Multiple Pregnancy Increases the Risk of Toxoplasma Gondii Infection

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MULTIPLE PREGNANCY INCREASES THE RISK OF *TOXOPLASMA GONDII*
INFECTION

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Abstract

Background. Congenital toxoplasmosis (CT) is an infectious disease caused by placental transfer of *Toxoplasma gondii* protozoa to the fetus, and can generate neurological and neurocognitive deficits, or death. As a mother's infection poses the risk of fetal transmission in about 30% of the cases, appropriate preventive strategies are required for infection-related risk factors. The present study assessed the prevalence of *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.** This cross-sectional study was conducted between April and December 2020. Pregnant women (n = 131) aged between 13 and 46 years, were recruited and evaluated for specific IgM and IgG antibody levels against *T. gondii*. These women also responded to a structured questionnaire, which was later used to determine the socioeconomic, environmental, gestational, clinical, and dietary patterns. **Results.** The prevalence of infection by *T. gondii* was 45.8% (n = 60); of these, multigravida women were more exposed to infection and were 2.6 times more likely to become infected with *T. gondii* compared to primigravida women (OR: 2.60; 95% CI: 1.25-5.39). A high prevalence of *T. gondii* seropositivity was identified in pregnant women in the region of Ouro Preto, Brazil, where part of the sample reported the absence of basic sanitation at home. **Conclusion.** Our data identified multigravida as a potential risk factor for gestational toxoplasmosis, based on the high positivity of anti-*T. gondii* serology in these women. Thus, educational and preventive measures must be intensified in both primigravida and multigravida women, to increase awareness regarding the potential risks of contact with *T. gondii*.

Key words: multiple gestations, pregnant, congenital toxoplasmosis, risk factors, Women's Health Services.

Introduction

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, 13]. 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 [14]. Treatment during pregnancy is related to a lower risk of infection and decreased severity in babies [15, 16]. Maternal health issues in Brazil have been discussed in 1980, with the implementation of different maternal health policies and programs [17], in addition to greater access to prenatal care in health units [18]. 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 [19, 20, 21, 22].

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 *T. gondii* infection can lead to improvements in preventive health strategies [23, 24]. In this context, the present study aimed to assess the prevalence of *T. gondii* infection and the factors associated with congenital toxoplasmosis in pregnant women with assistance from the Public Health Service at Ouro Preto, Brazil.

Materials and 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 $\geq$ 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 *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® electronic scale.

The income variable was classified based on the Brazilian minimum wage in *Reals* (R\$), R\$ 1,045.00 (US\$188) [25]. 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/m² and as “overweight” for BMI > 25.0 kg/m². 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 [26].

Microsoft Excel 2013 was used for data storage. Data analysis was performed using Stata (Stata Corp V.13, Texas, USA) [27]. Descriptive analysis included the distribution of frequencies and the absolute numbers of categorical variables. The prevalence of exposure and non-exposure to *T. gondii* associated with the 95% confidence interval were estimated for the included population. To verify the relationship between *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 *T. gondii* infection was 45.8% (n = 60) and in this group, multigravidae seemed to be more likely to be infected with *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 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 the evaluated pregnant women, 59.54% were in their second pregnancy; of these, 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, multigravida women were 2.6 times more likely to become infected with *T. gondii* than primigravida women (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 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 [28] but lack a water treatment system [29].

Regarding the factors associated with *T. gondii* infection, our results showed that multigravida presented as an important risk factor as multigravida women showed a 2.6 times greater risk of

being infected compared to primigravida women. In contrast, a recent study conducted in Turkey reported that primigravida pregnant women had a significantly higher risk ($P < 0.02$) of infection with the parasite compared to multigravida women [30]. However, in agreement with our result, Onduru & Aboud, (2021) [31] 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 multigravida women than in primigravidae. After adjusting for the effect of confusion and association in the multivariate model, the robustness of the multigestation 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 multigravida women to the parasite probably occurs due to continuous changes in the immunological mechanisms inherent to pregnancy, resulting from immune response suppression (due to the need for tolerance to the fetus) and/or due to hormonal 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 multi-pregnancy, when they are more vulnerable to parasitic infection.

Further, in the study by Berger et al. (2009) [32], 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 [33], 50.2% positivity in Mato Grosso do Sul [34], and 59.8% positivity in Porto Alegre [35]. In contrast, European countries, have demonstrated a tendency towards reduced seroprevalence in pregnant women [36]. The relevant risk factors include lack of basic sanitation, inadequate eating and hygiene habits, contact with contaminated animals and soil, territoriality, climatic characteristics [7, 33, 37, 38], and the presence of more virulent strains of *T. gondii* [40]. The greatest concern during parasitic infection during pregnancy is the damage it can cause to the fetus [40]. Thus, atypical strains of *T. gondii* circulating in Brazil [41], may also be associated with severe cases of ocular toxoplasmosis [42] 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 [43]. 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 [44]. 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 [45], mainly in developing countries.

One of the determining factors in the epidemiology of pathologies is the socioeconomic level [46]. According to Hotez (2008) [47], 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% [48]. 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 [49]. In the study by Chandrasena *et al.* (2016) [50], 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 [51].

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 [49, 52, 53]. Finally, this study demonstrated that the high prevalence of *T. gondii* seropositivity in pregnant women may be related to multigravida, 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.

Ethical 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.

Consent for publication:

All volunteers (women) of this research were informed and agreed to the publication of their anonymously data, as well as all authors read and agreed with the final version of this document.

Availability of data and materials

All data from this research, without identification of the participants, will be available under future request to the corresponding author.

Conflict of interest

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

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Authors' contributions

PVS (conceptualization, resource, writing-original draft preparation), DNMT (data curation, resource), BAAM (data curation, resource), VFAN (conceptualization and data curation), NSG (conceptualization, resource, writing-original draft preparation), AT (Supervision, conceptualization, writing-reviewing and editing).

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

Nutritional status before pregnancy	BMI (kg/m 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

496 *Source: Institute of Medicine (IOM-2009)*

497 **Table 2** - Univariate analysis with the characterization of pregnant women evaluated in 2020,
 498 Ouro Preto - MG, Brazil

Variables	N	%	Pregnant women		<i>P-value</i>
			Uninfected (n = 71)	Infected by <i>T. gondii</i> (n=60)	
Socioeconomic variables					
Age					
Adolescents (<18 years)	10	7.63	7	3	0.343 ^{&}
Adults (>18 years)	121	92.37	64	57	
Education					
< Complete high school	55	41.98	28	27	0.52*
> Complete high school	76	58.02	43	33	
Employment					
Unemployed	1	0.76	0	1	0.532 ^{&}
Employment bond	61	43.56	29	32	#
Student	15	11.45	10	5	0.252 ^{&}
Housewife	54	41.22	32	22	0.262 ^{&}
Income					
Up to 1 minimum wage	51	38.93	26	25	#
1-2 minimum wage	0	0	0	0	NA
2-3 minimum wage	69	52.67	38	31	0.657 ^{&}
>4 minimum wage	11	8.4	7	4	0.519 ^{&}
Habitation					
City	79	60.31	47	32	0.134*
District	52	39.69	24	28	
Environmental variables					
Access to treated water					
No	18	13.74	8	10	0.371*
Yes	113	86.26	63	50	
Access to sewer services					
No	14	10.69	7	7	0.739*
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	0.538 ^{\$}
No	43	32.82	28	15	0.407 ^{\$}
Animal waste disposed of in the house					
No	64	48.85	39	25	0.130*
Yes	67	51.15	32	35	
Gestational variables					
Gestational age					
First trimester	37	28.24	23	14	#
Second trimester	49	37.40	24	25	0.224 ^{\$}
Third trimester	45	34.35	24	21	0.421 ^{\$}
Pregnancy					

Primigravida	53	40.46	36	17	0.009*
Multigravida	78	59.54	35	43	
Neuro-ocular complications in the firstborn					
Yes	8	6.11	2	6	0.142 ^{&}
No	123	93.89	69	54	
Pre-gestational BMI					
Not Overweight	55	41.98	34	21	0.136*
Overweight	76	58.02	37	39	
Gestational weight gain					
Eutrophic	43	35.11	24	22	#
Low	44	33.59	27	17	0.379 ^{\$}
Excessive	41	31.30	20	21	0.752 ^{\$}
Clinical variables					
Parasitological examination of stools (N=78)					
Negative	67	85.90	40	27	0.056 ^{&}
Positive	11	14.10	3	8	
Blood pressure					
Normotensive	115	89.84	61	54	#
Hypertensive	5	3.91	1	4	0.148 ^{\$}
Hypotensive	8	6.25	8	0	NA
Dietary variables					
Handwashing before food preparation					
No	7	5.34	6	1	0.124 ^{&}
Yes	124	94.66	65	59	

Footnote: *Pearson's Chi-square test ^{\$}Chi-square partition test [&]Fisher's exact test. # Reference class
NA – not applicable when variables correspond to zero; IPE - Individual Protection Equipment

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	<i>T. gondii</i> -infected pregnant women variables			
	OR (IC 95%)	<i>P</i> -value	ORa (IC 95%)	<i>P</i> -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				
Primigravida (child-free)	1	0.010	1	0.018*
Multigravida	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. *Teste Hosmer-Lemeshow $R=0.865$.