

Evaluation of *Toxoplasma gondii* seroprevalence among women of reproductive age in South Backa district, Serbia

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Introduction

Toxoplasmosis is caused by an intracellular protozoan *Toxoplasma gondii* (*T. gondii*). It has been estimated that up to one third of the world's human population are infected by *T. gondii*. This parasite can infect humans or any other warm-blooded animals. Consumption of undercooked meat and exposure to oocyst contaminated soil or water are the two primary risk factors for human infection.

Infections in humans are often asymptomatic or a flu-like illness or lymphadenopathy. Primary infection acquired during pregnancy may result in severe damage to the fetus.

The prevalence of *T. gondii* infection in pregnant women varies substantially among countries. The prevalence typically increases with age and is higher in warm and humid areas. In European countries, seroprevalence of *T. gondii* varies from less than 20% in Scandinavian countries and the UK to more than 60% in southern Europe. In many countries it has declined sharply over the past three decades. More women are susceptible to infection, and the frequency of exposure to risk factors for infection may have increased.

Some countries, such as France and Austria, have introduced special health policies regarding this parasitic infection. Serologic screening of pregnant women and long-term follow up of congenitally infected children are taken into consideration. Universal antenatal screening is currently not performed in Serbia. Toxoplasmosis diagnosis is performed as part of the control of immunological status of women who are sent for this type of test because of current or future pregnancies.

Diagnosis of toxoplasmosis in humans is made by biological, serological, histological, or molecular methods, or by some combination of the above. Acute and latent *T. gondii* infections are mostly diagnosed by serological tests including detection of anti-*T. gondii* specific IgM and IgG antibodies, and avidity of *T. gondii*-specific IgG antibodies.

The aim of the study was to estimate toxoplasmosis seroprevalence among women of reproductive age in South Backa District and was to point to the significance of the IgG avidity test.

Materials and Methods

From January 2007 to May 2011, the prevalence of anti-toxoplasmosis antibodies were examined retrospectively in a population of 2796 women (aged 16 to 45). Data were used from the Protocol of virological analyses, Center of Virology, Institute of Public Health of Vojvodina. The subjects were grouped according to diagnosis. The study group consisted of 1374 pregnant women (mean age 29.03), 306 women with one or more abortion (mean age 30.72), 449 women suspected of toxoplasmosis - these were patients with diagnoses of lymphadenopathy referred by clinic doctors where the use of differential diagnoses was recommended to confirm their illness or women with acute toxoplasmosis (mean age 31.75), and 667 women of control group - these were women of reproductive age who were enrolled during routine control of agents of congenital infection (mean age 29.51).

Serum collection. Blood samples were obtained by venipuncture from women during their visits to the Institute of Public Health of Vojvodina or collected from patients during hospital treatment. The samples were centrifuged and then stored at 4 °C until analysis.

Laboratory tests. In determination of the presence and levels of *T. gondii* specific IgM and IgG antibodies in serum samples, commercially available ELISA kits were applied. The test was performed according to the producer's instructions using an automatic ELISA device. Results were interpreted only in qualitative terms, as positive or negative. Positive sera to specific IgM antibodies

were retested. Commercial Toxoplasmosis IgG Avidity test was used to differentiate between recent exposure to *T. gondii* and immune responses existing as results of past exposure. This test was also performed on an automatic ELISA device and evaluated according the manufacturer's instructions. High avidity was considered as more than 60%, low avidity for less than 40%, borderline avidity between 40%-60%. The IgG avidity test included 18 pregnant women.

Results

During the five-year period, 2796 women of reproductive age, (aged 16-45 years, mean 29.7 years) were enrolled. The overall *T. gondii* seroprevalence in the examined population was 16.2%. On the basis of specific IgM antibodies, acute infection was confirmed in 3.4% of the women. Within the group of pregnant women, *T. gondii* seroprevalence was 14.2%, while 3.2% of the women were confirmed to have acute infection.

The presence of IgG antibodies was strongly influenced by age of the examined women ($p=0.000$), but not significantly by studied group ($p=0.219$). However, rates of IgM antibodies were significantly influenced by both examined groups ($p=0.009$) and age ($p=0.003$).

The women of reproductive age were stratified into six age groups: 16-20 years, 21-25 years, 26-30 years, 31-35 years, 36-40 years, 41-45 years to measure the prevalence of specific IgG and IgM antibodies. When analyzing these results, it was observed that the seroprevalence of IgG antibodies varied according to age ($p=0.000$). Seroprevalence ranged from low rates in the age group 16-20 years to the highest rates in age group 36-40 in all studied groups, except women with spontaneous abortion, where the highest seroprevalence was observed in the age group 31-35 years. However, IgM positivity was present in the younger age group (26-30 years) ($p=0.0029$).

In order to identify the time of infection of *T. gondii*, the IgG avidity test was performed in 18 pregnant women. High avidity IgG antibodies were diagnosed in 15 (83.4%) pregnant women, indicating that the infection was acquired in the distant past, and 3 (16.6 %) women had low-avidity IgG antibodies suggesting a recent infection.

Discussion

Comparing these results with a study done in the previous period from 1988 to 2007 for the territory of Serbia, we observed a decrease in seroprevalence of the *T. gondii* (39% vs 16.2%). It is probable that this decrease correlates with qualitative changes in hygiene and dietary habits, as well as with improvements in food preservation quality or changes in nutrition.

The possibility that toxoplasmosis may be an important cause of single or repeated abortion has been analyzed in many studies. In the present study, the overall prevalence of IgG seropositivity among women with spontaneous abortion was 20.4% for IgG and 6.8% for IgM. These findings are in agreement with a past study conducted in Serbia.

In the present study, the seroprevalence increased with age, from 12.0% in the group aged 16-20 years, to 37.0% in the group aged 41-45. The reason might be increasing risk of exposure to oocysts with age.

Clinical signs of toxoplasmosis are nonspecific and are not sufficiently characteristic for a definite diagnosis. Detection of *T. gondii* antibody in patients may aid diagnosis. Serological diagnosis of acute toxoplasmosis has traditionally been made by detection of specific IgM antibodies or by the demonstration of a significant increase in specific IgG antibody levels, or both. Because specific IgM antibodies can persist for several months, or even years in some individuals following acute infection, interpretation of serological tests might be troublesome when acute toxoplasmosis is suspected. The diagnosis of chronic stage of infection or of past exposure to *T. gondii* is made by detection of specific IgG antibodies and the absence of the acute-phase markers IgM and IgA.

Introduction in serodiagnosis of toxoplasmosis infections measurement of IgG avidity has proved to be a highly useful procedure, especially in combination with conventional serological assays.

Conclusions

- Seroprevalence of *T. gondii* in women of reproductive age (16-45) was 16.2% and was 14.2% in the group of pregnant women.
- A high level of seronegativity in women of reproductive age indicates a risk of acute infection during pregnancy and also the risk of congenital toxoplasmosis.
- Based on the presented data, we proposed a health education program together with serological testing for all women of reproductive age.
- In order to identify the time of infection is necessary to perform *T. gondii* avidity test, which is important for pregnant women.

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