Seroprevalence of *Toxoplasma gondii* among general population in Bosnia and Herzegovina: 10-Years single center experience

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**A R T I C L E   I N F O**

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Toxoplasma gondii  
Seroprevalence  
Risk factors  
Women of reproductive age

**A B S T R A C T**

Objectives: The aim of this research was to determine the seroprevalence of *Toxoplasma gondii* in Bosnia and Herzegovina, with special reference to women of reproductive age in Bosnia and Herzegovina.

Methods: The retrospective cross-sectional study included 3685 participants tested at the Department of Microbiology and Molecular Diagnostics, University Clinical Hospital Mostar in the period from January 1, 2010 to December 31, 2019. The detection of IgG and IgM antibodies to *T. gondii* was based on the enzyme immuno-fluorescence test.

Results: In this study the total IgG prevalence of *T. gondii* was 18.7%, while only 1.7% were IgM positive. The IgG prevalence increased according to age of participants from 1.8% in 1-5 age group to 46.8% in participants older than 65 (p < 0.001). Out of total IgG positive participants, 62.8% came from rural areas (p < 0.001). An increased age (OR = 2.233) and rural residence (OR = 1.468) were found as risk factors for toxoplasmosis, whereas gender was not (OR = 1.021). Out of total 1514 women of reproductive age tested, the IgG prevalence of *T. gondii* was 15.8%, and IgM antibodies was present in 1.6% examined women.

Conclusions: This study showed a seroprevalence of *T. gondii* in Bosnia and Herzegovina, not only among the general population, but also among women of reproductive age. Results of the study suggest the need for more systematic preventive monitoring of pregnant women and preconception counselling for women of reproductive age.

1. Introduction

Toxoplasmosis represents an infestation by protozoan and intracellular parasite *Toxoplasma gondii* (*T. gondii*), and is one of the most prevalent zoonoses in humans.  

Although toxoplasmosis is usually asymptomatic, it can cause severe and life-threatening illness in some cases. Acute toxoplasmosis occurs after a few days of incubation and presents with symptoms similar to the flu (fever, fatigue, muscle pain, sore throat, and headache) or mononucleosis-like symptoms, often accompanied by myalgia and adenopathy.  

High-risk groups include pregnant women and immunocompromised individuals such as organ transplant recipients, cancer patients, and HIV-positive patients.  

If the infestation occurs during pregnancy, the *T. gondii* can be transferred vertically from the mother to the fetus, which can ultimately result in the development of congenital malformations. The appearance of congenital malformations mostly depends on the gestational age. Although vertical transmission most often occurs in the third trimester of pregnancy, the most severe clinical symptoms occur if the transmission happened in the first or second trimester. Symptoms of congenitally infected children vary from subclinical to very serious, such as chorioretinitis, intracranial calcification and hydrocephalus. Earlier studies also indicate a connection between *T. gondii* infestation and habitual abortions. Moreover, the data showed that women with a higher titer of antibodies, and especially those in whom *T. gondii* was isolated from the endometrium or placenta, had a greater tendency to repeated abortions.  

Serological diagnostics are one of the most commonly used methods for *T. gondii* screening. In humans, IgM antibodies to *T. gondii* are an early marker of the acute phase of disease and can be detected one week after infestation. However, given that IgM antibodies can persist for several months and years, and in pregnant women up to 18 months after an acute disease, this serological finding must be combined with...
epidemiological data on possible exposure to this pathogen. \(^8,9\) IgG antibodies to *T. gondii* appear 1–2 weeks after infestation with a peak 1–2 months after infestation and have a tendency to survive for life, although there is a gradual decrease in the level of the antibody titers over time. \(^3\)

The *T. gondii* seroprevalence varies worldwide, ranging from 16 to 40% in the United States, to 30% in the United Kingdom, in central and southern Europe to 50%, and in continental Europe up to 80%. \(^5,10\) The seroprevalence of toxoplasmosis has been declining in the Balkan countries for the last 40 years. This trend was observed in Greece, Serbia, Croatia, Northern Macedonia and Montenegro, with an average drop in seroprevalence 5–10% among verified cases. \(^13,15\) The occurrence of toxoplasmosis depends on a number of factors including food, water and soil quality, socioeconomic status, geographic location, access to health care facilities, and many others. \(^7\) Environmental contamination with oocysts is widespread and is most often associated with cats, which can excrete millions of oocysts. Oocysts have a very good adaptation to the environment in which they are found, especially in moist soil, and they can survive in it for months or even years. \(^7\) The most common way for humans to become infected is through the consumption of undercooked meat infected with bradyzoites, unwashed fruits and vegetables, or oocyst-infested water, and close contact with cats or cat litter. \(^18\)

Prevention of *T. gondii* infestation can be achieved by implementing basic epidemiological measures, such as raising awareness about personal hygiene, washing hands and other surfaces after handling raw meat, thorough washing of fruits and vegetables, and regular cleaning of cats and their environments. \(^2,19\)

The aim of this study is to determine the seroprevalence of *T. gondii* infestation over a ten-year period among the general population of Bosnia and Herzegovina and its relationship with demographic factors including age, sex, and place of residence.

2. **Materials and methods**

2.1. **Study design**

This retrospective epidemiological study included a total of 3685 participants seeking care at the University Clinical Hospital Mostar, a tertiary medical center and a referral center for the southern part of Bosnia and Herzegovina. The research comprised participants of both genders and aged from 1 to 91 in the period from January 2010 to December 2019 residing in all areas of southern part of the Bosnia and Herzegovina.

2.2. **Determining the seroprevalence of *T. gondii***

Serological screening for the presence of specific IgM and IgG antibodies was performed by Enzyme-linked fluorescence assay (ELFA) for IgG and IgM antibodies in accordance to the manufacturer’s instructions. The IgG level of 8 IU/ml was reported as positive, and ranging from 4 to 8 IU/ml was reported as doubtful. For IgM, the level of 0.65 IU/ml was considered positive, and ranging from 0.55 to 0.65 IU/ml was considered doubtful. This test is performed by a fully automated VIDAS machine that also automatically performs the calculation and interpretation of the results.

2.3. **Statistical data analysis**

Descriptive and analytical statistical methods were used in data processing. Data are presented as mean ± SD or median and number (percentage) for categorical variables. A chi-squared or Fisher’s exact test was used for the analysis of categorical data, as appropriate. *T. gondii* infestation risk factors were analysed by logistic regression. Odds ratios (OR) with 95% confidence intervals (CI) were computed and the Hosmer–Lemeshow goodness-of-fit test was performed to assess overall model fit. The statistical analyses were performed using SPSS version 23.0 software (SPSS Inc., Chicago, IL, USA).

3. **Results**

In a 10-year period, a total of 3685 participants were analysed for toxoplasmosis seroprevalence. A prevalence of *T. gondii* IgG was 18.7% (p < 0.001), while 1.7% of participants were IgM positive (p < 0.001) (Table 1). The *T. gondii* IgG prevalence increased according to the age of participants, with the lowest seroprevalence of 1.8% among participants of preschool age (1–5 age group), 2.5% in the 6–12 age group, 8.2% in 13–19 age group, 15.7% in 20–34 age group, 29.8% in 35–64 age group and the highest of 46.8% among participants older than 65 years (p = 0.001). The highest prevalence of *T. gondii* IgM was 3.3% in the age group 13–19, while the lowest was in participants younger than 6 years old and older than 65 (p = 0.003). There was no statistically significant difference between males and females, both in the prevalence of *T. gondii* IgG (p = 0.773) or IgM (p = 0.773). Of the IgG-positive participants, 62.8% came from rural areas (p = 0.001), while there was no statistically significant difference in the prevalence of IgM antibodies according to place of residence (p = 0.796, Fischer exact test). In order to test the distribution of IgG and IgM antibodies in relation to gender, age, and place of residence, logistic regression was performed. The analysis confirmed increased age (OR = 2.233; 95% CI = 2.038–2.446, p = 0.003) and rural residence (OR = 1.468; 95% CI = 1.321–1.682, p < 0.001) as risk factors for the appearance of IgG antibodies, while no significance was found for IgM prevalence in neither of all three categories (Table 1).

In this study, special attention on toxoplasmosis, was given to the women of reproductive age. From a total of 1514 tested sera of women of reproductive age, recent infestation with positive IgM was present in 1.6%, and there were no statistically significant differences between women of different age groups (p = 0.762), as well as those from urban and rural areas (p = 0.131). The overall prevalence of IgG antibodies to *T. gondii* in women of reproductive age was 15.8%. Statistically significant differences between IgG prevalence were found if different age groups were compared (p = 0.001); 11.5% in the 15–25 age group, in the 26–35 age group was 16.5%, while the prevalence was 21.8% in the 36–45 age group. There were no significant differences in the IgG prevalence among areas of residence (p = 0.129) (Table 2).

4. **Discussion**

In this study, we obtained data from the most extensive research on the seroprevalence of *T. gondii* infestation among the general population in Bosnia and Herzegovina, with a special focus on women of reproductive age. In a 10-year retrospectively investigated period, the total prevalence of IgG and IgM antibodies to *T. gondii* was 18.7% and 1.7%, respectively. The lower prevalence of IgM, as an indicator of recent infestation, is most likely a consequence of the asymptomatic or oligosymptomatic manifestation of the disease and less testing for *T. gondii* in the acute phase of the infection. Given that infestation with *T. gondii* in Bosnia and Herzegovina is routinely tested as a cause of infectious mononucleosis and as part of the TORCH, it is much more often detected as a previous infestation with positive IgG antibodies. IgG prevalence increased according to the age of participants, from participants of 1–5 age group (1.8%) with the lowest to the highest prevalence (46.8%) among participants older than 65 years. The highest prevalence of IgM antibodies was in the age group 13–19 with no statistically significant difference in prevalence of IgG or IgM antibodies between men and women. Statistically significant more IgG-positive participants came from rural areas, while there was no statistically significant difference in the prevalence of IgM antibodies according to place of residence. Also, the increased age and rural residence were confirmed as risk factors for the IgG prevalence of *T. gondii*, while such a difference was not found for IgM prevalence. (Table 1).

Previous research on this topic in Bosnia and Herzegovina was
conducted on blood-donor participants in 2015. The total prevalence in that period was 30.6%, which is quite higher compared to the prevalence in women of reproductive age.

Table 1 
<table>
<thead>
<tr>
<th>Demographic variables</th>
<th>Toxo IgG</th>
<th>Toxo IgM</th>
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<tbody>
<tr>
<td>Gender</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Male</td>
<td>2167 (58.8)</td>
<td>403 (18.6)</td>
</tr>
<tr>
<td>Female</td>
<td>1518 (41.2)</td>
<td>287 (18.9)</td>
</tr>
<tr>
<td>Overall</td>
<td>3685 (100)</td>
<td>690 (18.7)</td>
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Table 2 
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<thead>
<tr>
<th>Age groups</th>
<th>Toxo IgG</th>
<th>Toxo IgM</th>
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<tbody>
<tr>
<td>1-5</td>
<td>285 (7.7)</td>
<td>5 (1.8)</td>
</tr>
<tr>
<td>6-12</td>
<td>319 (8.7)</td>
<td>8 (2.5)</td>
</tr>
<tr>
<td>13-19</td>
<td>511 (13.9)</td>
<td>42 (8.2)</td>
</tr>
<tr>
<td>20-34</td>
<td>1272 (34.5)</td>
<td>200 (15.7)</td>
</tr>
<tr>
<td>35-64</td>
<td>1114 (27.5)</td>
<td>302 (29.8)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>284 (7.7)</td>
<td>133 (46.8)</td>
</tr>
<tr>
<td>Residence area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>2058 (55.8)</td>
<td>446 (21.7)</td>
</tr>
<tr>
<td>Urban</td>
<td>1627 (44.2)</td>
<td>244 (15.0)</td>
</tr>
</tbody>
</table>

Higher prevalence in rural areas can be explained by lifestyle, greater contact with infected animals, and contact with infected soil and vegetables during farming. The absence of a statistically significant difference in acute phase antibodies is in accordance to the fact that in the majority of immunocompetent persons, the acute infestation is mostly asymptomatic, and the majority of those infected are not tested in the acute phase of the infestation. In accordance with these results, we performed binary logistic regression, which confirmed older age and place of residence as a risk factor for Toxoplasma gondii infestation, although none of these parameters has been identified to be a risk factor for the IgM prevalence.

This study presents results of the Toxoplasma seroprevalence among general population in the past 10 years in Bosnia and Herzegovina, which is comparable to neighbouring countries and the surrounding countries of South-eastern Europe. The majority of positive respondents came from rural areas and were over the age of 65. The increase in the seroprevalence of toxoplasmosis in older age groups can be explained by longer exposure to potential carriers and the source of infestation. Living in rural areas has been shown to be a significant risk factor for Toxoplasma gondii infestation, possibly as a result of exposure to animal species that are potential carriers of T. gondii cysts or as a result of exposure to contaminated and uncooked food. Altogether, the risk of acquiring T. gondii infestation among the general population, and especially among women of reproductive age, is clear considering the role of this pathogen in the development of congenital malformations.
derived from a single center performed on participants seeking care in referral hospital and may not represent the overall population of Bosnia and Herzegovina. Furthermore, it would be interesting to check some other parameters, such as the number of household members, number and type of pets, educational status and place of employment. Also, it would be beneficial to monitor the clinical manifestations of acute *T. gondii* infection in the general population, and also in pregnant women or women of reproductive age. Additionally, the study presented no data on the pregnancy status of women participants nor the number of pregnancies and abortions associated with recent or past past infection. Therefore, further prospective investigation including all these clinical and epidemiological parameters should be provided.

5. Conclusions

In this study, we performed data from the most extensive epidemiological retrospective research on the seroprevalence of *T. gondii* infection among the general population in Bosnia and Herzegovina, with a special focus on women of reproductive age. Our study shows a rate of 18.7% seroprevalence of toxoplasmosis among the general population and 15.8% among women of reproductive age. Results from the current study indicate both rural area residences and increasing age as risk factors for acquiring toxoplasmosis. Special attention should be paid to women of reproductive age, considering congenital malformations as a potential risk for women of reproductive age in Bosnia and Herzegovina.

Ethical statement

All procedures were conducted according to ethical statements, as defined in the 1964 by the Helsinki Declaration and its later amendments. Since it was a retrospective analysis of the data base, informed patients’ consent was not required and there was no potential risk for identification of participants.

Author’s contribution

BS: Concept and desing of study, Aquisisation of data, Analysis and/or interpretation of data, Drafting and revising the manuscript; KM: Concept and desing of study, Aquisisation of data, Analysis and/or interpretation of data, Drafting and revising the manuscript; SJ: Concept and desing of study, Aquisisation of data, Drafting and revising the manuscript; JA: Concept and desing of study, Analysis and/or interpretation of data, Drafting and revising the manuscript.

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Author disclosure statement

The authors declare that they have no conflict of interest.

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References