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PhD THESIS

**SEROEPIDEMIOLOGY OF TOXOPLASMA GONDII
INFECTION IN PATIENTS WITH CARDIOVASCULAR
DISEASES IN WESTERN ROMANIA**

- ABSTRACT -

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ABSTRACT

Toxoplasma gondii has a worldwide distribution. Globally, it is estimated that one third of the human population is infected with *T. gondii*, and seroprevalence varies widely, between 0.5% and 87.7% (between countries and within a country) due to differences in the susceptibility of host, hygiene, dietary and cultural habits and climate (higher values were observed in low altitude areas with hot and humid climates). Information regarding the prevalence of *T. gondii* infection in patients with cardiovascular disease is extremely scarce. There was only one study conducted in Europe that identified a seroprevalence of 68% in patients with chronic heart failure from Turkey. In Romania, there are no data regarding the seroepidemiology of *T. gondii* infection in patients with cardiovascular diseases.

T. gondii infection in immunocompetent individuals, often asymptomatic, may sometimes present as myocarditis. Among cardiac complications of toxoplasmosis are listed arrhythmias (atrial and ventricular), pericardial effusions, cardiomyopathy, constrictive pericarditis, heart failure, and cardiac arrest. Studies conducted to date suggest that patients with a history of cardiovascular disease are more susceptible to *T. gondii* infection.

T. gondii triggers the development of an immune response in the body of the infected person, the titers of specific anti-*T. gondii* antibodies (type IgM, IgA, IgE and IgG) being detectable by serological tests, which can thus confirm the diagnosis.

This research evaluated for the first time the seroprevalence and potential risk factors associated with *T. gondii* infection among patients with cardiovascular diseases from five counties located in Western Romania (Arad, Bihor, Caraș-Severin, Hunedoara and Timiș), as well as the possible association between *T. gondii* infection and cardiac involvement. *T. gondii* seroprevalence was evaluated using the serological tests.

This doctoral thesis is structured in two parts: the general part and the special part.

The general part includes general data related to *T. gondii*: history, life cycle (in definitive hosts and intermediate hosts), transmission routes and risk factors, particularities of clinical manifestations of *T. gondii* infection (with emphasis on cardiovascular manifestations). Updated data regarding epidemiology of *T. gondii* infection in cardiovascular patients and blood donors are also presented.

The special part includes the objectives of the research, the materials and methods used, the results obtained and their integration in the general context of the information currently available in the international specialized literature, by comparing them with the results of the research conducted in different areas of the world. The case-control study (part of the present research) investigated the possible association between *T. gondii* infection and cardiovascular diseases, by comparing the results obtained in the group of cardiovascular

patients with those observed in the group of blood donors. The answers given by the study participants in the questionnaires specially created for this research were analyzed to evaluate the potential risk factors associated with *T. gondii* infection in cardiovascular patients.

The conclusions are presented in the last chapter of this thesis and underline the original contributions, the impact the results of this research can have on cardiovascular patients, the issues that have not been fully elucidated, while offering suggestions for future studies to support, develop and validate the results obtained so far.

Research objectives

Three main objectives were defined that governed this research:

- evaluation of the seroprevalence of anti-*T. gondii* antibodies (IgG and/or IgM) in patients with cardiovascular diseases from the Arad, Bihor, Caraș-Severin, Hunedoara and Timiș counties, establishing the acute or chronic status of *T. gondii* infection;
- identification of potential risk factors associated with *T. gondii* infection in patients with cardiovascular diseases;
- evaluation of a possible association between cardiac involvement and *T. gondii* infection.

Seroepidemiology of toxoplasmosis in patients with cardiovascular diseases from Western Romania

The evaluation was carried out in 1205 patients with cardiovascular diseases (aged between 19 and 94 years) admitted to the Institute of Cardiovascular Diseases Timișoara. To evaluate the potential risk factors associated with *T. gondii* infection, the answers provided by the study participants in the questionnaire specially created were analyzed. The sera of the study group were first tested for the presence of anti-*T. gondii* IgG and/or IgM antibodies, using the latex agglutination test. Positive sera were then tested for IgM antibody titer using the enzyme-linked fluorescence assay. In case of a positive result, IgG avidity was determined.

Seroprevalence of anti-*T. gondii* antibodies IgG and/or IgM was 52.12% (628/1205) in the group of cardiovascular patients. Of the 628 sera that were identified with anti-*T. gondii* antibodies present, 11 (1.75%) were identified with detectable titers of anti-*T. gondii* antibodies IgM. Testing for IgG avidity revealed increased avidity in all 11 cases with anti-*T. gondii* IgM type present.

The seroprevalence of anti-*T. gondii* antibodies increased with age in the study group, from 26.32% (5/19) in the age group 19-29 years to 59.77% (52/87) in the group of patients aged 80 years and more. Of the 502 cardiovascular patients from rural areas, 57.57%

(289/502) were identified with anti-*T. gondii* antibodies present. Among patients from urban areas the seroprevalence of anti-*T. gondii* antibodies was 48.22% (339/703). The seroprevalence of anti-*T. gondii* antibodies was 56.34% (240/426) in female cardiovascular patients and 49.81% (388/779) in males. The highest seroprevalence of anti-*T. gondii* antibodies was observed in the group of patients with dilated cardiomyopathy (66.66%) and the lowest among adults diagnosed with myopericarditis (30.00%).

Univariate analysis identified a significant association between *T. gondii* seropositivity and age, area of residence, sex, educational level, occupational status, owning cats, owning dogs, owning pets and toxoplasmosis awareness. Multiple logistic regression analysis of all these factors showed that only area of residence, sex, educational level, owning dogs, owning pets (cats and/or dogs) and toxoplasmosis awareness remained significantly associated with *T. gondii* seropositivity (**Table 1**).

Table 1. Evaluation of potential risk factors for *Toxoplasma gondii* infection by multiple logistic regression

Variables	OR (95% CI)	p
Area of residence		
Urban	1 (Ref.)	-
Rural	1,33 (1,04-1,69)	0,02
Sex		
Male	1 (Ref.)	-
Female	1,30 (1,01-1,66)	0,03
Educational level		
Primary/middle school	3,04 (1,83-5,03)	<0,001
High school	2,89 (1,83-4,54)	<0,001
University	1 (Ref.)	-
Owning dog(s)		
no	1 (Ref.)	-
yes	0,60 (0,40-0,90)	0,01
Owning pets: cat (cats) and/or dog (dogs)		
no	1 (Ref.)	-
yes	2,80 (1,59-4,93)	<0,001
Toxoplasmosis awareness		
no	1 (Ref.)	-
yes	0,62 (0,43-0,87)	0,007

The results revealed an increased prevalence of *T. gondii* infection in cardiovascular patients from Romania, with significantly higher seroprevalence in patients from rural areas and female patients. More than 80% of the study participants had no minimal information related to *T. gondii* and more than half of them were identified with anti-*T. gondii* antibodies present.

Evaluation of the seroprevalence of anti-*Toxoplasma gondii* antibodies in patients with cardiovascular diseases from Western Romania: a case-control study

In this study were evaluated 517 individuals: 256 patients diagnosed with cardiovascular diseases (included in the study group) and 261 blood donors (included in the control group). Study participants enrolled in the two groups (study group and control group) were selected so that there were no significant differences in age and gender between the 2 groups. For the simultaneous detection of the presence of anti-*T. gondii* antibodies IgG and/or IgM, the latex particle agglutination test was used.

Of the 256 cardiovascular patients included in the study group, 164 (64.06%) were identified with anti-*T. gondii* antibodies present. In the control group, of the 261 blood donors, 138 (52.88%) tested positive for anti-*T. gondii* antibodies.

In the group of patients with cardiovascular diseases, of the 141 individuals from urban areas, 56.74% (80/141) had detectable anti-*T. gondii* antibodies. Of the 115 patients from rural areas, 73.04% (84/115) were identified with anti-*T. gondii* antibodies present. In the control group, the analysis of the seroprevalence of anti-*T. gondii* antibodies according to the area of residence showed that of the 180 blood donors from urban areas, 49.44% (89/180) had detectable anti-*T. gondii* antibodies. Of the 81 blood donors from rural areas, 60.49% (49/81) were identified with anti-*T. gondii* antibodies present.

The seroprevalence of anti-*T. gondii* antibodies was 51.80% (43/83) in female cardiovascular patients and 69.94% (121/173) in male cardiovascular patients. In the blood donors' group, 47.87% (45/94) of women were identified with anti-*T. gondii* antibodies present and 55.69% (93/167) of men tested positive for anti-*T. gondii* antibodies.

In both cardiovascular patients and blood donors was observed an increasing trend of *T. gondii* seroprevalence with age. No significant difference in *T. gondii* seroprevalence was observed between the two groups (study and control groups) within the same age group. The seroprevalence of anti-*T. gondii* antibodies was higher in cardiovascular patients (56.74%; 80/141) compared to blood donors (49.44%; 89/180), with no significant difference ($p=0.21$). A similar result was observed in study participants from rural areas: the seroprevalence of anti-*T. gondii* antibodies was higher in cardiovascular patients (73.04%; 84/115) compared to blood donors (60.49%; 49/81), but the difference was not statistically significant ($p=0.06$). In the group of male study participants, the seroprevalence of anti-*T. gondii* antibodies was significantly higher in cardiovascular patients (69.94%; 121/173) compared to blood donors (55.69%; 93/167) ($p=0.006$). However, in the group of female study participants, no significant

difference was observed between the seroprevalence of anti-*T. gondii* antibodies in cardiovascular patients (51.80%; 43/83) and blood donors (47.87%; 45 /94) (p=0.6).

Statistical analysis showed a significantly higher seroprevalence of anti-*T. gondii* antibodies in the group of patients with unstable angina (67.56%; 50/74; p=0.02) and in the group of those diagnosed with arterial hypertension (82.35%; 14/17; p=0.01) compared to the control group.

The results of this case-control study show that the seroprevalence of anti-*T. gondii* antibodies was significantly higher in patients diagnosed with hypertension and unstable angina, suggesting that people diagnosed with these cardiovascular diseases may be more frequently infected with *T. gondii*.

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

In the international literature, few data are presented regarding the seroepidemiology of *T. gondii* infection in patients diagnosed with cardiovascular diseases. This scientific paper is a premiere for the medical community in terms of the new and important information it presents. For the first time, was evaluated the seroepidemiology of *T. gondii* infection in patients with cardiovascular diseases from five counties located in Western Romania (Arad, Bihor, Caraș-Severin, Hunedoara and Timiș). In addition, was assessed for the first time the potential association between *T. gondii* infection and cardiovascular disease. Therefore, all the objectives of this doctoral thesis were fulfilled.

The general conclusions that can be formulated by analyzing the results presented in this scientific paper are the following:

- more than half of the cardiovascular patients from Western Romania were identified with anti-*T. gondii* antibodies present;
- the highest seroprevalence of anti-*T. gondii* antibodies was observed in patients with dilated cardiomyopathy (66.66%) and the lowest in those with myopericarditis (30.00%);
- area of residence, sex, educational level, owning dogs, owning cats and/or dogs, and toxoplasmosis awareness remained significantly associated with *T. gondii* seropositivity after performing multiple logistic regression in cardiovascular patients from Western Romania;

- cardiovascular patients from rural areas have a 1.33 times higher risk of infection with *T. gondii* compared to those from urban areas;
- women with cardiovascular disease have a 1.30 times higher risk of infection with *T. gondii* than men diagnosed with cardiovascular disease;
- the risk of infection with *T. gondii* is 3.04 times higher in cardiovascular patients who only graduated primary/middle school and 2.89 times higher in those who graduated high school compared to those who reported having university studies;
- cardiovascular patients who reported owning pets (cat and/or dog) are 2.80 times more at risk of *T. gondii* infection compared to those who reported not owning such animals;
- most cardiovascular patients (86.22%) do not have basic knowledge related to toxoplasmosis and more than half of them (53.99%) were identified with anti-*T. gondii* antibodies present;
- the seroprevalence of anti-*T. gondii* antibodies was significantly higher in patients with unstable angina (67.56%) and those with hypertension (82.35%) compared to blood donors;
- the seroprevalence of anti-*T. gondii* antibodies was significantly higher in men with cardiovascular diseases (69.94%) compared to the seroprevalence observed in male blood donors (55.69%).

The results presented in this scientific paper represent a valuable starting point for future research to evaluate more detailed the impact of *T. gondii* exposure on the cardiovascular system and to elucidate the causal relationship between toxoplasmosis and heart disease.

This scientific paper is of utmost importance by identifying the risk factors associated with *T. gondii* infection, information that can be used to create effective prevention and control programs. Moreover, there is a need to increase toxoplasmosis awareness which could be achieved by widely distributing information related to the mode of infection with *T. gondii* and the potential risk that infection with this parasite poses to the cardiovascular system.