



Revista Brasileira de Parasitologia
Veterinária

ISSN: 0103-846X

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Colégio Brasileiro de Parasitologia
Veterinária
Brasil

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Revista Brasileira de Parasitologia Veterinária, vol. 20, núm. 2, abril-junio, 2011, pp. 111-
114

Colégio Brasileiro de Parasitologia Veterinária
Jaboticabal, Brasil

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Presence of antibodies against *Toxoplasma gondii*, *Neospora caninum* and *Leishmania infantum* in dogs from Piauí

Presença de anticorpos anti-*Toxoplasma gondii*, *Neospora caninum* e *Leishmania infantum* em cães do Piauí

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Received July 26, 2010

Accepted February 24, 2011

Abstract

This study aimed to evaluate the presence of antibodies against *Neospora caninum*, *Toxoplasma gondii* and *Leishmania infantum* in dogs attended at the Veterinary Hospital of the Federal University of Piauí, Northeastern Brazil, where there are no reports of the occurrence of *N. caninum* and *T. gondii* in dogs. Serum samples from 530 dogs of genders, different ages and breeds from the municipality of Teresina and nearby towns were analyzed using three indirect fluorescent antibody tests, each one targeting one of the three agents. The associations between the parasites and gender, breed and age of the dogs were assessed by the chi-square test ($p > 0.05$). The occurrence of antibodies to *N. caninum*, *T. gondii* and *L. infantum* was 3.2, 18.0 and 78.1%, respectively. *Toxoplasma gondii* was more frequently found in older dogs ($p < 0.05$) whereas *L. infantum* was more common in animals aged between 1 to 3 years ($p < 0.05$). In order to evaluate potential associations between the presence of anti-*N. caninum* and anti-*T. gondii* antibodies and *Leishmania* infection, 240 dogs were selected (120 positive and 120 negative for *Leishmania* spp.), based on serological and parasitological diagnoses. No association was found between *Leishmania* spp. and the coccidian parasites ($p > 0.05$). The results confirm the exposure of dogs to these parasites in the State of Piauí.

Keywords: *Leishmania infantum*, *Toxoplasma gondii*, *Neospora caninum*, Piauí, dogs.

Resumo

Este estudo teve como objetivo avaliar a presença de anticorpos contra *Neospora caninum*, *Toxoplasma gondii* e *Leishmania infantum* em cães atendidos no Hospital Veterinário da Universidade Federal do Piauí, Teresina, Piauí, onde não há relatos de ocorrência de *N. caninum* e *T. gondii* em cães. Amostras de soro de 530 cães de ambos os sexos, diferentes idades e raças do município de Teresina e cidades vizinhas, foram analisados utilizando três testes de imunofluorescência indireta para cada um dos três agentes. Associação entre os parasitas e o sexo, a raça e a idade dos cães foram analisadas pelo teste do qui-quadrado ($p > 0,05$). Ocorrência de anticorpos para *N. caninum*, *T. gondii* e *L. infantum* foi de 3,2, 18,0 e 78,1%, respectivamente. *Toxoplasma gondii* foi mais frequente em cães mais velhos ($p > 0,05$), enquanto *L. infantum* apresentou maior frequência em animais com idade entre um e três anos ($p > 0,05$). Para avaliar possíveis associações entre a presença de anticorpos anti-*N. caninum* e anti-*T. gondii* com infecção por *Leishmania*, outros 240 cães foram selecionados, sendo 120 positivos e 120 negativos para leishmaniose, por métodos parasitológicos e sorológicos. Nenhuma associação foi encontrada entre a positividade para *Leishmania* e para os coccídios ($p > 0,05$). Os resultados confirmam a exposição de cães a esses parasitas no Estado do Piauí.

Palavras-chave: *Leishmania infantum*, *Toxoplasma gondii*, *Neospora caninum*, Piauí, cães.

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Introduction

Neospora caninum is an intracellular protozoan with a wide host range and has emerged as a serious disease in cattle and dogs worldwide. Dogs are the definitive hosts, and neosporosis is a major cause of abortion in cattle (DUBEY et al., 1988).

Toxoplasmosis is caused by *Toxoplasma gondii*, an obligate intracellular and opportunistic parasite that affects all warm-blooded animals including humans. Due to the high rates of naturally infected dogs by *T. gondii* and its correlation with immunosuppressive diseases such as canine distemper and leishmaniasis (MORETTI et al., 2002), *T. gondii* is of great importance in this species. Although dogs are not definitive hosts of *T. gondii* they might eventually contribute to its mechanical dissemination (FRENKEL; PARKER, 1996; LINDSAY et al., 1997; SCHWARZ et al., 2005).

Leishmaniosis is caused by a digenetic parasite and can have different clinical forms, depending on the *Leishmania* species involved (GONTIJO; CARVALHO, 2003). In Brazil, the visceral form is caused by *Leishmania infantum* (= *L. chagasi*) of the *Leishmania donovani* complex (GONTIJO; MELLO, 2004). Dogs play an important role in the epidemiology of this disease, acting as reservoirs of the parasite. Even asymptomatic dogs can transmit the infection to humans through an arthropod vector (MOSHFE et al., 2009).

Recent studies conducted in Brazil have shown variation in the prevalence of anti-*T. gondii*, anti-*N. caninum* and anti-*Leishmania* spp. antibodies in dogs from different regions, ranging from 21.3 to 76.4% for *T. gondii* (SOUZA et al., 2003; CAÑÓN-FRANCO et al., 2004; AZEVEDO et al., 2005; DUBEY et al., 2007), 8.3 to 28.3% for *N. caninum* (reviewed by GENNARI et al., 2002, 2004; AZEVEDO et al., 2005; FIGUEREDO et al., 2008) and 3.4 to 51.5% for *Leishmania* spp. (DANTAS-TORRES et al., 2006; ABREU-SILVA et al., 2008; ALMEIDA et al., 2009). However, there were no studies of these parasites in dogs in the Northeastern State of Piauí.

The aim of this study was to evaluate the presence of antibodies anti-*N. caninum*, anti-*T. gondii* and anti-*L. infantum* in dogs and risk factors associated with these infections.

Material and Methods

A total of 530 serum samples from a collection of sera of the Laboratory of Animal Health at the Federal University of Piauí (UFPI), Teresina, Piauí, was examined from January 2007 to December 2008. Dogs with clinical signs of leishmaniosis were randomly selected. The dogs were of both genders (306 males and 224 females), aged two months to 12 years and of different breeds. The animals were from the urban area of the capital city Teresina and nearby towns.

An additional set of 240 serum samples from dogs with the same characteristics of the previous samples, and also collected in the UFPI Veterinary Hospital, was analyzed. However, these samples were collected in 2009 and half of them (120 dogs) were parasitologically and serologically positive for leishmaniosis and the other half were negative. These dogs were used to evaluate potential

associations between the presence of antibodies against *N. caninum* and *T. gondii* in *Leishmania* spp.-positive and negative groups.

Antibodies against *N. caninum* and *T. gondii* were determined by indirect immunofluorescence antibody test (IFAT) according to Dubey et al. (1988) and Camargo (1974), respectively. The sera were tested at a start dilution of 1:50 for *N. caninum* and 1:16 for *T. gondii*. In all reactions, positive and negative control sera were used.

The detection of antibodies against *Leishmania* spp. was performed according to the instructions of the manufacturer of IFAT-canine visceral leishmaniosis kit (Bio-Manguinhos, FIOCRUZ, MS, Brazil), and samples with titers ≥ 40 were considered positive.

For the parasitological diagnosis bone marrow and popliteal lymph node aspiration smears were prepared on slides, fixed with methanol and stained with Giemsa. The samples were analyzed using a 100 \times magnification for observation of amastigotes forms.

Lymph node and/or bone marrow tissues were punctured and aliquots of these tissues were seeded into tubes containing 2 mL of NNN medium (modified Novy-McNeal-Nicolle blood agar), added to Schneider medium for observation of promastigotes growth from Day 5 of cultivation (MONTEIRO et al., 1994).

For the statistical analyses, the chi-square test with a significance level of 5% was used to assess the associations between the occurrence of antibodies against each of the agents studied with gender, age and presence of co-infection. The associations between the presence of antibodies against *T. gondii* and *N. caninum* in dogs positive and negative to *Leishmania* spp. were analyzed using the same test.

Results and Discussion

The analysis of the 530 serum samples showed the following occurrence of antibodies: 18.0% (100/530) anti-*T. gondii*, 3.2% (17/530) anti-*N. caninum* and 77.9% (413/530) anti-*Leishmania* spp.

No association was found ($p > 0.05$) between the occurrence of anti-*N. caninum*, *Leishmania* spp. and *T. gondii* antibodies with gender and breed of the animals examined. These results are consistent with other Brazilian studies (SOUZA et al., 2002; TEIXEIRA et al., 2006) suggesting that male and female dogs have the same risk of infection by these parasites, although the level of exposure of males and females can also vary according to characteristics of each dog population (DANTAS-TORRES et al., 2006).

Regarding age, those dogs positive to *T. gondii* were more frequently older ($p < 0.05$) while *Leishmania* spp. infection was most commonly seen in animals aged between one to two years ($p < 0.05$) as shown in Table 1. Cañón-Franco et al. (2004) found in Monte Negro, Rondônia, Northern Brazil, that the occurrence of anti-*T. gondii* antibodies increased with animal age and they reported a high rate of postnatal exposure. This is similar to that seen in the present study, in which higher relative rates of infection were found with increasing dog age, however this increase was only significant in the group of dogs aged nine years ($p = 0.05$).

Although Piauí is an endemic area of leishmaniosis, there have been few studies in animal or humans (COSTA et al., 1990). An

Table 1. Occurrence (%) of antibodies against *Leishmania* spp., *Neospora caninum* and *Toxoplasma gondii* by age group in 530 dogs attended at the Veterinary Hospital, Federal University of Piauí, Northeastern Brazil, January 2007 to December 2008.

Age group (years old)	Total examined	Number (No.) positive dogs (%)		
		<i>Leishmania</i> spp.	<i>N. caninum</i>	<i>T. gondii</i>
< 1	89	64 (71.91) ^a	03 (3.51) ^a	12 (12.50) ^a
≥ 1 to < 3	167	144 (86.22) ^b	11 (6.63) ^a	22 (12.65) ^a
≥ 3 to < 5	146	116 (79.45) ^a	01 (0.69) ^a	30 (20.83) ^a
≥ 5 to < 9	97	70 (72.16) ^a	01 (1.05) ^a	23 (24.20) ^a
≥ 9	31	19 (61.29) ^a	01 (3.45) ^a	13 (41.38) ^a
Total	530	413 (77.92)	17 (3.20)	100 (18.00)

Different letters in the same column $p \leq 0.005$.

Table 2. Number (No.) of positive (+) and negative dogs (–) to anti-*Neospora caninum*, anti-*Toxoplasma gondii* and anti-*Leishmania* spp. antibodies and rates of potential co-infections.

Status	Number (No.) dogs	Rate (%)
<i>Leishmania</i> –, <i>T. gondii</i> –, <i>N. caninum</i> –	95	17.9
<i>Leishmania</i> –, <i>T. gondii</i> –, <i>N. caninum</i> +	0	0.0
<i>Leishmania</i> –, <i>T. gondii</i> +, <i>N. caninum</i> –	22	4.15
<i>Leishmania</i> +, <i>T. gondii</i> –, <i>N. caninum</i> –	321	60.5
<i>Leishmania</i> –, <i>T. gondii</i> +, <i>N. caninum</i> +	0	0.0
<i>Leishmania</i> +, <i>T. gondii</i> +, <i>N. caninum</i> –	75	14.1
<i>Leishmania</i> +, <i>T. gondii</i> –, <i>N. caninum</i> +	14	2.6
<i>Leishmania</i> +, <i>T. gondii</i> +, <i>N. caninum</i> +	3	0.5

Table 3. Occurrence (%) of anti-*T. gondii* and anti-*N. caninum* antibodies in positive (+) and negative (–) dogs for *Leishmania* spp. by parasitological and serological methods.

	Number (No.)	<i>Toxoplasma gondii</i>		<i>Neospora caninum</i>	
		+	–	+	–
<i>Leishmania</i> (+)	120	23 (19.16%)	97 (80.83%)	03 (02.50%)	117 (97.50%)
<i>Leishmania</i> (–)	120	18 (15.00%)	102 (85.00%)	04 (03.33%)	116 (96.60%)
Total	240	41 (17.08%)	199 (82.91%)	07 (02.91%)	233 (97.08%)

association was also found between age and seropositivity of dogs to *Leishmania* spp., with high occurrence in dogs aged between 1 to 3 years ($p < 0.05$). In Araçatuba, São Paulo, Southeastern Brazil, Gennari et al. (2006) reported an weak association ($p = 0.038$) with a higher number of 4-year-old positive dogs and Dantas-Torres et al. (2006), in Pernambuco, Northeastern Brazil, reported higher occurrence in dogs younger than 1 year.

No association was found between co-infection by *Leishmania* spp. and *T. gondii* and by *T. gondii* and *N. caninum* ($p > 0.05$). However an association between co-infection by *Leishmania* spp. and *N. caninum* ($p < 0.05$) was seen with all 17 *N. caninum*-positive dogs that were also *Leishmania* spp.-positive (Table 2). Gennari et al. (2006), in a study with dogs from Araçatuba, São Paulo, Southeastern Brazil, and Cringoli et al. (2002), with dogs from Southern Italy, also found an association between *L. infantum* and *N. caninum* antibodies.

As the samples in the present study were from dogs with suspected leishmaniosis, a new set of samples was analyzed, this time with the same number of positive ($n = 120$) and negative dogs ($n = 120$). This new sampling was carried out to confirm the results obtained in the analysis of associations between *Leishmania* spp. and the coccidian parasites. The results from these samples were different from those first analyzed and no differences were found ($p > 0.05$) between the proportions of positive dogs for *T. gondii* and *N. caninum* in the group of *Leishmania* spp.-positive and negative dogs (Table 3), which corroborates the results reported by Valadas et al. (2010) in the State of Pará, Northern Brazil. However, the rates of *T. gondii* (17%) and *N. caninum* (2.9%) antibodies found in these 240 new samples were similar ($p > 0.05$) to those found for *T. gondii* (18%) and *N. caninum* (3.2%) in our first evaluation.

There were other studies showing the presence of dogs positive to these parasites in other Northeastern States of Brazil. However, this is the first confirmation that dogs from Piauí were also exposure to *T. gondii*, *N. caninum* and *Leishmania* spp. Further studies focusing on the epidemiological aspects of these parasites in the region are necessary for better control them.

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