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Seroepidemiology and spatial distribution of *Toxoplasma gondii* in dogs from Ourinhos, São Paulo, Brazil

Soroepidemiologia e distribuição espacial de Toxoplasma gondii em cães de Ourinhos, São Paulo, Brasil

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ABSTRACT

Introduction: *Toxoplasma gondii* can infect all warm-blooded animals, including humans, which makes it a significant public health concern worldwide. Dogs can play a crucial role in the epidemiology of toxoplasmosis by acting as mechanical vectors and sentinel for environmental contamination. **Objectives:** This study aimed to assess the seroprevalence and spatial distribution of *T. gondii* in dogs from Ourinhos, São Paulo, Brazil. **Methods:** Blood samples from 602 dogs from different areas of Ourinhos municipality were collected during the rabies vaccination campaign. Information about the age, gender, breed of the dogs, and residential address of each tutor were collected. The indirect fluorescent antibody test (IFAT) was performed to evaluate the presence of antibodies against the parasite, while the spatial analysis was used to evaluate regions associated with seropositivity. **Results:** In total, 23.7% (143/602) of the samples tested positive, with no significant association observed between gender, age, or breed. Spatial analysis revealed a high concentration of positive cases in the municipality's most peripheral neighborhoods. **Conclusions:** These results indicate that dogs in Ourinhos, SP, Brazil, are exposed to T. *gondii*, and that peripheral neighborhoods likely have higher environmental contamination.

Keywords: Toxoplasmosis, One Health, Serologic Tests, Geographic Mapping.

RESUMO

Introdução: *Toxoplasma gondii* pode infectar todos os animais de sangue quente, incluindo humanos, o que o torna uma importatnte preocupação de saúde pública em todo o mundo. Os cães podem desempenhar um papel crucial na epidemiologia da toxoplasmose, atuando como vetores mecânicos e sentinelas para contaminação ambiental. **Objetivos:** Este estudo teve como objetivo avaliar a soroprevalência e a distribuição espacial de *T. gondii* em cães de Ourinhos, São Paulo, Brasil. **Métodos:** Amostras de sangue de 602 cães de diferentes áreas do município de Ourinhos foram coletadas durante a campanha de vacinação antirrábica. Informações sobre idade, sexo, raça dos cães e endereço residencial de cada tutor foram coletadas. O teste de imunofluorescência indireta (RIFI) foi realizado para avaliar a presença de anticorpos contra o parasita, enquanto a análise espacial foi utilizada para avaliar regiões associadas à soropositividade. **Resultados:** No total, 23,7% (143/602) das amostras foram consideradas positivas, não havendo associação significativa entre sexo, idade e raça. A análise espacial mostrou alta intensidade de animais positivos nos bairros mais periféricos do município. **Conclusões:** Esses resultados indicam que os cães de Ourinhos, Brasil, SP, estão expostos ao *T. gondii*, e bairros periféricos provavelmente apresentam maior contaminação ambiental.

Palavras-chave: Toxoplasmose, Saúde Única, Testes Sorológicos, Mapeamento Geográfico.

INTRODUCTION

Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii,* an intracellular coccidian protozoan that can infect all warm-blooded animals, including humans[1]. The parasite has a worldwide distribution, and it is estimated that around 30% of the human population is infected with *T. gondii*, making toxoplasmosis one of the significant global public health issues[2,3].

The *T. gondii* life cycle is facultative heteroxenous, and felids are the only definitive hosts, being able to shed oocysts through feces[4]. An infected cat sheds millions of oocysts, even in reinfected cats after years of first infection, contributing to environmental contamination[5]. Felids and the other animals are considered intermediate hosts and can be infected by horizontal (ingestion of sporulated oocysts or tissue cysts) or vertical (transplacental by tachyzoites) routes[1]. In humans, the transmission patterns have changed over the decades, and nowadays, most human outbreaks are linked to contaminated water and food with sporulated oocysts[6].

Dogs are important in the epidemiological chain of toxoplasmosis, as they have direct contact with humans and other domestic animals have the habit of eating or rolling in cat feces, known as xenosmophilia, which in turn allows them to carry oocysts on their skin and thus transmit them to humans[7]. Furthermore, dogs can act as mechanical vectors by ingesting oocysts and eliminating them infectious into the environment through their feces[8]. Thus, the present study aimed to evaluate the seroprevalence and spatial distribution of *T. gondii* in dogs from Ourinhos, São Paulo, Brazil.

METHODS

Study area and sampling

All procedures involving animals were approved by the Ethics Committee of the Centro Universitário das Faculdades Integradas de Ourinhos (approval number 26/2017). This study was conducted in the municipality of Ourinhos, São Paulo, Brazil (22°58′59″S, 49°51′25″W), which covers an area of 295.818 km² and has a population of 113,542 (Figure 1). Ourinhos is located in the southwestern region of São Paulo state, approximately 370 km from the state capital.

The sample size was calculated using EpiInfo software v. 7, considering a population of 15,000 dogs according to the Municipal Health Department; an estimated prevalence of 50%, and a confidence level of 95%, resulting in a sample size of 530 animals.

Dogs were sampled after owners' consent during the municipal vaccination campaign against rabies at 25 vaccination stations distributed across the municipality from September to October 2017. The blood samples (2 mL) were obtained by cephalic puncture, placed in a tube containing clot activator (BD Vacutainer®, Becton-Dickson, New Jersey, USA), and kept in isothermal boxes at 4-8 °C until transportation to the laboratory. A questionnaire about the characteristics of the animal was applied to each tutor. In the laboratory, the blood samples were centrifuged at 2,000 × g, and the obtained serum was stored at -20°C until the serological analysis.

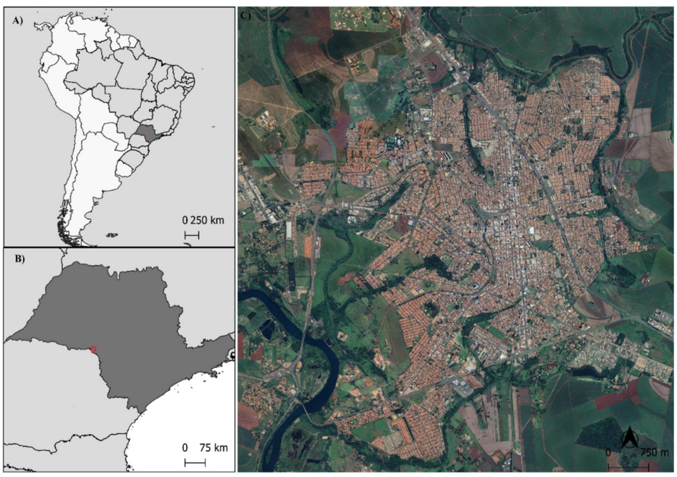

Figure 1. Map of the location where the study was conducted. A: Map of South America, highlight-ing Brazil (light gray) and São Paulo State (dark gray); B: map of São Paulo State highlighting the municipality of Ourinhos (in red); C: Map of the municipality of Ourinhos.

Serological analysis

The presence of antibodies IgG against *T. gondii* was evaluated by the indirect fluorescent antibody test (IFAT), as previously described[9]. Tachyzoites of the RH strain of *T. gondii* were used as antigens, and fluorescein isothiocyanate-labeled anti-dog IgG (Sigma-Aldrich, St. Louis, MO, USA) was used as the secondary antibody. Serum samples from dogs that were already known positive or negative for *T. gondii* were used as controls and included in all IFAT slides, which were mounted with carbonate-buffered glycerin (pH 9.5) and coverslips before observation under an epifluorescence microscope (Leica Microsystems, Germany). Only serum samples that exhibited fluorescence throughout the entire surface of the tachyzoites were considered positive. Serum samples from the dogs with titers ≥16 were considered positive, and the titer was determined by complete dilution of sera until no fluorescence reaction was observed[1].

Spatial analysis

The residence addresses of each tutor were georeferenced, and the points were determined by capturing the latitude and longitude coordinates provided by Google Earth. The Kernel intensity estimator was used in the analysis, which generated a density surface from a pre-established radius of 800 meters, allowing the visual detection of hot spots through statistical smoothing. This method has pointed out clusters in a spatial distribution, creating a continuous surface from the data[10]. The cartographic base in shapefile format, available on the Brazilian Institute of Geography and Statistics (IBGE) website, and the Qgis software version 3.38 (qgis.org) were used to create the maps.

Statistical analysis

The association between seropositivity and the variables sex (male or female), breed (pure or mixed breed), and age (up to 1 year, >1-5 years, >5 years, not reported) were calculated by the univariate analysis using the chi-square test. The strength of the association was determined by the odds ratio (OR) with a 95% confidence interval. P-values ≤ 0.05 were considered significant. All statistical analysis was performed using the Epi Info program.

RESULTS

Blood samples were collected from 604 dogs during the study period. Out of these, 47.5% (286/602) were males and 52.5% (316/602) female, with a predominance of mixed-breed dogs (68.3%; 411/602) than pure breed dogs (31.7%; 191/602). Regarding age, most of the animals were adults between one and five years old (49.3%; 297/602), followed by higher than five years (39.4%; 237/602), and puppies until one year old (7.6%; 46/602). The owners of 3.7% (22/602) animals did not know their age.

Of the 602 samples analyzed by IFAT, 143 (23.7%) were considered positive, with titer ranging from 16 (63; 10.5%), 64 (47; 7.8%); 256 (15; 2.5%), 1,024 (7; 1.2%), and 4,096 (11; 1.8%). There was no significant association between gender, age, and breed with seropositivity to *T. gondii* (Table 1).

Table 1. Univariate analyses model for the seropositivity for *Toxoplasma gondii* in dogs from Ourinhos, São Paulo State, Brazil, according to different variables.

Variables	Group	IgG anti-*Toxoplasma gondii* Positive/Sampled (%)	OR (CI 95%)	p-value
Gender	Male	76/286 (26.6%)	1.34 (0.92 – 1.95)	0.12
	Female	67/316 (21.2%)		
Breed	Pure	40/191 (20.9%)	0.79 (0.52 – 1.19)	0.26
	Mixed	103/411 (25.1%)		
Age (years)	0-1	10/46 (21.7%)	-	0.51
	>1-5	64/297 (21.5%)		
	>5	64/237 (27%)		
	Not informed	5/22 (22.7%)		

OR: odds ratio; CI: confidence interval.

The distribution map showed that seronegative and seropositive dogs were distributed across the municipality; however, the kernel map showed a higher intensity of seropositive dogs in the suburban areas (Figure 2).

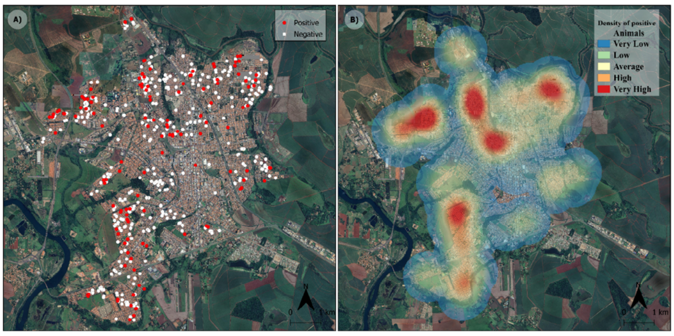

Figure 2. A: Distribution of dogs sampled according to their serological result by the indirect fluo-rescent antibody test (IFAT) for *Toxoplasma gondii* from Ourinhos municipality, Brazil. B: Kernal density estimation of seropositive dogs for *T. gondii*.

DISCUSSION

This study describes the first epidemiological and spatial distribution of seroprevalence of *T. gondii* in dogs from the studied region. In our study, the prevalence of seropositive animals was 23.7%. In Brazil, serological studies with a high number of samples have shown a prevalence of up to 70%. In other countries, previous studies showed a serological prevalence ranging from 1.9% to 98%[11]. These differences could be related to sample size, study location, test assay, cut-off, and epidemiological factors, such as environmental conditions and population socioeconomic development, which makes it difficult to compare the results of different studies[11]. In our study, we used the IFAT to evaluate the presence of antibodies against *T. gondii*. Although there are other test assays for toxoplasmosis in dogs, such as the enzyme-linked immunosorbent assay (ELISA); the indirect hemagglutination antibody test (IHA), the latex agglutination (LAT), and the modified agglutination test (MAT). The IFAT is the most widely used test since has the least cross-reactivity with other parasites and high sensitivity and specificity[1].

In the present study, we observed a higher percentage of seropositive dogs over five years old; however, this association was not statistically significant. This is similar to a previous study with dogs from Laguna, Santa Catarina, where adult dogs had higher seropositivity rates without significant association, suggesting that older animals had a higher chance of infection and, consequently, be seropositive[12]. No association was observed between gender and breed in animals from this study. Previous studies performed in Brazil and other regions worldwide also did not verify this association, even for hunting dogs[12-14]. However, a significant association between *T. gondii* infection and crossbreed dogs has already been described, probably due to anima access to the street and exposure to the contaminated environment[15-16]

In our study, all animals sampled were in adequate health conditions since they were eligible for rabies vaccination. Clinical toxoplasmosis as a primary disease in dogs is uncommon, with a low rate of morbidity and mortality; however, immunosuppression and co-infections can lead to clinical manifestation[17]. In this case, neuromuscular, cutaneous, and ocular signs have been described[11,17]. In a recent study from Argentina; serum samples from 7,238 dogs showing neurological clinical signs such as paraparesis, ascending paralysis, and tetraplegia showed a seropositivity rate of 35.7%, indicating that dogs are infected with *T. gondii* and toxoplasmosis should be included as a differential diagnosis for dogs presenting neuromuscular disorders[18]. In Brazil, a higher rate of seropositive dogs was found in the group with neurological disorders than the control group, with *T. gondii* seropositivity associated with peripheral neurological alteration[19].

The kernel map showed a cluster of seropositive in the peripheral neighborhoods of the municipality of Ourinhos, including housing complexes such as Padre Eduardo Murante and Helena Braz Vendramini, as well as the Jardim Anchieta neighborhood. These neighborhoods are characterized by generally deficient infrastructure and present notable differences in residential, social, and population profile. Previous studies have shown that dogs from owners living in areas with low socioeconomic levels are more likely to be positive, probably because of higher environmental contamination and stray animals[20]. Although the Municipal Human Development Index (IDH-M) is 0.778, which places the city in 145th position in Brazil, the total number of people in extreme poverty was approximately 1,169. In August 2024, 12,229 families were registered in the Brazilian Single Registry for Social Programs (CadÚnico)[21]. Furthermore, the animals in the present study were sampled during the public rabies vaccination campaign, which may represent a bias since animals from tutors with high purchasing power often undergo vaccination in private clinics.

Therefore, actions are needed to target locations with the highest risk of transmission and infection of toxoplasmosis between animals and humans. Furthermore, it is important to understand the environmental dynamics of maintenance of the protozoan in the indicated regions, and our results can strengthen the influence of the ecosystem context on the serological prevalence of *T. gondii* in dogs.

CONCLUSIONS

In conclusion, this study determined a seroprevalence of 23.7% of dogs from Ourinhos, SP, Brazil, with a higher density of seropositive animals in low socioeconomic levels of neighborhoods, indicating the circulation of this parasite in these environments. These results can support the development of public health to minimize the occurrence of *T. gondii* in dogs and other animals, including humans.

REFERENCES

1. Dubey JP. Toxoplasmosis of animals and humans. 2nd ed. Boca Raton: CRC Press; 2021. 564 p. doi:10.1201/9781003199373
2. Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: from animals to humans. Int J Parasitol. 2000;30(12–13):1217–58. doi:10.1016/S0020-7519(00)00124-7
3. Dubey JP. Outbreaks of clinical toxoplasmosis in humans: five decades of personal experience, perspectives and lessons learned. Parasit Vectors. 2021;14:263. doi:10.1186/s13071-021-04769-4
4. Dubey JP, Cerqueira-Cézar CK, Murata FHA, Kwok OCH, Yang YR, Su C. All about toxoplasmosis in cats: the last decade. Vet Parasitol. 2020;283:109145. doi:10.1016/j.vetpar.2020.109145
5. Zulpo DL, Sammi AS, Santos JR, Sasse JP, Martins TA, Minutti AF, et al. Toxoplasma gondii: a study of oocyst re-shedding in domestic cats. Vet Parasitol. 2018;249:17–20. doi:10.1016/j.vetpar.2017.10.021
6. Pinto-Ferreira F, Caldart ET, Pasquali AKS, Mitsuka-Breganó R, Freire RL, Navarro IT. Patterns of transmission and sources of infection in outbreaks of human toxoplasmosis. Emerg Infect Dis. 2019;25(12):2177–82. doi:10.3201/eid2512.181565
7. Lindsay DS, Dubey JP, Butler JM, Blagburn BL. Mechanical transmission of Toxoplasma gondii oocysts by dogs. Vet Parasitol. 1997;73(1–2):27–33. doi:10.1016/s0304-4017(97)00048-4
8. Schares G, Pantchev N, Barutzki D, Heydorn AO, Bauer C, Conraths FJ. Oocysts of Neospora caninum, Hammondia heydorni, Toxoplasma gondii and Hammondia hammondi in faeces collected from dogs in Germany. Int J Parasitol. 2005;35(14):1525–37. doi:10.1016/j.ijpara.2005.08.008
9. Camargo ME. Introdução às técnicas de imunofluorescência. São Paulo: Instituto Adolfo Lutz; 1973. 112 p.

10. Pfeiffer DU, Robinson TP, Stevenson M, Stevens KB, Rogers DJ, Clements ACA. Spatial analysis in epidemiology. Oxford: Oxford Academic; 2008. doi:10.1093/acprof:oso/9780198509882.001.0001

11. Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH, Yang Y, Su C. Toxoplasma gondii infections in dogs: 2009–2020. Vet Parasitol. 2020;287:109223. doi:10.1016/j.vetpar.2020.109223

12. Remor-Sebolt AP, Lima FR, Américo L, Padilha MAC, Chryssafidis AL, Moura AB. Occurrence of antibodies and epidemiological significance of Toxoplasma gondii and Neospora caninum infections in canine populations of Laguna, State of Santa Catarina. Vet Res Commun. 2024;48:3349–54. doi:10.1007/s11259-024-10462-5

13. Caramalac SM, Castilho PM, Lucas JI, Minutti AF, Garcia JL, et al. Seroprevalence of Toxoplasma gondii, Neospora caninum, and Leishmania spp. in hunting dogs from Mato Grosso do Sul, Brazil. Cienc Rural. 2021;51(5):e20200533. doi:10.1590/0103-8478cr20200533

14. Perin PP, Arias-Pacheco CA, Andrade LO, Gomes JS, Ferreira AFM, Pavaneli RO, et al. Toxoplasma gondii and Neospora caninum in invasive wild boars (Sus scrofa) and hunting dogs from Brazil. Int J Parasitol Parasites Wildl. 2024;24:100951. doi:10.1016/j.ijppaw.2024.100951

15. Huertas-López A, Sukhumavasi W, Álvarez-García G, Martínez-Subiela S, Cano-Terriza D, Almería S, et al. Seroprevalence of Toxoplasma gondii in outdoor dogs and cats in Bangkok, Thailand. Parasitol. 2021;148(7):843–9. doi:10.1017/S0031182021000421

16. Arruda IF, Millar PR, Barbosa AS, Abboud LCS, Reis IC, Moreira ASC, et al. Toxoplasma gondii in domiciled dogs and cats in urban areas of Brazil: risk factors and spatial distribution. Parasite. 2021;28:56. doi:10.1051/parasite/2021049

17. Calero-Bernal R, Gennari SM. Clinical toxoplasmosis in dogs and cats: an update. Front Vet Sci. 2019;6:54. doi:10.3389/fvets.2019.00054

18. Gos ML, Venturini MC, Felice L, Dellarupe A, Rambeaud M, Pardini L, et al. A 20-year serological survey of Toxoplasma gondii and Neospora caninum infection in dogs with neuromuscular disorders from urban areas in Argentina. Vet Parasitol. 2024;330:110235. doi:10.1016/j.vetpar.2024.110235

19. Caramalac SM, Leite BSNNC, Castilho PM, Minutti AF, Martins TA, et al. Anti-Toxoplasma gondii and anti-Neospora caninum antibodies in dogs with and without neurological signs. Cienc Rural. 2023;53(9):e20220133. doi:10.1590/0103-8478cr20220133

20. Olbera AVG, Fornazari F, Babboni SD, Rossi RS, Sevá AP, Latosinski GS, et al. Cumulative incidence and spatial distribution of dogs exposed to Toxoplasma gondii. Rev Bras Parasitol Vet. 2020;29(2):e000820. doi:10.1590/S1984-29612020025

21. Centro de Referência de Assistência Social. Painel do Cadastro Único. Brasília, DF: Ministério da Cidadania; 2024 [cited 2024 Sept 13]. Available from: https://cecad.cidadania.gov.br/painel03.php