Occurrence of antibodies anti-*Toxoplasma gondii* and anti-*Neospora caninum* in dogs from Natal, RN, Brazil

Ocorrência de anticorpos contra Toxoplasma gondii e Neospora caninum em cães de Natal, RN, Brasil

Marcos Gomes LOPES[1]; Maurício HERNANDEZ[1]; Júlia Tereza Ribeiro de LIMA[1]; José Henrique Hildebrand GRISI FILHO[1]; Solange Maria GENNARI[1]

[1] Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Medicina Veterinária Preventiva e Saúde Animal, São Paulo – SP, Brazil

Abstract

The occurrence of anti-*Toxoplasma gondii* and *Neospora caninum* in dogs from the municipality of Natal, RN, Brazil, was determined. Information about the presence of these coccidia in this species was not known. Blood samples were collected from 29 domiciled dogs which inhabit areas that surround two important protected areas of Atlantic Forest (Parque da Cidade and Parque das Dunas) and another 73 dogs that were sacrificed due to *Leishmania* spp. infection, in Center for Control of Zoonosis (CCZ) for the municipality of Natal. It was only possible to obtain information about the gender of dogs that live in the parks area. The presence of antibodies against each parasite was determined by indirect Fluorescence Antibody Test (IFAT), with a cutoff of 16 for *T. gondii* and 50 for *N. caninum*. Of the 102 dogs examined, 13 (12.7%, 95% CI 7.0-20.8%) were *T. gondii* positive and three (2.9%, CI 0.6-8.4%) for *N. caninum*. Association between: localities of obtaining samples (parks x CCZ) and sex of animals, with the occurrence of antibodies against each of the parasites, was determined by the Fisher exact test. For *T. gondii* association was found with males (p = 0.027) and dogs living close to parks (p = 0.008) had higher rates of infection. Associations were not observed in relation to *N. caninum.*

Keywords: Dogs. *Toxoplasma gondii. Neospora caninum*. Serology. Natal.

Resumo

A ocorrência de anticorpos anti-*Toxoplasma gondii* e anti-*Neospora caninum* foi determinada em cães do município de Natal, RN, onde informações sobre a presença destes coccídios nessa espécie não era conhecida. Para tanto, foram utilizados 29 cães domiciliados, que habitam áreas que circundam duas importantes Unidades de Conservação de Mata Atlântica presentes no município (Parque da Cidade e Parque das Dunas), e outros 73 cães que foram sacrificados no Centro de Controle de Zoonoses de Natal por serem positivos a *Leishmania* spp. Somente em relação aos cães que vivem próximos aos parques foi possível a obtenção de informações sobre o sexo dos animais. A presença de anticorpos contra cada um dos coccídios foi determinada com a Reação de Imunofluorescência Indireta com ponto de corte de 16 para *T. gondii* e 50 para *N. caninum.* Dos 102 cães examinados, 13 (12,7%, IC 95% 7,0-20,8) foram positivos para T. gondii e 3 (2,9%, IC 0,6-8,4%) para *N. caninum*. Associação entre: localidades de obtenção das amostras (parques x CCZ) e sexo dos animais com a ocorrência de anticorpos contra cada um dos parasitos, determinada através do teste exato de Fisher, foram positivas para *T. gondii,* com os machos (p = 0,027) e cães que habitam próximos aos parques (p = 0,008) apresentando maiores taxas de infecção. Associações não foram observadas em relação a *N. caninum.*

Palavras-chave: Cães. *Toxoplasma gondii. Neospora caninum.* Sorologia. Natal.

Introduction

Neospora caninum is a protozoan parasite of animals that have domestic dogs, dingo (*Canis familiaris*), coyote (*Canis latrans*) and grey wolf (*Canis lupus*) as its definitive hosts and many species of mammals and birds as intermediate hosts (MCALLISTER et al., 1998; KING et al., 2010; DUBEY et al., 2011). In Brazil, the prevalence of antibodies in dogs from Alagoas, Bahia, Paraíba, Piauí, Pernambuco, Maranhão, Amazonas, Pará, Rondônia, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná and São

Correspondence to:
Solange Maria Gennari
Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia,
Departamento de Medicina Veterinária Preventiva e Saúde Animal
Av. Prof. Dr. Orlando Marques de Paiva, 87 - Cidade Universitária
CEP 05508-270, São Paulo, SP, Brazil
e-mail: sgennari@usp.br

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Paulo showed occurrence values that ranged from 2.6% to 67.6% (revised by DUBEY, 2013). In Bahia, Brazil, Gondim et al. (2001) isolated the parasite, by bioassay in Mongolian gerbils, from the brain of a seven-year-old Collie that died with neurological signs (incoordination and hind-limb paresis).

Toxoplasma gondii is also a coccidian parasite with felids as the definitive host and warm-blooded animals as intermediate hosts. Until recently, *N. caninum* was confused with *T. gondii* because they are morphologically similar. However, *T. gondii* infection is widely prevalent in humans worldwide (DUBEY, 2010) and serologic surveys indicate that *T. gondii* infection is endemic in Brazil, where the occurrence in dogs, varies from 5% to 90% (revised by DUBEY et al., 2012).

In the municipality of Natal, the capital of the state of Rio Grande do Norte, two forest fragments stand out in terms of size and importance: the Parque Estadual das Dunas and the Parque da Cidade (FREIRE, 1990; CARVALHO, 2001). These forest fragments dramatically suffer the effect of unplanned urbanization, including the presence of small villages in the areas that surround the parks, with the introduction of domestic species into the wild ecosystem.

The aim of the present study was to investigate the presence of antibodies anti-*N. caninum* and anti-*T. gondii* in dogs that live in the villages surrounding the two parks of the city of Natal, and in dogs from different areas of the municipality. Information about seroprevalence of those parasites was not available in dogs from Natal.

Materials and Methods

The dogs were from small villages surrounding the Parque Estadual das Dunas de Natal (5°49'41"S and 35°11'25"W) that covers 1,172 hectares, and Parque da Cidade (5°50'45"S and 35°14'0"W) that covers approximately 64 acres. Both are areas of Atlantic Forest (RAMALHO; PIMENTA, 2010; NATAL, 2008) with some peculiarities. Dog samples were also obtained in August 2013 from the Control Center for Zoonosis (CCZ) from dogs from different regions of Natal, during euthanasia due infection by *Leishmania* spp., as preconized by regulation of the Brazilian Minister of Health (BRASIL, 2008).

Blood samples from a total of 29 domestic dogs (19 males and 10 females) were obtained from 26 residences, in which the owner allowed the collection, during August 2013. Only two dogs were young (<1-year). In CCZ, 73 dog samples were obtained and from those dogs no information was available regarding gender and age.

Blood was collected by puncture of the cephalic vein of each dog. The samples were centrifuged at 700 x g for 15 min to obtain the serum, that was then aliquoted, identified and stored at -20°C until transportation by air to the Faculty of Veterinary Medicine, University of São Paulo, São Paulo, where the analyses were performed.

An indirect fluorescent antibody test (IFAT) was used for investigation of anti-*T.gondii* antibodies, with the cutoff of 16 (LOPES et al., 2011). Tachyzoites of the *T. gondii* RH strain were used as the antigen, as described by Camargo (1974). For detection of anti-*N. caninum* antibodies, an IFAT using culture-derived tachyzoites of NC-1 isolate was used with a cutoff of 50 (SOUZA et al., 2002). Rabbit anti-canine IgG conjugate (Sigma, USA) was used in both tests. Sera were tested at 2-fold serial dilutions until the final dilution. Positive and negative *T. gondii* and *N. caninum* dog serum samples were added to each slide.

Associations between localities of sample collection (parks x CCZ) and occurrence of antibodies against *T. gondii* and *N. caninum*, and between gender and occurrence of antibodies against the parasites in the dogs from the parks, were analyzed by Fisher exact test. Values of $p < 0.05$ were considered statistically significant.

The project was approved by the Ethics Committee on Animal Use of the Institute of Biomedical Sciences, USP, protocol number 204.

Results and Discussions

Table 1 presents the occurrence of antibodies by locality and table 2 antibody titers against *T. gondii* and *N. caninum*.

The occurrence of *T. gondii* and *N. caninum* in the dogs analyzed was 12.7% (13/102) and 2.9% (3/102), respectively. Antibody titers ranged from 32 to 1,024 for *T. gondii* and from 100 to 400 for *N. caninum* and two dogs from the parks' area presenting antibodies against both parasites.

In the parks, where the gender of the animals was known, association was observed between gender and antibodies against *T. gondii* (p = 0.027). All eight *T. gondii* positive dogs were male.

Occurrence of *T. gondii* was also associated with locality of the sample, with association between positivity to *T. gondii* and dogs living around the parks (p = 0.008), indicating that *T. gondii* is well established in those environments. Fournier et al. (2014), studying the prevalence of *T. gondii* in wild and domestic animals from the same parks, found a high number of *T. gondii* seropositive animals and also the parasite was detected, by molecular methods, in tissue of those animals.

Occurrence was 4 to 5 times higher in the dogs (27.6%) from the parks when compared to the samples from CCZ (6.9%). It is important to note that the majority of the CCZ dogs were domiciled and not stray dogs, and the owner took them to be sacrificed due to leishmaniasis. This result is in agreement with studies from Gennari et al. (2006) with dogs from Araçatuba, SP, and from Ribeiro et al. (2011), with dogs from Belo Horizonte, that found, respectively, no association (p > 0.05) and a tendency of association (p = 0.053), between *Leishmania chagasi* positive dogs and occurrence of *T. gondii* infection.

No association was observed between *N. caninum* positivity and gender (p = 0.532) or origin of the dogs (p = 0.1939), and also no association was found

Table 1 – Number of dogs examined and positive to antibodies against *T. gondii* and *N. caninum* per locality – Natal, RN – 2013

Locality	**No. of dogs**	***T. gondii***			***N. caninum***		
	Examined	**Positive**	**Occurrence** (%)	CI (95%)	Positive	Occurrence (%)	CI (95%)
Parks	29	8	27.6	12.7-47.2	2	6.9	0.8-22.8
CCZ	73	5	6.8	2.3-15.3	1	1.4	0.03-7.4
Total	102	13	12.7	7.0-20.8	3	2.9	0.6-8.4

CCZ – Center for Control of Zoonosis; Parks – Parque das Dunas and Parque da Cidade; CI – Confidence Interval

Table 2 – Distribution of *T. gondii* and *N. caninum* antibody titer in dogs' residents around Parque das Dunas and Parque da Cidade and dogs euthanized in the Center for Control of Zoonosis (CCZ) – Natal, RN – 2013

	Titer	**P. das Dunas N = 17**	**P. da Cidade N = 12**	**CCZ N = 73**	**Total N = 102**
		Positive (%)	**Positive (%)**	**Positive (%)**	**Positive (%)**
T. gondii	32	2 (11.76)	2 (16.66)	1 (1.36)	5 (4.90)
	64	1 (5.88)	1 (8.33)	1 (1.36)	3 (2.94)
	128	1 (5.88)	0	3 (4.10)	4 (3.92)
	1024	1 (5.88)	0	0	1 (0.98)
N. caninum	100	0	0	1 (1.36)	1 (0.98)
	200	0	1 (8.33)	0	1 (0.98)
	400	1 (5.88)	0	0	1 (0.98)

N= number of dogs analyzed

between the occurrence of *T. gondii* and *N. caninum* in dogs living nearby Parque das Dunas (29.4% and 5.9% respectively) and Parque da Cidade (25.0% and 8.3%, respectively).

The values of occurrence found in the dogs from this study were lower than the majority of the studies conducted in Brazil with *T. gondii* (revised by DUBEY et al., 2012) and *N. caninum* (revised by DUBEY, 2013). According to information from owners, only four of the 29 dogs from the parks had free access to the streets and contact with the forest area. However, all dogs visit the streets and parks with their owners.

This is the first survey of occurrence of antibodies against *T. gondii* and *N. caninum* in Natal. The higher values found in the dogs living around the parks (especially in relation to *T. gondii*) means that the involved area represents an ecological niche for the parasite and a potential transmission risk for humans and wild animals that live in the area (CABRAL et al., 1998), once dogs serve as indicators of environmental contamination by oocysts of those parasites, and because of close association with humans (DUBEY et al., 2012).

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