

Seroprevalence of *Toxoplasma gondii* in domestic cats in tropical region of Serra da Tiririca State Park, Niterói, Rio de Janeiro

[Soroprevalência de *Toxoplasma gondii* em gatos domésticos na região tropical do Parque Estadual da Serra da Tiririca, Niterói, Rio de Janeiro]

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ABSTRACT

The present study aimed to determine the seroprevalence of anti-*T. gondii* antibodies in cats of the Serra da Tiririca State Park region using the IFAT to evaluate the sex and age variables. The cats were domiciled in the region of Serra da Tiririca State Park (PESET-RJ), Rio de Janeiro. Between 2015 and 2016, a total of 86 blood samples were collected from 35 female and 51 male semi-domiciled cats that were attended to by a spay-neuter program of UFF. Samples were evaluated for anti-*T. gondii* immunoglobulin G by the indirect immunofluorescence reaction (IFAT) with a cut-off point of 1:40. Of the samples analyzed, 29.07% (25/86) presented anti-*T. gondii* antibodies. There was no significant association ($P > 0.05$) between the variables sex and age of the animals with serology results. The results obtained in this study suggest that *T. gondii* has a considerable distribution among the cats of the studied region (PESET-RJ). This is the first report of the seroprevalence of *T. gondii* in cats from this area. Age and gender were not considered risk factors for infection by *T. gondii* in this group of animals studied.

Keywords: Toxoplasmosis; serology; cats; IFAT

RESUMO

O presente estudo teve como objetivo determinar a soroprevalência de anticorpos anti-*Toxoplasma gondii* em felinos da região do Parque Estadual da Serra da Tiririca (PESET-RJ), Rio de Janeiro, utilizando a IFAT para avaliar as variáveis de sexo e idade. Os gatos são oriundos da região do Parque Estadual da Serra da Tiririca (PESET-RJ), Niterói, Rio de Janeiro (RJ). Entre 2015 e 2016, um total de 86 amostras de sangue de felinos foi coletado de 35 fêmeas e 51 machos semidomiciliados, atendidos pelo Programa de Esterilização da UFF. As amostras foram avaliadas para pesquisa de anticorpos da classe imunoglobulina G (IgG) anti-*T. gondii* pela reação de imunofluorescência indireta (RIFI) com ponto de corte de 1:40. Das amostras analisadas, 29,07% (25/86) apresentaram anticorpos anti-*T. gondii*. Não se observou associação significativa ($P > 0,05$) entre as variáveis sexo e idade dos animais com os resultados da sorologia. Os resultados obtidos neste estudo sugerem que *T. gondii* possui uma distribuição considerável entre os felinos da região estudada (PESET-RJ). Este é o primeiro relato de soroprevalência de *T. gondii* em felinos desta área. Idade e sexo não foram considerados fatores de risco para infecção por *T. gondii* neste grupo de animais estudados.

Palavras-chave: toxoplasmose, sorologia, gatos, IFAT

INTRODUCTION

Toxoplasma gondii is a protozoan belonging to the family Sarcocystidae and the class Sporozoa. It is an obligatory intracellular parasite with the definitive host being domestic and wild felines and is one of the most widely studied parasites

due to its veterinary importance (Dubey *et al.*, 2016; Ahn *et al.*, 2019). The intermediary hosts of *T. gondii* include humans, other mammals, and birds that are infected through the ingestion of tissue cysts, soil and water contaminated by oocysts, or by congenital transmission, and the disease can affect a variety of cells from different tissues (Bastos *et al.*, 2014).

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Toxoplasmosis is a zoonotic disease of cosmopolitan distribution; moreover, it causes severe changes in fetuses, humans, and immunosuppressed populations (Wyrosdick and Schaefer, 2015). The determination of seroprevalence in cats is essential from the epidemiological point of view since there have been reports of outbreaks associated with infections derived from the presence of oocysts in the environment (Dubey et al., 2020).

Domestic and wild cats are important in the *T. gondii* cycle, as they are the only known definitive hosts. This contributes singularly to the perpetuation of the parasite (Wyrosdick and Schaefer, 2015; Dubey et al., 2020). However, the most common mode of infection of this host is through the ingestion of sporulated oocysts from the environment or by the tissue stages of the parasite through the practice of carnivorism (Gonçalves Netto et al., 2003). The sexual reproduction of the parasite occurs in the gastrointestinal tract of cats (Martorelli Di Genova et al., 2019), which eliminates oocysts through their feces in the environment. These oocysts sporulate and become infective after 1–2 days, depending on temperature and humidity (Wei et al., 2016).

In Brazil, there is a large population of wandering cats, and little is known about the prevalence of *T. gondii* in these animals in the country (Dubey et al., 2012). A single infected cat can eliminate millions of oocysts in the environment and may initiate an epidemiological outbreak of the disease (Dubey et al., 2020). The determination of seroprevalence is essential from the epidemiological point of view since there have been reports of outbreaks associated with infections derived from the presence of oocysts in the environment (Pena et al., 2006; Oliveira et al., 2000).

The detection of *T. gondii* in cats is important to establish continuous investigation and monitoring of the serological status. The importance is not only for updating the epidemiological survey for feline toxoplasmosis in a population of a specific region but also to alert the owner of that seropositive cat, as cats develop good immunity to oocyst re-excretion; however, there is no guarantee that cats will only eliminate oocysts in feces once in their lives (Dubey et al., 2020).

The present study aimed to determine the seroprevalence of anti-*T. gondii* antibodies in cats of the Serra da Tiririca State Park region using the indirect immunofluorescence technique (IFAT) to evaluate the sex and age variables. The felines could become infectious for other animals and humans living in this region. Therefore, the results of this study could be important since there is no data in the reviewed literature regarding the presence of this parasite in the studied region.

MATERIAL AND METHODS

This study was evaluated and approved by the Commission on Ethics for the Use of Animals of the Fluminense Federal University (UFF) under protocol number 793/2016 and by the Ethics Commission on the Use of Animals of the Federal Rural University of Rio de Janeiro (UFRRJ) under the protocol number 136/2014.

The samples were collected in the Serra da Tiririca State Park (PESET-RJ) (22°48'; 23°00'S and 42°57'; 43°02' W), area of environmental protection of lagoons and forests of the Municipality of Niterói, in the state of Rio de Janeiro, in the period from 2015 to 2016. The PESET is an area of integral coastal protection formed by the chain of mountains called Serra da Tiririca. Currently, PESET has an area of 34.92km² [3,492 hectares; (Parque..., 2019)].

A total of 86 serum samples from semi-domiciled domestic felines were used for the cat sterilization Project of the tropical region, between the period 2015 and 2016. Among the sample felines, 35 were females, and 51 were males. 3-mL blood samples were obtained from each animal by venipuncture (jugular or femoral), 10mL tubes (Vacutainer, BD Biosciences, Franklin Lanes, NJ, USA), stored in tubes without anticoagulant (EDTA), and then centrifuged (3,000 rpm for 10 min) to obtain an aliquot of each serum then conditioned in an isothermal container at 5°C. The samples were identified and maintained at -20°C, and the serology was performed by indirect immunofluorescence reaction (IFAT).

All the 86 serum samples were evaluated using an indirect fluorescent antibody test (IFAT) for demonstrate of antibodies to *T. gondii* in the felines. The samples were diluted 1:40 in

phosphate-buffered saline (PBS), pH 7.2, and prepared according to the manufacturer's instructions (Imunoteste®, Toxoplasma (RIFI) - Felino, Imunodot, Jaboticabal, SP, Brazil). After incubation in a humid chamber at 37°C for 30 minutes, the diluted samples and positive and negative control were washed three times with PBS and dried for 5 minutes. Then, the prepared anti-feline IgG conjugate was added with Evans blue solution and incubated for another 30 min in a dark room in the oven at 37°C. After further washing with PBS, the slides were assembled and observed under a Zeiss Axio Lab a1 fluorescence microscope with a 40× objective. Samples were considered positive when they showed evident fluorescence.

The animals were submitted to a general clinical examination and identified through an epidemiological survey that included lifestyle, age, sex, and type of feeding. The age of the animals was estimated according to data from the owners, and they were classified into kittens (up to 1-year-old), young (1- to 3-year-old), and adults (3- to 8-year-old).

Statistical analysis of the association of serology results by IFAT with sex and age was performed using the chi-square (χ^2) test, with a significance level of 0.05 (Vieira, 2015).

RESULTS AND DISCUSSION

This study is the first report on the frequency of anti-*T. gondii* antibodies in semi-domiciled cats from the tropical region of Serra da Tiririca State Park (PESET-RJ) in the city of Niterói (R.J.). A total seroprevalence of 29.07% (25/86) was observed (Table 1). Ribeiro *et al.* (2015) described a lower seroprevalence compared to the present study that reported a 10% prevalence of seropositivity in 100 samples of cats at FMVZ - Unesp Campus de Botucatu Veterinary Hospital (H.V). Also, in the city of Niterói, a study described a seroprevalence of 24.39% in a population of non-domiciled cats (GonçalvesNetto *et al.*, 2003).

When we compared the *T. gondii*-positive animals in terms of sex, 40% (10/25) of

seropositive females and 60% (15/25) of seropositive males. In terms of age, 32% (8/25) were kittens, 8% (2/25) were young, and 60% (15/25) of positive adults. No significant association ($p>0,05$) was observed between the sex and age of these animals with the serology results obtained in the IFAT.

Pereira *et al.* (2018) observed 21.9% of seropositivity among cats in a shelter and a set of condominiums in Rio de Janeiro. *Toxoplasma gondii* seroprevalence was 22.35% in feral cats at Trap-Neuter-Release clinics in Central Virginia, U.S.A. (Taetzsch *et al.*, 2018). However, in Fernando de Noronha, Brazil, a high prevalence of anti-*T. gondii* antibodies were found in the pet (71.26%) and feral cats (54.74%) (Magalhães *et al.*, 2017). The prevalence of *T. gondii* in cats depends on the animal's lifestyle (free access to the external environment or not), age, diet, diagnostic method, and geographical location (Pena *et al.*, 2006). In addition, the high prevalence of antibodies in cats indicates previous infection and excretion of *T. gondii* oocysts in the environment (Dubey *et al.*, 2020).

The results found in this study are also consistent with the data presented by Langoni *et al.* (2001), who described a 27% seroprevalence of *T. gondii* in a domiciled cat population, in the states of São Paulo and Paraná, using the indirect immunofluorescence technique with a dilution of 1:16. However, the dilution was different from that performed in the present study, making comparisons between studies difficult. Unlike the results of the present study, a project from the region corresponding to the Zoological Garden of the city of Rio de Janeiro conducted for three years indicated an index of high prevalence (92.1%) of *T. gondii* in felines in the first year of the study, with a decline in incidence in the following years (63.1% in the second year and 60.6% in the third year), among the total 75 samples studied. The authors pointed out that one of the essential factors for the fall in these indices was adopting the population control program in this area (Mendes-de-Almeida *et al.*, 2007).

Table 1. Seroprevalence of *Toxoplasma gondii* in domestic cats in tropical region of Serra da Tiririca State Park, Niterói-RJ according to sex, age, lifestyle, and type of feed

Number	Sex	Age	Lifestyle	Type of feeding	IFAT
1.	Female	Adult	semi-domiciled	not restricted	-
2.	Male	Adult	semi-domiciled	not restricted	-
3.	Male	Adult	semi-domiciled	not restricted	-
4.	Male	Adult	semi-domiciled	not restricted	-
5.	Male	Adult	semi-domiciled	not restricted	-
6.	Male	Adult	semi-domiciled	not restricted	-
7.	Male	Adult	semi-domiciled	not restricted	-
8.	Male	Adult	semi-domiciled	not restricted	-
9.	Female	Adult	semi-domiciled	not restricted	-
10.	Female	Adult	semi-domiciled	not restricted	-
11.	Female	Adult	semi-domiciled	not restricted	-
12.	Male	Adult	semi-domiciled	not restricted	-
13.	Female	Adult	semi-domiciled	not restricted	-
14.	Female	Adult	semi-domiciled	not restricted	-
15.	Female	Adult	semi-domiciled	not restricted	-
16.	Male	Adult	semi-domiciled	not restricted	-
17.	Male	Adult	semi-domiciled	not restricted	-
18.	Female	Adult	semi-domiciled	not restricted	-
19.	Female	Adult	semi-domiciled	not restricted	-
20.	Male	Adult	semi-domiciled	not restricted	-
21.	Male	Adult	semi-domiciled	not restricted	-
22.	Male	Adult	semi-domiciled	not restricted	-
23.	Male	Adult	semi-domiciled	not restricted	-
24.	Male	Adult	semi-domiciled	not restricted	-
25.	Female	Adult	semi-domiciled	not restricted	-
26.	Male	Adult	semi-domiciled	not restricted	-
27.	Female	Adult	semi-domiciled	not restricted	-
28.	Female	Adult	semi-domiciled	not restricted	-
29.	Female	Adult	semi-domiciled	not restricted	-
30.	Female	Adult	semi-domiciled	not restricted	-
31.	Female	Adult	semi-domiciled	not restricted	-
32.	Female	Adult	semi-domiciled	not restricted	-
33.	Male	Adult	semi-domiciled	not restricted	-
34.	Male	Adult	semi-domiciled	not restricted	-
35.	Male	Adult	semi-domiciled	not restricted	-
36.	Male	Adult	semi-domiciled	not restricted	-
37.	Female	Adult	semi-domiciled	not restricted	-
38.	Male	kittens	semi-domiciled	not restricted	-
39.	Male	kittens	semi-domiciled	not restricted	-
40.	Male	kittens	semi-domiciled	not restricted	-
41.	Female	kittens	semi-domiciled	not restricted	-
42.	Female	kittens	semi-domiciled	not restricted	-
43.	Female	kittens	semi-domiciled	not restricted	-
44.	Male	kittens	semi-domiciled	not restricted	-
45.	Female	kittens	semi-domiciled	not restricted	-
46.	Male	kittens	semi-domiciled	not restricted	-
47.	Female	kittens	semi-domiciled	not restricted	-
48.	Male	kittens	semi-domiciled	not restricted	-
49.	Female	kittens	semi-domiciled	not restricted	-
50.	Male	kittens	semi-domiciled	not restricted	-
51.	Female	kittens	semi-domiciled	not restricted	-
52.	Male	Young	semi-domiciled	not restricted	-
53.	Male	Young	semi-domiciled	not restricted	-
54.	Male	Young	semi-domiciled	not restricted	-
55.	Male	Young	semi-domiciled	not restricted	-
56.	Male	Young	semi-domiciled	not restricted	-
57.	Female	Young	semi-domiciled	not restricted	-
58.	Male	Young	semi-domiciled	not restricted	-
59.	Male	Young	semi-domiciled	not restricted	-
60.	Male	Young	semi-domiciled	not restricted	-

Seroprevalence of...

Number	Sex	Age	Lifestyle	Type of feeding	IFAT
61.	Male	Young	semi-domiciled	not restricted	-
62.	Female	Adult	semi-domiciled	not restricted	+
63.	Male	Adult	semi-domiciled	not restricted	+
64.	Male	Adult	semi-domiciled	not restricted	+
65.	Male	Adult	semi-domiciled	not restricted	+
66.	Female	Adult	semi-domiciled	not restricted	+
67.	Female	Adult	semi-domiciled	not restricted	+
68.	Female	Adult	semi-domiciled	not restricted	+
69.	Female	Adult	semi-domiciled	not restricted	+
70.	Male	Adult	semi-domiciled	not restricted	+
71.	Male	Adult	semi-domiciled	not restricted	+
72.	Male	Adult	semi-domiciled	not restricted	+
73.	Male	Adult	semi-domiciled	not restricted	+
74.	Male	Adult	semi-domiciled	not restricted	+
75.	Female	Adult	semi-domiciled	not restricted	+
76.	Male	Adult	semi-domiciled	not restricted	+
77.	Male	kittens	semi-domiciled	not restricted	+
78.	Male	kittens	semi-domiciled	not restricted	+
79.	Male	kittens	semi-domiciled	not restricted	+
80.	Female	kittens	semi-domiciled	not restricted	+
81.	Female	kittens	semi-domiciled	not restricted	+
82.	Female	kittens	semi-domiciled	not restricted	+
83.	Male	kittens	semi-domiciled	not restricted	+
84.	Male	kittens	semi-domiciled	not restricted	+
85.	Female	Young	semi-domiciled	not restricted	+
86.	Male	Young	semi-domiciled	not restricted	+
Total					29.07% (25/86)

The results presented in this study demonstrate the epidemiological importance of the parasite since these positive animals are in direct contact with humans and other animals of the region. Nonhuman primates are sensitive to *T. gondii*, and high mortality occurs when infected (Grumann *et al.*, 2017). A study on nonhuman primates from the PESET-RJ tropical region reported that these animals had direct contact with the local human population and domestic animals, frequently entering the houses and digging in the garbage, and may have some degree of contact with *T. gondii* oocysts (Molina *et al.*, 2017).

Based on the studies cited above, it can be suggested that domiciled cats fed with processed foods and restricted access to the street can reduce the exposure to *T. gondii* and possible infection (Bastos *et al.*, 2014). These factors were not observed in the animals used in this study since their owners allowed free access to the PESET-RJ tropical region environment, and feeding was not restricted. In addition, the infection may originate from the predation of other animals of the region, thereby exposing themselves to risk factors that may lead to *T. gondii* infection. Cats play an important role in the epidemiology of *T. gondii* infection in humans and other animals since they are the

definitive hosts and the only ones that eliminate oocyst in feces (Pena *et al.*, 2006; Martorelli Di Genova *et al.*, 2019).

In humans, risk factors for *T. gondii* infection are associated with hygiene, diet, cultural habits, and climate. One of the routes of disease and potential exposure, besides the lack of environmental sanitation and consumption of contaminated food, points to domestic felines in the environment and living with humans. This aspect further establishes the importance of seropositivity of these animals in the environment in which they live (Moura *et al.*, 2013). Prophylactic knowledge among the owners about the infection of *T. gondii* proves to be an important but not much-studied aspect as a preventive measure for the disease. Therefore, knowledge of these measures may reduce infection in both humans and their domestic animals (Vicente *et al.*, 2014).

Although the present study region constitutes an Environmental Protection Area of Lagoons and Forests of the Municipality of Niterói, basic sanitation is not ensured, which generates a public health problem due to the formation of sewage in the region (Barros, 2008). This problem may result in possible environmental contamination and diseases such as

toxoplasmosis in animals and humans living in the region. Studies demonstrate that untreated or unfiltered water consumption is a strong predictor of *T. gondii* seropositivity in endemic areas since the oocysts can remain viable for a long period in the water (Bahia-Oliveira et al., 2003). Also, the water was the source of transmission in Santa Maria, Rio Grande do Sul, of the largest outbreak of human toxoplasmosis ever identified in the world so far, with 809 confirmed cases (Minuzzi et al., 2020).

When we compared the *T. gondii*-positive animals in terms of sex, 40% (10/25) of seropositive females and 60% (15/25) of seropositive males. In terms of age, 32% (8/25) were kittens, 8% (2/25) were young, and 60% (15/25) were positive adults. No significant difference was observed when the sex ($p = 0.8729$) and age ($p = 0.4809$) of these animals were compared with the serology results obtained in the IFAT.

In the present study, there was no significant association with the sex and age of the animals. Similar results were obtained by Pena et al. (2006) and Bresciani et al. (2007), who reported no significant difference in the prevalence of infection between males and females, both of which are equally susceptible to *T. gondii*. According to (Dubey et al., 2020) seropositivity increases as the cats age increases, as there is strong evidence that postnatal toxoplasmosis infection occurs. Although this association was not significant in these studies, the parasite presents numerous routes for disease transmission and a complex life cycle, including several risk factors such as specific populations and exposure to the environment.

The presence of its definitive infected host in each environment has a significant serological correlation between cats and humans, not by direct contact but by environmental contamination due to the cats' ability to eliminate millions of oocysts in the environment, which further reinforces their importance in the life cycle of the parasite and dissemination of the disease independent of the sex and age of these animals (Oliveira et al., 2000; Dubey et al., 2020).

Indirect immunofluorescence reaction (IFAT) is a quick and practical technique with high

specificity (97.0%) and sensitivity (96.0%) for cat specimens. It is used for routine serological tests in this species and others (Baneth et al., 2016). Comparison of this technique with the indirect agglutination method showed small differences between the two methods, demonstrating the agreement results between the two serological tests with cats' samples (Silva et al., 2002). The IFAT serological diagnostic method for the detection of *T. gondii* antibodies used in this study was effective, quick, and simple.

A study on the seroprevalence of *T. gondii* in other animals from the state of Rio de Janeiro also showed a significant prevalence of the parasite in this region. In a more comprehensive study of horses from this state, a broad distribution of the parasite was observed in the regions studied, evidencing the presence of the parasite and its zoonotic potential (Venturi et al., 2017).

Sheep and goats are also species with epidemiological relevance in Rio de Janeiro, being important sources of parasite transmission to humans through the consumption of undercooked meat and causing reproductive losses in these species (Silva et al., 2013; Pereira et al., 2018).

The animals used in this study had access to the region of Serra da Tiririca State Park (PESET - RJ), acquiring direct contact with the biome belonging to the park and the people living in the region; the infected felines are potential sources of contamination of the environment and water. Thus, the interaction between domestic and wild animals and humans and the transmission of zoonotic agents such as *T. gondii* are approximated (Nunes et al., 2013).

CONCLUSION

The results obtained in this study suggest that *T. gondii* has a considerable distribution among the cats of the studied region (PESET-RJ). This is the first report of the seroprevalence of *T. gondii* in cats from this area. Age and gender were not considered risk factors for infection by *T. gondii* in this group of animals studied.

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