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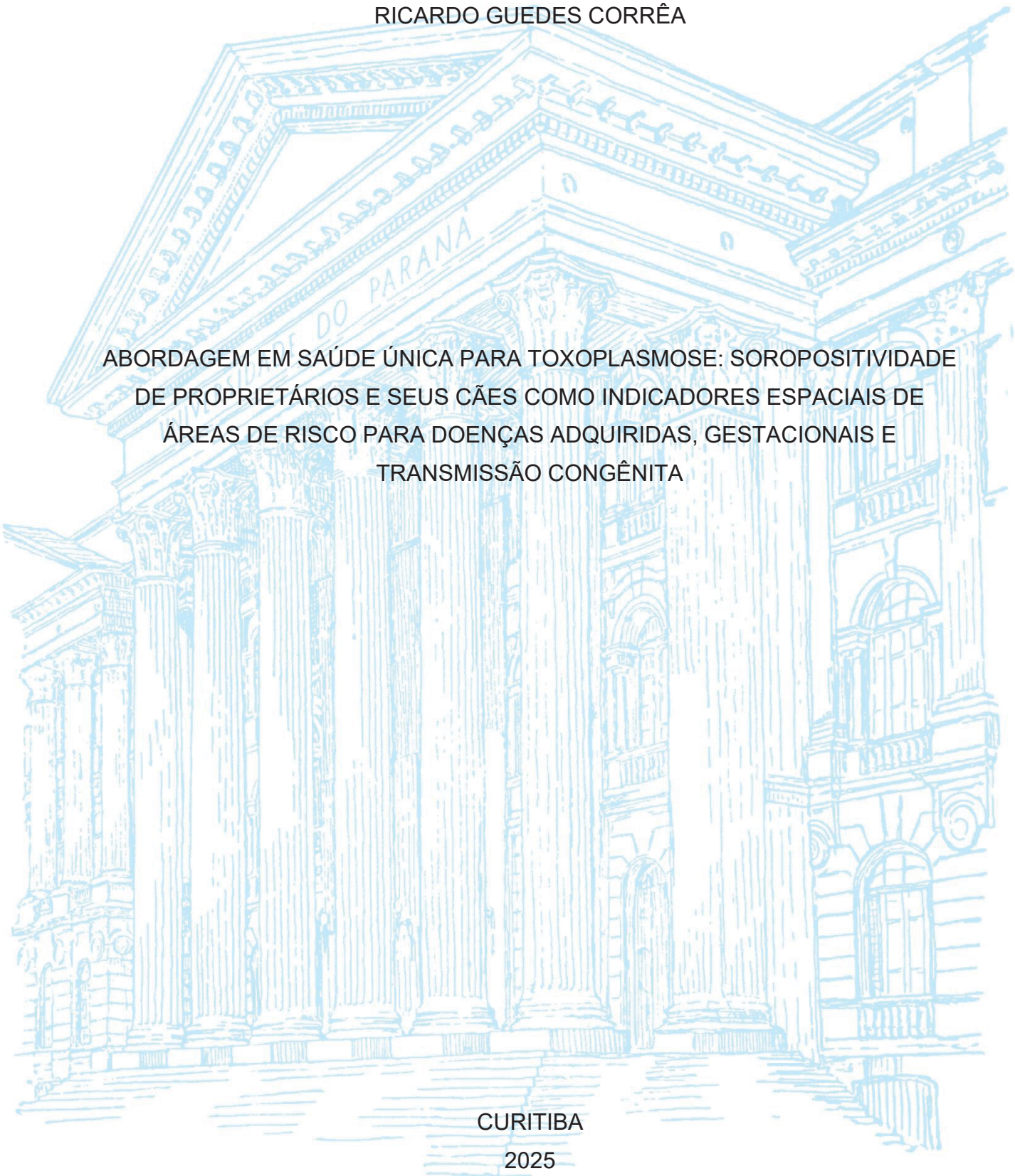

# ABORDAGEM EM SAÚDE ÚNICA PARA TOXOPLASMOSE: SOROPOSITIVIDADE DE PROPRIETÁRIOS E SEUS CÃES COMO INDICADORES ESPACIAIS DE ÁREAS DE RISCO PARA DOENÇAS ADQUIRIDAS, GESTACIONAIS E TRANSMISSÃO CONGÊNITA

CURITIBA

2025

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Dissertação apresentada ao curso de Pós-Graduação em Ciências Veterinárias, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ciências Veterinárias.

Orientador: Prof. Dr. Alexander Welker Biondo

Coorientadora: Dra. Natacha Sohn-Hausner

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# TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **RICARDO GUEDES CORRÊA**, intitulada: **ABORDAGEM EM SAÚDE ÚNICA PARA TOXOPLASMOSE: SOROPOSITIVIDADE DE PROPRIETÁRIOS E SEUS CÃES COMO INDICADORES ESPACIAIS DE ÁREAS DE RISCO PARA DOENÇAS ADQUIRIDAS, GESTACIONAIS E TRANSMISSÃO CONGÊNITA.**, sob orientação do Prof. Dr. ALEXANDER WELKER BIONDO, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 11 de Agosto de 2025.

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*“A mente que se abre a uma nova ideia jamais volta ao seu tamanho original.”*

*Albert Einstein*

## RESUMO

A toxoplasmose tem sido uma preocupação de saúde pública devido às associações diretas com vulnerabilidade socioeconômica e condições de vida inadequadas. Nesse sentido, o presente estudo teve como objetivo avaliar anticorpos contra *T. gondii*, casos históricos de toxoplasmose reportados e fatores de risco socioambientais associados em Pinhais, uma área completamente urbana de Curitiba, atualmente a oitava maior área metropolitana do Brasil. Os anticorpos anti-*Toxoplasma gondii* foram avaliados por meio de uma reação de imunofluorescência indireta (RIFI). Amostras de donos e cães também foram testadas por IFAT para anticorpos anti-*Leishmania spp.* e anti-*Trypanosoma cruzi*. No total, 20/135 (14,8%) pessoas e 13/133 (9,8%) cães de 25 domicílios diferentes foram considerados soropositivos para *T. gondii*. Todas as amostras foram soronegativas para *Leishmania spp*. e *Trypanosoma cruzi*. Embora nenhuma covariável significativa tenha sido encontrada no modelo de regressão, fatores de risco estatisticamente associados na análise bivariada incluíram o não uso de água pública ($p$ = 0,016) e consumo de leite cru ($p$ = 0,041) para os donos, e obesidade ($p$ = 0,028) e infestação por carrapatos ($p$ = 0,03) para os cães. Além disso, um aglomerado espacial de soropositividade para *T. gondii* tanto para os donos quanto para seus cães sobrepôs-se à localização dos casos históricos reportados de toxoplasmose adquirida, gestacional e congênita. Por fim, os resultados aqui mostraram a infestação por carrapatos como um indicador de risco socioambiental para exposição ao *T. gondii* no ambiente doméstico, e os cães podem ser usados como sentinelas para casos humanos de toxoplasmose.



Palavras-chave: Fatores de risco associados; domicílios; protozoários; animais sentinelas; carrapatos; zoonoses.

## ABSTRACT

Toxoplasmosis has been of public health concern due to direct associations with socioeconomic vulnerability and inadequate living conditions. Accordingly, the present study aimed to assess antibodies against *T. gondii*, historical reported toxoplasmosis cases and associated socio-environmental risk factors in Pinhais, a full urban area of Curitiba, currently the eighth biggest metropolitan area of Brazil. Anti-*Toxoplasma gondii* antibodies were assessed by an indirect immunofluorescence reaction (RIFI). Owner and dog samples were also tested by IFAT to anti-*Leishmania spp*. and anti-*Trypanosoma cruzi* antibodies. Overall, 20/135 (14.8%) persons and 13/133 (9.8%) dogs from 25 different households were considered seropositive to *T. gondii*. All samples were seronegative to *Leishmania spp.* and *Trypanosoma cruzi*. Although no significant covariates were found in the regression model, statistically associated risk factors in the bivariate analysis included no public water use ($p = 0.016$) and drinking raw milk ($p = 0.041$) for owners, and obesity ($p = 0.028$) and tick infestation ($p = 0.03$) for dogs. In addition, a spatial cluster of *T. gondii* seropositivity for both owners and their dogs overlapped the location of historic reported cases of human acquired, gestational and congenital toxoplasmosis. Finally, the results herein showed tick infestation as an indicator of socio-environmental risk for *T. gondii* exposure in the household environment, and dogs may be used as sentinels for human toxoplasmosis cases.



Keywords: Associated risk factors; households; protozoa; sentinel animals; ticks; zoonoses

## LISTA DE FIGURAS

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## LISTA DE ABREVIATURAS OU SIGLAS

| | |
|---|---|
| AIC | Akaike Information Criterion |
| CI | Confidence Interval |
| Cfb | Código da classificação climática de Köppen |
| GDP | Gross Domestic Product |
| HDI | Human Development Index |
| IFAT | Indirect Fluorescent Antibody Test |
| km² | Quilômetros quadrados |
| LIT | Liver Infusion Tryptose |
| mL | Mililitro |
| mg/mL | Miligrama por mililitro |
| mm | Milímetro |
| µL | Microlitro |
| OR | Odds Ratio |
| RIFI | Reação de Imunofluorescência Indireta |
| rpm | Revoluções por minuto |
| SINAN | Sistema de Informação de Agravos de Notificação |
| UHT | Ultra High Temperature |

## LISTA DE SÍMBOLOS

ºC Graus Celsius
º Graus
‘ Minutos
‘’ Segundos
> Maior que
< Menor que
= Igual a
% Percentagem

**SUMÁRIO**

## 1 INTRODUÇÃO

As doenças parasitárias permanecem entre as principais causas de morbidade e mortalidade em todo o mundo, particularmente na África, Ásia e América Latina (Meneguetti *et al*., 2020). Muitos desses patógenos foram incluídos no chamado grupo de Doenças Negligenciadas, que podem afetar quase 90% das pessoas com problemas de saúde do planeta, mas recebem menos de 10% de todos os recursos investidos em pesquisa, controle ou erradicação (Rocha, 2020). Entre elas, infecções por protozoários zoonóticos, como a toxoplasmose, têm sido de importância para a saúde pública devido à associação direta com a pobreza e condições de vida inadequadas (CDC, 2024). Embora essas doenças negligenciadas possam ter diminuído significativamente nos últimos anos, as áreas globais subdesenvolvidas ainda são responsáveis pela maior morbidade e mortalidade.

A toxoplasmose, causada pelo protozoário *Toxoplasma gondii* é uma das infecções zoonóticas mais disseminadas no mundo, com os gatos como únicos hospedeiros definitivos, pode causar graves danos a humanos e animais, particularmente em fetos (Hill & Dubey, 2016; Dubey, 2023). Embora não sejam os hospedeiros definitivos, os cães podem estar epidemiologicamente envolvidos na transmissão de *T. gondii* como sentinelas para doenças humanas (Frenkel & Parker, 1996; Lindsay *et al*., 1997; Bresciani *et al*., 2008; Leal & Domingues, 2014). Enquanto os órgãos humanos e de gatos mais afetados são os pulmões e os olhos, os cães apresentam mais comumente manifestações neurológicas (Hill & Dubey, 2016).

Apesar de doenças graves poderem ocorrer em mulheres grávidas e pacientes imunossuprimidos infectados (Capobiango *et al*., 2014; Almeria & Dubey, 2021), casos agudos têm sido geralmente limitados e registrados em baixas incidências (OPAS - Organização Pan-Americana da Saúde. Organização Mundial da Saúde. Escritório Regional para as Américas, 2024). Por outro lado, a infecção crônica tem sido estimada em uma ampla faixa de prevalência de 10 a 75% em vários países em todo o mundo (Woodhall *et al*., 2014). No Brasil, pesquisas sorológicas indicaram que aproximadamente 50% das crianças e 80% das mulheres em idade fértil apresentam anticorpos contra *T. gondii* (Dubey *et al*., 2012). Além da alta prevalência, o Brasil também foi responsável por 35,3% de todos os surtos relatados globalmente nos últimos 50 anos (Dubey *et al*., 2012), incluindo os dois maiores surtos registrados

até o momento, em 2001 e 2018 (de Moura *et al*., 2006; de Almeida, M. J. *et al*., 2011; Dal Ponte *et al*., 2019).

A fonte de infecção por *T. gondii* mais comum para hospedeiros intermediários (como humanos e cães) tem sido a ingestão de carne crua ou malcozida contendo bradizoítos, seguida pela ingestão de oocistos ambientais e transmissão transplacentária de taquizoítos (Hill & Dubey, 2016; Bahia-Oliveira *et al*. 2017; Dubey, 2023). No entanto, a transmissão oral por si só pode não explicar a ocorrência comum de toxoplasmose em uma variedade de hospedeiros, como herbívoros, aves e roedores selvagens (Ben-Harari, 2019; Adamska & Skotarczak, 2017). A manutenção dos parasitas *T. gondii* na natureza e as rotas de transmissão para hospedeiros domésticos e selvagens ainda não foram totalmente estabelecidas, com alguns estudos sugerindo que a manutenção pode ocorrer através de carrapatos (Sroka, J. *et al*., 2008; Skotarczak, 2016; Zhou *et al*., 2016).

Embora a toxoplasmose tenha sido diretamente associada à vulnerabilidade socioeconômica e condições inadequadas de vida, a distribuição espacial da soropositividade de proprietários e cães em relação aos casos atualmente notificados não foi realizada até o momento. Assim, o presente estudo teve por objetivo avaliar anticorpos contra *T. gondii* e fatores de risco associados em domicílios, proprietários e seus cães, e casos históricos de toxoplasmose notificados em Pinhais, uma área totalmente urbana de Curitiba, atualmente a oitava maior área metropolitana do Brasil.

## 2 ARTIGO PUBLICADO

## 2.1 ABORDAGEM DE SAÚDE ÚNICA PARA A TOXOPLASMOSE: SOROPOSITIVIDADE DE TUTORES E CÃES COMO INDICADORES ESPACIAIS DE ÁREAS DE RISCO PARA TRANSMISSÃO ADQUIRIDA, GESTACIONAL E CONGÊNITA

One Health Approach to Toxoplasmosis: Owner and Dog Seropositivity as Spatial Indicators of Risk Areas for Acquired, Gestational and Congenital Transmission

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**ABSTRACT:**

Background: Toxoplasmosis has been of public health concern due to direct associations with socioeconomic vulnerability and inadequate living conditions. Methods: Accordingly, the present study aimed to assess antibodies against *T. gondii*, historical reported toxoplasmosis cases and associated socio-environmental risk factors in Pinhais, a full urban area of Curitiba, currently the eighth biggest metropolitan area of Brazil. Anti-*Toxoplasma gondii* antibodies were assessed by an indirect immunofluorescence reaction (RIFI). Owner and dog samples were also tested by IFAT to anti-*Leishmania spp*. and anti-*Trypanosoma cruzi* antibodies. Results: Overall, 20/135 (14.8%) persons and 13/133 (9.8%) dogs from 25 different households were considered seropositive to *T. gondii*. All samples were seronegative to *Leishmania spp*. and *Trypanosoma cruzi*. Conclusions: Although no significant covariates were found

in the regression model, statistically associated risk factors in the bivariate analysis included no public water use ($p$ = 0.016) and drinking raw milk ($p$ = 0.041) for owners, and obesity ($p$ = 0.028) and tick infestation ($p$ = 0.03) for dogs. In addition, a spatial cluster of *T. gondii* seropositivity for both owners and their dogs overlapped the location of historic reported cases of human acquired, gestational and congenital toxoplasmosis. Finally, the results herein showed tick infestation as an indicator of socio-environmental risk for *T. gondii* exposure in the household environment, and dogs may be used as sentinels for human toxoplasmosis cases.

**Keywords:** associated risk ractors; One Health; protozoa; sentinel animals; ticks; zoonoses

### 2.1.1 INTRODUCTION

Parasitic diseases have remained among the main causes of morbidity and mortality worldwide, particularly in Africa, Asia, and Latin America [1,2]. Many of these pathogens have been included in the so-called neglected diseases group, which may affect nearly 90% of unhealthy habitants on the planet but receive less than 10% of all resources invested in research, control, or eradication [3]. Among them, zoonotic protozoan infections such as toxoplasmosis, leishmaniasis and Chagas disease have been of public health importance due to direct association with poverty and inadequate living conditions [4]. Although such neglected diseases may have significantly decreased in recent years, developing global areas have still accounted for higher morbidity and mortality. Toxoplasmosis, caused by the *T. gondii* protozoon and among the most dispersed zoonotic infections worldwide, and with cats as the only definitive hosts, may cause serious human and animal injuries, particularly in fetuses [5,6]. Although not the definitive hosts, dogs may be epidemiologically involved in *T. gondii* transmission as sentinels for human disease [7–9]. While the most affected human and cat organs are the lungs and eyes, dogs most commonly present neurological manifestations [6]. Despite pregnant women and infected immunosuppressed patients experiencing serious illness [5,10], acute cases have been generally limited and recorded in low occurrences [11]. On the other hand, chronic infection has been estimated in a wide prevalence range from 10 to 75% in several countries worldwide [12]. In Brazil, serosurveys have indicated that approximately 50% of children and 80% of childbearing-age women present antibodies against *T. gondii* [13]. In addition to high prevalence, Brazil has also accounted for 35.3% of all outbreaks reported globally in the past 50 years [13], including the two largest outbreaks recorded to date, in 2001

and 2018 [14–16]. The most common *T. gondii* infection source for intermediate hosts (such as humans and dogs) has been the ingestion of raw or undercooked meat containing bradyzoites, followed by the ingestion of environmental oocysts and the transplacental transmission of tachyzoites [6,17]. Nonetheless, oral transmission itself may not explain the common occurrence of toxoplasmosis in a variety of hosts, such as herbivores, birds, and wild rodents [18]. The maintenance of *T. gondii* parasites in nature and routes of transmission to domestic and wildlife hosts remain to be fully established, with some studies suggesting that maintenance may occur through ticks [19–21]. Although toxoplasmosis has been directly associated with socioeconomic vulnerability and inadequate living conditions, spatial distribution of owner and dog seropositivity on actual reported cases have not been conducted to date. Accordingly, the present study aimed to assess antibodies against *T. gondii*, historically reported toxoplasmosis cases and associated socio-environmental risk factors in Pinhais, a completely urbanized area of Curitiba, currently the eighth largest metropolitan area of Brazil.

## 2.1.2 MATERIALS AND METHODS

### 2.1.2.1 ETHICAL STATEMENT

The present study was approved by the National Research Ethics Commission, Brazilian Ministry of Health (protocol number 34934220.4.0000.0102/2020) and by the Ethics Committee on the Use of Animals at the Federal University of Paraná (protocol number 078/2019).

### 2.1.2.2 STUDY AREA

This study was carried out in Pinhais (25º25′57″S and 49º11′35″ W), Paraná state, southern Brazil, the eighth most populous metropolitan region in Brazil, with 3,731,769 inhabitants, and the second-largest metropolitan region nationwide, covering 16,581.21 km2. The area satisfied the Cfb Köppen climate classification, with annual mean temperature of 17ºC and annual rainfall of 1550 mm [22]. Pinhais has been divided into 15 neighborhoods and 4 hydrographic regions (Iraí, do Meio, Palmital and Atuba rivers) with different environmental and population characteristics [23].

### 2.1.2.3 SAMPLINGS AND TESTING

The sampling design was a convenience design, based on complaint protocols of household tick infestation reported to the Department of Health at Pinhais. Collections were performed by a multidisciplinary taskforce from April 2019 to November 2020. All volunteers signed an informed consent form before any information or blood samples

were obtained. Blood samples were collected in tubes with separating gel from owners by venipuncture of the median cubital vein by certified nurses, from in their dogs by venipuncture of the jugular or cephalic vein by certified veterinarians. Following collection, samples were centrifuged at 5000 rpm for 5 min, sera were separated and stored at–20ºC until serological analysis. Both owner and dog serum samples were tested for specific IgG antibodies against *Toxoplasma gondii*, *Leishmania spp*. and *Trypanosoma cruzi* by indirect fluorescent antibody test (IFAT) as previously described [24]. *T. gondii* tachyzoites (RH strain) were obtained by means of intraperitoneal inoculation of tachyzoites in Swiss mice and recovery of the suspensions 30 days after infection. The *Leishmania* major-like promastigotes (strain MHOM/SU/73/5-ASKH; Fiocruz IOC/L0581) and *T. cruzi* (strain Y) epimastigotes were cultivated in LIT medium (Liver Infusion Tryptose) supplemented with fetal bovine serum and antibiotic solution (Gentocin® 40 mg/mL) in solid medium maintained at 26 ºC, with fortnightly replications. Immunofluorescence slides were previously sensitized with the given protozoa, inactivated with 0.1% formaldehyde, and then stained for fluorescence using a specific commercial anti-IgG antibody (human or canine) conjugated with fluorescein isothiocyanate (Bethyl Laboratories, Inc., Montgomery, TX, USA). Readings were taken by a veterinary technician using an immunofluorescence microscope. Seropositive samples were established with a cutoff of antibody titers ≥ 16 for *T. gondii* screening up to 4096, ≥40 for *Leishmania spp*. and *T. cruzi* screening up to 640, and final serum titers were determined by the highest dilution with ≥50% parasites still fluorescing [25]. To ensure reliable results, positive and negative control sera were added to each slide in all readings.

**2.1.2.4 EPIDEMIOLOGICAL DATA COLLECTION**

Predefined epidemiological questionnaires based on previous studies and the published literature were provided to owner volunteers to complete concerning themselves and their dogs, to assess potential associated risk factors for *T. gondii*, *Leishmania spp*. and *T. cruzi* infection. These questionnaires contained close-ended questions about variables associated with likely exposure of owners and their dogs to pathogens. The questions were related to socioeconomic-environmental variables, personal sanitary habits and animal behavior, health, and management. Locations of all documented cases until December 2020 to the Pinhais municipality through the Notifiable Diseases Information System (SINAN, abbreviation of “Sistema de Informação de Agravos de Notificação” in Portuguese). The SINAN is responsible for notification, investigation and, in the case of communicable diseases, follow-up and

treatment. The diseases and conditions recorded by the SINAN are defined by the National Compulsory Notification List of diseases [26].

#### 2.1.2.5 STATISTICAL ANALYSIS

Data were descriptively analyzed at first with simple (n) and relative (%) frequency estimates for all variables in the database. Subsequently, the association with positive results (data from the questionnaires) was assessed using the chi-square test and estimated odds ratio (OR) and 95% confidence interval. Multiple logistic regression models were produced to obtain the profile of the cases. In multiple modeling, variables with $p < 0.20$ were selected to start the models. The method used for input and output of the variables was stepwise, starting from the most complex model to the simplest one. The criteria used to remain in the final model included changes >10% in the OR, improvement in the accuracy of the 95% CI, statistical significance, degrees of freedom and adjustment of the AIC of the model. Spatial analyses were carried out based on the georeferencing of the locations (addresses) of the protocols and reported cases by the SINAN and thematic maps and cluster analysis (kernel density) were produced. All analyses were performed in the R4.0.4 environment, with a minimum significance level of 5%.

### 2.1.3 RESULTS

#### 2.1.3.1 SEROLOGICAL ANALYSIS

Overall, 20/135 (14.8%) owner samples were seropositive for *T. gondii* with titers of 16 (80.0%), 64 (15.0%) and 256 (5.0%) (Table 1), while 13/133 (9.8%) dog samples were seropositive with titers of 16 (76.92%) and 64 (23.08%) (Table 2). All owner (0/135) and dog (0/133) samples were seronegative for *Leishmania spp.* and *T. cruzi*.

**Table 1.** Prevalence of IgG anti-*T. gondii* antibodies in owners.

| Variable | Result/Titer | N | % | 95% CI | |
|---|---|---|---|---|---|
| | | | | Lower | Upper |
| Seropositivity | Seropositive | 20 | 14.8 | 9.8 | 21.8 |
| | Seronegative | 115 | 85.2 | 78.2 | 90.2 |
| RIFI | 16 | 16 | 80 | 58.4 | 91.9 |
| | 64 | 3 | 15 | 5.2 | 36.0 |
| | 256 | 1 | 5 | 0.9 | 23.6 |

**Table 2.** Prevalence of IgG anti-*T. gondii* antibodies in dogs.

| Variable | Result/Titer | N | % | 95% CI | |
|---|---|---|---|---|---|
| | | | | Lower | Upper |
| Seropositivity | Seropositive | 13 | 9.77 | 5.8 | 16.0 |
| | Seronegative | 120 | 90.23 | 84.0 | 94.2 |
| RIFI | 16 | 10 | 76.92 | 49.7 | 91.8 |
| | 64 | 3 | 23.08 | 8.2 | 50.3 |

### 2.1.3.2 STATISTICAL ANALYSIS

Analysis of associated risk factors for *T. gondii* exposure was performed with logistic regression models made with the owner and dog serological results as the dependent Variable and with Applied epidemiological questionnaires as independent variables, with *p*-value < 0.2 in the bivariate analysis considered significant (Supplementary Tables S1 and S2). No final model was found with significant variables for both owner (Table 3) and dog (Table 4) seropositivity.

**Table 3.** Logistic regression models for owner exposure to *T. gondii* as the dependent variable.

| Variables | Mod1 | Mod2 | Mod3 | Mod4 | Mod5 | Mod6 |
|---|---|---|---|---|---|---|
| (Intercept) | 0.995 | 0.995 | 0.995 | 0.378 | 0.195 | 0.372 |
| When it rains, water accumulates inside the house: Yes | 0.115 | 0.115 | 0.118 | 0.103 | | |
| Occurred at home: Not bitten by ticks | 0.995 | 0.995 | | | | |
| Occurred at home: Yes | 0.995 | 0.995 | | | | |
| Occurred after visiting the forest: Not bitten by ticks | 0.999 | | | | | |
| Occurred after visiting the forest: Yes | 0.999 | | | | | |
| Time of year: Not bitten by ticks | 0.999 | 0.999 | 0.995 | | | |
| Time of year: Do not know | 0.992 | 0.992 | 0.992 | | | |
| Consumes raw or pasteurized milk: Does not drink milk | 0.156 | 0.156 | 0.123 | 0.052 | 0.058 | 0.058 |
| Consume raw or pasteurized milk: Pasteurized and/or UHT | 0.263 | 0.263 | 0.195 | 0.061 | 0.070 | 0.069 |
| Frequent contact with sand or earth: Yes | 0.041 | 0.041 | 0.057 | 0.071 | 0.110 | |

Mod = modifications.

**Table 4.** Logistic regression models for dog exposure to *T. gondii* as the dependent variable.

| Variables | Mod1 | Mod2 | Mod3 | Mod4 | Mod5 | Mod6 | Mod7 | Mod8 | Mod9 | Mod10 |
|---|---|---|---|---|---|---|---|---|---|---|
| (Intercept) | 0.305 | 0.355 | 0.728 | 0.234 | 0.136 | 0.016 | 0.005 | <0.001 | <0.001 | <0.001 |
| Breed: mixed | 0.018 | 0.025 | 0.029 | 0.034 | 0.045 | 0.044 | 0.064 | 0.041 | 0.071 | 0.097 |
| Body score | 0.724 | 0.752 | 0.917 | 0.920 | 0.960 | | | | | |

**Table 4.** *Cont.*

| Variables | Mod1 | Mod2 | Mod3 | Mod4 | Mod5 | Mod6 | Mod7 | Mod8 | Mod9 | Mod10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of ticks: 1 to 5 | 0.998 | | | | | | | | | |
| Number of ticks: 6 to 10 | 0.999 | | | | | | | | | |
| Number of ticks: >10 | 0.998 | | | | | | | | | |
| Tick collection locations: both | 0.999 | 0.727 | 0.857 | 0.822 | 0.889 | 0.881 | | | | |
| Tick collection locations: dog | 0.998 | 0.507 | 0.405 | 0.384 | 0.308 | 0.281 | | | | |
| Tick collection locations: no tick collection | 0.415 | 0.461 | 0.462 | 0.427 | 0.594 | 0.574 | | | | |
| Dog household location: backyard | 0.214 | 0.158 | 0.130 | | | | | | | |
| Dog household location: street | 0.997 | 0.997 | 0.997 | | | | | | | |
| Raw meat: yes | 0.072 | 0.068 | 0.101 | 0.141 | 0.119 | 0.115 | 0.120 | 0.093 | 0.093 | |
| Control ticks: yes | 0.360 | 0.389 | 0.202 | 0.233 | 0.208 | 0.206 | 0.234 | | | |
| Vaccination: both | 0.142 | 0.201 | 0.880 | 0.950 | | | | | | |
| Vaccination: antirabies | 0.193 | 0.246 | 0.945 | 0.874 | | | | | | |
| Vaccination: do not know | 0.890 | 0.883 | 0.323 | 0.209 | | | | | | |
| Vaccination: multipurpose | 0.995 | 0.995 | 0.995 | 0.993 | | | | | | |
| Deworming: do not know | 0.998 | 0.998 | | | | | | | | |
| Deworming: yes | 0.099 | 0.117 | | | | | | | | |
| Animal hygiene: dirty | 0.144 | 0.129 | 0.225 | 0.299 | 0.144 | 0.140 | 0.106 | 0.094 | | |

Mod = modifications.

Addresses were georeferenced based on the notification forms of patients residing in Pinhais for spatial assessment, visualization, and analysis of risk areas of each disease (Figure1). In the historical record, a total of 87 toxoplasmosis cases were reported by the SINAN between 2015 and 2020, with 28 cases of acquired, 43 of gestational, and 16 cases of congenital toxoplasmosis distributed in residents of 12 neighborhoods. In addition, nine human leishmaniasis cases were reported between 2007 and 2020 and distributed in six neighborhoods. Finally, 25 human Chagas cases were reported between 2013 and 2018 in residents of eight neighborhoods. Only three neighborhoods had no notification of any of the three surveyed diseases.

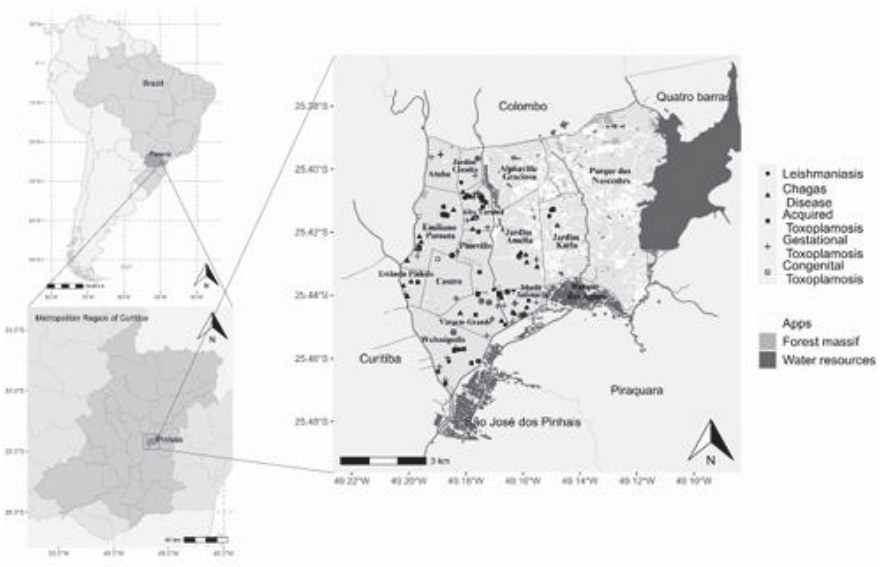


**Figure 1.** Location of human cases documented on the Notifiable Diseases Information System (SINAN) from 2007 to 2020 for acquired, gestational, and congenital toxoplasmosis, leishmaniasis, and Chagas disease in residents of Pinhais, Paraná, Brazil.

To verify the presence of toxoplasmosis clusters, a Kernel density analysis was performed with human seropositive location and overlapped with seropositive dogs. In addition, clusters was inversely verified, with Kernel density of seropositive dogs location 7 of 14 overlapped with the points with seropositive humans, and compared with the notified cases of toxoplasmosis in the municipality (Figure 2)

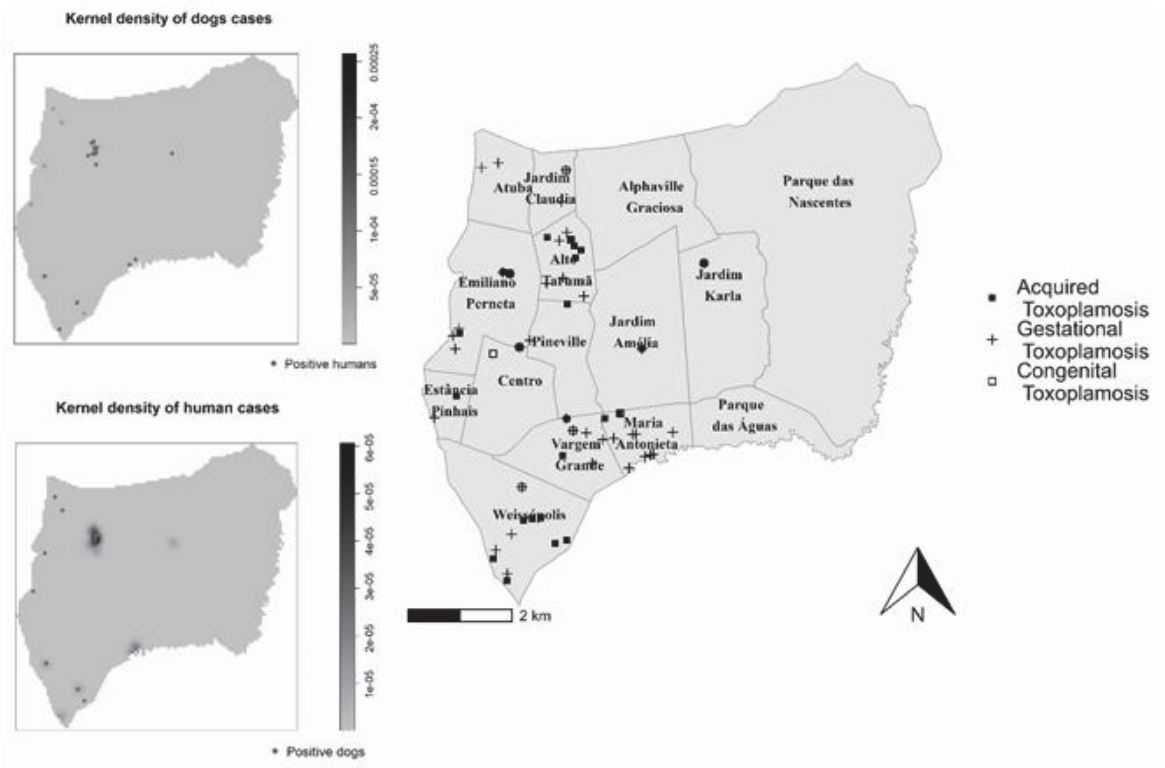


**Figure 2.** Spatial density of seropositive owners and dogs (kernel maps on the left) to *T. gondii* and historical records of human toxoplasmosis cases from 2007 to 2020.

### 2.1.4 DISCUSSION

The results found concerning antibodies to *T. gondii* in both human (14.8%) and canine (9.8%) samples, and none to *Leishmania* spp. and *T. cruzi*, corroborate those of previous serosurveys, with prevalent occurrences of human [27] and dog [28] toxoplasmosis, but no report to date of confirmed human or dog autochthonous cases in the Curitiba metropolitan area, Paraná state, of visceral leishmaniasis [29] or Chagas disease [28].

The relatively low human *T. gondii* seroprevalence herein (14.81%), compared to previous studies ranging from 59.8% to 72.6% in the general Paraná state population [30,31], may be due to regional characteristics, as seropositivity has varied from 10% to 90% world- wide [32]. As Curitiba has been considered the most sustainable city countrywide for years and is currently ranked as the eighth highest in population, sixth in gross domestic product (GDP) and tenth in human development index (HDI) out of 5565 municipalities in Brazil, this outcome may be the result of high density city infrastructure. Likewise, developed countries such as France have decreased their toxoplasmosis prevalence from 80% to about 30% between 1960 and 2016 due to increased knowledge and implementation of preventive actions by pregnant women

[33], such as changes in eating habits and the improvement in hygiene conditions [34]. In Brazil, human toxoplasmosis was included within the compulsory notification list in 2016 by the Ministry of Health, with nationwide integrated surveillance of gestational, congenital, and acquired toxoplasmosis and recommendation of serological prenatal screening [35].

Owner seropositivity to toxoplasmosis in the present study was associated as a risk factor to no public water use ($p$ = 0.016), as expected, since contamination by *T. gondii* oocysts present in untreated water may be one of the main infection routes [36], both in continental and island areas [37]. Oocysts may survive better in humid environments and are able to remain viable up to 200 days when kept in water between 10 °C and 25 ºC [38]. Another significant associated risk factor was drinking raw milk ($p$ = 0.041), which has also been considered a common route of *T. gondii* infection and increases infection probability [39]. As a limitation of the present study, consumption of pasteurized or UHT pasteurized milk data were not accessible, which may have impaired comparisons with the consumption of raw milk. In addition, due to difficulties in capture, restraint, potential scratching and biting, the city secretary of health denied cat samplings for this study, which should be further investigated. Although no significance was found for these covariates in the regression model, the ingestion of contaminated water, milk, or contaminated foods, has long represented an important route of toxoplasmosis transmission [38].

Dog seropositivity to toxoplasmosis in this study was comparable to that in previous Parana state studies, varying mostly by region with 16.3% prevalence at northern [31,40,41], and 67.02% at western [36], and 23.3% at eastern coastal [37] state regions, along with 30.7% in neighborhood [28] and 7.95% in hoarded dogs [42] at the state capital Curitiba. Nationwide, the seroprevalence of *T. gondii* in Brazilian domestic dogs has widely ranged from 5% to 88.52% [41,43–45], with rural and hunting dogs exhibiting a higher prevalence (34.3% and 31.2%) than urban dogs (19.7%) [46,47]. Worldwide, prevalence varies according to surveyed region [48], ranging from 64.7% to 78% in western Cuba [49], from 75.15% to 89.86% in west-central Ethiopia [50] and 17.3% to 34.7% in south-western China [51].

A body weight slightly above normal for dogs was a significant associated risk factor ($p$ = 0.028) to *T. gondii* seropositivity, which may indicate a closer human–dog interaction, as a significant association ($p$ = 0.008; OR = 2.81) was previously found between the presence of anti-*T. gondii* in dogs with their seropositive owners [37]. In addition, dog serological surveys may be important to assess the degree of infection spreading between humans and animals [52]. Finally, as shown herein, dogs may be good sentinels for assessing environmental contamination [53].

Interestingly, tick infestation herein was a significant variable ($p$ = 0.03) associated with *T. gondii* seropositivity in dogs, as the possibility of toxoplasmosis transmission by ticks has been proposed by previous reports [54,55]. Despite the lack of experimental evidence that *T. gondii* transmission can occur from infected ticks to their hosts, mechanical transmission through the ingestion of infected ticks may be an alternative transmission route [18,21], as *T. gondii* may survive in the body of ticks for more than 10 days [18,56].

*T. gondii* has been detected in several tick species in different countries [18], such as *Dermacentor reticulatus* and *Ixodes ricinus* in Poland [57], *Amblyomma* spp. in the Republic of Chad [58], *Haemaphysalis longicornis* in China [21], and *H. longicornis* and *Haemaphysalis flava* in Korea [59]. Moreover, a significant regional difference has been found in contamination within countries, which may reflect the difference in *T. gondii* environmental contamination [59]. In such a scenario, the present study may provide important information on potential environmental maintenance and alternative transmission routes of *T. gondii* by ticks, and further studies should be conducted to fully establish the transmission role of ticks in highly infested household settings. Regardless, ticks should be controlled and eradicated in any circumstance, as they are responsible for several tick-borne diseases of life-threatening impact for owners and their dogs.

As already mentioned, no case report of human or dog visceral leishmaniasis has been made in the Curitiba metropolitan area, Paraná state [29]. Human and dog cases of visceral leishmania have been mostly reported outside of urban areas and nearby rivers and recently deforested regions of the Paraná state [60], similar to other Brazilian states such as Minas Gerais [61], Piauí [62], Fortaleza [63], Mato Grosso [64] and Pernambuco [65], also associated with low-density infrastructure favoring vector growth, infection and transmission [66]. Thus, the leishmaniasis seronegativity herein may reflect the adequate infrastructure of the Curitiba metropolitan area, Paraná state, associated with the absence or low number of infected *Lutzomyia* spp. vectors due to unfavorable environmental conditions for vector maintenance such as climate and altitude [67]. In addition, the low number of cases found statewide may be a consequence of the mandatory euthanasia of seropositive dogs, considered the main *Leishmania* spp. reservoir in the domestic environment of Brazil [68]. Nationwide, prevalence to *Leishmania* spp. varies from 0.0027% to 32.5% [29,69] and from 2.0% to 4.0% for *T. cruzi* [70,71]. As the anti-*Leishmania* vaccination has been restricted by the Brazilian Ministry of Agriculture due to lack of efficacy, such vaccine status was not assessed in the dogs herein [72].

Expectedly, the present study showed an absence of owner and dog cases of Chagas disease, which mainly occurs in the northern Brazilian region with over 70% of reported new cases [73], mostly affecting socially vulnerable and uneducated people [74]. Even without seropositive samples for *T. cruzi* identified in our study and no autochthonous report of Chagas disease to date, Curitiba contains a series of forest fragments and city parks, providing a favorable environment for vector maintenance [75]. In addition, city surveillance reports in December, 2020 identified the presence of *Panstrongylus megistus* [62], a main transmission vector of *T. cruzi* in Brazil.

A major contribution of the present study is the combined spatial analysis of owner and dog *T. gondii* seropositivity. Through kernel density analysis, clusters of both human and dog *T. gondii* seropositivity overlapped with each other and overlapped with the historical cases reported by the Notifiable Diseases Information System (SINAN). Despite such distribution differences from a previous owner–dog toxoplasmosis study in the north [31] and similarities with another human toxoplasmosis survey in north-western Paraná state [76], both studies indicated socioeconomic vulnerability as an associated risk factor for *T. gondii* exposure.

Although the low prevalence found herein may reflect the better socioeconomic conditions of the municipality, the spatial clusters of owner and dog seropositivity overlapped the historical cases. Moreover, the kernel density maps were located in neighborhoods with a greater number of toxoplasmosis cases reported by SINAN. Despite previous studies in Brazil and Latin America showing an association and a three-fold-increased risk for toxoplasmosis in low-income populations, including pregnant women and dogs, affecting the geographic distribution of disease transmission [36,38,77–83], no study to date has proposed human and dog *T. gondii* seropositivity as spatial indicators of risk areas for actual transmission of acquired, gestational, and congenital toxoplasmosis.

Identification of toxoplasmosis risk areas may be crucial to support better diagnosis, control, and preventive programs, as these areas may lead to a greater probability of environmental *T. gondii* contamination and consequently an increase in human and animal infection [84]. Thus, future projections of human population growth and potential tropical change scenarios should be analyzed with disease risk altogether, particularly zoonoses, as human density increase in urban areas may be accompanied by an increase in density of domestic and feral cats, which are definitive hosts of *T. gondii* [85]. As an increase in intense rainfall events interspersed by longer periods of drought has been predicted to occur as a consequence of climate change in Curitiba [86], this new pattern may lead to a greater dispersion and oocyst uptake, due to the capacity of *T. gondii* to persist in the environment [38].

Moreover, global warming and other climate changes may expand the current distribution of ticks and other vectors such as sandflies, spreading to previously uninfected areas [87]. Surveys on the One Health approach, which characterized the human–animal– environment interface through intersectoral collaboration and multidisciplinary teams, have provided a more comprehensive understanding of disease cycles and associated public health risks [88,89]. In addition, the globalization of trade and travel may contribute to international pathogen spreading [90], demanding such holistic and borderless approaches.

As limitations, the IFAT applied herein may vary in accuracy among laboratories due to differences in sensitivity and specificity, influencing results and, therefore, restricting their comparisons [89]. Another limitation may be the under- or misreporting of diseases, particularly toxoplasmosis, possibly due to lack of diagnosis or misdiagnosis, inadequate medical records, patient failure to seek medical care, or even a deficiency in the local surveillance system, impairing their report to SINAN. Finally, although considered more visual than other analyses, the kernel approach may be limited by not considering the number of samples examined for assessment of heating areas [31].

Finally, despite other “One Health approach” studies on toxoplasmosis, our assessment of tick infestation in association with unrelated disease has shown an important indicator of socio-environmental risk for *T. gondii* exposure in the household environment, along with dogs as potential sentinels for human toxoplasmosis cases.

### 2.1.5 CONCLUSIONS

The present study has shown a low seroprevalence of *T. gondii* and no detection of anti-*T. cruzi* and anti-*Leishmania* spp. antibodies in the blood samples of

asymptomatic owners and their dogs in southern Brazil. Owners who did not use public water and drank raw milk, as well as dogs with a higher body score and tick infestation, were statistically more likely to be exposed to *T. gondii*. In addition, tick infestation in dogs was an associated risk factor for *T. gondii* exposure, indicating a direct risk by tick transmission or an indirect risk as an indicator of socioeconomical vulnerability. As toxoplasmosis in urban settings relies mostly on cats as definitive hosts, a further One Heath approach study should also consider the presence, number, and tick infestation of cats in such households.

The cluster of seropositive dogs to *T. gondii* overlapped the cluster of seropositive owners, both overlapping the historical reported cases of different toxoplasmosis presentations in the municipality. This One Health approach has indicated that owner and dog seropositivities act as spatial indicators of risk areas for acquired, gestational, and congenital transmission.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/tropicalmed9070143/s1, Table S1. Risk factors for *T. gondii* exposure in humans from Pinhais, Paraná, Brazil. Table S2. Risk factors for *T. gondii* exposure in dogs from Pinhais. Paraná state, Brazil.

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**Data Availability Statement:** Data are contained within the article.

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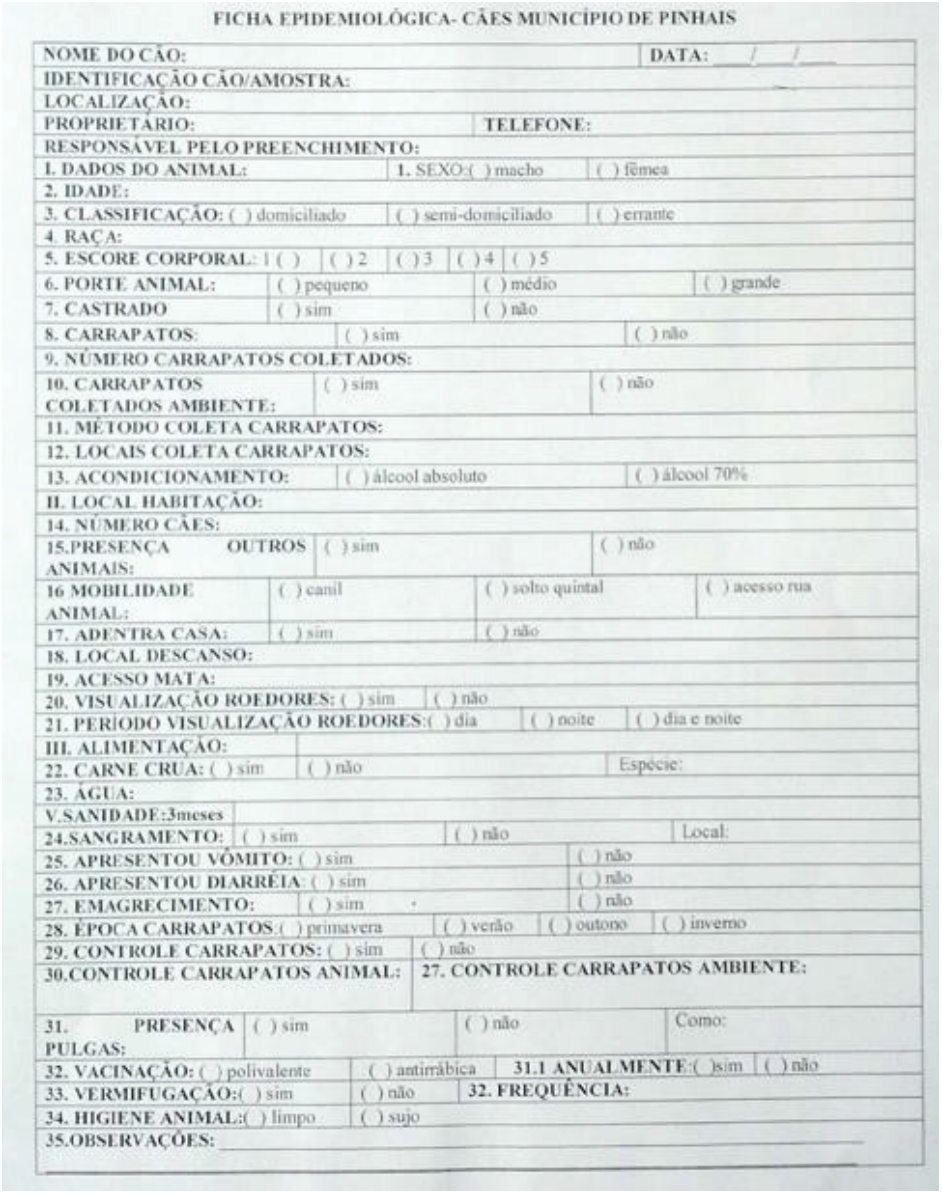
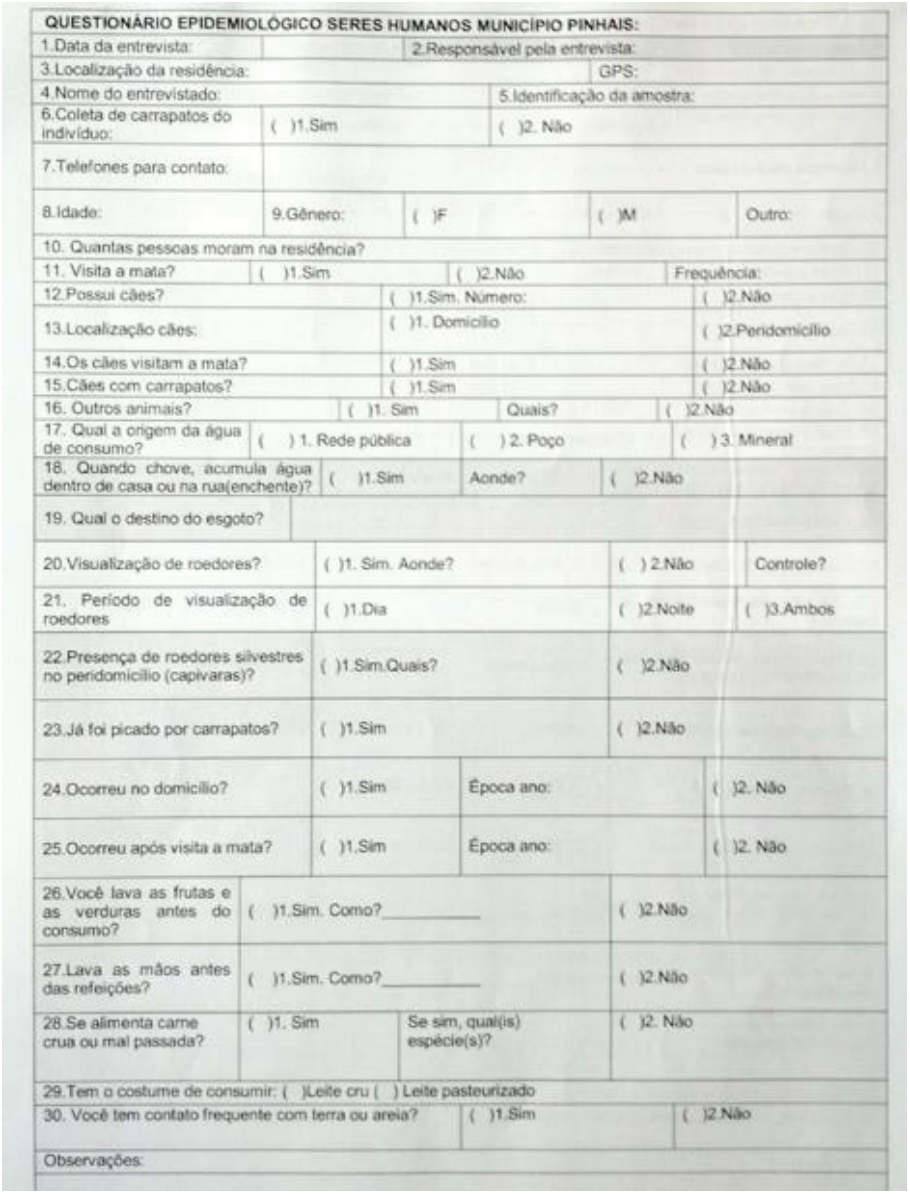
# ANEXO A - QUESTIONÁRIO EPIDEMIOLÓGICO - CÃES, PINHAIS, PR.



FICHA EPIDEMIOLÓGICA- CÃES MUNICÍPIO DE PINHAIS

| | | | | |
|---|---|---|---|---|
| NOME DO CÃO: | | | | DATA: ___/___/___ |
| IDENTIFICAÇÃO CÃO/AMOSTRA: | | | | |
| LOCALIZAÇÃO: | | | | |
| PROPRIETÁRIO: | | | TELEFONE: | |
| RESPONSÁVEL PELO PREENCHIMENTO: | | | | |
| I. DADOS DO ANIMAL: | | 1. SEXO:( ) macho | | ( ) fêmea |
| 2. IDADE: | | | | |
| 3. CLASSIFICAÇÃO: ( ) domiciliado | | ( ) semi-domiciliado | | ( ) errante |
| 4. RAÇA: | | | | |
| 5. ESCORE CORPORAL: 1 ( ) | ( ) 2 | ( ) 3 | ( ) 4 | ( ) 5 |
| 6. PORTE ANIMAL: | ( ) pequeno | | ( ) médio | ( ) grande |
| 7. CASTRADO | ( ) sim | | ( ) não | |
| 8. CARRAPATOS: | | ( ) sim | | ( ) não |
| 9. NÚMERO CARRAPATOS COLETADOS: | | | | |
| 10. CARRAPATOS COLETADOS AMBIENTE: | | ( ) sim | | ( ) não |
| 11. MÉTODO COLETA CARRAPATOS: | | | | |
| 12. LOCAIS COLETA CARRAPATOS: | | | | |
| 13. ACONDICIONAMENTO: | | ( ) álcool absoluto | | ( ) álcool 70% |
| II. LOCAL HABITAÇÃO: | | | | |
| 14. NÚMERO CÃES: | | | | |
| 15.PRESENÇA OUTROS ANIMAIS: | | ( ) sim | | ( ) não |
| 16 MOBILIDADE ANIMAL: | ( ) canil | | ( ) solto quintal | ( ) acesso rua |
| 17. ADENTRA CASA: | ( ) sim | | ( ) não | |
| 18. LOCAL DESCANSO: | | | | |
| 19. ACESSO MATA: | | | | |
| 20. VISUALIZAÇÃO ROEDORES: ( ) sim | | ( ) não | | |
| 21. PERÍODO VISUALIZAÇÃO ROEDORES:( ) dia | | ( ) noite | | ( ) dia e noite |
| III. ALIMENTAÇÃO: | | | | |
| 22. CARNE CRUA: ( ) sim | ( ) não | | Espécie: | |
| 23. ÁGUA: | | | | |
| V.SANIDADE:3meses | | | | |
| 24.SANGRAMENTO: ( ) sim | | ( ) não | | Local: |
| 25. APRESENTOU VÔMITO: ( ) sim | | | ( ) não | |
| 26. APRESENTOU DIARRÉIA: ( ) sim | | | ( ) não | |
| 27. EMAGRECIMENTO: ( ) sim | | | ( ) não | |
| 28. ÉPOCA CARRAPATOS:( ) primavera | | ( ) verão | ( ) outono | ( ) inverno |
| 29. CONTROLE CARRAPATOS: ( ) sim | | ( ) não | | |
| 30.CONTROLE CARRAPATOS ANIMAL: | | 27. CONTROLE CARRAPATOS AMBIENTE: | | |
| 31. PRESENÇA PULGAS: | ( ) sim | | ( ) não | Como: |
| 32. VACINAÇÃO: ( ) polivalente | | ( ) antirrábica | 31.1 ANUALMENTE:( )sim | ( ) não |
| 33. VERMIFUGAÇÃO:( ) sim | | ( ) não | 32. FREQUÊNCIA: | |
| 34. HIGIENE ANIMAL:( ) limpo | | ( ) sujo | | |
| 35.OBSERVAÇÕES: | | | | |

# ANEXO B - QUESTIONÁRIO EPIDEMIOLÓGICO - SERES HUMANOS, PINHAIS, PR



| QUESTIONÁRIO EPIDEMIOLÓGICO SERES HUMANOS MUNICÍPIO PINHAIS: | | | | |
|---|---|---|---|---|
| 1.Data da entrevista: | | 2.Responsável pela entrevista: | | |
| 3.Localização da residência: | | | GPS: | |
| 4.Nome do entrevistado: | | 5.Identificação da amostra: | | |
| 6.Coleta de carrapatos do indivíduo: | ( )1.Sim | ( )2. Não | | |
| 7.Telefones para contato: | | | | |
| 8.Idade: | 9.Gênero: | ( )F | ( )M | Outro: |
| 10. Quantas pessoas moram na residência? | | | | |
| 11. Visita a mata? | ( )1.Sim | ( )2.Não | Frequência: | |
| 12.Possui cães? | ( )1.Sim. Número: | | ( )2.Não | |
| 13.Localização cães: | ( )1. Domicílio | | ( )2.Peridomicílio | |
| 14.Os cães visitam a mata? | ( )1.Sim | | ( )2.Não | |
| 15.Cães com carrapatos? | ( )1.Sim | | ( )2.Não | |
| 16. Outros animais? | ( )1. Sim | Quais? | ( )2.Não | |
| 17. Qual a origem da água de consumo? | ( ) 1. Rede pública | ( ) 2. Poço | ( ) 3. Mineral | |
| 18. Quando chove, acumula água dentro de casa ou na rua(enchente)? | ( )1.Sim | Aonde? | ( )2.Não | |
| 19. Qual o destino do esgoto? | | | | |
| 20.Visualização de roedores? | ( )1. Sim. Aonde? | | ( ) 2.Não | Controle? |
| 21. Período de visualização de roedores | ( )1.Dia | | ( )2.Noite | ( )3.Ambos |
| 22.Presença de roedores silvestres no peridomicílio (capivaras)? | ( )1.Sim.Quais? | | ( )2.Não | |
| 23.Já foi picado por carrapatos? | ( )1.Sim | | ( )2.Não | |
| 24.Ocorreu no domicílio? | ( )1.Sim | Época ano: | ( )2. Não | |
| 25.Ocorreu após visita a mata? | ( )1.Sim | Época ano: | ( )2. Não | |
| 26.Você lava as frutas e as verduras antes do consumo? | ( )1.Sim. Como?_____ | | ( )2.Não | |
| 27.Lava as mãos antes das refeições? | ( )1.Sim. Como?_____ | | ( )2.Não | |
| 28.Se alimenta carne crua ou mal passada? | ( )1. Sim | Se sim, qual(is) espécie(s)? | ( )2. Não | |
| 29.Tem o costume de consumir: ( )Leite cru ( ) Leite pasteurizado | | | | |
| 30. Você tem contato frequente com terra ou areia? | | ( )1.Sim | ( )2.Não | |
| Observações: | | | | |

# ANEXO C - PROTOCOLO DE RIFI

fmvz - unesp
FACULDADE DE M...NÁRIA E ZOOTECNIA - CAMPUS DE BOTUCATU
DEPARTAMENTO DE HIGIENE VETERINÁRIA E SAÚDE PÚBLICA
Disciplina de Zoonoses

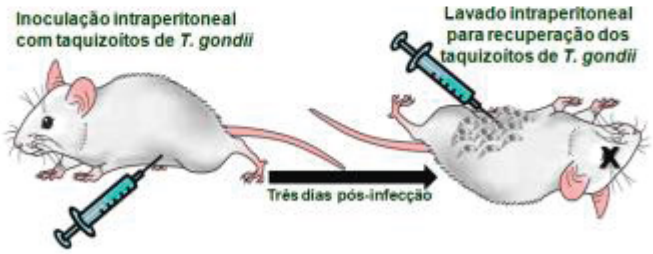
**SÚMULA DE AULA PRÁTICA: DIAGNÓSTICO LABORATORIAL PARA TOXOPLASMOSE.**
**RODÍZIO DE AULAS PRÁTICAS DO 4º ANO. 2018**

**Prof. Titular Helio Langoni**

A prova de imunofluorescência indireta é uma das técnicas imunológicas recomendadas e muito utilizada para o diagnóstico da toxoplasmose. Pode ser utilizada para se verificar a prevalência de anticorpos IgM ou IgG anti-*Toxoplasma gondii*. O teste de ELISA também é recomendado, entretanto, não realizamos essa técnica na rotina do laboratório. Descrevemos, portanto, a técnica de imunofluorescência indireta – RIFI.

**Prova de imunofluorescência indireta:**

**Sensibilização das lâminas** (fixação do antígeno – taquizoítos de *Toxoplasma gondii*). A solução antigênica é obtida a partir de lavados peritoniais de camundongos, previamente inoculados intraperitonialmente, com 1 mL de suspensão rica em taquizoítos de *Toxoplasma gondii* (Figura 1). Após a obtenção do lavado, o mesmo é transferido para tubo de centrífuga (50 ml), adicionando-se, em igual volume solução de formol 0,2% (9,8 ml de solução salina + 0,2 ml de formol puro). A seguir, incuba-se a temperatura de 37 ° C por 30 minutos, homogeneizando-se suavemente, por inversão, a cada 10 minutos.




A seguir, centrifuga-se a suspensão antigênica inativada, a 3000 rpm por 10 minutos e descarta-se o sobrenadante. Ao pellet, ou sedimento, acrescenta-se 2 - 3 ml de solução salina

# ANEXO D - PROTOCOLO DE RIFI



tamponada (SST) 0,01M pH 7,2. Homogeneiza-se em vórtex e centrifuga-se novamente a 3000 rpm por 10 minutos. Descarta-se o sobrenadante e, ao sedimento, adiciona-se 1 - 2 ml de SST 0,01M pH 7,2, observando-se a sua concentração em lâmina, de tal forma que a solução contenha de 30 a 40 taquizoítos por campo, pipetando-se 50 µl em uma lâmina, cobrindo-se com lamínula 24 x 60 mm para proceder a contagem dos taquizoítos.

As lâminas, utilizadas na RIFI, são compostas de duas fileiras de seis poços, fixados com antígeno, procedendo-se a adição, em cada um dos poços 10 µl da solução antigênica, e espera-se de 2 – 3 minutos para ocorrer a fixação dos taquizoítos na lâmina e, a seguir, retira-se o excesso por aspiração, restando uma fina película sobre cada poço ou perfuração a ser utilizada na reação. Depois de secas a temperatura ambiente, as lâminas são estocadas em caixa de madeira ou laminários, e são mantidas sob temperatura de congelamento entre -18ºC a -20ºC.

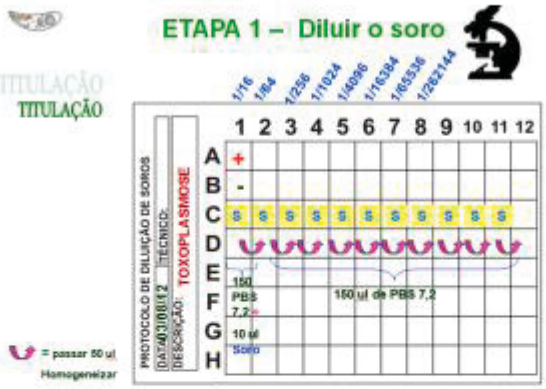
**Material utilizado:** Como material para a pesquisa de anticorpos anti-*Toxoplasma gondii*, utiliza-se amostra de no mínimo 0,5 ml de soro. Quando for enviada amostra de sangue, este deve ser devidamente dessorado, e se necessário centrifugado. O soro deve ser acondicionado em eppendorf identificado, e mantido sob temperatura de congelamento até o seu processamento.
No momento do exame, a amostra de soro deve ser diluída em SST 0,01M pH7,2 nas diluições 1:16, 1:64, 1:256, e assim por diante, em quantas diluições forem necessárias. Em microplaca devidamente identificada, pipeta-se 150 microlitros de SST 0,01M pH7,2 nas suas perfurações, formando uma fileira. O número de perfurações preenchidas, é definido, de acordo com as diluições a serem testadas.

Adicionar a primeira perfuração respectiva ao soro teste, 10 microlitros do soro, obtendo-se a diluição 1:16. Após homogeneização, transferir 50 microlitros dessa primeira diluição para a segunda perfuração, obtendo-se a diluição 1:64. Proceder da mesma forma para as demais perfurações obtendo-se diluições em quadruplicada. Ao final, (após a homogeneização) desprezar os 50 microlitros, referentes a última diluição testada. Na mesma microplaca, proceder da mesma maneira para os soros controles positivo e negativo, pipetando-se 10 microlitros de soro sabidamente positivo e 10 microlitros de soro sabidamente negativo, respectivamente (Etapa 1).

# ANEXO E - PROTOCOLO DE RIFI

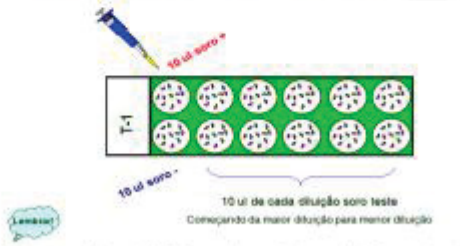


## ETAPA 1 – Diluir o soro




Na lâmina fixada previamente com o antígeno, distribui-se 10 microlitros de cada diluição do soro em cada poço ou perfuração, em fileira, ordenados em forma crescente de diluição, pipetando-se inversamente, ou seja, da maior diluição para a menor, protocolando-se o esquema adotado. Fazer o mesmo com o controle positivo e o negativo (Etapa 2).

## ETAPA 2 – Adicionar soro na lâmina

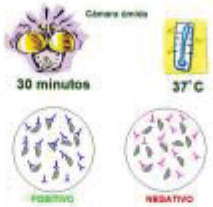



A lâmina deve ser incubada à 37ºC, em câmara úmida por 30 minutos (Etapa 3).

# ANEXO F - PROTOCOLO DE RIFI



ETAPA 3 – Incubar




A seguir, realizar 2 a 3 lavagens em SST 0,01M pH7,2 por 10 minutos cada. Primeiro, deve-se escorrer a lâmina com a solução e depois colocar em uma cuba contendo a solução, mantendo-a por 10 minutos. A seguir, despreza-se o conteúdo da cuba, preenchendo-se novamente com a solução, mantendo-se a lâmina na mesma por mais 10 minutos.

A seguir, acrescenta-se à reação o conjugado que nada mais é do que anticorpo anti-IgG específica para a espécie examinada (ou pesquisada) conjugado com o isotiocianato de fluoresceína. Este conjugado deve ser diluído de acordo com o seu título previamente estabelecido, em solução de azul de Evans a 20 mg%, também previamente preparada, que no momento do uso deve ser diluída a 1:5 ou seja 1 ml de azul de Evans mais 4 ml de SST 0,01M pH7,2 (Etapa 4).

ETAPA 5 – Adicionar conjugado na lâmina

Volume = n poços x 10 (cte) x n lâminas. Ex.:
Volume= 12 x 10 x 1 = 120 ul

Título do conjugado
Ex: (1/200)
1 ---------- 199

Azul de Evans / PBS (1/5)
1 ---------- 4
40 ---------- 160 (Total 200)
Preciso de 199, então, tiro 1 ul.
Adiciono 1 ul de conjugado.

O conjugado é então adicionado no volume de 10 µl em cada uma das diluições. A lâmina é então incubada à 37C, em câmara úmida por 30 minutos (etapa 5) e a seguir lavada em SST

# ANEXO G - PROTOCOLO DE RIFI



0,01M pH7,2 de 2 a 3 vezes durante 10 minutos cada, adotando-se o mesmo procedimento descrito anteriormente.


Após a secagem das lâminas com o auxílio de ventilador ou secador de cabelos, colocar duas gotas de solução glicerinada pH 8,5 sobre a lâmina, cobrindo-se com lamínula. Proceder a leitura em microscópio de imunofluorescência, com objetiva de 40X e ocular de 10X. Após a leitura dos controles, sem discordância com o resultado esperado, proceder a leitura das amostras em teste, considerando-se como ponto final da reação a maior diluição do soro, em que ainda houver fluorescência completa e intensa na borda de pelo menos 50% dos taquizoítos, sendo este então o título de anticorpos, que o animal apresenta, e que é expresso em UI, por exemplo 256 UI.