

Occurrence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in cats with outdoor access in São Luís, Maranhão, Brazil

Ocorrência de anticorpos anti-*Toxoplasma gondii* e anti-*Neospora caninum* em gatos com acesso à rua em São Luís, Maranhão, Brasil

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Abstract

The present study aimed to investigate the frequency of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in cats with outdoor access in São Luís, Maranhão, Brazil. The presence of IgG anti-*T. gondii* and anti-*N. caninum* antibodies was tested using the Indirect Immunofluorescent Antibody Test (IFAT). IgG anti-*T. gondii* and anti-*N. caninum* antibodies were detected in 101 (50.5%) and 54 (27%) sampled cats, respectively. The titers of anti-*T. gondii* antibodies ranged from 40 (cut-off) to 2560. On the other hand, the titers of anti-*N. caninum* antibodies ranged from 25 (cut-off) to 400. Twenty-seven cats (13.5%) were shown to be seropositive for both parasites. Seventy-four cats (34%) were seropositive only for *T. gondii*. Twenty-two cats (11%) were seropositive only for *N. caninum*. The present study showed that cats with outdoor access in São Luís, Maranhão, are exposed to *T. gondii* and *N. caninum*.

Keywords: *Toxoplasma gondii*, *Neospora caninum*, cats, serology.

Resumo

O presente estudo objetivou verificar a frequência de anticorpos anti-*Toxoplasma gondii* e anti-*Neospora caninum* em gatos com acesso à rua em São Luís, Maranhão, Brasil. A presença de anticorpos IgG anti-*T. gondii* e anti-*N. caninum* foi verificado pela Reação de Imunofluorescência Indireta (RIFI). Anticorpos IgG anti-*T. gondii* e anti-*N. caninum* foram detectados em 101 (50,5%) e 54 (27%) gatos amostrados, respectivamente. Os títulos de anticorpos anti-*T. gondii* variaram de 40 (ponto de corte) a 2560. Por outro lado, anticorpos anti-*N. caninum* variaram de 25 (ponto de corte) a 400. Vinte e sete gatos (13,5%) mostraram-se soropositivos para ambos os parasitas. Setenta e quatro gatos (34%) foram soropositivos somente para *T. gondii*. Vinte e dois gatos (11%) foram soropositivos somente para *N. caninum*. O presente estudo demonstrou que gatos com acesso à rua em São Luís, Maranhão, são expostos ao *T. gondii* e *N. caninum*.

Palavras-chave: *Toxoplasma gondii*, *Neospora caninum*, gatos, sorologia.

Introduction

Toxoplasmosis is a zoonotic protozoan disease caused by the apicomplexan parasite *Toxoplasma gondii* (TENTER et al., 2000). This disease is acquired principally by eating food or drinking water contaminated with oocysts or by ingestion of tissue containing *T. gondii* cysts. Cats and related felids are the

definitive hosts, because they are the only animal species that excrete resistant oocysts into the environment (JACKSON; HUTCHINSON, 1989). *Toxoplasma gondii* has a wide range of intermediate hosts, including humans and several animal species, particularly mammals and birds (TENTER et al., 2000). On the other hand, *Neospora caninum*, a related coccidian protozoon, was first identified from the brain of a dog (DUBEY et al., 1988a, b). To date, only domestic dogs, coyotes (*Canis latrans*) and dingoes (*Canis lupus dingo*) have been recognized as definitive hosts for *N. caninum* (MCALLISTER et al., 1998a, b; LINDSAY et al., 1999; GONDIM et al., 2004; KING et al., 2010). Regarding

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economic importance in livestock, *N. caninum* is recognized as an important cause of abortion in cattle (DUBEY; LINDSAY, 1996). Most likely because cats may only play a minor role in the epidemiology of *N. caninum* infection, there are only a few reports on naturally acquired seropositivity to *N. caninum* among cats (DUBEY et al., 2002; FERROGLIO et al., 2005; BRESCIANI et al., 2007; HORNOK et al., 2008). The present study aimed to investigate the frequency of anti-*T. gondii* and anti-*N. caninum* antibodies in cats with outdoor access in São Luís, Maranhão, Brazil.

Material and Methods

1. Sample collection

Between October 2008 and January 2009, serum samples were collected by venipuncture in the jugular and/or cephalic vein from 200 peridomestic cats (*Felis catus*) in peripheral areas of São Luís, state of Maranhão. The sampled cats were of both genders and different breeds and ages. All the cats appeared to be healthy at the time of sample collection. To facilitate blood collection, the cats were chemically immobilized using xylazine (1 mg/kg, intramuscularly).

2. Serological tests for *T. gondii* and *N. caninum*

The presence and level of IgG anti-*T. gondii* and anti-*N. caninum* antibodies were tested using the Indirect Immunofluorescent Antibody Test (IFAT). The antigenic substrate for *T. gondii* consisted of purified tachyzoites that were obtained by means of peritoneal lavage of previously infected mice, as described by Camargo (1964).

The antigen substrate used in preparing slides to detect antibodies against *N. caninum* by means of IFAT was produced using isolate NC-1 (DUBEY et al., 1988a, b). For this, CV-1 cells were cultured in RPMI medium (Sigma, St. Louis, MO, USA), supplemented with 2% fetal calf serum (BFS). Three days after infection, parasites were harvested from mononuclear cell layers using 1% trypsin treatment, and were passed into another flask with CV-1 cells. The *N. caninum* tachyzoites that were recovered were used as the antigen substrate (FURUTA et al., 2007).

All serum samples were screened at serial dilutions in phosphate-buffered saline (PBS, pH 7.2), using cutoffs of 1:40 and 1:25 for *T. gondii* and *N. caninum*, respectively. Cat serum samples that were negative for *T. gondii* and *N. caninum* and samples naturally infected with these parasites, from the serum bank of the Immunoparasitology Laboratory, Department of Veterinary Pathology, Unesp, Jaboticabal, SP, were also used in the serological reactions. Briefly, slides with diluted serum samples were incubated at 37 °C in a moist chamber for 45 min, washed three times in PBS (pH 7.2) for 5 min, and air-dried at room temperature. IgG anti-cat conjugate labeled with fluorescein isothiocyanate (Sigma, St. Louis, MO, USA) was diluted at 1:64 in accordance with the manufacturer's instructions and was then added to each well. These slides were incubated again, washed, dried and overlain with buffered glycerin (pH 8.7), covered with glass coverslips, and examined under a fluorescence microscope (Olympus BX60).

Results

IgG antibodies against *T. gondii* and *N. caninum* were detected in 101 (50.5%) and 54 (27%) sampled cats, respectively. IgG antibody titers against *T. gondii* ranged from 40 (cutoff) to 2560. On the other hand, IgG antibody titers against *N. caninum* ranged from 25 (cutoff) to 400. Twenty-seven cats (13.5%) showed IgG antibodies for both *T. gondii* and *N. caninum*. Seventy-four cats

Table 1. Number of cats seropositive for *T. gondii* and *N. caninum*, according to IgG antibody titers.

Titers for <i>T. gondii</i>	Number of cats seropositive for:				Titers for <i>N. caninum</i>
	<i>T. gondii</i> (only)	<i>T. gondii</i> (but also seropositive to <i>N. caninum</i>)	<i>N. caninum</i> (but also seropositive to <i>T. gondii</i>)	<i>N. caninum</i> (only)	
			14	11	25
40	18	9	11	8	50
80	8	5	1	2	100
160	18	4	0	0	200
320	8	4	1	1	400
640	11	2			
1280	10	3			
3560	1	0			
Total	74	27	22	27	

(34%) were seropositive only for *T. gondii*. Twenty-two cats (11%) were seropositive only for *N. caninum* (Table 1). Seventy-seven cats (38.5%) were seronegative for both parasites.

Discussion

The incidence of toxoplasmosis and neosporosis in urban areas is closely related to environmental contamination with oocysts. Direct measurement of environmental contamination by means of oocyst counting is unfeasible for technical reasons (MEIRELES et al., 2004). One interesting alternative for measuring *T. gondii* and *N. caninum* dissemination is by analyzing the seroprevalence in free-living urban animals, which are thus used as sentinels. In this regard, the seroprevalence of *T. gondii* and *N. caninum* in animals could be an indirect indicator of the degree of parasite spreading in urban and rural areas.

The present study showed that cats in São Luís, state of Maranhão, are exposed to *T. gondii* and *N. caninum*. The prevalence of *T. gondii* antibodies (50.5%) in the cats of the present study was higher than what was found in previous studies conducted in Brazil, in the states of Santa Catarina (DALLA ROSA et al., 2010); São Paulo (SOGORB et al., 1972; LUCAS et al., 1998; SILVA et al., 2002; MEIRELES et al., 2004; PENA et al., 2006; BRESCIANI et al., 2007); Rio Grande do Sul (PINTO et al., 2009); and Rio de Janeiro (GONÇALVES NETO et al., 2003). On the other hand, the prevalence found in our study was lower than what was found among cats in Paraná (GARCIA et al., 1999) and Rondônia (CAVALCANTE et al., 2006). However, because different techniques and cutoff values were used, comparisons may generate misunderstandings (BRESCIANI et al., 2007).

The majority of the positive cats showed *T. gondii* antibodies at titers of 1:40. Lower titers may suggest latent infection (LUCAS et al., 1998). It is difficult to draw conclusions from interpreting a single serological test on cats and, therefore, paired serological tests are desirable. Titers higher than 1,024 usually represent strong determinants of toxoplasmosis with or without clinical signs of disease (BRESCIANI et al., 2007). Here, 11 cats showed antibody titers higher than 1,024. Furthermore, the level of *T. gondii* antibodies is not associated with oocyst shedding levels (OMATA et al., 1990).

The prevalence of antibodies against *N. caninum* found in the present study was similar than what was reported among cats in Araçatuba, state of São Paulo (cutoff > 1:16; BRESCIANI et al., 2007), and in Italy (cutoff > 1:80; FERROGLIO et al., 2005). On the other hand, it was higher than what was found by Dubey et al. (2002) in Brazil (11.9%; cutoff > 1:40), and by Hornok et al. (2008) in Hungary (0.6%; cutoff > 1:40). The possibility of cross-reactions with *T. gondii* was ruled out, given that serological cross-reactivity often occurs with soluble antigens; in the case of IFAT, it does not occur (DUBEY et al., 1996). Also, considering the fact that the lesions caused by *N. caninum* in cats are similar than those found in cats with toxoplasmosis (DUBEY et al., 1990), and the moderately high prevalence found in the present study, neosporosis should be a differential diagnosis for cats with neurological clinical signs.

Diet and access to the outdoor environment have been incriminated as important factors for cat infection (LUCAS et al., 1998). In our study, the sampled cats had free access to the outdoor environment, and probably had the opportunity to hunt small prey, thus becoming more susceptible to infection by *T. gondii* than are cats that are exclusively kept indoors. Small birds, like pigeons and sparrows, or rodents that live in the synanthropic environment, could have been the possible prey hunted by cats. Recently, it was found that pigeons (*Columba livia*) and sparrows (*Passer domesticus*) can act as intermediate hosts for *T. gondii* and *N. caninum* (MINEO et al., 2009; GONDIM et al., 2010). Rodents are part of the life cycle of *T. gondii*, acting as prey containing *T. gondii* cysts for cats (HUTCHISON; DUNACHIE, 1971). Huang et al. (2004) found from PCR that 5.8% of their rat sample were positive for *N. caninum* and suggested that rats could serve as a reservoir of infection. On the other hand, *Rattus norvegicus*, a common synanthropic rat species, could be considered to be a difficult prey for cats. Moreover, garbage food is more available than birds in urban areas (LUCAS et al., 1998).

The sampled cats must have had access to food found in domestic garbage, which is usually food similar to what is prepared for human consumption. *Toxoplasma gondii* and *N. caninum* cysts may be found in leftovers of meat for human consumption that is available in garbage. However, such meats are under sanitary control, and hence, neosporosis and toxoplasmosis from these sources were not investigated in the present survey. The seroprevalence of *T. gondii* among chickens in Brazil ranges from 39% to 66% (DA SILVA et al., 2003; DUBEY et al., 2003; DUBEY et al., 2006; DE OLIVEIRA et al., 2009); and among cattle, from 1% (GONDIM et al., 1999) to 71% (SANTOS et al., 2009). On the other hand, the seroprevalence of *N. caninum* among cattle in Brazil ranges from 14.3% (GUIMARÃES et al., 2004) to 91.2% (GUEDES et al., 2008). Intake of water contaminated by oocysts of *N. caninum* and *T. gondii* may also have played a role in transmission of these coccidia among the sampled cats.

The present study showed that cats in São Luís, Maranhão, with outdoor access, are exposed to *T. gondii* and *N. caninum*. While the role of cats as the definitive hosts in the epidemiology of toxoplasmosis is already well defined, the importance of these animals in the epidemiology of neosporosis in Brazil has not been determined yet.

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