

**Association between seroprevalence of IgG anti-*Toxoplasma gondii* and risk factors for infection among pregnant women in Climério de Oliveira Maternity, Salvador, Bahia, Brazil**

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**ABSTRACT**

Maternal *Toxoplasma gondii* (*T. gondii*) infection can lead to spontaneous abortion, fetal malformation, and numerous sequelae in the newborn, including visual and hearing impairment. *T. gondii* serology was determined in pregnant women and their newborns who were referred to *Climério de Oliveira Maternity*, Federal University of Bahia, and the possible associations with the risk factors for maternal infection were investigated. This cross-sectional study was conducted from January to December 2014 and included 712 pregnant women, aged 15 to 49 years, and 235 newborns. Seroprevalence was determined by the detection of *T. gondii*-specific immunoglobulin (Ig) M (IgM) and IgG antibodies using enzyme-linked immunoassay. In addition, mothers completed a questionnaire that focused on socioeconomic aspects, and survey data were tabulated using the SPSS statistical software program (version 21.0 for Windows). The seroprevalence rate for specific IgG antibodies was 51% (362) among mothers and 93% (219) among neonates. All the mothers and their newborns were negative for specific-IgM antibodies. Higher age group and lower maternal education were the only risk factors statistically associated with the presence of specific IgG. In summary, high levels of specific IgG antibodies were observed in pregnant women. The knowledge of risk factors for toxoplasmosis can help in the orientation of newly pregnant women.

**KEYWORDS:** Toxoplasmosis. Prevalence. Infection. Pregnant women.

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**INTRODUCTION**

The protozoan *Toxoplasma gondii* (*T. gondii*), causative agent of toxoplasmosis, was first identified at the Pasteur Institute in Tunis, Tunisia, by the French researchers Nicolle and Manceaux and in Brazil by Splendore, in 1908, according to Weiss and Dubey<sup>1</sup>. This intracellular parasite can infect different tissues in a variety of vertebrate animals, including humans<sup>2</sup>. The ability of this parasite to multiply within all nucleated cells of hosts is noteworthy<sup>3</sup>. Members of the *Felidae* family are considered definitive hosts, whereas birds, livestock, and the great majority of homoeothermic vertebrates are intermediate hosts<sup>4</sup>. In addition to being a zoonosis, toxoplasmosis is a systemic infectious disease. It is associated with all continents and most types of climate, with a higher prevalence in hot and humid climate<sup>5</sup>. This infection can be transmitted by the passage of one of the forms of *T. gondii* (i.e., oocysts, bradyzoites, and trophozoites) through inhalation, ingestion, transfusion, and organ transplantation from one individual to another or vertically from the infected mother to the fetus or newborn<sup>6</sup>. Depending on the immunological

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competence of the host, signs and symptoms may range from absent to severe. Immunocompetent individuals usually present an initial or acute phase of asymptomatic disease<sup>7</sup>. The percentage of these individuals varies between 80% and 90%. Nevertheless, in immunocompromised individuals, disease manifestations can be severe<sup>8</sup>.

The occurrence of a *T. gondii* infection during gestation deserves special attention from public health agencies, whether individually or collectively. Pregnant women who are *T. gondii* seronegative raise the greatest concern. A first-time *T. gondii* infection during the gestational period without the protection of specific IgG antibodies may compromise embryogenesis, fetal development and increase neonatal morbidity and mortality. According to Higa *et al.*<sup>9</sup>, the prevalence of infection in the population and the frequency of contacts with possible sources of contamination are factors associated with acquisition of the infection. Remington *et al.*<sup>10</sup> have suggested the possibility of vertical transmission of toxoplasmosis resulting from a reactivation of the disease in chronically infected mothers caused by the expressive reduction of immunity. Immunocompetent people are usually asymptomatic. Thus, infection in pregnant women often goes undetected, and a possible maternal-fetal transmission may be recognized with the appearance of early or late sequelae in the neonate<sup>11</sup>.

This work aimed to establish the seroprevalence for toxoplasmosis in mothers and their respective newborns who were referred to the *Climério de Oliveira Maternity* (MCO), Federal University of Bahia (Salvador-Bahia), Brazil, from January to December 2014, and to associate maternal seroprevalence with risk factors of infection.

## MATERIAL AND METHODS

The present study is part of a project entitled “Agents of congenital infection in pregnant women in Salvador, Bahia: research on the prevalence and analysis of risk factors.” This study was approved by the Ethics Committee of the Federal University of Bahia (UFBA), School of Nursing, in 2013, under the protocol N° 384.209.

This cross-sectional study was carried out from January to December 2014 at the *Climério de Oliveira Maternity*, UFBA, Salvador, Bahia. The study participants were 712 pregnant women from Salvador and other municipalities in the State of Bahia, assisted by the Unified Health System (SUS), and 235 newborn infants (NB) from seropositive mothers. Pregnant women with insufficient blood samples to perform tests were excluded from the study.

Pregnant women were informed on the nature of the study and those who agreed to participate signed the

consent form and completed the questionnaire addressing socioeconomic aspects and risk factors of contracting *T. gondii* infection.

For the detection of IgG antibodies, a third-generation indirect enzyme-linked immunoassay (recombinant antigen) was used, and IgM was detected according to the manufacturer’s instructions (Symbiosys, Leme, *State of São Paulo*, Brazil). Optical densities were measured by using the 450-nm and 630-nm filters and the ALISEI automated system (Seac Radim, Italy).

After data tabulation, SPSS statistical software (version 21.0 for Windows) was used for analyses. The normality of continuous data was evaluated using the Kolmogorov-Smirnov test. The chi-square test was used for comparisons between proportions, and the level of statistical significance was set at  $p \leq 0.05$ .

## RESULTS

All the participants (712 pregnant women aged 15 to 49 years and 235 newborns) presented negative anti-*T. gondii* IgM antibodies. Of the total maternal sera analyzed, 362 were positive for specific IgG and 350 were negative, resulting in a seroprevalence rate of 51%.

Among two hundred and thirty-five newborns of seropositive mothers, IgG anti-*T. gondii* was positive in 219 (93%) and negative in 16 (7%).

Table 1 shows that the presence of anti-*T. gondii* antibody was positively associated with the level of education and the age group of pregnant women who participated in the study.

## DISCUSSION

The seroprevalence of 51% for *T. gondii* infections found in pregnant women is consistent with the results of Dubey *et al.*<sup>12</sup>. These authors mentioned a variation of anti-*T. gondii* IgG seroprevalence in pregnant women, in the Brazilian regions, ranging from 50% to 80%. In 2002, Nascimento *et al.*<sup>13</sup> carried out a study in pregnant women who attended SUS in Salvador, came from different cities of Bahia and reported a seroprevalence of 64.9%. However, the seroprevalence in pregnant women from Porto Alegre was 59.8% as reported in 2003 by Varella *et al.*<sup>14</sup>. In 2012, in the city of Aracaju, Sergipe, Tavares *et al.*<sup>15</sup> reported a IgG anti-*T. gondii* seroprevalence of 43.5% in pregnant women who attended SUS, which was a much lower rate than the ones reported by previously mentioned studies. Prevalence rates also differ between countries. Flatt and Shetty<sup>16</sup> reported, in a study published in 2012, an IgG seroprevalence rate of 17.32% for *T. gondii* in pregnant

**Table 1** - Anti-*T. gondii* IgG in pregnant women versus risk factors for infection

Risk factors for infection by <i>T. gondii</i>	Dosage IgG anti- <i>T. gondii</i>				p-value
	Positive	Negative	Total	OR (95% CI)	
<b>Do you breed dogs and/or cats?</b>					0.365
Yes	182 (51.6%)	171 (48.8%)	353		
No	168 (48.1%)	181 (51.9%)	349		
<b>Do you consume undercooked meat?</b>					0.678
Yes	88 (58.5%)	84 (48.8%)	172		
No	264 (49.3%)	269 (50.7%)	531		
<b>Contact with soil / manure?</b>					0.170
Yes	86 (58.5%)	61 (41.5%)	147		
No	264 (47.5%)	292 (52.5%)	556		
<b>Education</b>					
Complete 3rd grade	12 (38.7%)	19 (61.3%)	31		
Incomplete 3rd grade	19 (38.8%)	30 (61.2%)	49		0.995
Complete 2nd grade	159 (49.1%)	165 (50.9%)	324		0.272
Incomplete 2nd grade	59 (46.5%)	68 (53.5%)	127		0.497
Complete 1st grade	25 (46.3%)	29 (53.7%)	54		0.149
Incomplete 1st grade	74 (63.8%)	42 (36.2%)	116		0.010*
Illiterate	2 (100.0%)	0 (0.00%)	2		0.197
<b>Family Income (minimum wage)</b>					0.330
< 1	20 (43.5%)	26 (56.5%)	46		
1 – 3	298 (51.0%)	286 (49.0%)	584		
4 a 6	30 (47.6%)	33 (52.4%)	63		
> 6	2 (25.0%)	6 (75.0%)	8		
<b>Place of residence</b>					0.331
Capital	303 (49.1%)	314 (50.9%)	617		
Interior	46 (54.8%)	38 (45.2%)	84		
<b>Age Range (years)</b>					
15 – 24	99 (39.6%)	151 (60.4%)	250	Ref.	
25 – 34	172 (53.6%)	149 (46.4%)	321	1.76 (1.25 – 2.46)	< 0.010*
35 – 44	74 (59.7%)	50 (40.3%)	124	2.26 (1.45 – 3.50)	< 0.010*
≥ 45	5 (62.5%)	3 (35.5%)	8	2.54 (0.59 – 10.87)	0.208

**Source:** Research data. \*Significant association ( $p < 0.05$ )

women in London. In Kinshasa, the Democratic Republic of Congo, this rate was 80.3%, according to the study of Yobi *et al.*<sup>17</sup>. The specific IgG seroprevalence reported by Yad Yad *et al.*<sup>18</sup> in a study involving pregnant women in the Southern province of Khuzestan, Iran, was 27.3%.

According to Yobi *et al.*<sup>17</sup>, the hygienic conditions in the environment contribute to a lesser or greater exposure to the parasite and, by extension, to the seroprevalence in each region.

As expected, in this study, there was a high seropositivity rate of anti-*T. gondii* IgG in the newborn infants of

seropositive pregnant women (93%) due to the passage of these maternal antibodies through transplacental routes.

The lack of the detection of anti-*T. gondii* IgM among the neonates indicates that there was no vertical transmission of toxoplasmosis. This finding is consistent with the fact that specific IgM was not detected in any of the pregnant women who participated in the study, an indicator of a *T. gondii* primary infection or reactivation, which could increase the risk of vertical transmission. Andrade *et al.*<sup>19</sup> reported a case of vertical transmission in a mother who had retinochoroiditis due to *T. gondii* reactivation during

the gestational period, representing a rare case of congenital infection.

In the analysis of the risk factors, an association between anti-*T. gondii* IgG seropositivity and dog and/or cat breeding was not demonstrated. Our finding corroborates data of Flatt and Shetty<sup>16</sup>, involving 2,610 pregnant women in the city of London, United Kingdom, and Porto *et al.*<sup>20</sup>, involving 503 pregnant women attending a maternity ward in Recife, State of Pernambuco, Brazil, between October 2004 and April 2005. Although Tavares *et al.*<sup>15</sup> reported a positive association in a study involving 395 pregnant women from Aracaju, State of Sergipe, Brazil, who attended SUS in 2011.

The habit of ingesting undercooked meat was not a risk factor for toxoplasmosis, which agrees with the study of de Quadros *et al.*<sup>21</sup>, involving 148 pregnant women. However, an outbreak of toxoplasmosis due to the ingestion of raw meat occurred in the city of São Paulo, State of São Paulo, Brazil, where six patients acquired acute toxoplasmosis, including a pregnant woman; despite maternal treatment, fetal death occurred<sup>22</sup>.

In our study, the habit of handling soil or manure did not constitute a risk factor for toxoplasmosis as evidenced by antibodies against *T. gondii*. The difference in the seropositivity of the pregnant women who handled these materials (58.5%) and those who did not (47.5%) was not statistically significant. A similar result was demonstrated in a study by Porto *et al.*<sup>20</sup> who reported that pregnant women who had a habit of handling soil (16.8%) versus those who did not (24.5%) did not generate a statistical significance ( $p = 0.07$ ).

Regarding the family income, the seropositivity of participants with a family income below one minimum wage (43.5%) was similar to those whose income was greater than six minimum wages (25%). A similar result was reported by Rhodes and Shetty<sup>16</sup> but they argued that the difference was not statistically significant because only eight patients had income of more than six minimum wages. It was not possible to indicate in this research whether the family income increment would make a difference in this population.

As for the place of residence, Yad Yad *et al.*<sup>18</sup> reported no significant differences in the specific IgG seroprevalence in a rural area ( $p < 0.5$ ) versus an urban area ( $p = 0.06$ ). These results are possibly due to the scarcity of studies involving these variables, given that most of the work is carried out in developed countries where social equality is more prevalent.

*T. gondii* seropositivity showed a negative association with educational level, with a decreasing tendency of seropositivity as the level of education increased (Table 1) and revealed a significant statistical correlation ( $p = 0.01$ ). This finding is in line with the results of Varella *et al.*<sup>14</sup> and Porto *et al.*<sup>20</sup> ( $p = 0.0013$ ); both studies demonstrated

lower rates of toxoplasmosis seropositivity in pregnant women with eight or more years of education (26.1%) than in those with up to seven years of study (12.6%). On the other hand, de Quadros *et al.*<sup>21</sup> demonstrated that there were no differences for specific IgG production among pregnant women who had not completed primary education compared to those who had completed high school. This divergence probably reflects differences in habits of the studied populations and the negative association could be explained by better hygiene habits and a consequent reduction in the degree of exposure to *T. gondii*. Pregnant women with a higher educational level have better access to information.

This study demonstrated a IgG anti-*T. gondii* seroprevalence of 51% in pregnant women and of 93% in their newborn infants. There was no detection of serological markers of primary maternal infection (IgM) in pregnant women included in the study. Among the neonates of these chronically infected mothers, none had anti-*T. gondii* IgM. Risk factors that had a statistically significant association with *T. gondii* infection were education level and age group. The fact that several risk factors were not associated with *T. gondii* infection might depend on characteristics and habits of different populations.

Besides contributing with important information about the vertical transmission of toxoplasmosis, risk factors involved and adoption of preventive measures, this study raises a question about the seronegative status of 7% of the newborn infants of IgG-positive mothers. Although IgG can cross the placenta, not all subclasses (e.g., IgG1, IgG2, IgG3, and IgG4) are equally efficient in this process; for example, IgG2 does not easily cross the placenta. This curious finding deserves further investigation. It is noteworthy that not all newborns of mothers seropositive for IgG anti-*T. gondii* tested positive for this serological marker. This finding shows that there is not always a transplacental transfer of maternal IgG to the fetus, which can be explained by the different structural and functional characteristics of IgG subclasses, such as the ability to cross the placenta. The IgG2 isotype is least effective at crossing the placental barrier. Although the lack of IgG maternal transmission to the fetus could be related to the antibody titer; that is, women who had higher IgG concentration would be more likely to generate a naturally acquired passive immunity in their newborns<sup>23</sup>. Future studies are needed to clarify these issues.

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