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PhD THESIS

**PREVALENCE OF TOXOPLASMA GONDII INFECTION IN
WOMEN OF REPRODUCTIVE AGE FROM WESTERN
ROMANIA. THE ROLE OF ANTI-*TOXOPLASMA GONDII* IGA
ANTIBODIES IN THE DIAGNOSIS OF TOXOPLASMOSIS**

- A B S T R A C T -

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ABSTRACT

Recent epidemiological studies have shown that the protozoan *Toxoplasma gondii* has a global distribution, being widely spread among the female population in their fertile period of life. Transmission routes are varied, such as consumption of contaminated water, unwashed fruits or vegetables contaminated with *T. gondii* oocysts, consumption of contaminated meat that has not been sufficiently thermally prepared, containing cysts, blood transfusions, intravenous drug use and organ transplants.

The spread of *T. gondii* can be estimated by performing serological tests to detect anti-*T. gondii* antibodies, which, if present, demonstrate the existence of infection. A positive result obtained after determining immunoglobulin G (IgG) type antibodies against specific *T. gondii* antigens indicates past contact with the parasite, as well as a degree of risk in case of immunosuppression, of the parasite transitioning from the latent cyst state to the active form, with the possibility of affecting the fetus.

It is estimated that about one-third of the global population is infected with *T. gondii*. Seroepidemiological studies have shown that the prevalence of anti-*T. gondii* IgG antibodies varies depending on the geographical area studied. In Europe, the prevalence of this parasitic infection in the general population ranges between 10-50%, depending on the geographical area studied. In Romania, information on the seroepidemiology of toxoplasmosis is limited and epidemiological studies have been conducted on small population groups.

The research was carried out in three counties in western Romania: Arad, Bihor and Timiș. The seroprevalence of anti-*T. gondii* IgG antibodies was evaluated for the first time on large population groups of women of reproductive age.

Following the determination of seroprevalence, the sera of a group of women of reproductive age with detected anti-*T. gondii* IgG antibodies were investigated. Immunoglobulin M (IgM) and immunoglobulin A (IgA) anti-*T. gondii* were determined to evaluate the role of IgA in the diagnosis of toxoplasmosis.

This doctoral thesis is composed of 3 parts: the general part, the special part and the conclusions.

The general part includes information on the history of the parasite, the life cycle in definitive and intermediate hosts, the epidemiology of toxoplasmosis, including the transmission routes of *T. gondii* infection and seroprevalence, clinical forms of toxoplasmosis, serological diagnosis and lastly, the interpretation of anti-*T. gondii* antibodies in women of reproductive age.

The special part includes the motivation, purpose, objectives of the research, the materials and methods used for the research, the results obtained and their inclusion in current medical literature, as well as comparisons between the results obtained for women of reproductive age from the counties of Arad, Bihor, Timiș and the specialized literature. The last part consists of the analysis of IgA and IgM test results, as well as results of the avidity anti-*T. gondii* IgG antibodies in women with detectable anti-*T. gondii* IgG, followed by discussions on the results obtained.

The conclusions represent the last chapter of this thesis which highlights the personal contributions, the impact that this research may have on women of reproductive age, unresolved issues and directions in which research should be continued.

Research objectives

This research was carried out following two main objectives:

1. determining the seroprevalence of anti-*T. gondii* IgG antibodies in women of reproductive age from western Romania: Arad, Bihor and Timiș counties
2. evaluating the role of anti-*T. gondii* IgA antibodies in establishing the immunodiagnosis in women of reproductive age.

Determination of the seroprevalence of anti-*T. gondii* IgG antibodies in women of reproductive age from western Romania

Three seroepidemiological studies were conducted in Arad, Bihor and Timiș counties to assess the prevalence of *T. gondii* infection in women of reproductive age in each of the three counties, as well as a general evaluation of the prevalence of specific anti-*T. gondii* IgG antibodies in women of reproductive age from western Romania.

Determination of specific anti-T. gondii IgG antibodies in women of reproductive age from Arad county

The evaluation was conducted on 2626 women of reproductive age. The sera from the study group were tested for the presence of specific anti-*T. gondii* IgG antibodies using the chemiluminescence technique.

The seroprevalence of anti-*T. gondii* IgG antibodies was 41.16% (1081/2626) in the study group. Of the 1293 women from urban areas, 36.12% (467/1293) tested positive for anti-*T. gondii* IgG antibodies. The seroprevalence of IgG antibodies in women from rural areas was 46.06% (614/1333).

The results obtained indicated an increased prevalence of *T. gondii* infection in this population group, with significantly higher prevalence rates in rural areas. The evaluation indicated a trend of increasing seroprevalence with age.

Determination of specific anti-T. gondii IgG antibodies in women of reproductive age from Bihor county

The study was conducted on a group of 1935 women. The sera were tested to assess the presence of specific anti-*T. gondii* IgG antibodies using chemiluminescence technique.

The seroprevalence of anti-*T. gondii* IgG antibodies was 36.48% (706/1935) in the study group. Out of the 1299 women from urban areas, 30.95% (402/1299) were identified with IgG antibodies present. In the group of women from rural areas, the seroprevalence of IgG antibodies was 47.79% (304/636).

The results obtained in Bihor county indicated an increased prevalence of *T. gondii* infection compared to the European average. The prevalence of toxoplasmosis in rural areas was significantly higher than that recorded in urban areas.

Determination of specific anti-T. gondii IgG antibodies in women of reproductive age from Timis county

The study was conducted on a group of 1317 women. Testing to identify the presence of specific anti-*T. gondii* IgG antibodies was performed using chemiluminescence technique.

Of the 1317 women enrolled in the study, 46.09% (607/1317) tested positive for anti-*T. gondii* IgG antibodies. Among women from rural areas, the prevalence of *T. gondii* infection was 51.76% (339/655). Among women from urban areas, 40.48% (268/662) were detected with anti-*T. gondii* antibodies present.

This seroepidemiological research confirmed the existence of a high prevalence of toxoplasmosis in Timis county, which was significantly higher in rural areas compared to urban areas. Results indicated different seroprevalence rates depending on age groups, the seroprevalence tended to increase with age. The study conducted in Timis county also demonstrated that exposure to *T. gondii* infection begins in childhood.

Assessment of the prevalence of specific anti-T. gondii IgG antibodies in women of reproductive age from western Romania

The analysis was based on the results of seroepidemiological investigations carried out in the counties of Arad, Bihor and Timis between January 1, 2016 and February 1, 2019.

A total of 5878 women of reproductive age (15-45 years) from the counties of Arad, Bihor and Timiș were evaluated. To identify the presence of specific anti-*T. gondii* IgG antibodies, the chemiluminescence technique was used.

The overall seroprevalence of *T. gondii* was 40.73% (2394/5878). The women enrolled in the study were divided into six age groups: 15-19 years, 20-24 years, 25-29 years, 30-34 years, 35-39 years and 40-45 years. Of the 138 women in the 15-19 age group, 24.64% (34/138) tested positive for anti-*T. gondii* IgG antibodies. The *T. gondii* seroprevalence was 38.60% (325/842) in the 20-24 age group, 39.56% (845/2136) in the 25-29 age group, 41.62% (710/1706) in the 30-34 age group and 40.69% (330/811) in the 35-39 age group. Among women aged 40-45 years, 61.22% (150/245) were identified with IgG antibodies present. *T. gondii* seroprevalence was 34.94% (1137/3254) in women from urban areas and 47.9% (1257/2624) in those from rural areas (**Table 1**).

Table 1. Prevalence of anti-*T. gondii* IgG antibodies in women from Western Romania by age group and place of origin

Age group (years)	No. females tested positive/No. females investigated (%)		Total
	Urban	Rural	
15–19	7/59 (11,86%)	27/79 (34,18%)	34/138 (24,64%)
20–24	104/328 (31,71%)	221/514 (43%)	325/842 (38,6%)
25–29	360/1115 (32,29%)	485/1021 (47,5%)	845/2136 (39,56%)
30–34	397/1082 (36,69%)	313/624 (50,16%)	710/1706 (41,62%)
35–39	192/536 (35,82%)	138/275 (50,18%)	330/811 (40,69%)
40–45	77/134 (57,46%)	73/111 (65,77%)	150/245 (61,22%)
Total	1137/3254 (34,94%)	1257/2624 (47,9%)	2394/5878 (40,73%)

The results show that the seroprevalence of toxoplasmosis in western Romania is among the highest in Europe and exposure to *T. gondii* infection is significant from childhood.

The evaluation of the studies carried out in the counties of Arad, Bihor and Timiș revealed a significantly higher *T. gondii* seroprevalence in women of reproductive age from rural areas compared to those from urban areas.

The research results indicated different percentage rates of seroprevalence depending on age groups, prevalence of toxoplasmosis having an increasing trend with age .

Evaluation of the role of anti-*Toxoplasma gondii* IgA antibodies in the diagnosis of toxoplasmosis

A total of 1317 women of reproductive age (15-45 years) from Timiș County were evaluated. Testing for the identification of specific anti-*T. gondii* IgG antibodies was performed using the chemiluminescence technique, and the evaluation of specific anti-*T. gondii* IgM antibodies was carried out using the enzyme-linked fluorescence assay (ELFA). The ELISA test was used to identify anti-*T. gondii* IgA antibodies. The binding intensity of specific IgG antibodies to the multivalent *T. gondii* antigen was assessed using the avidity test by the ELFA technique.

Of the 607 study participants with positive results for IgG antibodies, 58 (9.56%) were identified with IgM and/or IgA antibodies present and the IgG avidity test was performed for these

individuals. The remaining 549 (90.44%) study participants did not have specific IgM and IgA antibodies and were considered chronically infected with *T. gondii*.

Among the women identified with anti-*T. gondii* IgG antibodies present, 8.90% (54/607) (95%CI: 6.88-11.43) tested positive for IgM antibodies, 1.65% (10/607) (95%CI: 0.90-3.01) for IgA antibodies, also 0.99% (6/607) (95%CI: 0.45-2.14) for both IgM and IgA antibodies.

The results of the IgG avidity tests showed that 12.07% (7/58) had low or equivocal avidity, as follows: 6.90% (4/58) had low avidity and 5.17% (3/58) had an equivocal result. Among the study participants who were identified with IgM and IgA antibodies present, 66.67% (4/6) had low or equivocal IgG avidity, compared to 6.25% (3/48) of women who tested positive for IgM antibodies and negative for IgA antibodies with low or equivocal IgG avidity ($p=0.001$).

A decreasing trend in IgA seroprevalence has been observed, in parallel with an increase in IgG avidity, from 75% (3/4) in women with low avidity (values ranging from 0.0 to 0.19) to 11.76% (6/51) in those with high avidity (≥ 0.3) ($p=0.01$). Of the 51 women identified with high avidity (≥ 0.3), only 11.76% (5/51) tested positive for IgA antibodies compared to 92.16% (47/51) of women with IgM antibodies present ($p<0.001$).

Serologic screening of women of reproductive age showed that anti-*T. gondii* IgA antibodies are rarely detected, compared to IgM antibodies. These results suggest that IgA testing should not be routinely performed as a screening method. However, in individuals with both IgG and IgM anti-*T. gondii* antibodies present, IgA testing may improve the detection rate of a recently acquired toxoplasmosis, considering that detectable titers of IgA and IgM argue are highly suggestive for a recent *T. gondii* infection.

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

There is limited information in the international literature on *T. gondii* seroprevalence in women of reproductive age. Most of the published studies included seroepidemiological assessments conducted in pregnant women. This thesis is the first research study in Romania that evaluates *T. gondii* seroprevalence in women of reproductive age (15-45 years) from three counties in the western part of the country (Arad, Bihor and Timis). Moreover, the role and importance of IgA testing as an adjunctive marker in serologic screening for *T. gondii* infection and the diagnosis of acute infection in women of reproductive age are investigated for the first time, with the objectives of the doctoral thesis being met.

In Romania, there is no implemented screening program and no obligation for official reporting of data on *T. gondii* infection in women of reproductive age and pregnant women. The information

available so far is limited. From this perspective, the present scientific research brings new and important data on the epidemiology of *T. gondii* infection in women of reproductive age in western Romania.

The main conclusions derived from the results presented in this doctoral thesis are the following:

1. the prevalence of *T. gondii* infection in women of reproductive age in western Romania (40.73%) is among the highest in Europe;
2. more than half of the women of reproductive age in western Romania are exposed to the risk of *T. gondii* infection and the consequences can be devastating if primary infection occurs during pregnancy;
3. *T. gondii* seroprevalence varies from one county to another, with the highest rate observed in Timis county (46.09%), followed by Arad county (41.16%) and Bihor county (36.48%);
4. there is a significant association between the environment of origin and the prevalence of *T. gondii* infection, with higher rates in women from rural areas compared to those from urban areas;
5. *T. gondii* seroprevalence increases with age, with significantly higher values in the 40-45 age group compared to the 15-19, 20-24, 25-29, 30-34, and 35-39 age groups;
6. Anti-*T. gondii* IgA antibodies are less frequently detected compared to IgM antibodies;
7. assessing the presence of IgA antibodies is not strictly necessary to be included in the serological screening for determining the immunological status against *T. gondii* infection;
8. a recent infection with *T. gondii* is more likely to be diagnosed in women with both IgM and IgA antibodies present, compared to women in whom only IgM antibodies have been identified;
9. testing for IgA can significantly improve the detection rate of a recently acquired *T. gondii* infection;

The results of the studies conducted in this doctoral thesis provide an overview of the magnitude of the prevalence of *T. gondii* infection among women of reproductive age in western Romania and can contribute to the development of health policies at the local as well as national level. The research carried out for detecting anti-*T. gondii* IgA antibodies in women of reproductive age demonstrated the serological marker role that this test has in the diagnosis of acute infection in this population group.