

Occurrence of *Neospora caninum* and *Toxoplasma gondii* antibodies in dogs from rural properties surrounding a biological reserve, Espírito Santo, Brazil

Ocorrência de anticorpos anti-*Neospora caninum* e *Toxoplasma gondii* em cães de propriedades rurais do entorno de uma reserva biológica, Espírito Santo, Brasil

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Abstract

The aim of this study was to determine the presence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in serum samples of 187 dogs from 30 rural properties surrounding Córrego do Veado Biological Reserve, Espírito Santo State, Brazil. The Reserve is one of the last lowland Atlantic Forest remnants of the region, surrounded by agriculture farms and cattle pastures. The presence of IgG antibodies was determined by the Indirect Fluorescent Antibody Test for *T. gondii* (cut-off 1:16) and *N. caninum* (cut-off 1:50). Positive samples were diluted 2-fold until the last positive dilution. Antibodies to *T. gondii* were found in 77 (47.05%) dogs and antibodies to *N. caninum* in 22 dogs (11.76%) and one only dog was positive for both infections. No association between *T. gondii* and *N. caninum* infection and sex was observed ($p>0.05$). Control measures to prevent those infections in dogs that living surrounding the reserve and that had contact with wild animals are important to avoid the introduction of *N. caninum* in wild animals. This was the first study of frequency of occurrence of *T. gondii* and *N. caninum* in dogs from Espírito Santo, Brazil.

Keywords: *Neospora caninum*, *Toxoplasma gondii*, dogs, Atlantic Forest.

Resumo

O objetivo deste estudo foi determinar a presença de anticorpos contra *Neospora caninum* e *Toxoplasma gondii* em amostras de soro de 187 cães de 30 propriedades rurais do entorno da Reserva Biológica Córrego do Veado, Espírito Santo, Brasil. A Reserva é um dos últimos remanescentes de Mata Atlântica da região, cercada por fazendas com agricultura e pastagens. A presença de anticorpos IgG foi determinada pela reação de imunofluorescência indireta para *T. gondii* (ponto de corte de 1:16) e para *N. caninum* (ponto de corte de 1:50). As amostras positivas foram diluídas na base dois e tituladas. Anticorpos para *T. gondii* foram encontrados em 77 (47,05%) cães e para *N. caninum* em 22 (11,76%). Um cão foi positivo para ambas as infecções. Não houve associação entre a infecção pelo *T. gondii* e *N. caninum* e o sexo ($p>0,05$). Medidas de controle, para prevenir essas infecções em cães que vivem no entorno da reserva e com possibilidade de contato com animais selvagens, são importantes para evitar a introdução de *N. caninum* nos animais selvagens. Este foi o primeiro relato da frequência de anticorpos contra estes coccídios em cães do Espírito Santo, Brasil.

Palavras-chave: *Neospora caninum*, *Toxoplasma gondii*, cães, Mata Atlântica.

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Toxoplasma gondii is a coccidian parasite found worldwide. It infects virtually all warm-blooded animals, including humans but only cats (domestic and wild) are its definitive hosts (DUBEY, 2010). *Neospora caninum* is also a coccidian parasite with a similar host range but canids are definitive hosts (MCALLISTER et al., 1998; GONDIM et al., 2004; KING et al., 2010; DUBEY et al., 2011). It causes mortality in dogs (DUBEY & LINDSAY, 1996) and abortion in cattle (ANDERSON et al., 1995; CORBELLINI et al., 2002).

In Brazil *T. gondii* is a common parasite, and the infection in dogs ranges from 2.6% to 90% (revised by DUBEY et al., 2012; FOURNIER et al., 2014; LOPES et al., 2011, 2015; RAIMUNDO et al., 2015). Also common, *N. caninum* is found to infect dogs in rates that go from 2.6% to 67.6% (revised by DUBEY, 2013).

In the State of Espírito Santo, information related to these parasites in dogs is not available. In this State, in the municipality of Pinheiros, is located the Córrego do Veado Biological Reserve, a protected area (2.357 ha) and one of the last lowland Atlantic Forest remnants of the region. Agriculture and cattle farms surround all the Reserve. Companion animals, mostly dogs, are often present and, because they are close to the forest, they can easily get in contact with wild animals. The objective of this study was to determine the frequency of occurrence of antibodies to *T. gondii* and *N. caninum* in dogs living in rural environment close to a forest matrix, in the state of Espírito Santo, where no data is available.

The present study was conducted in 30 rural properties surrounding the Córrego do Veado Biological Reserve (Figure 1), municipality of Pinheiros, State of Espírito Santo, southeastern region of Brazil (40°08'48"S, 18°20'33"W).

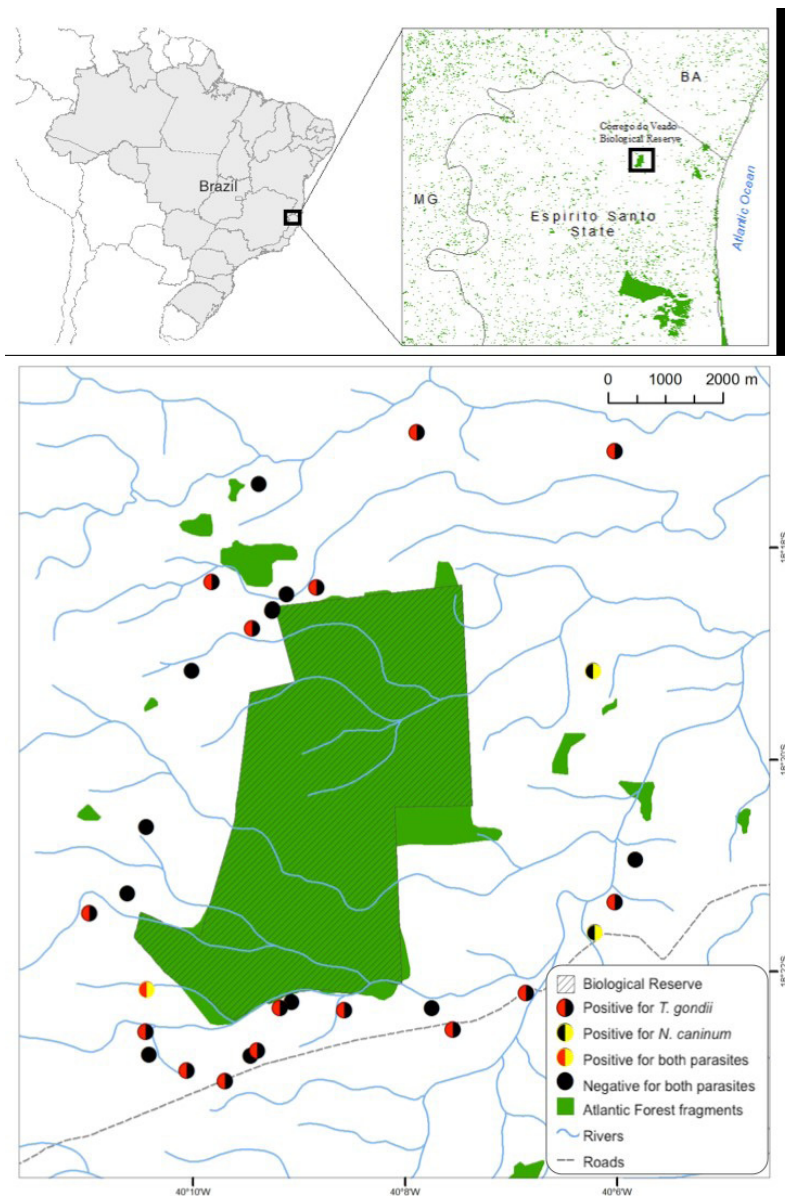


Figure 1. Geographical localization of Córrego do Veado Biological Reserve, municipality of Pinheiros, State of Espírito Santo, Brazil. Circles indicate rural properties with dogs tested for antibodies against *Toxoplasma gondii* and *Neospora caninum*, with: (red semicircle) positive for *T. gondii*; (yellow semicircle) positive for *N. caninum*; and (black semicircle) negative for both.

Serum samples were collected from all 187 dogs (70 females and 117 males) that were present in the farms on the day of the visit from January 2011 to November 2012. The blood was collected from a jugular or cephalic vein and serum was kept at -20 °C until analyses. The presence of IgG antibodies against *T. gondii* and *N. caninum* was determined by means of an Indirect Fluorescent Antibody Test (IFAT) with a cut-off of 1:16 for *T. gondii* (CAÑÓN-FRANCO et al., 2004) and 1:50 for *N. caninum* (SOUZA et al., 2002). All positive samples were diluted serially 2-fold until the last positive dilution. Positives and negatives samples were added in each slide.

The Pearson's chi-square test (χ^2) was applied to evaluate whether the presence of antibodies against both parasites is associated with gender of dogs.

The handling of the dogs was previously authorized by the owners and the study was approved by the Ethics Committee for Animal Use of the Faculty of Veterinary Medicine, University of São Paulo, Brazil.

Antibodies to *T. gondii* were found in 77 (47.05%) of 187 dogs from 16 of 30 farms with titers of 32 in six dogs, 64 in 13 dogs, 128 in 18 dogs, 256 in 14 dogs, 512 in 22 dogs and 1.024 in four dogs. Also, 22 dogs (11.76%) from three of the 30 farms tested positive for *N. caninum* antibodies, with titers of 100 in two dogs, 200 in five dogs, 800 in seven dogs, 12,800 in six dogs and 25,600 in two dogs. Sampling sites are shown in Figure 1.

Infection rates were not statistically different among female and male dogs: 32.86% (23/70) female dogs and 46.15% (54/117) male dogs were positive for *T. gondii* ($X^2=0.47$; $df=1$; $p>0.05$); and 11.4% (8/70) of female and 11.9% (14/117) of male tested positive for *N. caninum* ($X^2=0.52$; $df=1$; $p>0.05$). One dog presented antibodies for both coccidian.

The results demonstrate that *T. gondii* and *N. caninum* are distributed along the farms surrounding the Córrego do Veado Reserve, in Espírito Santo State, Brazil. The farms from where the dog's samples were collected, originally, were all Atlantic Rainforest territory, that is the biome with the highest degree of degradation and is currently restricted to remnants (ICMBio, 2000; ACOSTA et al., 2014). In the Reserve natural fauna and flora are preserved, and dozens of species of animals and plants that are under threat of extinguishment are present (ICMBio, 2000; CHIARELLO, 2000).

As show in Figure 1, around 50% of the farms surrounding the Reserve presented positive dogs to one or two studied coccidian. The farm dogs and cats, definitive hosts for *N. caninum* and *T. gondii*, respectively, easily can go to the Reserve territory and leave fecal oocysts in the area. Studies showed that both avian and mammals' species can clinically react diversely to *T. gondii* infection and for some species the parasite could be fatal (DUBEY, 2010). With *N. caninum* much less information is available in wild animals in the world and in Brazil (revised by ALMERIA, 2013).

In relation to *T. gondii*, an important zoonotic agent, the dogs are only intermediate hosts and, in this study, they represent sentinels of the environmental contamination. Due the proximity of the farms with the protected areas, special care should be introduced to reduce the wildlife infection possibilities. The occurrence of 47.05%, found in the rural dogs from Espírito Santo, is in

agreement with results found in other parts of Brazil (revised by DUBEY et al., 2012; LOPES et al., 2011).

N. caninum infections are higher in rural dogs, probably related to availability of dead cattle carcasses and abortion products (DUBEY, 2013) in those areas. In the present study the occurrence of 11.76% is in the range of the results found in Brazilian dogs from rural areas (reviewed by DUBEY, 2013).

This is the first survey of frequency of occurrence of antibodies to *T. gondii* and *N. caninum* in dogs in the State of Espírito Santo, Brazil.

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