

A decreasing trend in *Toxoplasma gondii* seroprevalence among pregnant women in Romania - results of a large scale study

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Abstract. Toxoplasmosis is a zoonotic infection caused by the obligate intracellular apicomplexan parasite *Toxoplasma gondii* (*T. gondii*). *T. gondii* infection is a cause of congenital infection worldwide. Primary infection or the reactivation of latent infection during pregnancy may lead to fetal infection and to congenital toxoplasmosis syndrome. Seropositive pregnant women are generally protected from maternal-fetal transmission of *T. gondii*, although exceptions exist. The aim of our study was to analyze the dynamics of *T. gondii* seroprevalence during a 10-year period and to correlate it with age and demographic features of pregnant women. We tested 6,889 pregnant women in Timisoara, Romania, for IgG-anti-*T. gondii* antibodies, in two successive periods: i) 2008-2010 (group 1: 1,457 participants); and ii) 2015-2018

(group 2: 5,432 participants). For each participant, data on age and area of residence were collected. Our results showed that in the Western Region of Romania *T. gondii* seroprevalence in pregnant women declined from 43.79 to 38.81% in the last ten years. This trend was observed in both urban (40.53 vs. 34.85%) and rural areas (52.22 vs. 46.22%). A higher seroprevalence rate was found in rural than in urban areas. In addition, we found an increasing tendency of seroprevalence related to the age of pregnant women.

Introduction

Toxoplasmosis is a zoonotic infection caused by the obligate intracellular apicomplexan parasite *Toxoplasma gondii* (*T. gondii*) (1,2). *T. gondii* can infect both birds and mammals (3). However, the only hosts where the parasite's sexual reproduction takes places are the members of the Felidae family: Both domestic and wild cats (4).

Scientific reports showed that around one third of global population is infected with *T. gondii* (4). The contamination could occur by ingestion of sporulated oocysts present in contaminated soil, water or food (5). Ingestion of raw or undercooked meat from infected animals is the primary source of infection (5). Vertical transmission from infected mother to fetus is also possible (6), as well as transmission via blood products and graft tissue (7). A large European study found that traveling outside Europe and the United States of America or Canada is also considered a risk factor predictive of acute infection in pregnant women besides the ingestion of undercooked meat (lamb, beef, game), contaminated water or contact with contaminated soil (8).

T. gondii is a parasite with specific strategies to avoid the host's immune defenses and to establish a life-long infection that may influence in various modes the infected hosts (9). In

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immunocompetent persons infections are merely asymptomatic and self-limiting. In immunocompromised individuals and in the developing fetus, however, *T. gondii* may cause life-threatening infections (10). Congenital toxoplasmosis is caused by transplacental infection of the fetus. This could determine a spontaneous abortion, fetal demise, intrauterine growth restriction, hydrocephalus, encephalitis, neurological, ocular or auditive diseases, inflammation or cardiovascular diseases (6,11). Also, infection in pregnancy could be associated with preterm birth (12). The risk of vertical transmission is correlated with the gestational age at the time of infection. It is approximately 15% at 13, 44% at 26, and 71% at 36 weeks of pregnancy (13). Even if the risk of fetal infection is directly correlated with gestational age, the severity of fetal damage inversely correlates with gestational age, at the time the infection occurs (14).

The global incidence of congenital toxoplasmosis is approximately 190,000 cases each year with a rate of 1.5 cases/1,000 births. The incidence varies in different regions from 0.5-1.6/1,000 births in Europe, 0.4-3.4/1,000 births in America to 2.0-2.4/1,000 births in Africa (15,16).

The global prevalence of toxoplasmosis is estimated between 25-30% (4,15). Within low prevalence areas (between 10-30%) are: North America, North of Europe, South-East Asia and Sahelian Africa (16). Areas with a moderate seroprevalence (30-50%) are Central and South of Europe (16). High seroprevalence areas are Latin America and Tropical Africa (15,16).

Childbearing and pregnant women from different geographical zones showed different seroprevalence between 9% in the United Kingdom (17) and 48.7% in Bruxelles, Belgium (18). In 2015 in Romania, a study by Olariu *et al* (19) showed a seroprevalence of 57.6% in women at childbearing age (20).

The aim of our study was to analyse the dynamics of *T. gondii* seroprevalence during a 10-year period and to correlate it with age and demographic features of pregnant women.

Patients and methods

Study design, settings and patients. A cross-sectional study involving 6,889 adult pregnant women was performed in Timisoara, Romania in two successive periods: i) 2008-2010 (group 1: 1,457 patients at City University Emergency Hospital, Timisoara, Romania); and ii) 2015-2018 (group 2: 5,432 patients at Bioclinica Laboratory srl, Timisoara, Romania). Patients were enrolled according to a consecutive-case population base. We collected data for each patient, namely age and area of residence.

Ethical issues. Approval from the Institutional Board of the 'Victor Babeş' University of Medicine and Pharmacy (Timisoara, Romania; approval no. 848/06.04.2011) was obtained to perform this study. The present study meets the ethical guidelines, including adherence to the legal requirements of the study country. Informed consent was obtained from each patient.

Serological testing. The IgG- anti-*T. gondii* antibodies titer was determined by the immune-chemiluminescence method using

Table I. Demographic characteristics of participants.

Characteristics	Group 1 (2008-2010) n=1,457	Group 2 (2015-2018) n=5,432
Area of residence [n (%)]		
Urban	1,051 (71.15)	3,541 (65.19)
Rural	406 (27.87)	1,891 (34.11)
Age (years)	28 (6) ^a	29 (7) ^a

^aP<0.001.

an Immulite One Machine (Diagnostic Products Corporation) and commercial tests (Siemens Healthcare Diagnostics Products) for group 1 patients (2008-2010) and by chemiluminescent microparticle immunoassay (CMIA) method using an Architect i1000SR engine (Abbott) and commercial tests (Abbott) for group 2 patients (2015-2018). According to cut-off values we stratified patients into two categories: Those with positive test values of IgG antibodies; and those with negative or inconclusive IgG test values.

Statistical analysis. Data were stored in the Astraia database (Astraia Software GmbH) and Microsoft Office Excel (Microsoft Corporation). InStat GraphPad Prism Software (GraphPad Software, Inc.) was used for statistical analysis. The results are presented as medians and (interquartile ranges). Mann-Whitney-U, respectively Fisher's exact tests (proportions) were used to assess the differences between groups. Cochran-Armitage test (Chi-square test for trend) was used to evaluate the association between seroprevalence rate and age of pregnant women. P<0.05 was considered to indicate a statistically significant difference.

Results

Demographic characteristics of pregnant women. Among women in group 1, 71.15% (1,051/1,457) declared to be from urban and 27.85% (406/1,457) from a rural area, while of women in group 2, 65.19% (3,541/5,432) were from urban and 34.11% (1,891/5,432) from rural areas (Table I).

Pregnant women from group 1 (2008-2010) were younger than those from the group 2 (2015-2018): 28 years (n=6) vs. 29 years (n=7), (P<0.001; Table I).

Seroprevalence results. Of the 1,457 women included in group 1, 638 (43.79%) were tested positive, 812 (55.73%) negative and 7 (0.48%) inconclusive, while of the 5,432 women included in the group 2, 2,108 (38.81%) were tested positive, 3,210 (59.09%) negative and 114 (2.10%) inconclusive for the presence of IgG-anti-*T. gondii* antibodies.

Women from urban areas showed lower seroprevalence rates compared with those from rural areas: Group 1 (2008-2010) 40.53 vs. 52.22% (P<0.001) or group 2 (2015-2018) 34.85 vs. 46.22% (P<0.001; Table II).

A decreasing trend was found of IgG-anti-*T. gondii* seroprevalence in pregnant women. In the first tested period

Table II. Seroprevalence to *Toxoplasma gondi* according to the area of residence and age of pregnant women.

Variables	Group 1: 2008-2010, n=1,457	Group 2: 2015-2018, n=5,432	P-value
Overall seroprevalence	638 (43.79%)	2,108 (38.81%)	<0.001
Area of residence			
Urban	426/1,051 (40.53%)	1,234/3,541 (34.85%)	<0.001
Rural	212/406 (52.22%)	874/1,891 (46.22%)	<0.05
P-value	<0.001	<0.001	
Age interval, years			
18-26	216/527 (40.99%)	606/1,595 (37.99%)	
27-35	346/836 (44.98%)	1,172/3,078 (38.07%)	
≥36	46/94 (48.94%)	330/759 (43.47%)	
P-value	0.074	0.035	

the seroprevalence was found to be 43.79% compared with 38.81% in the second tested period ($P<0.001$). This trend was observed in pregnant women from both urban [40.53 vs. 34.85% ($P<0.001$)] and rural [52.22 vs. 46.22% ($P=0.02$)] areas (Table II).

We found an increasing tendency of seroprevalence with the age of pregnant women, thus women older than 36 years had a higher seroprevalence rate than women aged 27-35 years or younger than 26 years. The difference was observed in both groups, however a significant value was found only in the second group ($P=0.035$), while in the first group the significance was borderline ($P=0.074$; Table II).

Discussion

We found that 62.3 and 56.26% of pregnant women from group 1, and respectively from group 2 were susceptible to a primary *T. gondii* infection in our region. In low seroprevalence regions the risk of contacting an infection is low but if a contamination occurs, the risk of primary infection is high. On the contrary, in high prevalence regions the risk of a pregnant women to contract a primary infection is low because the majority of pregnant women are seropositive. However, in the context of iatrogenic immunosuppression or of a disease that causes immunosuppression, a reinfection may occur. Also, contamination with other *T. gondii* strains may lead to a reinfection in an immunocompetent organism (21).

The global *T. gondii* seroprevalence in the general population is approximately 25-30% (4,15). Studies from the United States of America (USA) showed that the prevalence in the USA has continued to decline from 14 to 23% (1988-1994), to 9% (1999-2004) and 6% (2009-2010) (22,23). Research from other regions showed a general seroprevalence approximately 44% in France (24), between 51 and 72% in Latin America and between 5 and 35% in Asia, China and Korea (25).

Data on childbearing age women showed a seroprevalence of 9% in Great Britain (17), between 16 and 29% in Spain (26), approximately 24% in North of Portugal (27), 29.1% in Croatia (28), 44% in France (29) and 19% in Italy (30). Other studies showed a seroprevalence of 9.1% in 2009 in the USA (22). Thus, showed that pregnant women from our region

fit in a moderate level of seroprevalence. However, in Europe only women from France showed a higher seroprevalence (29).

A study conducted by Olariu *et al* in 2008 showed a seroprevalence of 57.6% in women of childbearing age (19). Our results showed a decreasing trend in the seroprevalence among pregnant women from our region from 43.72% (2008-2010) to 38.70% (2016-2018). Likewise, a decrease in the seroprevalence rate was found in the USA from 14.9% (1988-1994), to 11.1% (2004) and to 9.1% (2009-2010) (22). Studies by Villena *et al* reported in France a decrease in seroprevalence in pregnant women from 66% (1980), to 54% (1995) and 44% (2003) (29). Similarly, researchers from Netherlands showed a decline in seroprevalence in pregnant women from 32.5% (1995-1996) to 18.5% (2006-2007) (31). Data on seroprevalence from many other European countries showed a similar decreasing trend: Austria [from 48% (1970) to 31-35% at present] (32,33), Italy [from 31% (2001) to 21% in (2005)] (15), Portugal [from 31.4% (2005) to 24.4% (2010)] (30), and Poland [from 41.8% (2004) to 37.8% (2012)] (34).

The decreasing course of seroprevalence was found in both women from rural (52.22 vs. 46.22%) and urban areas (40.53% vs. 34.85%) and could be explained by an improvement of socioeconomic and hygiene condition in the past ten years.

Our study showed a higher *T. gondii* seroprevalence in pregnant women from rural compared with women from urban areas, in both groups: Group 1 (52.22 vs. 40.53%) and group 2 (40.53 vs. 34.85%). Such results are in line with a previous study by Olariu *et al* which established the same trends in women (76 vs. 55.3%) (35) and in children (18.4 vs. 14.7%) (36). Other countries showed also higher seroprevalence rates in people from rural compared with urban areas: Mexico 32 vs. 12% (37) or Argentina 36.4 vs. 26.8% (38). This variation can be attributed to the lower socio-economic level in some rural areas compared with urban ones, as well as to the less easy access to some services, such as running water, in some of these areas. An epidemiological profile with an increased risk of seropositivity for *T. gondii* is represented by a lower educational status, a lower economic level, without an occupation outside the household, and people living in a household without running water resources. These risk factors are more common in rural and suburban areas, areas with poor

socioeconomic conditions with more frequent contact with animals, and eating habits, which may explain the association with a higher seroprevalence (39,40).

Our results confirm the studies of Vilibic-Cavlek in Croatia and Olariu in Romania, showing an age-related increase in seroprevalence (28,35).

It should be noted that a limitation of the study could be the fact that the two institutions in which the patients were tested might be slightly different in accessibility. The clinical hospital is a public institution while the Bioclinica laboratory is a private institution. This might, theoretically, indicate a different social structure of the patients tested in the two institutions/groups: 2008-2010 and 2015-2018. However, we consider that this aspect could be negligible because in both institutions the tests were done at a similar cost, so that the effect of patient selection on economic and social criteria is probably minimal. Future studies should, therefore, confirm this decreasing trend in seroprevalence for *T. gondii*. We include screening for TORCH agents along with first and second trimester screening in antenatal care program (41-43).

In conclusion, in the Western Region of Romania *T. gondii* seroprevalence in pregnant women has declined from 43.79 to 38.81% in the past ten years. This trend was found in both urban (40.53 vs. 34.85%) and rural areas (52.22 vs. 46.22%). A higher seroprevalence rate was found in rural than in urban areas. In addition, we found an increasing tendency of seroprevalence related to the age of pregnant women. Future studies should, therefore, confirm this decreasing trend in seroprevalence for *T. gondii*.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the first author on reasonable request.

Authors' contributions

DBN, SM, DN, CM, DB and TVC were involved in the conception of the study and the interpretation of the data. DM, DBN, SM, IC, FG, MC and AD contributed to the acquisition of the data and performed statistical analysis. IC performed serological testing. DBN, DN, IC, FG and TVC wrote the manuscript. SM, DM, MC, DB and AD revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study meets the ethical guidelines, including adherence to the legal requirements of the study country. The study was approved by the Institutional Board of the 'Victor Babes' University of Medicine and Pharmacy (Timisoara, Romania;

approval no. 848/06.04.2011). Informed consent was obtained from each patient.

Patient consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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