Seroprevalence and risk factors of *Toxoplasma gondii* infection among pregnant women

Gholamreza Barzgar1 #, Ehsan Ahmadpour2 #, Mohammad Hasan Kohansal3,4, Sirous Mehrani Moghaddam2, Tohid Jafari Koshki5, Aleksandra Barac6, Veeranoot Nissapatorn7, Alok K Paul8, Jelena Micić9

1 Department of Parasitology and Mycology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2 Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3 School of Medicine, Bam University of Medical Sciences, Bam, Iran
4 Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
5 Department of Statistics and Epidemiology, Faculty of Health and Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran
6 Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia
7 School of Allied Health Sciences and Research Excellence Center for Innovation and Health Products (RECIHP), Walailak University, Nakhon Si Thammarat, Thailand
8 School of Pharmacy and Pharmacology, University of Tasmania, Hobart, TAS 7001, Australia
9 Clinic for Gynecology and Obstetrics, Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

# Authors contributed equally to this work.

**Abstract**

Introduction: *Toxoplasma gondii* is an obligate intracellular parasite affecting a broad range of warm-blooded animals, including humans. Infection acquired during pregnancy can be transmitted to the fetus and leading to serious problems such as spontaneous abortion, stillbirth, or severe mental and/or physical handicaps in the child. The purpose of this study was to investigate the seroprevalence of *Toxoplasma* infection and related risk factors in pregnant women.

Methodology: The study enrolled 1200 serum samples of pregnant women from February-November 2017. Then the samples were tested for the presence of anti-*T. gondii* antibodies (Ab) using enzyme-linked immunosorbent assay.

Results: Out of the 1200 samples, 381 (31.7%) and 41 (3.4%) subjects were positive for IgG and IgM Ab, respectively. Among the evaluated risk factors, the seroprevalence of *Toxoplasma* infection was not related to the occupation in a significant way. However significant relationship was observed with factors such as; contact with soil, cats, consumption of raw washed vegetables, and washed hands before meals.

Conclusions: According to the results, more than two-thirds of pregnant women are susceptible to *Toxoplasma* infection, hence training health care programs should be provided to prevent infection.

**Key words:** *Toxoplasma gondii*; seroprevalence; congenital; ELISA.


Copyright © 2024 Barzgar et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Introduction**

Toxoplasmosis is a zoonotic disease caused by the obligate intracellular parasite *Toxoplasma gondii* (*T. gondii*) [1]. Members of the feline family are considered final hosts, while other vertebrates, such as birds and livestock, serve as intermediate hosts [2-3]. The disease can be acquired through various routes, including ingestion of uncooked meat containing tissue cysts, ingestion of oocysts, congenital transmission, and organ transplants [2,4,5]. Toxoplasma infection is usually asymptomatic; however, it can develop into acute, chronic, or symptomatic forms depending on the individual’s immune status [6-7]. Since infection acquired during pregnancy can be transmitted to the fetus, sometimes the disease displays serious symptoms. Clinical symptoms of congenital infection include stillbirth, microcephaly, hydrocephalus, miscarriage, and severe fetal neurological disorders [8-10]. Studies have estimated that at least one-third of the world’s population has a positive immune response to
Toxoplasma gondii [11]. The prevalence rates of infection vary in different parts of the world and depend on a variety of factors, such as dietary habits, host susceptibility, geographic location, and culture [12-13]. It is indeed important to recognize Toxoplasma infection before or in the early stage of conception to prevent or decrease the risk of congenital infection. Serological tests are routinely used for determining Toxoplasma-specific IgG and IgM antibodies (Ab) [14-16]. Due to the worldwide distribution of T. gondii and its potential for opportunistic infection, it is necessary to assess the prevalence of the infection and its associated risk factors. Therefore, we investigated the rate of anti-Toxoplasma seropositivity and the related risk factors in pregnant women.

Methodology

Study design and data collection
The present cross-sectional study aimed to determine the seroprevalence of T. gondii in pregnant women. A total of 1200 pregnant women in their first trimester were randomly selected for the study. Structured questionnaires, consisting of simple closed questions, were used to collect demographic information and assess risk factors such as age, educational level, contact with cats, and consumption of unwashed raw vegetables.

Sample collection and questionnaire
The samples were collected from pregnant women (without any other manifestations of infectious or non-infectious diseases) between February and November 2017. Informed consent was obtained from all individuals participating in the study. Approximately 3 mL of blood sample was collected from the brachial vein of each participant (aged 18-42 years old) under sterile conditions using a venipuncture, disposable syringe, and needle. The blood samples were transferred to the parasitology department, where sera were separated and stored at -20 °C until analysis (Figure 1). Serological tests for T. gondii were performed by enzyme-linked immunosorbent assay (ELISA) with the Pishtaz Teb Commercial Kit (Pishtaz Teb Diagnostics, Tehran, Iran) (cut-off > 0.15) following the manufacturer’s instructions.

Data analysis
Data analysis was performed using SPSS software (Version 11.0, Chicago, IL, USA) to assess the relationship between infection and potential risk factors. Bivariate analysis by chi-square test and Fisher test was used to estimate the association between seropositivity and risk factors. A p-value of less than 0.05 (p < 0.05) was considered statistically significant.

Ethics approval
Ethical approval for this study was obtained from Bam University of Medical Sciences, Bam, Iran by number (IR.MUBAM.REC.1399.015) in 2020.4.25.

Results
Out of the 1200 serum samples (mean age: 30 ± 12 years old), 381 (31.7%) tested positive for anti-T. gondii IgG Ab, and 41 (3.4%) samples were positive for anti-T. gondii IgM Ab (Table 1). The highest seroprevalence (38.73%), was found in women aged between 28 and 32 while those under 22 exhibited the lowest seroprevalence (16.35%).

Table 1. Seropositivity of Anti-Toxoplasma IgG and IgM antibodies from ELISA in different age group.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number (N)</th>
<th>IgG positive N (%)</th>
<th>IgG negative N (%)</th>
<th>IgM Positive N (%)</th>
<th>IgM negative N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-22</td>
<td>214 (17.8)</td>
<td>35 (16.35)</td>
<td>179 (83.65)</td>
<td>5 (2.33)</td>
<td>209 (97.66)</td>
</tr>
<tr>
<td>23-27</td>
<td>274 (22.8)</td>
<td>83 (30.29)</td>
<td>191 (69.71)</td>
<td>9 (3.28)</td>
<td>265 (96.72)</td>
</tr>
<tr>
<td>28-32</td>
<td>284 (23.7)</td>
<td>110 (38.73)</td>
<td>174 (61.27)</td>
<td>16 (5.63)</td>
<td>268 (94.37)</td>
</tr>
<tr>
<td>33-37</td>
<td>207 (17.2)</td>
<td>75 (36.23)</td>
<td>132 (63.76)</td>
<td>5 (2.41)</td>
<td>202 (97.58)</td>
</tr>
<tr>
<td>38-42</td>
<td>221 (18.4)</td>
<td>78 (35.29)</td>
<td>143 (64.71)</td>
<td>6 (2.71)</td>
<td>215 (97.29)</td>
</tr>
<tr>
<td>Total</td>
<td>1200 (100)</td>
<td>381 (31.75)</td>
<td>819 (68.25)</td>
<td>41 (3.41)</td>
<td>1159 (96.59)</td>
</tr>
</tbody>
</table>
The result of current study showed that *T. gondii* seroprevalence was not age-dependent (*p* > 0.05). Among the factors studied, hand washing showed a statistically significant association with positive anti-*Toxoplasma* IgG Ab in the study group (*p* < 0.001) (Table 2). Additionally, the consumption of raw vegetables, contact with soil, and cats showed significantly higher prevalence in exposed women (*p* < 0.001). Other demographic factors such as “education level” and “occupation” showed a *p* > 0.05 (Table 2).

**Discussion**

An epidemiological study was conducted to determine the seroprevalence of *T. gondii* infection and associated risk factors of pregnant women. The findings of the study showed that the seropositivity of anti-*Toxoplasma* IgG and IgM Ab was 31.7% and 3.41%, respectively. These results align with those reported by Soltani *et al.*, who found a prevalence of 32.95% for the seropositivity of anti-*Toxoplasma* IgG antibodies among women referred to clinics in southwest Iran [17]. Recent studies have reported infection rates of 74.5%, 58.3%, and 35.1% among reproductive-age women in Brazil, Turkey, and Sudan, respectively [18-20]. In Iran, the estimated prevalence of *Toxoplasma* infection in pregnant women is 41%, determined using a randomized model. Furthermore, IgG and IgM antibodies were obtained at rates of 38% and 4%, respectively [10]. In Iran, the highest and lowest incidences of *T. gondii* were reported in the Southern and Eastern parts, respectively [21]. The results of the current study compared to other studies conducted in Iran showed the prevalence of IgG and IgM Ab in this study were 6.3% and 0.59% lower than the average prevalence in Iran [10].

Anti-*Toxoplasma* IgG and IgM Ab are routinely screened using serological methods, particularly ELISA [21]. An increased anti-*Toxoplasma* IgG Ab can indicate either a primary infection or a reactivation of chronic toxoplasmosis, which is more common in immunocompromised patients (e.g., HIV patients, organ transplant recipients, and individuals with chronic diseases) and is associated with severe manifestations. On the other hand, Anti-*Toxoplasma* IgM antibodies, are a specific indicator of newly acquired infection [22-23]. As a result, anti-*Toxoplasma* IgG and IgM Ab testing is a common method for detecting chronic (latent) or acute (recently acquired) infection phases. Seronegative pregnant women who have not been exposed to Toxoplasma infection are also at risk, as antibodies are protective against congenital toxoplasmosis. Therefore, screening for anti-*Toxoplasma* Abs before pregnancy is required for disease prevention and control [10,23]. The age of women is an important factor in the consequences of infection. Therefore, the study groups consisted of women aged between 18 to 42 years, as this period corresponds to the highest pregnancy rate. The results of the present study showed the highest prevalence of anti-*Toxoplasma* IgG and IgM Ab in the 28-32 age groups. Previous Similar studies have also demonstrated correlation between higher seroprevalence and increasing, likely due to greater exposure as individuals grow older [15,22]. A study was conducted in Norway to update the prevalence of *Toxoplasma* IgG Ab among pregnant women. The result of the study showed a low prevalence of

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. Persons</th>
<th>No. Positive (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>778</td>
<td>238 (30.5)</td>
<td>0.77</td>
<td>0.60-0.98</td>
<td>0.03</td>
</tr>
<tr>
<td>College studies (ref.)</td>
<td>422</td>
<td>143 (33.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laborer</td>
<td>465</td>
<td>162 (34.8)</td>
<td>1.26</td>
<td>0.98-1.61</td>
<td>0.07</td>
</tr>
<tr>
<td>Non laborer (ref.)</td>
<td>735</td>
<td>219 (29.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cats in home or neighborhood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>405</td>
<td>150 (37)</td>
<td>1.52</td>
<td>1.18-1.97</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No (ref.)</td>
<td>795</td>
<td>221 (27.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact with soil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>280</td>
<td>116 (41.4)</td>
<td>1.74</td>
<td>1.32-2.31</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No (ref.)</td>
<td>920</td>
<td>265 (28.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consumption of raw washed vegetables with disinfectant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>201</td>
<td>88 (43.7)</td>
<td>1.87</td>
<td>1.37-2.57</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No (ref.)</td>
<td>999</td>
<td>293 (29.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand washing before meals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>921</td>
<td>249 (27)</td>
<td>0.41</td>
<td>0.31-0.54</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No (ref.)</td>
<td>279</td>
<td>132 (47.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Toxoplasma infection in women is influenced by various factors, including climate, personal hygiene, and diet. Studies have shown that the prevalence of Toxoplasma infection varies in different regions of Iran, with Tabriz province located in the northwest having a lower infection rate compared to other regions with colder climates [21]. The risk of infection is also affected by demographic factors such as age, educational level, and the habit of hand washing after contact with soil. The presence of domestic cats and the consumption of raw vegetables are significant risk factors [25].

In the study conducted in Tabriz, 35.6% of pregnant women were found to be seropositive for Toxoplasma gondii. The results showed that women who consumed half-cooked lamb and raw vegetables were more likely to be seropositive compared to those who consumed fully cooked food [26]. Furthermore, the study revealed that women who washed their hands after contact with soil had a lower seroprevalence of Toxoplasma infection [25].

The findings suggest that soil exposure, cats, and poor personal hygiene may significantly increase the risk of infection. Understanding Toxoplasma risk factors may be beneficial for the prevention and control of the infection.

Conclusions

The need for global efforts to educate women about the potential of T. gondii transmission and the adverse effects of this parasitic disease is necessary, especially before meals. Washing hands before meals is an important factor that increases the risk of Toxoplasma contamination. The percentage of contaminated hand washers (27%) was much lower than that of non-washers (47.3%). According to previous studies, the seroprevalence of infection in Tabriz was lower than the average infection rate in Iran (39.3%) [21]. A study conducted in 2014 in east Iran revealed a 29.35% prevalence of toxoplasmosis in pregnant women [24].

The climate parameter is another important factor in Toxoplasma prevalence. The appropriate condition for oocyst sporulation and survival in the environment plays an important role in the infection rate and disease burden. Iran has seasonal variation so the weather condition varies in the provinces of this country. Studies have shown that a low prevalence of Toxoplasma infection belongs to cold and hot climates and a high prevalence belongs to wet and mild climates. Tabriz province is located northwest of Iran with cold climates which might be one of the reasons contributing to the low prevalence Toxoplasma infection [21,25].

Furthermore, our findings revealed that the proportion of Toxoplasma in women exposed to contaminated vegetables was higher (43.7%) than in women who were not exposed (29.3%). A study conducted in Mexico City found a significant increase in the number of women who consumed half-cooked lamb and raw vegetables compared to those who consumed it fully cooked [26]. Mizani et al. found the same results in Iranian women’s systematic review and meta-analysis [25]. Another study was conducted in northern Ethiopia to evaluate seroprevalence and risk factors of Toxoplasma gondii among pregnant women. Results of the study showed 35.6% of pregnant women were found to be positive for Ab specific to T. gondii. The most significant risk factors associated with T. gondii were age, educational level, the habit of hand washing after contact with garden soil or domestic animals, presence of domestic cats, history of contact with domestic dogs, and consumption of raw vegetables [27].

Also, the study surveyed Tanzania to evaluate risk factors of toxoplasmosis. Overall T. gondii seroprevalence was reported at 44.5%. Two risk factors including consumption of raw and having regular contact with soil were both associated with T. gondii Ab status [28]. Toxoplasma infection can also be contracted by eating unwashed vegetables or drinking water that has been contaminated with soil or exposed to cat oocysts. Variations in Toxoplasma seroprevalence are related to the dietary habits of any given population [25]. Indeed, seronegative women during pregnancy are vulnerable to toxoplasmosis infection, which can be fatal if raw vegetables are consumed. As a result, some demographic factors such as climate, general knowledge, and eating habits are linked to T. gondii seroprevalence [15]. More research is required to complete the information flow, and a larger sample size should be implemented in multiple organizations. One-third of the pregnant women in Tabriz tested positive for T. gondii infection. Despite this, the absence of Ab in the sera of 68.3% of study participants raised concerns about congenital toxoplasmosis during pregnancy. Given the low seroprevalence of IgG and IgM Ab in the area, pregnant women must be aware of the disease's risk factors in order to reduce their risk of infection.

Conclusions

The findings suggest that soil exposure, cats, and poor personal hygiene may significantly increase the risk of infection. Understanding Toxoplasma risk factors may be beneficial for the prevention and control of the infection.

Acknowledgements

We are thankful to our colleagues and laboratory coworkers who provided expertise that greatly assisted the research and all the participants.

Authors’ contributions

The study was designed and directed by Ehsan Ahmadpour, Aleksandra Barac, Veeranoot Nissapatorn and Mohammad Hasan Kohansal. Material preparation and sample collection were performed by Sirous Mehrani Moghaddam, Gholamreza Barzgar and Mohammad Hasan Kohansal. Data analysis was performed by Tohid Jafari Koshki, Alok K. Paul and Jelena Micić. The first draft of the manuscript was written by Mohammad Hasan Kohansal and Ehsan Ahmadpour and reviewed by, Aleksandra Barac, Veeranoot Nissapatorn, Alok K. Paul and Jelena Micić. All authors read and approved the final manuscript.

References


Corresponding authors
Mohammad Hasan Kohansal
School of Medicine, Bam University of Medical Sciences,
Bam, Iran
Tel: +98 413 5428595,
Fax: +98 413 337 3745
Email: kohansalhasan@gmail.com

Jelena Micić
Clinic for Gynecology and Obstetrics,
Clinical Center of Serbia, Faculty of Medicine,
University of Belgrade,
Belgrade, Serbia
Email: jdmicic@yahoo.com

Conflict of interests: No conflict of interests is declared.