

***Toxoplasma gondii*, *Neospora caninum* and *Leishmania amazonensis* antibodies in domestic dogs in the western Brazilian Amazon region**

***Toxoplasma gondii*, *Neospora caninum* e *Leishmania amazonensis* em cães domésticos da Amazônia Ocidental Brasileira**

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Abstract

Blood samples from 99 domestic dogs were collected in the urban area (n = 33) and rural area (n = 66) of the municipality of Lábrea, state of Amazonas, Brazil. Among the rural samples, 40 were collected in riverine communities and 26 were collected in Indian communities, both along the Purus River. During the sampling, a questionnaire was applied to obtain information relating to sex, age, living in dryland or seasonally flooded areas, access to the streets (for urban dogs) and access to forests. The presence of antibodies against *Toxoplasma gondii*, *Neospora caninum*, *Leishmania infantum chagasi* and *Leishmania amazonensis* was detected by means of the indirect fluorescent antibody test (IFAT) with cutoffs of 16, 50, 40 and 40, respectively. The association of each qualitative variable, with each parasite and positivity for each of them, were evaluated using χ^2 test ($p < 0.05$). *T. gondii*, *N. caninum* and *L. amazonensis* antibodies were found, respectively, in 61.6%, 7.1% and 8.1% of the animals examined. None of the samples were positive for *L. infantum chagasi*. The variable of living in an urban area ($p = 0.041$) presented an association with occurrence of *T. gondii* and the positivity for *T. gondii* increased with age of the dogs ($p = 0.0006$). The variable of male sex presented an association with *N. caninum* ($p = 0.018$) and no variable presented any association with *L. amazonensis*. The antibody titers against *T. gondii* ranged from 64 to 32,768; against *N. caninum* from 100 to 800; and against *L. amazonensis* from 40 to 640. Both *T. gondii* and *L. amazonensis* are important zoonotic agents and are active in this region. The municipality studied, and especially its riverine communities, has geographic, social and ecological peculiarities. This region requires greater attention from the authorities for controlling these agents.

Keywords: *Toxoplasma gondii*. *Neospora caninum*. *Leishmania* spp.. Amazon. Dogs.

Resumo

Amostras de sangue de 99 cães domiciliados foram coletadas no meio urbano (n = 33) e rural (n = 66) do município de Lábrea, estado do Amazonas, Brasil. Dentre as amostras rurais, 40 foram obtidas em comunidades ribeirinhas e 26 em comunidades indígenas, ambas ao longo do rio Purus. Durante a amostragem foi aplicado um questionário com informações sobre sexo, idade, viver em áreas secas ou alagadiças, acesso ou não às ruas (cães urbanos) e acesso à floresta. A presença de anticorpos contra *Toxoplasma gondii*, *Neospora caninum*, *Leishmania infantum chagasi* e *Leishmania amazonensis* foi detectada pela reação de imunofluorescência indireta (RIFI) com ponto de corte de, respectivamente, 16, 50, 40 e 40. Associação entre as variáveis qualitativas e a positividade para cada um dos parasitos foi avaliada pelo teste do χ^2 ($p < 0,05$). A ocorrência de animais com anticorpos contra *T. gondii*, *N. caninum* e *L. amazonensis* foi de, respectivamente, 61,6%; 7,1% e 8,1%. Nenhuma amostra foi positiva para *L. infantum chagasi*. A variável viver em área urbana ($p=0,041$) apresentou associação com ocorrência de *T. gondii* e o número de cães positivos a *T. gondii* aumentou com a idade ($p = 0,0006$). A variável sexo (machos) apresentou associação com *N. caninum* ($p = 0,018$) e nenhuma variável apresentou associação com *L. amazonensis*. Os títulos de anticorpos contra *T. gondii* variaram de 64 a 32.768; contra *N. caninum* de 100 a 800 e contra *L. amazonensis* de 40 a 640. *T. gondii* e *L. amazonensis* são dois agentes de importantes zoonoses e encontram-se ativos na região. O município estudado, especialmente as comunidades ribeirinhas, possuem peculiaridades geográficas, sociais e ecológicas distintas e necessitam maior atenção das autoridades para o controle desses agentes.

Palavras-chave: *Toxoplasma gondii*. *Neospora caninum*. *Leishmania* spp.. Amazonas. Cães.

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Introduction

Toxoplasma gondii is a coccidian parasite for which felids are the definitive host and warm-blood animals are the intermediate ones. It is responsible for one of the most important zoonotic diseases (DUBEY, 2010). Antibodies against *T. gondii* have been found in canine serum samples worldwide and in Brazil the occurrence rate ranges from 5.2% to 90% (reviewed by DUBEY et al., 2012). How dogs become infected with *T. gondii* is unknown, but the higher prevalence in stray and farm dogs than in pets has suggested that consumption of infected prey is an important source of infection (SOUZA et al., 2003).

Neospora caninum is an important cause of abortion among cattle worldwide. Canids, including dogs (*Canis lupus familiaris*), coyotes (*Canis latrans*), dingoes (*Canis lupus dingo*) and gray wolves (*Canis lupus*) (MCALLISTER et al., 1998; GONDIM et al., 2004; KING et al., 2010; DUBEY et al., 2011) play an important role in the epidemiology of this parasite because they are the definitive host, shedding the environmentally resistant oocysts in their feces. Epidemiological studies have reported the presence of dogs as a risk factor for bovine neosporosis (PARÉ et al., 1998; SAWADA et al., 1998; WOUUDA et al., 1999). In Brazil, several studies have reported occurrences of antibodies against *N. caninum* in dogs living in both rural and urban areas. The values, measured by means of the indirect fluorescent antibody test (IFAT ≥ 50), range from 4.5% to 45% (reviewed by GENNARI, 2004, and by DUBEY; SCHARLES, 2011).

The genus *Leishmania* comprises about 30 different species that infect mammals. These parasites are transmitted by various blood-sucking invertebrates of the family Psychodidae, which are distributed in tropical and subtropical regions (ASHFORD, 2000; DESJEUX, 2004). They can infect humans and cause different clinical symptoms, among which visceral leishmaniasis is the most severe.

Domestic dogs are considered to be the main reservoir of *L. infantum chagasi* in urban areas and are the main epidemiological source of the parasites, causing visceral leishmaniasis among humans (LAINSON, 2010). Moreover, dogs can be infected by *L. amazonensis*, which is one of the cutaneous leishmaniasis causal agents (TOLEZANO et al., 2007; HOFFMANN et al., 2012). In Brazil, cutaneous leishmaniasis is a disease of great public health importance, with clinical features ranging from an unapparent form to cases of mucosal and cartilage injury (BRASIL, 2010; GRAMICCIA; GRADONI, 2005).

Studies conducted in Italy (CRINGOLI et al., 2002) and in Brazil (GENNARI et al., 2006) found an association between occurrences of antibodies against *L. infantum chagasi* and seropositivity for *N. caninum*. No association was observed between *L. infantum chagasi* and *T. gondii* (GENNARI et al., 2006). These authors reported that immunosuppression due to *Leishmania* infection was the most likely cause of *N. caninum* infection. However, no studies relating to *L. amazonensis* and positivity for *T. gondii* or *N. caninum* are available.

Although several surveillance studies relating to *T. gondii*, *N. caninum* and *Leishmania* spp., have been conducted among dogs in Brazil, no study has been undertaken in the state of Amazonas or, particularly, among riverine communities of the Amazon basin, which have geographical, social and ecological peculiarities. Therefore, the present study aimed to determine the occurrence of *T. gondii*, *N. caninum*, *L. infantum chagasi* and *L. amazonensis* antibodies in

domestic dogs living in communities along the Purus River and in the urban area of the municipality of Lábrea, Amazonas, Brazil.

Material and Methods

Study area

Blood samples from domestic dogs were collected in the urban and rural areas of Lábrea, a municipality located in the southern part of the state of Amazonas, Brazil. The climate of that region is humid equatorial (annual precipitation > 2,000 mm), with a mean temperature above 25 °C throughout the year (SILVA et al., 2008). The municipality has a typical Amazon rainforest landscape, with some land permanently flooded (*igapós*), some land submerged only during rainy seasons (*várzeas*) and other areas of land that are never submerged (*low plains*). The municipality encompasses an area of 68,229 km², with a population of 41,600 inhabitants living mostly in the urban area. Its rural population mostly comprises

riverine families (living on the banks of the Purus River). Indian villages also exist in the rural area of the municipality.

Dog sampling

A total of 99 dogs were sampled, of which 33 were in the urban area and 66 in the rural area of Lábrea, as previously described (SOARES et al., 2014). Among the rural dogs, 40 were owned by people living in riverine communities and 26 were owned by Indians (Table 1). The 33 urban dogs were randomly selected according to their availability in homes during visits. The 66 rural dogs encompassed all the canine populations that were present in each of the six riverine communities and two Indian villages during the visits. During the sampling, a questionnaire was applied to the owners and information on the animals was obtained in relation to: sex, age, rearing mode (restrained or free-roaming), living in dryland or seasonally flooded areas and access to forests.

Table 1 – Geographic localities, number of dogs examined and number of dogs seropositive for *T. gondii*, *N. caninum* and *L. amazonensis*. Lábrea, state of Amazonas – Brazil – 2013

Locality	Coordinates		Living area	Main landscape	No. of sampled dogs	<i>T. gondii</i> Positive (%)	<i>N. caninum</i> Positive (%)	<i>L. amazonensis</i> Positive (%)
	South	West						
Urban area	07°15'55"	64°48'08"	Urban	Low plain	33	25 (75.7)	3 (9.1)	4 (12.1)
Rural areas*								
Carmelitas	07°16'51"	64°50'10"	Riverine	Várzea	3	3 (100.0)	1 (33.3)	0
Boca do Ituchi	07°18'36"	64°50'51"	Riverine	Várzea	6	4 (66.6)	0	0
Maciari	07°16'57"	64°51'05"	Riverine	Várzea	5	2 (40.0)	0	0
Samaúma	07°18'50"	65°08'39"	Riverine	Várzea	6	4 (66.6)	1 (16.7)	1 (16.7)
Santa Rosa	07°20'28"	64°59'50"	Riverine	Várzea	12	4 (33.3)	0	1 (8.3)
Bacural	07°15'02"	64°53'03"	Riverine	Igapó	8	5 (62.5)	1 (12.5)	0
Jarauara	07°16'42"	65°10'31"	Indian village	Low plain	15	10 (66,6)	0	1 (6.7)
Araça	07°18'54"	64°55'56"	Indian Village	Low plain	11	4 (36,3)	1 (9.1)	1 (9.1)
Total					99	61 (61.6)	7 (7.1)	8 (8.1)

*Banks of Purus River

Blood was collected from each dog by means of venipuncture. The serum was separated by means of centrifugation, identified and kept frozen until tested using serological methods.

The procedures of this study had previously been approved by the Animal Ethics Committee of the

Faculty of Veterinary Medicine, University of São Paulo (protocol no. 1667/2009).

Serological analyses

An indirect fluorescent antibody test (IFAT) was used to detect *T. gondii* antibodies, with a cutoff value of 1:16 (SILVA et al., 1997). Tachyzoites of the RH strain of *T. gondii* were used as an antigen, as described by Camargo (1974). To detect *N. caninum* antibodies, IFAT with culture-derived tachyzoites of the NC-1 isolate (DUBEY et al., 1988) was used with a cutoff value of 1:50.

To detect *L. infantum chagasi* and *L. amazonensis* antibodies, IFAT was performed using a cutoff of 1:40 (BRASIL, 2010). Promastigote forms of *L. infantum chagasi* and *L. amazonensis* were used as the antigen, as described by Ferrer et al. (1995).

Serum reacting at the cutoff dilution of each of the four antigens was retested at twofold serial dilutions until the last positive dilution. A rabbit anti-canine IgG conjugate labeled with fluorescein (Sigma-Aldrich, Bellefonte, PA, USA, catalog n-A6792) was used in all the tests, and appropriate positive and negative control serum samples were added to each slide.

Statistical analyses

The association of each qualitative variable with seropositivity (for *T. gondii*, *N. caninum* and *L. amazonensis*) was evaluated by the Mann-Whitney test. The median age (qualitative variable) was compared between seropositive and seronegative animals using the Mann-Whitney test. The significance level in both tests was 5% and the SPSS 17 software was used to perform the analysis.

Results

Table 1 presents the geographical localities of the animals and occurrences of antibodies against *T. gondii*, *N. caninum* and *L. amazonensis*.

Toxoplasma gondii, *N. caninum* and *L. amazonensis* antibodies were found, respectively, in 61 (61.6%), 7 (7.1%) and 8 (8.1%) of the 99 dogs examined. None of the samples were positive for *L. infantum chagasi*.

The category of qualitative variable associated with seropositivity for *T. gondii* was the urban area ($p = 0.041$). Since all the rural dogs had contact with forest areas, the association observed between *T. gondii* seropositivity and being kept away from forests ($p = 0.041$) was the same association as between seropositivity and living in the urban area. The qualitative variable associated with seropositivity for *N. caninum* was sex (male) ($p = 0.018$), while for *L. amazonensis*, none of the variables analyzed presented any association with the infection.

Twelve (36.36%) of the 33 young dogs (from 0 to 1 year old) and 49 (74.24%) of the 66 adult dogs (> 1 year of age) were found to be positive for *T. gondii*. The median age was greater among dogs that were seropositive for *T. gondii* ($p = 0.0006$). Two young dogs (6.0%) and five adult dogs (7.5%) were found to be positive for *N. caninum*, and this difference was not significant ($p > 0.05$). Two young dogs (6.1%) and six adult dogs (9.0%) presented *L. amazonensis* antibodies, and this difference was not significant ($p > 0.05$).

Associations between seropositivity and access to the streets were only analyzed in relation to the urban area. No such associations with *T. gondii*, *N. caninum* and *L. amazonensis* occurrence were observed.

All the dogs in the urban area that were sampled lived in dryland areas. However, 26 dogs in the rural area lived in dryland areas and 40 in seasonally flooded areas. Using only the samples from rural dogs, no association between the occurrence values, for each parasite, and living in dryland or seasonally flooded areas was observed ($p > 0.05$). These results are summarized in Table 2.

Table 2 – Association between qualitative variables and dogs' seropositivity for *T. gondii*, *N. caninum* and *L. amazonensis* – Lábrea – 2013

Variable	<i>T. gondii</i>	P	<i>N. caninum</i>	P	<i>L. amazonensis</i>	P
Sex						
Female	24/43 (55.8)	0.298	0/43 (0.0)	0.018	5/43 (11.6)	0.446
Male	37/56 (66.1)		7/56 (12.5)		3/56 (5.3)	
Age (years)						
0-1	12/33 (36.4)	0.0006	2/33 (6.1)	0.887	2/33 (6.1)	0.887
> 1	49/66 (74.2)		5/66 (7.6)		6/66 (9.0)	
Locality						
Urban area	25/33 (75.7)	0.041	3/33 (9.1)	0.683	4/33	0.671
Rural area	36/66 (54.5)		4/66 (6.1)		4/66	
Living in dryland area						
No	39/59 (66.1)	0.365	4/59 (6.8)	0.791	6/59 (10.2)	0.589
Yes	22/40 (55.0)		3/40 (7.5)		2/40 (5.0)	
Urban area						
Access to streets						
No	7/8 (87.5)	0.217	1/8 (62.5)	1.000	0/8 (0.0)	0.560
Yes	18/25 (72.0)		2/25 (8.0)		4/25 (16.0)	
Rural area						
Dryland area	14/26 (53.8)	0.862	1/26 (3.8)	0.655	2/26 (7.7)	0.920
Seasonally flooded area	22/40 (55.0)		4/40 (7.5)		2/40 (5.0)	

The *T. gondii*, *N. caninum* and *L. amazonensis* antibody titers are presented in Table 3. For *T. gondii*, approximately 50% of the positive animals presented high IgG antibody titers (1024 and 2048). Only one dog presented antibodies against all three of the

parasites examined. Positivity for *L. amazonensis* and *T. gondii* was observed in six dogs and all dogs positive for *N. caninum* were also positive for *T. gondii*. An IgG antibody titer of 400 for *N. caninum* was the most common result.

Table 3 – Distribution of *T. gondii*, *N. caninum* and *L. amazonensis* antibody titers among dogs (N = 99) in the municipality of Lábrea, western Amazon region – Brazil – 2013

<i>T. gondii</i>		<i>N. caninum</i>		<i>L. amazonensis</i>	
Titer	No. positive	Titer	No. positive	Titer	No. positive
64	4	100	2	40	1
128	7	200	1	80	4
256	5	400	3	160	2
512	6	800	1	640	1
1024	16				
2048	15				
4096	3				
8492	3				
32768	2				
TOTAL	61		7		8

Discussion

All dogs presented during the visits to the riverine communities were sampled and the urban dogs were sampled in different sites of the municipality by a convenience sample. It was possible to collect samples once for each community or site visited; however, due to the very typical profile presented in the studied

location and as no data is available in the region, the results, despite the relatively low number of dogs, are very important for the region.

The results indicate that dogs in this part of the Amazon region are exposed to *T. gondii*, *N. caninum* and *L. amazonensis* infection.

None of the animals examined presented antibodies against *L. infantum chagasi* and 8.1% of the animals were positive to *L. amazonensis*.

The tripanosomatides, and not only the species of *Leishmania*, may have similar antigens (SUNDAR; RAI, 2002). The use of recombinant antigens is often not able to minimize cross-reactions because some proteins are very similar in the tripanosomatides and may cause cross-reactions (SUNDAR; RAI, 2002). Among the standard methodologies, only TESA blot proved extremely sensitive and specific, but this test is not commercially available (UMEZAWA et al., 2009). As cross-reactivity can occur, tests for two antigens were conducted; however, all animals were negative for *L. infantum chagasi* and some animals were positive for *L. amazonensis*. This fact demonstrates that, despite the expected cross-reactivity, probably these kinds of reactions were not observed in this study.

Occurrences of leishmaniasis have been correlated with deforestation processes, with subsequent colonization by competent vector species (DOURADO et al., 1989). The dogs sampled in this study lived in the municipality of Lábrea, which has a low degree of human disturbance. A study conducted in the same region recorded the existence of 32 species of sand flies, but none of these was identified as a vector of *L. infantum chagasi* (SILVA et al., 2014). However, the occurrence rate of cutaneous leishmaniasis in the Brazilian Amazon region is very high, especially in the state of Amazonas, and this favors infection among domestic animals (GUERRA et al., 2003).

Although cutaneous leishmaniasis is of wild origin, cases of this disease have been reported in urban environments within endemic areas. Dogs can be considered to be possible reservoirs of this disease, given that cutaneous leishmaniasis caused by *L. amazonensis* has been described in Brazil, among domestic dogs in São Paulo and Paraná (LAINSON, 2010; TOLEZANO et al., 2007; HOFFMANN et al., 2012).

The constant deforestation process that the Amazon region is suffering may favor the increased incidence of leishmaniasis in this region. Dogs have been neglected with regard to epidemiological studies on cutaneous leishmaniasis, but their presence seems to favor exchanges between vectors, wild animals, man and *Leishmania* species (LAINSON, 2010).

Seropositivity for *T. gondii* presented an association with the living area, such that dogs in the urban area of the municipality presented higher occurrences. The great majority of the dogs in urban areas in this part of the Amazon region have access to areas where cats are also present, like yards and streets. Thus, it is likely that this urban environment is more contaminated by *T. gondii* oocysts than rural environments. No association ($p > 0.05$) was observed between free access to the streets and positivity for *T. gondii*.

The system of flood and drought that is seen in the Purus River and in a large part of the rural area (várzeas and igapós) probably dilutes the environmental contamination by oocysts and, therefore, the animals' exposure. However, when the dogs in the rural area that were living in seasonally flooded and inundated areas were compared, it was not possible to find any association ($p > 0.05$), thus indicating that the real risk factor for *T. gondii* infection was probably the environment (rural versus urban).

The *T. gondii* occurrence rate found among the dogs sampled in the present study (61.6%) was lower than the rates of 76.5% and 69.8% that were observed, respectively, among dogs in the urban area of Monte Negro, Rondônia (CAÑÓN-FRANCO et al., 2004) and in urban and rural areas of the state of Pará (VALADAS et al., 2010), which are both in the Amazon region. The increase in the occurrence rate with increasing age of the dogs observed in the present study was also found among the dogs in Monte Negro (CAÑÓN-FRANCO et al., 2004), thus indicating the existence of postnatal exposure to *T. gondii*.

For *N. caninum*, the occurrence rate of 7.1% was also lower than the rates of 8.3% found in Rondônia (CAÑÓN-FRANCO et al., 2003) and 8.4% found in the state of Pará (VALADAS et al., 2010). The seropositivity values obtained were similar in dogs of different ages and localities (urban and rural). The only variable associated with the presence of *N. caninum* antibodies was the sex of the dogs, such that all seven of the positive dogs were males. Very few studies conducted with dogs found association

between presence of *N. caninum* antibody and gender. Gózd et al. (2011), in Poland, observed higher seropositivity in female dogs; however, the gender seems to have little importance in the epidemiology of *N. caninum* infection in dogs (GENNARI, 2004).

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