Seroprevalence and risk factors associated with *T. gondii* infection in pregnant individuals from a Brazilian Amazon municipality

Rafaela dos Anjos Pinheiro Bogoevich Morais [a,b,*], Ediclei Lima do Carmo [b],
Clea Nazaré Carneiro Bichara [c], Bruna Ramos dos Santos [b,d],
Kaio Willy Silva da Silveira [b,d], Marinete Marins Póvoa [a,b]

[a] Curso de Pós-graduação em Biologia de Agentes Infecciosos e Parasitários, Instituto de Ciências Biológicas da Universidade Federal do Pará, Belém, Pará, Brazil
[b] Seção de Parasitologia, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brazil
[c] Universidade do Estado do Pará, Belém, Pará, Brazil
[d] Programa Institucional de Bolsas de Iniciação Científica, Instituto Evandro Chagas, CNPq, Ananindeua, Pará, Brazil

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ABSTRACT

The aim of this study was to determine the prevalence and to identify the risk factors associated with *T. gondii* infection in pregnant individuals living in the Ponta de Pedras municipality, Marajó Archipelago, State of Pará, where an outbreak of toxoplasmosis occurred in 2013. From 2014 to March 2017, a cross-sectional study was conducted, including 555 pregnant individuals aged 13- to 42-years-old. Serological tests (enzyme immunoassays) were performed, and socioenvironmental and behavioral information were obtained through the application of a questionnaire. A prevalence of 68.3% was detected, and older age, having contact with soil and living in an urban area were the risk factors associated with seropositivity. The study confirmed the high prevalence of infection among pregnant individuals in the region. The association of the infection with the variables of residential area and contact with soil indicates that there was environmental contamination by *T. gondii* oocysts in the municipality.

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1. Introduction

Toxoplasmosis is a zoonotic infection caused by *Toxoplasma gondii*, a protozoan with a worldwide distribution. Humans can become infected by the ingestion of raw or undercooked meat containing tissue cysts or the consumption of food or water contaminated with sporulated oocysts.

Epidemiological studies conducted in different countries have shown that the prevalence of infection can vary among continents. In Estonia and Germany, the prevalence was around 55% (Wilking et al., 2016; Lassen et al., 2016), in China, from 3.4%

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to 17.5% (Pan et al., 2017), in the United States of America, it was around 11.4% (Jones et al., 2018), while in Brazil, is high, ranging from 52.6% to 72.3% (Costa et al., 2018; Prestes-Carneiro et al., 2013).

In general, toxoplasmosis is asymptomatic or presents moderate symptoms with a benign evolution that are self-limiting. Severe manifestations are mainly common in immunocompromised individuals, newborns with a congenital infection or immunocompetent individuals infected with an atypical parasite and/or virulent strains (Demar et al., 2012).

Congenital transmission occurs when tachyzoites from the maternal circulation infect the fetus, which can lead to abortion or severe ocular or neurological lesions that are manifested at birth or throughout the individual's life. Dubey et al. (2012) (Dubey et al., 2012) reported that in Brazil, approximately 35% of the children affected by congenital toxoplasmosis presented neurological problems, including hydrocephalus, microcephaly, mental retardation and ocular lesions.

The incidence of congenital toxoplasmosis varies from 0.5 to 3.4 cases per 1000 live births in some regions of Europe and South America, respectively (Torgerson and Mastroiacovo, 2013). In Brazil, investigations based on neonatal screening performed in some states, such as Mato Grosso do Sul, Pará and Rondônia, have shown that this rate ranges from 0.2 to 2 cases per 1000 neonates (Bichara et al., 2012; Neto et al., 2010).

Considering the clinical importance of congenital toxoplasmosis, determining the serological profile of pregnant individuals and the factors that may be related to the occurrence of cases is important for defining strategies for the prevention and control of gestational/congenital toxoplasmosis.

In 2013, an outbreak of toxoplasmosis was reported in the Ponta de Pedras municipality in Brazilian Amazon, which included 90 individuals aging from 11 months to 64 years old, infection on pregnant individuals and ocular and multivisceral toxoplasmosis were not detected. The toxoplasmosis cases were associated to the ingestion of açaí (*Euterpe oleracea*) juice, widely consumed in the region (Morais et al., 2017). The epidemiological surveillance of toxoplasmosis was proposed due to the risk of infection and the possibility of the circulation of virulent strains in the region. Other outbreaks have been reported in Brazil (Silva et al., 2019; Almeida et al., 2011) and also in northern Brazil (da Saúde, 2011; Carmo et al., 2010) and in Amazon region (Demar et al., 2007), reinforcing the concern with the circulation of virulent lineages in the region, as there are reports of persistence of symptoms, severe manifestations and death.

Thus, the present study is an epidemiological study conducted following the outbreak and aimed to determine the seroprevalence and to identify the risk factors associated with *T. gondii* infection in study participants.
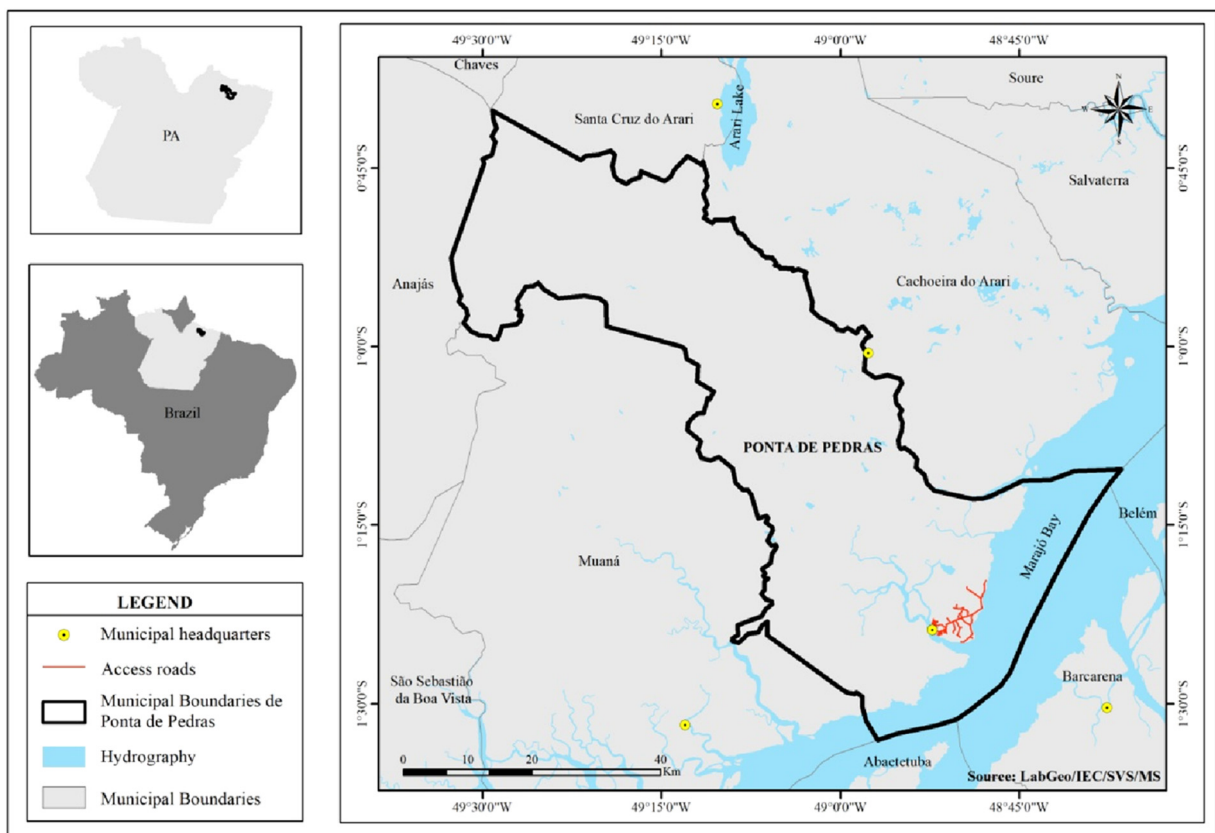

Fig. 1. Map of the Ponta de Pedras municipality, State of Pará, Brazil.

2. Methods

This study was approved by the Ethics Committee of the Evandro Chagas Institute on August 26, 2014 (CAAE 1630514.3.0000.0019/No. 764.785). The sample collection and technical procedures were only initiated after the adult participants had signed an informed consent form and for the children group their parents or guardians signed, in accordance with the Brazilian regulations.

2.1. Study area

The study was carried out in the Ponta de Pedras municipality (01°23″42′S, 48°52″18′W), located in the Marajó Archipelago, State of Pará, Brazilian Amazon (Fig. 1). The municipality has an area of 3,365,148 km², and its territory is divided into areas of lowland and solid ground. The climate is generally hot and humid, with an average annual temperature of 27 °C and an average rainfall of 3000 mm per year. The estimated residential population is 25,999 inhabitants, 49% living in an urban area, and only 20% of the population has adequate sanitation and sewage conditions (Instituto Brasileiro de Geografia e Estatística, 2010; Lima et al., 2017).

2.2. Study design and samples

Considering the number of pregnant individuals seen at the Prenatal Care Program of the Ponta de Pedras municipality (300/year), seroprevalence of 65% (Ferreira et al., 2009), the adopting of a confidence level of 95%, margin of error of 5% and a nonadherence to the study of 10%, a minimum sample of 157 pregnant individuals was calculated by the program Epi Info version 7.2.2.1-Centers for Disease Control and Prevention (CDC).

From August 2014 to March 2017, a cross-sectional study was conducted, including 555 pregnant individuals seen at the Prenatal Care Program at any gestational period. Approximately 5 mL of venous blood was collected from each pregnant individual. Serum aliquots were obtained by centrifugation and stored at −20 °C until the serological tests were performed.

2.3. Questionnaire

The pregnant individuals answered a structured epidemiological questionnaire covering categorical variables as the place of residence, the type of water consumed, the consumption of raw/undercooked meat, the consumption of fruits and vegetables, contact with soil (occupational activity with land handling, cultivating home garden, cleaning yard), contact with animals, age group, gestational age and prior knowledge on toxoplasmosis transmission (consumption of raw or undercooked meat, consumption of raw vegetables or contaminated water, contact with materials potentially contaminated with cat feces).

2.4. Serological analysis

Serum samples were tested for anti-*T. gondii* IgG and IgM antibodies by indirect and immunocapture immunoenzymatic assay (ELISA) (Symbiosis Diagnóstica Ltda., Leme, Brazil), respectively, following the procedures and by calculating the cut-off point according to the manufacturer's recommendations. In addition to the positive and negative controls available in the kit, laboratory reference controls were included.

2.5. Statistical analysis

To evaluate the prevalence and risk factors associated with *T. gondii* seropositivity, descriptive and inferential statistical methods were used. To identify the risk factors, a bivariate analysis (chi-square test) was initially used. The variables that had a p-value < 0.2000 in the bivariate analysis were selected for analysis with a multivariate logistic regression model. The logistic regression was performed with the stepwise forward method and the factors that had showed association with the dependent variable (positive for anti-*T. gondii* IgG antibodies) were identified during the succession of steps. The data were analyzed with two statistical programs, BioEstat program version 5.3 and Epi Info version 7.2.2.1.

Table 1
Serological profile for anti-*T gondii* IgG and IgM antibodies in 555 pregnant individuals seen at the Prenatal Care Program of the Ponta de Pedras municipality, Pará, Brazil, from August 2014 to March 2017.

Serological profile	AF	RF (%)	95% CI
IgG positive/IgM negative (chronically infected)	374	67.4	63.4–71.2
IgG negative/IgM negative (seronegative)	176	31.7	28.0–35.7
IgG positive/IgM positive (possible acute/recent infection)	05	0.9	0.39–2.1
Total	555	100	–

AF: absolute frequency; RF: relative frequency; CI: confidence interval.

Table 2

Bivariate analysis between the studied variables and anti-*T. gondii* IgG seropositivity of pregnant individuals seen at the Prenatal Care Program of the municipality of Ponta de Pedras, Pará, Brazil, from August 2014 to March 2017.

Variables	N	IgG positive	%	OR (CI 95%)	p-Value
Residential area					
Urban	364	258	70.9	1.4 (1.0–2.0)	0.0864
Rural	191	121	63.4		
Lived in a riverside area					
Yes	147	97	66.0	0.8 (0.6–1.3)	0.5511
No	408	282	69.1		
Age group					
13–20	248	146	58.9	Ref.	<0.0001
21–30	242	180	74.4	2.0 (1.4–3.0)	
>30	65	53	81.5	3.1 (1.6–6.1)	
Gestational age					
1° trimester	295	204	69.2	Ref.	0.6324
2° trimester	226	150	66.4	0.88 (0.6–1.3)	
3° trimester	34	25	73.5	1.2 (0.5–2.8)	
Consumed well water					
Yes	141	95	67.4	0.9 (0.6–1.4)	0.8691
No	414	284	68.6		
Consumed river water					
Yes	93	57	61.3	0.7 (0.4–1.1)	0.1423
No	462	322	69.7		
Drank filtered or boiled water					
Yes	184	127	69.0	1.0 (0.7–1.5)	0.8901
No	366	249	68.0		
NR	5	3	60.0		
Consumed raw/undercooked meat					
Yes	166	107	64.5	0.8 (0.5–1.1)	0.2432
No	389	272	69.9		
Consumed hunted meat					
Yes	317	219	69.1	1.1 (0.7–1.5)	0.7730
No	228	154	67.5		
NR	10	6	60		
Washed fruits/vegetables					
Yes	496	338	68.1	0.8 (0.3–2.1)	0.7963
No	19	14	73.7		
NR	40	27	67.5		
Consumed açaí juice					
Yes	542	371	68.4	1.3 (0.4–4.2)	0.8199
No	13	8	61.5		
Consumed fruit pulp					
Yes	494	335	67.8	0.7 (0.3–1.6)	0.5155
No	32	24	75.0		
NR	29	20	69.0		
Cats in the house					
Yes	224	159	71.0	1.2 (0.8–1.8)	0.3035
No	331	220	66.5		
Contact with cats					
Yes	326	226	69.3	1.1 (0.8–1.6)	0.5936
No	229	153	66.8		
Dogs with free access to street					
Yes	289	205	70.9	1.3 (0.9–1.9)	0.1265
No	250	161	64.4		
NR	16	13	81.2		
Soil contact					
Yes	201	149	74.1	1.5 (1.1–2.3)	0.0329
No	354	230	60.7		
Gets into the forest/woods					
Yes	223	157	70.4	1.3 (0.9–1.8)	0.2690
No	280	183	65.4		
NR	52	39	75		
Prior knowledge on toxoplasmosis transmission					
Yes	78	59	75.6	1.5 (0.8–2.5)	0.2196
No	449	305	67.9		
NR	28	15	53.6		

n: number; OR: odds ratio; CI: confidence interval; Ref.: reference; NR: no response.

3. Results

The age range of the 555 studied pregnant individuals was 13 to 42 years, with a mean age of 22.6 ± 6.0 years. Regarding the serological analysis, 374 pregnant individuals were chronically infected, 176 seronegative, and 5 presented a profile suggestive of an acute/recent infection (Table 1).

Of the latter group, three were in the first gestational trimester, and two were in the second gestational trimester, and all were referred to the referral hospital located in the municipality of Belém. IgG antibodies were detected in 379 pregnant individuals, resulting in a prevalence of 68.3% (379/555; 95% CI: 64.4–72.2%).

The majority of the pregnant individuals were aged between 13- and 20-years-old (44.7%, 248/555), living in an urban area (65.6%, 364/555), and had no prior knowledge about toxoplasmosis transmission (85.2%, 449/527). In regard to the gestational period, 295 (53.2%) of the pregnant individuals were in the first trimester, 226 (40.7%) were in the second trimester and 34 (6.1%) were in the third trimester.

In the bivariate analysis, a statistically significant difference was observed in IgG seroprevalence by age group ($p < 0.0001$) as well as by whether the individual reported contact with soil. The estimated seroprevalence for each age group showed that 58.9% (146/248) of the pregnant individuals aged 13 to 20 years and 81.5% (53/65) over 30 years had already been exposed to *T. gondii*. This group of pregnant individuals had higher odds to be seropositive than the ones aged 13 to 20 years (OR: 3.1; 95% CI: 1.6–6.1), and those who had soil contact in relation to those who did not (OR: 1.5; 95% CI: 1.0–2.3) (Table 2).

In the multivariate analysis, the association of the seroprevalence of *T. gondii* infection was analyzed with respect to the variables age, residential area, consumed river water, dogs with access to the street and contact with soil. The final logistic regression model identified older age ($p < 0.0001$), urban residential area ($p = 0.026$) and reported contact with soil ($p = 0.0085$) as risk factors (Table 3).

4. Discussion

The prevalence of anti-*T. gondii* antibodies in pregnant individuals was 68.3%, a little lower to the ones already reported in Amazon region populations (Bóia et al., 2008; Silva et al., 2015) and higher to those registered in pregnants from Salvador, State of Bahia (51%) (Avelar et al., 2017), Divinópolis, Minas Gerais State (38%) (Nascimento et al., 2017) and Lages, Santa Catarina State (16%) (Quadros et al., 2015).

The prevalence of toxoplasmosis in pregnant individuals is very variable, ranging from below 5% in China (Deng et al., 2018) to above 80% in the Democratic Republic of Congo (Doudou et al., 2014); in Brazil, Dubey et al. (2012) (Dubey et al., 2012) showed that there was a range of 50% to 80%, according to the region of origin. This fact may be related to the cultural differences, population habits and climatic conditions of the regions. In the Amazon region, the hot and humid climate favors the sporulation and survival of oocysts that are easily disseminated in the environment (Carmo et al., 2016).

It is very important to determine the baseline seroprevalence, since in the areas of high prevalence the chance of a seronegative individual acquiring an infection during pregnancy is very high (Torgerson and Mastroiacovo, 2013). In this study, 31.7% of the pregnant individuals were seronegative (IgM and IgG nonreactive) and thus were at risk of acquiring an infection and transmitting it to the fetus.

In relation to trimester of gestation, 46.8% (260/555) were in the second and third trimesters of gestation. In Brazil, it is recommended that the serological test be performed at the beginning of gestation, and if a susceptibility profile is detected, the test must be performed again in the 2nd and 3rd gestational trimesters. These measures aim to diagnose and treat the infection without a long delay to minimize the risk of fetal infection. According to Viellas et al. (2014), 77.4% of pregnant individuals in the northern region of Brazil started prenatal care at <13 weeks of gestation (first trimester); thus, the Ponta de Pedras municipality is not in line with the regional average and urgently needs to improve the health care of pregnant individuals.

As for the risk factors, *T. gondii* infection was associated with age ($p < 0.0001$), as two age ranges, 21 to 30 and >30 years of age, had the highest percentage of seropositive pregnant individuals, 74.4% and 81.5%, respectively. Age is related to a greater chance of acquiring the infection due to exposure. The same association was observed in studies conducted in Salvador (Bahia state, Brazil) (Avelar et al., 2017), Fortaleza (Ceará state, Brazil) (Sroka et al., 2010) and Umuarama (Paraná state, Brazil) (Caetano et al., 2017). In Amazonas and Tocantins states (Brazil), although age was not a factor associated with *T. gondii* infection, a seropositivity increase was observed as the age increased (Rocha et al., 2015; Vitaliano et al., 2015).

In the studied municipality, the percentage of seronegative pregnant individuals (31.7%, 176/555) is worrisome, especially combined with the fact that 80.9% of the patients reported a lack of prior knowledge about toxoplasmosis transmission

Table 3

Final multiple logistic regression model between the studied variables and anti-*T. gondii* IgG seropositivity of pregnant individuals seen at the prenatal care program of the Ponta de Pedras municipality, Pará, Brazil, from August 2014 to March 2017.

Variables	OR	CI 95%	p-Value
Age (≥21 years)	2.2	1.5–3.2	<0.0001
Urban residential area	1.5	1.1–2.7	0.0260
Contact with soil	1.7	1.1–2.5	0.0085

OR: odds ratio; CI: confidence interval.

mechanisms, highlighting the importance of education in the prevention of maternal infection and thus reducing exposure to risk factors (Lopes-Mori et al., 2011).

The statistical analysis results indicated the association of infection with soil contact and living in urban areas. In Burkina Faso and Sri Lanka, an association of infection with living in urban areas was also observed, which was justified by the urbanization process of developing countries associated with poverty, overpopulation, unsanitary conditions and inadequate water supply system (Bamba et al., 2017; Iddawela et al., 2017). However, in other Brazilian areas, it has been observed that residing in rural areas is a risk factor for *T. gondii* infection, suggesting that low socioeconomic status, difficulty in accessing health services, high exposure and little knowledge about disease transmission result in a high prevalence (Avelar et al., 2017; Lopes-Mori et al., 2013). A further association was found between being seropositive and having contact with soil, which demonstrates that there is a contamination of the municipality soil by oocysts of *T. gondii*, which are released by felines.

Although no association between presence of cats and infection was detected, the frequency of this animal in urban area can support the association of infection and living in an urban area. In Brazil, this association was found in Paraná state (Caetano et al., 2017) but not in Ilhéus (Bahia state) (Costa et al., 2018). Canatto et al. (2012) (Canatto et al., 2012) and Hanmer et al. (2017) (Hanmer et al., 2017) had showed that the density of domestic cats is higher in urban areas and consequently the level of *T. gondii* oocyst contamination is also higher.

In this present study, transmission proves to be caused mainly by oocysts, which reveals the high dispersion of this infective form in the municipality. This is consistent with the previous outbreak in with the source of infection was açaí juice. Studies conducted in Haiti (Caribbean) and Chile (South America) had showed that environmental conditions can favor the spread of oocysts and ingestion in the first years of life (Demar, 2001; Munoz-Zanzi and Campbell, 2016).

It is important to note that residing in riverside communities was not associated with *T. gondii* infection. In these locations, persistent rain, especially in the months from December to May, cause river flooding, which makes it difficult to raise animals in the peridomicile, including domestic cats, and dilutes environmental contamination, reducing the probability of contamination by oocysts of this area (Vitaliano et al., 2015). On the other hand, the annual cycle of flooding and drought in the region can promote the dispersion of oocysts released by domestic or wild cats directly into the flow of the river water.

Although untreated water consumption was not an associated risk factor, river contamination with *T. gondii* oocysts is a possibility, as it was already described in studies involving aquatic mammals in the region (Santos et al., 2011). Another important fact is that the possibility of reinfection by sylvatic strains has already been suggested (Elbez-Rubinstein et al., 2009), which shows the need for the implementation of primary prevention measures, including among pregnant individuals who have been infected previously.

Studies on different sentinel species such as chicken, wild boar, wild cervids and shellfish have been conducted in different countries (Marangi et al., 2015; Witkowski et al., 2015). In the Brazilian Amazon, studies with poultry and synanthropic wild animals, especially small rodents, as well as water and food collected within the forest (such as açaí) could provide important information regarding environmental contamination and circulating strains in the region, since the risk factors identified in this study indicate that there is environmental contamination with oocysts, which probably use the same route of infection in humans and animals.

5. Conclusions

High seropositivity for *T. gondii* infection was observed in pregnant individuals from the Ponta de Pedras municipality, and the associated risk factors were older age, living in an urban area and having soil contact. These results demonstrate the need for the screening and monitoring of pregnant individuals in the studied municipality for *T. gondii* infection to prevent gestational/congenital toxoplasmosis.

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