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

Maria do Socorro Costa de Oliveira Braga*; Marcos Rogério André; Márcia Mariza Gomes Justi; Carla Roberta Freschi; Márcia Cristina Alves Teixeira; Rosangela Zacarias Machado

1Departamento de Clínicas Veterinárias, Universidade Estadual do Maranhão – UEMA, São Luís, MA, Brasil
2Departamento de Patologia Veterinária, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista – UNESP, Jaboticabal, SP, 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. On the other hand, os títulos de anti-N. caninum variaram de 25 (ponto de corte) a 400. Vinte e sete gatos (13,5%) foram 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
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>
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<tr>
<th>Titers for <em>T. gondii</em></th>
<th><em>T. gondii</em> (only)</th>
<th><em>T. gondii</em> (but also seropositive to <em>N. caninum</em>)</th>
<th><em>N. caninum</em> (but also seropositive to <em>T. gondii</em>)</th>
<th><em>N. caninum</em> (only)</th>
<th>Titers for <em>N. caninum</em></th>
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<td>1280</td>
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<td>3560</td>
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<td>74</td>
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Table 1. Number of cats seropositive for *T. gondii* and *N. caninum*, according to IgG antibody titers.
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.

**Acknowledgements**

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**References**


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