Occurrence of anti-*Toxoplasma gondii*, *Neospora caninum* and *Leptospira* spp. antibodies in opossums (*Didelphis* spp.) in São Paulo State, Brazil

Ocorrência de anticorpos anti-Toxoplasma gondii, anti-Neospora caninum e anti-Leptospira spp. em gambás (Didelphis spp.) no estado de São Paulo, Brasil

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Abstract

Opossum (*Didelphis* spp.) is an omnivorous marsupial native to the Americas that shows synanthropic behavior in urban areas. Despite its proximity to domestic animals and humans, knowledge of its participation in the epidemiology of some zoonotic agents is substantial. This study aimed to determine the presence of antibodies against *Toxoplasma gondii*, *Neospora* spp. and *Leptospira* spp. in blood samples collected from opossums in 18 municipalities in the state of São Paulo, Brazil, between 2003 and 2008. Blood samples from 343 opossums: *Didelphis aurita* (n = 256) and *Didelphis albiventris* (n = 87) were obtained. These were tested to detect antibodies against *T. gondii,* using the modified agglutination test (MAT-Toto; cutoff ≥ 25); *Neospora* spp., using the indirect fluorescent antibody test (IFAT; cutoff ≥ 25); and *Leptospira* spp., using the microscopic agglutination test (MAT-Lepto; cutoff ≥ 100). Frequency of anti-*T. gondii*, *Neospora* spp. and *Leptospira* spp. antibodies were in 22.7%, 1.5% and 3.5%, respectively. The serogroups-serovars of *Leptospira* spp. presenting positive MAT-Lepto reactions were: Autumnalis-Butembo; Mini-Mini; Ballum-Castellonis; Icterohaemorrhagiae-Icterohaemorrhagiae; Icterohaemorrhagiae-Copenhageni and Grippotyphosa-Grippotyphosa or Bananal. This study demonstrated that these zoonotic agents are circulating in opossum populations in the state of São Paulo. Therefore, investigations regarding the role of marsupials in the epidemiology of each of these diseases should be conducted, especially to understand the behavior of these animals as zoonosis maintenance hosts.

Keywords: Seroprevalence. Toxoplasmosis. Neosporosis. Leptospirosis. Opossum.

Resumo

O gambá (*Didelphis* spp.) é um marsupial onívoro nativo das Américas que apresenta comportamento sinantrópico em áreas urbanas. Apesar da sua proximidade com animais domésticos e o homem, o conhecimento da sua participação na epidemiologia de alguns agentes zoonóticos é fundamental. Este estudo objetivou determinar a presença de anticorpos contra *Toxoplasma gondii*, *Neospora* spp. e *Leptospira* spp. em amostras de sangue colhidas de gambás de 18 municípios do estado de São Paulo, Brasil, entre 2003 e 2008. Foram obtidas amostras sanguíneas de 343 gambás: *Didelphis aurita* (n = 256) e *Didelphis albiventris* (n = 87). As amostras foram testadas para detecção de anticorpos contra *T. gondii,* utilizando o teste de aglutinação modificado (TAM-Toxo; ponto de corte ≥ 25); *Neospora* spp., utilizando a reação de imunofluorescência indireta (RIFI; ponto de corte ≥ 25); e *Leptospira* spp., utilizando a soroaglutinação microscópica (SAM-Lepto; ponto de corte ≥ 100). As frequências de anticorpos contra *T. gondii*, *Neospora* spp. e *Leptospira* spp. foram 22,7%, 1,5% e 3,5%, respectivamente. Os sorogrupos-sorovares de *Leptospira* spp. que apresentaram soropositividade foram: Autumnalis-Butembo; Mini-Mini; Ballum-Castellonis; Icterohaemorrhagiae-Icterohaemorrhagiae; Icterohaemorrhagiae-Copenhageni e Grippotyphosa-Grippotyphosa ou Bananal. Esse estudo demonstrou que esses agentes estão circulando em populações de gambás no estado de São Paulo. Desta forma, investigações que visam determinar o papel dos marsupiais na epidemiologia de cada doença devem ser conduzidas, especialmente visando o entendimento do comportamento desses animais como hospedeiros dessas zoonoses.

Palavras-chave: Soroprevalência. Toxoplasmose. Neosporose. Leptospirose. Gambá.

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Introduction

Toxoplasma gondii is one of the most common parasitic infections in humans and other warm-blooded animals. This coccidian is prevalent worldwide and is of veterinary and medical importance (TENTER et al., 2000; MONTOYA; LIESENFELD, 2004; DUBEY, 2010). Humans became infected through ingestion of tissue cysts from undercooked meat, consumption of food or drink contaminated with oocysts or as result of congenital infection (MCLEOD et al., 2000; TENTER et al., 2000).

Neospora caninum, the etiological agent of neosporosis, is a protozoon responsible for reproductive signs in several animal hosts, especially cattle and dogs. Its definitive host is certain species of canids that shed oocysts in their feces (MCALLISTER et al., 1998; GONDIM et al., 2004; KING et al., 2010; DUBEY et al., 2011). Abortion is the main clinical manifestation of neosporosis, especially among cattle. It has long been suspected that *N. caninum* has a life cycle in wild (i.e. sylvatic) animals, as neosporosis has been detected in several wild animal species (GONDIM et al., 2004).

Leptospirosis is a worldwide zoonotic disease that occurs in wildlife living in both rural and urban areas (LINS; LOPES, 1984; FAINE, 1999). Some animals are accidental hosts, but others, such as marsupials, are maintenance hosts because they present no clinical signs of the disease but can be infected and may harbor leptospires in their kidneys, thus eliminating the microorganisms in their urine over a long period of time. In this manner, these microorganisms can contaminate water sources, food and the environment in which they live, acting as sources of infection for other animal species (FAINE, 1999).

Experimental infection of *D. marsupialis* with serovar Grippotyphosa showed that this host species was moderately susceptible (REILLY, 1970). In Peru, the serovars Huallaga (serogroup Djasiman), Rupa rupa (serogroup Sejroe), and Tingomaria (serogroup Cynopteri) were isolated from *D. marsupialis* (HIDALGO; SULZER, 1984). Carusi et al. (2009), in Buenos Aires, Argentina, found that the rate of *Leptospira* spp. infection in *D. albiventris* was 13%.

In Brazil, the serovars Brasiliensis, Grippotyphosa, Ballum, and Swajzak were isolated from *D. marsupialis* (LINS; LOPES, 1984). The serovar Guaratuba (SANTA ROSA et al., 1975) and the serovars Ballum and Grippotyphosa (CORDEIRO et al., 1981) were isolated from *Philander opossum.* The serovars Mangus and Pomona were isolated from *D. albiventris* (CORDEIRO et al., 1981). An unidentified serovar of *Leptospira borgpetersenii* was isolated in Jaboticabal, São Paulo, from *D. albiventris* (SILVA et al., 2013), while in the state of Rio Grande do Sul (JORGE et al., 2012), the serovar Castellonis was identified in this marsupial.

Serological surveys conducted in Brazil on free-living wild opossums (*D. marsupialis* or *D. albiventris)* detected that they were reactive to serovars Ballum, Bataviae, Bratislava, Copenhageni, Icterohaemorrhagiae, Grippotyphosa, Panama, Patoc and Szwajzak, (SANTA ROSA et al., 1975; CORDEIRO et al., 1981; CALDAS et al., 1992; SILVA et al., 2015). Opossums can live in the same environment used by other synanthropic animals that are *Leptospira* spp. reservoirs, such as *Rattus norvegicus*, *Rattus rattus* and *Mus musculus*, and may increase the dispersion of this agent in nature (FAINE, 1999).

The present study aimed to investigate the presence of antibodies against *T. gondii*, *Neospora* spp. and *Leptospira* spp. in serum samples collected from

opossums in 18 municipalities in the state of São Paulo, Brazil.

Materials and Methods

Opossums

The opossums were caught in 18 municipalities in the state of São Paulo, southeastern region of Brazil: Barueri, Biritiba-Mirim, Cotia, Guarujá, Itapetininga, Itapevi, Mogi das Cruzes, Osasco, Pedreira, Piracicaba, Pirassununga, Ribeirão Pires, Salesópolis, Santos, São Bernardo do Campo, São Paulo, Sorocaba and Suzano (Figure 1).

Blood samples were collected between May 2003 and May 2008, from two species of opossum: *Didelphis aurita* (n = 256) and *Didelphis albiventris* (n = 87). The animals were caught, using Sherman and Tomahawk traps, with fruit or other bait to attract the animals (NOWAKI, 1991). The opossums were physically restrained and sedated using ketamine 15-30 mg/kg injected intramuscularly (MOORE, 1984). Serum samples were collected from the caudal vein or by intracardiac puncture. These samples were identified and stored at -20°C. The animals were released in the same area in which they had been caught.

This study was approved by the Ethics Committees of the Faculty of Veterinary Medicine, University of São Paulo (protocol 301/2003) and the Brazilian Institute for the Environment and Renewable Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, IBAMA) (nos. 02027.023912/2002, 02027.002317/2005-21, and 2027.000562/2006-84).

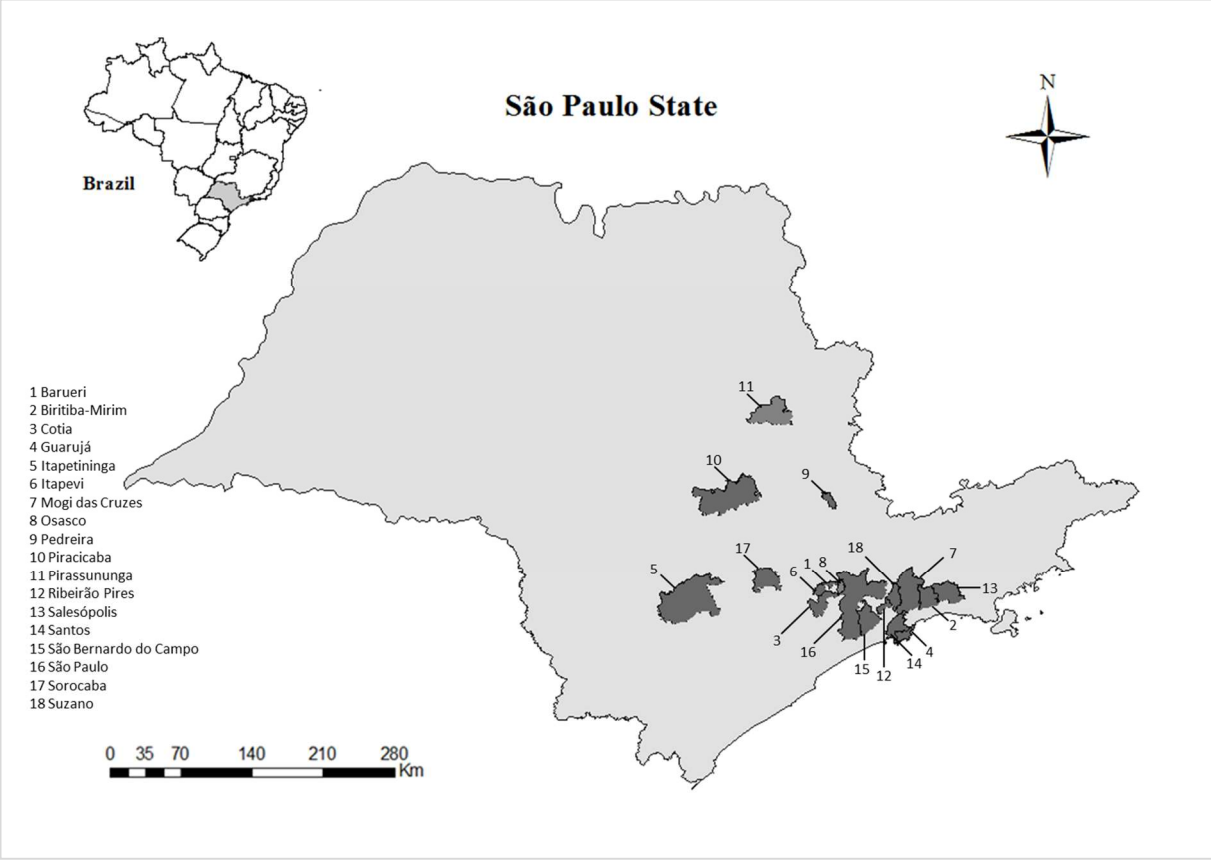

Figure 1 – Geographic locations of the 18 municipalities in which the opossums were caught. State of São Paulo, Brazil, from samples obtained between 2003 and 2008

Serological techniques

• Modified agglutination test (MAT-Toxo)

The modified agglutination test, using formalin-fixed whole tachyzoites, was used to detect antibodies against *T. gondii* (DUBEY; DESMONTS, 1987). The samples were tested at dilutions of 1:25 (YAI et al., 2003). Positive samples were retested in twofold serial dilutions until the maximum positive titer. Positive and negative controls were used in all reactions.

• Indirect fluorescent antibody test (IFAT)

The indirect fluorescent antibody test for *N. caninum* was performed as described by Paré et al. (1995), and the dilution of 1:25 was taken to be the cutoff (YAI et al., 2003). The NC-1 strain of *N. caninum* was used as antigen (DUBEY; BEATTIE, 1988). Opossum antiserum labeled with fluorescein isothiocyanate in a goat antibody was produced in the Zoonosis Control Center of the municipality of São Paulo, SP. This antiserum was used at a dilution of 1:100. The cutoff was 25 and positive serum samples were tested in twofold serial dilution until the maximum positive titer. Positive and negative controls were used in all reactions.

• Microscopic agglutination test (MAT-Lepto)

Leptospiral antibodies were detected by the microscopic agglutination test (GALTON et al., 1965), using 24 reference serovars: Australis, Bratislava Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes Hardjo, Wolfii, Shermani, Tarassovi, Andamana, Patoc, Sentot and Mini; and also three strains isolated in Brazil: An-776 serogroup Bataviae, serovar Brasiliensis, isolated from *D. marsupialis* (SANTA ROSA et al., 1972); M9-99 serogroup Icterohaemorrhagiae, serovar Copenhageni, isolated from *Rattus norvegicus* (MIRAGLIA et al., 2013); and 2A-CAP, serogroup Grippotyphosa, serovar Bananal, isolated from *Hydrochoerus hydrochaeris* (AHMED et al., 2006). The serum samples were first tested with the whole antigen collection at a dilution of 1:100 and then titrated with the reacting antigens in a double dilution series. The titer was the reciprocal of the highest dilution that presented 50% of agglutinated leptospires.

Results

Anti-*T. gondii* antibodies (MAT-Toxo ≥ 25) were found in 22.7% (78/343) of all opossums, and in 20.7% (53/256) of the *D. aurita* specimens and 29% (25/86) of the *D. albiventris* specimens. The titers ranged from 25 to 6,400 (Table 1). The anti-*T. gondii* antibody titers from *D. aurita* were 25 (16 opossums), 50 (10), 100 (12), 200 (11), 400 (3) and 800 (1); and from *D. albiventris* were 100 (2), 200 (7), 400 (10), 800 (3), 1,600 (2) and 6,400 (1). No positive animals were found in the municipalities of Pirassununga and Salesópolis (Table 2).

Anti-*N. caninum* antibodies were found in 1.5% (5/342) of the opossums. The antibody titers were 25 (1), 50 (2) and 100 (2). One *D. aurita* opossum from São Paulo showed both anti-*T. gondii* and anti-*N. caninum* antibodies.

Table 1 – Serological evaluation of *T. gondii, N. caninum* and *Leptospira* spp. antibodies in 343 opossums in the state of São Paulo, Brazil, from samples obtained between 2003 and 2008

Etiological agent	Positive samples (occurrence %)	Titer range
Toxoplasma gondii[a]	78 (22.7)	25 to 6,400
Neospora caninum[b]	5 (1.5)	25 to 100
Leptospira spp.[c]	11 (3.5)	100 to 800

[a] Modified agglutination test, [b] indirect fluorescence antibody test, [c] microscopic agglutination test

Table 2 – Seropositive / tested serum samples from opossums, analyzed for the presence of antibodies against *T. gondii*, *N. caninum* and *Leptospira* spp., according to municipality in the state of São Paulo, from samples obtained between 2003 and 2008

Municipality	*D. aurita*			*D. albiventris*			Total		
	T. gondii	*N. caninum*	*Leptospira* ssp.	*T. gondii*	*N. caninum*	*Leptospira* ssp.	*T. gondii*	*N. caninum*	*Leptospira* ssp.
Barueri	0/2	0/2	0/2	0/0	0/0	0/0	0/2	0/2	0/2
Biritiba-Mirim	1/12	0/12	1/12	0/1	0/1	0/1	1/13	0/13	1/13
Cotia	0/1	0/1	0/1	0/0	0/0	0/0	0/1	0/1	0/1
Guarujá	6/13	0/13	1/13	0/0	0/0	0/0	6/13	0/13	1/13
Itapetininga	0/0	0/0	0/0	0/1	0/1	0/1	0/1	0/1	0/1
Itapevi	0/1	0/1	0/1	0/0	0/0	0/0	0/1	0/1	0/1
Mogi das Cruzes	3/16	0/16	2/16	0/0	0/0	0/0	3/16	0/16	2/16
Osasco	0/2	0/2	0/2	0/0	0/0	0/0	0/2	0/2	0/2
Pedreira	2/14	0/14	1/14	0/0	0/0	0/0	2/14	0/14	1/14
Piracicaba	0/0	0/0	0/0	3/17	0/17	0/17	3/17	0/17	0/17
Pirassununga	0/0	0/0	0/0	0/12	0/12	0/12	0/12	0/12	0/12
Ribeirão Pires	2/7	0/7	2/7	0/0	0/0	0/0	2/7	0/7	2/7
Salesópolis	0/5	0/5	0/5	0/0	0/0	0/0	0/5	0/5	0/5
Santos	0/13	0/13	0/13	0/0	0/0	0/0	0/13	0/13	0/13
São Bernardo do Campo	12/27	0/27	0/27	1/2	0/2	0/2	13/29	0/29	0/29
São Paulo	18/114	5/114	1/114	0/0	0/0	0/0	18/114	5/114	1/114
Sorocaba	9/28	0/28	1/28	21/54	0/54	3/54	30/82	0/82	4/82
Suzano	0/1	0/1	0/1	0/0	0/0	0/0	0/1	0/1	0/1
Total	**53/256**	**5/256**	**9/256**	**25/87**	**0/87**	**3/87**	**78/343**	**5/343**	**12/343**

Anti-*Leptospira* spp. antibodies were found in 3.5% (12/343) of the opossums: nine specimens of *D. aurita* and three of *D. albiventris.* The antibody titers ranged from 100 to 800. The proportions of animals presenting higher titers with reference-strain serovars were: Butembo (3/10), Mini (3/10); Castellonis (2/10); Icterohaemorrhagiae (1/10) and Grippotyphosa (1/10). Two specimens of *D. albiventris* (numbers 187 and 249) presented higher titers with a local strain: M9/99 of Copenhageni serovar (isolated from a synanthropic *R. norvegicus* that was caught in São Paulo Zoo, Brazil). The specimen of *D. aurita* (opossum #266) that presented a positive reaction with the reference serovar Grippotyphosa showed the same titer of 800 with a local strain: 2A-CAP of the Bananal serovar (isolated from a specimen of the wild rodent *Hydrochoerus hydrochaeris* in São Paulo, Brazil). Animals reacting with the serovar Butembo were found in Mogi das Cruzes (#74 and #76) and Biritiba Mirim (#125); Mini in Pedreira (#94), Guarujá (#247) and Sorocaba (#252); Castellonis in Ribeirão Pires (#169 and #170); Grippotyphosa in São Paulo (#266) and M9/99 (Copenhageni) in Sorocaba (#187 and #249).

Discussion

Studies involving zoonotic agents in opossums are scarce. The seroprevalence of *T. gondii* in *Didelphis* spp. has been described in the Americas, showing values ranging from 13 to 37.3% (HILL JUNIOR et al., 1998; THOISY et al., 2003; YAI et al., 2003; MITCHELL et al., 2006; FORNAZARI et al., 2011; SIQUEIRA et al., 2013). In the city of São Paulo, Brazil, Yai et al. (2003) used the same serological technique and cutoff, and found a prevalence of 20.4%, such that 82 of the 396 animals were positive for *T. gondii*, i.e. a result very similar to that found in the present study (22.74%).

Antibodies against *N. caninum* were found in 21.2% (84/296) of the specimens of *D. marsupialis* in the city of São Paulo (YAI et al., 2003). However, because the geographic distribution of *D. marsupialis* is limited to the Amazon area, the host species described in this study may be *D. aurita*. Seropositive opossums in the present study originated from the city of São Paulo, with an antibody frequency of 4.4% (5/114), which was lower than the value previously found using the same technique and cutoff (YAI et al., 2003).

Although the number of *D. aurita* specimens reacting to *Leptospira* spp. was higher than the number of *D. albiventris* specimens, the occurrence rates for each animal species were very close: *D. aurita* (9/256; 3.51%) and *D. albiventris* (3/88; 3.41%); the two opossums species examined, seemed to present the same susceptibility to leptospiral infection. Out of the 18 different localities in the state of São Paulo that were included in the investigation, positive opossums were found in seven: Mogi das Cruzes (n = 2), Pedreira (n = 1), Biritiba Mirim (n = 1), Ribeirão Pires (n = 1), Sorocaba (n = 4), Guarujá (n = 1) and São Paulo (n = 1). These results demonstrated that the distribution of infection was different in each opossum population examined. Animals reactive to five Leptospira serogroups were found: Autumnalis (n = 3); Mini (n = 3); Icterohaemorrhagiae (n = 3); Ballum (n = 2) and Grippotyphosa (n = 1). Out of the three animals reactive to the Icterohaemorrhagiae serogroup, two of them (#187 and #249) presented higher titers for M9-99, a strain of Copenhageni serovar isolated in Brazil, showing that the inclusion of local strains could enhance MAT-Lepto sensitivity, as previously observed by Sarmento et al. (2012). In Brazil, the serovar Copenhageni is currently the main agent responsible for cases of leptospirosis in humans (SAKATA et al., 1992). Strains of the serogroup Autumnalis were isolated from sheep in Rio Grande do Sul (SILVA et al., 2007) and from cattle in Rio de Janeiro (MARTINS et al., 2015), and opossums could be considered their reservoir hosts. The serovars Ballum and Icterohaemorrhagiae have already been isolated from opossums, respectively, in the states of Pará (LINS; LOPES, 1984) and São Paulo (SANTA ROSA et al., 1975).

In conclusion, this study reinforces the presence of these zoonotic agents in opossum populations in the state of São Paulo. Therefore, investigations regarding the role of marsupials in the epidemiology of each disease should be conducted, especially to understand the behavior of these animals as zoonosis maintenance hosts in urban and rural areas.

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