Multiparity as a risk factor for congenital toxoplasmosis: a cross-sectional study

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Background

Congenital toxoplasmosis (CT) is caused by placental transfer of Toxoplasma gondii to the fetus, which can generate neurological, neurocognitive deficits, or death. Appropriate preventive strategies are required for infection-related risk factors. This study assessed the prevalence of T. gondii infection and the factors associated with CT in pregnant women with assistance from the Public Health Service at Ouro Preto, Brazil.

Methods

This cross-sectional study was conducted between April and December 2020. Pregnant women (n=151) aged between 13 and 46 years, were recruited and evaluated for specific IgM/IgG antibody levels against T. gondii. A structured questionnaire was applied to determine the socioeconomic, environmental, gestational, clinical, and dietary patterns.

Results

The prevalence of T. gondii was 45.8% (n = 60) in which multiparas revealed to be more exposed to infection and were 2.6 times more likely to become infected with the parasite compared to primiparas, (odds ratio, OR=2.60; 95% confidence interval, CI=1.25-5.39). A high prevalence of T. gondii seropositivity was found to be related to the absence of basic sanitation at home. In conclusion, multiparas constitute risk factor for CT.

Conclusions

Educational and preventive measures should be intensified in uninfected multiparas to raise awareness about the potential risks of contact with T. gondii.

Congenital toxoplasmosis (CT) is an infectious disease caused by placental transfer of Toxoplasma gondii protozoa to the fetus and can result in neurological and neurocognitive deficits or death.1,2 Most cases are asymptomatic in immunocompetent individuals, even though some patients may experience self-limiting symptoms such as febrile conditions, lymphadenopathy, hepatosplenomegaly, and eventual skin rash.3,4 The pathogenesis of toxoplasmosis varies by strain and host susceptibility based on an individual's genetic traits,5 causing significant damage and even death in immunocompetent adults.6 In pregnant women, placental transference of the protozoan is related to severe toxoplasmosis in neonates, which may be related to the genetic characteristics of T. gondii isolates prevalent in animals and humans.7

T. gondii infection is predominantly widespread in tropical climates, especially at low altitudes, as the oocysts survive better in these environments.8,9 It is estimated that approximately 51.4% of pregnant women worldwide are infected with the parasite.10 In Brazil, T. gondii infection ranges from 50–80% in women of reproductive age.11

Maternal serological screening during prenatal care enables the identification of infected and vulnerable pregnant women for follow-up as a means to reduce the risk of fetal infection.12 The severity of congenital toxoplasmosis is inversely related to the gestational period at the time of infection. At the beginning of pregnancy, transmission is low, but the pathology can be severe. At the end of pregnancy, high transmission occurs, but with a lower risk of miscarriage.13 Treatment during pregnancy is related to a lower risk of infection and decreased severity in babies.14,15 Maternal health issues in Brazil have been discussed in 1980, with the implementation of different maternal health policies and programs,16 in addition to greater access to prenatal care in health units. In Italy, Slovenia, France, and Austria, the prenatal program is mandatory, whereas in the United States of America, lack of screening has contributed to greater disease severity, which is only observed at birth.17-20

Epidemiological research in urban and rural environments shows geographical territory together with the perception of the disease spread. Thus, detection of risk factors...
associated with \textit{T. gondii} infection can lead to improvements in preventive health strategies.\textsuperscript{21,22} In this context, the present study aimed to assess the prevalence of \textit{T. gondii} infection and the factors associated with congenital toxoplasmosis in pregnant women with assistance from the Public Health Service at Ouro Preto, Brazil.

METHODS

A cross-sectional study was conducted with pregnant women between April and December 2020, with assistance from the Public Health Service at Ouro Preto, Brazil.

After signing the assent form (for children’s parents or responsible girls < 18 years old) or consent form (for women ≥ 18 years old), the non-probabilistic sample, by convenience, included 131 pregnant women, regardless of age. After being recruited, specific IgM and IgG antibody levels against \textit{T. gondii} were determined. The women also responded to a structured questionnaire that was used to obtain information regarding the socioeconomic (age, education, occupation, income, and place of residence), environmental (access to water, sewage network, urban waste collection, contact with domestic animals, animal disposals at home, gardening, use of personal protective equipment (PPE) in gardening, handwashing after using the bathroom), gestational (gestational age, number of pregnancies, neuroocular complications of the firstborn, abortion, eclampsia, body mass index (BMI) pre-gestational and gestational weight gain), clinical (stool parasitological examination, type of protozoan in stool, blood pressure, kidney disease, urine culture, and types of bacteria), and food (consumption of unpasteurized milk, raw vegetables and meat, fruit/vegetable washing process, cross contamination in meal preparation, handwashing before food preparation) conditions. Their weight (kg) and height (cm) were recorded using a stadiometer, which was attached to the Welmy\textsuperscript{®} electronic scale.

The income variable was classified based on the Brazilian minimum wage in \textit{REAIL} (R$), R$ 1,045.00 (US$188). The cutoff point for the education variable was 12 years of study for categorizing women who had studied until high school. The pre-gestational body mass index variable was classified as "not overweight" for BMI < 24.9 kg/m\textsuperscript{2} and as "overweight" for BMI > 25.0 kg/m\textsuperscript{2}. The gestational weight gain variable was classified as shown in Table 1.

Stool samples were collected and sent to the Inflammation Immunobiology Laboratory (LABIIN) for conservation by the addition of mercury, iodine, and formaldehyde (MIF). Subsequently, the samples were sent to the Clinical Analysis Laboratory of the same institution for analysis using the Hoffman, Pons, and Janer method for parasite identification.\textsuperscript{23}

Microsoft Excel 2013 was used for data storage. Data analysis was performed using Stata (Stata Corp V.13, Texas, USA).\textsuperscript{24} Descriptive analysis included the distribution of frequencies and the absolute numbers of categorical variables. The prevalence of exposure and non-exposure to \textit{T. gondii} associated with the 95% confidence interval were estimated for the included population. To verify the relationship between \textit{T. gondii} exposure and the explanatory variables, univariate logistic regression was used, expressed by the odds ratio adjusted for age. The explanatory variables that presented a p-value < 0.20 in the univariate analysis, were selected for multivariate analysis. The forward data entry method was chosen wherein all variables selected in the univariate analysis were inserted simultaneously in the model and were then removed individually based on the probability of a likelihood-ratio statistic based on conditional parameter estimates, starting from the least significant. The final model included all variables that were statistically associated with outcomes, showing a p-value ≤ 0.05. The Hosmer-Lemeshow test was used to analyze or adjust the model.

RESULTS

Of the 131 pregnant women evaluated, 59.54% reported being in their second pregnancy; however, the prevalence of \textit{T. gondii} infection was 45.8% (n = 60) and in this group, multi-paras seemed to be more likely to be infected with \textit{T. gondii} (P < 0.009). The average age of this group of women was 27.3 ± 6.6 years. The participants’ sociodemographic, gestational, clinical, and nutritional data, as well as BMI distribution are shown in Table 2.

Most of the pregnant women reported that they had completed high school education (58.02%), had a job (43.56%), had an income between two and three minimum wages (52.67%), lived in the urban area of the city (60.31%), and had access to basic sanitation such as running water (86.26%), a sewage network (89.31%), and urban waste collection (97.71%) at their residence. Half of the participants reported having contact with domestic animals (50.38%). Among the pregnant women, 25.19% had gardening habits, and of these, 84.85% did not wear gloves. Further, 51.91% of

Table 1. Recommended weight gain according to pre-pregnancy maternal body mass index (BMI)

| Nutritional status before pregnancy | BMI (kg/m\textsuperscript{2}) | Weight gain during pregnancy (Kg) | Weight gain per week in the 2nd and 3rd trimester (Kg) |
|------------------------------------|-------------------------------|---------------------------------|---------------------------------|
| Underweight                        | <18.5                         | 12.5 – 18                       | 0.5                             |
| Eutrophic                          | 18.5-24.9                     | 11 – 16                         | 0.4                             |
| Overweight                         | 25.0-29.9                     | 7 – 11.5                        | 0.3                             |
| Obesity                            | ≥30.0                         | 5 - 9                           | 0.2                             |

Source: Institute of Medicine (IOM-2009)
Table 2. Univariate analysis with the characterization of pregnant women evaluated in 2020, Ouro Preto - MG, Brazil

| Variables                  | N   | %     | Pregnant women                                      | P-value |
|----------------------------|-----|-------|-----------------------------------------------------|---------|
|                            |     |       | Uninfected (n = 71)                                  |         |
|                            |     |       | Infected by *T. gondii* (n=60)                        |         |
| Socioeconomic variables    |     |       |                                                     |         |
| Age                        |     |       |                                                     |         |
| Adolescents (<18 years)    | 10  | 7.63  | 7                                                   | 0.343***|
| Adults (>18 years)         | 121 | 92.37 | 64                                                  |         |
| Education                  |     |       |                                                     |         |
| < Complete high school     | 55  | 41.98 | 28                                                  | 0.52*   |
| > Complete high school     | 76  | 58.02 | 43                                                  | 0.252** |
| Employment                 |     |       |                                                     |         |
| Unemployed                 | 1   | 0.76  | 0                                                   | 0.532** |
| Employment bond            | 61  | 43.56 | 29                                                  | 32      |
| Student                    | 15  | 11.45 | 10                                                  | 0.252** |
| Housewife                  | 54  | 41.22 | 32                                                  | 0.262** |
| Income                     |     |       |                                                     |         |
| Up to 1 minimum wage       | 51  | 38.93 | 26                                                  | 25      |
| 1-2 minimum wage           | 0   | 0     | 0                                                   | 0       |
| 2-3 minimum wage           | 69  | 52.67 | 38                                                  | 31      |
| >4 minimum wage            | 11  | 8.4   | 7                                                   | 4       |
| Habitation                 |     |       |                                                     |         |
| City                       | 79  | 60.31 | 47                                                  | 32      |
| District                   | 52  | 39.69 | 24                                                  | 28      |
| Environmental variables    |     |       |                                                     |         |
| Access to treated water    |     |       |                                                     |         |
| No                         | 18  | 13.74 | 8                                                   | 10      |
| Yes                        | 113 | 86.26 | 63                                                  | 50      |
| Access to sewer services   |     |       |                                                     |         |
| No                         | 14  | 10.69 | 7                                                   | 7       |
| Yes                        | 117 | 89.31 | 64                                                  | 53      |
| Contact with pets          |     |       |                                                     |         |
| Yes                        | 66  | 50.38 | 31                                                  | 35      |
| Pet in the house (but without direct contact) | 22  | 16.79 | 12                                                  | 10      |
| No                         | 43  | 32.82 | 28                                                  | 15      |
| Animal waste disposed of in the house | No | 64  | 48.85                                             | 39      |
| Yes                        | 67  | 51.15 | 32                                                  | 35      |
| Gestational variables      |     |       |                                                     |         |
| Gestational age            |     |       |                                                     |         |
| First trimester            | 37  | 28.24 | 23                                                  | 14      |
| Second trimester           | 49  | 37.40 | 24                                                  | 25      |
| Third trimester            | 45  | 34.35 | 24                                                  | 21      |
| Pregnancy                  |     |       |                                                     |         |
| Primipartity               | 53  | 40.46 | 36                                                  | 17      |

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the pregnant women reported that they did not wash their hands after bathroom use.

Regarding clinical conditions, most of the women evaluated were normotensive and did not have kidney disease (95.42%). Parasitological examination of feces showed 14.10% of positivity, in which 72.73% presented the *Entamoeba coli* protozoa. Among the pregnant women who underwent urine culture (n = 128), *Escherichia coli* was present in 36.36% of the samples.

Among those pregnant women in their second pregnancy; 96.95% had no history of eclampsia, 19.08% reported having a prior abortion, 6.11% reported neurological complications in their firstborn child, and 58.02% were “overweight” before pregnancy. Further, 37.40% were in the second trimester of the current pregnancy, and the weight inadequacy was 33.59% of the evaluated sample.

Regarding the volunteers’ eating habits (dietary variables), most of the sample consumed pasteurized milk (89.31%), raw vegetables (99.24%), and raw/undercooked meat (67.94%). Overall, 50.38% reported the habit of cleaning/washing fruits and vegetables under running water. When investigating cross-contamination, we observed that 75.57% washed their knives before reusing them. The volunteers reported that they routinely washed their hands before preparing meals (94.66%).

The Table 3 demonstrates the odds ratios for women infected with *T. gondii*. Among all factors, multiparous were 2.6 times more likely to become infected with *T. gondii* than primiparous (OR: 2.60; 95% CI: 1.25-5.39). This result was observed even after adjusting the odds ratio for age.

The variables of access to garbage collection services, gardening habits, use of PPE in gardening, hand washing after using the bathroom, abortion, eclampsia, type of protozoan in the stool, kidney disease, urine culture and presence of bacteria, consumption of unpasteurized milk, consumption of raw vegetables, consumption of raw meat, fruit and vegetable washing process, and cross-contamination during meal preparation did not show any statistically significant differences.

**DISCUSSION**

This study was carried out in the Ouro Preto microregion, which is one of the most important historic cities in the
Table 3. Multivariate analysis: Risk or protection factors associated with *T. gondii* infection in pregnant women evaluated in 2020 at Ouro Preto, MG, Brazil.

| Variables                                         | T. gondii-infected pregnant women variables | Gross OR (IC 95%) | p       | Adjusted OR (IC 95%) | P-value |
|---------------------------------------------------|--------------------------------------------|-------------------|---------|----------------------|---------|
| **Social variables**                              |                                            |                   |         |                      |         |
| Habitation                                        |                                            |                   |         |                      |         |
| City                                              |                                            | 1                 | 0.704   | 1                    | 0.685   |
| District                                          |                                            | 0.78 (0.23-2.70) | 0.77 (0.22-2.70) |         |         |
| **Environmental variables**                       |                                            |                   |         |                      |         |
| Animal waste disposed of in the house             |                                            |                   |         |                      |         |
| No                                                |                                            | 1                 | 0.552   | 1                    | 0.566   |
| Yes                                               |                                            | 0.72 (0.24-2.12) | 0.73 (0.25-2.15) |         |         |
| **Gestational variables**                         |                                            |                   |         |                      |         |
| Pregnancy                                         |                                            |                   |         |                      |         |
| Primiparity                                       |                                            | 1                 | 0.010   | 1                    | 0.018   |
| Multiparity                                       |                                            | 2.60 (1.25-5.39) | 2.50 (1.17-5.36) |         |         |
| Children complications                            |                                            |                   |         |                      |         |
| No                                                |                                            | 1                 | 0.664   | 1                    | 0.664   |
| Yes                                               |                                            | 1.74 (0.14-21.53) | 1.74 (0.14-21.53) |         |         |
| Pre-gestational BMI                               |                                            |                   |         |                      |         |
| Without overweight                                |                                            | 1                 | 1       |                      | 0.397   |
| With overweight                                   |                                            | 0.62 (0.20-1.92) | 0.408   | 0.60 (0.18-1.95)     |         |
| **Clinical variables**                            |                                            |                   |         |                      |         |
| Parasitic examination in stools (N=78)            |                                            |                   |         |                      |         |
| Negative                                          |                                            | 1                 | 0.370   | 1                    | 0.366   |
| Positive                                          |                                            | 2.45 (0.34-17.38) | 2.47 (0.35-17.6) |         |         |
| Blood pressure                                    |                                            |                   |         |                      |         |
| No                                                |                                            | 1                 | 0.183   | 1                    | 0.203   |
| Yes                                               |                                            | 4.51 (0.49-41.67) | 4.23 (0.45-39.17) |         |         |
| Dietary variables                                 |                                            |                   |         |                      |         |
| Handwashing before food preparation               |                                            |                   |         |                      |         |
| No                                                |                                            | 0.74 (0.05-11.25) | 0.827   | 0.75 (0.05-11.54)    | 0.838   |
| Yes                                               |                                            | 1                 | 1       |                      |         |

Footnote: ORa - age-adjusted odds ratio. Logistic Regression

central region of Minas Gerais state in Brazil. The economic activities revolve around mineral extraction, metallurgical industry, tourism, and agriculture. Despite increasing tourist and financial activities, this city has precarious water treatment with 75.6% of the households having adequate sanitation but lack a water treatment system.

Regarding the factors associated with *T. gondii* infection, our results showed that multiparas presented as an important risk factor as they showed a 2.6 times greater risk of being infected compared to primiparas. In contrast, a recent study conducted in Turkey reported that primipara women had a significantly higher risk (*P* < 0.02) of infection with the parasite compared to multipara women.\(^{25}\) However, Onduru & Aboud, (2021)\(^{26}\) reported that in the Temeke district, Dar es Salaam, Tanzania, the probability of a pregnant woman giving birth to a child with a toxoplasmosis was 28.5 times higher in multiparas than in primiparas. After adjusting for the effect of confusion and association in the multivariate model, the robustness of the multipara variable increased to 40.89 times more likely for pathology in the child. There is great cultural diversity among studies from different countries and Brazil, as the culture seems to show overprotection vis-à-vis the firstborn, and through the observation and personal reports of pregnant women, the subsequent pregnancies seem to be more “peaceful,” with less stress and more experience. Such facts may contribute to less food care and greater exposure to risk factors such as contact with infected soil and untreated water. As biological explanations, we present the following points in our results: greater vulnerability of uninfected multiparas to the parasite probably occurs due to continuous changes in the immunological mechanisms inherent to pregnancy, resulting from a partial immune response suppression (due to the need for tolerance to the fetus) and/or due to hormonal
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imbalances characteristic of the gestational condition. Further, unfavorable living conditions of the population in underdeveloped countries with less financial acquisition for larger families can result in a greater chance of infection in women, especially during multiparity, when they are more vulnerable to parasitic infection.

Further, in the study by Berger et al. (2009), a predisposition to T. gondii infection was observed in multigravida French pregnant women from 1995 to 2003, thus consolidating the variable as a risk factor for the signs and symptoms of toxoplasmosis.

Effective screening programs are essential for implementing public health policies. In our study, we showed 45.8% positivity for anti-T. gondii IgG, and other studies carried out in different regions of Brazil showed 45% positivity in the central-west region of Minas Gerais, 50.2% positivity in Mato Grosso do Sul, and 59.8% positivity in Porto Alegre.

In contrast, European countries, have demonstrated a tendency towards reduced seroprevalence in pregnant women. The relevant risk factors include lack of basic sanitation, inadequate eating and hygiene habits, contact with contaminated animals and soil, territoriality, climatic characteristics, and the presence of more virulent strains of T. gondii. The greatest concern during parasitic infection during pregnancy is the damage it can cause to the fetus. Thus, atypical strains of T. gondii circulating in Brazil, may also be associated with severe cases of ocular toxoplasmosis from congenital infection.

High rates of toxoplasmosis seroprevalence are expected in rural areas such as farms, due to the presence of domestic cats and the common presence of intermediate hosts, such as commensal rodents. Despite the possibility of the infectious form spreading in these areas, the present study found no interference from the geographical location in the disease spread. The municipality where our study was conducted had surface and underground water collection points and water treatment. For historical and bureaucratic reasons, there was no treatment of water meters, making it impossible to control water consumption in this region.

Although this study did not present statistical significance in relation to basic sanitation, consumption of untreated water is considered a risk factor for the transmission of oocysts, mainly in developing countries.

One of the determining factors in the epidemiology of pathologies is the socioeconomic level. According to Hotez (2008), seroprevalence in the upper, middle, and lower socioeconomic strata in Brazil was 23%, 62%, and 84%, respectively. By combining sanitation and socioeconomic status, the prevalence rate in the adult population can reach 90%. In the present study, more than a third of the pregnant women survived on up to a minimum wage; however, there was no significant difference in the serotype of T. gondii.

Consumption of undercooked/roasted meat is common in more than half of the group studied, whereas in the United Kingdom, pregnant women are warned about the consumption of unpasteurized milk and undercooked meat, and risk assessment for T. gondii infection is carried out individually during prenatal care. In the study by Chandrasena et al. (2016), with pregnant women in Sri Lanka, almost all women had the habit of washing their hands after handling raw meat. Although meat consumption is high, the meat is well cooked and prepared with traditional sauces, reducing the risk of parasite infection. In a study conducted in Western Iran, the prevalence of pregnant women infected with T. gondii was high among those who had consumed undercooked eggs and meat as well as unwashed vegetables.

In general, the diverse prevalence (20–90%) of T. gondii infection can be understood by distinction between the populations studied, diagnostic methods, and specific risk factors that facilitate disease spread, including nutritional insecurity and/or contact with vector animals for T. gondii infection. Finally, this study demonstrated that the high prevalence of T. gondii seropositivity in pregnant women may be related to multiparity, which should be considered a risk factor for toxoplasmosis.

CONCLUSIONS

Based on our data, we propose rigorous monitoring of pregnant women during prenatal care, in both primigravida and multigravida women, using screening tests, the provision of guidance to pregnant women on the risk factors for T. gondii infection. To target the relevant risk factors, it is essential to develop educational programs in communities together with preventive measures in the public health service that must be maintained, in addition to reinforcing the application of public health policies.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Research Ethics Committee of the Universidade Federal de Ouro Preto under the protocol CAAE: 23467219.7.0000.5150. All women were informed about the research, as well as read and assigned the consent to their participation.

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AUTHORSHIP CONTRIBUTIONS

PVS: conceptualization, resource, writing-original draft preparation, DNMT: data curation, resource, writing-original draft preparation, BAAM: data curation, writing-origi-
COMPETING INTERESTS

The authors declare that the research was conducted without conflict of interest.

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