

Presence of anti-*Toxoplasma gondii*, -*Neospora caninum*, -*Leishmania* spp. and -*Ehrlichia canis* antibodies in free-ranging maned wolves (*Chrysocyon brachyurus*) in the northeastern region of the state of São Paulo, Brazil

Presença de anticorpos anti-*Toxoplasma gondii*, -*Neospora caninum*, -*Leishmania* spp. e -*Ehrlichia canis* em lobos-guará (*Chrysocyon brachyurus*) de vida livre na Região Nordeste do Estado de São Paulo, Brasil

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Abstract

The maned wolf (*Chrysocyon brachyurus*), an inhabitant of the Brazilian savanna, is considered the largest canid of South America and is classified as a near-threatened species by the International Union for Conservation of Nature (IUNC). The purpose of this study was to investigate the presence of antibodies against *Toxoplasma gondii*, -*Neospora caninum*, -*Leishmania* spp., and -*Ehrlichia canis* in free-ranging maned wolves in the northeastern region of the state of São Paulo, Brazil. Of the 17 serum samples tested by the indirect fluorescent antibody test (IFAT), 88.2% (15/17), 17.6% (3/17) and 52.9% (9/17) showed anti-*T. gondii*, -*Leishmania* spp., and -*E. canis* antibodies, respectively. None of the studied maned wolves tested positive for *N. caninum* antibodies. Our results indicated the exposure of free-ranging maned wolves to the agents in question. The presence of industrial complexes, extensive agriculture and habitat fragmentation in the northeastern region of the state of São Paulo puts these wild animals in proximity to urban areas, possibly contributing to the transmission of diseases between wild and domestic animals and human beings.

Keywords: Maned wolf. *Toxoplasma gondii*. *Neospora caninum*. *Leishmania* spp.. *Ehrlichia canis*.

Resumo

O lobo-guará (*Chrysocyon brachyurus*) habita o ecossistema de Cerrado e é considerado o maior cánido da América do Sul e uma espécie ameaçada de extinção pela "International Union for Conservation of Nature" (IUNC). O objetivo desse estudo foi investigar a presença de anticorpos anti-*Toxoplasma gondii*, -*Neospora caninum*, -*Leishmania* spp. e -*Ehrlichia canis* em lobos-guará da região nordeste do estado de São Paulo, Brasil. Das 17 amostras de soro testadas por meio da reação de imunofluorescência indireta (RIFI), 88,2% (15/17), 17,6% (3/17) e 52,9% (9/17) apresentaram anticorpos anti-*T. gondii*, -*Leishmania* spp. e -*E. canis*, respectivamente. Todos os animais testados foram soronegativos para *N. caninum*. Esses resultados indicam a exposição dos lobos-guará dessa região aos agentes pesquisados. A presença de um complexo industrial, agricultura extensiva e fragmentação de habitat na região nordeste do estado de São Paulo, favorece a proximidade desses animais silvestres a ambientes urbanos o que pode contribuir para a transmissão de doenças entre os animais silvestres, domésticos e o homem.

Palavras-chave: Lobo-guará. *Toxoplasma gondii*. *Neospora caninum*. *Leishmania* spp.. *Ehrlichia canis*.

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Introduction

The maned wolf (*Chrysocyon brachyurus*) is considered the largest canid of South America and inhabits the Brazilian savanna. The species is classified as “near threatened” by the International Union for Conservation of Nature (IUCN) in the Red List of 2008 (RODDEN et al., 2008) and “vulnerable” by the Brazilian Institute of the Environment and Renewable Natural Resources [Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, IBAMA] (CHIARELLO et al., 2008).

Toxoplasma gondii and *Neospora caninum* are intracellular protozoan parasites that can infect a large variety of hosts. Felids and some canids are the definitive hosts of *T. gondii* and *N. caninum*, respectively (DUBEY; BEATTIE, 1988; DUBEY, 2013). *T. gondii* has been isolated from many species of domestic and wild animals (DUBEY, 2010), unlike *N. caninum*, for which the isolation of viable parasites is rare (DUBEY; SCHARES, 2011).

Visceral leishmaniasis (VL) is a zoonotic disease caused by *Leishmania (Leishmania) infantum chagasi* and mainly transmitted by the phlebotomine *Lutzomyia longipalpis* (SANTOS et al., 1998). A large diversity of mammals can be infected by *Leishmania* spp., whereas domestic dogs are the main reservoir of *L. infantum chagasi* in the domestic environment. Wild canids such as the crab-eating fox (*Cerdocyon thous*), hoary zorro (*Lycalopex vetulus*), bush dog (*Speothos venaticus*) and maned wolf may play a role in VL epidemiology in the wild environment

(FIGUEIREDO et al., 2008; LUPPI et al., 2008; FERREIRA et al., 2013).

Ehrlichia canis is the primary etiological agent of canine monocytic ehrlichiosis (CME), a tick-borne disease transmitted by *Rhipicephalus sanguineus*. This multisystemic disease manifests itself in acute, subclinical and chronic forms (YU et al., 2007; STICH et al., 2008). *E. canis* may affect domestic dogs around the world (YU et al., 2007).

Anti-*T. gondii*, -*N. caninum* or -*Leishmania* sp. antibodies have been reported in wild and/or captive maned wolves in Brazil by few serological surveys (VITALIANO et al., 2004; CURI et al., 2006; CURI et al., 2010). To complement these results, the purpose of this study was to investigate the presence of antibodies against *T. gondii*, *N. caninum*, *Leishmania* spp. and *E. canis* in free-ranging maned wolves in the northeastern region of the state of São Paulo, Brazil.

Material and Methods

Ethics committee

All animals were handled in accordance with protocols approved by the Brazilian Institute of Natural Resources and Environment (IBAMA; Process n. 02027.005551/00-86).

Study area and sample collection

The study area was the northeastern region of the state of São Paulo, Brazil, including the Jataí Ecological Station in Luíz Antônio County. The maned wolves were trapped from 2000 to 2003.

An investigative survey conducted with residents around the study area was performed to identify the presence of the maned wolf and to determine the trails used by the animals. The locations where animals were trapped are shown in figure 1. Modified traps were placed along the trails (DIETZ, 1984). All 17 trapped animals (nine males and eight females) were anesthetized by intramuscular injection using a blowgun and homemade darts loaded with a combination of tiletamine hydrochloride and

zolazepam hydrochloride (Zoletil® 100, Virbac®), at 7.0 mg/Kg as described by Novaes (1997).

Blood samples were collected from the cephalic vein, and serum samples were separated by centrifugation and kept frozen at -20°C until the

serological analyses. After sample collection, the animals were kept inside the traps until they had completely recovered from anesthesia, and then released to their habitat.

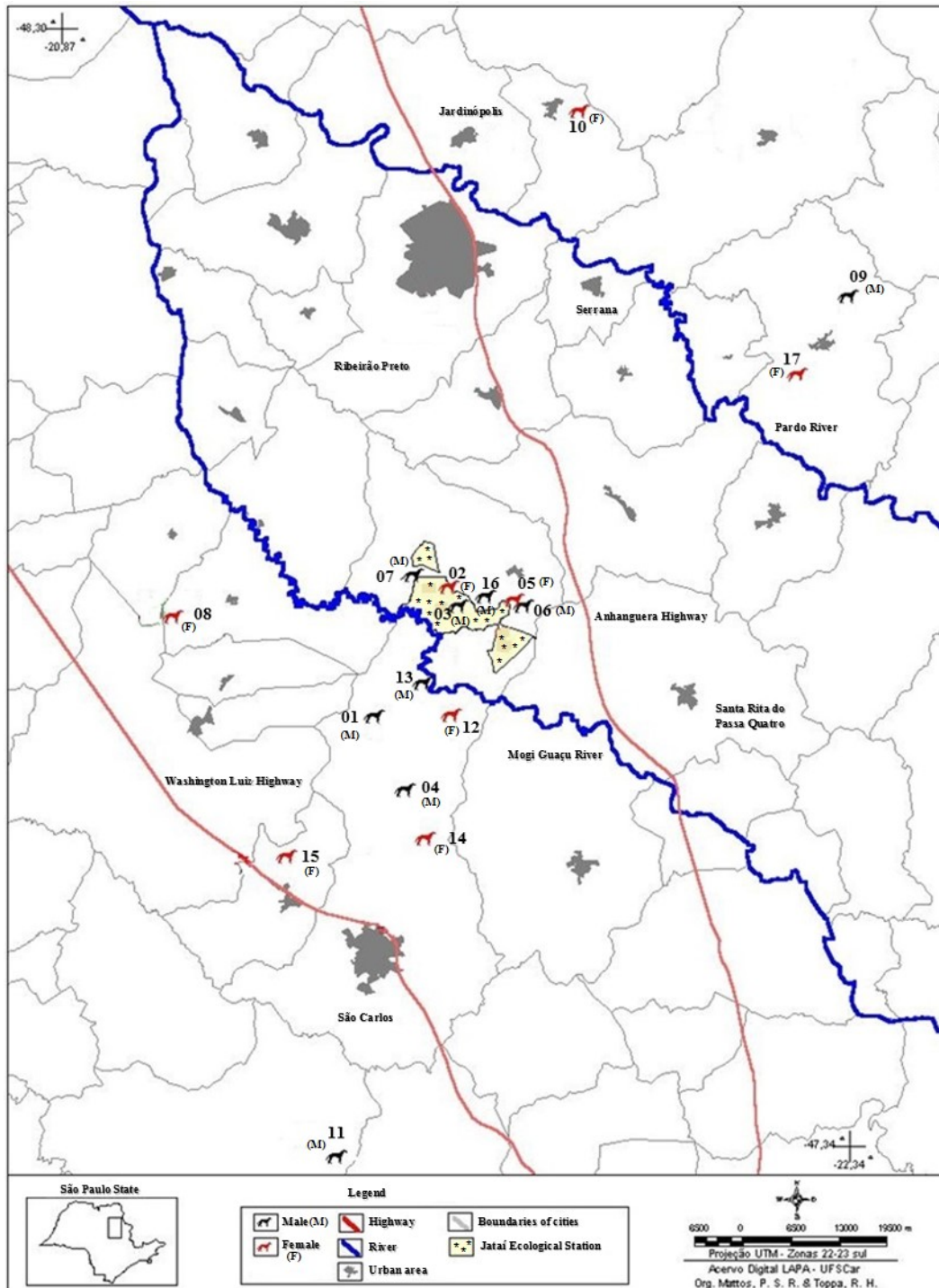


Figure 1 – Places of the capture and identification of free-ranging maned wolves (*Chrysocyon brachyurus*) in the northeastern region of the state of São Paulo, Brazil
Source: (MATTOS, 2003)

Serological analysis

Serum samples were tested using the indirect fluorescent antibody test (IFAT) to detect antibodies against tachyzoites of *T. gondii* (titers ≥ 16), tachyzoites of *N. caninum* (≥ 50), promastigotes of *Leishmania* sp. (≥ 40), or *E. canis*-infected DH82 cells (≥ 80). The RH strain of *T. gondii*, the NC-1 strain of *N. caninum*, strain 154 of *L. amazonensis*, and strain 153 of *L. infantum chagasi* (from the Brazilian Collection of Trypanosomatids [Coleção Brasileira de Tripanossomatídeos]), and the São Paulo strain of *E. canis* (AGUIAR et al., 2008) were used as antigens. Positive and negative controls from domestic dogs were used in each slide. Heterologous conjugate anti-dog IgG labeled with fluorescein isothiocyanate (FITC) (Sigma-Aldrich®, St. Louis, MO, USA) was used as described by Silva et al. (2005).

Results

The presence of antibodies against *T. gondii*, *N. caninum*, *Leishmania* spp., or *E. canis* observed in maned wolves from the northeastern region of the state of São Paulo is summarized in table 1. Of the 17 maned wolves tested, 15 showed antibodies against at least one of the agents under investigation. Anti-*T. gondii* antibodies were detected in 88.2% (15/17) of the animals, anti-*Leishmania* sp. in 17.6% (3/17), anti-*E. canis* in 52.9% (9/17), and all maned wolves tested seronegative for *N. caninum* (0/17). Cross-reaction between anti-*L. amazonensis* and -*L. infantum chagasi* antibodies is common (VALE et al., 2009), and seropositive results only for the genus (*Leishmania* spp.) were considered in this study.

Table 1 – Titers of antibodies against *Toxoplasma gondii*, *Neospora caninum*, *Leishmania* spp., and *Ehrlichia canis* from free-ranging maned wolves (*Chrysocyon brachyurus*) from the northeastern region of the state of São Paulo – Brazil – 2015

Animal ID	Gender	Titers			
		Anti- <i>T. gondii</i>	Anti- <i>N. caninum</i>	Anti- <i>Leishmania</i> spp.	Anti- <i>E. canis</i>
1	M	256	<50	80	80
2	F	32	<50	40	80
3	M	128	<50	80	160
4	M	128	<50	<40	160
5	F	256	<50	<40	160
6	M	128	<50	<40	320
7	M	256	<50	<40	<80
8	F	128	<50	<40	80
9	M	128	<50	<40	≥ 1280
10	F	512	<50	<40	80
11	M	128	<50	<40	<80
12	F	128	<50	<40	<80
13	M	1024	<50	<40	<80
14	F	<16	<50	<40	<80
15	F	256	<50	<40	<80
16	M	<16	<50	<40	<80
17	F	256	<50	<40	<80

M = male, F = female

All animals seropositive to *Leishmania* spp. or *E. canis* were also seropositive to *T. gondii*. Of all the animals seropositive for *T. gondii*, six were

seropositive only for *T. gondii* (40%, 6/15), six for *T. gondii* and *E. canis* (40%, 6/15) and three for *T. gondii*, *Leishmania* spp. and *E. canis* (20%, 3/15).

Discussion

In the present study, antibodies against *T. gondii* were found in most of the free-ranging maned wolves tested. Of the animals, 88.2% (15/17) were seropositive for *T. gondii*, while none was seropositive for *N. caninum*. Our results were similar to those of other serological surveys. Curi et al. (2012) and Proença et al. (2013) found 75% (6/8) and 100% (3/3) of seropositive results for *T. gondii* (modified agglutination test, MAT) in free-ranging maned wolves from the state of Minas Gerais and the Federal District, Brazil, respectively. In both studies, all animals were seronegative for *N. caninum* (IFAT). However, anti-*N. caninum* antibodies (IFAT) were detected in captive maned wolves in zoos in the Czech Republic (SEDLÁK; BÁRTOVÁ, 2006) and Brazil (MATTOS et al., 2008), with occurrence of 16.6% (1/6) and 28.6% (4/14), respectively. As part of the management, captive carnivorous animals are fed raw meat that could be a source of *T. gondii* and *N. caninum* infection. The raw meat should be previously frozen to reduce the risk of infection, as suggested by Silva et al. (2007).

The presence of high levels of antibodies against *T. gondii* indicates exposure of these maned wolves to different sources of *T. gondii*, such as environmental contamination by sporulated oocysts and prey infected with tissue cysts. The diet of the maned wolf is variable and fluctuates according to season, availability of food, and location (MOTTA-JUNIOR et al., 1996; RODRIGUES et al., 2007). Rodents, birds, and wolf's fruit (*Solanum lycocarpum*) are the main components of the diet, but occasionally small and medium-sized mammals can also become prey (QUEIROLO; MOTTA-JUNIOR, 2007). When capturing the maned wolves in this study, two of the animals (IDs 8 and 9) were seen searching for food in dumps. This situation may increase the chances of transmission of infectious and parasitic diseases from domestic animals to maned wolves (MURRAY et al., 1999).

The northeastern region of the state of São Paulo is a non-endemic area for VL, but the vector *Lu. longipalpis* is present in this area and cases of canine and human visceral leishmaniasis are increasing in the state of São Paulo year by year (CIARAVOLO et al., 2015). In our findings, three animals had anti-*Leishmania* spp. antibodies, which is consistent with other studies (CURI et al., 2006; JUSI et al., 2011). In the physical examination, none of the maned wolves showed skin lesions or clinical alterations that suggested clinical disease due to *Leishmania* spp. It is important to emphasize that wild animals, whether free-ranging or captive, can be reservoirs of *Leishmania* spp. without clinical signs and become an important source of infection for human and domestic animals (ACHA; SZYFRES, 2003). On the other hand, wild canids can be reservoirs of other trypanosomatids, such as *Trypanosoma cruzi* (ROCHA et al., 2013). Therefore, the possibility of cross-reaction in serologic tests among members of Trypanosomatidae family cannot to be ruled out, as has been suggested by some authors (LUCIANO et al., 2009).

Antibodies against *E. canis* had the second highest frequency of occurrence in this survey. Of the 17 maned wolves studied, nine were seropositive for *E. canis* (more than 50% of the animals tested). Ehrlichiosis in domestic dogs is well understood (LABRUNA et al., 2007). Nevertheless, there are few surveys of the occurrence of anti-*Ehrlichia* sp. antibodies in wild canids (PARAS et al., 2012). In Brazil, Arrais (2013) found 18.2% (16/87) of seropositive results (IFAT) for *E. canis* in maned wolves from the Serra da Canastra National Park in the state of Minas Gerais. The *R. sanguineus* tick was not found in the maned wolves; however, ticks of the genus *Amblyomma* were detected (data not shown). Therefore, the epidemiology of species of *Ehrlichia* in wild canids in Brazil requires further investigation (COSTA JUNIOR et al., 2007).

In conclusion, this survey found that free-ranging maned wolves from the northeastern region of the state of São Paulo, Brazil, have been exposed to *T. gondii*, *E. canis* and, possibly, to *Leishmania* spp. The nature of this region, with its large industrial area, extensive agriculture, and habitat fragmentation, contributes to the contact of maned wolves with suburban areas and farms. These environmental circumstances can increase the risk of transmission of pathogens, especially zoonotic agents, between domestic and wild animals and human beings.

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