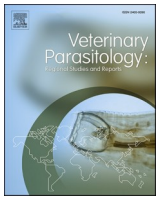
Veterinary Parasitology: Regional Studies and Reports 53 (2024) 101063

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr



Short Communication

# *Toxoplasma gondii* seroprevalence and risk factors in cats in Rio de Janeiro

Leila Maria de Carvalho Alves [a], Edwards Frazão-Teixeira [b,c], Celso Guimarães Barbosa [d], Daniel Guimarães Ubiali [a], Cristiane Divan Baldani [a], Heloísa Justen Moreira Souza [a], Andreza Amaral da Silva [e], Andressa Ferreira da Silva [a,*]

[a] *Programa de Pós-graduação em Medicina Veterinária – PPGMV, Universidade Federal Rural do Rio de Janeiro UFRRJ - km 7 da BR-465, Brazil*
[b] *Laboratório de Biologia Estrutural, IOC, FIOCRUZ, Brazil*
[c] *Laboratório de Sanidade Animal, CCTA, UENF, Brazil*
[d] *DEMAT - Departamento de Matemática - UFRRJ, Brazil*
[e] *DMCV – Departamento de Medicina e Cirurgia Veterinária - UFRRJ, Brazil*

ARTICLE INFO

*Keywords:*
Animals
coccidia
IgG
Toxoplasmosis

ABSTRACT

Seroprevalence studies on cats are essential for monitoring the occurrence of *Toxoplasma gondii* infection. The present research investigated anti-*T. gondii* antibodies, risk factors, clinical signs, hematology and serum biochemistry in cats from different regions of Rio de Janeiro. An overall 18.7% (17/91) of the cats were seroreactive, and age was associated with increased chances of seroprevalence of anti-*T. gondii* antibodies. Clinical signs, hematology and serum biochemistry parameters did not help achieve an *antemortem* diagnosis of cat toxoplasmosis. The parasite circulates in cats from three major regions of Rio de Janeiro, and the present data set will contribute to future epidemiological studies in this endemic state of Brazil.

## 1. Introduction

Toxoplasmosis is an important disease that affects humans and warm-blooded animals, and anti-*Toxoplasma gondii* antibodies have been found in the sera of animals from all continents (Chemoh et al., 2018; Gonzales et al., 2022; Khalife and El Safadi, 2023). Infection occurs after ingestion of food or water contaminated with oocysts shed in the environment within the feces of infected felids, the definitive hosts (Dubey et al., 1970), or through consumption of undercooked tissues of infected animals containing cysts, called intermediate hosts. During the oocyst shedding period of two weeks, cats can release about 20 million oocysts (Dubey, 1995), which undergo sporulation in the environment and become infective in favorable environmental conditions for 48 to 72 h (Dubey et al., 2011). It is widely reported that cats only shed oocysts after initial infection with *T. gondii,* but experimental studies have shown that repeat oocyst shedding can occur (Zhu et al., 2022).

Toxoplasmosis is a worldwide-distributed infection caused by *Toxoplasma gondii,* which causes a wide range of clinical syndromes in humans, mammals and birds (Sander et al., 2018). The infection is often subclinical in cats or manifests only as non-specific signs (Dubey, 2022; Pereira et al., 2018). The increased number of cats in Brazilian homes leads to closer contact with humans, favoring the transmission of zoonotic diseases (Arruda et al., 2021).

Monitoring the serological status of specific antibodies is essential for further epidemiological inference of feline toxoplasmosis. Although cats can develop an excellent immunological response and reduce the chances of oocyst re-shedding, it is not uncommon during their lifetime (Dubey et al., 2020). In addition to the even higher urgency to study the association of cat ownership, its intrinsic socioeconomic association and the frequency of *T. gondii* to antibodies (Arruda et al., 2021), the present study investigated the seroprevalence of IgG anti-*T. gondii antibodies*, clinical signs, hematological and serum biochemical parameters and risk factors in cats from Rio de Janeiro.

## 2. Material and methods

### 2.1. Sampling and clinical examination

The study was performed conveniently, following the routine veterinary appointments of the Feline Department of the Veterinary Hospital of Universidade Federal Rural do Rio de Janeiro, UFRuralRJ, Brazil. The 91 domestic cats included in this research were those of which tutors agreed to participate during the second semester of 2018 and the first semester of 2019.

* Corresponding author.
*E-mail address:* andressafsilva@ufrrj.br (A.F. da Silva).

https://doi.org/10.1016/j.vprsr.2024.101063
Received 29 November 2023; Received in revised form 9 June 2024; Accepted 10 June 2024
Available online 12 June 2024

Blood samples were collected from all animals following a physical examination performed as recommended by Feitosa (2008), measuring general physical parameters such as weight, body temperature, skin and coat condition and appearance, body condition score, color of the mucosas, degree of hydration, palpation of lymph nodes, capillary refill time, auscultation and heart rate, respiratory rate and palpation of abdominal organs. A 4 mL whole blood sample was collected through venipuncture and distributed into two tubes of 2 mL: one with Ethylene diamine tetraacetic acid (EDTA) for hematological analysis and another without anticoagulant to separate the serum for serological testing and biochemical analysis.

### 2.2. Hematological and biochemical analysis

Specific leukometry and cell morphology were measured using an optical microscope (Nikon® Eclipse E200) and the Poch100/Sysmex® cell counter. Enzyme activities were measured using the automatic analyzer A15/Biosystem® for alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, urea and creatinine parameters.

### 2.3. Questionnaires

History of the animals and questions applied to tutors included factors considered of possible risk for animals' infections, including age, sex, breed, number of animals per residence, access to the outdoors, diet, place of excretion, meat intake, frequency of litter box or yard cleaning, hunting habits and location (North, West and Baixada Fluminense).

### 2.4. Modified agglutination test (MAT)

Serologic testing of cats was performed by the MAT, according to Dubey and Desmonts (1987). In short, sera were diluted in phosphate-buffered saline (PBS) pH 7.2 and those with agglutinating activity at a dilution of 1:25 were considered positive. Two-fold additional dilutions were tested for titration of positive sera until 1:3200 (Silva et al., 2001).

The antigen was constituted by formalin-fixed *T. gondii* tachyzoites of the RH strain, and the test was performed in U-shaped 96-well plates, where 25 μL of the antigen solution was mixed with 25 μL of the previously diluted sera. Positive and negative controls were included, and plates were incubated at 37 °C for 12 h. Results were based on the sedimentation profile of the tachyzoite suspension, where the formation of a blue button means negative and a clear bottom means positive.

### 2.5. Statistical analysis

Different statistical tests were used to assess the diversity of factors with MAT results. The Chi-Square Test ($X^2$) was used to verify the association of seroprevalence and anti-*T. gondii* antibodies and potential risk factors, the Student's *t*-test to verify association with complete blood count and serum biochemistry. Fisher's exact and G-test were used to assess the statistical association with clinical signs. The BioEstat statistical program (Ayres et al., 2007) and a significance level of 5% were used in the analyses.

## 3. Results and discussion

The overall seroprevalence for the presence of anti-*T. gondii* antibodies in cats of these three major mesoregions of Rio de Janeiro was 18.7% (17 of 91). Similar MAT results for antibodies anti-*T. gondii* was found by Bolais et al. (2017) in Rio de Janeiro and Gonzales et al. (2022) in Lima, Peru. Out of 17 seropositive animals of our study, titers varied from 25 to 3200, and most of the animals presented with titers equal to or higher than 100, which might be associated with persistent infection in cats from this endemic area of Brazil (Dubey and Thulliez, 1989). Titers of the 17 seropositive animals are distributed as follows: five animals had a titer of 25 (29.4%), three of 50 (17.6%), two animals with a titer of 100 (11.8%), three of 200 (17.6%), one of 400 (5.9%) and three with a titer of 3200 (17.6%). We did not take a further blood sample of these cats, but *T. gondii*-specific IgG levels stayed high for years.

The factors investigated in this research are detailed in Table 1. Out of 10 analyzed possible risk factors, age was the only significant parameter for this cat population ($p < 0.05$). Thus, cats older than ten years have a higher probability of infection, which is consistent with prolonged contact with the highly contaminated environment of Rio de Janeiro state (Da Silva et al., 2003). Bolais et al. (2017), studying cats from a public cat shelter and a private residential area in the seaside district of Rio de Janeiro, under the same serotest, found a low frequency of positive animals among those aged 24 months or less, what is consistent with our rationale. All other factors assessed in the present study were not statistically significant. The chances of acquiring the infection and the time of environmental exposure to the parasite are also important regarding human toxoplasmosis (Rezende et al., 2019).

Stray cats might be the sentinels for such studies in urban areas once exposed to similar risks of *Toxoplasma* infection to humans. *Toxoplasma gondii* spreading in a metropolitan area was evaluated by seroprevalence in free-living cats and dogs (Meireles et al., 2004). The serum testing in the present study could be interpreted as an immune response or infection, considering the definite host epidemiological role of cat species but remembering the critical role of the cat as an intermediate host in the cycle.

Hunting habits are common among felids, including domestic cats, which might represent significant potential as a risk factor for infection by *T. gondii* (Dubey, 2022). In our study, many cats were not stray but had access to the outdoors. Semi-domiciled cats have access to industrialized food offered by their owners, which reduces natural habits such as hunting for feeding purposes, thus decreasing the chances of infection

**Table 1**
Chi-square test for factors associated to the occurrence of anti- *Toxoplasma gondii* antibodies in domestic cats in Rio de Janeiro, Brazil.

| Factors | Positive** | Negative** | % | *P*-value |
|---|---|---|---|---|
| **Age** | | | | |
| ≤10 years | 11 | 62 | 15.1 | 0.010* |
| >10 years | 6 | 7 | 46.2 | |
| **Sex** | | | | |
| Male | 11 | 34 | 24.4 | 0.145 ns |
| Female | 5 | 36 | 12.2 | |
| **Residence type** | | | | |
| House | 15 | 58 | 20.5 | 0.755 ns |
| Apartment | 2 | 10 | 16.7 | |
| **Outdoors access** | | | | |
| Yes | 5 | 31 | 13.9 | 0.229 ns |
| No | 7 | 20 | 25.9 | |
| **Region** | | | | |
| North | 0 | 5 | 0.0 | |
| West | 2 | 15 | 11.8 | 0.551 ns |
| Baixada Fluminense | 13 | 65 | 16.7 | |
| **Hunting habit** | | | | |
| Yes | 7 | 32 | 17.9 | 0.931 ns |
| No | 6 | 26 | 18.8 | |
| **Meat intake** | | | | |
| Yes | 8 | 23 | 25.6 | 0.767 ns |
| No | 7 | 24 | 22.6 | |
| **Cats per residence** | | | | |
| One | 4 | 20 | 16.7 | 0.677 ns |
| >1 | 13 | 50 | 20.6 | |
| **Defecation site** | | | | |
| Outside the residence | 9 | 28 | 24.3 | 0.205 ns |
| Litter box | 8 | 49 | 14.0 | |
| **Cleaning the cat litter box (times a week)** | | | | |
| ≤1× per week | 7 | 27 | 20.6 | 0.775 ns |
| 3× per week | 7 | 32 | 17.9 | |

ns not significative (P > 0.05).
* significative (P ≤ 0.05).
** The sampling categories differed due to incomplete epidemiological forms response.

by *T. gondii*. However, the risk of infection remains since contact with an environment contaminated by oocysts is also a source of infection to which stray and semi-domiciled cats are exposed (Pinto et al., 2009), besides the increased chances of hunting intermediate hosts like birds and rodents.

Males and females had the same chances of infection by *T. gondii*, similar to the results found by Bolais et al. (2017). Living in a house or apartment did not affect the infection of the cats by *T. gondii*. Cats bred inside apartments are generally less likely to be exposed to synanthropic animals and possible intermediate parasite hosts. Dubey et al. (2020) stated that the prevalence of *T. gondii* infection will vary according to the animal and population lifestyle, as it occurs in cats that hunt their food, such as feral and stray cats.

Hunting and eating meat were surprisingly not considered risk factors, which conflicts with information gathered by Dubey (2022), in which Thai cats, for instance, had lower seropositivity rates due to the eating habits of the predominantly vegetarian population. This situation is analogous to the case in which cats eat fresh or cooked meat.

The coexistence of more than one cat in the environment allowed us to infer that there would be a possibility of infection through ingesting oocysts shed by others recently infected. However, our results corroborate with data reviewed by Dubey (2022), who concluded that oocyst infection in cats is not pathogenic due to inefficient excystation of sporozoites in cat's gut. Also, the defecation site and the frequency at which the litter box was cleaned were fundamental for reducing environmental contamination and interrupting the life cycle of *T. gondii*.

The regions of Rio de Janeiro state investigated in the present study - North, West and Baixada Fluminense - did not influence cat seroprevalence. In these three regions, majorly located in the metropolitan area of Rio de Janeiro, public health authorities provide its residents with the Pest Control Program, in which routine treatments and inspections are frequently performed for pest control inside residences, schools, daycare centers and hospital units. Also, they monitor public areas such as vacant lots, water galleries, riverbanks, parks, and low-income urban regions to control the proliferation of rats (Rio de Janeiro City Hall, 2020). Therefore, while preventing potentially infected prey for felines, authorities contribute to the reduced association of these metropolitan regions in Rio de Janeiro and the presence of cats seropositive for anti-*T. gondii* antibodies.

There was no significant association ($p > 0.05$) between clinical signs and seroprevalence of antibodies anti-*T. gondii*. Among the 17 cats identified as positive in the present research, six present with dyspnea. Calero-Bernal and Gennari (2019) reported dyspnea associated with pneumonia in cats with the respiratory system affected by *T. gondii*. The clinical signs of toxoplasmosis are generally non-specific. We also did not find significant differences between clinical manifestations displayed by seropositive cats who were seronegative for antibodies anti-*T. gondii*. The same is true for the hematological profile. The analyzed variables were not significantly associated with *T. gondii*-specific antibodies in cats ($p > 0.05$). On the other hand, although serum biochemistry analysis showed no significant difference ($p > 0.05$) for the variables albumin, alkaline phosphatase (FA), urea, and creatinine (Weiss et al., 2010), ALT enzyme activity was significantly lower for seropositive cats. However, the reference values were within the expected range for the species (Kaneko et al., 2008). These results mean no clinical concern (Dubey, 2022). Veterinarians should become aware of borderline results for ALT activity for cats living in areas known to be endemic for human and animal toxoplasmosis. Serum biochemistry results such as urea, serum creatinine, alkaline phosphatase, serum calcium, and bilirubin are generally unnecessary for feline toxoplasmosis diagnosis.

We conclude that *T. gondii* circulates among cats in the North, West and Baixada Fluminense regions of Rio de Janeiro, and this set of data will be helpful for future epidemiological measures to control toxoplasmosis in this endemic area.

## Financial support

We thank the financial support by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES), Brazil financing code 001 and the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), Brazil.

## Committee on Ethics

This Research was approved in 2018 by the Committee on Ethics in the Use of Animals (CEUA) of the Veterinary Institute from Federal Rural University of Rio de Janeiro (UFRRJ), register 5936110418.

## CRediT authorship contribution statement

**Leila Maria de Carvalho Alves:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Edwards Frazão-Teixeira:** Methodology. **Celso Guimarães Barbosa:** Formal analysis. **Daniel Guimarães Ubiali:** Methodology, Investigation. **Cristiane Divan Baldani:** Methodology. **Heloísa Justen Moreira Souza:** Methodology. **Andreza Amaral da Silva:** Supervision. **Andressa Ferreira da Silva:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The datasets used and analyzed during the current study are available from the corresponding author upon request.

## References

Arruda, I.F., Millar, P.R., Barbosa, A.D.S., Abboud, L.C.S., Reis, I.C., Moreira, A.S.D.C., Guimarães, M.P.P., Amendoeira, M.R.R., 2021. *Toxoplasma gondii* in domiciled dogs and cats in urban areas of Brazil: risk factors and spatial distribution. Parasite 28, 56.

Ayres, M., Ayres Jr., M., Ayres, D.L., Santos, A.A.S., 2007. BioEstat. Aplicações estatísticas nas áreas das ciências bio-médicas. Belém, Software versão 5.3.

Bolais, P.F., Vignoles, P., Pereira, P.F., Keim, R., Aroussi, A., Ismail, K., Dardé, M.L., Amendoeira, M.R., Mercier, A., 2017. *Toxoplasma gondii* survey in cats from two environments of the city of Rio de Janeiro, Brazil by Modified Agglutination Test on sera and filter-paper. Parasit. Vectors 10, 88.

Calero-Bernal, R., Gennari, S.M., 2019. Clinical toxoplasmosis in dogs and cats: an update. Front.Vet. Sci 26, 54.

Chemoh, W., Sawangjaroen, N., Nissapatorn, V., Sermwittayawong, N., 2018. Vet. Parasitol. Reg. Stud. Report, 13, pp. 105–109.

Da Silva, D.S., Bahia-Oliveira, L.M., Shen, S.K., Kwok, O.C., Lehman, T., Dubey, J.P., 2003. Prevalence of *Toxoplasma gondii* in chickens from an area in southern Brazil highly endemic to humans. J. Parasitol. 89, 394–396.

Dubey, J.P., 1995. Duration of immunity to shedding of Toxoplasma gondii oocysts by cats. J. Parasitol. 81, 410–415.

Dubey, J.P. (Ed.), 2022. Toxoplasmosis of Animals and Humans, 3ed. CRC Press, New York.

Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Eq. Vet. J. 9, 337–339.

Dubey, J.P., Thulliez, P., 1989. Serologic diagnosis of toxoplasmosis in cats fed *Toxoplasma gondii* tissue cysts. J. Am. Vet. Med. Assoc. 194, 1297–1299.

Dubey, J.P., Miller, N.L., Frenkel, J.K., 1970. The *Toxoplasma gondii* oocyst from cat feces. J. Exp. Med. 132, 636–662.

Dubey, J.P., Ferreira, L.R., Martins, J., Jones, J.L., 2011. Sporulation and survival of *toxoplasma gondii* oocysts in different types of commercial cat litter. J. Parasitol. 97, 751–754.

Dubey, J.P., Cerqueira-Cézar, C.K., Murata, F.H.A., Kwok, O.C.H., Yang, Y.R., Su, C., 2020. All about toxoplasmosis in cats: the last decade. Vet. Parasitol. 283, 1–30.

Feitosa, F.L. (Ed.), 2008. Semiologia veterinária: a arte do diagnóstico. Editora Roca, São Paulo, pp. 313–331.

Gonzales, C., Vargas-Calla, A., Gomez-Puerta, L.A., Robles, K., Lopez-Urbina, M.T., Gonzalez, A.E., 2022. Seroprevalence of *toxoplasma gondii* and associated risk factors in cats from Lima. Peru.Vet. Parasitol. Reg. Stud. Report. 31, 100733.

Kaneko, J.J., Harvey, J.W., Bruss, M.L., 2008. Clinical Biochemistry of Domestic Animals, , 6th editionvol. 493. Academic Press, San Diego, pp. 889–895.

Khalife, S., El Safadi, D., 2023. Seroprevalence and risk factors of *Toxoplasma gondii* infection in slaughtered chickens in Tripoli, Lebanon. Vet. Parasitol. Reg. Stud. Report. 46, 100941.

Meireles, L.R., Galisteo Jr., A.J., Pompeu, E., Andrade Jr., H.F., 2004. Toxoplasma gondii spreading in an urban area evaluated by seroprevalence in free-living cats and dogs. Trop. Med. Int. Health 9, 876–881.

Pereira, P.F., Barbosa, A.S., Santos, A.L.C., Bolais, P.F., Dardé, M., Amendoeira, M.R.R., 2018. Toxoplasma gondii: infection among shelter and stray cats in Rio de Janeiro, Brazil. Rev. Bras. Parasitol. Vet. 27 (3), 401–408.

Pinto, L.D., Araujo, F.A.P., Stobb, N.S., Marques, S.M.T., 2009. Seroepidemiology of *toxoplasma gondii* in domestic cats treated in private clinics of Porto Alegre, Brazil. Cien. Rural. 39 (8), 2464–2469.

Rezende, H.H.A., Storchilo, H.R., Avelar, J.B., Silveira, M.B., Vinaud, M.C., Castro, A.M. D., 2019. Frequência de anticorpos anti-*Toxoplasma gondii* em gatos errantes (*Felis catus domesticus*) em Goiânia, Goiás, Brasil. Mult. Sci. J. 2 (1), 86–88.

Rio de Janeiro City Hall, 2020. https://carioca.rio/servicos/controle-de-roedores/. Accessed on May 30th 2024.

Sander, V., Angel, S., Clemente, M., 2018. A Comprehensive review of *Toxoplasma gondii* biology and host-cell interaction: challenges for a plant-based vaccine. https://doi.org/10.1007/978-3-319-90137-4_4.

Silva, J.C., Ogassawara, S., Adania, C.H., Ferreira, F., Gennari, S.M., Dubey, J.P., Ferreira-Neto, J.S., 2001. Seroprevalence of *toxoplasma gondii* in captive neotropical felids from Brazil. J. Vet. Parasitol. 103, 217–224.

Weiss, D.J.K., Wardrop, J., Schalm, J.O.W., 2010. Schalm's Veterinary Hematology, 6th ed. Wiley-Blackwell, Ames Iowa.

Zhu, S., Shapiro, K., Van Wormer, E., 2022. Dynamics and epidemiology of *toxoplasma gondii* oocyst shedding in domestic and wild felids. Transbound. Emerg. Dis. 69, 2412–2423. https://doi.org/10.1111/tbed.14197.