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Short communication

Prevalence of antibodies against *Toxoplasma gondii* among Brazilian white-eared opossums (*Didelphis albiventris*)

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ABSTRACT

Considering that *Toxoplasma gondii* is a parasite of global importance which affects several animal species including humans, the current study aimed to investigate the prevalence of antibodies against *T. gondii* among 72 white-eared opossums (*Didelphis albiventris*) from Botucatu Municipality (22°53′S 48°26′W), São Paulo State, Brazil. The investigation was carried out from January 2008 to December 2009, when the animals had their blood samples collected and subjected to the modified agglutination test (MAT); 12 specimens had brain samples bioassayed in mice. Seroprevalence was 5.5% ($n = 4$) and bioassays were negative. Older animals had higher prevalence of antibodies against *T. gondii*. Opossums in closer contact with the urban environment are likely more exposed to *T. gondii* than animals from the sylvatic environment.

1. Introduction

Toxoplasma gondii is an intracellular parasite of worldwide distribution which can infect several animal species including humans. In general the infection does not progress to disease, but severe clinical signs eventually develop. Considering humans, the importance of *T. gondii* is great when affecting immunosuppressed individuals or pregnant women, occurring problems like encephalitis, eye disorders and mental retardation (Tenter et al., 2000). In animals, toxoplasmosis can be also responsible for abortion and death, especially in pigs, sheep and goats, leading to losses in animal production (Dubey, 2009a,b).

Once infected, several wild animal species can sporadically develop toxoplasmosis. However, two groups of animals are highly sensitive to *T. gondii*: neotropical primates and Australian marsupials (Silva, 2006), to which toxoplasmosis is lethal. Thus, preventive measures are essential in conservation programs involving these animals. The study of toxoplasmosis in wild animals can also provide information related to the epidemiology of *T. gondii* in different environments. Another important aspect is wild animal hunting and consumption, which may represent a route of infection for *T. gondii* in humans (Tenter et al., 2000). Thus, studies involving toxoplasmosis in wild animals are important for both human and animal health.

The white-eared opossum (*Didelphis albiventris*) is a marsupial that inhabits the largest part of South America and is able to adapt to different types of biotopes (Vieira, 2006; Cerqueira and Tribe, 2007). It can develop synantropic habits, living in urban areas in close contact

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with domestic animals and humans. Therefore, white-eared opossum can be a good indicator of environmental contamination by *T. gondii*. Hunting and consumption of wild animals, including opossums, are frequent in small cities in Brazil, and these animals could be a source of infection (Silva et al., 2008). Previous studies have reported infection by *T. gondii* in American marsupials (Ferraroni and Marzochi, 1980; Hill et al., 1998; Smith and Frenkel, 1995; Thoisy et al., 2003; Mitchell et al., 2006; Dubey et al., 2009), especially those of the genus *Didelphis*, but only a few works included South American species.

A current problem involving studies with marsupials of the *Didelphis* genus is species identification. According to Cerqueira and Tribe (2007), there are five species of this genus in South America: white-eared opossum (*D. albiventris*), big-eared opossum (*Didelphis aurita*), common opossum (*Didelphis marsupialis*), Andean white-eared opossum (*Didelphis pernigra*) and Guianan white-eared opossum (*Didelphis imperfecta*). These species are very similar morphologically, which frequently leads to misidentification. This can be observed in studies describing a species that does not occur in the cited area. Thus, the literature must be carefully analyzed when the study subject is opossum. In São Paulo State, there are only two species, white-eared opossum and big-eared opossum, which can be easily identified by the color of the fur and ears. The present study aimed to investigate *T. gondii* infection among white-eared opossums.

2. Materials and methods

The study was carried out in Botucatu Municipality (22°53′S 48°26′W, 804 m altitude, 20.7 °C mean temperature, 1358.6 mm rainfall/year), São Paulo State, Brazil. The animals were divided into two different groups according to their origin. Group A was constituted of opossums from the urban area, while Group B was constituted of opossums from a protected forest area located in a ~1200 ha farm, both in Botucatu Municipality.

Group A animals were sent to the Center for Medicine and Research of Wild Animals (CEMPAS), School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, between January 2008 and December 2009. They were manually captured by the Department of Environmental Surveillance, Botucatu Municipal Government, or delivered by local citizens who had found the animal in their own house or wounded on the streets. Group B animals were captured by using live and pitfall traps, with bananas as bait. A total of 12 captures of five days a month were performed between July 2008 and July 2009.

The animals of both groups were anesthetized with an intramuscular injection of ketamine (100.0 mg/mL, Dopalen®, Vetbrands Group Ltd., Jacareí, Brazil) and xylazine (20.0 mg/mL, Anasedan®, Vetbrands Group Ltd., Jacareí, Brazil), at the doses of 40.0 mg/kg and 4.0 mg/kg, respectively. Blood samples were collected by intracardiac puncture or from the caudal vein. After centrifugation, the serum was stored at −20 °C until analysis. Animal age was classified according to Macedo et al. (2006), who established four categories for didelphid marsupials based on their tooth eruption: newborn, mother-dependent (class I); sexually immature subadults (class II); sexually mature subadults (class III); and adults (class IV). After completely recover from anesthesia, the animals were released in the wild.

Serum samples were tested through modified agglutination test (MAT) with formalin-fixed tachyzoites, produced on the laboratory's own premises, in 30 day-old female Swiss mice inoculated with RH strain and Sarcoma TG-180 cells (ATCC CCRFS-180 II), as described by Desmonts and Remington (1980). The samples were first tested at 1:25 and 1:100 dilutions. Positive samples were further tested at 2-fold dilutions until the final titer. Positive and negative controls were used, and animals showing titers equal to or higher than 25 were considered positive. There is no information about MAT sensitivity and specificity for toxoplasmosis diagnosis in opossums (Yai et al., 2003) however, Patton and Funk (1992) reported that the MAT worked well to measure antibodies against *T. gondii* in a similar species of opossum (*Didelphis virginiana*).

Brain samples from dead or euthanized opossums were bioassayed in mice. The entire brain (approximately 2 g) of each animal was triturated, digested in acid pepsin, neutralized with bicarbonate, washed and subcutaneously inoculated into 10 adult female Swiss mice (1 mL/mouse). Forty-five days after inoculation, blood samples of each mouse were collected from the retrorbital sinus, and the serum was processed using the MAT as previously described. The bioassay was considered positive if at least one mouse presented titer equal to or higher than 25.

Serological results and animal data were analyzed through Chi-square or Fischer's Exact Test by using Epi-Info 3.5.1. software. Results were considered significant when $P \leq 0.05$.

3. Results

A total of 72 white-eared opossums were used: 60 in Group A and 12 in Group B. As regards age and sex, the number of animals in each group is shown in Table 1. There were no data recorded for eight animals, which were then excluded from the statistical analysis. The 10 class-I animals were from 2 different mothers and the latter could not be sampled because they were found dead. The prevalence of seropositive animals was 5.55% ($n = 4$), all from Group A (Table 1), with titers ranging from 25 to 100. Of these four animals, two were male and two female; three were class IV and the remaining one was class III. Class III opossums had higher prevalence of antibodies. For mouse bioassay, 12 white-eared opossums were studied, 11 from Group A and one from Group B. Of these 12 animals, only one was positive in the MAT (titer 25). All bioassays were negative.

4. Discussion

The capture of only 16 animals in the forest was unexpected (four were not sampled thus, twelve were used). Either the population density for white-eared opossums in the studied area is low, or the adopted method for the captures was not efficient. The population of white-eared opossum is likely larger in the urban area than in the wild,