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[4], 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
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 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. Subclinical infection is commonly observed for infection occurring in the final trimester, which can be confirmed by serology.

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. Therefore, understanding the factors associated with infection in a specific population is extremely relevant, since not all forms of parasite transmission are equally important and the serology of the pregnant women and that of their pet animals can be confirmed by serology. However, diagnosis after the 30th week requires administration of spiramycin immediately after diagnosis in pregnant women, although diagnosis after the 30th week requires administration of pyrimethamine, sulfadiazine or folinic acid, due to the inherent risks caused to the fetus by the infection.

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, and serological monitoring of pregnant women during the prenatal period. 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, 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, and an area of 1,584.693 km².

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 or jugular 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 chemiluminescent 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 and 1:64 for cats. Furthermore, a cut-off point of 1:16 was used for the detection of IgM antibodies in cats. 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 -F4263, Sigma-Aldrich®) 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.

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 was used for the amplification of *T. gondii* DNA: Tox4 (CGCTGCAGGAGGAACAGGAAATGTTG) and Tox5 (CGCTGCAGACACAGTGCATCTGGATT) primers were used to amplify a 529 bp fragment (GeneBank N0 LFI46527) 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 mM PCR buffer (200 mM Tris-HCl (pH 8.0), 500 mM KCl), 1.5 mM of MgCl₂, 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 min 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/other meats: 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             | 21                  | 20.6  | 3.12 (1.66-5.84) | < 0.001 |
| < 25 years old (Ref)             | 52             | 42                  | 44.7  |                     |         |
| **BHU**                          |                |                     |       |
| Urban                            | 31             | 21                  | 40.4  | 0.61 (0.31-1.18)   | 0.19   |
| Peri-urban or rural area (Ref)   | 102            | 42                  | 29.2  |                     |         |
| **Level of education**           |                |                     |       |
| Primary School (Ref)             | 57             | 16                  | 21.9  |                     |         |
| Secondary school + further education | 76              | 47                  | 38.2  | 0.45 (0.23-0.88)   | 0.03   |
| **Monthly family income**        |                |                     |       |
| ≤ 1 minimum salary (Ref)        | 90             | 41                  | 31.3  |                     |         |
| > 1 minimum salary               | 43             | 22                  | 33.8  | 0.89 (0.47-1.68)   | 0.84   |
| **Origin of drinking water**     |                |                     |       |
| Public network or mineral (Ref)  | 124            | 55                  | 30.7  |                     |         |
| Other sources: well, mine or river/stream | 9           | 8                   | 47.1  | 0.5 (0.18-1.36)    | 0.27   |
| **Sewage destination**           |                |                     |       |
| Public network                   | 46             | 20                  | 30.3  | 1.14 (0.6-2.1)     | 0.82   |
| Others: cesspit, open air or river/stream (Ref) | 87       | 43                  | 33.1  |                     |         |
| **Garbage destination**          |                |                     |       |
| Public collection (Ref)          | 126            | 59                  | 31.9  |                     |         |
| Wasteland or yard                | 7              | 4                   | 36.4  | 0.82 (0.23-2.91)   | 0.98   |
| **Presence of wasteland close to home** |          |                     |       |
| Yes                              | 70             | 29                  | 29.3  | 1.30 (0.71-2.38)   | 0.47   |
| No (Ref)                         | 63             | 34                  | 35.1  |                     |         |
| **Flooded areas close to home**  |                |                     |       |
| Yes                              | 22             | 17                  | 43.6  | 0.54 (0.26-1.10)   | 0.13   |
| No (Ref)                         | 111            | 46                  | 29.3  |                     |         |
| **Having a vegetable garden at home** |          |                     |       |
| Yes                              | 23             | 11                  | 32.4  | 0.99 (0.45-2.18)   | 0.86   |
| No (Ref)                         | 110            | 52                  | 32.1  |                     |         |
| **If the participant prepares the food** |          |                     |       |
| Yes                              | 120            | 48                  | 28.6  | 2.88 (1.28-6.51)   | 0.016  |
| No (Ref)                         | 13             | 15                  | 53.6  |                     |         |
| **Presence of rodents**          |                |                     |       |
| Yes                              | 77             | 31                  | 28.7  | 1.42 (0.78-2.59)   | 0.32   |
| No (Ref)                         | 56             | 32                  | 36.4  |                     |         |
| **Presence of cats in the residence** |          |                     |       |
| Yes                              | 113            | 45                  | 28.5  | 2.26 (1.10-4.66)   | 0.04   |
| No (Ref)                         | 20             | 18                  | 47.4  |                     |         |
| **Number of cats**               |                |                     |       |
| One (Ref)                        | 24             | 9                   | 27.3  |                     |         |
| Two or more                      | 16             | 5                   | 23.8  | 1.20 (0.34-4.24)   | 0.97   |
| **Age of the cat(s)**            |                |                     |       |
| ≤ 1 years (Ref)                  | 16             | 5                   | 23.8  |                     |         |
| > 1 years                        | 22             | 8                   | 26.7  | 0.86 (0.24-3.12)   | 0.92   |
| **Whether the cat leaves the house/apartment** |          |                     |       |
| Yes                              | 28             | 11                  | 28.2  | 0.70 (0.16-2.97)   | 0.89   |
| No (Ref)                         | 11             | 3                   | 21.4  |                     |         |
| **Whether the cat is fed raw or rare meat** |          |                     |       |
| Yes                              | 13             | 2                   | 13.3  | 3.12 (0.60-16.09)  | 0.28   |
| No (Ref)                         | 25             | 12                  | 32.4  |                     |         |
| **Presence of dogs in the house/apartment** |          |                     |       |
| Yes                              | 51             | 30                  | 37.0  | 0.68 (0.37-1.25)   | 0.28   |
| No (Ref)                         | 82             | 33                  | 28.7  |                     |         |
| **If the participant has ever had a blood transfusion** |          |                     |       |
| Yes                              | 3              | 3                   | 50.0  | 0.46 (0.09-2.35)   | 0.61   |
| No (Ref)                         | 130            | 60                  | 31.6  |                     |         |
| **If the participant has received previous orientation on toxoplasmosis** |          |                     |       |
| Yes                              | 28             | 23                  | 45.1  | 0.46 (0.24-0.90)   | 0.03   |
| No (Ref)                         | 105            | 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                | 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               |      |
| Washing the meat board before using it to chop vegetables | 128 | 68.4 | 59 | 31.6 | 1.74 (0.45-6.70) | 0.66 |
| 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 | 64 | 69.6 | 28 | 30.4 | 1.16 (0.63-2.12) | 0.74 |
| If the participant has ever assisted in animal births | 69 | 66.3 | 35 | 33.7 |      |
| If the participant has ever assisted in the slaughter of cows/pigs/sheep/goats | 3 | 75 | 1 | 25 | 1.33 (0.13-13.35) | 0.75 |

CI: confidence interval; Ref: reference.
TABLE 3: Final non-conditional logistic regression model of factors associated with *Toxoplasma 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 Brazil25,26,27. Although previous studies have been conducted regarding the occurrence of toxoplasmosis in the state of Bahia28,29, 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 measures27,30. 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 studies31,32,33.

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...
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\(^36\). 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.

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.

References

1. Tenter AM. Toxoplasma gondii in animals used for human consumption. Mem Inst Oswaldo Cruz. 2009;104(2):364-69.
2. Robert-Gangneux F. Is it not only the cat that did it? How to prevent and treat congenital toxoplasmosis. J Infect. 2014;68(Suppl 1):S125-33.
3. Dubey JP, Jones JL. Toxoplasma gondii infection in humans and animals in the United States. Int J Parasitol. 2008;38(11):1257-78.
4. Montoya JG. Laboratory diagnosis of Toxoplasma gondii infection and toxoplasmosis. J Infect Dis. 2002;185(Suppl 1):S73-82.
5. Hill D, Dubey JP. Toxoplasma gondii: transmission, diagnosis and prevention. Clin Microbiol Infect. 2002;8(10):634-40.
6. Jones JL, Dargelas V, Roberts J, Press C, Remington JS, Montoya JG. Risk factors for Toxoplasma gondii infection in the United States. Clin Infect Dis. 2009;49(6):878-84.
7. Petersen E. Toxoplasmosis. Semin Fetal Neonatal Med. 2007;12(3):214-23.
8. Vaz RS, Rauli P, Mello RG, Cardoso MA. Toxoplasmosis congenital: uma doença negligenciada? Atual política de saúde pública brasileira. Field Actions Sci Rep. 2011;3(1):0-8.
9. Bollani L, Stronati M. II neonato con toxoplasmosi congenita: clinica, terapia e follow-up. J Pediat Neonatal Individualized Medicine. 2014;3(1):e030104.
10. Garcia JL, Navarro IT, Ogawa L, Oliveira RC. Seroepidemiologia da toxoplasmosis em gatos e cães de propriedades rurais do município de Jaguapeitá, Estado do Paraná, Brasil. Cienc Rural.1999;29(1):99-04.
11. Edelhofer R, Prossinger H. Infection with Toxoplasma gondii during pregnancy: seroepidemiological studies in Austria. Zoonoses Public Health. 2010;57(1):18-26.
12. Dubey JP, Lindsay DS. Neosporis, toxoplasmosis and sarcocystosis in ruminants. Vet Clin North Am Food Anim Pract. 2006;22(3):645-71.
13. Mitsuka-Breganó R, Lopes-Mori FMR, Navarro IT. org. Toxoplasmosese adquirida na gestação e congênita: vigilância em saúde, diagnóstico, tratamento e condutas [online]. Londrina: EDUEL, 2010. 62 p.
14. Ministério da Saúde (MS). Secretaria de Atenção à Saúde. Departamento de Ações Programáticas Estratégicas. Gestação de alto risco: Manual técnico. 5ª edição. Brasília: MS; 2012. 302 p.
15. Instituto Brasileiro de Geografia e Estatística (IBGE). Censo demográfico [Internet]. 2010 [updated 2017 June 20; cited 2017Nov 20]. Available from: https://cidades.ibge.gov.br/brasil/ba/ilheus/panorama
16. Instituto Brasileiro de Geografia e Estatística (IBGE). Área da unidade territorial [Internet]. 2016 [updated 2017 June 20; cited 2017 Nov 20]. Available from: https://cidades.ibge.gov.br/brasil/ba/ilheus/panorama
17. Langoni H, Fornazari F, Silva RC, Monti ET, Villa FB. Prevalence of antibodies against Toxoplasma gondii and Neospora caninum in dogs. Braz J Microbiol. 2013;44(4):1327-30.
18. Silva NM, Lourenço EV, Silva DA, Mineiro JR. Optimization of cut-off titres in Toxoplasma gondii specific ELISA and IFAT in dog sera using immunoactivity to SAG-1 antigen as a molecular marker of infection. Vet J. 2002;163(1):94-98.
19. Moura AB, Trevisani N, Quadros RM, Ledo G, Souza AP, Sartor AA. Anticorpos contra Toxoplasma gondii em gatos apreendidos pelo Centro de Controle de Zoonoses de Lages, SC. Arch Vet Sci. 2015;20(1):01-07.
20. Rosa LD, Moura AB, Trevisani N, Medeiros AP, Sartor AA, Souza AP. et al. Toxoplasma gondii antibodies on domiciled cats from Lages municipality, Santa Catarina State, Brazil. Vet Parasitol Reg Stud Reports. 2010;19(4):268-69.
21. Sroka J, Karamon J, Dutkiewicz J, Fatla AW, Cencek T. Prevalence of Toxoplasma gondii infection in cats in southwestern Poland. Ann Agric Environ Med. 2018;25(3):576-80.
22. Munhoz AD, Hage SB, Cruz RDS, Calazans APF, Silva FL, Albuquerque GR. et al. Toxoplasmosis in cats in northeastern Brazil: Frequency, associated factors and coinfection with Neospora caninum, feline immunodeficiency virus and feline leukemia virus. Vet Parasitol Reg Stud Reports. 2017;8:35-38.
23. Carlos RSA, Albuquerque GR, Bezerra R.A, Sicupira PML, Munhoz AD, Lopes CWG. Ocorrência de anticorpos anti-Toxoplasma gondii e principais fatores de risco associados à infecção canina na região de Ilhéus-Itabuna, Estado da Bahia. Rev Bras Med Vet. 2010;32(2):115-21.
24. Homan WL, Vercammen M, Braekelee J, Verschuren H. Identification of a 200- to 300-fold repeatitive 529 bp DNA fragment in Toxoplasma gondii, and its use for diagnostic and quantitative PCR. Int J Parasitol. 2000;30(1):69-75.
25. Alves JAB, Oliveira LAR, Oliveira MFB, Araújo RM, Santos RCS, Abud AFC. et al. Prevalência de anticorpos anti-Toxoplasma gondii em mulheres grávidas. Rev enferm UERJ. 2009;17(1):107-10.
26. Barbosa IR, Holanda CMCX, Andrade-Neto VF. Toxoplasmosis screening and risk factors amongst pregnant females in Natal, northeastern Brazil. Trans R Soc Trop Med Hyg. 2009;103(4):377-82.
27. Sroka S, Bartelheimer N, Winter A, Heukelbach J, Ariza L, Ribeiro H. et al. Prevalence and risk factors of toxoplasmosis among...
pregnant women in Fortaleza, Northeastern, Brazil. Am J Trop Med Hyg. 2010;83(3):528-33.

28. Nascimento I, Carvalho S, Cardozo N, Asfora S, Campos A, Menezes S et al. Estudo da prevalência de anticorpos anti-Toxoplasma gondii em mulheres grávidas no Estado da Bahia. R Ci Méd Biol. 2002;1(1):12-15.

29. Avelar MV, Martinez VO, Moura DL, Barros IA, Primo AAS, Duarte AO et al. Association between seroprevalence of IgG anti-Toxoplasma gondii and risk factors for infection among pregnant women in Cimério de Oliveira Maternity, Salvador, Bahia, Brazil. Rev Inst Med Trop Sao Paulo. 2017;59:e90.

30. Silva MG, Vinaud MC, Castro AM. Prevalence of toxoplasmosis in pregnant women and vertical transmission of Toxoplasma gondii antibodies in patients from basic units of health from Gurupi, Tocantins, Brazil, from 2012 to 2014. PLoS One. 2015;10(11): e0141700.

31. Nóbrega OT, Karnikowski MGO. An estimation of the frequency of gestational toxoplasmosis in the Brazilian Federal District. Rev Soc Bras Med Trop. 2005;38(4):358-60.

32. Avelino MM, Campos D Jr, Parada JCB, Castro AM. Pregnancy as a risk factor for acute toxoplasmosis seroconversion. Euro J Obstet Gynecol Reprod Biol. 2003;108(1):19-24.

33. Figueiró-Filho EA, Lopes AHA, Souza Jr VG, Botelho CA, Figueiredo MS et al. Toxoplasmose aguda: estudo da frequência, taxa de transmissão vertical e relação entre os testes diagnósticos materno-fetais em gestantes em estado da Região Centro-Oeste do Brasil. Rev Bras Ginecol Obstet. 2005;27(8):442-49.

34. Carellos EVM, Andrade GMQ, Aguiar RALP. Avaliação da aplicação do protocolo de triagem pré-natal para toxoplasmose em Belo Horizonte, Minas Gerais, Brasil: estudo transversal em puêrperas de duas maternidades. Cad Saude Publica. 2008;24(2):391-01.

35. Cademartori BG, Farias NAR, Brod CS. Soroprevalência e fatores de risco à infecção por Toxoplasma gondii em gestantes de Pelotas, sul do Brasil. Rev Panam Infectol. 2008;10(4):30-5.

36. Cong W, Dong XY, Meng QF, Zhou N, Wang XY, Huang SY et al. Toxoplasma gondii infection in pregnant women: A seroprevalence and case-control study in Eastern China. Biomed Res Int. 2015;2015:170278.

37. Dias RCF, Lopes-Mori FMR, Mitsuka-Breganô R, Dias RAF, Tokano DV, Reiche EMV et al. Factors associated to infection by Toxoplasma gondii in pregnant women attended in Basic Health Units in the city of Rolândia, Paraná, Brazil. Rev Inst Med Trop Sao Paulo. 2011;53(4):185-91.

38. Lopes-FRM, Mitsuka-Breganô R, Gonçalves DD, Freire RL, Karigyo CJT, Wedy GF et al. Factors associated with seropositivity for anti-Toxoplasma gondii antibodies in pregnant women of Londrina, Paraná, Brazil. Mem Inst Oswaldo Cruz. 2009;104(2):378-82.

39. Branco BHM, Araújo SM, Falavigna-Guilherme AL. Prevenção primária da toxoplasmose: conhecimento e atitudes de profissionais de saúde e gestantes do serviço público de Maringá, estado do Paraná. Sci Med. 2012;22(4):185-90.

40. Kompalic-Cristo A, Nogueira SA, Guedes AL, Frota C, González LF, Brandão A et al. Lack of technical specificity in the molecular diagnosis of toxoplasmosis. Trans R Soc Trop Med Hyg. 2004;98(2):92-5.

41. Khalifa K el-S, Roth A, Roth B, Arasteh KN, Janitschke K. Value of PCR for evaluating occurrence of parasitemia in immunocompromised patients with cerebral and extracerebral toxoplasmosis. J Clin Microbiol. 1994;32(11):2813-19.

42. Câmara JT, Silva MG, Castro AM. Prevalência de toxoplasmose em gestantes atendidas em dois centros de referência em uma cidade do Nordeste, Brasil. Rev Bras Ginecol Obstet. 2015;37(2):64-70.

43. Bezerra RA, Carvalho FS, Guimarães LA, Rocha DS, Silva FL, Wenceslau AA et al. Comparison of methods for detection of Toxoplasma gondii in tissues of naturally exposed pigs. Parasitol Res. 2012;110(2):509-14.

44. Spagnol FH, Paranhos EB, Oliveira LLS, Medeiros SM, Lopes CWG, Albuquerque GR. Prevalência de anticorpos anti-Toxoplasma gondii em bovinos abatidos em matadouros do estado da Bahia, Brasil. Rev Bras Parasitol Vet. 2009;18(2):42-45.

45. Guimarães LA, Bezerra RA, Rocha DS, Albuquerque GR. Prevalence and risk factors associated with anti-Toxoplasma gondii antibodies in sheep from Bahia state, Brazil. Rev Bras Parasitol Vet. 2013;22(2):220-24.

46. Rocha DS, Moura RLS, Maciel BM, Guimarães LA, O’dwyer HNS, Munhoz AD et al. Detection of Toxoplasma gondii DNA in naturally infected sheep’s milk. Genet Mol Res. 2015;14(3):8658-62.

47. Ribeiro LA, Santos LKNSS, Brito Jr PA, Maciel BM, Silva AV, Albuquerque GR. Detection of Toxoplasma gondii DNA in Brazilian oysters (Crassostrea rhizophorae). Genet Mol Res. 2015;14(2):4658-65.