Pesq. Vet. Bras. 46:e07825, 2026
DOI: 10.1590/1678-5150-PVB-7825

Original Article
Small Animal Diseases

Seroprevalence of anti-*Toxoplasma gondii* antibodies, associated factors, and spatial analysis in domiciled dogs from the metropolitan region of Rio de Janeiro, Brazil[1]

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ABSTRACT.- Gomes COE, Athayde-Gusmão AE, Santos LMCA, Coelho BP, Frazão-Teixeira E, Ubiali DG, Silva AF. **Seroprevalence of anti-*Toxoplasma gondii* antibodies, associated factors, and spatial analysis in domiciled dogs from the metropolitan region of Rio de Janeiro, Brazil**. *Pesquisa Veterinária Brasileira 46:e07825, 2026.* Faculdade de Medicina Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 Km 7, Campus Seropédica, RJ 23890-000, Brazil. E-mail: andressafsilva@ufrrj.br

Toxoplasmosis is a zoonotic disease of major public health importance caused by *Toxoplasma gondii*. This study aimed to determine the seroprevalence of anti-*T. gondii* antibodies in domiciled dogs from the metropolitan region of Rio de Janeiro, Brazil, identify associated factors, and evaluate the spatial distribution of seropositive cases. A total of 47 dogs attended at a veterinary center located in the Pavuna neighborhood were evaluated using the indirect immunofluorescence assay (IFAT), with a cutoff titer of 1:40. Epidemiological data were obtained through a structured questionnaire applied to dog owners. The overall seroprevalence was 38.3% (18/47). A significant association was observed between seropositivity and contact with cats in the household environment ($p \leq 0.05$). No significant association was observed between seropositivity and area of origin. No significant associations were observed with sex, age, type of diet, consumption of raw meat, type of environment, or scent-rolling behavior (rolling in feces or decomposing organic matter). These findings suggest previous exposure to *T. gondii* among dogs from the studied area and reinforce the importance of epidemiological and spatial analyses for understanding exposure patterns.

INDEX TERMS: Indirect immunofluorescence assay (IFAT), risk factors, toxoplasmosis, serology, dogs.

[1] Received on March 19, 2026.
Accepted for publication on July 13, 2026.

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RESUMO.- [**Soroprevalência de anticorpos anti-Toxoplasma gondii, fatores associados e análise espacial em cães domiciliados da região metropolitana do Rio de Janeiro, Brasil.**] A toxoplasmose é uma zoonose de grande importância para a saúde pública, causada por *Toxoplasma gondii*. Este estudo teve como objetivo determinar a soroprevalência de anticorpos anti-*T. gondii* em cães domiciliados da região metropolitana do Rio de Janeiro, Brasil, identificar fatores associados e avaliar a distribuição espacial dos casos soropositivos. Um total de 47 cães atendidos em um centro veterinário localizado no bairro de Pavuna foram avaliados por meio da reação de imunofluorescência indireta (RIFI), com título de corte de 1:40. Os dados epidemiológicos foram obtidos por meio de um questionário estruturado aplicado aos tutores dos cães. A soroprevalência geral foi de 38,3% (18/47). Observou-se associação significativa entre a soropositividade e o contato com gatos no ambiente domiciliar ($p \leq 0,05$). Não foi observada associação significativa entre a soropositividade e a área de origem. Também não foram observadas associações significativas com sexo, idade, tipo de dieta, consumo de carne crua, tipo de ambiente ou comportamento de rolamento em odores (rolar em fezes ou matéria orgânica em decomposição). Esses achados sugerem exposição prévia a *T. gondii* entre os cães da área estudada e reforçam a importância das análises epidemiológicas e espaciais para a compreensão dos padrões de exposição.

TERMOS DE INDEXAÇÃO: Imunofluorescência indireta (RIFI), fatores de risco, toxoplasmose, sorologia, cães.

INTRODUCTION

Toxoplasmosis is a zoonotic disease with worldwide distribution and major public health relevance, caused by the obligate intracellular protozoan *Toxoplasma gondii*. Felids are the only definitive hosts of the parasite, whereas a wide range of vertebrate species, including humans, act as intermediate hosts (Huertas-López et al. 2021, Dubey 2022). Canids are considered important environmental sentinels for indirectly detecting environmental contamination by *T. gondii* oocysts (Ferreira et al. 2016, Silva et al. 2025). This is mainly because domiciled dogs often share environments and habits similar to those of their owners and may therefore reflect household and peridomestic exposure to sources of infection such as unfiltered water, contaminated soil, and improperly prepared food (Arruda et al. 2021). In addition, it is not uncommon for dogs to be fed leftovers or homemade food, which may represent a potential route of exposure to the parasite and may also indicate a shared risk with humans (Dubey 2022).

Although dogs are not definitive hosts and do not shed oocysts in their feces, they may act as mechanical carriers of *T. gondii* due to their habit of rolling in feces, carcasses, or contaminated environments, potentially transporting oocysts adhered to their fur and contributing to the indirect dissemination of the parasite (Rezaei et al. 2021). Furthermore, behaviors such as coprophagy may increase the risk of infection, especially in dogs that share the same environment with cats that circulate in the peridomicile, increasing the likelihood of exposure to oocysts present in the environment (Dubey et al. 2020). Following infection, dogs may develop necrotizing lesions associated with tachyzoite proliferation in different tissues (Pereira et al. 2022). Most immunocompetent dogs tend to remain asymptomatic; however, immunosuppressed animals are more likely to develop clinical manifestations compatible with toxoplasmosis (Calero-Bernal & Gennari 2019). The main clinical signs described in symptomatic dogs include fever, weight loss, lethargy, and focal or generalized lesions, and may involve lymphadenitis, encephalitis, pneumonia, myocarditis, and retinochoroiditis (Arruda et al. 2021).

Serological analyses are widely performed in dogs worldwide, and a range of tests can be used to detect anti-*T. gondii* antibodies (Dubey et al. 2020). In Brazil, which is considered endemic for toxoplasmosis, a total of 60 serological studies involving dogs have been reported. Among these studies, the indirect fluorescent antibody test IFAT, recognized as a reliable diagnostic method, has been the most commonly used, followed by the modified agglutination test MAT and the enzyme-linked immunosorbent assay ELISA. In addition, the indirect hemagglutination assay IHA has also been used, although less frequently than the other aforementioned tests (Silva et al. 2025).

Dogs are among the most common companion animals in Brazilian households, and according to data from the Brazilian federal government, the estimated dog population in the country is approximately 62.2 million (Brasil 2025). Given the epidemiological relevance of this species, the present study aimed to perform a clinical and seroepidemiological assessment of domiciled dogs, investigating seropositivity for anti-*T. gondii* antibodies and its association with risk factors related to infection in a microregion of the state of Rio de Janeiro, Brazil.

MATERIALS AND METHODS

Ethical approval. This study was submitted to the Ethics Committee on the Use of Animals (CEUA) of the "Instituto de Veterinária" (IV), "Universidade Federal Rural do Rio de Janeiro" (UFRRJ), and approved under protocol number CEUA no. 9957151221.

Study area. The study was conducted at the "Centro Integrado de Terapia Veterinária" (CITVET), located in the Pavuna neighborhood, municipality of Rio de Janeiro, Brazil. The animals included in the study originated from the metropolitan region of the state of Rio de Janeiro, including the municipalities of Rio de Janeiro, São João de Meriti, and Nova Iguaçu.

Laboratory procedures. Laboratory analyses were performed at the "Laboratório de Pesquisa Apicomplexa" (LAPA), IV-UFRRJ.

Animals and sampling criteria. A convenience sample of 47 dogs of different breeds, sizes, ages, and both sexes was selected as they arrived for routine veterinary care. All animals were domiciled and were accompanied by their owners at the time of consultation. Inclusion criteria were body weight between 5 and 20 kg and owner authorization for participation in the study.

Clinical evaluation. All dogs underwent complete clinical examination to support the diagnosis of toxoplasmosis and other comorbidities. During anamnesis, the owner's personal information was collected, and all data were recorded in individual clinical forms corresponding to each animal. The physical examination included general observation, cardiac and pulmonary auscultation, abdominal palpation and percussion, neurological assessment, and locomotor evaluation. When necessary, complementary diagnostic tests were requested to investigate specific pathological conditions.

Blood collection and serum storage. After clinical evaluation, approximately 1 mL of blood was collected from each animal by puncture of the cephalic vein using a scalp needle, a disposable 3 mL syringe, and a Vacutainer tube with clot activator. Samples were processed for serum separation and stored at −20 °C until serological testing for anti-*Toxoplasma gondii* antibodies.

Indirect immunofluorescence assay (IFAT). Serum samples were analyzed by indirect immunofluorescence assay IFAT for the detection of anti-*T. gondii* antibodies, using the commercial kit Imunoteste® *Toxoplasma gondii* RIFI Canino, Imunodot Diagnósticos, Brazil. This kit is commercially indicated for the *in vitro* diagnosis of canine toxoplasmosis by indirect immunofluorescence and includes positive and negative control sera supplied by the manufacturer. Serum samples were centrifuged at 5,000 rpm for 15 minutes at room temperature. Dilutions were prepared starting at 1:40, using 1 µL of serum diluted in 39 µL of 1× phosphate-buffered saline PBS. Immunofluorescence slides containing 12 wells were used. Ten microliters of the negative control were added to the sixth well, and 10 µL of the positive control supplied with the kit were added to the seventh well. Ten microliters of each diluted serum sample were added to the remaining wells, allowing 10 samples to be tested per slide. Slides were incubated in a humid chamber at 37 °C for 30 minutes and washed three times with 1× PBS for 5 minutes each. Subsequently, 10 µL of Evans blue solution, prepared by mixing 14 µL of Evans blue with 140 µL of conjugate, was added to each well. Slides were incubated again in a humid chamber at 37 °C for 30 minutes, followed by three additional washes in 1× PBS for 5 minutes each. After drying, slides were mounted with buffered glycerin and coverslips and examined under a Zeiss Axio Lab.A1 microscope at 400× magnification. Reactions were considered positive when complete peripheral fluorescence was observed in more than 50% of tachyzoites. Partial or apical fluorescence patterns were considered negative. The positive and negative controls supplied by the commercial kit were included in each reaction.

Epidemiological questionnaire. During the consultation, an epidemiological questionnaire was administered to dog owners to identify the main risk factors associated with toxoplasmosis in the animals' household environment. The questionnaire included open- and closed-ended questions addressing age group, sex, housing environment, feeding practices, origin, contact with cats, hunting behavior, and scent-rolling behavior (rolling in feces or decomposing organic matter). The collected data were subsequently evaluated in association with the serological results.

Statistical analysis. Questionnaire data were initially analyzed using descriptive statistics. Associations between seropositivity and categorical variables were assessed using Pearson's chi-square test when expected frequencies were adequate, and Fisher's exact test was applied for 2 × 2 comparisons with small expected frequencies. Statistical analyses were performed using BioEstat 5.0 software, adopting a 95% confidence level ($p \leq 0.05$), according to Ayres et al. (2007). Crude odds ratios (OR) and 95% confidence intervals (95% CI) were calculated only for dichotomous variables. Variables with multiple categories and sparse data or zero cells were presented descriptively and were not subjected to inferential analysis.

Spatial analysis and mapping. Location maps were generated to indicate the geographic origin of each animal, as well as a Kernel density map to identify areas of higher risk within the study region. Addresses collected during the consultations were converted into geographic coordinates using Google Maps. Thematic

maps were created using cartographic data and Rio de Janeiro shapefiles obtained from the "Instituto Brasileiro de Geografia e Estatística" (Brazilian Institute of Geography and Statistics – IBGE 2019, 2022) and the DATA.RIO database. Spatial interpolation of origin points and Kernel density estimation were performed using QGIS software version 3.16.0 (2019), adopting the "Sistema de Referência Geocêntrico para as Américas" (Geocentric Reference System for the Americas – SIRGAS 2000). In addition, an inset map of Brazil was included to indicate the location of the study area within the country.

RESULTS

An overall seropositivity of 38.3% (18/47) for anti-*Toxoplasma gondii* antibodies was observed. Seropositivity rates and the evaluated risk factors associated with *T. gondii* infection in the studied dogs are presented in Table 1.

Regarding sex, 42.5% (20/47) of the animals were males and 57.5% (27/47) were females. Seropositivity was 40.0% (8/20) among males and 37.0% (10/27) among females. No significant association was observed between seropositivity and sex ($p = 0.8364$).

With respect to breed distribution, the following proportions were observed: 48.9% (23/47) mixed-breed dogs, 4.3% (2/47) Chow Chow, 10.6% (5/47) Shih Tzu, 2.1% (1/47) Beagle, 4.25% (2/47) German Shepherd, 4.25% (2/47) Yorkshire Terrier, 2.1% (1/47) Labrador Retriever, 2.1% (1/47) Pinscher, 8.5% (4/47) French Bulldog, 4.25% (2/47) Poodle, 6.4% (3/47) Pit Bull, and 2.1% (1/47) Dachshund. The number of seropositive dogs according to breed was as follows: 47.8% (11/23) among mixed-breed dogs, 100% (2/2) among German Shepherds, 100% (1/1) among Pinschers, 75.0% (3/4) among French Bulldogs, and 50.0% (1/2) among Poodles. The distribution of seropositive dogs according to breed is presented descriptively in Table 1.

Regarding age, 10.7% (5/47) were puppies up to one year old, 27.6% (13/47) were young dogs between one and three years, 42.6% (20/47) were adults between three and seven years, and 19.1% (9/47) were older than seven years. Seropositivity was 60.0% (3/5) among puppies, 53.85% (7/13) among young dogs, 25.0% (5/20) among adult dogs, and 33.3% (3/9) among older dogs. No significant association was observed between seropositivity and age ($p = 0.2689$).

Contact with cats was reported for 36.2% (17/47) of the dogs, whereas 63.8% (30/47) did not live with cats. Among dogs that had contact with cats, 58.8% (10/17) were seropositive, while among those without contact, 26.7% (8/30) were seropositive. A significant association was observed between seropositivity and contact with cats ($p = 0.0297$).

Four dogs (8.5%, 4/47) exhibited scent-rolling behavior (rolling in feces or decomposing organic matter), whereas 91.5% (43/47) did not. Among dogs exhibiting this behavior, 25.0% (1/4) were seropositive, while 39.5% (17/43) of those without this behavior were seropositive. No significant association was observed between scent-rolling behavior and seropositivity ($p = 0.5565$).

Regarding feeding practices, 70.2% (33/47) of the dogs were fed commercial dog food, whereas 29.8% (14/47) were fed partially or entirely homemade food. Among dogs fed commercial food, 39.4% (13/33) were seropositive, while among those receiving homemade food, 35.7% (5/14) were seropositive. No significant association was found between type of diet and seropositivity ($p = 0.8119$).

Additionally, raw meat consumption was reported in 31.9% (15/47) of the dogs, while 68.1% (32/47) did not receive raw meat. Among dogs fed raw meat, 53.3% (8/15) were seropositive, whereas 31.3% (10/32) of dogs not fed raw meat were seropositive. No significant association was observed between raw meat consumption and seropositivity ($p = 0.1492$).

Evaluation of the living environment revealed that 25.5% (12/47) of the dogs spent most of the time on soil or grass, while 74.5% (35/47) lived in cemented or paved areas. Among dogs living on soil/grass, 58.3% (7/12) were seropositive, whereas 31.4% (11/35) of those living on paved areas were seropositive. No significant association was observed between living environment and seropositivity ($p = 0.0900$).

The geographic origin of the animals was distributed as follows: 68.1% (32/47) from Pavuna, 2.1% (1/47) from Costa Barros, 8.5% (4/47) from Anchieta, 2.1% (1/47) from Vila da Penha, 2.1% (1/47) from São Cristóvão, 8.5% (4/47) from São João de Meriti, and 8.5% (4/47) from Nova Iguaçu. Among these locations, Pavuna showed a seropositivity of 34.4% (11/32) and represented the largest number of animals included in the study. The single dog from Costa Barros was seropositive. Anchieta and Nova Iguaçu each contributed four animals, three of which were seropositive, resulting in a seropositivity rate of 75.0% (3/4) in both locations. No significant association was observed between seropositivity and area of origin ($p = 0.0541$).

Kernel density mapping indicated the area with the highest concentration of seropositive dogs (Fig. 1).

Clinical findings showed that 27.65% of dogs presented no clinical signs (13/47). Gastrointestinal alterations were observed in 21.27% (10/47), ehrlichiosis was diagnosed in 14.9% (7/47), dermatological

alterations were observed in 14.9% (7/47), canine distemper was diagnosed in 6.4% (3/47), allergic reactions were observed in 6.4% (3/47), neurological alterations were observed in 4.25% (2/47), endocrinopathy was diagnosed in 2.1% (1/47), and ophthalmic alterations were observed in 2.1% (1/47).

DISCUSSION

Rio de Janeiro is considered an endemic state for toxoplasmosis, although there is still a limited number of studies evaluating *Toxoplasma gondii* infection in domestic dogs in this region. Previous evidence indicates that high population density and the presence of household waste accumulation in residential yards are factors associated with the circulation of the parasite (Silva et al. 2025). In the present study, 38.3% of dogs presented anti-*T. gondii* antibodies. Kernel density mapping identified areas with a greater concentration of sampled seropositive dogs; however, no statistically significant spatial difference was observed among the evaluated areas (IBGE 2022).

Ornellas (2017), in a study conducted at the Campus Fiocruz Mata Atlântica (CFMA), located in a nearby area, reported a seroprevalence of 37.2% for *T. gondii* using the same diagnostic method, supporting the findings of the present study and reinforcing that the municipality of Rio de Janeiro may represent an area with substantial transmission potential. In contrast, Leal (2014), also in Rio de Janeiro, reported a seroprevalence of 10.44% for anti-*T. gondii* antibodies, suggesting an increase in the proportion of seropositive dogs over the last few years when compared with more recent data. Cunha et al. (2016) evaluated dogs from the municipalities of Cachoeiras de Macacu, Guapimirim, Itaboraí, Magé, Resende, Seropédica, and Silva Jardim, also in the state of Rio de Janeiro, and reported 46.08% seropositivity, which is higher than that observed in the present study. It is important to note that most areas included in their study were less urbanized, with predominance of rural and peri-urban environments, which may represent a relevant risk factor for parasite transmission. Additionally, population habits such as feeding dogs homemade food and providing unfiltered water may also contribute to increased exposure. Furthermore, Cunha et al. (2016) used an indirect enzyme-linked immunosorbent assay (ELISA), which is generally considered more sensitive and is often used as a screening method, which may partially explain the higher prevalence reported.

Regarding diet, no significant association was observed between feeding type and seropositivity in the present study. However, it was reported that approximately one third of the dogs received raw meat. This practice, particularly when meat originates from unknown or inadequate sources, may negatively affect animal health and should be discouraged. In addition, 35.7% of dogs fed homemade food were seropositive, which may reflect an increased risk of horizontal transmission through food, considered one of the most important routes of infection for both humans and animals (Silva et al. 2016). Several studies (Cunha et al. 2016, Arruda et al. 2021, Freitas et al. 2022, Dini et al. 2024, Silva et al. 2025) have identified feeding practices as relevant risk factors for *T. gondii* infection in domestic dogs. In this context, veterinarians play an essential role in advising owners on proper food preparation, including meat freezing and cooking as prophylactic measures. Pinto-Ferreira et al. (2021) reported that non-sporulated oocysts are more sensitive to cold inactivation, being inactivated at −21 °C after one day. In contrast, sporulated oocysts require exposure to the same temperature for 28 days. Regarding heat treatment, the same study demonstrated that oocysts are inactivated after exposure to 50 °C for 30 minutes, 55–60 °C for 15 minutes, 70 °C for 2 minutes, 80 °C for 1 minute, and 90 °C for 30 seconds.

Sex was not identified as a risk factor in the present study, suggesting that infection affects males and females similarly. Ferreira et al. (2016) evaluated 598 dogs from a veterinary hospital using indirect immunofluorescence (IFA) for detection of anti-*T. gondii* antibodies and found no correlation between sex and seropositivity, corroborating the present findings. Although age was not statistically associated with seropositivity, more than half of young dogs aged between one and three years were seropositive. Arruda et al. (2021) reported that dogs in this age group have a higher probability of infection compared to other age categories. This may be related to the more active and exploratory behavior of young dogs, which may increase exposure to infection sources, such as hunting, contact with feces from other animals, or even ingestion of fecal material.

Dogs with cat contact in their household environment showed a significantly higher probability of *T. gondii* seropositivity. Fernandes et al. (2018) and Dini et al. (2024) reported that cohabitation with cats is a relevant factor for toxoplasmosis prevalence in dogs, with an estimated 1.57-fold increased risk. Therefore, hygienic measures such as appropriate cleaning of litter boxes are essential to reduce environmental contamination with sporulated oocysts, especially considering that dogs may exhibit coprophagic behavior (Dubey et al. 2020). Although the present study did not evaluate factors such as yard sanitation, socioeconomic conditions, accumulation of debris, or deficient infrastructure, Benitez et al. (2017) associated dog and human

seropositivity with these variables. Arruda et al. (2021) also suggested that walking dogs outdoors with their owners may increase exposure risk and contribute to higher prevalence rates.

The study has some methodological limitations. The use of convenience sampling, based on dogs seen at a single veterinary center, may introduce some bias, since animals that seek clinical care are not necessarily representative of the general dog population in the metropolitan region of Rio de Janeiro (Dohoo et al. 2009, Thrusfield & Christley 2018). Dogs presented to veterinary clinics are generally owned animals and, consequently, may have greater access to animal health resources, which may influence management conditions, housing, and exposure to sources of infection (Arruda et al. 2021). In addition, the sample size of 47 animals limits the statistical power to detect associations with less prevalent variables and prevents generalization of the results to the broader population (Dohoo et al. 2009, Perez 2021). The predominance of animals from the Pavuna neighborhood (68.1%) also restricts the spatial representativeness of the data. Thus, the results presented here should be interpreted as indicative of exposure patterns in the sampled population, rather than as prevalence estimates for the region as a whole.

Primary toxoplasmosis in dogs is considered uncommon, and in most cases the disease is associated with immunosuppression and lack of vaccination against canine distemper virus, as well as neurological manifestations such as seizures, tremors, ataxia, and paresis related to encephalomyelitis. In addition to neurological signs, dogs may develop noise sensitivity, myositis, muscle wasting, limb stiffness, and ocular disease such as conjunctivitis, uveitis, endophthalmitis, and chorioretinitis (Calero-Bernal & Gennari 2019). In the present study, some seropositive dogs had diagnoses such as canine distemper, neurological alterations, and ophthalmic alterations. In this context, serological testing for anti-*T. gondii* antibodies may be recommended in animals undergoing prolonged corticosteroid therapy, since immunosuppression induced by corticosteroids may contribute to the development of acute toxoplasmosis (Salant et al. 2021).

CONCLUSION

The present study detected anti-*Toxoplasma gondii* antibodies in domiciled dogs from the metropolitan region of Rio de Janeiro, with an overall seropositivity of 38.3%. This finding indicates previous exposure to the parasite among the animals evaluated. However, it does not provide direct evidence of active parasite circulation in the sampled areas, since the agent itself was not investigated. In addition, the place where exposure occurred cannot be determined, as information on previous residence history or movement of the animals to other areas was not available. Contact with cats was significantly associated with seropositivity, suggesting that household or peridomestic coexistence with felids may be related to exposure. However, no statistically significant spatial difference was observed among the areas evaluated. Considering the exploratory design, limited sample size, and cross-sectional nature of the study, these findings should be interpreted with caution. Further studies with larger populations, broader geographic coverage, detailed epidemiological histories, and complementary diagnostic methods are needed to understand better the epidemiology of *T. gondii* exposure in domiciled dogs from urban areas of Rio de Janeiro.

Acknowledgments.- This study was financed in part by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES), Brasil. This work was also supported by "Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro" (FAPERJ E26/204.436/2025-315444; FAPERJ E26/210.886/2024-295779).

Conflict of interest statement.- The authors declare that they have no conflicts of interest.

Credit author statement.- CrediT. Christiane O.E. Gomes: Investigation, Data curation, Writing – original draft. Anna Elisa Athayde-Gusmão: Writing – review & editing. Leila Maria C.A. Santos: Writing – review & editing. Beatriz P. Coelho: Writing – review & editing. Edwards Frazão-Teixeira: Supervision, Writing – review & editing. Daniel G. Ubiali: Review & editing. Andressa F. Silva: Conceptualization, Methodology, Supervision, Project administration, Writing – review & editing.

Data availability statement.- The data that support the findings of this study are available from the corresponding author upon reasonable request.

Editor-in-Chief.- Fabiano José Ferreira de Sant'Ana.

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Figures

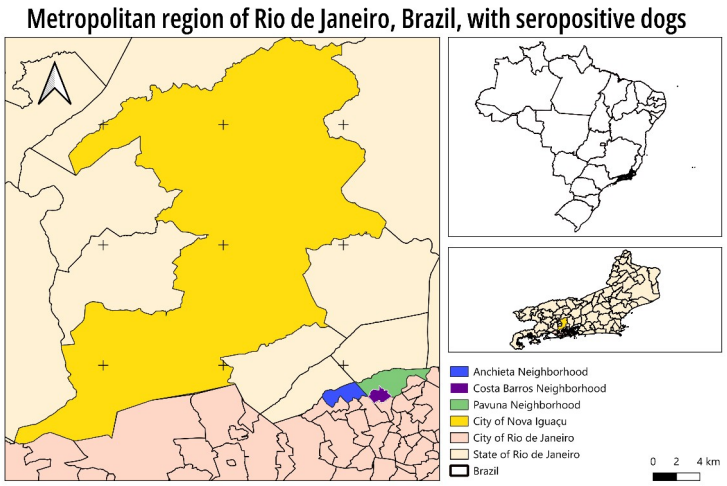

Fig. 1. Kernel density map of dogs seropositive for *Toxoplasma gondii* according to their area of origin in the metropolitan region of Rio de Janeiro, Brazil. The map was generated using QGIS version 3.4 with cartographic data obtained from IBGE (2022) and DATA.RIO. The inset map indicates the location of the study area within Brazil.

Table 1. Seropositivity for *Toxoplasma gondii* according to epidemiological and clinical variables in domiciled dogs from the metropolitan region of Rio de Janeiro, Brazil

Variable	Category	Seropositive/total n (%)	OR (95% CI)	*p*-value
Sex	Male	8/20 (40.0)	1.13 (0.35–3.72)	0.8363
	Female	10/27 (37.0)		
Breed	Mixed-breed	11/23 (47.8)	ND	ND
	German Shepherd	2/2 (100.0)		
	Pinscher	1/1 (100.0)		
	French Bulldog	3/4 (75.0)		
	Poodle	1/2 (50.0)		
	Other breeds[†]	0/15 (0.0)		
Age group	Puppies (≤ 1 year)	3/5 (60.0)	ND	ND
	Young (1–3 years)	7/13 (53.8)		
	Adult (3–7 years)	5/20 (25.0)		
	Older (> 7 years)	3/9 (33.3)		
Contact with cats	Yes	10/17 (58.8)	3.93 (1.11–13.85)	0.0293*
	No	8/30 (26.7)		
Scent-rolling behavior[‡]	Yes	1/4 (25.0)	0.51 (0.05–5.32)	1.0000
	No	17/43 (39.5)		
Diet type	Commercial dog food	13/33 (39.4)	0.85 (0.23–3.13)	0.8124
	Homemade food	5/14 (35.7)		
Raw meat consumption	Yes	8/15 (53.3)	2.51 (0.71–8.86)	0.1466
	No	10/32 (31.3)		
Living environment	Soil/grass	7/12 (58.3)	3.05 (0.79–11.80)	0.1675
	Cement/paved floor	11/35 (31.4)		
Area of origin	Pavuna	11/32 (34.4)	ND	ND
	Anchieta	3/4 (75.0)		
	Nova Iguaçu	3/4 (75.0)		
	Costa Barros	1/1 (100.0)		
	Other locations[¶]	0/6 (0.0)		
Clinical findings	No clinical signs	6/13 (46.2)	ND	ND
	Gastrointestinal	2/10 (20.0)		
	Ehrlichiosis	4/7 (57.1)		
	Dermatological	2/7 (28.6)		
	Canine distemper	2/3 (66.7)		
	Neurological	2/2 (100.0)		

Variable	Category	Seropositive/total n (%)	OR (95% CI)	*p*-value
	Other pathologies[±]	0/5 (0.0)		

OR = Odds ratio, CI = confidence interval, ND = not applicable; * $p \leq 0.05$; [†] Other breeds: Chow Chow (n = 2), Shih Tzu (n = 5), Beagle (n = 1), Yorkshire Terrier (n = 2), Labrador Retriever (n = 1), Pit Bull (n = 3), and Dachshund (n = 1); [‡] Scent-rolling behavior: rolling in feces or decomposing organic matter; [¶] Other locations: Vila da Penha (n = 1), São Cristóvão (n = 1), and São João de Meriti (n = 4); [±] Other pathologies: allergic reactions (n = 3), endocrinopathy (n = 1), and ophthalmic alterations (n = 1); Pearson's chi-square test was used when expected frequencies were adequate; Fisher's exact test was used for 2 x 2 comparisons with small expected frequencies; ORs and *p*-values were calculated only for dichotomous variables; Variables with multiple categories and sparse data were presented descriptively only.