)	8 (16.3)	7.3-29.7

*pairwise comparisons incorporating Bonferroni's correction (i.e. multiplying each *p* value by the number of comparisons; ND category not included); †calculated only between female and male; §calculated only between juvenile and adult; CI: 95% confidence interval; ND: not determined.

Information on *T. gondii* infection in neotropical primates in Brazil is limited, with most reports from captive animals (Epiphanio et al., 2003; Antoniassi et al., 2011; Minervino et al., 2017). In addition to high susceptibity of some species of primates to clinical toxoplasmosis, they can act as sentinels to *T. gondii* infection (Leite et al., 2008).

In all genera of primates surveyed here, at least one animal was seropositive. Although seroprevalence was higher in genus *Sapajus*, no statistically significant difference was detected. According to Garcia et al. (2005) and regarding wild animals, the genus *Sapajus* (formerly *Cebus*) presented higher seroprevalence than the genus *Alouatta*. This higher value may be justified by the more terrestrial behavior of those animals, which increases the probability of contact with *T. gondii* sporulated oocysts. A lower value (9.7%) in the genus *Alouatta* is probably due to their arboreal way of life and to the essentially folivorous-frugivorous food.

Also by using the MAT, but in captive animals, Minervino et al. (2017) found a higher occurrence (52.2%) in New World non-human primates from the Amazon region. On the other hand, no antibodies to *T. gondii* were found in recently captured neotropical primates from Niterói, Rio de Janeiro state, Brazil studied by Molina et al. (2017). Hence, the scarcity of information on the prevalence of *T. gondii* in free-living primates and the use of different methods to diagnose specfic antibodies, makes it difficult to compare the results of the present study with other published studies worldwide (reviewed by Dubey, 2010).

In the genus *Callithrix* a seronegativity of 91.7% suggests that these animals had no contact with *T. gondii* or died of acute toxoplasmosis, with no time to produce IgG antibodies (Catão-Dias et al., 2013). The causes of mortality of the animals that arrived dead or that died at AMC have not been confirmed and therefore we cannot infer about the real pathogenic role of *T. gondii*.

Primates can be sentinels for human infection, as they have access to human sites and eat the same food potentially contaminated with soil oocysts. In addition, in some geographical areas, primates are hunted and used as food themselves, which represents another mode of transmission (da Silva et al., 2014).

CONCLUSIONS

Considering the life cycle of *T. gondii* and the high susceptibility of neotropical primates, it is relevant to emphasize the need for strict sanitary measures to protect captive animals that may be under the threat of extinction. Fighting for the conservation of these primates is imperative, since they are subject to sev-

eral diseases that are potentially fatal to them and can drastically reduce their populations(Santos et al., 2014).

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