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Major Article

Frequency and factors associated with *Toxoplasma gondii* infection in pregnant women and their pets in Ilhéus, Bahia, Brazil

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Abstract

Introduction: Toxoplasmosis is an asymptomatic disease that can lead to systemic disease in the fetus of pregnant women with primary infection. This study aimed to determine the prevalence of toxoplasmosis, associated factors, and correlation between the serology of pregnant women and their pets, in the municipality of Ilhéus, Bahia, Brazil. **Methods:** This cross-sectional study was conducted in 196 pregnant women and their cats or dogs (n=89). Semi-structured interviews were conducted and serum samples from the pregnant women were tested to detect IgM and IgG antibodies against *Toxoplasma gondii,* and avidity tests were performed for IgM-positive samples. The serum collected from pets were tested for IgG antibodies, and IgM antibodies in cats. A non-conditional logistic regression analysis was performed to identify infection-associated factors. **Results:** IgG and IgM antibodies were detected in 67.9% (133/196) and 1.5% (3/196) samples, respectively, for women with an avidity of over 60%. Age ≥ 25 and the presence of cats in the vicinity were found to be associated with infection, while the level of education and previous orientation toward prevention of toxoplasmosis were protective factors in pregnant women. IgG antibodies were detected in 46.1% (41/89) of the animals, and cats were found to be negative for IgM. For the animals, age ≥ 1 year was a factor associated with infection. There was no correlation between serology of the pregnant women and the animals (p=0.15). **Conclusions:** An elevated prevalence of toxoplasmosis was detected in the region. Therefore, the adoption of preventive measures by public healthcare bodies is recommended.

Keywords: *Toxoplasma gondii*. Gestation. Risk factor. Diagnosis.

INTRODUCTION

Toxoplasmosis is a zoonosis of great importance to both human health and veterinary medicine. It is caused by the protozoan *Toxoplasma gondii,* which uses Felidae as its definitive host and other warm-blooded animals as its intermediary hosts[1]. *Toxoplasma gondii* infection in humans and carnivorous animals occurs through various mechanisms such as through the ingestion of sporulated oocysts in food, soil, or water, through the consumption of viable parasite cysts present in raw or rare meat, or through trans-placental infection[2,3].

The majority of human and animal *T. gondii* infections are asymptomatic[3], and only non-specific clinical signs of toxoplasmosis are usually present. Immunocompromised humans or animals may develop ophthalmic[4] or neurological alterations[5], while primary infection in pregnant women may lead to infection in the fetus, with the appearance of microcephaly[6], inflammatory lesions with permanent neurological damage[7], or fetal death. The effect of infection

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on the fetus or the newborn child depends on the stage of pregnancy at which the infection occurred. If infection occurs in the first trimester, the newborn child may present serious signs of toxoplasmosis such as hydrocephaly, or depending on the age of the fetus, more extreme consequences that may lead to abortion or stillbirth[8]. Subclinical infection is commonly observed for infection occurring in the final trimester, which can be confirmed by serology[9].

Toxoplasma gondii is a cosmopolitan pathogen; hence, there might be a correlation between the serology of humans and their domesticated animals (cats or dogs), suggesting a common point of infection[10]. Therefore, understanding the factors associated with infection in a specific population is extremely relevant, since not all forms of parasite transmission are equally important in the epidemiology of a disease, in different locations or cultural groups[11].

Asymptomatic evolution of toxoplasmosis, which is observed in the majority of the population, makes it a highly neglected disease. The public must therefore be made aware of preventative actions such as washing hands after handling mud, washing raw vegetables before eating, avoiding eating raw or rare meat[12], and serological monitoring of pregnant women during the prenatal period[13]. The Ministry of Health recommends the use of spiramycin immediately after diagnosis in pregnant women, although diagnosis after the 30th week requires administration of pyrimethamine, sulfadiazine or folinic acid[14], due to the inherent risks caused to the fetus by the infection.

The aim of the present study is to determine the prevalence of factors associated with toxoplasmosis in pregnant women treated at Basic Healthcare Units in Ilhéus, in the state of Bahia, Brazil, and in their respective domesticated animals. Additionally, we also aimed to verify the correlation between the serology of the pregnant women and that of their pet animals (cats and dogs).

METHODS

Study design and ethical considerations

This was a cross-sectional study comprising 196 pregnant women who were being treated at Basic Healthcare Units (BHU), as well as 61 dogs and 28 cats. The research project was approved by the Santa Cruz State University Ethics Committee for Research in Human Beings (CAAE 15703113.5.0000.5526) and the Ethics Committee for the use of Animals (CEUA/UESC 09/2013). The blood samples were collected between February and December 2017, in the municipality of Ilhéus (latitude 14º47"S; longitude 39º02"W), which has a population of 184,236[15] and an area of 1,584.693 km² [16].

Inclusion criterion for participation in the study was pregnancy in any gestational trimester. Consent was obtained from pregnant women who were attending the healthcare service. Participants of the study signed Informed Consent Forms (ICF) in duplicates, one of which was to be returned to the participant. In case the participating pregnant woman was under the age of 18, an Informed Assent Form (IAF) was signed by the participant and an ICF by her guardian.

Samples and data collection

Blood samples were collected from pregnant women in different gestational periods from 14 BHUs, belonging to the Teotônio Vilela, Basílio, Hernani Sá, Salobrinho, Nossa Senhora da Vitória, Conquista, Iguape, and Malhado neighborhoods. An 8 mL sample of blood was collected by puncturing the cephalic vein and half the sample was stored in tubes without EDTA to obtain the serum for serological analysis. The remaining blood was stored in tubes with EDTA for molecular identification of the parasite. Blood samples were also collected from cats and dogs living with the pregnant women participating in the study. A 5 mL sample of blood was collected by puncturing the cephalic or jugular vein. Two milliliters of blood were stored without EDTA to obtain serum and the remaining sample was stored with EDTA for molecular identification of the parasite.

A semi-structured interview was conducted with the participants to monitor their dietary, cultural habits and socioeconomic characteristics which could be used to identify factors associated with *T. gondii* infection. Following the interview, blood samples were collected from the participants and their pets. A similar interview was conducted regarding the handling and habits of the pets from which the blood was collected.

Serology for *Toxoplasma gondii*

The sera from the pregnant women were tested using the IgG/IgM detection kit against *T. gondii*, based on a chemiluminescence immunoassay (CLIA) using Architect (Abbott®) technology. An avidity test for IgG antibodies was also conducted for IgM positive samples using a chemiluminescent microparticle immunoassay. The samples were collected every three months until the end of the gestation period, including from those participants who presented with an initial negative serology result but were interested in proceeding with a follow-up.

The animal sera were tested through an indirect fluorescent antibody test (IFAT) for the detection of IgG antibodies, with a cut-off point of 1:16 for dogs[17,18] and 1:64 for cats[19,20]. Furthermore, a cut-off point of 1:16 was used for the detection of IgM antibodies in cats[21]. The antigens for IFAT were produced using tachyzoites of the RH strain of *T. gondii,* maintained in cell cultures. The anti-dog (Anti-Dog IgG -F7884, Sigma-Aldrich®) and the anti-cat IgG FITC-conjugated antibodies (Anti-Cat IgG-F4262, Sigma-Aldrich®) were used with a conjugate dilution of 1:32 for dogs and 1:128 for cats. The anti-cat IgM FITC-conjugated antibody (Anti-Feline IgM heavy chain specific-VRMD®) was also used according to the manufacturer's protocol. An epifluorescence microscope (OLYMPUS, BX51®) was used to conduct the measurements. Samples with complete fluorescence around the tachyzoites were considered positive. The positive and negative controls were obtained from previous studies in the region[22,23].

Extraction of genomic DNA

The blood samples were stored at -20 ºC until genomic DNA extraction using a commercial kit (PureLink® Genomic DNA kit, Invitrogen®), following manufacturer's protocol.

DNA was extracted only from IgG positive (human and pets) blood samples. The extracted DNA was quantified using spectrophotometry (NanoDrop®) and then stored at -20°C until polymerase chain reaction (PCR) was performed.

Polymerase chain reaction

The following protocol[24] was used for the amplification of *T. gondii* DNA: Tox4 (CGCTGCAGGGAGGAAGACGAAAGTTG) and Tox5 (CGCTGCAGACACAGTGCATCTGGATT) primers were used to amplify a 529 bp fragment (GeneBank N0 AFI46527) from different regions of the *T. gondii* genome. The reaction mix contained 5 µL extracted DNA, added to 20 µL of a mixture of 0.5 µM of each primer (Invitrogen®), 0.2 mM of each dNTP (Invitrogen®), 10× PCR buffer (200 mM Tris-HCl (pH 8.0), 500 mM KCl), 1.5 mM of $MgCl_2$, and 1.25 U of Taq DNA polymerase (Invitrogen®), and ultra-pure water for a total volume of 25 µL. A 7 min cycle at 94 °C was used for denaturation followed by 33 1 min cycles at 94 °C for further denaturation, 1 min at 55 °C for annealing and 1 min at 72 °C for extension, followed by a final extension of 10 mins at 72 °C. The PCR products were subjected to electrophoresis on a 2% agarose gel and stained with SYBR safe DNA loading dye (Invitrogen®). Ultra-pure water was used as a negative control.

Data tabulation and analysis

The following variables were used for statistical modelling of the data obtained from the participants: age (< 25 years old / ≥ 25 years old), BHU (urban/peri-urban or rural area), level of education (primary school/secondary school or further education), monthly family income (≤ 1 minimum salary/ > 1 minimum salary), origin of drinking water (public network or mineral/other sources: well, mine or river/stream), sewage destination (public network/others: cesspit, open air or river/ stream), garbage destination (public collection/wasteland or yard), presence of wasteland close to home (yes/no), flooded areas close to home (yes/no), having a vegetable garden at home (yes/no), if the participant prepares the food (yes/no), presence of rodents (yes/no), presence of cats in the residence (yes/no), number of cats (one/ two or more), age of the cat(s) (≤ 1 year/ > 1 year), whether the cat leaves the house/apartment (yes/no), whether the cat is fed raw or rare meat (yes/no), presence of dogs in the house/apartment (yes/no), handling of sand/mud (yes/no), fishing or swimming habits (yes/no), consumption of meat (yes/no), consumption of raw or rare meat (yes/no), the kind of raw meat consumed (beef/others: pork, lamb/mutton or chicken), consumption of raw kibbeh (yes/no), consumption of oysters (yes/no), consumption of rare barbecued meat (yes/ no), washing the meat board before using it to chop vegetables (yes/no), washing the meat board with soap and water (yes/no), consumption of fruit (yes/no), consumption of raw vegetables (yes/no), consumption of farm milk (yes/no), boiling milk (yes/no), origin of the milk (cow/goat), consumption of fresh cheese (yes/no), if the participant has ever lived on a ranch/ smallholding/farm (yes/no), if the participant has ever assisted in animal births (yes/no), if the participant has ever assisted in the slaughter of cows/pigs/sheep/goats (yes/no), if the participant has ever had a blood transfusion (yes/no), if the participant has received previous orientation on toxoplasmosis (yes/no). The variable presence of IgG antibodies against *T. gondii* was considered the outcome variable.

For the purposes of statistical modelling, the variables for the domestic animals were characterized as follows: age of the animal (≤ 1 year/ > 1 year), sex (female/male), leaves the house/apartment (yes/no), alimentation (kibble/kibble and food or only food), fed with raw or rare meat (yes/no), origin of drinking water (public network or mineral/ other sources: well, mine or river/stream), and hunting (yes/no). The variable presence of IgG antibodies against *T. gondii* was considered the outcome variable.

The data from the epidemiological investigation of the participants and their animals were entered into the EPI INFO 3.5.1® statistical package and analyzed using the chi-squared statistical test with Yates correction or Fisher's exact test. The variables with a *p*-value ≤ 20% were selected and subjected to collinearity analysis using the Spearman correlation coefficient, employing the Bioestat 5.0® statistics program, and an initial logistic regression model was constructed. The final model was constructed by removal of the variables (backward model) in accordance with *p*-values adjusted by the Hosmer & Lemshow test. The significance level for a variable to remain in the final model was 5%.

To verify the serological correlation of the participant and their domestic animals, a Spearman correlation coefficient was performed using the Bioestat 5.0® statistical program.

RESULTS

Among the 196 participants in the study, 67.9% (133/196 IC: 60.8-74.3%) showed prevalence of IgG antibodies against *T. gondii* and 1.5% (3/196 IC: 0.3-4.4%) showed prevalence of IgM antibodies. All the participants with positive IgM serological results showed >60% avidity for IgG antibodies and had their blood first collected in the third trimester of pregnancy. None of the IgG positive samples showed the presence of parasite DNA in the blood.

Among the 63 participants who showed negative IgG/IgM results, 45 were in the 1st and 2nd trimesters of gestation and 18 among these (40%) consented to being monitored until the end of the gestation period. None of these women showed positive results in the remaining monitoring period.

Tables 1 and **2** present all the variables of the bivariate analysis. In the final logistic regression model (**Table 3**), it can be observed that age >25 and the presence of cats in the residence were factors associated with infection, while having a higher level of education (secondary school or further education) and receiving previous orientation on toxoplasmosis were factors associated with prevention.

A total of 55 pregnant women (55/196= 28%) had pet animals. Among the 89 samples from cats and dogs, 46.1% (41/89 IC: 35.4-57%) showed a prevalence of IgG antibodies against *T. gondii* with 50% (14/28 IC: 30.3-69.4%) being in cats and 44.3% (27/61 IC: 31.5-57, 6%) in dogs. No felines

TABLE 1: Bivariate analysis of socio-demographic characteristics associated with *Toxoplasma gondii* infection in pregnant women treated at BHUs in the municipality of Ilhéus, Bahia.

Variables	Pregnant women				Odds ratio (95% CI)	*P*
	Positives %		Negatives %			
Age						
≥ 25 years old	81	79.4	21	20.6	3.12 (1.66-5.84)	< 0.001
< 25 years old (Ref)	52	55.3	42	44.7		
BHU						
Urban	31	59.6	21	40.4	0.61 (0.31-1.18)	0.19
Peri-urban or rural area(Ref)	102	70.8	42	29.2		
Level of education						
Primary School (Ref)	57	78.1	16	21.9		
Secondary school + further education	76	61.8	47	38.2	0.45 (0.23-0.88)	0.03
Monthly family income						
≤ 1 minimum salary (Ref)	90	68.7	41	31.3		
> 1 minimum salary	43	66.2	22	33.8	0.89 (0.47-1.68)	0.84
Origin of drinking water						
Public network or mineral (Ref)	124	69.3	55	30.7		
Other sources: well, mine or river/stream	9	52.9	8	47.1	0.5 (0.18-1.36)	0.27
Sewage destination						
Public network	46	69.7	20	30.3	1.14 (0.6-2.1)	0.82
Others: cesspit, open air or river/stream (Ref)	87	66.9	43	33.1		
Garbage destination						
Public collection (Ref)	126	68.1	59	31.9		
Wasteland or yard	7	63.6	4	36.4	0.82 (0.23-2.91)	0.98
Presence of wasteland close to home						
Yes	70	70.7	29	29.3	1.30 (0.71-2.38)	0.47
No (Ref)	63	64.9	34	35.1		
Flooded areas close to home						
Yes	22	56.4	17	43.6	0.54 (0.26-1.10)	0.13
No (Ref)	111	70.7	46	29.3		
Having a vegetable garden at home						
Yes	23	67.6	11	32.4	0.99 (0.45-2.18)	0.86
No (Ref)	110	67.9	52	32.1		
If the participant prepares the food						
Yes	120	71.4	48	28.6	2.88 (1.28-6.51)	0.016
No (Ref)	13	46.4	15	53.6		
Presence of rodents						
Yes	77	71.3	31	28.7	1.42 (0.78-2.59)	0.32
No (Ref)	56	63.6	32	36.4		
Presence of cats in the residence						
Yes	113	71.5	45	28.5	2.26 (1.10-4.66)	0.04
No (Ref)	20	52.6	18	47.4		
Number of cats						
One (Ref)	24	72.7	9	27.3		
Two or more	16	76.2	5	23.8	1.20 (0.34-4.24)	0.97
Age of the cat(s)						
≤ 1 years (Ref)	16	76.2	5	23.8		
> 1 years	22	73.3	8	26.7	0.86 (0.24-3.12)	0.92
Whether the cat leaves the house/apartment						
Yes	28	71.8	11	28.2	0.70 (0.16-2.97)	0.89
No (Ref)	11	78.6	3	21.4		
Whether the cat is fed raw or rare meat						
Yes	13	86.7	2	13.3	3.12 (0.60-16.09)	0.28
No (Ref)	25	67.6	12	32.4		
Presence of dogs in the house/apartment						
Yes	51	63.0	30	37.0	0.68 (0.37-1.25)	0.28
No (Ref)	82	71.3	33	28.7		
If the participant has ever had a blood transfusion						
Yes	3	50.0	3	50.0	0.46 (0.09-2.35)	0.61
No (Ref)	130	68.4	60	31.6		
If the participant has received previous orientation on toxoplasmosis						
Yes	28	54.9	23	45.1	0.46 (0.24-0.90)	0.03
No (Ref)	105	72.4	40	27.6		

CI: confidence interval; **Ref:** reference; **BHU:** Basic Healthcare Unit.

TABLE 2: Bivariate analysis of eating habits and behaviors associated with *Toxoplasma gondii* infection in pregnant women treated at BHUs in the municipality of Ilhéus, Bahia.

Variables	Pregnant women				Odds Ratio (95% CI)	*P*
	Positives	%	Negatives	%		
Handling of sand/mud						
Yes	26	78.8	7	21.2	1.94 (0.79-4.76)	0.20
No (Ref)	107	65.6	56	34.4		
Fishing or swimming habits						
Yes	44	74.6	15	25.4	1.58 (0.80-3.13)	0.25
No (Ref)	89	65	48	35		
Meat eating						
Yes	132	68.4	61	31.6	4.33 (0.38-48.65)	0.50
No (Ref)	1	33.3	2	66.7		
Eating raw or rare meat						
Yes	34	66.7	17	33.3	0.90 (0.45-1.78)	0.89
No (Ref)	98	69.0	44	31		
The kind of raw meat eaten						
Beef (Ref)	27	65.9	14	34.1		
Others: pork. lamb/mutton or chicken	7	70.0	3	30.0	1.21 (0.27-5.41)	0.90
Eating raw kibbeh						
Yes	22	75.9	7	24.1	1.58 (0.64-3.94)	0.43
Não (Ref)	111	66.5	56	33.5		
Eating oysters						
Yes (Ref)	40	63.5	23	36.5		
No	93	69.9	40	30.1	0.75 (0.40-1.41)	0.46
Eating rare barbecued meat						
Yes	67	62.0	41	38.0	0.54 (0.29-1.01)	0.07
No (Ref)	66	75.0	22	25.0		
Washing the meat board before using it to chop vegetables						
Yes	128	68.4	59	31.6	1.74 (0.45-6.70)	0.66
No (Ref)	5	55.6	4	44.4		
Washing the meat board with soap and water						
Yes	108	69.7	47	30.3	1.38 (0.62-3.05)	0.56
No (Ref)	20	62.5	12	37.5		
Eating fruit						
Yes	130	67.7	62	32.3	0.70 (0.07-6.86)	0.82
No (Ref)	3	75.0	1	25		
Eating raw vegetables						
Yes	86	69.9	37	30.1	1.29 (0.69-2.38)	0.52
No (Ref)	47	64.4	26	35.6		
Drinking farm milk						
Yes	78	72.2	30	27.8	1.56 (0.85-2.85)	0.20
No (Ref)	55	62.5	33	37.5		
Boiling milk						
Yes	74	74.0	26	26.0	2.85 (0.66-12.21)	0.29
No (Ref)	4	50.0	4	50.0		
Origin of the milk						
Cow	77	72.6	29	27.4	2.65 (0.16-43.86)	0.93
Goat (Ref)	1	50.0	1	50.0		
Eating fresh cheese						
Yes	42	72.4	16	27.6	1.36 (0.70-2.66)	0.47
No (Ref)	91	65.9	47	34.1		
If the participant has ever lived on a ranch/ smallholding/farm						
Yes	64	69.6	28	30.4	1.16 (0.63-2.12)	0.74
No (Ref)	69	66.3	35	33.7		
If the participant has ever assisted in animal births						
Yes	3	75	1	25	1.33 (0.13-13.35)	0.75
No (Ref)	61	69.3	27	30.7		
If the participant has ever assisted in the slaughter of cows/pigs/sheep/goats						
Yes (Ref)	2	100.0	0	0		
No	62	68.9	28	31.1	0	0.87

CI: confidence interval; **Ref:** reference.

TABLE 3: Final non-conditional logistic regression model of factors associated with *T. gondii* infection in pregnant women treated at BHUs in the municipality of Ilhéus, Bahia.

Variable	Category	Odds ratio (95%CI)	*P*
Age	≥ 25	3.81 (1.94-7.48)	0.001
	< 25 (Ref)		
Level of education	Primary school (Ref)		
	Secondary school or Further education	0.43 (0.21-0.88)	0.02
Presence of cats in the residence	Yes	2.42 (1.10-5.31)	0.03
	No (Ref)		
In the participant has received previous orientation on toxoplasmosis	Yes	0.46 (0.22-0.95)	0.04
	No (Ref)		

Likelihood: 215.92; **CI:** confidence interval; **Ref:** reference.

showed positive serology for IgM antibodies against *T. gondii*. There was no correlation between the serological result of the human participants and that of their animals r=-0.15 (p=0.15). No serological-positive sample from animals had amplification of parasite DNA in the blood.

Table 4 presents the variables of the bivariate analysis of the samples collected from the animals. In the final logistic regression model (**Table 5**), it can be observed that age ≥ 1 year was a factor associated with infection.

DISCUSSION

The elevated seroprevalence of toxoplasmosis found in the present study was similar to that identified in some regions of North-East Brazil[25,26,27]. Although previous studies have been conducted regarding the occurrence of toxoplasmosis in the state of Bahia[28,29], knowledge about the prevalence and risk factors for specific locations is necessary, as different factors may be associated with seropositivity in different areas. Understanding these differences would enable the development of more specific prevention and control measures[27,30]. There was no incidence of new cases among the pregnant women in the present study. During the process of obtaining informed consent, the study participants were informed of preventative and prophylactic measures. Therefore, it is possible that this knowledge prevented the occurrence of new cases. In fact, raising the public's awareness of this disease through the dissemination of informative material and lectures has been recommended in previously conducted longitudinal studies[31,32,33].

The serum samples of all the pregnant women with IgM-positive serology were tested for avidity to determine the period of *T. gondii* infection. However, as the participants were already in their third trimester, the result of the test enabled the

TABLE 4: Bivariate analysis of factors associated with *Toxoplasma gondii* infection in pet animals of pregnant women treated at BHUs in the municipality of Ilhéus, Bahia.

Variables	Animals (dog and cat)				Odds ratio (95% CI)	*P*
	Positives %		Negatives %			
Age of the animal						
≥ 1 year	29	60.4	19	39.6	3.69 (1.52-8.96)	0.003
< 1 year (Ref)	12	29.3	29	70.7		
Sex						
Female	18	55.0	22	45.0	0.92 (0.40-2.14)	0.98
Male (Ref)	23	46.9	26	53.1		
Leaves the house/apartment						
Yes	27	45.8	32	54.2	0.96 (0.40-2.33)	0.88
No (Ref)	14	46.7	16	53.3		
Alimentation						
Kibble (Ref)	8	53.3	7	46.7		
Kibble and food or only food	33	44.6	41	55.4	0.70 (0.23-2.14)	0.74
Fed with raw or rare meat						
Yes	20	57.1	15	42.9	2.1 (0.88-4.97)	0.14
No (Ref)	21	38.9	33	61.1		
Origin of drinking water						
Other sources: well. mine or river/stream (Ref)	2	40.0	3	60.0		
Public network or mineral	39	46.4	45	53.6	0.77 (0.12-4.84)	0.86
Hunting habits						
Yes	21	44.7	26	55.3	0.89 (0.38-2.05)	0.95
No (Ref)	20	46.6	22	52.4		

CI: confidence interval; **Ref:** reference.

TABLE 5: Final non-conditional logistic regression model of factors associated with *T. gondii* infection in pet animals of pregnant women treated at BHUs in the municipality of Ilhéus, Bahia.

Variable	Category	Odds ratio (95%CI)	*P*
Age of the animal	≥ 1 year	3.69 (1.52-8.96)	0.004
	< 1 year (Ref)		

Likelihood: 114.02; **CI:** confidence interval; **Ref:** reference.

conclusion that infection had not occurred in the past 4 months, as there was an increase in the affinity of specific IgG antibodies, characterized by a possible delayed or chronic infection (high avidity)[4]. Thus, these women were referred to the Reference Center of the municipality for follow-up of the newborn child's health. Trimestral serological follow-ups of the pregnant women initiated in the first trimester is essential, along with increased awareness, as it enables the performance of tests while the women are still in their early stages of pregnancy[34]. This would assist in early diagnosis of infection[35] and in determining if the infection occurred during pregnancy.

Risk factors identified in the present study indicate that advancing age leads to greater vulnerability of pregnant women to infectious forms of the parasite, corroborating previously reported findings[36,25]. Additionally, the presence of cats in the residence or in the surrounding neighborhood (strays) increases the likelihood of pregnant women to come in contact with sporulated oocysts present in the environment as observed in previous studies[37]. It is important to raise awareness regarding the necessary precautions when handling feline feces during gestation[26], especially when the serological status is unknown.

The level of education was also a relevant factor in infection as observed in the present study and others[38]. Previously it has been found that individuals with low levels of education (≤ 8 years of study) presented a higher prevalence for *T. gondii*, demonstrating the importance of education in informing the population of health risks[39].

Prior orientation for prevention of toxoplasmosis was considered a protective factor for pregnant women. Thus, training of healthcare professionals in informing the population of the risks, preventative actions, and means of toxoplasmosis transmission may lead to a reduction in disease prevalence. Raising awareness about the disease is essential for seronegative pregnant women and also for seronegative women who wish to get pregnant[40].

As expected, the blood samples of pregnant women that were seropositive were negative for PCR. Due to the low sensitivity of PCR[41], positive results are only obtained during the acute phase of the disease, when there is parasite dissemination in the blood[42].

The lack of association between seropositivity in pregnant women and behavioral habits such as handling sand, swimming or fishing, and eating fruits or raw vegetables, corroborated with previous findings[43]. Moreover, previous studies carried out in the same region with pigs[44], cows[45], sheep[46,47] oysters[48], cats[22], and dogs[23], corroborated the frequency of toxoplasmosis found in the present study among pets of pregnant women, emphasizing the risk to public health caused by the direct ingestion of oocysts or tissue cysts present in raw or rare meat.

The absence of correlation between the presence of antibodies against *T. gondii* in pregnant women and their respective animals (cats and dogs) indicated that the sources or time of infection are distinct in these populations, contrary to previous findings[10].

Despite the lack of seropositivity in the acute phase of infection and the lack of oocysts release in the environment, the high positivity of IgG antibodies against *T. gondii* in the animals up to 1 year post-infection and the absence of IgM antibodies against *T. gondii* in cats, which are definitive hosts of toxoplasmosis, suggests that the study participants were exposed to infectious forms of the parasite early on. In addition, infections in canines is of importance to public health, as it is described as a sentinel for environmental contamination, indicating a risk to humans[49].

During the study period, there were 496 pregnant women being treated in at least one of the 14 BHUs. Among these, 196 (39.52%) participated in this study. Participation of the pregnant women in this present study was low, despite extensive promotion of this project through posters, manuals, and informative folders in the BHUs. Additionally, information about the project was passed on to the pregnant women by nurses, during monthly prenatal assessment, and by community health agents during home visits, along with the use of audio communication through cars with loud speakers, emphasizing the importance of the disease to the health of the pregnant woman and her child, and the importance of promoting general health in the community. It is possible that the low participation is a result of a lack of knowledge regarding toxoplasmosis and its consequences, especially for the fetus.

In this context, the implementation of a pregnancy follow-up program and inclusion of serology for toxoplasmosis as a routine prenatal exam led to a decrease in the prevalence of the disease in the city of Rolândia, in Paraná[38], and a consequent reduction in the treatment of pregnant women for toxoplasmosis in the municipality of Londrina[30]. Although serology for toxoplasmosis is among the exams requested during prenatal assessment, only 26.8% of pregnant women treated at BHUs undergo this test. The lack of information on toxoplasmosis and the ignorance regarding the serological status renders pregnant women susceptible to infection during the gestational period. These data reinforce the necessity to adopt measures to provide information and orientation to the population on the prevention of toxoplasmosis. Since low positivity was observed

in pregnant women who received some orientation regarding disease prevention, this highlights the efficacy of increasing awareness, corroborating previous findings[43].

The high prevalence found in pregnant women participating in the study and the early infection in animals suggests that there is widespread exposure to the agent in the region. The results found in the present study may support and serve as a stimulus for public bodies to adopt, implement, and amplify preventative measures, especially in vulnerable populations with low levels of education.

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Conflict of interest

The authors declare that there is no conflict of interest.

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